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EXPERIMENTS ON INDUCED BREEDING OF THE GREY MULLET, *MUGIL CEPHALUS LINNAEUS**

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and Mou-Chun Lin³⁾

Abstract

This paper is the sixth report of the Working Team on Artificial Propagation of Mullet. The team consisted of the research personnel of several fisheries institutions in Taiwan and was first organized in 1963. The site for work in the 1969-1970 season was Tungking Marine Laboratory (also known as Tungking Shrimp Culture Center) in south Taiwan.

The grey mullet, *Mugil cephalus* Linnaeus, is one of the important commercial fishes in Taiwan, and is especially valued for its roe. For stocking in fresh and brackish water ponds, the fingerlings are normally caught from estuarine waters along the west coast of the island from December to March. Recently, the catch of fingerlings from natural waters has been poor and a shortage of supply resulted. In order to produce fingerlings by artificial propagation, the team has carried out experiments for six years during the mullet spawning season. Significant success was achieved in the experiment from November 1969 to February 1970. A total of 431 hatched larvae were obtained, measuring 3.28 cm and weighing 0.34 g on the 45th day. They were about 1.5 times larger than the fingerlings normally collected from natural waters and were robust and strong. Some of them were stocked in fish ponds and grew to 28.1 cm and 217.4 g by the 200th day.

1. Introduction

The grey mullet is one of the important commercial fishes in Taiwan. Mulletts, mainly of the four and five year class measuring 42-45 cm, migrate southward for spawning in schools. After entering the Taiwan Strait they

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head towards the middle region of the west coast of Taiwan and then proceed southward along the coast line. During this period from December to January, they are captured and the roe is dried for local consumption and export to Japan.

Mullet fingerlings are caught from estuaries along the west coast during the period from December to March and are used for stocking either fresh or brackish water ponds. In recent years, the catch of mullet fingerlings from estuarine waters has been poor and mullet farmers often face the problem of shortage of mullet fingerlings for stocking their ponds. In order to supply enough fingerlings to fish farmers, a team of workers consisting of personnel from the Taiwan Fisheries Research Institute, Institute of Fishery Biology of National Taiwan University and Taiwan Fisheries Bureau, have carried out experiments on the artificial propagation of mullet each winter since 1963. The work was first carried out at Sanwei, Kaohsiung Hsien. A research fellow of the Rockefeller Foundation Fish Culture Research Project in Taiwan joined the team in 1968. Dr. S. W. Ling, FAO Regional Fish Culturist for Asia & Far East, participated in the experiment in 1968/69 and 1969/70 seasons when the work was shifted to the Tungkang Marine Laboratory (also known as Tungkang Shrimp Culture Center). In the winter of 1969, Dr. Z. H. Shehadeh of the Oceanic Institute, Makapuu Oceanic Center, Hawaii, U. S. A. came and worked with the team under a Sino-US Science Cooperation Project.

In the first and second seasons of research, the work was mainly on selection, transportation and impoundment of the spawners and hormone treatment (Tang *et al.*, 1964; Team of Artificial Propagation of Mullet, 1965). In the third season, rearing of larvae was started (Team of Artificial Propagation of Mullet, 1966). In the next two seasons, effort was made to improve the method of rearing larvae and controlling environmental factors (Team of Artificial Propagation of Mullet, 1967; Liao *et al.*, 1969). Finally, in the fifth season of the experiments, i.e. from December 1968 to February 1969, 2 larvae survived for 30 days; they measured 1.0 and 1.1 cm and were covered with scales. The reason for their death was the lack of adequate warming equipment, with the result that they died when the water temperature dropped suddenly (Liao *et al.*, 1969).

In the sixth season, 19 spawners received hormone treatment, of which 12 ovulated successfully and the eggs of 9 of them hatched. A total of 431 larvae survived and measured 3.28 cm and weighed 0.34 g on the 45th day.

They were about 1.5 times larger than those collected from natural waters. These were robust and strong, suitable for stocking.

2. Materials and Methods

2.1 Spawner

A special raft (7.55 m x 1.34 m), constructed of 8 plastic pipes (16.5 cm in diameter) and fitted with an outboard motor of 113 cc (Mitsubishi Model S6), was used for going out to buy uninjured mullet from the catches of professional fishermen. To minimize injury to the fish, the fishermen were asked to release the fish gently into rectangular bags made of plastic pipe frames lined with nylon cloth. The technicians from the Laboratory selected the male or female fish in the bags and examined the stage of maturity. If they decided to buy the fish, they put it in a thick blue or black plastic bag of 60 cm x 90 cm. One to three spawners were held in each bag, which was filled with 30 liters of sea water and then inflated with oxygen. The bags with fish were then taken ashore to the stock tanks.

2.2 Stock tanks

Of the four outdoor tanks of 5m x 7m x 1.5m in size, one was divided into two parts with a nylon net for holding the spawners after hormone treatment. As required, the spawners were taken out for examination of their condition. Fresh sea water was pumped in with a 2-horse-power motor through a filter pipe inserted about two meters below the surface of the beach about 10 meters from the tide line. The flow speed in each tank was about 50 l/min.

2.3 Hormone treatment

According to the experience of the previous five years (Tang *et al.*, 1964; Team of Artificial Propagation of Mullet, 1965, 1966, 1967; Liao *et al.*, 1969), it had been found best to give the first injection within one hour after stocking in the tanks and the second injection within the next twenty-four hours. The total dosage for each fish was 2.75 to 5 mullet pituitary glands mixed with 20 to 50 Rabbit Units of Synahorin. In addition, most of the spawners received 50 to 150 mg of Vitamin E. Some of the spawners were placed in 50 ppm aureomycin solution for 40 minutes for sterilization.

The pituitary glands used were collected from large matured male and gravid female mullets soon after capture or immediately after death. The pituitary glands were preserved in acetone and stored in a refrigerator.

2.4 Egg collection and artificial fertilization

Usually the eggs expelled by the spawner within 40 to 50 hours after the first injection had more chance to be successfully fertilized (Liao *et al.*, 1969). To determine as closely as possible the ovulating time, the spawners were netted and examined every one to two hours. In carrying out artificial propagation, the mullet lost scales easily and became too weak to tolerate repeated manipulations during its breeding season. Therefore, they could not be kept for more than three days after hormone treatment.

The best response to hormone treatment was found in one spawner whose belly became extended in little more than ten hours after the first injection. On touch by hand, the belly felt loose and soft. When it was ready to ovulate, slight pressure on the anus would make the eggs flow from the genital pore. The ideal case was the so-called "water eggs", i.e., a large amount of eggs flowing out at the moment the spawner was caught. Eggs obtained in either of these ways were suitable for artificial fertilization. To strip the fish, one person held the tail of fish with his left hand and the trunk between his right arm and body, wiped the water from the fish body and pointed the fish head obliquely upward with the abdomen downward. A second person lightly squeezed the abdomen from the pelvic fin to the anus with his left hand and held a plastic basin with his right hand to receive the eggs. If the fish was ready, eggs would flow out. It would be best to have a third person hold the snout of the fish at the same time.

Fertilization was generally carried out by the dry method. Stripping the milt was done in a manner similar to stripping the ova. However, the male mullet was always smaller in size so one person could hold and squeeze it to let the sperms fall onto the eggs in a plastic basin. Meanwhile, a second person mixed the eggs and sperms carefully with a goose feather and rinsed them several times with sea water. Fertilization was then completed. Sperms in water do not survive for more than one minute. Therefore quick, skillful action was needed.

2.5 Incubation patterns

Hatching was done in round plastic tanks of 0.5 and 1.0 ton capacity in flowing and stagnant water. In the flowing water type of hatching, two to three conical hatching nets were hung in the water, and their tail ends connected with a plastic pipe to supply the continuous upward flow of fresh sea water so that the fertilized eggs would continue to roll in the net. In the other type of hatching, the tanks were aerated, and once the water became stale, it was changed. Usually when there were fertilized eggs in

the tanks, a siphon pipe was used to let water in and out at the same time so that water quality would not change sharply.

2.6 Rearing of larvae

The larvae were reared both indoors or outdoors. Plastic tanks of 0.5 ton were used indoors and large tanks of 5m x 7m x 1.5m (actual water depth 0.6 to 1.0 m) outdoors. The latter were covered with plastic plates to prevent sudden changes of temperature. Water in the rearing tanks was refreshed gradually.

2.7 Food for larvae

Prepared materials such as cooked egg yolk, soy-bean milk, ground pig liver, flour, milk powder, albumin from eggs, fish meal, yeast and artificially fertilized oyster eggs and trochophore larvae as well as rotifers, copepods, *Artemia* nauplii were used as food for the larvae.

3. Results

3.1 Spawners

The first spawner was obtained on December 19, 1969 and the 19th one on January 11, 1970. They weighed from 1.8 kg to 2.5 kg and measured 51.5 cm to 61.0 cm. Twelve of them ovulated, and the eggs from 9 of the 12 hatched with a percentage of 47.4% (Table I) which is more than four times higher than the 10% of the previous year (Liao *et al.*, 1969).

Table I The response of spawners to hormone treatment.

Response	Ovulated and eggs hatched	Ovulated but eggs not hatched		No ovulation			Mistaken in sex identification	Total
		Fertilized but no cleavage	Unfertilized	Responsive but not spawning	Died from injury	No response		
Number of treated spawner	9*	1	2	0	3	2	2	19
%	47.4	5.3	10.5	0	15.8	10.5	10.5	100

* Including one fish which was found to have spawned naturally in the tank and the remaining eggs of which were artificially fertilized and hatched well.

Also shown in Table I, three spawners died of internal injury and the sex of two was wrongly identified. The workers were successful in keeping most of the spawners alive with the help of accumulated experience.

3.2 Hormone treatment

Twelve spawners out of 19 ovulated after hormone treatment, making a high percentage of 63.2%. Among these, the eggs of 9 spawners hatched (one ovulated naturally and the eggs were artificially fertilized). Two spawners did not show any response at all to hormone treatment.

Table 2 shows the dosage of injection and response of the 12 spawners. Spawner No.1 showed the slowest response. It took 67 hours and 15 minutes after the first injection to achieve ovulation. Spawner No.2 showed the most rapid response. Spawner No.3 received the least hormonal material.

In general, most of the fish received hormone treatment of 3 to 3.5 mullet pituitary glands combined with 30 RU Synahorin. All of them except No.11 received an injection of Vitamin E. The 9 which hatched all received injection of 150 mg Vitamin E. It took 40 to 51 hours for spawners other than No.1 and No.2 to respond. It took 67 hours for No.1 and 31 hours for No.2.

The time between two injections was 19 to 24 hours in spawners No.1 to No.14. It was shortened to 12 hours in No.16 and 19. The time it took to obtain eggs was not shortened.

3.3 Egg collection and fertilization

As shown in Table 2, it took 40-50 hours for spawners other than No.1 and No.2 to release the eggs. Spawner No.2 ovulated in the stock tank 30 hours and 40 minutes after the first injection and was caught for artificial fertilization within the next 15 minutes. However, more than 85% of the eggs had been expelled and could not be fertilized.

Fertilization rate varied from 20 to 90%, averaging less than 70%. The low rate was due to the quality of the eggs themselves.

3.4 Hatching

As shown in Table 2, hatching rate of No.9 and No.10 was as low as 5%, because inadequate aeration during the late period of hatching caused the oxygen content to become low. Generally, the hatching rate was high.

Water temperature was quite stable. Therefore, the time it took to hatch seldom varied. It took 34-38 hours to hatch at water temperature of 23-24.6°C and 49-54 hours at 22.5-23.7°C, with salinity of 30.1-33.8‰ in both cases.

3.5 Larvae and rearing of larvae

The newly hatched larvae measured 2.08-3.40 mm and were unable to swim actively; however, they were capable of weaving or making darting

Table 2 The response of spawner to hormone treatment, ovulation and hatching.

Individual number	Injection				Ovulating time after receiving the initial injection	Fertilization %	Hatching		Hatching time after fertilization (W.T.°C, S %)	Remarks
	1st. injection		2nd. injection				Date	%		
	Date	Dosage	Date	Dosage						
1	19-12-69 16:00	2P+10RU + 150VE	20-12-69 16:10	2P+20RU	22-12-69 11:15	10	24-12-69 17:00	100	53 hrs 45mins (23.7-22.7, 33.99-30.08)	No response occurred following the first two injections. However, a response was noted in the evening to third injection of 1P+20RU at 14:25, Dec. 21. The belly became greatly enlarged on Dec. 22 at 11:15. The eggs were collected and fertilized artificially. During the hatching period, the water was gradually freshened. The number of hatched eggs was 5×10 ⁴ .
2	20-12-69 16:20	2P+10RU + 150VE	21-12-69 14:20	1.5P + 20RU	21-12-69 23:15	90	23-12-69 15:25	100	40 hrs 10mins (24.0-24.7, 32.49-33.29)	The belly became swollen after the first injection. It spawned naturally at 23:00, Dec. 21. The remaining eggs which were artificially fertilized at 23:15 were hatched in hanging net.
3	20-12-69 16:30	1P+10RU + 150VE	21-12-69 14:30	1.5P + 20RU	22-12-69 15:10	40	24-12-69 17:00	100	49 hrs 50mins (23.7-22.5, 33.72-33.32)	Because of syringe trouble, the second injection was only half completed. 10 minutes later, another injection of 1P and 20RU was added. The water was gradually freshened during hatching. The number of hatched eggs was 6×10 ⁴ .
6	21-12-69 16:20	1P+10RU + 150VE	22-12-69 12:10	2P+20RU	23-12-69 12:30	0.5	—	—	—	Discarded because of low fertilization rate.
9	28-12-69 16:00	2P+10RU + 150VE	29-12-69 14:30	1.5P + 20RU	30-12-69 17:10	60	1-1-70 11:30	5	42 hrs 20 mins (23.0-24.0, 32.95-33.58)	The hatching rate was affected by bad water quality which was caused by faulty aeration in the later hatching period. Before this accident, the embryo developed normally.
10	28-12-69 16:05	2P+10RU + 150VE	29-12-69 14:35	1.5P + 20RU	30-12-69 16:30	60	1-1-70 11:30	5	43 hrs (23.0-24.0, 33.35-32.76)	"
11	28-12-69 16:10	1P+10RU	29-12-69 14:40	2P+20RU	30-12-69 19:35	60	—	—	—	Unfertilized.
12	28-12-69 16:15	1P+20RU + 150VE	29-12-69 14:45	2P+20RU	30-12-69 14:30	90	1-1-70 5:00	100	38 hrs 30mins (24.0-24.5, 32.5-33.22)	Spawning was very good. Release of the "water eggs" was induced by gently pressing the belly of the fish. Part of fertilized eggs which were put in the hanging net hatched out, but the rest failed to hatch probably because of faulty aeration.
13	3-1-70 14:00	2P+10RU + 150VE	4-1-70 12:20	1.5P + 20RU	5-1-70 10:25	10	7-1-70 11:30	70	49 hrs 5mins (23.0-22.5, 33.61-32.51)	Enough aureomycin was added to the tank water to produce a concentration of 50 ppm for sterilization. Spawning was poor. Fertilization rate was rather low. 26×10 ³ fertilized eggs were moved to tanks (5m×7m×1.5m) No.5 and No. 6.
14	4-1-70 17:20	2P+10RU + 150VE	5-1-70 16:00	1.5P + 20RU	6-1-70 16:15	40	8-1-70 11:30	100	43 hrs 15mins (22.8-23.2, 33.01-34.62)	At 11:00 Jan. 8, 9×10 ³ fertilized eggs were moved to tanks No.5 and No.6
16	8-1-70 17:10	1P+50VE	9-1-70 5:10	2P+20RU	10-1-70 9:00	40	—	—	—	The eggs were nontransparent. Large amounts of oil globules were present.
19	11-1-70 20:30	2P+10RU + 150VE	12-1-70 8:30	1.5P + 20RU	13-1-70 15:30	20	15-1-70 1:30	100	34 hrs (23.0-24.0, 33.23-33.83)	At 7:50 Jan. 13, body cavity was opened and the eggs removed from the ovaries for artificial fertilization. The fertilization rate was approximately 30%. 4×10 ⁴ fertilized eggs were moved to tanks No.5 and No.6 at 22:00 Jan. 14.

P: Pituitary of mullet (pieces)

RU: Synahorin (Rabbit Unit)

VE: Vitamin E (mg)

movements for a distance of 0.5-1.0 cm. Then they drifted on the surface of the water for a short period and slowly sank to the bottom. On the third day, formation of the mouth occurred, and artificially fertilized oyster eggs were given as food to the larvae. As often as possible, seasoned stagnant sea water was used during the larval stages to simulate natural environment. "Green water" and artificially cultured diatoms were added respectively to keep the water clear and as compensatory food. "Green water" is produced by letting natural sea water, enriched with organic fertilizers, stand in an outdoor tank (5m x 7m x 1.5m) until a rich growth of micro-organisms occurs, giving the water a definite greenish color. It seems the larvae were able to feed upon oyster eggs, blue green algae, and ciliates (8-30 μ) which can also be found in green water to fulfill their nutritional requirements. Due to the size increase of the four-day old larvae, yeast and albumin from eggs were added. It was noted that some larvae began to die on the third day and most had died by the fourth day. Phototaxis was demonstrated in the surviving larvae. The remaining larvae were found in the non-turbulent upper water layer and did not hunt for food in the dark. Oyster larvae were given continuously until the ninth day. On the sixth day, rotifers and copepods collected from fish ponds were added. It was observed that the larvae fed on rotifers and copepods which were about 100 μ in length. However, it was rather difficult to select and use only rotifers and copepods of this size. Many adult rotifers and copepods gave rise to mass production of their own larvae in the rearing water of the mullet larvae. The amount of food available was usually maintained at a concentration of 50 to 70 organisms/liter.

The rearing water was gradually refreshed from the sixth or seventh day. The mullet larvae became active, moved up or down as day and night passed, and had good appetite. For example, 10 copepods of 170 μ x 395 μ were found in one stomach. In addition to the above-mentioned prepared food, fish meal was added on the sixth day. Rapid growth was not observed before the ninth day (3.71-3.91 mm), but growth was easily detected by visual observation. A maximum length of 4.8 mm was recorded on the 11th day. Also at this stage they became darker and larger with distended abdomen. Strong phototactic responses were demonstrated in that the larvae preferred the upper or middle levels of the tank during the day and the lower levels during the evening and night. Some larvae died during this stage. Some stabilization of the survival rate occurred on the 13th day with

the largest larvae surviving. The smaller larvae that survived were found to be floating on the water and contributed to the low survival rate of 1.55% for the total number of larvae on the 13th day. The survival rate dropped to 0.68% by the 18th day. On this day, some of the morphological characteristics were as follows: average total length 6.6 mm, scattered body spots, and a silvery green color from the abdomen to the anus (scales seemed to be appearing). The skin color followed a diurnal cycle in which the color changed from brown in the night and early morning to black during the day. Swimming took place only on the upper level of the tank. The food of suitable size for the larvae after the 18th day varied between rotifers and adult copepods.

Artemia nauplii were given after the 20th day in addition to prepared food and copepods, although the mullet larvae were reluctant to feed on *Artemia* until the 22nd or the 23rd day. On the 21st day, the larvae appeared to be light brown or silvery green in color and still showed phototaxis during the day. At night, they floated on the water surface. On the 22nd and 24th day, the mullet larvae measured 8.8 mm and 10.9 mm respectively. In this period the mullet larva consumed 244-833 *Artemia* per day. On the 24th day, the larvae assumed a silvery white color with the appearance of some scales. During this stage, active swimming was observed in the upper level of the tank, and any sound or action would cause them to dive to the middle level, remain for a short period, and drift back towards the top. They were scattered when floating until a light was installed, at which time grouping took place. The larvae were inclined to swim against the water current. On the 26th day, they measured 1.285 cm in length. On the 27th day, they looked grayish white instead of silvery white. On the 28th day, the survival rate decreased to 0.31% and the larvae measured 1.5 cm in length and acquired a silvery green color, scales, and fin rays. The larvae at this stage preferred *Artemia* and large copepods to the prepared food. The rearing water was freshened to a salinity of 20.66 ‰. A very rapid rate of growth was observed after the larvae measured 1.5 cm. From the 29th to 32nd day, the fingerlings ate 655-2071 *Artemia* per day and fed only during the daytime (Fig. 1). They became very sensitive and difficult to catch. The swimming level was lowered to the middle or bottom part of the tanks. At night, they ascended to the surface of the water and were easily frightened. On the 34th day, they measured 2.22 cm and appeared grass green. They grouped and swam at the bottom in daytime, but scattered

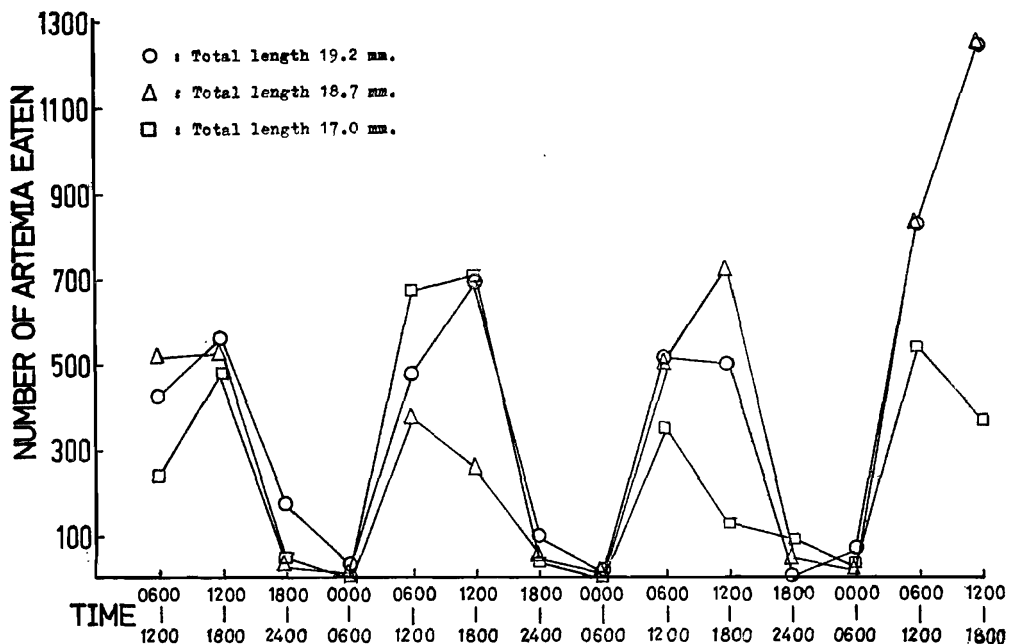


Fig. 1 Feeding rhythm of three different size larvae of 29-32 days old.

and stayed on the surface at night. Within a few days following the 37th day, they changed the time of feeding, eating only in the evening. From the 38th and 39th day on, they were sensitive to light. They dispersed when light was projected on the water, and no longer grouped under light at night. On the 40th and 45th day, they measured 2.76 cm and 3.28 cm respectively with a weight of 0.34g on the 45th day. At this time, the fingerlings were 1.5 times larger than the fry normally collected from the sea and were strong enough and suitable for stocking.

Still water was used in both the plastic tank of 0.5 ton and the 5m x 7m x 1.5m outdoor tank for rearing the larvae. Starting from the 33.50-32.27‰ in the early stage of the larvae, salinity was reduced by freshening the tank water down to 4.15‰ and the outdoor tank water to 15.10‰. The oxygen content of the water was maintained at as high a level as possible by constant aeration during the entire rearing period. For comparison, water with and without large amounts of blue green algae or diatoms was used in rearing. The results showed that the former was better for rearing mullet larvae.

As the larvae grew, prepared food, artificially fertilized oyster eggs, rotifers, copepods and *Artemia* were given in this order or in combination (Fig. 2). Those larvae in the outdoor tank were not given *Artemia*, but

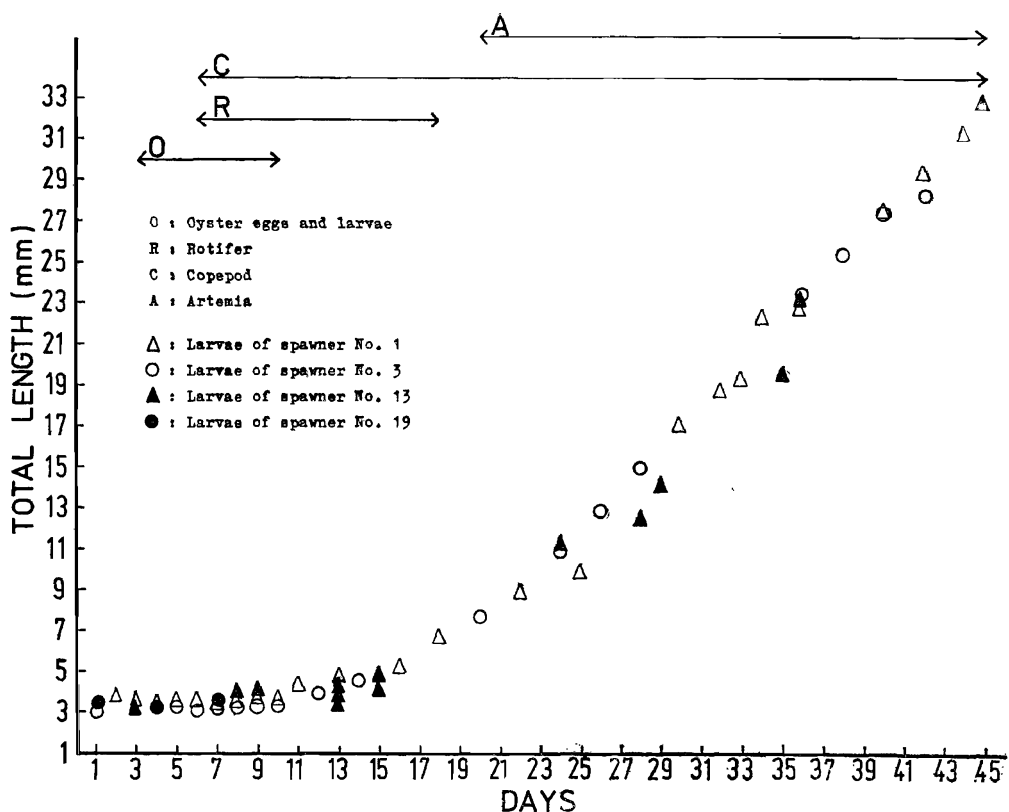


Fig. 2 The growth of larvae and food supply.

were given copepods collected from fish ponds. Even though the roofing of the outdoor tank was destroyed by a typhoon, causing the water temperature to drop and thus decreasing the survival rate to 0.75%, this survival rate was still more than twice the 0.31% rate of the indoor 0.5 ton plastic tank. Apparently, the large outdoor tank was better for rearing the fingerlings.

In the course of this work in 1969-1970, 431 fingerlings were produced, of which 280 in plastic bags filled with oxygen were transported to the Wushantou Freshwater Fish Propagation Center without any mortality (90 on the 44th day and 190 on the 55th day). The growth and survival of this batch of fingerlings were not satisfactory due to poor management (Fig. 3). A second batch of fingerlings was stocked in a brackish water pond near the Laboratory on March 1, i.e., the 54th day after hatching, and good results were obtained. They measured 20.1 cm and weighed 82.3g on the 151st day and 28.1 cm and 217.4 g on the 200th day (Figs. 3 and 4). A third batch was reared in 0.5 ton freshwater tank in the Laboratory. Growth was not good. On May 17, i.e., the 129th day, they measured only 8.9 cm

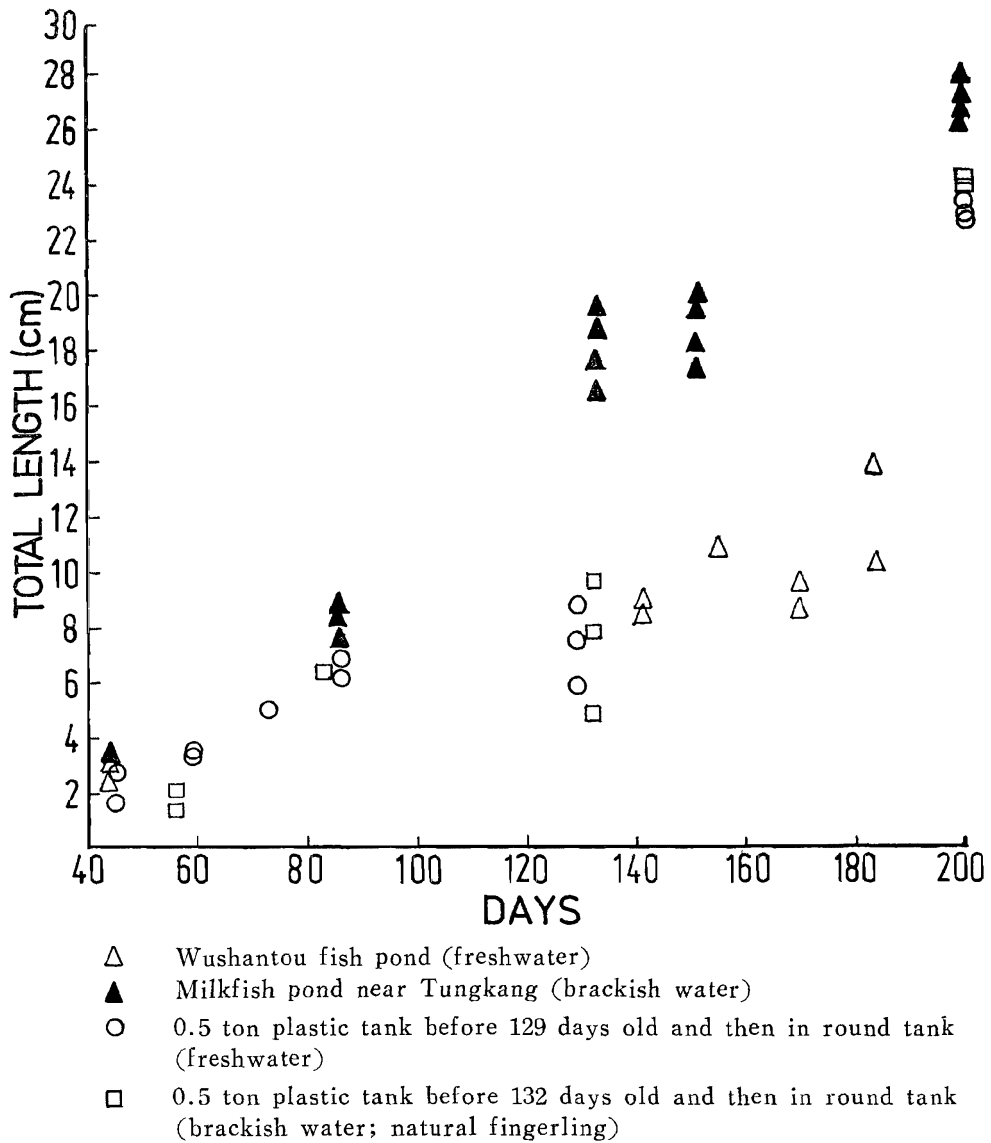


Fig. 3 The growth of young mullet older than 45 and 55 days in various tanks.

and weighed only 6.26 g due to the lack of space in the small tank. On the next day, 45 of them were stocked in round tank A with a diameter of 8 m together with 72 milkfish (two-year old). Prepared food was given twice daily. On the 200th day, they measured 23.6 cm and weighed 138.1 g (Figs. 3 and 4). For comparison, a fourth batch of 30 day-old fingerlings, caught from the sea and bought on January 31, was stocked in an indoor 0.5 ton plastic tank. On May 13, they measured 9.8 cm and weighed 7.41 g. They were stocked in round tank B of the same size as round tank A together

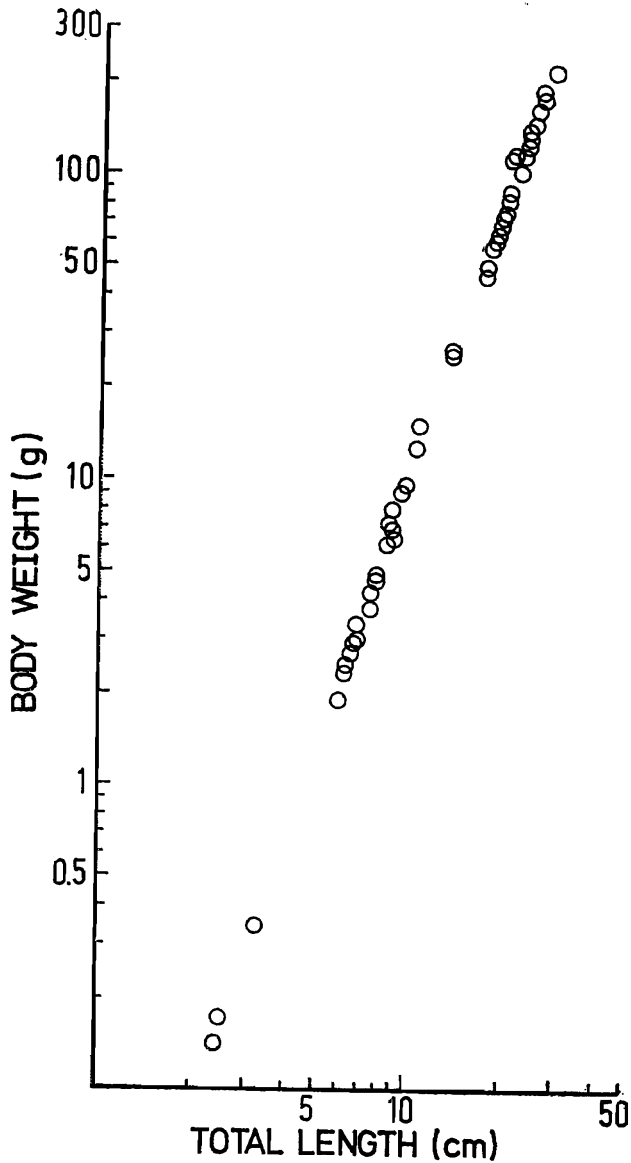


Fig. 4 Relationship of total length to weight of reared young mullet.

with 75 milkfish of the same age. Water in tank B was brackish rather than fresh. Identical food rations were given. On July 20, i.e., the 200th day, they measured 24.4 cm and weighed 136.3 g (Figs. 3 and 4).

4. Discussion

The first spawner was collected on December 19, 1969 and the last one on January 11, 1970. A total of 19 spawners was collected. The fishing

season lasted only 24th days. All spawners for this reasearch were obtained by purchase from fishing boats. As mullets usually suffered injury while being captured, especially in the spawning season, the work was difficult. Two mullets were wrongly sexed and three were found injured internally. However, with continuous improvements, particularly with the use of the specially constructed raft, the work was mostly successful (Liao *et al.*, 1969).

