CHINESE - AMERICAN
JOINT COMMISSION ON RURAL RECONSTRUCTION

Fisheries Series: No. 5

INDUCED SPAWNING OF CHINESE CARPS BY PITUITARY INJECTION IN TAIWAN

(A Survey of Technique and Application)

By

Shu-yen Lin

Taipei, Taiwan, China
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FOREWORD

Immediately after the discovery of naturl spawning of Chinese carps in 1959 in the Ah Kung Tien Reservoir in southern Taiwan, the Joint Commission on Rural Reconstruction assisted the Taiwan Fisheries Research Institute to conduct a survey to confirm this discovery, estimate the number of available spawners in the reservoir, and determine the season and place the spawning occurred. In the ensuing years, JCRR gave further assistance to the Institute to carry out experiments in artificial propagation of this important pond fish. Success led to further success, until the practice is now practically commercialized. It is estimated that the Chinese carp fry produced in 1965 by induced spawning will be sufficient to meet the entire need of the fish farmers in Taiwan. Many enquiries have been received by the Division concerning the techniques used in this work, and many visitors have come to Taiwan to observe the procedures. Mr. S. Y. Lin, Senior Specialist of the Division has written this report, which describes the method in detail and suggests many improvements. I feel sure that this report will serve as important reference and aid to fish culturists in those part of the world where the Chinese carps are cultured as well as in some countries where the grass carp as a weed eradicator is being used.

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Acting Chief
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INDUCED SPAWNING OF CHINESE CARPS BY PITUITARY INJECTION IN TAIWAN

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By
S. Y. Lin

Senior Specialist, Joint Commission on Rural Reconstruction

The Chinese carp production in ponds in mainland China was estimated at one to two million metric tons per annum. The fishes that support such production are known as:

1. The common carp, *Cyprinus carpio* Linn.
2. Silver carp, *Hypophthalmichthys molitrix* (Val.)
3. Big head, *Aristichthys nobilis* (Richardson)
4. Grass carp, *Ctenopharyngodon idella* (Val.)
5. Black carp, *Mylopharyngodon piceus* (Richardson)
6. Mud carp, *Cirrhina molitorella* (C. and V.)

The common carp heads this list not because it is the most important species for pond production but because of its virtual seniority in the history of domestication. Of the total pond production in mainland China the common carp amounts to only a little over five per cent. In some ponds the stocking rate of common carp is about ten per cent, but in many others no common carp is used for stocking at all (Lin, 1954). Among fishes of natural lakes and rivers in China, common carp is indeed very common, and yet its commercial production has never been higher than the other Chinese carps.

In Fanli's book, the "Classic of Fish Culture", was described the method of common carp culture in ponds as practiced in 500 B.C. But the practice of culture of other Chinese carps was only briefly mentioned many centuries later in the literature of the Han and Tang Dynasties (300 to 800 A.D.)

The cause for the delay of domestication of the grass carp, silver carp, big head, black carp and mud carp in ponds lay apparently in the difficulty of obtaining fingerlings for stocking. It took time to discover that the eggs and fry which the Chinese carps, with the exception of the common carp, annually produced by natural spawning in the rivers, could be collected for culture. Once the existence of the eggs and fry, in abundance, of the Chinese carps was known, the technique of collecting, hatching, and rearing of the fry was systematically developed.

This may explain how the fish fry industry has been established in mainland China after the domestication of the common carp, but in Taiwan the situation is different. Here on the island, before reservoirs were artificially built, were no natural rivers and lakes large enough to accommodate the Chinese carps for spawning even if they had the chance to get there, and therefore there were no fish fry available, except the common carp, from the local waters.

The farmers came to live in Taiwan from the mainland many centuries ago, and by virtue of their inherent love for fish culture, were prone to establish ponds as a part of their farming activities. Many new techniques were developed in the special circumstances for the culture of local species such as the milkfish, *Chanos chanos,* and grey mullet, *Mugil cephalus,* but they could not forget their traditional culture of the Chinese carps. To solve the problem of fry supply for stocking they were compelled to import fry of grass carp, silver carp, big head, and mud carp every year from the mainland.

As every one can see the supply of fish fry by importation is not only costly but also uncertain. However highly desirable it had been to improve the situation, there was no solution until the induced spawning of the Chinese carp by pituitary injection became successful in 1962, which in turn led to the accomplishment of commercialized fish fry production by induction in 1965. Such achievement is attributed mainly to industriousness and progressive spirit of the fish farmers and the undefatigable efforts made by the technical workers of the Taiwan Fisheries Research Institute and the Taiwan Fisheries Bureau as well as continued technical and financial assistance of JCRR. The present survey was made therefore in acknowledgement of their efforts and in the hope that this report may serve as the basis for further technical improvement of the induction methods.

Establishment and Equipment

There are all kinds of establishments for induced reproduction of the Chinese carps existing in Taiwan; some of them are elaborate, well-planned and almost complete with facilities for all phases of the work while many others are incomplete and poorly equipped.

As an illustration, the Wu-Shan-Tou Hatchery in Tainan County designed by C. C. Cheng, Fisheries Specialist of the Taiwan Fisheries Bureau, is described with respect to principal installations and equipment; meanwhile a few different types of equipment designed and employed in other hatcheries are included for comparison. This Wu-Shan-Tou Hatchery is comparatively small in size and inexpensive in construction, having the advantage of plentiful water supply, better soil and quality of water (table 1), and a favourable topography for pond construction. It is worthy to note that the high pH value together with abundant calcium and bicarbonate in solution is extremely healthy for fish growth and reproduction, particularly the grass carp and mud carp, which thrive best in slightly alkaline water.