As for hormone treatment, 12 out of the 19 fish ovulated at an ovulating rate of 63.2%. The hatching rate of eggs reached 47.4%, which, though not high, was over four times greater than that of the previous year (Liao *et al.*, 1969). However, no conclucision can yet be made as to the best method of hormone treatment. The fishing season of mullet in Taiwan was too short to obtain more spawners for comparative treatment. Each one of the 6 of the 9 ovulating spawners received 3.5 mullet pituitary glands combined with 30 Rabbit Units of Synahorin. In general, this seems to be a good dosage, although it might be better to use more pituitary glands and less Synahorin in the first injection, and less pituitary glands and more Synahorin in the second injection. The time between two injections was cut down to 12 hours as in spawners No. 16 and No. 19 (Table 2). Nevertheless, this did not shorten the ovulating time. Basically 24 hours seem to be acceptable. More study is necessary on hormone dosage and ratio as well as the interval between two injections. Nine ovulating spawners received an additional injection of 150 mg Vitamin E with the hormone material. The effect of this treatment has not been determined. The spawners were reared for a longer time to reach the ovulating stage, except the three injured internally. They were active with one or two exceptions. The eggs of two spawners ovulated did not hatch (No.11 and No.16). No.11 did not receive any injection of Vitamin E, and No.16 received only 50 mg Vitamin E. Neither showed any effect on hatching of their eggs. Further study seems appropriate on the effects and dosage of Vitamin E.

There was no problem with male mullets, 90% of which could be successfully stripped. They were seldom treated with hormone. Two males were mistaken for females and received injections. One of them expelled sperms automatically just after the second injection. The other expelled sperms easily when it was squeezed. Injection of hormone is convenient and advantageous provided there are sufficient quantities of pituitaries on hand.

As for hatching, high dissolved oxygen content, minimum variation in water temperature and salinity kept the hatching rate over 70%. An exception

was found in the hatching rate of eggs of No.9 and No.10, which was only 5%. This was due to inadequate aeration and consequent low dissolved oxygen content.

In using the hanging hatching nets in running water, the adjustment of water flow is very important. If the flow of water is not adequate, the eggs do not roll in the nets, and then some of the eggs sink to close the inlet hole resulting in damage to all of the eggs. In the non-flowing water system, aeration is necessary. It is better to have heavy aeration so that eggs can be rolled in the tank. Fertilized eggs seem unaffected by violent agitation caused by the water flow. Further, the water quality, especially when the fertilization rate is low, needs constant attention. Water should be changed often. When the tank contains fertilized eggs, a siphon is used to change the water gradually, avoiding a sharp change in water quality. After hatching of the eggs, the water should not be changed. Therefore, a suitable rearing environment should be provided before hatching. If rearing is continued in the same tank, "green water" or diatoms should be piped into the tank in order to clean the water which had been fouled by hatching. Alternatively, hatched eggs can be moved to a prepared rearing tank which is filled with seasoned stagnant sea water. Both methods were used this time and change in water quality was avoided as much as possible. This resulted in a better record of rearing for the first few days after hatching.

Rearing the larvae is the most important part of this work. For the convenience of discussion, the rearing record and development of larvae are listed in Table 3. During the whole rearing procedure, there were two critical periods, the third to fourth day which sometimes extended to the seventh to eighth day, and the 11th to 13th day. During the second critical period, the survival rate was less than 2%. Larvae continued to die and the survival rate was less than 1% on the 18th day. The survival rate was not stable until the 24th day. By that time, they measured 1.09 cm, looked silvery white, preferred *Artemia* nauplii, and scales appeared. Rearing came to an end when the above characteristics were noted.

Gradually freshened water was used throughout the entire rearing period. The two larvae that reached 1 cm in last year's experiment were reared in gradually freshened water (Liao *et al.*, 1969). Observations on the development of larvae in natural environment showed that they had the habit of congregating in river mouths. With growth they tend to migrate to fresh water. Starting with the sixth or seventh day, the water for rearing was

Table 3 Development of larvae.

Days after hatching	Total length (mm)	Food	Observation on the behavior of the larvae.
1	2.08-3.40	—	No swimming ability.
3	3.34-3.54	O. Bg. Ci.	Mouth developed. Feeding began. First critical period.
4	3.20-3.53	O. P.	Phototaxis exhibited. Aggregated at water surface at night.
6	3.12-3.46	O. P. R. Co.	Up and down migration according to daytime and night. Fed only in daytime. Commencement of freshening rearing water.
11-13	4.25-4.80	P. R. Co.	Survival rate dropped to 1.55%. Second critical period. Darkening of body color. Strong phototoxic response exhibited.
18	6.6	P. R. Co.	Survival rate dropped to 0.68%. Black spots scattered on the body. Silvery green appearance from belly to anus.
20-21	7.65	P. Co. A.	Daytime phototoxic response shown. Floated on water surface at night. Body color was brown or silvery green.
24	10.9	P. Co. A.	Silvery white scales appeared. Swam in upper level in daytime and liked to swim against aeration. Scattered on surface but aggregated under light.
26-27	12.85	P. Co. A.	Grayish white appearance.
28	15.0	Co. A.	All scales and fin rays well developed. Silvery green in color. Prefer <i>Artemia</i> . Survival rate 0.31%.
29-32	18.6	Co. A.	Very easily scared. Swam in middle and lower level of water in daytime. Floated on surface at night.
34	22.2	Co. A.	Swam on the bottom in daytime. Floated separately at night. Grass green color.
37	23.1	Co. A.	Feeding habits slightly changed. Ate in evening.
38-39	25.35	Co. A.	Sensitive to light. Aggregation under light at night ceased.
45	32.8	Co. A.	Salinity dropped to 4.15%. High environmental resistance. Suitable for stocking.

O. : Oyster eggs and larvae

Bg. : Blue-green algae

Ci. : Ciliata

P. : Prepared materials that consist of albumin from eggs, yeast and fish meal

R. : Rotifer

Co. : Copepod

A. : Nauplius of *Artemia*

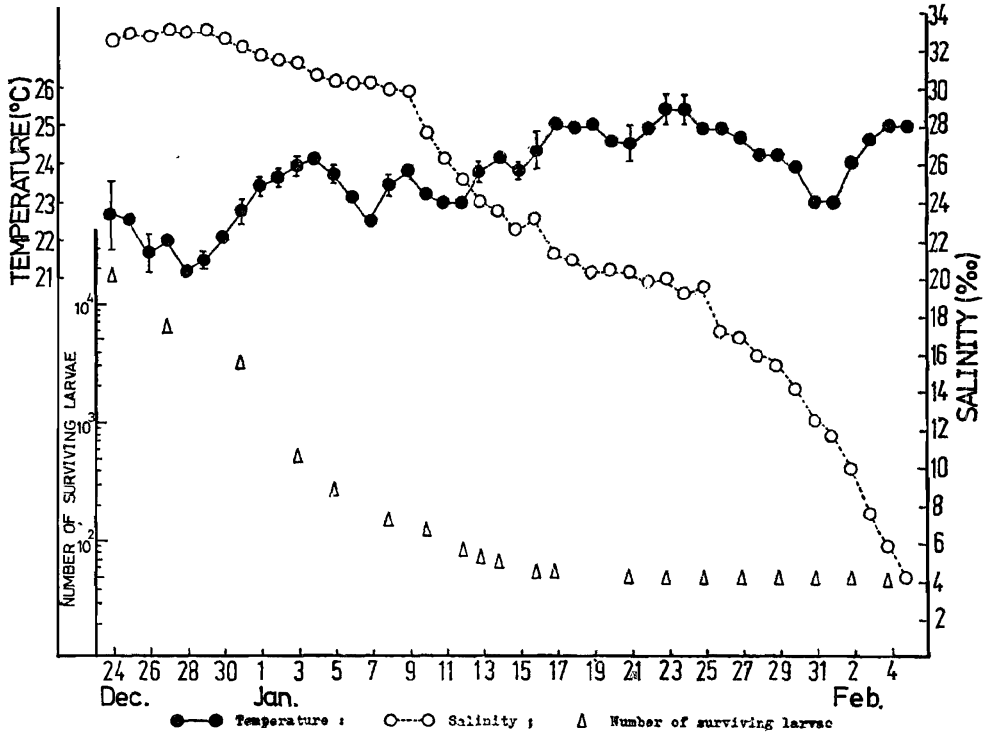


Fig. 5 Water temperature, salinity and the number of surviving larvae in 0.5 ton plastic round tank.

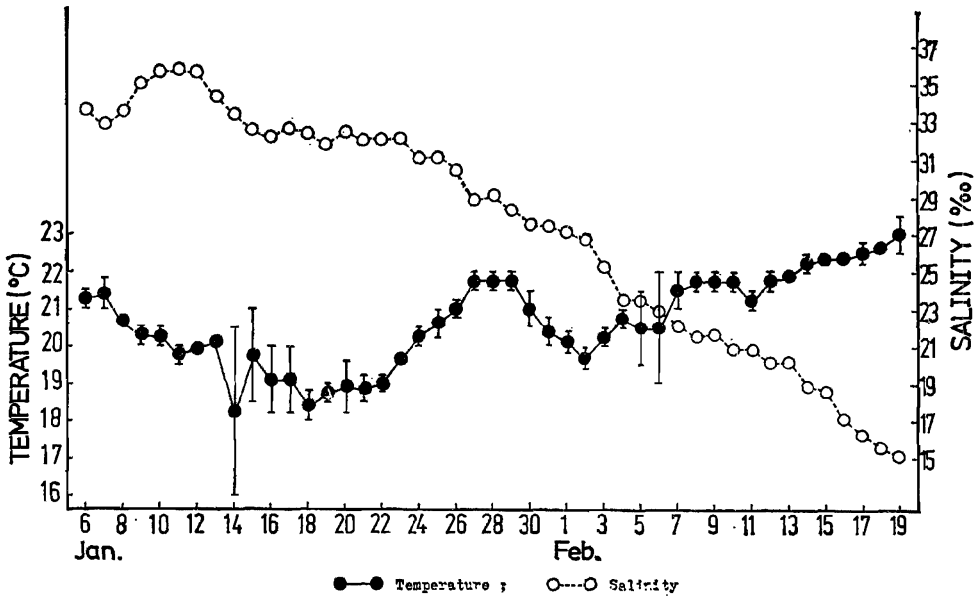


Fig. 6 Variation of water temperature and salinity during rearing period in 5m x 7m x 1.5m outdoor tank.

freshened. Figure 5 shows the freshening in the tank. It can be divided into three stages. The freshening was very slow in the first ten days, changing from 32.84‰ to 29.70‰ in twelve days. The reduction was 10.6‰ for the next 15 days and was 15‰ for the last 12 days. On the 45th day, it was 4.15‰. Figure 6 shows the freshening in the large tank. The freshening process was slightly slower in the tank but more rapid than the process used in the previous year (Liao *et al.*, 1969). There is no doubt that it is advantageous to rear the larvae in freshened sea water. Initiation and freshening rate are some of the problems encountered. A further study of these problems is needed.

For comparison, water containing large amount of blue green algae or diatoms and water not containing these were used. The result showed that the former was better in rearing the mullet fry. The reason is that algae can prevent the water from fouling and can be used as compensatory food. "Green water" is full of blue green algae and also ciliates which can be used as food for a certain period. Further investigation is needed to determine why the seasoned non-flowing water is suitable for rearing.

As for larval food, Figure 2 shows that artificially fertilized oyster eggs, rotifers, copepods and *Artemia* were used in this order and in combination as the larvae grew. The quality, size, and motility of the food are important factors in this rearing technique. Density of given food is also important. A density of 50-100 organisms/cc of oyster eggs and its trochophore stage larvae is easy to maintain, but hard in the case of copepods. Rotifers in heavily aerated tanks tend to float on the surface, and thus are not ideal larval food. Rotifers are too small and adult copepods are too large for 18-day-old larvae measuring about 6.6 mm. Barnacle larvae of stage 1 and 2 may be used in future experiments. When *Artemia* were given in the previous year, the mullet larvae showed no tendency to eat them. They were afraid of the reddish brown color and finally died from starvation and cold temperature (Liao *et al.*, 1969). Therefore, *Artemia* were given early in this experiment so that larvae would become familiar with them. On the first and second day, the mullet larvae were scared and retreated. Within a few days, however, they began eating the *Artemia*. After the initial feeding on *Artemia*, there was no fear of insufficient food and rearing became easier. This time, copepods were given instead of *Artemia* to the larvae in large outdoor tanks. Local fish ponds had abundant populations of copepods and so a density of 50-70 organisms/liter could be maintained easily with the supply from them.

As for prepared food, starting from the third day, albumin from eggs, yeast, and fish meal were given in addition to oyster eggs and rotifers. The results are not clearly understood, but, at least, they seem to serve as compensatory food. Experience so far does not indicate that prepared food can be used alone. They foul the water. Further study needs to be made to develop some good prepared food.

A careful examination was made when the rotifers and copepods from fish ponds were given in order to prevent undesirable organisms from getting into the water. However, eggs and larvae of fish lice were not completely screened out. Many mullet larvae were infected with parasites and injured. More attention should be paid to this problem and probably medicinal baths should be given to help eradicate fish lice.

Although great care was given, the survival rate in the indoor plastic round tanks of 0.5 ton was only 0.31%. Less care was given to the mullet larvae in the larger outdoor tanks, and only copepods from fish ponds were added. Furthermore, the roofing was blown off by typhoon causing the water temperature to drop suddenly, resulting in a decrease in the survival rate of the larvae. However, the survival rate (0.75%) was still higher than that in the smaller tanks. This indicates that the large tanks are better for rearing of the young mullet larvae.

The newly hatched larvae up to the 13th day could not tolerate direct or high intensity sunshine. On the 17th and 18th day, black pigments appeared, and the larvae had more resistance. All plastic tanks were indoors. Some received direct sunshine and this resulted in less desirable rearing conditions. The optimum of light intensity should be investigated in order to reduce mortality. There is no direct relationship between the two critical periods and content of dissolved oxygen. On the contrary, high content of dissolved oxygen resulted in increased mortality. Silvery green larvae measuring 0.5-1.2 cm were very sensitive to fluctuations of environmental factors. If at all possible, the larvae should not be disturbed. The attempt to find the color of light to which the larvae were most attracted was unsuccessful owing to the varying intensities of light used. Since larvae have the habit of eating in the daytime, growth and survival rate can be promoted by illuminating the tanks at night. There are three possible reasons for low survival rate, food quality, water quality, and the condition of the larvae. Before or after the formation of certain organs, the larvae may require more nutrients, microquantities of inorganic elements or even special water quality.

All of these need to be studied. For example, the reasons for the critical periods need to be determined and then more efficient methods of rearing evolved.

The survival rate of those larvae during transportation to Wushantou and stocking in pond was 100%. The smallest one measured 2 cm. The transportation containers were thick, non-transparent plastic bags. The number of larvae in each bag was dependent on the distance of transportation, content of oxygen, and size of the larvae. They were moved in winter when water temperature was not too high. Simple inflation with oxygen made transportation over short distance possible. The size limit of the larvae which can be transported is not known. The fingerlings should not be stocked in water of higher salinity than the original. They should be stocked in water of lower salinity. Their growth after being stocked is shown in Figure 3. On the 200th day, the fish in brackish-water pond measured 28.1 cm and weighed 217.4 g (Figs. 3 and 4). If things go well, they will become the first artificially propagated spawner within two or three years.

Before hatching, eggs should be kept in tanks with aerated water or tanks prepared for rearing. The larvae should not be moved immediately after hatching. Seasoned sea water should be used for rearing and should be gradually freshened. Sharp changes in water temperature and quality should be avoided. The nature of the food should correspond to the developmental stage of the larvae.

Artificial propagation of the grey mullet became successful after six years of research. There are many problems to be further studied, such as maturity of spawners, hormone treatment, physiological and ecological needs of fertilized eggs and larvae, food for larvae, especially prepared foods, etc. In addition, the rearing of the spawner is one of the most important problems at the present time. It was reported that spawners grown in fish ponds were able to ovulate and the eggs could be hatched there (Yashouv *et al.*, 1969). A large fish pond should be built or a good coastal area should be screened off somewhere south of Tungkan for this test. No wintering equipment will be needed there. Good food will help the mullets to mature. Thus a systematic and complete culture could be obtained.

5. Summary

The spawning mullet schools usually migrate off the coast of southern Taiwan in winter. From December 1969 to January 1970, live spawners

were obtained from the fishermen on the fishing ground and then treated with hormones for study of artificial propagation. The results obtained are as follows:

(1) Nineteen spawners were obtained during the experimental period. After hormone treatments of 2.75 to five mullet pituitary glands combined with 20 to 50 Rabbit Units of Synahorin, 12 spawners ovulated (63.2%) and the eggs from nine of them hatched (47.4). It was the most successful experiment in the last six years.

(2) Among the nine spawners, six of them were given 150 mg Vitamin E in addition to 3.5 mullet pituitary glands combined with 30 Rabbit Units of Synahorin.

(3) During the experimental period, the water temperature was relatively stable. Therefore, the range of hatching time was narrow, between 34 and 54 hours. Hatching took place in 34-38 hours if incubated at water temperature of 23-24.5°C, and 49-54 hours at 22.5-23.7°C with salinity of 30.1-33.8‰ in both cases.

(4) Seasoned sea water was used in rearing. Rapid freshening was made starting on the sixth day after hatching with salinity reduced from 32.8‰ to 4.15‰ within 45 days.

(5) The food supply was modified according to the development of the larvae. Artificially fertilized oyster eggs, rotifers, copepods collected from fish ponds, and *Artemia* nauplii were used in this order. The amount of food given depended on the amount consumed. Usually 50-100 organisms/cc of oyster eggs and its trochophore stage larvae were given, but the concentration of rotifers and copepods was maintained at 50-70 organisms/l. On the 23th and 24th day, one larva of 1 cm long would ingest 244-833 *Artemia* per day. From 655 to 2071 *Artemia* per day were ingested by each larva of 29 to 32 days old measuring 1.8 cm in length.

(6) The critical periods were the third to fourth day and the 11th to 13th day. After these stages, the survival rate was below 2%, but was below 1% on the 18th day. Stabilization of the survival rate began on the 24th day. At this stage, the larvae reached a total length of 1.09 cm, was silvery white in color, developed scales, and took *Artemia* nauplii as the favorite food.

(7) Two kinds of tanks were used for rearing. Those reared in small tanks had a survival rate of 0.31% on the 45th day. Those reared in large outdoor tanks, in spite of a sudden drop in water temperature, still had a

survival rate of 0.75%. Thus, it is obvious that the large tanks are more suitable than small ones for rearing mullet larvae.

(8) A total of 431 larvae survived on the 45th day. They each weighed 0.34 g and were 3.28 cm in length, about 1.5 times larger than the larvae collected in natural waters, and had high resistance to environmental changes. The reared larvae were divided into several groups and stocked in fish ponds and tanks. The best growth obtained on the 200th day after hatching was 28.1 cm in length and 217.4 g in weight.

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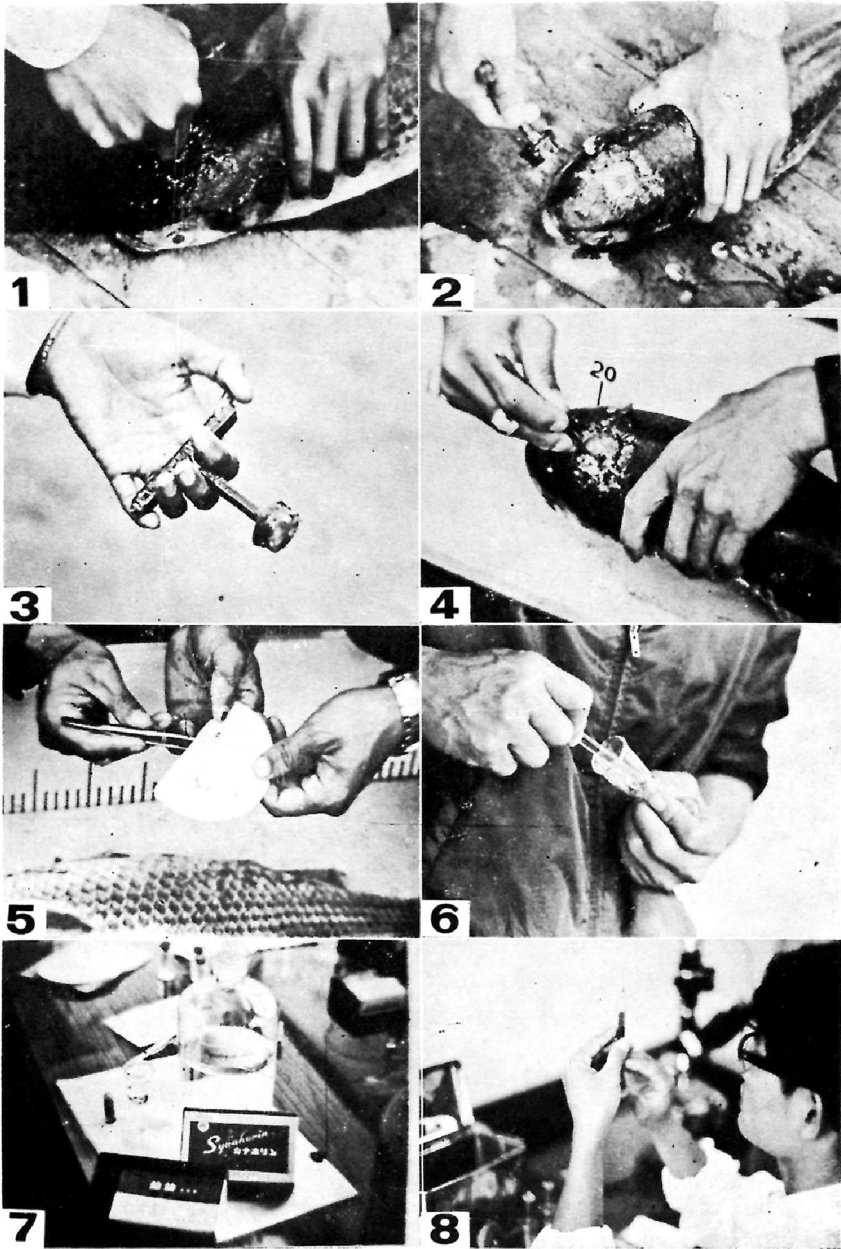


PLATE I

Plate I. Hypophysation technique & preparation before injection.

- Fig.1 Removal of the cranium. Fig.2 Location of pituitary gland.
 Fig.3 Tool used to remove the cranium.
 Fig.4 Removal of pituitary gland. Fig.5 Cleaning the pituitary gland.
 Fig.6 Finely grinding the gland in a tissue grinder.
 Fig.7 Addition of Synahorin & Vitamin E to the pituitary suspension
 in physiological saline. Fig.8 Ready for injection.

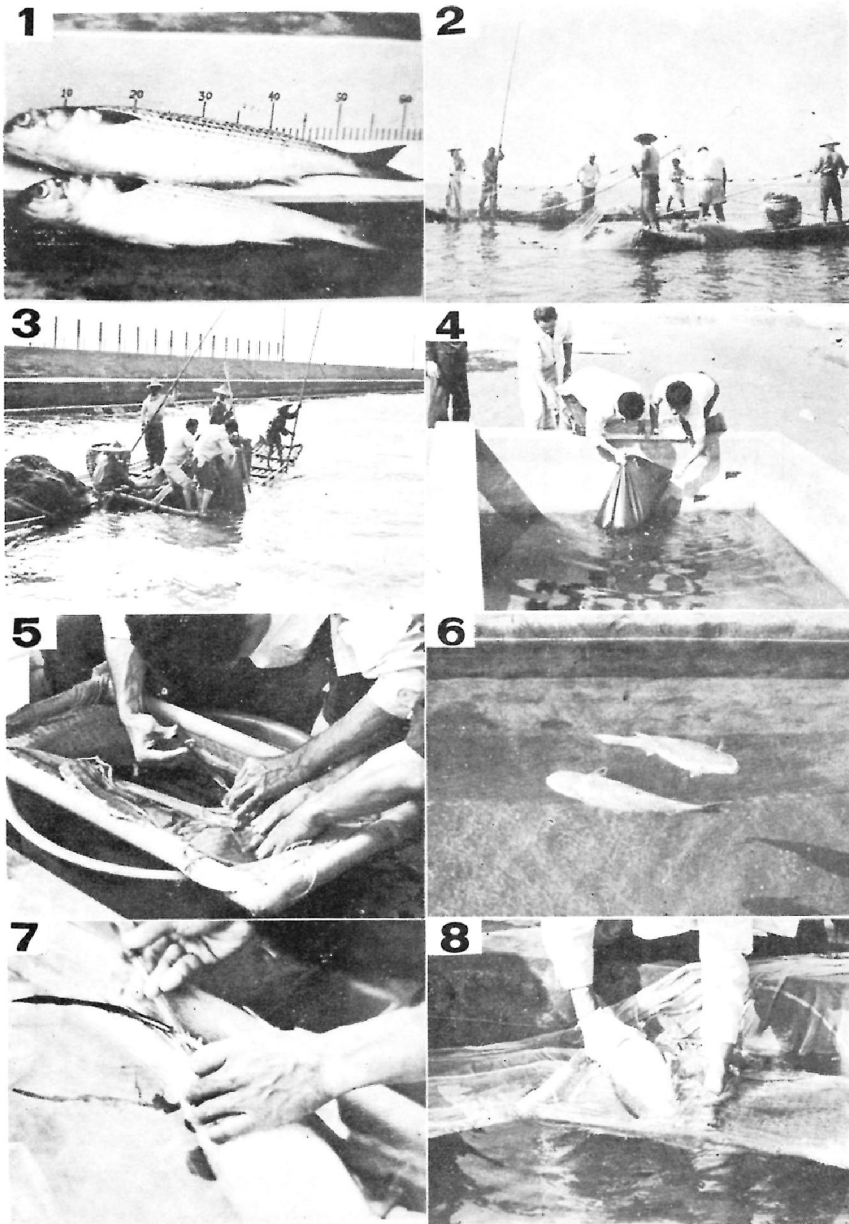


PLATE II-1

Plate II. Procedure of induced spawning & culture.

Plate II-1

Fig.1 Mature mullets (female above, male below). Fig.2 Operation of a purse seine. Fig.3 Selection of mature mullets. Fig.4 Stocking tank for induced spawning. Fig.5 Pituitary injection given to female mullet. Fig.6 Mature female after injection. Fig.7 Examining the condition of ovulation. Fig.8 Handling the female spawner gently in water.

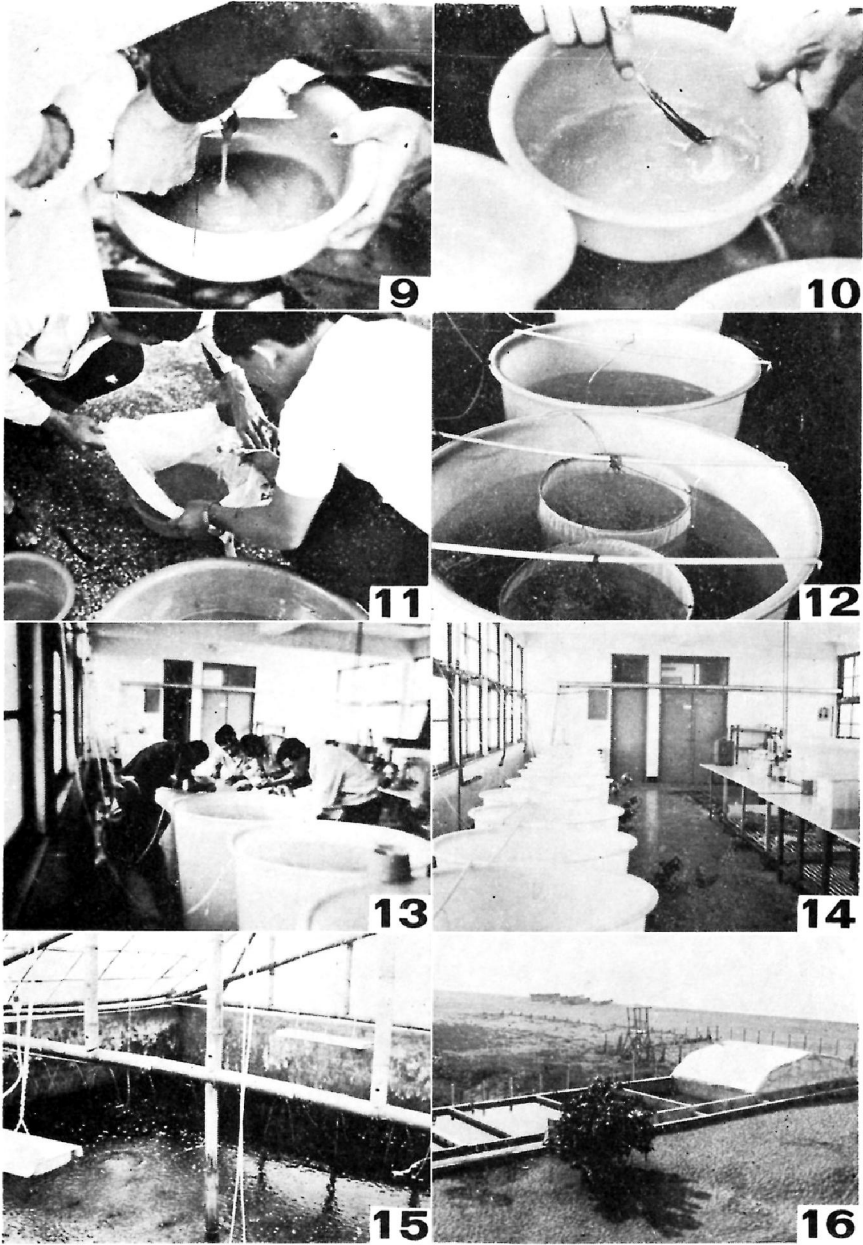


PLATE II-2

Plate II-2

Fig.9 Stripping the spawner for eggs. Fig.10 Artificial fertilization. Fig.11 Washing away sperm fluid, mucous and feces from the fertilized eggs. Fig.12 Hanging nets in hatching tank with running water. Fig.13 Picking out the unfertilized eggs. Fig.14 Indoor plastic tanks and heating apparatus. Fig.15 Freshening system in large tank. Fig.16 Large tanks (5m x 7m x 1.5m) for stocking spawner and rearing larvae.

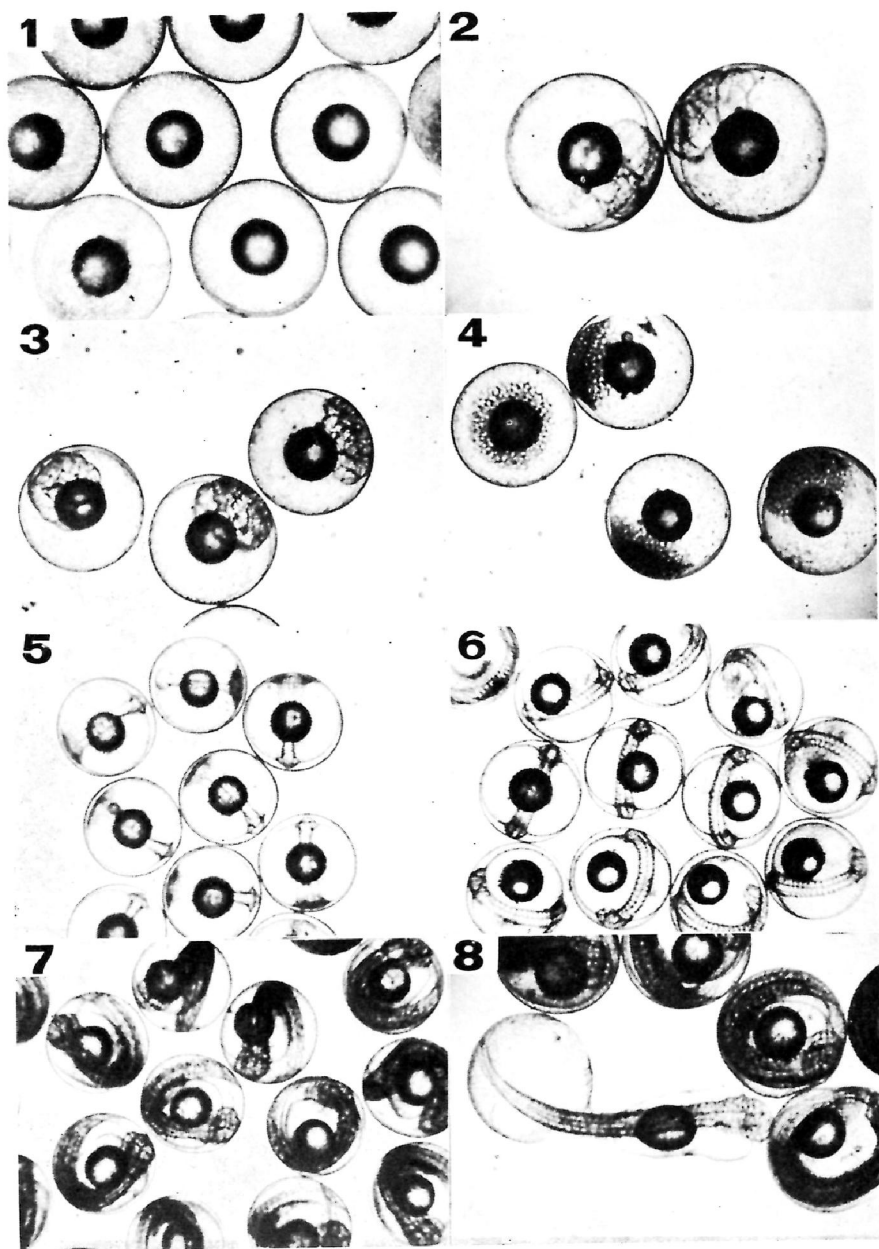


PLATE III-1

Plate III. Embryogenesis & growth.

Plate III-1

Fig.1 Eggs just after spawning. Fig.2 2nd cleavage (4 cells stage).
 Fig.3 5th cleavage (32 cells stage). Fig.4 Late segmentation stage.
 Fig.5 Optic vesicle and myotome formation stage. Fig.6 Brain differentiation stage.
 Fig.7 Optic cup & membranous fin formation stage.
 Fig.8 Hatching stage (an embryo just emerged from chorion).

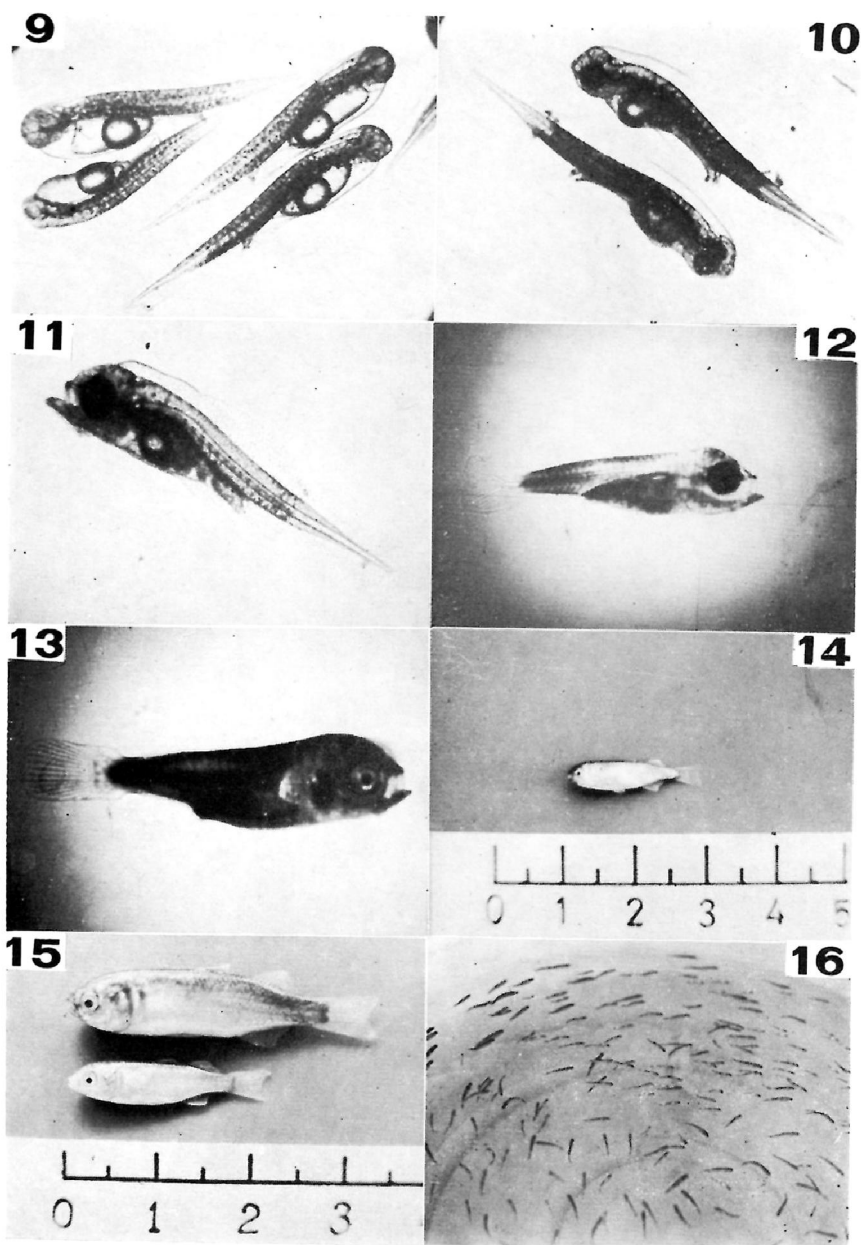


PLATE III-2

Plate III-2

Fig.9 8-10 hours after hatching. Fig.10 25-30 hours after hatching.
 Fig.11 4 days after hatching. Fig.12 16 days after hatching (5.5 mm
 in length). Fig.13 20 days after hatching (7.65 mm in length). Fig.14
 33 days after hatching (1.915 cm in length). Fig 15 Comparison of
 artificially propagated fingerling (above, 45 days after hatching) with
 fingerling (below) collected from natural waters. Fig.16 Fingerlings
 swimming in tank.

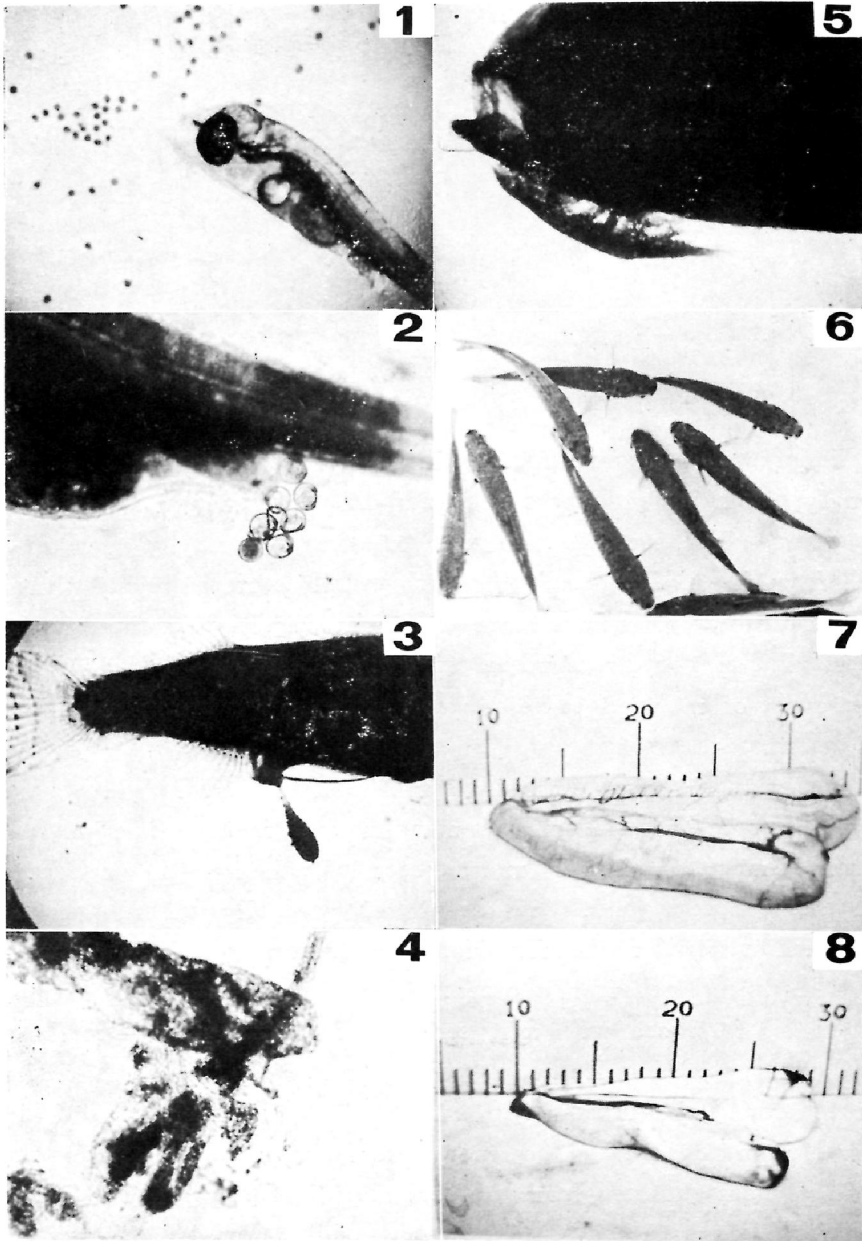


PLATE IV

Plate IV. Feeding & others.

Fig.1 Larva and its food (oyster eggs and trochophore stage larvae).

Fig.2 Discharging oyster larvae from anus.

Fig.3 Discharging copepod from anus. Fig.4 Feces of larva.

Fig.5 Parasitic fish lice on larva. Fig.6 200th day young mullets.

Fig.7 Ovaries. Fig.8 Testes.

淡水魚塭烏魚人工繁殖試驗初步報告

PRELIMINARY REPORT ON INDUCED BREEDING OF POND-REARED MULLET, *MUGIL CEPHALUS* LINNAEUS

廖一久 • 李棟樑 • 林美雲 • 羅明川

I-Chiu Liao, Dong-Liang Lee, Mee Yoon Lim and Ming-Chuan Lo*

Abstract

Artificial propagation of the grey mullet (*Mugil cephalus* Linnaeus) was successfully accomplished after seven years of experimental work conducted in the winter. In the course of these experiments, mature spawners were obtained from fishing boats at sea, which usually suffered injury while being captured. Weather conditions increased the difficulties of obtaining mature spawners. In order to overcome these difficulties and to establish a more reliable source of mature spawners, work on induced breeding of pond-reared mullet was started.

A three-year old spawner measuring 41.5 cm in standard length and 1.2 kg in weight (post-ovulation measurement) from a freshwater pond was used in this experiment. Before hormone treatment, the spawner was reared in a freshwater concrete pond, with sea water gradually added, and the fish was fed with artificial feedstuff. Hormone treatment was the same as for those spawners obtained from the sea. After three injections, the eggs were discharged and fertilized with milt stripped from mature sea mullet. The fertilized eggs hatched well. The rearing of larvae was carried out in 1.0 and 0.5 ton round plastic tanks.

Larvae from pond-reared spawner were found to be easier to rear than those of spawners obtained from the sea. By February 22, 717 larvae had survived for 35 days with a standard length of 2.32 cm. The survival rate was 1.48%.

Although the result obtained was only that of one spawner, it had proved that pond-reared mullet could be used as spawner in artificial propagation and that the difficulty of obtaining mature spawners could be overcome to a large extent.

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前 言

本省烏魚人工繁殖試驗開始於民國52年(1963年)，其中56年中斷一年，迄今已有七年歷史，至民國59年始告初步成功¹⁾。歷年來種魚皆捕自產卵洄游至東港沿岸之魚羣，但每每受天候海況等因素之影響。又捕獲時魚體常受損傷，不易獲得理想的種魚。因此，爲了獲得健全可用的種魚及確保其來源穩定必須在魚塢中從事烏魚種魚之人工繁殖。

此次獲得淡水魚塢養成之烏魚一尾，經賀爾蒙處理結果，順利排卵並孵出幼魚。至2月22日即孵出後第35天，共存活幼魚717尾，體長2.32cm，存活率達1.48%，其活力及成長情形不遜於天然種魚之人工繁殖。

此次雖因情況所限，僅一尾試驗成功，然而足以證明魚塢確可養成種魚以供人工繁殖。茲將試驗經過敘述如下，以供今後從事此項試驗之參考。

材 料 及 方 法

種魚：59年10月19日由東港附近之淡水魚塢購得已飼育三年以上之大形烏魚10尾。其中4尾在漁民捕起時死亡，另5尾在活運途中及移放蓄養池前後，因受傷亦相繼死亡。最後僅存活1尾，蓄養於直徑2.10m，水深0.95m之圓形水泥池中。最初三天蓄養於淡水中，此後逐漸注入海水。蓄養期間之平均水溫爲21.2°C，鹽分濃度爲28.6‰。每日投給混合飼料(含魚粉20%，米糠20%，花生餅25%，酵母25%，綜合維他命2%及維他命E1%)約50g。蓄養約三個月後於60年1月15日移至大蓄養池(2.5m×7m×1.5m)，準備施予賀爾蒙處理。此魚肥大健壯，活力遠較天然烏魚爲強，體表黏液多，以手按捺其腹部有柔軟感，成熟情形不遜於天然洄游至東港沿岸之種魚。

賀爾蒙處理：一如施於天然種魚者^{1, 2)}，此次共用6個烏魚腦下腺，混合40家兔單位生殖腺刺激賀爾蒙(Synahorin)另加150mg維他命E，分三次注射。

人工授精：亦同施於天然種魚者^{1, 2)}，採用乾導法，精液則採自海中之成熟雄魚。

孵化：孵化用之海水鹽分濃度爲28.6~29.4‰，水溫爲23.0~23.9°C。孵化期中予以充分打氣，俾卵粒不斷在槽內滾動。至肉眼可識別卵中確已形成胎胚，即受精後約26小時，以手掌用力攪水使其迴轉，俾使受精卵及不受精卵分離。通常不受精卵易沉於水底，俟其沉底後，用虹吸管抽除之。此後將受精卵分移各槽(所用之塑膠水槽爲0.5及1.0噸二型)，並加入綠水以穩定水質。

幼魚培育：採用止水式，但依水質之狀態時加綠水，並漸漸加淡。培育期間之水溫爲19.9~23.2°C，鹽分濃度29.3~23.4‰。

餌料：使用人工授精牡蠣卵及其幼虫、輪虫、撓腳類，豐年蝦幼虫及人工配合飼料等。

結 果

種魚經如第一表所示之賀爾蒙處理，於注射第3針後，經1小時又45分，即自然產卵於池中。迅即捕起施予人工授精，約得235,200粒卵，其中受精者約53,700粒，受精率爲22.8%。另產於蓄養池之卵，撈起後，即予人工授精，但不見受精。

卵徑0.90~0.96mm，油球約0.38mm，與採自天然種魚之卵並無差異。

孵化率高達90%以上。在鹽分濃度28.6~29.4‰，水溫23.0~23.9°C之下，37小時又45分即孵化。

剛孵出之幼魚，全長約2.7mm(附圖一)，體色較天然種魚所產之幼魚爲白。第3天，口部形成，開始攝食牡蠣卵及其幼虫，此時全長約爲3.35mm。至第12天，除輪虫及撓腳類外，開始授予若干剛孵出之豐年蝦幼虫。此時全長已達4.15mm，體高0.85mm，喜聚集於槽壁，似有強烈之趨光性。翌日即第13天，飽食者腹部呈紅色，白天浮游於較上層，夜間則下沉，常逆水而游。第17天左右，幼魚生長開

Table 1. The response of the spawner to hormone treatment, ovulation and hatching.

Ser. No.	Injection		Ovulation		Fertilization %	Hatching		Remarks
	Date	Dosage	Date	%		Date	%	
1st. inj.	15-1-71 18:30	2P +20 RU +150V. E.	17-1-71 21:45	70	22.83	19-1-71 11:30	90	No response to 1st injection. Beginning of enlargement of the belly with 2nd injection. 1 hr and 45 mins after the 3rd injection, the spawner was found to discharge eggs in pond, and artificial fertilization was done immediately. 24% of all eggs, i. e., 235,200, were obtained.
2nd. inj.	16-1-71 18:00	2P +20 RU	Time after the initial injection			Time after fertilization (W. T. °C, S ‰)		
3rd. inj.	17-1-71 19:45	2P	51 hrs 15 mins			37 hrs 45 mins (23.0-23.9°C 28.6-29.4‰)		

P: Pituitary of mullet (pieces) RU: Synahorin (Rabbit Units) V.E.: Vitamin E. (mg)

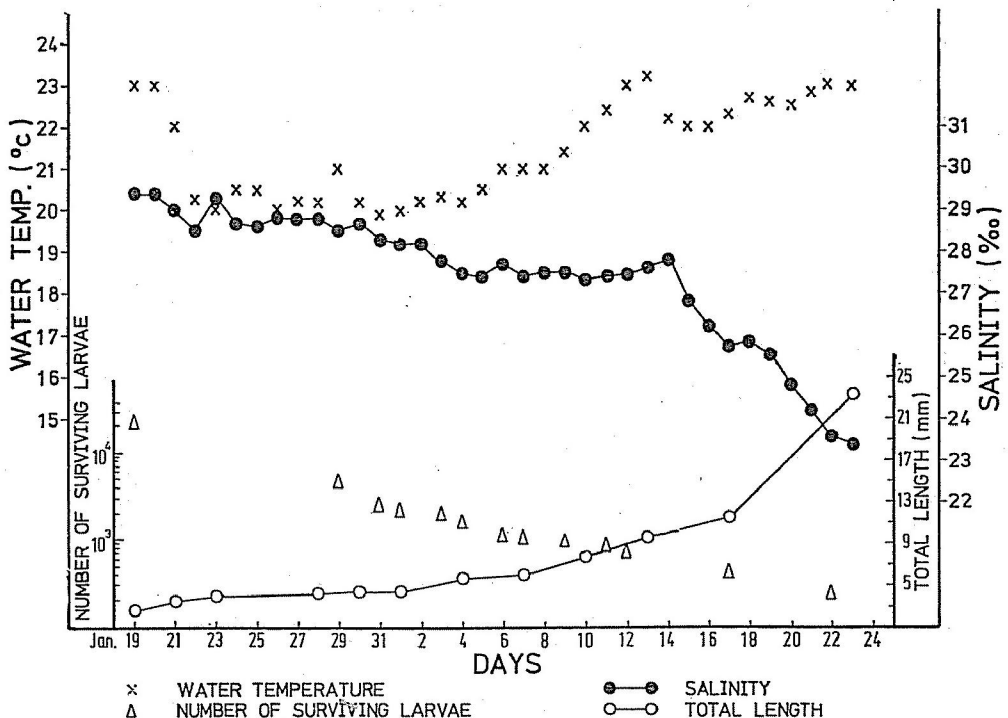


Fig. 1 Water temperature, salinity, total length of larvae and number of surviving larvae in 0.5 ton tank.

始呈現大小參差不齊現象，大者全長已達 6.34mm，但小者僅 0.85mm。鱗片陸續於第22天左右出現，此時大者全長為 7.2mm，幼魚易受驚動且易死亡。至第25天，鱗片幾已覆被全身，體色銀白，背部稍黑，聞聲即驚恐而游入下層。第28天後魚羣晝夜間之游層分明，晝間在中、下層，夜間漂浮於表層，僅於晝間攝食，食量驚人，成長亦極速。至第35天全長已達2.32cm 存活者717尾幼魚，存活率為1.48%。幼魚肥滿健壯，已可供放養於魚塢養殖。

討 論

此次所得之種魚，係於淡水魚塢（佔地約10多公頃）長大者，年齡約三歲。此魚賀爾蒙處理前3個月之成熟度，即漁塢捕獲時之成熟度，可由與此魚同時捕獲之其他8尾之記錄推測（第2表），其 G.S.I. (Gonadosomatic index) 在 0.4~4.2之間。據 Kesteven³⁾ 指出 G.S.I. 7~20始達成成熟期第5期。因此，此魚當時可視為未達成成熟階段。但飼養約3個月後，再經注射6個烏魚腦下腺，混合40家兔單位生殖腺刺激賀爾蒙(Synahorin)，51小時15分即達成促進產卵之目的。可見 G.S.I. 值約2者，經3~4個月後似可作為種魚。Yashouv⁴⁾ 亦指出淡水或半鹹淡水魚塢中飼育者，其 G.S.I. 有時可高達19~20。但此次所投飼之混合飼料，較一般魚塢所投之飼料為佳。因此產卵前所需營養問題尚待今後進一步之研討。

又該種魚之健康情形，遠較捕自海中之種魚為佳，體表黏液多，鱗片不易脫落。經三次注射處理，魚體毫無損傷，捕起施予人工排卵後其鱗片亦能保持原狀，但兩天後即死亡，檢查結果係因擠卵時過份用力，導致卵巢受傷所致，否則定可繼續飼養，待其明年抱卵。一般海中捕獲者，體表黏液不多，鱗片易於脫落，往往打下第1針後不到50小時即死亡。通常捕回後不加賀爾蒙處理，亦難飼育。淡水或海水之不同環境對存活率影響不大，此次捕自淡水魚塢者，其存活率亦僅10%。此尾之健康情形或可歸因於自魚塢購回後，3個月內所給與富於營養之混合飼料，惟此點仍待進一步之研究。

此次捕自魚塢之大形烏魚皆為雌魚。據董⁵⁾指出河游至臺灣沿岸之產卵羣之性別約為♂1.8: ♀1。一般海產之烏魚雄多於雌，而此次捕魚之網具係圍網之一種，網目不大絕無選別之可能，然捕到之烏魚均為雌性，留下一大疑問，有待今後之調查及研討。

Table 2. Measurements of pond-reared mullet

Individual No.	T.L. (cm)	F.L. (cm)	S.L. (cm)	B.W. (gm)	G.W. (gm)	Sex	Maturity	
							G.S.I.*	G.I.**
1	53.74	48.72	43.31	1763	25.73	♀	1.45	3.17
2	47.43	43.87	38.35	1231	15.09	♀	1.22	2.68
3	49.45	45.70	40.35	1343	8.22	♀	0.60	1.25
4	50.95	46.10	40.60	1295	6.41	♀	0.40	0.96
5	48.80	44.05	33.60	1219	6.90	♀	0.56	1.82
6	50.69	46.33	40.17	1346	38.87	♀	2.88	5.99
7	51.47	46.78	41.28	1537	10.97	♀	0.71	1.56
8	50.22	46.28	40.82	1410	59.62	♀	4.22	8.77

* G.S.I.: Gonadosomatic Index

** G.I.: Gonad Index

此次受精率僅為22.8%，不甚理想。檢討結果或與注射第3針有關。注射第2針後原已有反應。第3針之注射，導致提早排卵，以致1小時又45分後即自然產卵於池中。當時雖即捕起施行人工授精，但部份卵粒顯然為過早之卵，其他似甚正常，但亦不受精，可能亦為未完全成熟之卵。據此，欲適時予以人工授精至為困難，尤其捕自海中之烏魚，為使其在存活時間內順利達成產卵，有時難免施予過量之賀爾蒙，而引起類似上述之現象。此次採用之種魚其活力旺盛不虞體力不支，或可作少量處理，使反應和緩以增加受精率。惟欲把握適時人工授精，則對種魚即將產卵之某種徵候之識別仍待今後之觀察及研究。為達成省力的原則，同時提高受精率起見，在此試驗期間曾數次嘗試將數尾雄魚與處理後之雌魚置於同槽，以期自然交配，但未見成功。今後亟需研討促其產卵之機構及設置促其產卵之生態條件。

孵出幼魚之體色較得自海中捕獲種魚者為白，然健康情形良好，雖經孵化後第3天左右及第9~13天左右之二次危險期，但孵化後至第35天之存活率為1.48%，自第13天至第35天者則達12.0%。此次試驗發現，得自魚塢養成種魚之幼魚較得自海中種魚之幼魚易於飼養，其對水質，水溫等環境變化之抵抗力顯然較強，這一點和母魚之原在魚塢養成，即家畜化現象，是否有關，有待今後之研討。又此次培育過程中，自第17天左右起，幼魚體積之差略漸趨明顯，甚至相差7倍之多，其原因或由於飼料充足，故攝食力強之先天優良者越吃越大所致。

如上所述，有待今後研討之問題頗多。此次成果僅得自一尾，但由於此次試驗之成功，足可證實魚塢確可養成種魚以供人工繁殖。去年人工繁殖之烏魚苗，至今一年已成長為體長34cm，體重0.7kg。如悉心照料，則2~3年後可望成長為種魚，再予以賀爾蒙處理，則可實現烏魚養殖「完全人工控制」之目標。

摘 要

1. 供試之烏魚，約3年大，體長41.5cm，體重1.2kg（產卵後予以秤量），G.S.I. 為25左右。賀爾蒙處理前約3個月移自淡水魚塢，漸漸加入海水，並投以富於營養之混合飼料，結果體肥且健，成熟情形不遜於天然洄游至東港附近沿海之種魚。

2. 前後予以3次賀爾蒙處理，共使用6個烏魚腦下腺，混合40家兔單位生殖腺刺激賀爾蒙，(Synahorin)，另加150mg維他命E，結果首次處理後51小時又15分即產卵。

3. 卵徑0.90~0.96mm，油球約0.38mm，與採自天然種魚之卵並無差異。

4. 受精卵在鹽分濃28.6~29.4‰，水溫23.0~23.9°C之下，37小時又45分即孵化。

5. 剛孵化出之幼魚全長約2.7mm，體色較白，但活力甚強，在逐漸淡化之飼育水中培育，孵出後第35天，體長已達2.32cm。共存活717尾魚苗，存活率為1.48%。

6. 此次雖因情況所限，供試魚僅有一尾，然而足可證實魚塢確可育成種魚以供人工繁殖。

附 記

本試驗所供試之種魚，承東港陳清泉先生之熱心協助提供，始得順利進行工作。又本試驗由承農復會陳組長同白，袁技正柏偉之全力支持，聯合國糧農組織漁業專家林紹文博士之鼓勵，及東港海產種苗繁殖中心各位同仁之鼎力協助，因是有成，謹於此一併致謝。

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PHOSPHORUS DYNAMICS IN CHUPEI FISH PONDS

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Introduction

In natural aquatic environments phosphorus is not present in large amount. Therefore, it is often a limiting factor for the growth of organisms (Hutchinson, 1957). Lin and Chen (1966) demonstrated the effectiveness of superphosphate in improving fish production in the freshwater ponds and reservoirs of Taiwan. Since that time the use of superphosphate in fish ponds has become widespread in Taiwan. Liaw (1969) demonstrated that a combination of alkalinity and phosphorus concentration may be used as an index of fish production in freshwater ponds. Lin (1969) observed that chlorophyll increased in amount after application of superphosphate in fish ponds. Additional research must be undertaken to determine the optimum dose of superphosphate and the method and optimum time of its application. It is also necessary to study the complete role of phosphorus in fish ponds including the water, soil and food chain.

At the Chupei Fish Culture Station a long-term project has been in progress since 1968 to study the optimum dose of superphosphate for fish pond fertilization. The authors of this paper participated in this project by determining the chemical content of the water and soil as well as calculating the primary production. This paper is only concerned with the role of phosphorus in the water of these fish ponds. Samples of pond bottom soil were also analyzed and studied in relation to changes in phosphorus concentration in the water.

Description of Experimental Ponds

All ten ponds used in the experiments received water from a well located near the Station. All ponds were less than 1,200m² in area and less than 1m deep. They were divided into two groups. Ponds A-1 to A-5 were located in front of the Station and were slightly deeper than B-1 to B-5 which were located at the back of the Station. The size, depth and location of all ten ponds are shown in Figure 1.

In 1969, in order to measure the effect of fertilization on the ponds'

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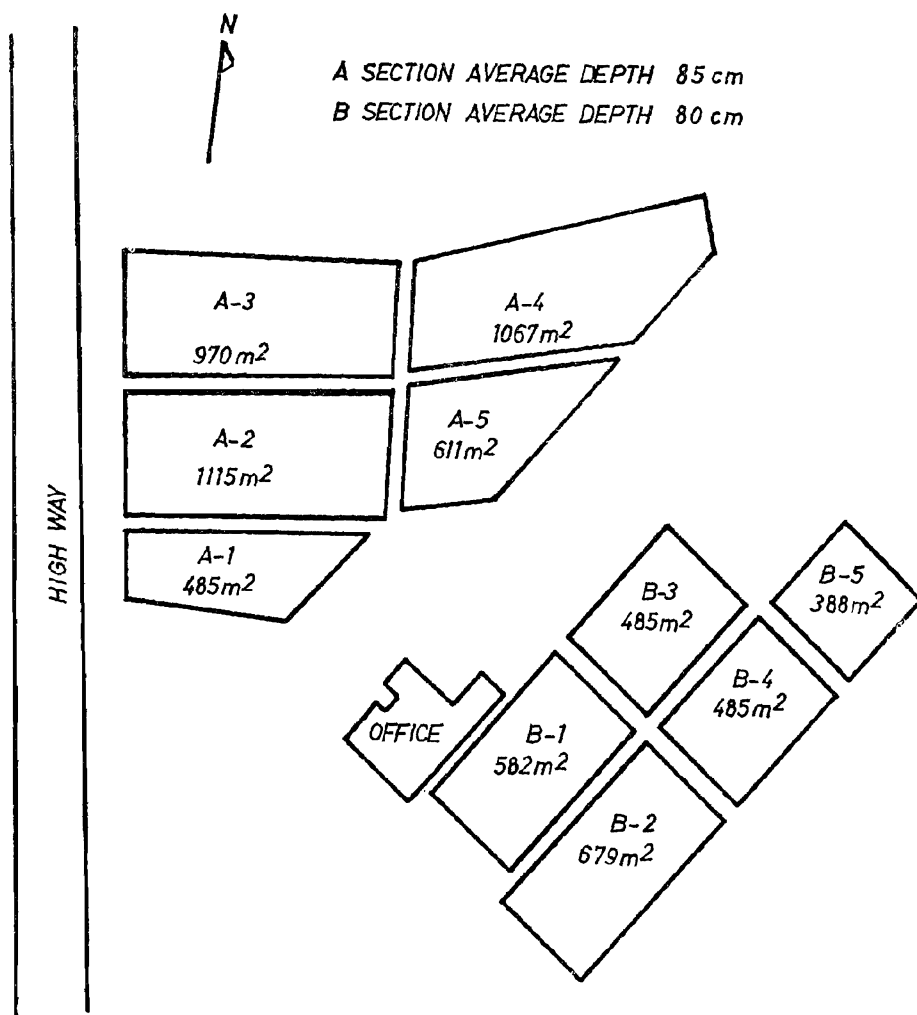


Fig. 1 Depth, area and location of Chupei fish ponds.

ecosystem, various amounts of superphosphate were added to eight of the ten ponds twice weekly. Ponds A-1 and B-1 were kept as controls. In addition, ponds B-2, B-3, B-4 and B-5 received a total of 1950 kg/ha/year of ammonium sulphate applied in equal amounts twice weekly. To compare the relative productivity of these ten ponds, no fertilizer of any kind was added from February to June 1970. However, from July through November 1970 the ponds were treated with different amounts of N-P-K fertilizer (Table 1).

Polyculture of fish was practiced in both 1969 and 1970. The fish cultured in these experiments included silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), crucian carp (*Carassius carassius*), big head (*Aristichthys nobilis*),

Table 1. Soluble phosphorus concentration (ppm) and amount of superphosphate applied (kg/ha)

	1969		1970			
	PO ₄ -P	super-phosphate	Mar. to June		July to Dec.	
			PO ₄ -P	Super-phosphate	PO ₄ -P	Superphosphate in N-P-K fertilizer
A ₁	0.039±0.021	0	0.011±0.007	0	0.110±0.159	720
A ₂	0.160±0.065	1,040	0.055±0.062	0	0.093±0.067	326
A ₃	0.312±0.119	2,040	0.123±0.177	0	0.100±0.055	274
A ₄	0.600±0.253	6,090	0.393±0.266	0	0.123±0.055	480
A ₅	0.760±0.305	10,240	0.446±0.245	0	0.224±0.371	0
B ₁	0.044±0.035	0	0.015±0.012	0	0.070±0.131	720
B ₂	0.220±0.079	1,040	0.062±0.062	0	0.094±0.096	480
B ₃	0.305±0.074	2,040	0.050±0.050	0	0.040±0.031	504
B ₄	0.581±0.136	6,090	0.181±0.118	0	0.231±0.181	411
B ₅	0.929±0.339	10,240	0.353±0.239	0	0.054±0.037	0

and the perch or sea bass (*Lateolabrax japonicus*).

Method

I. Water

Chemical analysis of the water in the ten experimental ponds was made every two weeks from February through November 1970. pH, chlorophyll, primary production of O₂ and PO₄-P determinations were made from samples of pond water obtained after the regular semi-weekly fertilization treatments. Chiu (1969 and 1970, unpublished) determined weekly the total phosphorus and organic phosphorus in the water of these ten ponds.

(A) pH values were measured in situ by Darmark pH M28 meter.

(B) Chlorophyll was determined by spectrophotometric method (Richards and Thompson, 1968).

(C) Gross production of O₂ was determined (McConell 1962).

(D) PO₄-P was determined by the modified single solution method (Murphy and Riley, 1968).

II. Soil

Each of the ten ponds was divided into 9, 15 or 18 sections depending on the size of the ponds. From each of these sections soil samples were collected. The samples from each pond were put together to form three

combined samples, which were mixed thoroughly before analysis. The makeup of each of these three combined samples was determined by the direction of inflow or outflow of water over the original section. The soil in each of the combined samples was analyzed three times, except for particle size which was analyzed just once. The purpose was to determine the average pH value and phosphorus concentration of each combined sample. The soil in each of the ten ponds was collected at three separate dates, February 5, July 2, and November 27 in 1970 and brought to the laboratory for analysis.

In order to measure pH of the soil obtained on February 5, 20 grams of bottom soil was thoroughly mixed with 20 ml of de-ionized water and the pH. immediately determined. On July 15 and November 27 the pH of the combined sample was determined in the field without addition of de-ionized water. Particle size was determined using the hydrometer method (Black 1965). For soluble phosphorus determination as well as for calcium phosphorus, iron phosphorus and aluminum phosphorus determinations, the flow sheet for soil phosphate fraction system was used to extract the phosphate from the soil (Jackson 1962). The phosphorus concentration of the extract was measured by the modified single solution method (Murphy and Riley 1968).

Result and Discussion

I. Phosphorus content in the water

Phosphorus concentration in the water of the Chupei fish ponds in the 1969 period has been reported by Chiu (1970). When the pond water was sampled twice weekly from February to November, the lowest average concentration of phosphorus was in ponds A-1 and B-1 (0.039 ± 0.021 ppm and 0.044 ± 0.035 ppm). This low concentration was due to the fact that no superphosphates were added to these ponds in the period. The highest average concentration of phosphorus was found in ponds A-5 and B-5 (0.760 ± 0.305 ppm and 0.929 ± 0.339 ppm). This higher concentration was due to the twice weekly addition of superphosphates to the pond water (the 1969 total was 10,230 kg per hectare of pond surface). The phosphorus concentrations in the other six ponds were between these extreme values. Their phosphorus concentrations were strongly affected by the amount of superphosphate added.

From the data (Chiu 1971, unpublished) in Table 1, we found that there was also a relationship between phosphorus concentration and the amount of

superphosphate added to these ponds in 1969. No superphosphate was added to these ponds from February to June 1970. The two ponds with the lowest phosphorus concentrations in 1970 were the same as in 1969, A-1 and B-1 (0.011 ± 0.007 ppm and 0.015 ± 0.012 ppm). The two ponds with the highest phosphorus concentrations in 1970 were the same as in 1969, A-5 and B-5 (0.446 ± 0.245 ppm and 0.353 ± 0.239 ppm).

Phosphates in N-P-K fertilizers were added to all ponds except A-5 and B-5 in the July to December 1970 period, but in amounts lower than in the 1969 period. The average phosphorus concentration in all ten ponds was higher in the July to December 1970 period than in the February to June 1970 period, but lower than in the 1969 period. The average phosphorus concentration in these ten ponds in the July to December 1970 period was lowest in B-3 (0.040 ± 0.031 ppm) and highest in B-4 (0.231 ± 0.181 ppm). No relationship between the amount of phosphate added to these ponds in this period and the phosphorus concentration in the water could be found. The phosphorus concentration in the water of each of these ponds during the 1969 and 1970 period is summarized in Table 1.

In 1932 Yoshimura developed a scheme for classifying ponds on the basis of phosphorus content (reported by Liaw 1969). The ten fish ponds at Chupei were classified according to this scheme and it was found that the phosphorus concentration of the ponds depended on the amount of superphosphate added (Table 2). Using Yoshimura's scheme we can say that the "phosphorus fertility" in these ten ponds ranked from good to excessive in 1969, from low to excessive in the February to June 1970 period, and from fair to good in the July to December 1970 period. However, there seems to

Table 2. Classification of water from the Chupei fish ponds on the basis of phosphorus content

Phosphorus (ppm)	Phosphorus fertility	Ponds		
		1969	Mar. to June	1970 July to Dec.
0.000~0.200	Low		A ₁ B ₁ B ₂	
0.021~0.050	Fair	A ₁ B ₁	B ₃ B ₄	B ₃
0.051~0.100	Good		A ₂ B ₅	A ₂ A ₃ B ₁ B ₂ B ₅
0.110~0.200	Very good	A ₂	A ₃	A ₁ A ₄
0.21 ~more	Excessive	A ₃ A ₄ A ₅	A ₄ A ₅	A ₅ B ₄
		B ₂ B ₃ B ₄ B ₅		

be no positive relationship between the "fertility" of these ponds and their fish production. For example, in 1969 the highest fish production was from ponds A-3, A-4 and A-5 which were rated as excessive in "phosphorus fertility", but B-2, with excessive "fertility", had very low fish production (Lin 1970).

During the period from July 29 to December 17, 1970, the phosphorus concentration in the water showed continuing reduction. The average concentration in the ten ponds was reduced from a high of 0.335 ppm on July 29

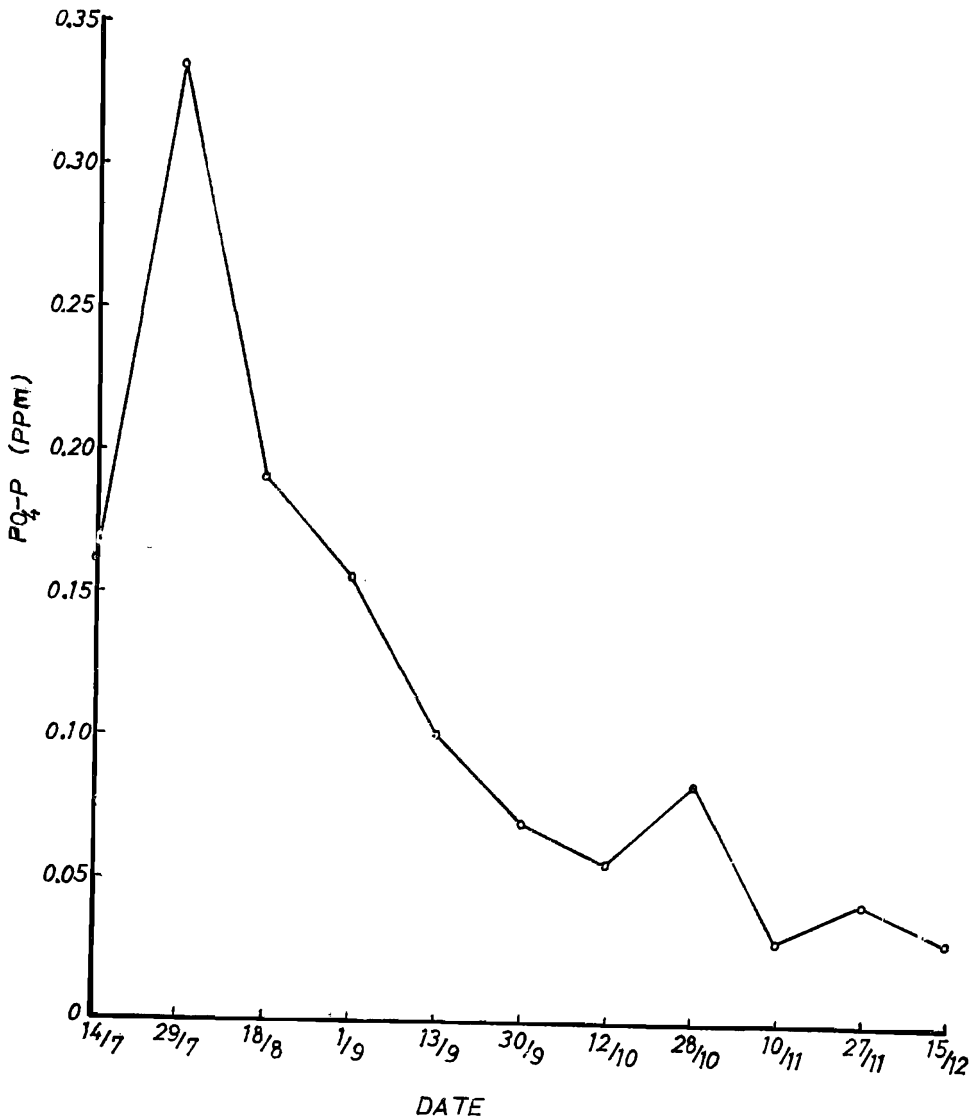


Fig. 2 Monthly variation of phosphorus in Chupei fish ponds from July to December, 1970.

to a low of 0.029 on December 15 (Figure 2). No relationship between this reduction in phosphorus and fluctuations of other nutrients and minerals such as calcium was found.

Figure 3 shows the relationships between average phosphorus concentration and increasing amounts of superphosphates. Averages for ten ponds were computed for each week in 1969 and in the February to June 1970 period. It was found that the different concentrations of the inorganic phosphorus, organic phosphorus and the total phosphorus in the water were due to differences in amount of fertilization in these ponds in 1969. During the 1970 period, when no additional superphosphates were applied, the phosphorus concentration in the water still differed from pond to pond but at a lower level than in 1969. This difference was due to the amount of phosphorus leached out from the soil.

II. Phosphorus content in pond soil

Phosphorus concentrations in bottom soil of the Chupei fish ponds were determined three times in 1970 (on February 5, July 2, and November 27). Soluble phosphorus (water soluble and loosely bound phosphorus), calcium phosphorus, iron phosphorus and aluminum phosphorus were calculated. These data along with the percentage of each kind of phosphorus in the soil

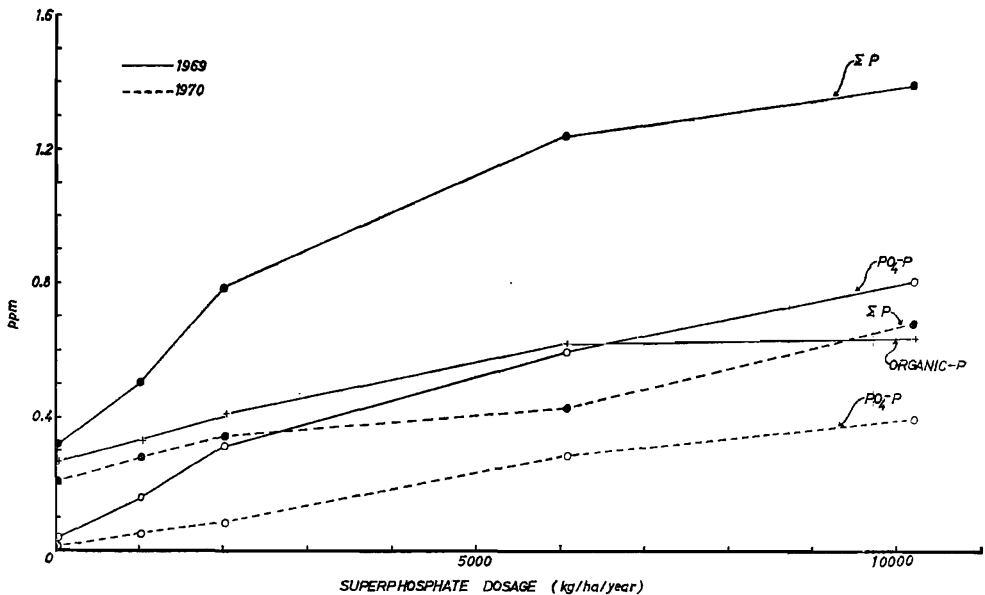


Fig. 3 Relationship between superphosphate dosage in 1969 and average concentration of PO_4-P , organic-P and total phosphorus (ΣP) among the ten ponds from Feb. to Dec. in 1969, compared to those from March to June in 1970.

Table 3. Inorganic phosphorus content of bottom soil in Chupei fish ponds (mg P/kg soil)

	Sol.-P			Al-P			Fe-P			Ca-P			Sum		
	Feb.	July	Nov.	Feb.	July	Nov.	Feb.	July	Nov.	Feb.	July	Nov.	Feb.	July	Nov.
	A-1	3.2	0.6	0.5	5.9	57.8	52.6	49.9	105.0	21.2	276.6	203.0	1.4	335.6	366.4
A-2	5.0	0.6	0.4	11.7	27.6	35.7	103.3	86.0	43.4	194.0	124.3	2.6	314.0	238.5	82.1
A-3	107.5	1.0	0.8	15.8	45.8	58.6	161.6	135.4	100.3	181.6	178.8	8.6	466.5	361.0	168.3
A-4	68.9	3.2	1.3	56.8	87.2	133.2	284.2	236.3	191.8	373.1	241.0	7.7	783.0	567.7	334.0
A-5	241.9	4.4	3.0	55.5	193.1	23.1	608.5	352.3	419.0	536.3	396.9	31.6	1,442.2	946.7	476.7
B-1	87.0	1.0	0.7	15.6	46.0	66.8	297.9	103.5	156.5	243.4	90.4	5.2	643.9	240.9	229.2
B-2	0.6	1.3	0.5	12.4	44.9	44.2	161.7	137.1	131.8	177.4	82.6	6.4	352.1	265.4	182.9
B-3	1.3	0.9	0.3	18.8	58.7	39.7	184.7	145.6	148.6	218.8	82.5	7.7	423.6	287.7	182.9
B-4	1.6	1.0	0.5	33.4	92.5	71.6	367.3	267.7	197.9	244.0	175.1	14.5	646.3	536.3	281.5
B-5	2.9	0.7	0.7	51.6	40.4	82.5	423.5	159.8	178.9	234.2	114.9	8.3	712.2	315.8	270.4
Ave.	51.0	1.5	0.9	27.8	69.4	60.8	264.3	172.9	158.9	267.9	168.9	9.40	611.9	412.6	228.6
%	4.3			12.6			47.5			35.6			100		

are shown in Table 3.

Soluble phosphorus

On February 5 the soluble phosphorus concentrations in ponds A-3, A-4, A-5 and B-1 ranged from 68.9 to 241.9 ppm. The other six ponds all had concentrations below 5 ppm. The cause of these concentration differences was not known.

On July 2, four months after the beginning of the 1970 experiment, the soluble phosphorus concentrations in the ten ponds ranged from 0.6 to 4.4 ppm. This means that most of the soluble phosphorus in the pond soil in February went into the pond water by July and was available to the growing phytoplankton,

On November 27, nine months after the beginning of the 1970 experiment, the soluble phosphorus concentrations in the ten ponds ranged from 0.3 to 3.0 ppm. The soils of the ten ponds showed continued reduction in the soluble phosphorus concentration, even though varying amounts of N-P-K fertilizers were added to the water in the period from July to November. Since the amounts of phosphorus added to the water in this period were much lower than that in 1969, the build-up of soluble phosphorus in the soil did not occur.

Calcium phosphate

On February 5 the calcium phosphorus concentrations in the soil of the ten ponds ranged from 177.4 to 536.3 ppm. On July 2 they ranged from 82.5 to 396.9 ppm, and on November 27 they ranged from 1.4 to 31.6 ppm. The average drop for the ten ponds was 99.0 ppm for the February to July period and 159.6 ppm for the July to November period.

Iron phosphorus

On February 5 the iron phosphorus concentrations in the soil of the ten ponds ranged from 49.9 to 608.5 ppm, on July 2 they ranged from 85.0 to 352.3 ppm, and on November 27 they ranged from 21.2 to 419.0 ppm. The average drop for the ten ponds was 100.7 ppm for the February to July period and 13.9 ppm for the July to November period.

Aluminum phosphorus

On February 5 the aluminum phosphorus concentrations in the soil of the ten ponds ranged from 5.9 to 56.8 ppm, on July 2 they ranged from 27.6 to 193.1 ppm, and on November 27 they ranged from 23.1 to 133.2 ppm. The average concentration of the ten ponds was 27.8 ppm on February 5, 69.4 ppm on July 2, and 60.8 ppm on November 27. The fact that

average aluminum phosphorus concentration increased in the February to July period while other phosphorus complexes decreased may be explained by suggesting that phosphorus was transferred from other phosphorus complexes in this period. Not all the decrease in phosphorus is explained by this transfer, since large amounts were used up by fish and other organisms in the ponds ecosystem.

In 1970, the different concentrations of the inorganic phosphorus in the soil were due to the differences in the amount of fertilization in these ponds in 1969, and, therefore, varying amounts of phosphorus deposits in the bottom soils (Figs. 4, 5, 6 and 7). However, pond A-1, to which no

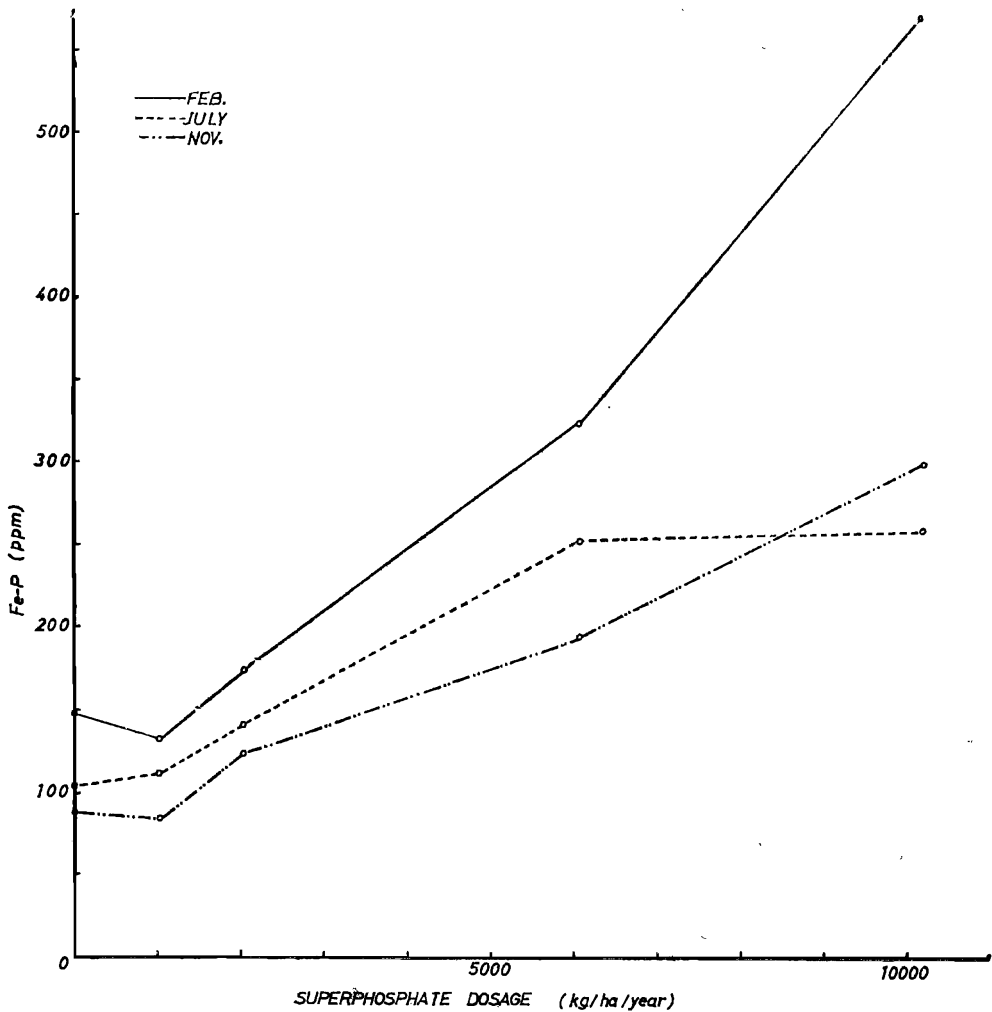


Fig. 4 Relationship between total amount of superphosphate dosage in 1969 and the iron phosphorus content of bottom soil in 1970.

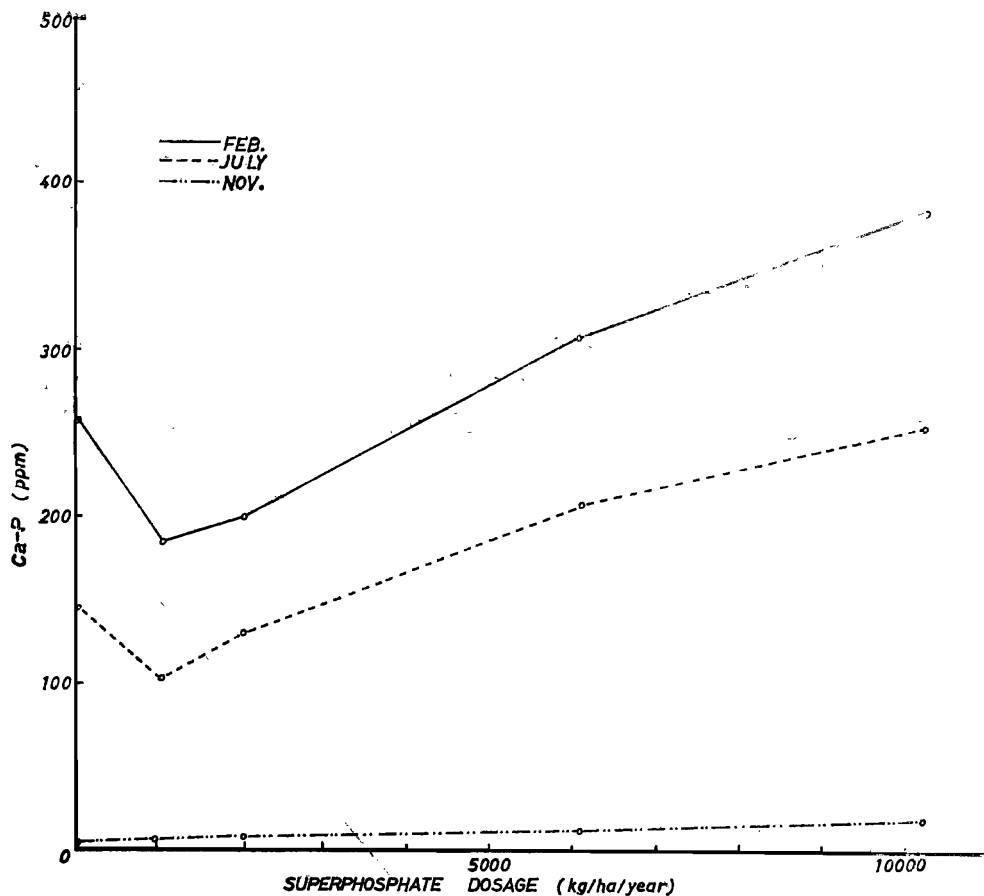


Fig. 5 Relationship between total amount of superphosphate dosage in 1969 and calcium phosphorus content of bottom soil in 1970.

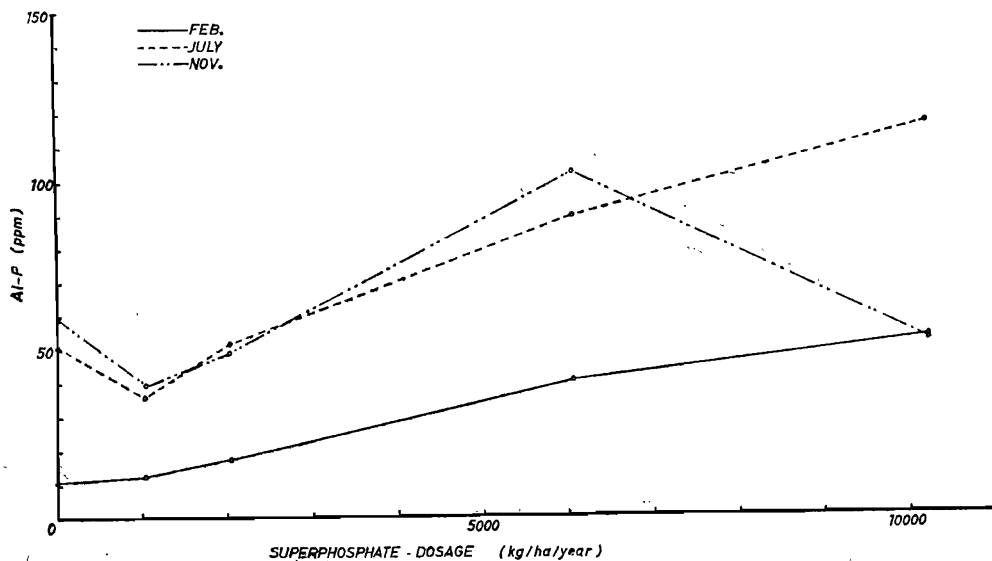


Fig. 6 Relationship between total amount of superphosphate dosage in 1969 and aluminum phosphorus content of bottom soil in 1970.

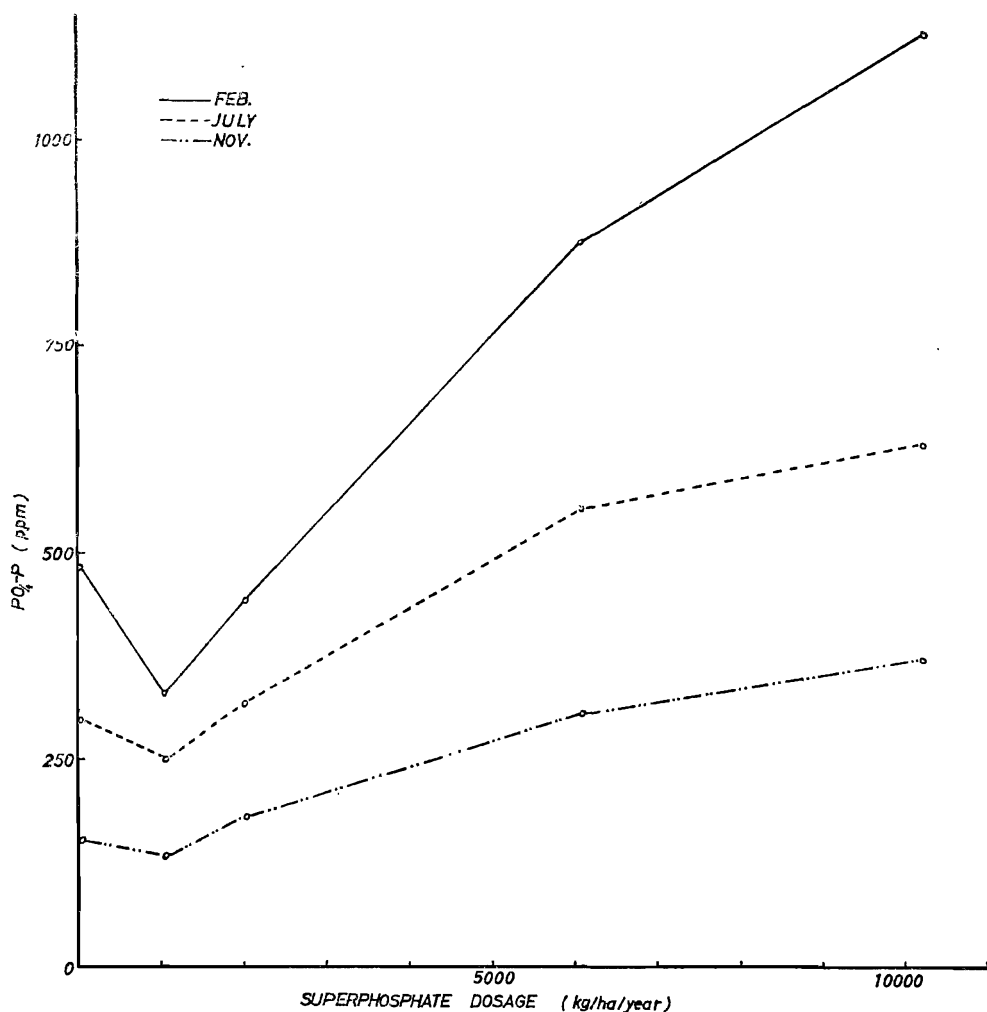


Fig. 7 Relationship between total amount of superphosphate dosage in 1969 and the sum of four kinds of inorganic phosphorus content of bottom soil in 1970.

superphosphate was added in 1969, had a higher concentration of calcium phosphorus in its soil than did ponds A-2 and A-3 which received no superphosphate. This may be explained by the fact that in the two years prior to 1969 pond A-1 received 3,000 kg/ha more superphosphate than A-2 and 2,200 kg/ha more superphosphate than A-3.

From the data of particle size analysis (Table 4), we found that the gradients of the three combined samples from one pond were different from one another except those of A-3. In ponds B-1 and B-3, the sand percentage of the three samples were all below 50 and the lower ones had higher phosphorus content. In the other ponds, samples containing half or more

Table 4. The percentage of particle size and phosphorus content of the soil samples in Chupei fish ponds on Feb. 5, 1970

Pond	Particle size			Phosphorus content	Ave. of PO ₄ -P
	Clay	Silt	Sand		
A-1	27	49	24	353.4	325.6
	27	43	30	403.5	
	20	30	50	249.8	
A-2	21	49	30	420.0	314.0
	19	33	48	257.6	
	23	27	50	264.5	
A-3	19	35	46	530.2	466.5
	17	37	46	490.7	
	19	33	48	378.5	
A-4	19	41	40	676.6	783.0
	19	37	44	1,117.6	
	10	17	73	554.9	
A-5	27	59	14	1,912.1	1,442.2
	17	21	62	1,059.7	
	10	17	73	1,354.8	
B-1	36	56	8	689.8	6,431.9
	23	47	30	837.9	
	24	36	40	403.9	
B-2	32	42	26	334.2	352.1
	25	44	31	376.7	
	21	31	48	345.7	
B-3	43	48	9	578.8	423.6
	27	34	39	341.8	
	23	26	41	350.2	
B-4	21	26	53	419.7	646.3
	—	—	—	—	
	—	—	—	—	
B-5	26	34	40	920.7	712.2
	23	34	43	814.0	
	16	29	55	401.7	

sand had a lower phosphorus content than the average of three samples. This seems to indicate that particle size affects the adsorption of phosphorus in the soil.

A relationship between the phosphorus concentration in the water and the total of the four kinds of phosphorus which we measured in the soil is

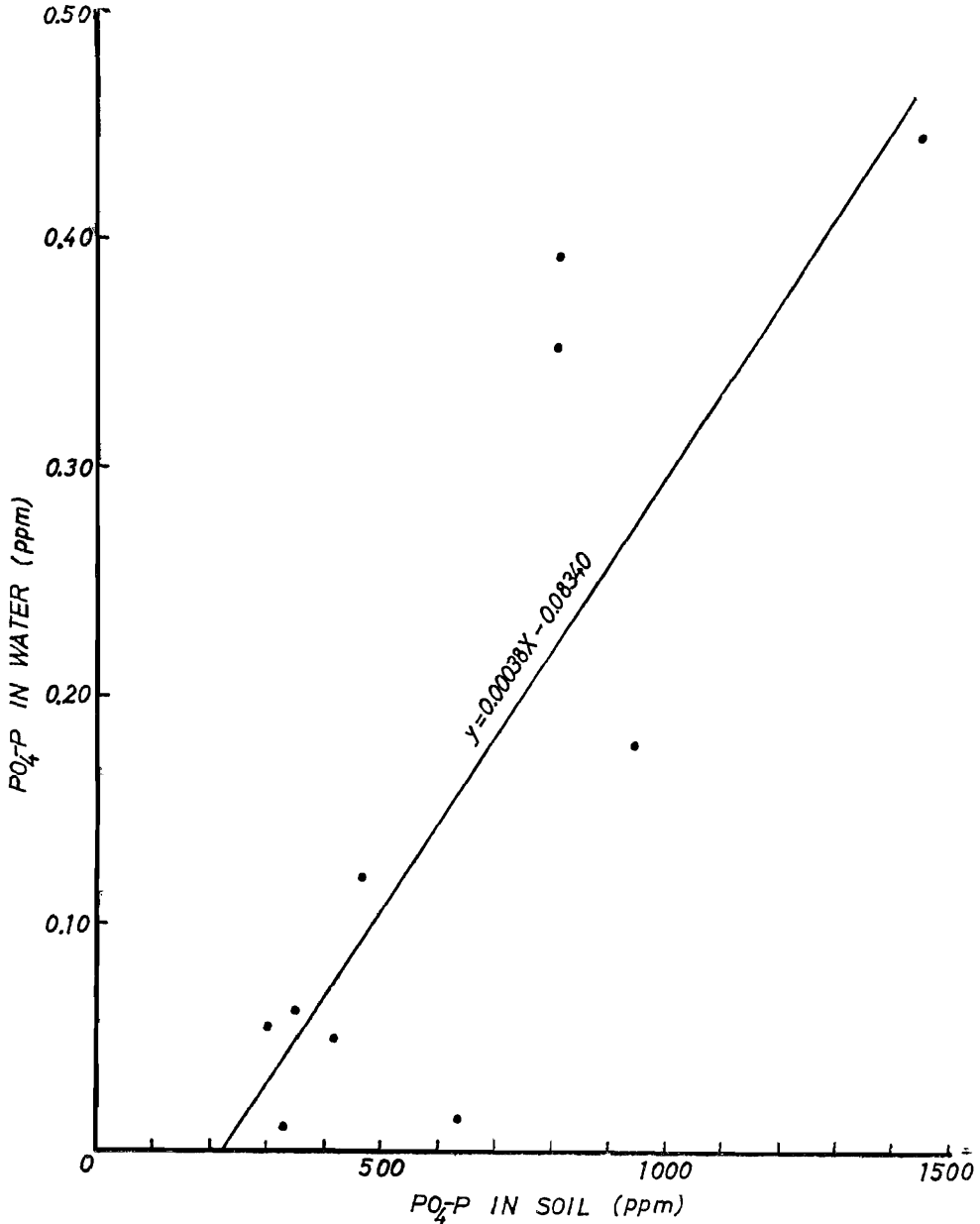


Fig. 8 Relationship between phosphorus content of bottom soil and phosphorus content in water of Chupei fish ponds when fertilizers were not added.

established by analysis of our data. If no fertilizers are added, this relationship shows that the concentration of phosphorus in the water is roughly proportional to the concentration of phosphorus in the soil (Figure 8).

III. Equilibrium between pond water and bottom soil

This equilibrium can be said to exist in a pond which is not artificially fertilized. Fertilization upsets this equilibrium. Phosphorus added to ponds in fertilization is lost from the water in three ways: (1) uptake by phytoplankton and other organisms; (2) adsorption into the bottom soil in the form of iron or aluminum phosphorus; and (3) precipitation as calcium phosphate through a reaction utilizing calcium carbonate.

1. Uptake of phosphorus by phytoplankton and other organisms

Phosphorus is one of the major nutrients for normal growth of algae. S. Y. Lin (1968) demonstrated that a suitable phosphorus concentration must be maintained to insure an optimum production of phytoplankton and domesticated fish. C. N. Lin (1969), working with the fish ponds of Taoyuan and Chupei, demonstrated that fertilization with phosphorus increased chlorophyll concentrations. If additional phosphorus is not applied, the concentration of phosphorus in the water will decrease.

The correlation coefficients between average phosphorus concentration and chlorophyll concentration or net gross production among the ten ponds in this study are shown in Table 5. The relationship between chlorophyll and phosphorus concentration varied with the ponds. The relationship in ponds A-2 and B-5 was significant ($P=0.05$) while no statistical significance

Table. 5. Correlation coefficient between PO_4 -P concentration and chlorophyll concentration or net gross production of O_2 in each pond from July to Dec. 1970

r Item	Ponds									
	A ₁	A ₂	A ₃	A ₄	A ₅	B ₁	B ₂	B ₃	B ₄	B ₅
PO_4 -P vs. Chl.	-0.621	0.764**	0.398	0.439	-0.375	-0.400	0.316	-0.119	-1.141	0.803**
PO_4 -P vs. Net gross production of O_2	0.096	0.822**	0.573	0.818**	-0.059	0.184	0.781**	0.328	0.425	0.657*

* $P=0.05$ $r=0.602$

** $P=0.01$ $r=0.735$

could be demonstrated in the other eight ponds. However, the relationship between average phosphorus concentration and chlorophyll concentration was significant ($P=0.05$) when the data from all ten ponds were combined. The relationship between net gross production and concentration of phosphorus also varied with the ponds studied. The relationship in ponds A-2, A-4, B-2 and B-5 was significant ($P=0.05$) while no statistical significance could be demonstrated in the other six ponds. However, the relationship between gross production and phosphorus was significant ($P=0.05$) when the data from all ten ponds were combined.

Therefore, there appear to be relationships between chlorophyll concentration or gross production and phosphorus concentration in the individual ponds if no other environment factors interfere.

Using the data presented in Table 6, we can calculate the following two regression equations Figure 9 and Figure 10, which show the relationship of chlorophyll concentration or gross production and phosphorus concentration.

Table 6. Average concentration of PO_4 -P, chlorophyll and O_2 gross production in the ten ponds in Chupei from July to December, 1970 ppm

	14/7	29/7	18/8	1/9	13/9	30/9	12/10	28/10	10/11	27/11	15/12
PO_4 -P	0.161 ± 0.138	0.335 ± 0.385	0.191 ± 0.142	0.156 ± 0.093	0.101 ± 0.069	0.070 ± 0.052	0.056 ± 0.047	0.083 ± 0.068	0.029 ± 0.026	0.042 ± 0.044	0.029 ± 0.012
Chl	98.86 ± 110.979	122.36 ± 140.403	172.54 ± 146.902	98.06 ± 78.678	84.14 ± 93.390	117.09 ± 88.880	86.03 ± 65.515	115.01 ± 114.581	—	71.53 ± 85.172	31.10 ± 22.857
O_2 Production	22.34 ± 12.236	23.80 ± 13.056	21.43 ± 9.159	17.30 ± 6.378	20.97 ± 8.940	8.51 ± 3.432	19.66 ± 7.234	7.27 ± 3.211	8.16 ± 3.738	7.38 ± 4.703	5.33 ± 2.363

1. Chlorophyll concentration = a chlorophyll constant plus the amount due to phosphorus concentration
 $= 50.300 + 356.399$ of phosphorus $r = 0.679^*$
2. Gross production (of O_2) = an oxygen constant plus the amount due to phosphorus concentration
 $= 5.281 + 86.433$ of phosphorus $r = 0.796$

From these data, we can demonstrate that a higher concentration of phosphorus is necessary to maintain chlorophyll concentration and gross production.

2. Phosphorus adsorption by bottom soil

Oxidized ferric compounds such as ferric hydroxide, ferric phosphate

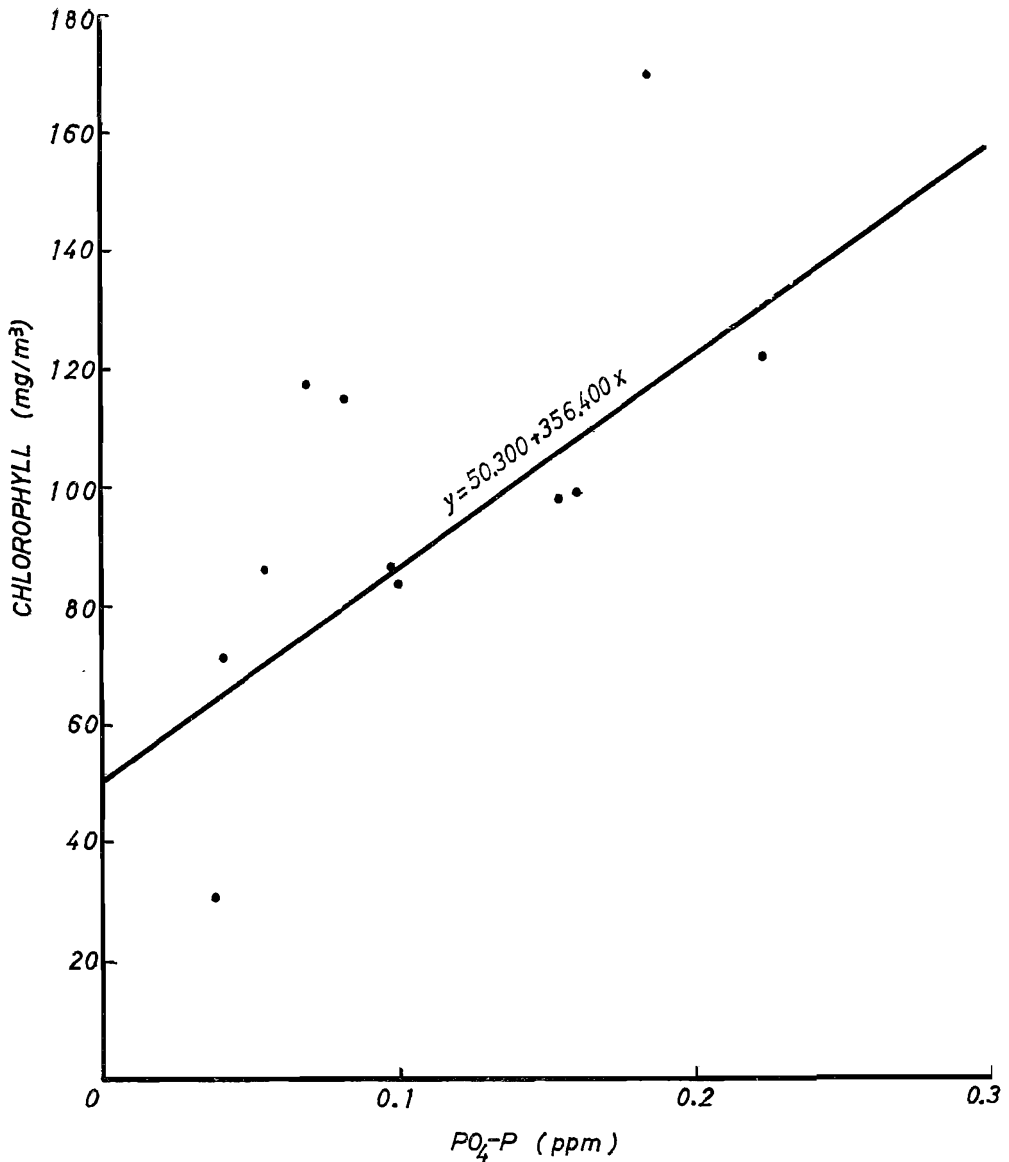


Fig. 9 Relationship between average phosphorus and chlorophyll concentration of the ten ponds from July to December, 1970.

and ferric-organic-complex compounds adsorb various quantities of phosphorus. However, phosphorus adsorption by these compounds can only take place when pH values are less than 8 (Hepher 1958).

In fish ponds pH values of lower layers of bottom soil are often below 8 and are generally lower than the upper layers of bottom soil. This is due to decomposing organic materials in the lower layers which have higher carbon dioxide concentration (Hutchinson 1957). Lin (1969) found the same

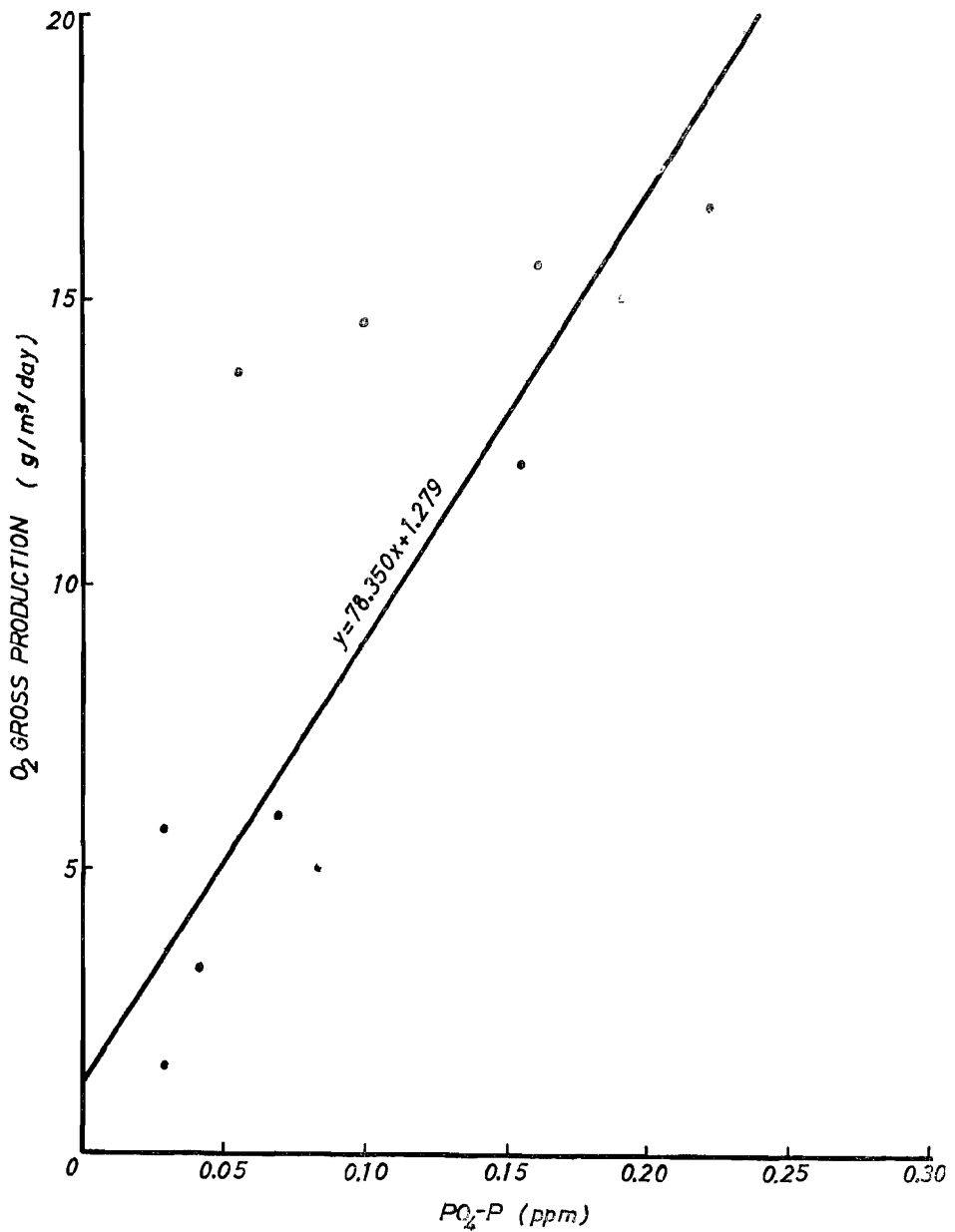


Fig. 10 Relationship between average phosphorus and O₂ gross primary production of the ten ponds from July to December, 1970.

Table 7. pH value of bottom soil in Chupei fish ponds in 1970

	A-1	A-2	A-3	A-4	A-5	B-1	B-2	B-3	B-4	B-5
Feb.	7.7	7.5	7.7	7.6	7.5	7.5	7.2	7.3	7.2	7.7
July	7.2	7.5	7.6	7.7	7.9	7.5	7.6	7.5	7.5	7.6
Dec.	7.3	7.4	7.3	7.7	7.5	7.4	7.4	7.3	7.5	7.4

phenomenon in the Taoyuan fish ponds. In the Chupei fish ponds we found that the pH values in the bottom soil ranged between 7 and 8 (Table 7). Therefore, the phosphorus in the water could be adsorbed by ferric compounds. For example, the determinations completed in 1970 showed that an average of 47.5% of all phosphorus measured in the bottom soil were iron phosphorus (Table 3). It is also clear that when increased levels of superphosphates are added to ponds, a corresponding increase in adsorption of iron phosphorus will occur in the bottom soil. For example, in 1969 in ponds A-2 and B-2, 1,040 kg/ha/year of superphosphate was added and the corresponding iron phosphorus values were 103.3 and 161.7 ppm, while in ponds A-5 and B-5, 10,230 kg/ha/year of superphosphate was added and the corresponding iron phosphorus values were 608.5 and 423.5 ppm.

Iron phosphorus can be released from the soil into the water if the soil becomes reduced. Table 8 shows the concentration of organic matter and the percentage change in organic matter and percentage change in iron phosphorus among the ten ponds in the year 1970. Figure 11 shows the ratio between organic matter and iron phosphorus as it changed in these ponds in 1970. From these data we found that there was a positive relationship between these two factors in the bottom soil.

Table 8. Organic matter and iron phosphorus content of bottom soil and percentage change in the periods from February to July and from July to November 1970

Pond	Organic matter content			Percentage change in organic matter		Iron phosphorus content			Percentage change in iron phosphorus	
	Feb.	July	Nov.	Feb.- July	July- Nov.	Feb.	July	Nov.	Feb.- July	July- Nov.
A-1	2.03	2.69	3.54	-32.5	—	49.9	105.0	21.2	-110.4	—
A-2	2.33	1.60	2.50	31.3	—	103.3	86.0	43.4	16.8	—
A-3	2.18	1.97	1.98	9.6	-0.5	161.6	135.4	100.3	16.2	25.9
A-4	1.92	1.48	1.56	22.9	-5.4	248.2	236.3	191.8	16.9	18.8
A-5	2.23	1.66	1.83	25.6	-10.2	608.5	352.3	419.0	42.1	-18.9
B-1	2.12	1.69	2.01	20.3	-18.9	297.9	103.5	156.5	65.3	-51.2
B-2	2.35	1.84	1.77	17.4	8.8	161.7	137.1	131.8	15.2	3.8
B-3	2.51	1.87	1.52	25.5	18.7	184.7	145.6	148.6	21.2	2.1
B-4	1.99	1.17	1.03	30.4	12.0	367.3	267.7	197.9	27.1	26.1
B-5	1.68	1.17	1.03	30.4	12.0	423.5	159.8	178.9	62.3	12.0

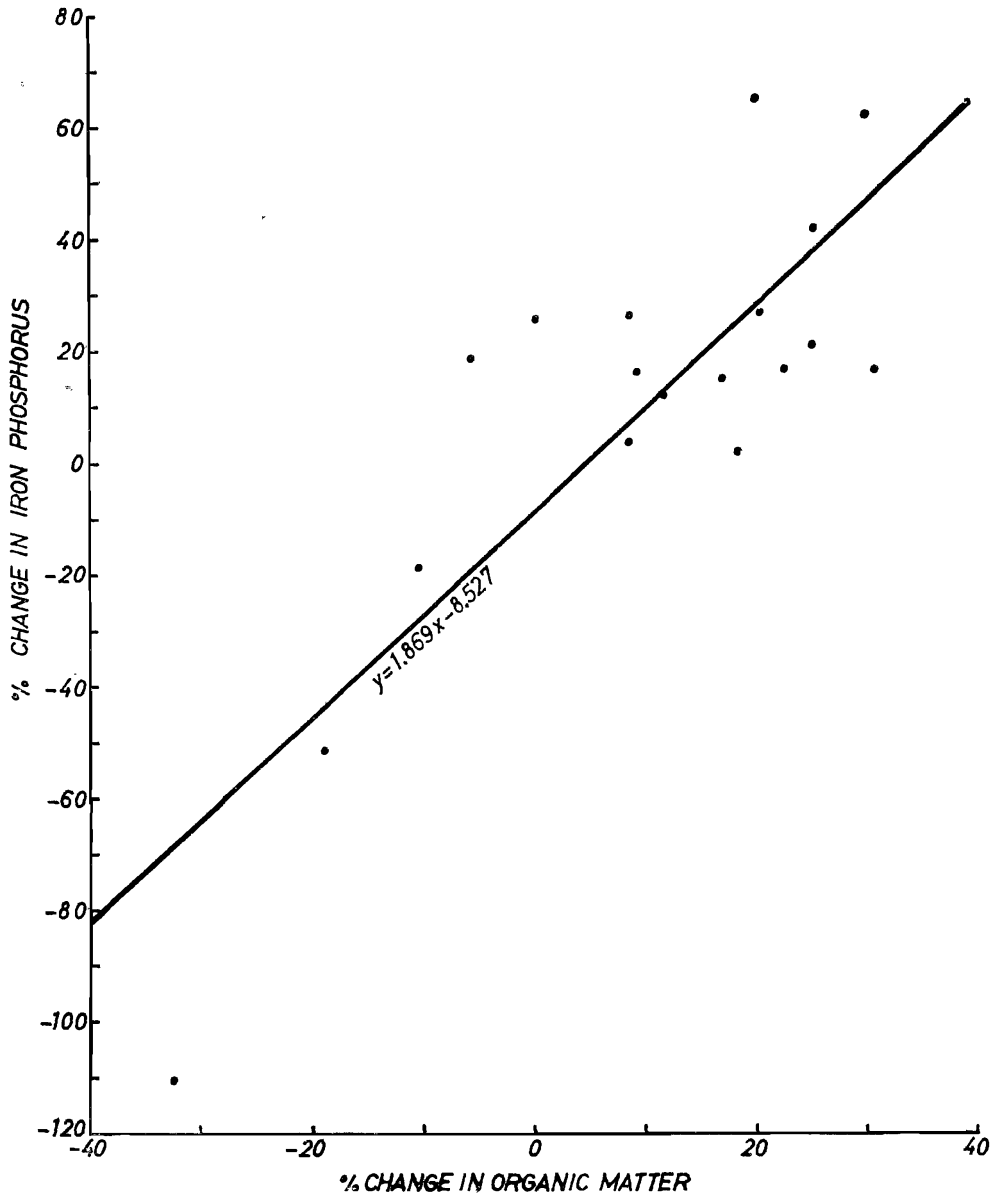


Fig. 11 Relationship between percentage change in organic matter and change in iron phosphorus content of bottom soil from February to June and from July to November, 1970.

Organic detritus from the ponds' food chain settles to the bottom and forms organic matter in the bottom soil. Reducer organisms in the soil break down this organic material and decrease its amount. To break down larger quantities of organic material these reducer organisms use increasing amounts of oxygen and thus reduce the bottom soil including the reduction

of iron phosphorus.

Ferric complexes, ferric hydroxide and ferrous phosphate are all related to phosphorus availability in the soil. Ferric hydroxide has the ability to adsorb phosphorus onto its molecule surface and ferrous phosphate contains phosphorus. Mortimer (1942 as reported in Hayes, 1958) indicated that the reduction of normally insoluble ferric complexes such as ferric hydroxide and ferrous phosphate resulted in the release of ferrous iron and other reducing materials from the bottom soil into the water. Therefore, as the organic material in the soil decreases, the iron phosphorus will also decrease. The biotic activity of reducer organisms will be involved in the release or accumulation of iron phosphorus in the soil.

3. Precipitation of calcium phosphate through a reaction utilizing calcium carbonate.

Hepher (1958) in Israel demonstrated that much of the phosphorus which was added to fish ponds by fertilization was precipitated as calcium phosphate through a reaction utilizing calcium carbonate. He pointed out that this reaction was pH sensitive. As photosynthetic activity increased in the pond water and raised the pH, calcium phosphate precipitation would increase especially in a pond with high calcium content.

In 1969, the average yearly pH value measured at 10:00 a.m. in the Chupei fish ponds ranged from 8.6 to 9.5 (Chiu 1970, unpublished). In 1970, the lowest average pH value for the ten Chupei fish ponds occurs at about 6:00 a.m. and ranged from 8.18 to 9.30. Since respiration exceeds photosynthesis in the 12 hour period before 6:00 a.m., CO₂ concentration in the pond water was highest at this time. This high concentration of CO₂ will lower the pH of the pond water.

From September to November, 1969, the average calcium content in ponds A-3, A-4, B-3 and B-4 ranged from 25.957 to 36.710 ppm (Lin 1970). This was similar to that in the same ponds and the same period in 1970. In 1970, the average yearly calcium content in the ten ponds ranged from 22.95 to 38.44 ppm and only two of these ponds averaged below 30 ppm (Lin unpublished). Since the saturation of calcium in water is about 15 ppm, higher amount of phosphates added in fertilization will be precipitated as calcium phosphate.

On February 5, 1970, the average concentration of calcium phosphorus in the bottom soil was 234.2 ppm. This represents 47.26% of the total phosphorus measured. On July 15, the calcium phosphorus concentration in

the soil was 114.9 ppm and on November 27, it was 8.3 ppm. The calcium phosphorus concentration was highest on February 5, immediately after the heavy fertilization period of 1969.

Summary

1. The phosphorus concentration in the water of the ten Chupei fish ponds depended on the concentration of phosphorus in the bottom soil if no fertilizers were added to the ponds. The addition of superphosphate or N-P-K fertilizers will affect the above relationship. For example, when N-P-K fertilizers were added to the pond water from July to December of 1970, the relationship between phosphorus concentration in the pond water and that in the bottom soil was not significant. When heavy doses of superphosphate fertilizers were added to the pond water in 1969, increases in phosphorus concentration in the bottom soil occurred even though water phosphorus concentration and fish production were maintained at about the same level. Pond water phosphorus concentration was in relation to various amounts of superphosphate added.

2. The relationship between the average concentration of phosphorus and chlorophyll or primary production in the pond water from July to December in 1970 was significant. It was demonstrated that a higher concentration of phosphorus was necessary to maintain a higher chlorophyll concentration or a higher gross production.

3. The pH values of the Chupei fish ponds were all above 8, while the pH in the bottom soils were all between 7 and 8. The average yearly calcium content in the Chupei fish ponds ranged from 22.95 to 38.44 ppm. Both the high pH and high calcium concentration enabled more phosphorus to be precipitated from the water into the bottom soil. The pH value of less than 8 in the soil is suitable for fixing the phosphorus into iron phosphorus.

4. An analysis of soil samples for particle size indicated that the soil was not homogenous. It was also found that differences in bottom soil among the ten ponds existed. The particle size in the bottom soil may affect the amount of phosphorus which could be adsorbed.

5. The increase in average aluminum phosphorus concentration of the ten ponds was observed in the February to July period, during which other phosphorus were found to be decreasing. However, the sum of the four groups of phosphorus, Sol.-P, Ca-P, Fe-P, Al-P, in the soil was much

higher in February 1970 than that in November 1970. Since no superphosphate was added to the pond water from February to July in 1970, the fish culture food chains must obtain their phosphorus from the bottom soil. From July to December 1970, phosphorus was obtained from the bottom soil and added N-P-K fertilizers.

6. There was a positive relationship between the changing ratio of organic matter and the iron phosphorus in these ponds. The biotic activity of reducer organism may be involved in the release or accumulation of iron phosphorus in the soil.

7. Heavy fertilization in some of the ten ponds throughout 1969 increased the amounts of inorganic phosphorus in the bottom soils measured in 1970.

From the above it can be concluded that for optimum fish culture a balance among soil phosphorus concentration, water phosphorus concentration, and the addition of various phosphorus fertilizers must be maintained.

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淡水魚池施肥試驗研究

磷、氮肥料對浮游生物量與魚產之關係

FERTILIZER EXPERIMENTS IN FRESHWATER FISH PONDS— RELATION OF PHOSPHORUS AND NITROGEN TO PLANKTON AND FISH PRODUCTION

Hong-siong Ong*

Abstract

The purpose of this experiment was to find the effect of phosphorus and nitrogen fertilizers on the seasonal succession of plankton and fish production in ponds. Two series, A and B, each consisting of five ponds (four treated and one as control), were used. It was found that the monthly fluctuation of phytoplankton production was different from that of zooplankton, as zooplankton became abundant from February to May and phytoplankton were dominant in the months of July to November. In ponds of series A, fish production and phytoplankton abundance were in linear relationship, but in series B, their relationship was irregular. There was no clear relationship between the abundance of zooplankton and fish yield in either series of the experimental ponds. Because of the fact that water supply to the experimental ponds was mixed with the drainage from some rice fields and thus contaminated with both inorganic and organic fertilizers, there was no clear-cut effect of different dosages of fertilizers applied on fish production. Among the ponds of series A, the one that maintained a PO_4 -P level of 0.1-0.29 had the highest yield of fish. The addition of nitrogen fertilizer to ponds of series B resulted in a tremendous increase in bluegreen algae but failed to increase fish production, probably due to the low value of bluegreen algae as forage for Chinese carps and further because the bluegreen algae in abundance suppressed the growth of the diatoms and green algae.

In fish ponds, adequate growth of phytoplankton in general improves

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water quality and provides better conditions for fish growth, and it is possible that Chinese carps thrived better in ponds of series A with deeper water than those of series B.

引 言

施肥養魚的目的在以最少之成本獲得較高的魚產；這是每一位養殖業者的企盼，亦是一般專家的理想。要達到此一理想的目標，必須考慮到生物的、化學的、及物理的各種因素的相互關連。本試驗旨在訂定一標準淡水魚塢施肥方法，以增加魚產。

本試驗之設計與進行，原由林書顏教授主持，先以竹北五個池作為試驗，決定磷肥之各種不同濃度對於魚產之影響；後農復會又邀請日本淡水區研究所專家里見至弘博士，伊藤時夫，杉目宗尙及東井純一，相繼來竹北分所作技術性指導。里見至弘博士等認為氮素對於施肥養魚有其重要性，故又另設計五個池作磷肥加施氮肥之研究，以探討加施氮肥對魚產之關係。

施肥養魚試驗研究計劃於1969年1月18日開始實施，由竹北分所長劉嘉剛策劃，督導；技士吳樂藩負責魚池施肥與管理；彭弘光與邱長吉負責水質分析；筆者司浮游生物研究工作。

材 料 與 方 法

本試驗設計利用10個魚池作為試驗；分A、B兩區各5個池即(A₁、A₂、A₃、A₄、A₅及B₁、B₂、B₃、B₄、B₅)，A₁與B₁池不施任何肥料，作為對照池。A區之A₂至A₅各池施各級磷肥(過磷酸鈣[CaH₄(PO₄)₂])，全年分80次施給；B區之B₂至B₅各池則加施氮肥(硫酸銨[(NH₄)₂SO₄])，全年分78次施給；如表一。

表一 A、B區各池預定施肥濃度概況

池 號	磷 肥 濃 度 (ppm)	氮 肥 濃 度 (ppm)
A ₁		
A ₂	0.05~0.09	
A ₃	0.10~0.29	
A ₄	0.30~0.59	
A ₅	0.60~1.00	
B ₁		
B ₂	0.05	0.5
B ₃	0.10	0.5
B ₄	0.30	0.5
B ₅	0.60	0.5

浮游生物的採集，配合水質部分進行。浮游生物研究之方法，可分為兩個步驟：

(→)野外工作：浮游生物水樣之採集於每週一、四上午十時在每一池之中央，分表層(距水面20公分處)，底層(距池底面20公分處)，全層(水面至池底面之處)三處分別採水。底層浮游性植物之水樣，由軟質橡皮管吸取注入250cc之塑膠瓶中；全層浮游性植物及浮游性動物水樣則用一圓形鐵桶(直徑20cm，高100cm)採集，將圓形鐵桶之鐵板徐徐斜向水裏放下至池底面，然後對準鐵板之中軸，將圓形桶殼徐徐套入至池底面置妥之鐵板，再將螺絲轉緊，並量其水體積之高度，然後將鐵桶由池中提出水面，放置於已備好之圓形塑膠桶中(直徑35cm，高45cm)，將鐵桶中柱之螺絲鬆開，水即注入桶內，

然後將 250cc 之塑膠瓶放入桶中盛滿作為全層浮游性植物之樣品，同時又從桶中取出 500cc 水作為水質分析用。桶內剩水全部傾入 Hansen 式浮游生物網內（2 號節網布）過濾，作為浮游性動物之採集樣品。

採水之同時，對於天氣的變化，水色、水溫等加以記錄，以作參考。

(二)室內工作：將採自 10 個池之 40 個樣品（浮游性植物 30 個，浮游性動物 10 個）帶回試驗室。固定於 5% 福馬林防腐液中，若藍藻類多者因不易沉澱，必需加多倍之福馬林原液。然後將動、植物浮游生物標本分別靜置於樣品架上，候廿四小時後始可作定量用。浮游性動物之定量用有刻度（0.1~10cc）大型 100cc 沉澱管使之沉澱，候廿四小時後始可紀錄其沉澱量之體積。浮游性植物先用 50cc 大型離心管以 3,500 R.P.M. 離心 20 分鐘使之沉澱，至 250cc 標本離心完畢為止；然後將所有沉澱物取出倒入已校正過之有刻度（0.01~2cc）小型離心管再作最後一次離心，廿分鐘後取出讀其沉澱量之體積。在離心前需將原始樣品分別量其水之體積，以便計算其每公升水或每立方米水浮游性植物之含量；其後再利用顯微鏡觀察動、植物浮游生物之種類組成。

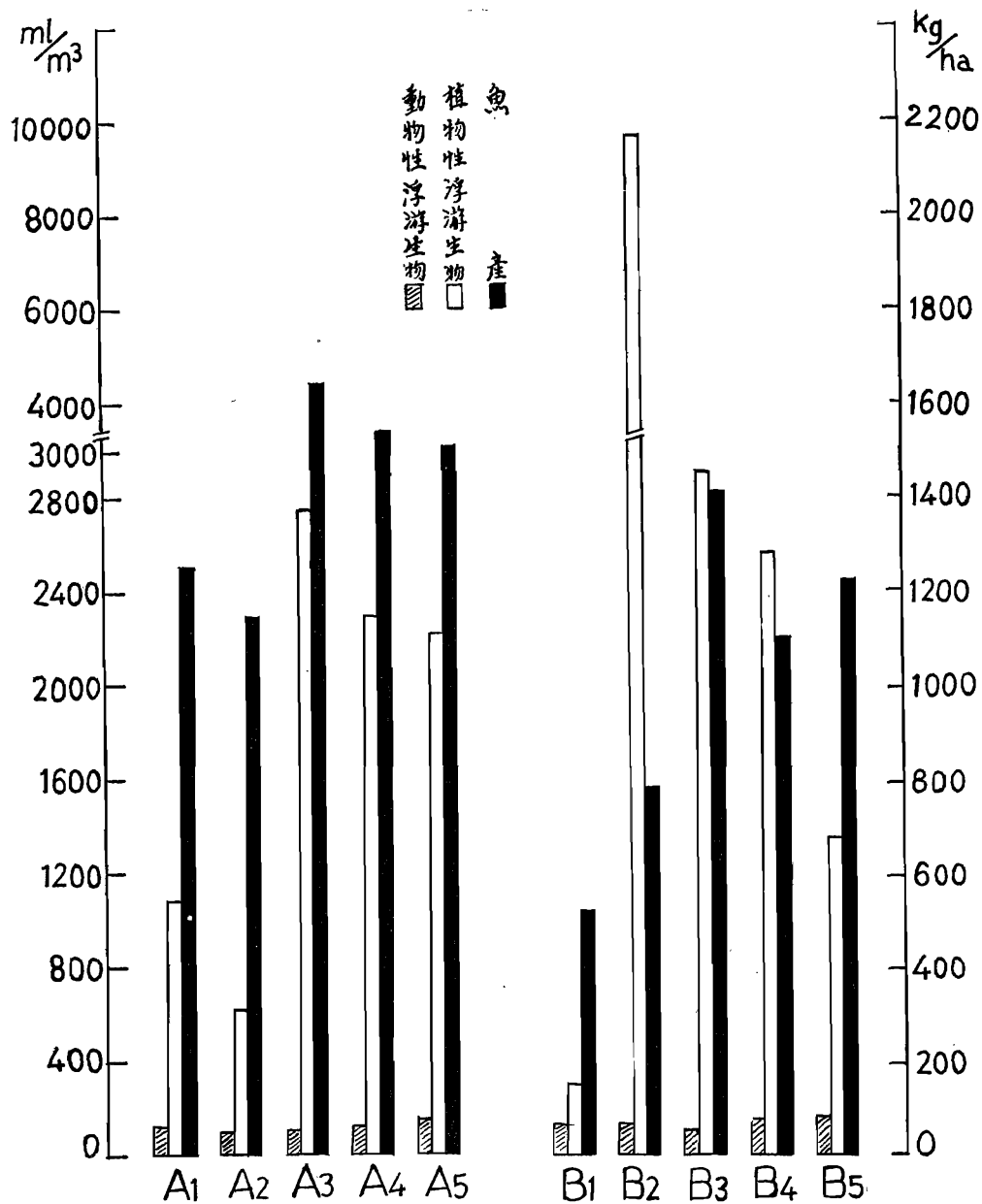
表二 植物性浮游生物月平均之含量值（A 區）

單位：cc/m³

日期	池號	A ₁	A ₂	A ₃	A ₄	A ₅
一	月	11.4	10.3	11.2	16.2	13.6
二	月	15.0	9.9	8.3	15.1	42.5
三	月	13.0	8.4	8.9	11.0	55.0
四	月	19.4	14.1	13.3	29.0	19.9
五	月	89.8	20.4	23.9	59.0	110.3
六	月	71.1	22.5	28.8	61.8	126.6
七	月	49.2	26.1	44.1	163.7	308.6
八	月	169.6	48.6	262.6	444.4	487.0
九	月	201.2	90.6	411.8	542.3	462.6
十	月	170.8	135.5	927.3	330.8	232.9
十一	月	282.7	239.7	1009.1	627.8	361.8

結 驗 與 討 論

表二，為 A 區試驗池植物性浮游生物一至十一月各月平均之比較變化。A₃池在 A 區中藻類生產量最高，其全年總含量為每立方米水 2,749.3cc 之沉澱量，因而魚產量亦高，每公頃單位產量達 1,664.02 公斤。魚產量與植物性浮游生物量在 A 區魚池中成正相關之關係，如表六及圖一所示。而 A₃池的磷肥濃度在 0.1~0.29ppm，故可證明施適量之磷肥濃度，可促使魚產增高。根據 Moyle (1946)，磷的池水含量以 0.1~0.2ppm 最好，0.2 以上則過量，故 A₄池的磷肥濃度在 0.30~0.59ppm 的魚產量較 A₃池為低；A₅池的磷肥濃度在 0.60~1.00ppm 時之魚產量又較之 A₄池魚產量為低，因此可證明施肥量太多並無益處，且不經濟，但施肥量太少亦不好，如 A₂池磷之濃度為 0.05~0.09ppm，其魚產在各施肥池中屬最低，單位生產量僅 1,149.60 公斤，浮游性植物的含量也最低。故磷之不足，影響植物性浮游生物的發生，且並不如 A₃、A₄、A₅ 各池的浮游性植物在夏天以後溫度高時有顯著增加。至於不施肥的對照池 A₁池亦有相當高的魚產量，較 A₂池有施肥者為高，這是因為 A₁池本身所含有的肥度很高，且磷肥有殘餘效果 (Mortimer, 1954)，可能一部份由稻田經水流帶入該池。一般來說，A 區各池的浮游性植物之多寡與魚產成正相關趨勢；但和施放磷肥之多少無明顯之關係（圖 1）。



圖一 A區與B區各池浮游生物及魚產年總產量之比較

Fig. 1 Comparison of the total plankton quantity and fish yield in ponds A and B series.

表三 植物性浮游生物月平均之含量值 (B區)

單位: cc/m³

日期	池號	B ₁	B ₂	B ₃	B ₄	B ₅
一	月	11.9	11.9	14.5	13.2	17.2
二	月	12.5	13.3	14.3	18.3	21.1
三	月	11.7	18.5	13.5	17.9	24.7
四	月	17.5	20.1	21.4	55.3	24.4
五	月	52.6	48.9	165.5	82.1	44.1
六	月	22.0	96.2	128.2	119.1	26.7
七	月	28.0	2,931.2	168.9	137.0	37.7
八	月	21.2	3,112.5	765.5	377.9	199.8
九	月	53.1	951.9	538.5	589.8	402.3
十	月	32.4	661.2	454.0	454.6	319.1
十一	月	35.2	1,829.7	626.9	701.3	237.5

表三, 為B區試驗池植物性浮游生物各月平均值之比較。B₃池磷之濃度維持在(0.1~0.29ppm)並加施氮肥(0.5ppm), 其浮游性藻類發生量雖不如B₂池之高, 但魚產量在B區為最高, 可見磷肥維持在(0.1~0.29ppm)的施肥量在B區中最適合, 但與單用磷肥 0.1ppm~0.29ppm 濃度之A₃池比較, 則魚產遜於A₃池。由此可知加施氮肥於池水中, 有減產之情形, 與美國、以色列之試驗結果相同(Lin, 1969)。B₄池(磷肥濃度0.3~0.59ppm及0.5ppm氮肥)之浮游性藻類產量較B₃池為低, 而魚產則更低。B₅池施磷肥濃度為 0.6~1.00ppm 及 0.5ppm氮肥)之浮游藻類產量較B₄池為低, 每立方米水含量為 1,519.70cc, 而單位魚產量為 1,231.19公斤, 比之B₄池為高, 而B₂池(施磷肥, 濃度 0.05~0.09ppm 及0.5ppm氮肥)之浮游性藻類產量較各施肥池為高, 每立方米水含有9,824.40cc. 而魚產量則為最低, 每單位魚生產量僅有785.57公斤, 如表六及圖一所示。故在B區魚產量和植物性浮游生物並無明顯之關係存在。

表四 動物性浮游生物月平均之含量值 (A區)

單位: cc/m³

日期	池號	A ₁	A ₂	A ₃	A ₄	A ₅
一	月	12	8	7	9	11
二	月	17	13	7	23	31
三	月	13	10	5	26	33
四	月	16	8	8	9	19
五	月	20	8	18	17	11
六	月	18	16	28	14	12
七	月	9	9	12	7	10
八	月	7	6	10	8	9
九	月	6	5	7	6	6
十	月	4	7	4	3	8
十一	月	3	3	5	4	4

表五 動物性浮游生物月平均之含量值 (B區)

單位: cc/m³

日期	池號	B ₁	B ₂	B ₃	B ₄	B ₅
一 月		10	8	9	7	11
二 月		19	18	14	24	20
三 月		24	12	14	16	10
四 月		44	26	11	30	45
五 月		8	17	16	27	38
六 月		9	15	13	22	20
七 月		6	10	9	9	8
八 月		4	9	5	6	5
九 月		4	6	5	4	4
十 月		2	4	3	3	2
十一 月		2	4	4	3	2

動物性浮游生物量的變化，A區以A₅池為最高，年單位總含量每立方米水中為154cc，依次為A₄、A₁、A₃、A₂；B區則以B₅池佔最高含量，每立方米水中含有165cc，依次為B₄、B₁、B₂、B₃，如表六。一般而言攝食動物之魚類如鱸、鯉在A區之生長較B區為佳如表七。故不論在A、B區魚池，動物性浮游生物之現存量和魚產間並無一定關係。表四、表五表示各池浮游性動物量的季節變化，可以看出在溫暖的春季大量繁殖，尤以B區之B₅、B₁、B₄及B₂在四月時相續達到最高峯。B₃池沒有太大變化；B₁池五月時已急降，所有B區魚池在七月以後全部急降，一直維持到試驗終了。而A區之A₅、A₄在二月時大量繁殖，至三月達到最高峯，四月急降；但A₃在六月才達到最高峯，其他各池在養殖期間雖有變化，但並不太明顯。所有A區魚池到七月以後和B區魚池一樣亦低降，而沒多大變化。比較A、B二區之浮游性動物量之變化，可知B區魚池之變化頗為一致，在四月及五月為最多；A區魚池之變化不規則，有些魚池形成二個高峯，但相同的是不論A、B二區魚池在七月以後，動物性浮游生物逐漸下降至年底，這可能是夏天陽光太強，水温高，影響浮游性動物的棲息與生存（西條八東，1962）及浮游性動物被魚類所食之結果；且七月以後植物性浮游生物大量繁殖，因此減少池水之溶存有機物，使動物性浮游生物之食料缺乏而影響其現存量，因而鯉在A、B區各施肥池之生產量，一般不如不施肥池（Lin and Chen, 1966）。

表六 A區與B區各池浮游植物及動物年總產量與魚年總產量比較

種 類	池 號					總 計
	A ₁	A ₂	A ₃	A ₄	A ₅	
浮游植物 ml/m ³ /year	1,093.2	626.1	2,749.3	2,301.1	2,220.8	8,990.5
浮游動物 ml/m ³ /year	124	93	111	126	154	608
浮游植物 + 動物	1,217.2	719.1	2,860.3	2,427.1	2,374.8	9,598.5
魚 產 量 ky/ha/year	1,256.91	1,149.60	1,664.02	1,568.23	1,521.77	7,160.53
種 類	池 號					總 計
	B ₁	B ₂	B ₃	B ₄	B ₅	
浮游植物 ml/m ³ /year	298.1	9,695.4	2,911.2	2,566.5	1,354.7	16,825.9
浮游動物 ml/m ³ /year	132	129	103	151	165	680
浮游植物 + 動物	430.1	9,824.4	3,014.2	2,717.5	1,519.7	17,505.9
魚 產 量 ky/ha/year	524.57	785.57	1,421.65	1,100.00	1,231.19	5,062.98

表七 A、B兩區各試驗池魚產量收成之比較

單位：kg/ha

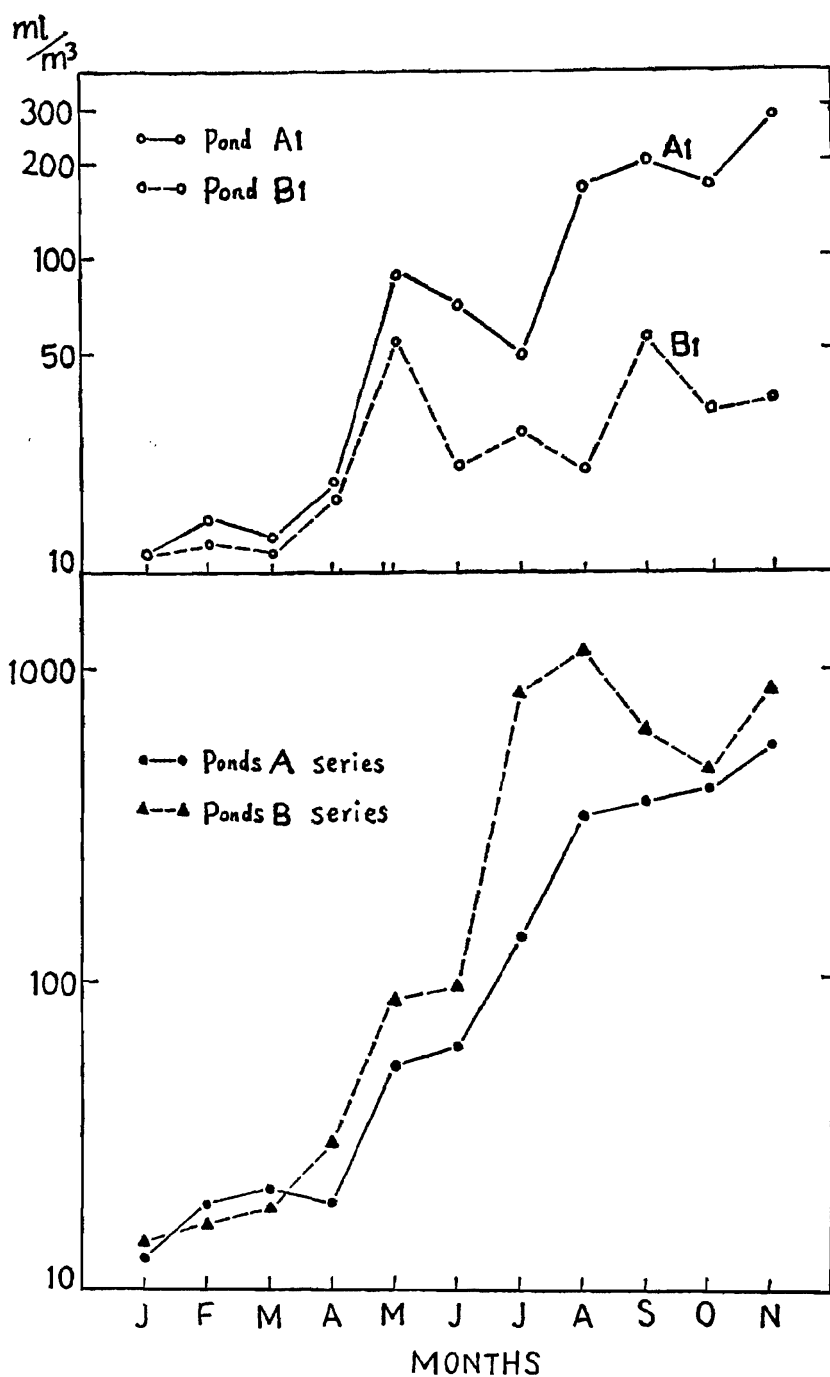
魚別	池號	A區						B區					
		A ₁	A ₂	A ₃	A ₄	A ₅	總計	B ₁	B ₂	B ₃	B ₄	B ₅	總計
鱧		643	444	856	800	742	3,485	193	245	649	501	596	2,184
鱮		157	112	174	184	131	758	17	46	120	115	111	409
鮭		38	42	63	29	21	193	25	14	38	26	24	127
鯉		194	227	208	137	741	840	138	48	101	151	201	639
河內	鮑	89	77	143	119	115	543	48	63	97	96	135	439
雜	魚	137	247	221	299	439	1,343	103	369	417	220	164	1,273
總	計	1,258	1,149	1,665	1,568	1,522	7,162	524	785	1,422	1,109	1,231	5,071

表八 A、B兩區魚類之肥滿度及各池魚類之平均肥滿度比較

魚別	池號	A區					平均文	B區					平均文
		A ₁	A ₂	A ₃	A ₄	A ₅		B ₁	B ₂	B ₃	B ₄	B ₅	
鱧		10.35	12.09	12.16	10.67	11.34	11.32	11.11	9.56	12.09	11.76	11.04	11.11
鱮		11.50	11.62	15.31	11.15	12.09	12.33	10.69	13.30	12.32	11.44	12.62	11.67
鮭		11.06	12.98	10.41	9.53	9.84	10.76	8.35	12.59	9.98	10.69	9.38	10.20
鯉		14.95	17.07	16.52	15.65	16.11	15.96	15.90	16.93	16.52	19.53	17.75	17.33
河內	鮑	16.90	16.74	20.40	20.05	16.93	18.20	16.63	15.88	15.86	20.95	19.97	17.86
平均	X	12.95	14.10	14.96	13.31	13.26		12.54	13.25	13.35	14.87	14.15	
A、B區各總平均文		13.71						13.63					

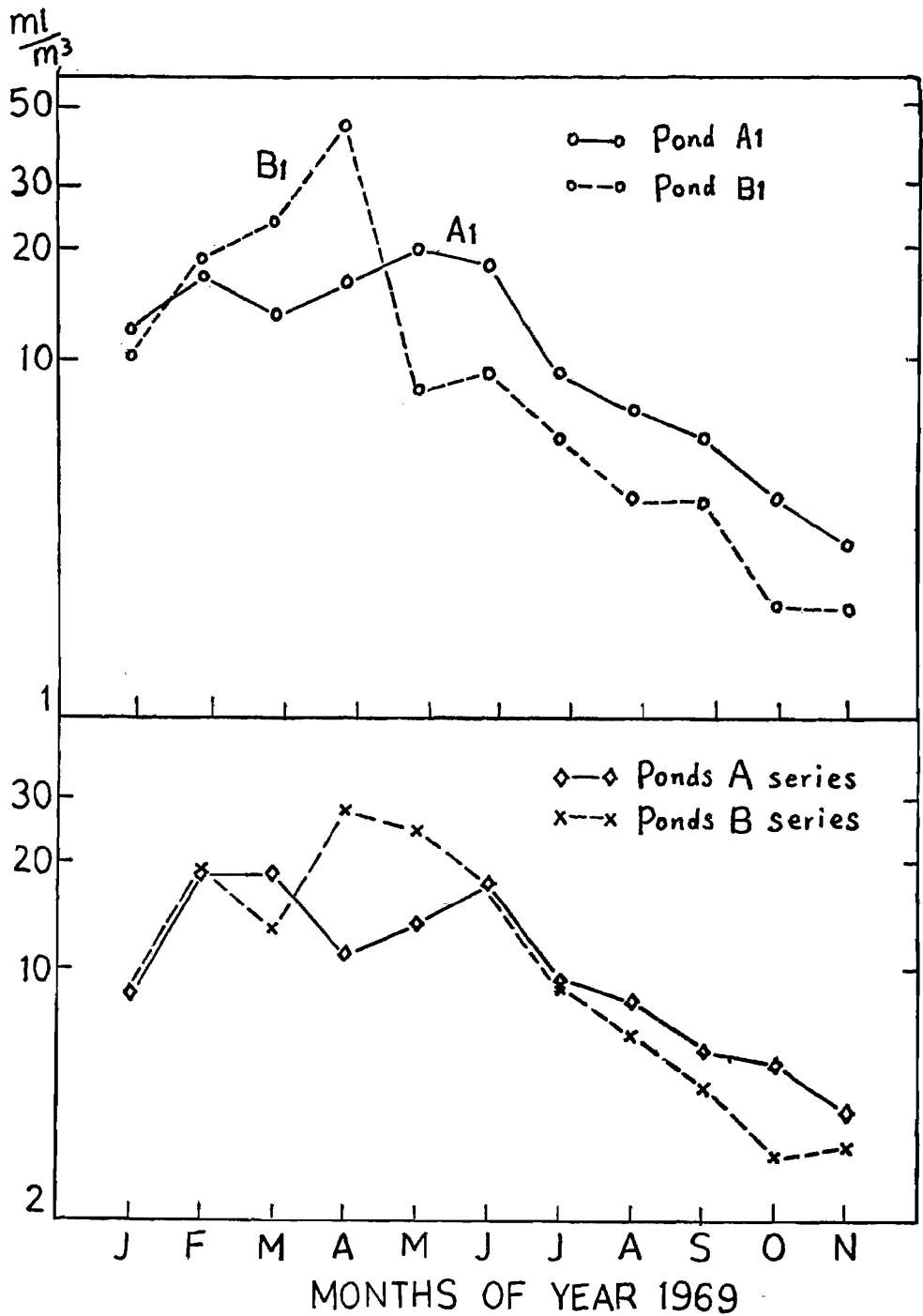
在本試驗主要是放養鱧、鱮、河內鮑，故植物性浮游生物的發生量對其生長有極重要之關係，而動物性浮游生物的影響較小。浮游植物未大量發生前，浮游性動物先大量發生，以後植物性浮游生物大量發生，動物性浮游生物才急降。故不論A、B二區魚池，動物性和植物性浮游生物有明顯之負相關存在。一般來說，植物性浮游生物的發生依着水温之升高，施肥量之多寡就直接影響其發生，魚亦開始生長。如A₃池B₃池之藻類較多，鱧魚產量亦最高，如表七；其肥滿度亦較其他各池為高，如表八，此乃因鱧魚對磷肥有顯著反應。另值得注意的是施無機磷肥可直接影響綠藻類及硅藻類之大量繁殖，因其易於消化，富營養（Lin S. Y., 1966），如其繁殖量足夠魚之攝食，能促使魚之生長加速，因魚之生長最快在夏季，其新陳代謝作用快，需足夠有營養之藻類為其食用。如磷肥施用太少，如A₂池，則藻類繁殖不足以供給魚類之所需，致使池水成貧瘠狀態。如施太多磷肥亦不好，因磷肥過多時致使細菌及藻類對氮（N）及碳（C）之要求亦高，因而引起缺乏，而使藻類減產；如含磷量在0.3~0.59ppm及0.6~1.00ppm之A₄、A₅池非但藻類無法繼續繁殖，且魚產亦跟着減產之現象；林正男（1968）亦證明此點。

磷肥加施氮肥雖可促使植物性浮游生物大量繁殖，但對魚產並不能增加，反而產量較低，如B₂池為例。一般證明藻類繁殖過量時，將產生很多弊害，尤其是藍藻類之發生。筆者將B₂池浮游性藻類作定性分析，每立方米水中含有藍藻類之 *Phormidium tenue*, *Merismopedia convoluta* 及 *Merismopedia punctata*, *Chroococcus cinneticus* 與 *Chroococcus turgidus*，以及其他微量之藍藻類如 *Anabaena* sp., *Coelosphaerium* sp., *Aphanocapsa* sp., *Microcystis* sp. 及 *Oscillatoria* sp. 共佔89.40%。綠藻類佔5.40%；硅藻類只佔2.04%。由此可證藍藻類在B₂池中佔最高總含量；而此種藻類在腐敗後，會產生大量有機質，致使水質變壞，同時產生大量H₂S, NH₄OH及沼氣等，其濃度過大時，影響水中生物之生長，一方面水中之有機質含量太大，使水中缺氧，因有機物分解時需要氧之供應，



圖二 比較A區與B區及其兩對照池之植物性浮游生物平均值月變化。A區 (A₂~A₅) 施用過磷酸鈣 [CaH₄(PO₄)₂] ; B區 (B₂~B₅) 施過磷酸鈣加施硫酸銨 [(NH₄)₂SO₄]。

Fig. 2 Comparison of the mean phytoplankton succession in two control ponds A₁ and B₁ and ponds A series with superphosphate application and B series with superphosphate plus ammonium sulfate.



圖三 比較A區與B區及其兩對照池之動物性浮游生物平均值月變化。A區(A₂~A₅)施用過磷酸鈣 [CaH₄ (PO₄)₂] ; B區(B₂~B₅)施用過磷酸鈣加施硫酸銨 [(NH₄)₂SO₄]。

Fig. 3 Comparison of the mean zooplankton succession in two control ponds A₁ and B₁ and ponds of A series with superphosphate application and of B series with superphosphate and ammonium sulfate.

故水中的溶解氧往往不敷魚類所需用時則會大量死亡。鯉之死亡率達58.3%，可能與此有關。若不至此，亦會抑制魚類之生長。尤以B₂、B₃、B₄各池之藻類繁殖年總含量已超過A₂、A₃、A₄各池如表六，其魚產量比之甚低；其原因是池中加施氮肥易助長藍藻類如*Phormidium*, *Oscillatoria*, *Merismopedia*, *Chroococcus*, *Anabaena*, *Coelosphaerium*, *Aphanocapsa*, *Microcystis* 等及綠藻類之 *Chlorella* sp., 因其細胞壁厚，魚食之則不易消化，營養價值甚少，且其在水表面繁殖，阻礙他種藻類如矽藻類之發生 (Lin, 1966)。E. A. Savin (1962 與 1963) 在俄國研究鱖魚的食性亦證明藍藻類如 *Anabaena*, *Coelosphaerium*, *Oscillatoria*, *Merismopedia* 不易被魚消化。筆者曾於1968年研究鱖、鱒，鱖之食性，發現B池有大量繁殖之藍藻類尤其是 *Anabaena* sp. 和 *Coelosphaerium* sp., *Aphanocapsa* sp., *Microcystis* sp. 等，鱖、鱒魚之生長效果不佳；而A池塘則僅有綠藻類如 *Scenedesmus* sp. 等與矽藻類大量發生，魚類較B池塘生長率高。由此亦可證明鱖、鱒魚對藍藻類不易消化。因之可知增施氮肥會促使藍藻類過量繁殖，反而對魚之生長有不良影響。

魚池大小與水深亦影響浮游生物繁殖之密度、營養之分佈及魚類活動之範圍，此亦B區魚產量低之一原因。魚池較大者，魚類所能利用之食料之總量較多。據伊藤時夫、東井純一等 (1957) 在日本的鹽田平之溜池作試驗亦獲此一結論。如A₃、A₄和B₃、B₄之浮游生物量沒有顯著之差異，但魚產量普遍地提高，乃因水位較B區為高，如表九。A區平均水深為75cm，B區平均水深為66.5cm，相差約10cm。在一般施肥養魚池，其水位以1米最佳，故本試驗未能達到最高魚產和此亦有關。

表九 A區與B區平均水深與其各池平均水深之比較

池 號	A ₁	A ₂	A ₃	A ₄	A ₅	B ₁	B ₂	B ₃	B ₄	B ₅
水 深 cm	72.2	81	72.1	70.6	78.2	65.2	67.1	66.6	62.2	71.6
A、B區水深	75cm					66.5cm				

摘 要

(1)本試驗自1969年1月至11月，每期二次測定竹北魚池 (A區為施磷肥，B區為施磷及加施氮肥) 浮游生物量之季節變化。

(2)不論A區或B區，動物性浮游生物量及植物性浮游生物量有明顯之負相關。動物性浮游生物一般A區在二月至三月，B區在四月至五月為最多；而植物性浮游生物在七至十一月最多。

(3)A區魚池，植物性浮游生物及魚產量有明顯之正相關；B區魚池，植物性浮游生物與魚產量則無顯著之關係存在。

(4)不論A、B區魚池，動物性浮游生物和魚產無明顯之關係。

(5)有若干魚池由於先天環境之不同，及難於控制藻類生長；故施放肥料之多少和魚產無明顯之關係。

(6)A區魚池，水中磷量以保持0.1~0.29ppm 為最適合，太少或太多，則成不足或浪費，並引起其他物質之缺乏，以致於減產。

(7)B區魚池中，氮肥增加浮游生物量，其中尤以B₂池最顯著，但增加之藻類量非魚類所能完全消化吸收，且藻類腐敗後可能引起各種弊害，故魚產量亦不高，氮肥之效果不佳。

(8)在魚池環境中，浮游生物為左右水質和魚產量之主要因素。

(9)水深亦為一影響魚產量之重要因素；B區池水淺，亦為魚產量較少之一原因。

附 記

本報告得以完成，全賴農復會漁業組陳組長同白，林顧問書顏、袁技正柏偉；省水產試驗所鄧所長

火土等之策勵、督導；竹北分所各位同仁之協助，陳弘成、吳榮富等先生提供寶貴之意見，筆者在此一致謝。

再本文承蒙林教授書額在美國百忙之中親為修改，筆者謹此致最大之謝忱。

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INCREASE OF BENTHIC ALGAE IN MILKFISH PONDS BY APPLICATION OF SILICATE

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Abstract

Conditions provided in the winter preparation of the milkfish ponds are favorable for bottom algal growth, especially the diatoms which might reach 90% of the total bottom algal population. Such continuous growth would eventually deplete the nutrients of the pond, and in Taiwan the problem of silicate deficiency is critical. An experiment was designed and carried out, therefore, to test if this deficiency could be made up with silicate application. Two ponds were treated with sodium silicate and two others used as control. The result was that the ponds with added Na_2SiO_3 yielded chlorophyll 1.72 to 2 times as much as that of the control. In milkfish ponds the limiting level of $\text{SiO}_3\text{-Si}$ for algal production is 0.25 ppm, but during the winter preparation of ponds $\text{SiO}_3\text{-Si}$ might be depleted to somewhere between 0 and 0.4 ppm. Inference from experimental data shows that by an application of 100 kg/ha of Na_2SiO_3 , the $\text{SiO}_3\text{-Si}$ concentration could be raised to above 0.25 ppm, but besides this essential element, organic matter is also needed to bring down the pH value and to supply other elements for algal growth.

Introduction

The milkfish ponds in Taiwan produce some 1900 kg/ha/year on the average. To maintain this high yield, one of the important measures to be taken in pond management is to grow and maintain an adequate layer of bottom algae during the months of December to March when the ponds are lying fallow. Then in April when water temperature rises above 20°C, the ponds are filled with water and stocked with milkfish fingerlings. The bottom algae, which should be 3 or 4 mm thick by this time will continue to grow for a few more months until July or August and provide sufficient forage for the fast growing milkfish. Upon analysis, it is found that the bottom algae in the winter months consisted mostly of diatoms and bluegreen

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algae; of the diatoms, *Nitzschia* and *Pleurosigma* are dominant, especially the former species which may reach as high as 90%. In the summer months, however, more bluegreen algae will thrive.

There are many favorable conditions for the growth of bottom algae. Temperature is one, tillage and fertilization with organic and inorganic substances are the others. Even the regulation of the depth of water and manipulation of the rate of fingerling stocking and harvesting will affect the growth of bottom algae and consequently the milkfish production. As the present study concerned only silicate as nutrient to algae, a review of literature on the question of activism of silicate in water and its role in algae production was first made and an experiment was designed and carried out to verify the points in question.

Review of Literature

In describing the different phases of milkfish culture in Taiwan and in trying to link up scientific information with traditional practices, Lin (1968) emphasized the importance of silica in milkfish pond fertilization. Whiford and Schumacher (1968) recorded the better growth of bluegreen algae in warm water, while Carl (1940), Rao (1955), Blum (1956) and Venkateswarlu (1969) mentioned that most diatoms thrived better at low temperature. Lund (1949) discussed the relationship of nitrate, phosphate and silicate to the growth of diatoms. Hutchinson (1944) and Smyth (1955) found that diatoms required a large amount of silicate to grow, and the requirement of nitrate and phosphate was not so great. In the decomposition of dead diatoms only a very small amount of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ is released in water (King and Davidson, 1933), and the concentration of N and P and N/P ratio in water bear little relation to the fluctuation of the diatom population (Rao, 1955). There is no relationship at all between the multiplication of diatoms and the concentration of $\text{NO}_3\text{-}$ (Ketchum, 1939).

Okuda and Takahashi (1967) described from experiments in literature that rice and diatoms required silicate to grow although silicate was not a requirement essential for plants in general. The supply of silicate is absolutely necessary for diatom multiplication. The cell wall of some diatoms contains as much as 96.5% of silica; provided that other nutrients are equally abundant, diatoms grow directly in proportion to the concentration of silicon which no other elements can replace (Lewin, 1962). Bluegreen algae require much less silicate for growth (Lewin, 1962). In seas and

lakes the concentration of silicate is usually in reverse proportion to the abundance of diatoms, for when other favorable conditions are present, rapid growth of diatoms will take up silicate very rapidly to leave a minimum silicate level in water (Lund, 1949; Roy, 1955; Hutchinson, 1957; Venkateswarlu, 1969). Chu (1942) also stated that silicate in sea water was generally low. The disappearance of silicate from water is due mainly to (1) combination with positively charged bivalent ions to form insoluble compounds and with organic substances to form insoluble organosilicates (Hauser, 1957), and also with $\text{Fe}(\text{OH})_3$ and $\text{Al}(\text{OH})_3$ to form insoluble compounds of iron and aluminum (Hutchinson, 1957), but the formation of Mg-silicate and Ca-silicate is most common, because Mg and Ca are abundant in brackish and sea waters, (2) reduction of silicate by H^+ of water, H_2CO_3 and organic acids to insoluble silica and thus rendering it unavailable to the assimilation of the diatoms (Hutchinson, 1957), (3) adherence of silicate to soil particles to help liberation of phosphate into water (Okuda and Takahashi, 1967) and to leach out, and (4) utilization of silicate by diatoms. Hutchinson (1957) estimated that diatoms were capable of absorbing up to 3/4 of the silicate added to water; Lewin (1962) stated that diatoms could assimilate double as much silicate as would be usually required to build a cell wall of double thickness if the concentration of silicate was sufficiently high in water.

On the other hand, the concentration of silicate can be increased first through a natural process in the form of leachable and exchangeable silicate (Round, 1957), because the concentration of silicate in both water and soil tends to reach a state of balance; if silicate concentration is low in water but high in soil, silicate will be liberated into water until equilibrium is reached; secondly if insoluble silica is rich in the soil, high pH and high temperature will increase the solubility of silica (Patrick, 1948; Hutchinson, 1957 and Eitel, 1954), for alkaline water tends to transform silica into soluble orthosilicate or silicate (Lewin, 1955a); and in the third place dead diatoms are a good source of silicate but its liberation has no direct relation to bacteria action (Sverdrup *et al*, 1949). There is a definite ratio between phosphate and silicate liberation as mentioned by Grill and Richards (1964). Silicate liberation from dead diatoms is a slow process starting a few days after death and taking about five months to complete (King and Davidson, 1933; Grill and Richards, 1964). In contrast to the process of its liberation from dead diatoms and soil, the up-take of silicate by growing

diatoms and by returning back to the soil is rapid, resulting in a low concentration of silicate in water (Hutchinson, 1944).

As a limiting factor in diatom multiplication, Chu (1942) defined a concentration of 0.4 ppm of silica as being the minimum, while Pearsall (1932), Lewin (1955) and Lund (1949) stated that 0.5 to 0.6 ppm of silica would depress diatom growth. However, high concentration of silicate in alkali water inhibits the propagation of diatoms. Chu (1942) found that diatoms stopped growing at a concentration of 54 ppm of silica and King and Davidson (1933) said that they died at 100 ppm. The results of an experiment led Lin (1968) to believe that when the silica concentration in milkfish ponds drops below 0.25 ppm, silicate fertilizer should be immediately added in order to maintain a high level of bottom algae growth and the application should be done on several occasions in small dosages at a time.

Lund (1949) discovered that the application of silicate to freshwater did not necessarily result in diatom bloom, because, as Mommaerts (1969) stated, silica concentration in freshwater is usually higher than that in sea water. Liaw (1969) also noted that silica content in the inland waters of north Taiwan was 5.06 ppm which was above the general need for algal growth. Adequate quantity of silica in the cell wall increases resistance to the attack of bacteria and fungi (Patrick, 1948).

The Experiment: Material and Method

Four small ponds, each of 0.375 ha at the Tainan Fish Culture Station were selected for the experiment carried out in 1968-1969. The design and treatments of the ponds are shown in Table 1.

Table 1 Dosage and application date of silicate in the experimental ponds.

Pond No.	Area (ha)	Fertilizer dosage	Application date of silicate
32	0.375	Chicken manure 240 kg	
35	0.375	Chicken manure 240 kg	
34	0.375	Chicken manure 240 kg Sodium silicate 40 kg	Dec. 19.....20 kg Dec. 25.....10 kg Jan. 510 kg
36	0.375	Chicken manure 240 kg Sodium silicate 20 kg	Dec. 20.....10 kg Jan. 510 kg

Ponds Nos. 34 and 36 were treated with Na_2SiO_3 and Nos. 32 and 35 used as control. All the experimental ponds were drained on November 16, 1968. When the pond bottom dried and cracked on December 9, 1968, 240 kg of chicken manure was applied to each of the ponds. On December 12 sea water was admitted to a depth of 12 cm in all the ponds, which were then allowed to dry again by evaporation. Bottom algae kept on growing during this evaporation period and whenever water was admitted again. No more fertilizers were added to ponds Nos. 32 and 35, but Na_2SiO_3 was added to Nos. 36 and 34 as shown in Table 1. Turbidity, silicate content, pH value and bottom algae quantity were determined once daily at 9 a.m. and pH and air temperature at 3 p.m.

Determination of silicate and nitrite contents and chlorophyll amount was made according to methods described in the "Manual of Sea Water Analysis" by Strickland (1960). Phosphate and ammonia were determined by methods described in "Apparatus and Methods of Oceanography" by Burne (1959). The pH value was determined by using the glass electrode pH meter, Model HM-5, and turbidity by HACH water analysis kit.

Results

Figure 1 shows the daily fluctuation of $\text{SiO}_3\text{-Si}$ concentration in the experimental ponds ($\text{SiO}_3\text{-Si} \times 2.14 = \text{silica}$). Ponds No. 32 and No. 35 had no added silicate, the water contained a low level of $\text{SiO}_3\text{-Si}$ of 0.1-0.4 ppm, reaching the lowest point at 0.025 ppm in 4-10 days after the admission of new sea water. With added silicate to Nos. 34 and 36 the $\text{SiO}_3\text{-Si}$ content remained comparatively high, reaching 2.6 ppm at the time of silicate application and dropping to pre-application level of 0.25 ppm after 3-6 days. Silicate content was highest in No. 34, because more Na_2SiO_3 was added.

Chlorophyll content is used to indicate the bottom algae production which, as shown in Fig. 2, was low at an average of 82.9 mg/m^2 and 85.1 mg/m^2 in ponds Nos. 32 and 35 respectively. In No. 34 when 20 kg of silicate was added on Dec. 19, chlorophyll content was increased from 80 to 132 mg/m^2 . Again on Dec. 25, with 10 more kg of silicate added, chlorophyll was further increased from 113 to 179 mg/m^2 .

Figure 4 and Table 3 show the daily difference of pH at 9 a.m. and 3 p.m. between ponds No. 36 and No. 35, and Fig. 5 shows the difference between ponds No. 34 and No. 32. These two figures point not only to the higher pH value in silicate fertilized ponds than in unfertilized ponds,

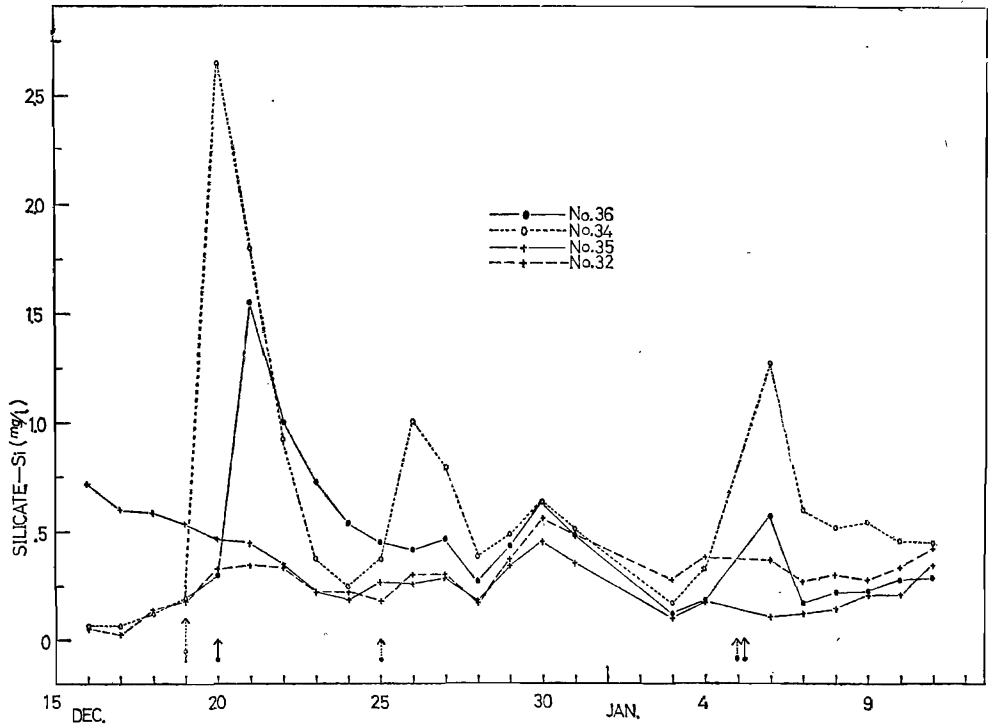


Fig. 1 Daily fluctuation of SiO₃-Si concentration in the experimental ponds. The length and positions of arrows indicate the dosage and time of silicate treatment.

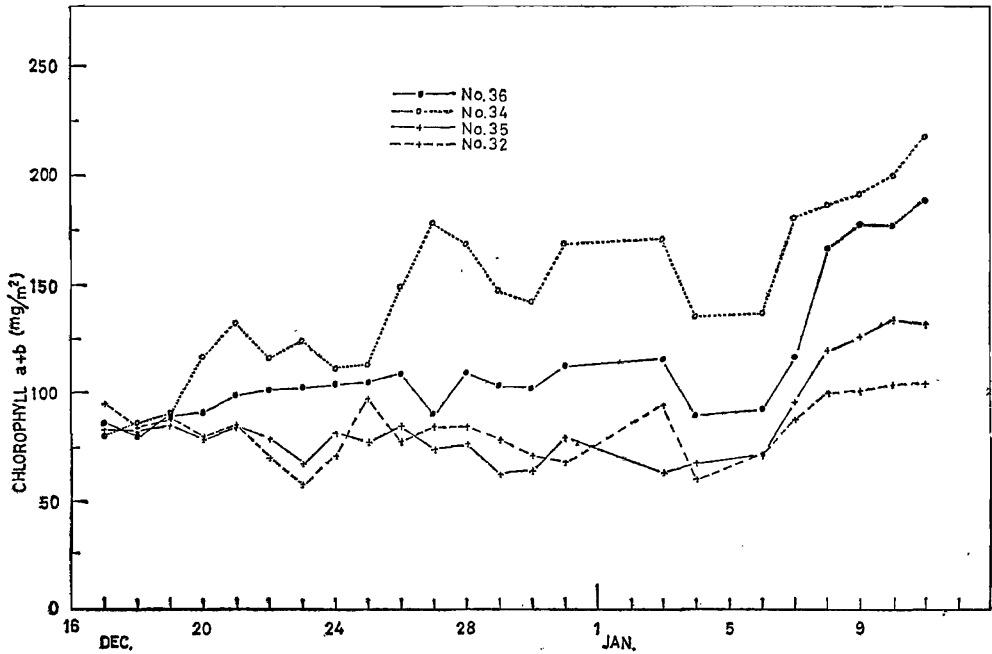


Fig. 2 Daily fluctuation of benthic algae production in the experimental ponds.

but also sharper pH increase from morning to afternoon in the fertilized ones. All these phenomena can be interpreted as better bottom algae production in the silicate fertilized ponds than in the unfertilized.

Turbidity of pond water affects bottom algae growth to a great extent. The cause of turbidity in milkfish ponds is usually due to plankton growth, as a result of which bottom algae are destroyed. Fig. 6 shows that pond No. 32, without silicate application, had a higher turbidity of 30-50

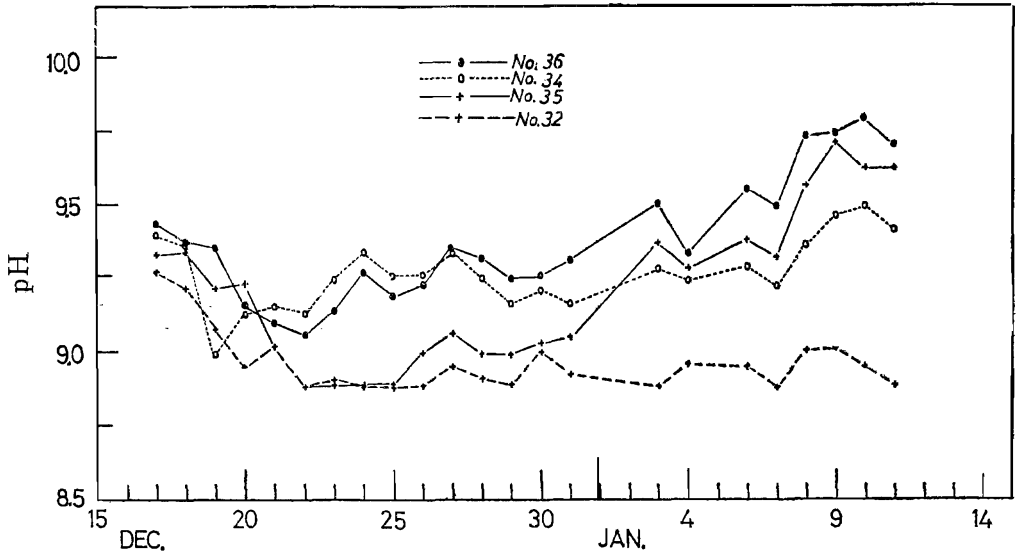


Fig. 3 Daily fluctuation of pH value of the four ponds at 3:00 p.m.

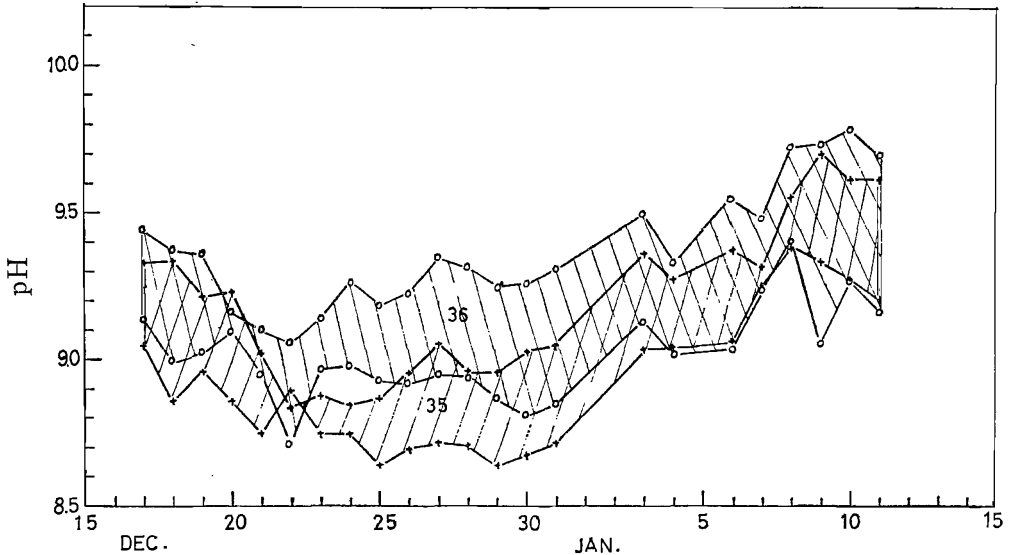


Fig. 4 Daily difference of pH value between ponds No. 36 and No. 35 at 9:00 a.m. and 3:00 p.m.

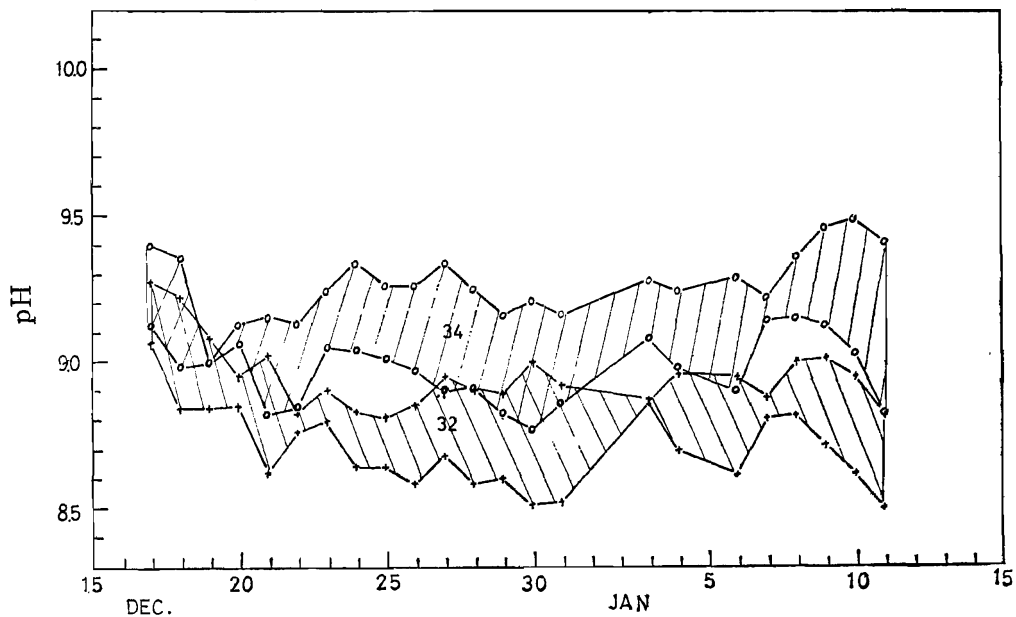


Fig. 5 Daily difference of pH value between ponds No. 34 and No. 32 at 9:00 a.m. and 3:00 p.m.

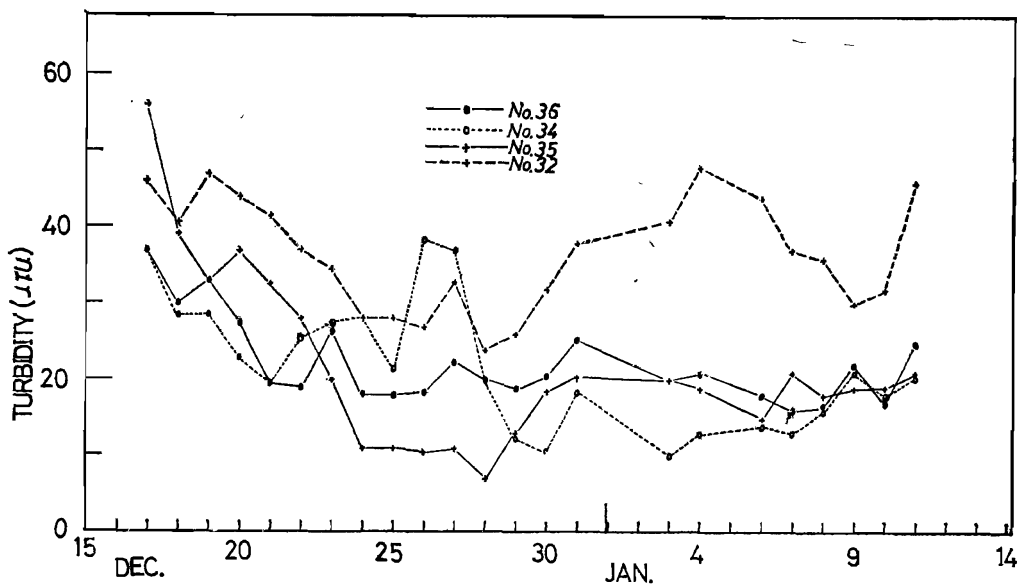


Fig. 6 Daily fluctuation of turbidity in the experimental ponds.

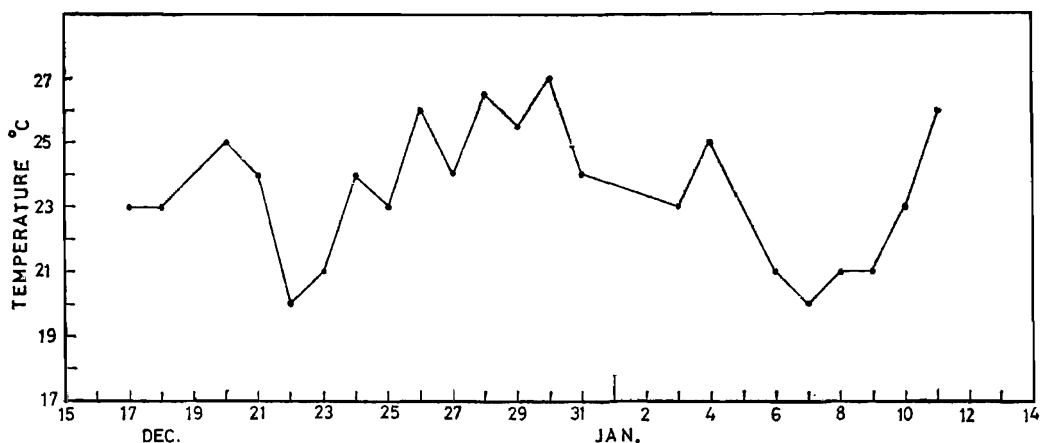


Fig. 7 Daily variation of air temperature at 3:00 p.m. in Tainan during this study.

J.T.U. than those with silicate added. Temperature also affects bottom algae growth. As shown in Fig. 7, the air temperature in January was generally lower than that in December, so the algae (or the diatom) growth was also better in January. On January 5, again 10 kg of silicate was added to No. 34 and chlorophyll content was increased from 135 mg/m² to 218 mg/m². Similar increment of chlorophyll concentration was also found in No. 36 with application of silicate.

The results of t-test with significance at 1% level by pairing method shows the positive effect of silicate fertilization to bottom algae growth (Table 2). The slight increase of bottom algae in No. 35 where no silicate was added was evidently due to the continuous seepage that carried extra silicate from the canal to the pond.

Figure 3 points to the fluctuation of pH value at 3 p.m. of the four ponds. High pH value indicated more O₂ content in those ponds with silicate fertilization as illustrated by pH 9.0-9.5 in No. 34 and No. 36 against 8.9 in No. 32 and No. 35 in the early part of the experiment period. The pH value in No. 35 became high in the latter part of the experiment due to additional silicate carried in through seepage. Photosynthesis was

Table 2 Significance test of benthic algae production in the four experimental ponds by pairing method.

Treatment	Pond Nos.					
	34-36	34-35	34-32	36-35	36-32	35-32
t value	6.57**	9.20**	10.79**	8.20**	5.66**	0.72

** significant at 1% level

most active during the hours from 10:00 to 14:00 and this was considerably increased by adequate silicate supply, resulting in more CO₂ up-take and more O₂ liberated in water to increase the pH value. Table 4 shows the quality of water in the four experimental ponds.

Table 3 Mean pH values at 9 a.m. and 3 p.m. and the difference between morning and afternoon values.

Pond No.	35	32	34	36
9 a.m.	8.926	8.703	9.971	9.017
3 p.m.	9.193	8.951	9.267	9.374
Difference	0.268	0.248	0.296	0.351

Table 4 Chemical quality of water in different ponds

Date of examination	December 15			Jan. 10
Pond No.	NH ₄ -N (ppm)	NO ₂ -N (ppm)	PO ₄ -P (ppm)	Salinity (‰)
35	0.36	0.004	0.37	60
36	0.27	0.0025	0.38	72.5
34	0.285	0.0034	0.34	70.6
32	0.255	0.0038	0.26	68

Discussion and Conclusion

The practice of tillage, fertilization and fallowing with periodic flooding and evaporation to dryness of the bottom of milkfish ponds is evidently a proper process to accumulate nutrients and to prepare a firm bottom texture for the growth of bottom algae. The low temperature in winter particularly favors the growth of diatoms, but, to obtain a maximum growth, adequate supply of silicate is necessary.

Sea water in the canal contains a considerable amount of silicate, usually above 1 ppm of SiO₃-Si, but a few days after its admission to the ponds, rapid multiplication of diatoms and other algae assimilates most of the silicate and leaves a concentration in water even as low as 0.0125 ppm. At this low level of concentration, diatoms are unable to grow any further as shown in the control ponds Nos. 32 and 35.

The purpose of applying chicken manure equally to the four experimental ponds was to provide adequate concentration of nitrogen and phosphorus as

well as organic matter, CO_2 , organic acids and possibly vitamins to the need of bottom algae growth.

Now the only factor that remained to be determined was whether silicate constitutes an essential nutrient for bottom algae growth, and this has been proved positive in the present experiment, as the chlorophyll content in ponds No. 34 and No. 36 with added silicate increased from 80 to 218 mg/m^2 , whereas in ponds No. 32 and No. 35 without silicate application the chlorophyll content remained 82-85 mg/m^2 throughout the experimental period.

Another point of significance in the results of the present experiment is that bottom algae increased in direct proportion to the quantities and times of silicate applications as illustrated particularly in the results of pond No. 34 with three silicate applications. In this pond, one application of 20 kg of Na_2SiO_3 was made on Decemebur 19, another 10 kg on Dec. 25 and the last 10 kg on Jan. 5, and, as a result, chlorophyll content increased 52, 66 and 83 mg/m^2 respectively.

The findings of this experiment can be applied to milkfish pond management with profit. It is suggested, therefore, to carry out further experiments on a large scale with several ponds each of 4 to 6 hectares. In the treated ponds 20-40 kg of Na_2SiO_3 would be divided into four or more portions or doses to be applied in December; and similar doses and manner of application will be made to the same ponds in the following months from January to July. The quantity of silicate and time of application will depend, of course, on the firmness of the algal bed, water supply and other conditions.

Meanwhile, it should be noted that, in pond No. 34, when silicate was applied on Dec. 19, the $\text{SiO}_3\text{-Si}$ concentration was observed to be 2.5 ppm, but after 5 days it dropped to 0.25 ppm. Such rapid loss of silicate in water was chiefly due to assimilation by diatoms for propagation. If no silicate was further added, its concentration might drop close to zero as revealed by analysis of the pond water. On the basis of this information, the traditional practice of keeping old water for the sake of preserving fertility appears to be a mistake. If efficient filters can be installed to keep the undesirable organisms out, it would be highly advisable to partly drain the pond and let in fresh sea water, for as nutrients are depleted after water is left standing in the pond for more than 10 days, the admission of fresh sea water rich in nutrients could only be beneficial.

Acknowledgement

This project was carried out at the Tainan Fish Culture Station with financial support from the Rockefeller Foundation. I wish to thank Professor S. Y. Lin for his valuable comments and suggestions on the design and work of the experiment. I am also grateful to Messrs. T. P. Chen and P. W. Yuan of the Fisheries Division of J.C.R.R., Y. S. Liang and, H. T. Ting of the National Taiwan University, and. H. C. Hsieh & the staff of the Tainan Fish Culture Station for their assistance and encouragement.

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NOTES ON ORGANISM CAUSING BROWN WATER IN MILKFISH PONDS

Shih-lin Shen*

and

Young-Meng Chiang**

Abstract

Formation of brown water in milkfish ponds used for wintering purpose was found to have been caused by a bloom of *Olisthodiscus carterae*.

In southern Taiwan, the water in brackish-water ponds used for wintering of the milkfish, especially in the old ponds, frequently turns brown in winter. The appearance of the brown water, when it becomes very thick, tends to have a lethal effect on milkfish (Lin, 1968).

In the winter of 1969 the writers collected samples of this brown water and found that the color was caused by a species of brown pigmented flagellate, identified as *Olisthodiscus carterae* Hulburt (1956).

The genus *Olisthodiscus* was established by Carter in 1937 based on one species, *O. luteus*, and was placed in the Xanthophyceae (Leadbeater, 1969). Recently Hulburt (1965) added two new brackish water species, *O. carterae* and *O. magnus*, to the genus.

Leadbeater (1969), based on fine structural study and recent biochemical work on *Olisthodiscus luteus*, suggested that the genus should be transferred from the Xanthophyceae tentatively to the Chrysophyceae.

Olisthodiscus carterae Hulburt was described by Hulburt from specimens collected from brackish waters in the vicinity of Woods Hole, Massachusetts, and this is the second time that this species was reported.

Under normal conditions of rapid growth both in ponds and uni-algal culture, the organisms are very active. They are ovoid to subovoid in shape and more or less compressed. But in some cultures they tend to change their size and shape and become sub-globular, cylindrical or polyhedral. They become sluggish and settle down to the bottom of the culture, become globular and eventually die.

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Olisthodiscus carterae Hulburt (Figures 1~5). Cells moderate-sized, without cell wall, somewhat ovoid to subovoid in outline, more or less compressed, anterior end wider than posterior end, dorsal margin more convex than ventral in side view, sometimes irregular along margin, the

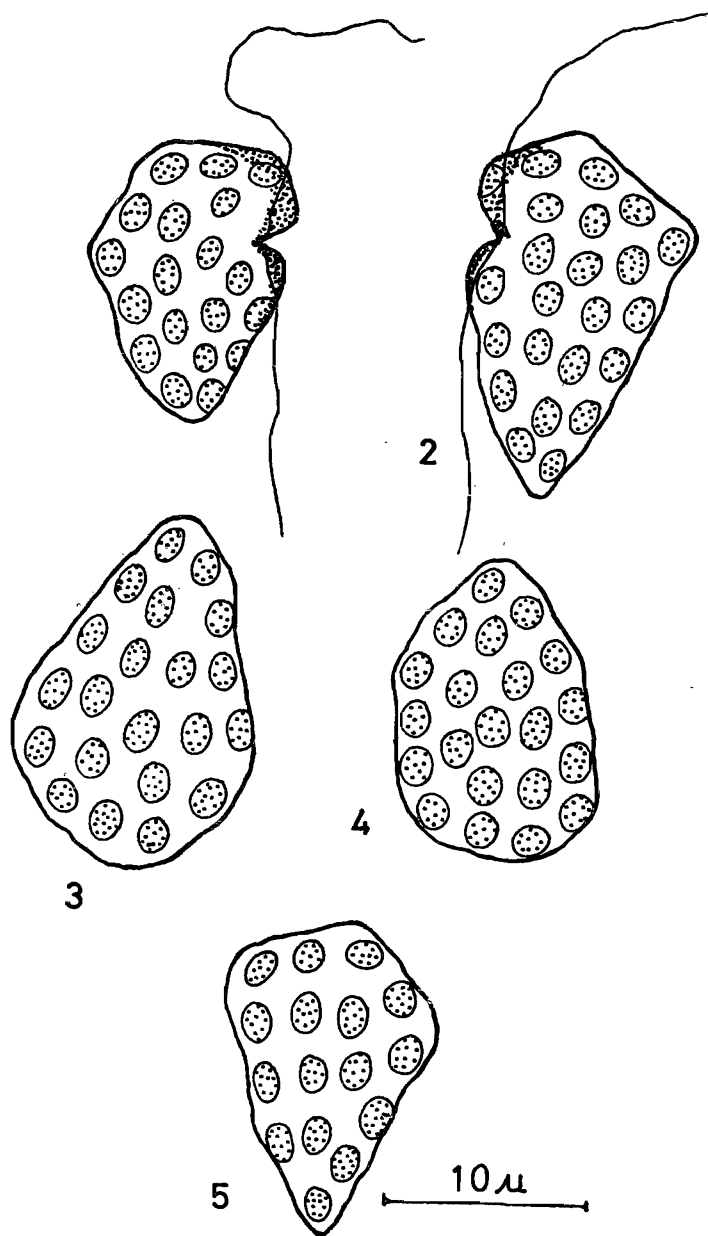


Fig. 1-5. *Olisthodiscus carterae* Hulburt.

surface of the cell sometimes uneven, with two lumps on the ventral side forming a longitudinal and somewhat shallow transverse furrow. Length 15-23 μ , width 10-15 μ . Flagella two, one projecting anteriorly, the other trailing, inserted ventrally at the end of the longitudinal furrow. Chromatophores globular to elliptical, 5 to 27, brown, peripherally placed except none present on ventral side. Granules occasionally present.

Our organism seems identical to Hulburt's except for the size of the cells. In our specimens the size of the cell is 15 to 23 μ in length and 10 to 15 μ wide. Hulburt describes them as 8 to 12 μ 10 long and 5 to 8 μ wide. The number of the chromatophores per cell in our organism (5 to 27) is also greater than that of Hulburt's (8 to 16).

Acknowledgements

The authors wish to express their appreciation to Dr. Edward M. Hulburt of Woods Hole Oceanographic Institution for his assistance in the identification of *Olisthodiscus carterae*.

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A DISEASE OF THE GRASS CARP (*CTENOPHARYNGODON IDELLUS*) AND ITS CHEMOTHERAPEUTICAL CONTROL

Wilson Sheng-yu Wu*

The grass carp, *Ctenopharyngodon idellus*, is one of the principal species reared in freshwater ponds in Taiwan. It is susceptible to a disease and each year a large number die, thereby greatly reducing the profit to the fish farmers.

This disease of the grass carp shows up with the following symptoms: loss of appetite, swimming slowly near the surface of the water, a slight reddening and swelling of the anus, some yellowish mucus in the abdomen around the intestines, and the inflammation of the stomach with engorged blood vessels (Fig. 1).

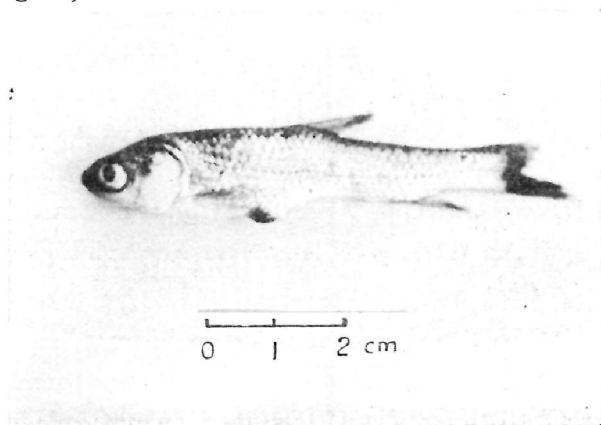


Fig. 1 Diseased *Ctenopharyngodon idellus*

The fish farmers feed rice bran, ground bean cake and duckweed (*Lamna*) to the fish and use night soil as fertilizer. So it is necessary to examine the water and feed in an effort to look for the pathogen.

This study was prompted by the desire to find the causal organism of this disease and the methods of control.

Materials and Methods

Diseased and healthy grass carp were collected from the fish ponds in the North Ward and the An-nan Ward of Tainan City, Yungkang Hsiang of

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Tainan Hsien, and Hu-*nei* Hsiang of Kaohsiung Hsien. These fish were brought back to the Microbiology Laboratory of the National Taiwan University in Taipei in clean plastic bags kept in thermos bottles with ice.

Immediately upon arrival at the Laboratory, the healthy and the diseased fish were examined. Bacteria were isolated from the mucus in the abdomen, liver and blood, and also from the intestine by sterile cotton swabs and streaked on different agar plates with a loop needle.

One of the isolates (A-2) was cultured in broth in an Erlenmeyer flask for 24 hours and then inoculated into healthy fish which had been brought back to Taipei and kept in large tanks.

The inoculation was carried out by two methods: First by putting a culture of the A-2 bacteria on rice bran which served as food for the healthy grass carp; and secondly by intramuscular injection.

Water collected from the ponds in which the fish were reared was cultured on agar plates by the dilution plate method and by the Miles and Misra surface method in order to learn what bacteria were dominant in the pond water.

The identification of the bacteria isolated from both diseased and healthy fish was made according to Bergey's Manual of Determinative Bacteriology and the Standard Descriptive Chart prepared by the Committee on Bacteriological Technique of the Society of American Bacteriologists.

Results

(1) 14 species of bacteria were isolated and identified from the diseased fish, as shown in Table 1. One species was consistently found in the diseased fish but not in the healthy fish. This was culture No. A-2 which was selected as the possible causal organism and used in the inoculation tests.

(2) The result of inoculation test has been that by using the first method, i.e. feeding the A-2 culture on rice bran to 34 grass carp, four or five days after inoculation with the bacteria, one of the healthy fish was found dead. Ten days after inoculation, three more were found dead and 20 others were sick, but the remaining 10 seemed to be healthy. The 25 grass carp used as control were all in normal condition. By using the second method, i.e. intramuscular injection to 47 healthy grass carp, the fish began to die one after another for 25 days following the inoculation, and 36 were found dead 45 days after the inoculation; of the remaining 11, five were sick and six appeared in normal condition.

(3) The results of the action of some of the antibiotics on the pathogen A-2 are shown in Table 2.

Table 2

Chemotherapeutic agents	Inhibition zone (cm)			Note
	Low conc.	Medium conc.	High conc.	
Sulfisoxazol	2.4	2.7	3.2	
Colistin	2.0	2.1	2.1	
Kanamycine	1.9	2.3	2.7	
Dihydrostreptomycin	1.8	2.3	2.7	
Tetracycline	2.5	2.6	2.8	
Chloramphenicol	3.0	3.2	4.1	
Leucomycin	×	1.3	2.0	
Oleandomycin	×	1.2	2.0	
Erythromycin	1.1	1.6	2.2	
Penicillin	×	×	×	No inhibition
Streptomycin	1.9	2.2	2.4	
Trisulfa pyrimidines	2.1	2.2	2.4	
Auromycin	2.2	2.6	2.8	
Terramycin	2.2	2.6	2.9	
Bacitracin	×	1.1	1.2	
Declomycin	2.1	2.3	2.9	
Ampicillin	×	×		No inhibition
Cephalothin	—	—	2.8	
Cephaloridine	—	—	2.8	
Nafcillin	×	—	—	No inhibition
Lincomycin	×	—	—	No inhibition
Methicillin	×	—	—	No inhibition
Sulfamerazine	1.6	1.9	2.1	

Discussion

(1) The A-2 organism is *Aeromonas punctata* (Zimmermann), which is a gram negative, short rod (0.6-0.8×0.9-1.4 μ) monotrichous bacteria (Fig. 2 and Fig. 3).

It is the pathogen that causes the edema in the grass carp. Sometimes, a fish can first be infected by *Aeromonas* and secondarily infected by other

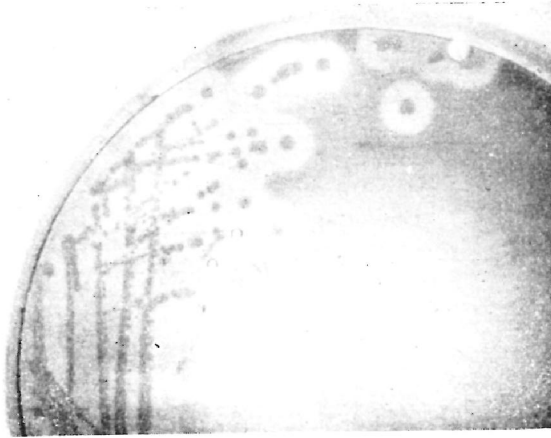


Fig. 2 Hemolysis of *Aeromonas punctata*

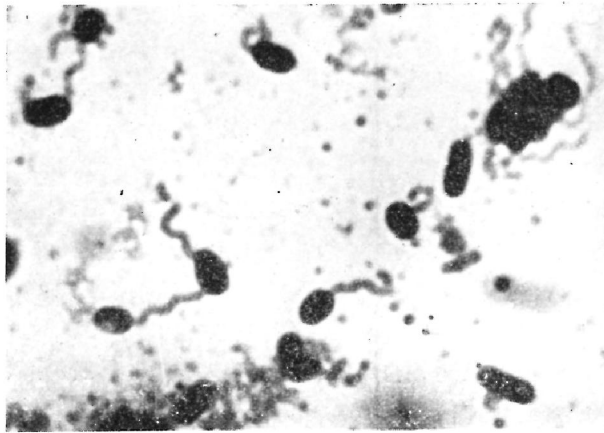


Fig. 3 Flagella stain of *Aeromonas punctata*

pathogens (22). It is the opinion of Goncarov, Tomasec *et al.* (4) that a virus is the primary cause of certain diseases, and the bacteria come into play only as secondary invaders.

(2) *Aeromonas punctata* was found in the water from the ponds in which the fish were reared. This bacteria is always associated with duckweed which is found in ponds in which the carp, eel and other fishes are present.

(3) Penicillin interferes with the incorporation of the N-acetylmuramic acid peptide from a carrier within the bacteria cell to its position in the mucopeptide polymer (6) structure that normally comprises the rigid bacterial cell wall. But *Aeromonas punctata* is not sensitive to penicillin.

(4) Chloramphenicol (Fig. 4) was found to be the most efficient

antibiotic in this study. The inhibition zone of sensitivity disk test was the largest, as shown in Table 2. The site of inhibition of chloramphenicol blocks protein synthesis (5) and has been shown to involve the transfer of amino acids from the transfer RNA component of RNA to the peptide-bond forming site in the ribosome (11, 18).

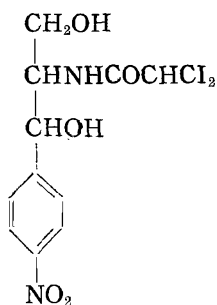


Fig. 4 Chloramphenicol

(5) *Aeromonas punctata* is also sensitive to heavy metal ions, such as copper. This phenomenon is known as the oligodynamic action.

This investigation has revealed that *Aeromonas punctata* is associated with the disease of grass carp and that chloramphenicol is effective in inhibiting the growth of this pathogen, but since this is an expensive antibiotic, there is need to find a more economical method of control.

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STUDIES ON FEEDING AND PROTEIN DIGESTIBILITY OF SILVER CARP, *HYPOPHTHALMICHTHYS MOLITRIX*, (C. & V.)

Wan Chiang*

Introduction

Hypophthalmichthys molitrix (Cuvier & Valenciennes), a herbivorous freshwater fish, is widely distributed in Southeast Asia, South China, and Taiwan, and is one of the most important fishes cultured in ponds. However, a species of such high economic value is relatively poorly understood, especially regarding feed digestibility, although it has been studied by a few workers such as Fang (1928), Kobayasi (1929a, 1929b, cited from S.Y. Lin 1969), Nikolsky (1963), S. Y. Lin (1968, 1969), Y. W. Hou (1969), *et al.*

In the study on the digestibility of the feed of fish, Maynard and Loosli suggested in 1959 an indirect method, making use of an inert reference substance as its indicator. Nose (1960 b), and Y. S. Lai (1966) pursued this method and selected Cr_2O_3 as an indicator. In silver carp, however, the digestibility of phytoplankton could not be determined easily by Cr_2O_3 . An attempt was made in this study to use dietary chlorophyll instead. Particular attention was given to trophogenic aspects and morphological factors. Comparisons between silver carp and its related species were also made in this investigation.

Material and Methods

The silver carp used in the investigation was obtained partly from the fertilized ponds of the Chupei Fish Culture Station which were cleared at the end of November 1968 and partly from cement ponds ($100 \times 110 \times 72 \text{ cm}^3$) in which the fish were fed with algae containing 3-5% of Cr_2O_3 .

The techniques employed were as follows:

A. Chemical properties of the pond-water.

1. Alkalinity, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, SiO_2 : Determined by standard methods for the examination of water and waste water (American Public Health Association 1965).

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2. $\text{PO}_4\text{-P}$: Determined by a modified single solution method of Murphy and Riley (Murphy & Riley 1962).
 3. K^+ , Na^+ , Mg^{++} , Ca^{++} : Determined by Hilger-Watts method, technical data sheets, directly by Uvispek atomic absorbance spectrophotometric MK 9 (H 700).
 4. Chlorophyll: By Richard and Thompson's method for determination of chlorophyll a, b, c at three different wave lengths (Richard & Thompson 1968).
- B. Feeding periodicity: By Keast and Welsh's method (Keast & Welsh 1968).
 - C. State of nourishment: By weighing the collected fish and their dried tract-contents and then calculating the ingested amount of food per gram of body weight.
 - D. Digestive rate: By measuring the lengths of the entire empty gut of the fish at regular intervals.
 - E. The pH value of the digestive tract: By BTS test paper.
 - F. Analysis of the tract-contents: By microscopic examination of the fore-and hind-gut contents.
 - G. Analysis of the phytoplankton in the ponds: By microscopic examination of the minute vegetable organisms collected with a plankton net.
 - H. Chlorophyll digestibility: Indicated by Cr_2O_3 , Nose's method (Nose 1960b).
 - I. Protein digestibility: Indicated by chlorophyll, Nose's method (Nose 1960b).

Experimental Results

A. Pond condition

The fish ponds at Chupei Station were drained of water at the end of November, 1968, and were dried under the sun for three months. The fry of big head, grass carp, common carp and silver carp were planted in the refilled ponds. At the end of November, 1969, the ponds were again cleared. There were ten ponds in all, classified into Groups A and B according to the fertilizers used. The area, depth, and fertilizers used are listed in Table 1.

Every other week from September to November in 1969, water of the five ponds, two chosen at random from Group A, two from Group B and

one from the unfertilized ponds, was analyzed. The water drawn up from various depths of the ponds with a plastic cylinder 10 cm in diameter and 100 cm in height and poured into a 500 cc plastic vessel was brought to the limnological laboratory of the National Taiwan University for analysis. The results are given in Table. 2.

Table 1. Condition and treatment of experimental ponds at Chupei Station.

Pond No.	Area (m ²)	Mean Depth (cm)	Annual Fertilization (kg/ha)	
			CaH ₄ (PO ₄) ₂	(NH ₄) ₂ SO ₄
A-1	485	73.4	0	0
A-2	1,115	80.9	1,040	0
A-3	970	76.3	2,040	0
A-4	1,067	70.3	6,090	0
A-5	611	78.2	10,230	0
B-1	582	64.0	0	0
B-2	485	67.0	1,040	1,950
B-3	679	66.5	2,040	1,950
B-4	485	62.3	6,090	1,950
B-5	388	71.7	10,230	1,950
C	211	37.0	0	0

Table 2. Analysis of water of experimental ponds.

Nutrients \ Ponds					
	A ₁	A ₂	B ₁	B ₂	C
Mg ⁺⁺	6.519 ± 1.231	11.924 ± 2.951	14.123 ± 4.542	13.249 ± 8.861	16.634 ± 3.299
Ca ⁺⁺	25.519 ± 1.936	34.676 ± 7.823	34.708 ± 6.433	34.708 ± 7.487	32.664 ± 10.32
Na ⁺	10.180 ± 1.125	12.161 ± 1.984	12.051 ± 2.155	12.365 ± 2.307	12.744 ± 3.412
K ⁺	11.481 ± 0.311	2.014 ± 0.401	1.700 ± 0.473	1.781 ± 0.241	2.032 ± 0.793
SiO ₂	11.437 ± 4.105	10.526 ± 3.125	12.607 ± 3.322	14.311 ± 3.360	14.685 ± 2.303
PO ₄ -P	0.351 ± 1.223	0.563 ± 0.331	0.271 ± 0.089	0.504 ± 0.120	0.416 ± 0.975
NO ₃ -N	0.251 ± 0.014	0.005 ± 0.006	0.015 ± 0.017	0.010 ± 0.012	0.008 ± 0.007
NO ₂ -N	0.005 ± 0.006	0.003 ± 0.001	0.009 ± 0.008	0.004 ± 0.003	0.035 ± 0.008
Alkalinity	49.816 ± 7.599	86.197 ± 18.80	80.288 ± 17.00	74.193 ± 18.52	90.974 ± 30.08
Chlorophyll a	0.096 ± 0.079	0.067 ± 0.038	0.117 ± 0.046	0.112 ± 0.047	0.059 ± 0.051

B. Feeding periodicity

At intervals of six hours from 9:00 a.m. on the first day to 3:00 a.m.

on the following day, eight silver carp and eight common carp were weighed and the lengths of their body, entire tract and empty tract were measured. Thus, the percentage of the latter two could be calculated. The tract-contents placed in a 90-100°C oven for twenty-four hours were also weighed and compared with body weight. The results are shown in Figs. 1 and 2.

The silver carp were fed once a day at 4:00-5:00 a.m., while the common carp were fed twice daily at 4:00-5:00 a.m. and 4:00-5:00 p.m.

C. State of nourishment

Eight silver carp and eight common carp were chosen from each of the eight ponds, sixty-four fish in all. The weight of the tract-contents of each carp was determined by the above mentioned method and compared

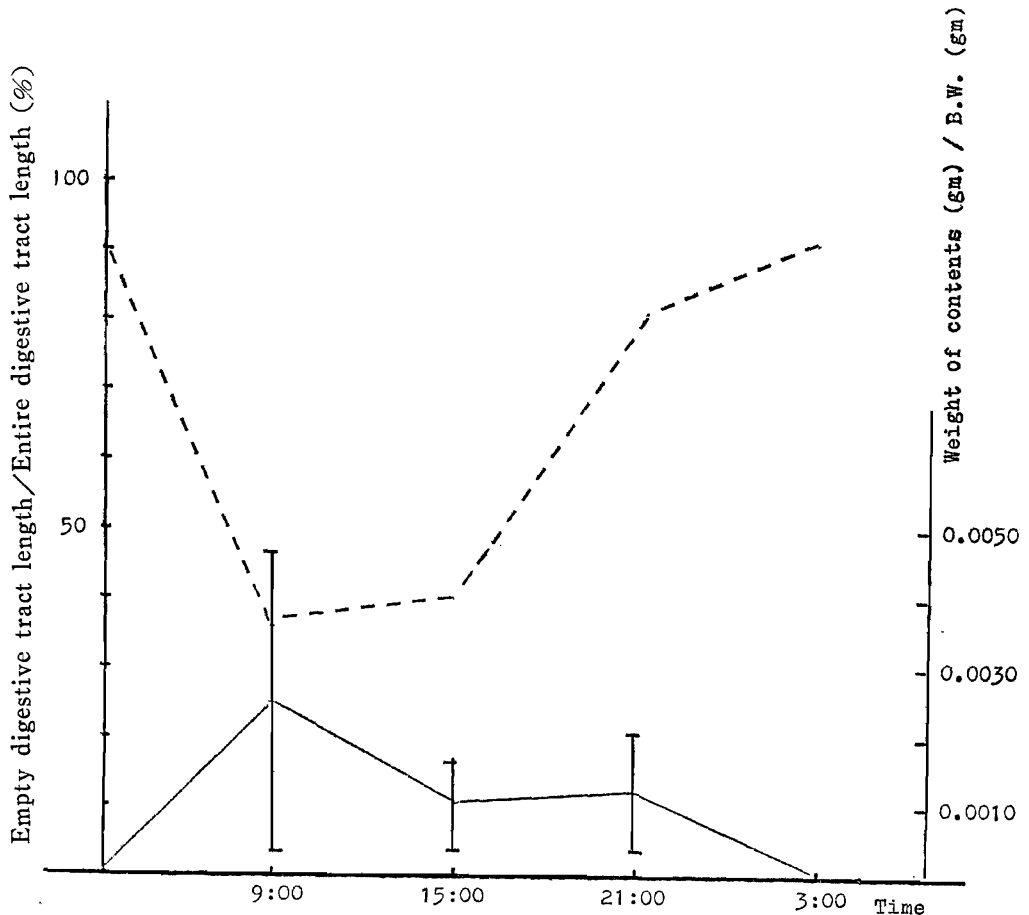


Fig. 1 Feeding periodicity of silver carp. The broken line refers to the percentage of empty digestive tract and the solid line to the weight of the digestive tract contents per gram of body weight.

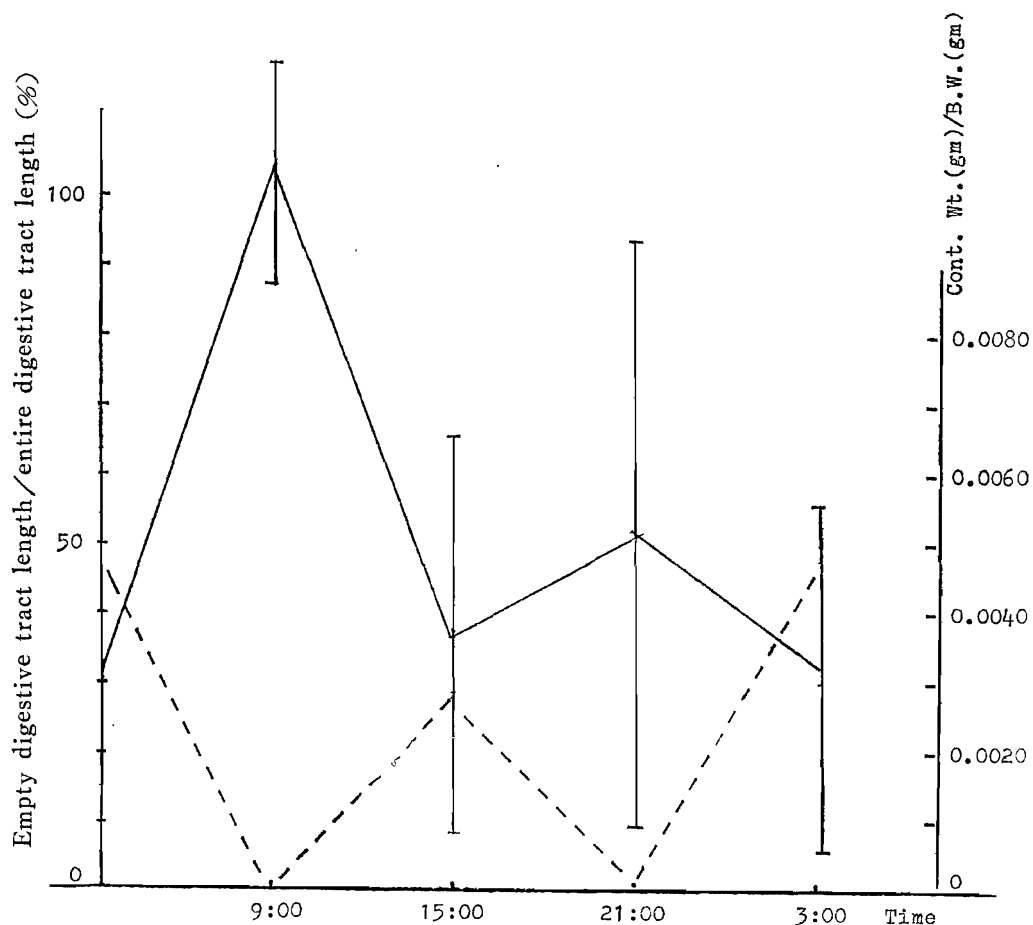


Fig 2 Feeding periodicity of common carp. The broken line refers to the percentage of empty digestive tract and the solid line to the weight of the digestive tract contents per gram of body weight.

Table 3. State of nourishment of silver carp

Observations	Body Wt.(gm)	Empty Gut %	Cont. Wt(gm)	Ratio: Cont. Wt. to B.W.
1	1,370	21.3	3.288	0.24
2	1,470	0	4.166	0.28
3	1,330	0	6.118	0.46
4	1,460	3.8	6.570	0.45
5	1,260	3.2	1.134	0.09
6	1,300	0	3.900	0.30
7	1,310	0	6.812	0.52
8	1,500	8.6	14.250	0.95

TABLE 4. State of nourishment of common carp

Observations	Body Wt.(gm)	Empty Gut %	Cont. Wt(gm)	Ratio: Cont. Wt. to B.W.
1	235	0	1.833	0.78
2	225	0	1.733	0.77
3	220	0	1.694	0.77
4	224	0	1.837	0.83
5	200	0	2.900	1.45
6	240	0	4.200	1.75
7	210	0	0.441	0.21
8	145	0	6.989	4.82

with its body weight. The results are given in Tables 3 and 4.

D. Digestive rate

Thirty carp, 15-30 cm in length and 40-450 gm in weight, were evenly placed in five plastic vessels ($68 \times 47 \times 36$ cm³) filled with underground water. After fasting for three days and becoming accustomed to the environment of the vessels, the carp were fed cultured algae amounting to 15% of body weight. At two-hour intervals, the digestive rates of five carp were determined by comparing the anterior empty tract length with the entire tract length. The results are given in Fig. 4.

On the average, it took more than ten hours for silver carp to completely digest its food,

E. The pH value of the digestive tract

The digestive tracts of ten carp were each divided into three segments, the pH values of which were determined by BTS test paper.

There was little variation in the segmental pH values. They were neutral, weakly acidic, or weakly basic, ranging from 6.8 to 8.3 (Table 5).

F. Relationship between the digestive tracts and body lengths.

Ninety carp were grouped with respect to body length. Both digestive tract length and body length were measured and their relationship is graphed in Fig. 3.

A regression line was found to exist between digestive tract length (Y) and body length (X), the line being $Y = 9.449 \times -102.098$ and the correlation coefficient being $r = 0.959$. There was thus a positive correlation between tract and body length, when the latter was 15-50 cm.

Table 5. pH value of the digestive tract of silver carp

Observations	Body Weight gm	Body Length cm	pH Value Fore-gut	pH Value Mid-gut	pH Value Hind-gut
1	809	40.0	7.3	8.3	7.9
2	580	34.8	7.5	8.0	7.8
3	542	34.4	7.0	8.0	7.8
4	522	33.7	7.2	8.1	7.8
5	509	33.6	7.6	8.2	7.8
6	510	33.5	7.0	8.0	8.2
7	220	24.6	7.0	8.2	7.8
8	158	21.6	6.9	8.2	8.0
9	150	20.9	7.0	8.1	7.8
10	148	21.4	6.8	8.0	7.9

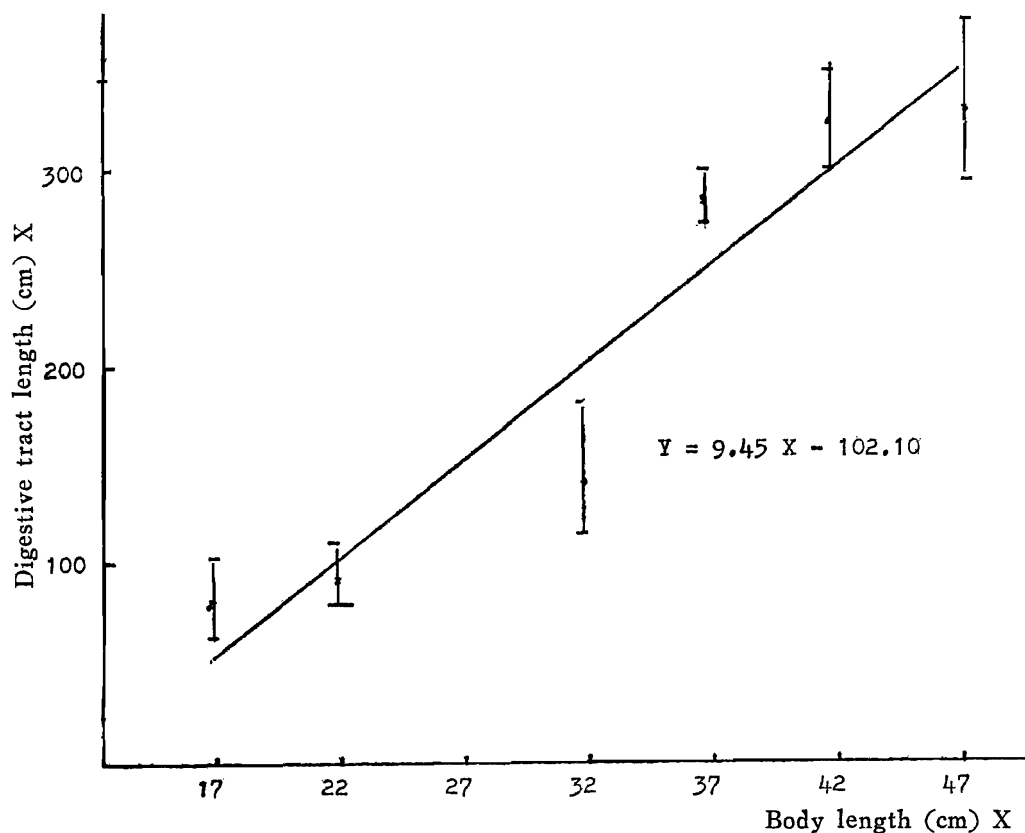


Fig. 3 Relationship between the digestive tract length and body length of silver carp.

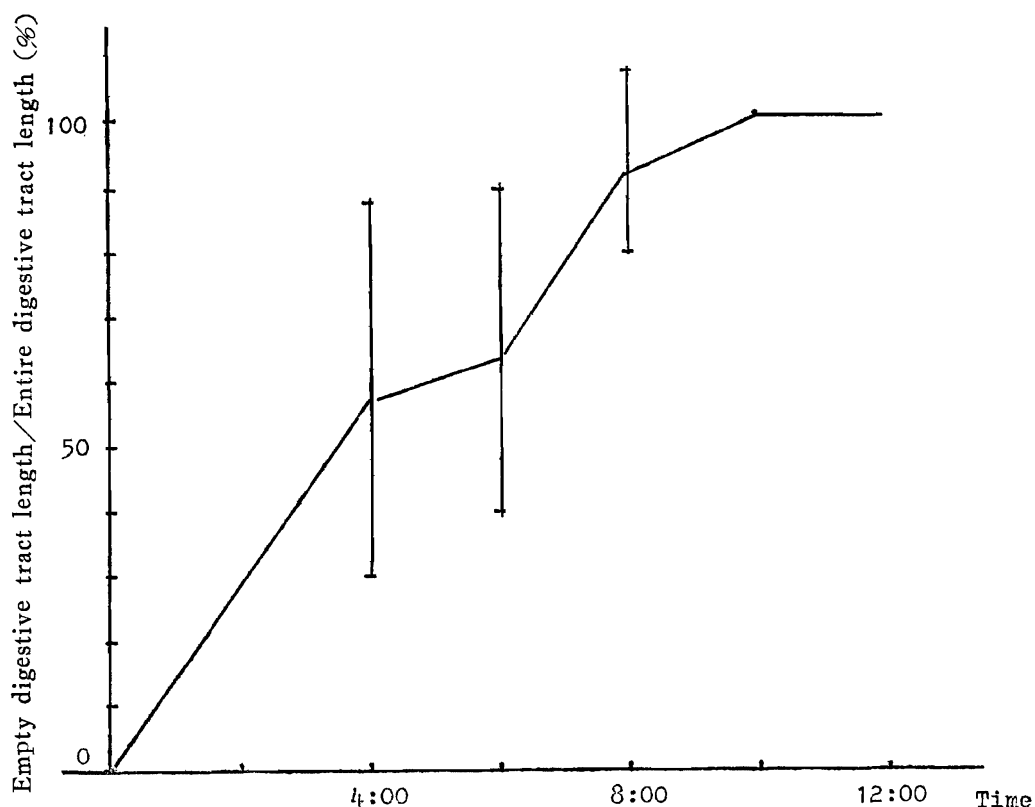


Fig. 4 Digestive rate of silver carp.

G. Analysis of the contents of digestive tracts

The fore-gut contents of sixty-three carp were preserved in 5% formalin. After agitation, 1 cc of each of the preserved contents was divided into several fractions which were examined under a microscope. This process was repeated three times. The mean value of the data obtained is given as follows:

Cyanophyceae 62.3%—*Anacystis*, single cell algae.

Bacillariophyceae 10.6%—*Navicula*, *Frustulia*.

Chlorophyceae 11.9%—*Scenedesmus*, *Ankistrodesmus*, *Selenastrum*,
Tetraedron, *Pediastrum*.

Detritus 13.0%.

Others 2.2%.

The hind-gut contents were analyzed in the same way and found to consist of a large percentage of Bacillariophyceae and a small amount of other unidentified substances. Therefore, it could be safely concluded that silver carp digested Cyanophyceae and Chlorophyceae more easily than

Bacillariophyceae.

H. Analysis of the phytoplankton in the ponds.

Every ten days during the investigation, five liters of water obtained from the central portion of each experimental pond was filtrated through a No. 25 phytoplankton net, condensed and preserved with 5% formalin in a 50 cc plastic vessel. Analysis was made in the same way as that described in G and the following results were obtained:

Protozoa 5.70%—*Eudorina*, *Euglena*, *Trachelomonas*.

Cyanophyceae 39.16%—*Anacystis*, single cell algae.

Bacillariophyceae 32.80%—*Melosira*, *Navicula*, *Frustulia*, *Synedra*, *Eunotia*, *Gyrosigma*.

Chlorophyceae 22.34%—*Docidium*, *Scenedesmus*, *Tetraedron*, *Pedias-trum*, *Spirogyra*, *Gloeocystis*, *Crucigenia*, *Ankistrodesmus*, *Actinas-trum*, *Schroederia*, *Selenastrum*, *Chodatella*.

I. Chlorophyll digestibility

Three hours after feed was given to the fish in the cement ponds on the day when the fish had been reared for fifteen days, the contents of the first and last segments of the digestive tracts of ten carp were obtained to determine the amount of Cr_2O_3 and chlorophyll per gram of diet. The contents dried for twenty-four hours in a 90°C–100°C oven and treated according to Furukawa and Tsukahara's method (Furukawa & Tsukahara 1966) were determined for the values of Cr_2O_3 , while those dried at 5°C in the shade and treated following Richard and Thompson's method (Richard & Thompson 1952) were determined for the values of chlorophyll a. Digestibility was then calculated by the following formula (Maynard & Loosli 1956):

$$\text{Digestibility} = 100 - 100 \left(\frac{\% \text{ indicator in feed} \times \% \text{ nutrient in feces}}{\% \text{ indicator in feces} \times \% \text{ nutrient in feed}} \right)$$

In case the contents obtained could not be treated in time, Doxtater's refrigeration method (Doxtater 1963) was used before the determination of digestibility. The results are given in Table 6.

The results showed that chlorophyll could not be digested in the digestive tract of silver carp.

J. Protein digestibility

Thirty-six carp were divided according to sex and weight into six groups. The digestive tract of each carp was divided into nine segments. Two portions of contents were obtained from each segment. One portion

Table 6. Chlorophyll digestibility of silver carp

Chromium Trioxide (mg/g)		Chlorophyll a (mg/g)		Digestibility %
Intestinum		Intestinum		
Primum	Ultimum	Primum	Ultimum	
0.175	0.196	1.035	1.205	-0.07
0.191	0.211	0.840	0.979	-0.05
0.134	0.165	0.326	0.344	+0.14
0.226	0.300	0.150	1.411	+0.76
0.141	0.165	0.344	0.326	+0.09
0.129	0.192	1.567	3.454	-0.48
0.141	0.177	1.263	1.781	-0.12
0.128	0.318	0.394	0.524	-0.86
0.146	0.155	1.190	1.954	-0.54
0.154	0.191	0.313	0.618	-0.59

Table 7. Protein digestibility of silver carp

Body Weight (gm)	Males			Females		
	500-900	200-400	20-50	700-900	200-400	20-40
Body Length (cm)	35-40	25-30	10-15	35-40	25-30	10-15
Digestibility	91.79	95.36	62.54	73.72	94.43	71.09
	97.32	97.60	91.65	78.24	70.56	86.15
	71.16	96.31	71.35	66.15	83.42	78.09
	81.45	88.40	83.00	85.59	97.09	94.28
	77.15	87.13	67.63	62.97	94.07	63.33
	—	91.42	83.29	—	85.92	87.88
	—	—	90.29	—	95.53	—
	Mean	83.77	92.70	78.62	73.33	88.77

was used for determining the content of chlorophyll a in a gram of diet by the above method, and the other one for determining that of protein by Osmund Holm-Hansen's method (Holm-Hansen 1968). Protein digestibility was calculated by Maynard and Loosli's formula and the results are recorded in Table 7. The protein amounts are given in Table 8.

The protein digestibility of silver carp ranged from 73.33% to 92.70%, varying little between sexes, but affected by the size of fish. The group of 200-400 gm had the highest value, that of 20-40 gm the medium, and

that of 500-900 gm the lowest.

Silver carp digest protein most easily in the fore-gut, less in the mid-gut, and the least in the hind-gut.

Table 8. Segmental protein amount (mg/g tract-contents)

Body Weight (g) Body Length(cm)	Males		Females		
	500-900	200-400	700-900	200-400	20-50
	35-40	25-30	35-40	25-30	10-15
1	22.271 ± 2.584	26.303 ± 14.244	35.554 ± 14.456	29.767 ± 5.477	34.127 ± 14.09
2	17.400 ± 2.280	15.095 ± 4.966	28.596 ± 9.623	23.571 ± 7.526	21.998 ± 8.726
3	16.560 ± 3.775	17.966 ± 6.529	25.972 ± 8.043	23.860 ± 7.041	19.482 ± 7.356
4	14.720 ± 6.847	15.615 ± 6.850	23.607 ± 13.82	19.861 ± 7.140	19.292 ± 8.449
5	14.260 ± 2.057	15.046 ± 4.334	23.676 ± 1.929	22.316 ± 6.194	15.257 ± 6.442
6	12.076 ± 5.482	14.353 ± 4.559	18.710 ± 5.513	21.600 ± 3.000	17.679 ± 7.801
7	8.950 ± 6.483	12.465 ± 5.161	19.052 ± 5.283	18.364 ± 5.986	14.354 ± 7.616
8	9.198 ± 6.932	11.340 ± 5.260	16.364 ± 7.281	16.459 ± 5.951	15.444 ± 6.596
9	5.054 ± 9.712	7.495 ± 3.462	17.188 ± 7.052	13.619 ± 7.302	10.787 ± 5.367

Discussion

It is clear from the present analysis of the pond-water (Table 2) and plankton as well as the past data obtained by W. K. Liaw (1969a, 1969b) and Y. S. Lin (1970a, 1970b) that the ponds of the Chupei Station are eutrophic ones which abound with nutrient elements for the silver carp.

A. Feeding

1. Feeding time

The feeding time varies from fish to fish. For example, yellow perch feeds at 7:00 a.m. and 6:00 p.m.; rock bass at 5:00 p.m.; pumpkinseed at 5:00-7:30 a.m. and 3:00-5:30 p.m.; banded killifish at 1:00-5:00 p.m.; and bluegill, continuously (Keast & Welsh 1968). The present investigation shows that the feeding time of silver carp is 4:00-5:00 a.m. (Fig. 1), four hours before the peak of the feeding curve (Keast & Welsh 1968), while that of the cohabiting common carp is 4:00-5:00 a.m. and 5:00 p.m.

2. State of nourishment

The state of nourishment is expressed as the ratio of the dry weight of the contents of the digestive tract to the body weight. The experiments made by others have led to an awareness of the close relationship of the state of nourishment to the location of the laboratory (Ishiwata 1968a),

satiation amount, starving state, feeding enticement, dietary components (Ishiwata 1968b, Kevern 1969), inter- or intra-competition (Seaburg & Moyle 1964), fish size, water temperature (Ishiwata 1968), etc. For example, skipjack tuna has a value of 8.6% (Magnuson 1969), sunfish 1-2% (Seaburg & Moyle 1964), coho salmon and trout 1.8-3.6% (Laarman 1969), and carp 3.9% (Kevern 1966). It is noteworthy that the difference is of minimal significance, if all of the values fall inside the 95% confidence limits. From the results given in Tables 3 and 4, we know that silver carp has a lower mean value of 0.41%, while the cohabiting common carp has a mean value of 1.42%.

An experiment to determine the relationship between the size of fish and the state of nourishment was carried out by Keast and Welsh in 1968 giving the following results: The state of nourishment of 10-20 cm carp was 6%, while that of 25-30 cm was 2%. These results apparently indicate that the larger the size of fish, the less vigorous the state of nourishment becomes. In this study, however, such a relationship was not found, because the determination of the state of nourishment was made in 40-45 cm fish, but not in the smaller ones.

A practical and accurate method for the determination of daily feeding amount remains obscure. Darnell and Meierotto's method (Darnell & Meierotto 1962) met with failure in this investigation, because of the anorexia of silver carp and the alteration of its feeding time during the course of this experiment and manipulation (Ishiwata 1968a).

3. Digestive rate

Fish takes 5-8 hours to empty the stomach. For example, flounder takes 8 hours to do so (Miller 1967) and eel takes 8-10 hours to empty the digestive tract (K. L. An 1968). As for the silver carp studied, food arrived at the mid-gut four hours after feeding and it took an average of ten hours to find remnants at the gut-end. However, a few silver carp moved its food in the gut with a velocity about two and a half times the average and took only four hours to empty the gut, while some did not give any feces at the gut-end even ten hours after feeding. Based on these findings, it is evident that the digestive rate of silver carp varies a great deal.

4. Food contents

Silver carp is one of the phytoplanktophagous fish bearing spongiform gillrakers well suited for filtering minute plankton or organisms in the

water (S.Y. Lin 1968). Observations made by Nikolsky in 1963 has led to the concept that the food contents in a phytoplanktrophagous fish depend upon the gillraker structure, oral position, and components and distribution of foodstuffs. The environment of the present experiment resulted in the following ingested contents in the silver carp: 62.3% of Cyanophyceae (mainly *Anacystis*). 11.9% of Chlorophyceae (mainly *Scenedesmus* and *Ankistrodesmus*), 10.6% of Bacillariophyceae (mainly *Navicula* and *Frustulia*), and a small portion of fragments and unidentified particles.

Table 9. Analysis of components of food in the water and gut

<i>Components</i>	<i>Water</i>	<i>Food Contents</i>
Protozoa	5.70 %	0
Chlorophyceae	22.34 %	11.9 %
Bacillariophyceae	32.80 %	10.6 %
Cyanophyceae	39.16 %	62.3 %
Detritus	0	13.0 %
Others	0	2.2 %

From the fact that the ponds of the Chupei Station abound with net plankton and nannoplankton which the gillrakers can filter, it is logical to deduce that silver carp must have digested these organisms so completely or to such an extent that hardly any remained in the digestive tract.

It can be seen from Table 9 and from examination of the remnants at the gut-end that silver carp is selective about its food, favoring Cyanophyceae most, relishing Chlorophyceae much, and showing aversion to Bacillariophyceae.

There is little convincing evidence in the present investigation to support the view held by Nikolsky in 1963 and by Smith and Page in 1969 that food contents vary a great deal among the fish congeneric but different in size, owing to a constant change of gillraker structure with age, or to verify the concept of Nikolsky in 1963 that silver carp digested phytoplankton only during the adult period, but absorbed zooplankton in its early life.

The fact that the digestive tract of silver carp is longer than its entire body favors strongly the findings of Nikolsky in 1963 and Ochiai in 1968. Both found that food contents could influence the tract length. Herbivores have guts often several times the size of the fish, because of the alimentary nature. The relationship between digestive tract length is illustrated graphically in Fig. 3.

B. Digestibility

Changes in pH value after feeding, for example, the reduction of pH from 4.4-7.5 to 2.2-2.4 in the digestive tract of fed pike, was observed by Nikolsky in 1963. However, there were minimal changes in the silver carp's digestive tract, whose anterior, middle, and posterior segments all had a value of 6.8-8.3. Under such circumstances, chlorophyll of phytoplankton was not destroyed easily, although acid could convert a little of the chlorophyll. The study revealed the fact that a gradual slight increase of chlorophyll in the gut from the first to the ninth segment occurred, and a negligible value of chlorophyll digestibility determined by the indicator Cr_2O_3 suggested that chlorophyll was not digested in the silver carp tract. As a result, chlorophyll could be used directly as an indicator for the determination of protein digestibility.

Protein digestibility differs considerably among the different foodstuffs. For example, that of goldfish on a chlorophyceous diet was 54%-63% (Nose 1960) as compared to 23.06%-69.43% of milkfish on miscellaneous foodstuffs (Y. S. Lai 1965). Other species also showed various degrees of protein consumption (Nose 1963, 1966, 1967). As far as silver carp feeding on phytoplankton was concerned, it had a mean value of 73.33%-92.70%, showing no significant difference between the two sexes by t-test, but values varying a great deal due to weight by F-test [$F=5.72$ greater than $F_{33}^2(0.55)=3.28$] from 92.70%-88.77% in the 200-400 gm carp to 80.13-78.62% and 83.77%-73.33% in the 20-45 gm and 500-900 gm respectively.

Protein digestibility of silver carp is in accord with observations by Nose in 1964 on that of crayfish and with the results that proteins are absorbed fast from the anterior segment of the digestive tract, less fast from the middle, and little from the posterior.

It appears obvious that quite a few factors may influence the assimilation of protein. However, opinion is sharply divided as to the exact influence of the factors. There is no general agreement, for example, on the ichthyological role of carbohydrate. It was thought by Tunison, Brockway, Maxwell (1942-1944), Hanaoka (1948), Inaba *et al.* (1963), and Kitamikado (1964) that diet-carbohydrate could enhance protein digestibility, but it was believed by Nose (1966, 1967) that the latter was directly proportional to the quantity of diet-protein itself instead of that of carbohydrate. On the other hand, Maynard and Loosli (1956) were inclined to attribute it to the size of fish, feeding amount, and fibrinous substances, but did not presume

it had anything to do with the diet-protein concentration. Hence it would be of interest to determine whether the protein digestibility of silver carp is much the same as above and to investigate whether chlorophyll is an ideal indicator in the study of herbivorous fishes.

Summary

1. The fish ponds of the Chupei Station are eutrophic and rich in various nutrient elements and algae.
2. Silver carp digests Cyanophyceae easily but Bacillariophyceae with difficulty.
3. Silver carp is a diurnal fish and feeds at 4:00 a.m.-5:00 a.m.
4. It takes ten hours on an average to change ingesta into excreta.
5. pH in the digestive tract of the silver carp ranges from 6.8 to 8.3.
6. There is a positive correlation between digestive tract length (Y) and body length (X). The regression line: $Y=9.449 \times -102.098$, $r=0.959$.
7. The indigestible chlorophyll instead of Cr_2O_3 is used as an indicator for the determination of protein digestibility.
8. Protein digestibility of silver carp ranges from 73.33% to 92.70%.
9. Protein is digested mostly in the fore-gut, secondarily in the mid-gut, and little in the hind-gut.
10. There is no difference in digestibility between sexes.
11. Protein digestibility varies with the size of fish: Maximal 200-400 gm, intermediate 20-45 gm, and minimal 500-900 gm.

Acknowledgements

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NOTES ON EXPERIMENTAL REARING OF SWAMP EEL

Chia-kang Liu*

The swamp eel, *Fluta alba* (Zuiew), is valued by the Chinese as food. It is caught mainly from rice paddies and has never been cultured, except that some are impounded to meet market demands in the off-season.

In 1969 and 1970, the Chupei Fish Culture Station of the Taiwan Fisheries Research Institute carried out experiments on its culture to determine (1) whether it could grow by artificial feeding, (2) the feed conversion ratio, and (3) whether it could be induced to take food in the day time, it being normally a night feeder.

The experiments were conducted from September 9, 1969 to August 31, 1970 in three concrete tanks of one square meter in area. The water temperature varied from 10°C to 26°C during this period. The feeds consisted of earthworms in the initial stage of the first half of the experimental period. Later, minced trash fish was added. During the second half of the experimental period, mixed feed formulated for Japanese eel was added, while the earthworm diet was gradually reduced and finally withheld altogether.

In the first half of the experimental period, the tank bottom was filled with a layer of mud to simulate natural habitat and provide a hiding place for the eel during the day. In the second half of the experimental period, the mud layer was removed and water hyacinth was placed in the tank to cover about one half of the surface area.

Unfortunately, due to faulty equipment, one of the three tanks was flooded one night, and most of the eels escaped during the latter part of the experimental period. Thus, the results obtained were neither complete nor conclusive. They are shown in the following table:

	Sept. 9, 1969		Aug. 31, 1970		Total wt. of feed	Increase in wt.
	No.	Total wt.	No.	Total wt.		
Tank A.	35	1,675 g	35	1,950 g	5,373 g	276 g
Tank B	38	743 g	18*	600 g	6,718 g	-143 g
Tank C	139	1,250 g	128	1,600 g	8,790 g	350 g

* Eels escaped due to flooding of tank.

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The experiments did show:

1. The swamp eel could grow by artificial feeding. The feed conversion ratio, however, was very high, 19.5:1 in Tank A and 25.2:2 in Tank C.
2. The swamp eel could be trained to feed during the day.
3. Feeding activity of the swamp eel slowed down when the water temperature was down to 16°C and ceased entirely when it dropped to 12° or 13°C.
4. The amount of feed given should be from 3 to 5 % of body weight. Excessive amount of feeds given, as in this experiment, resulted in very high feed conversion ratio.

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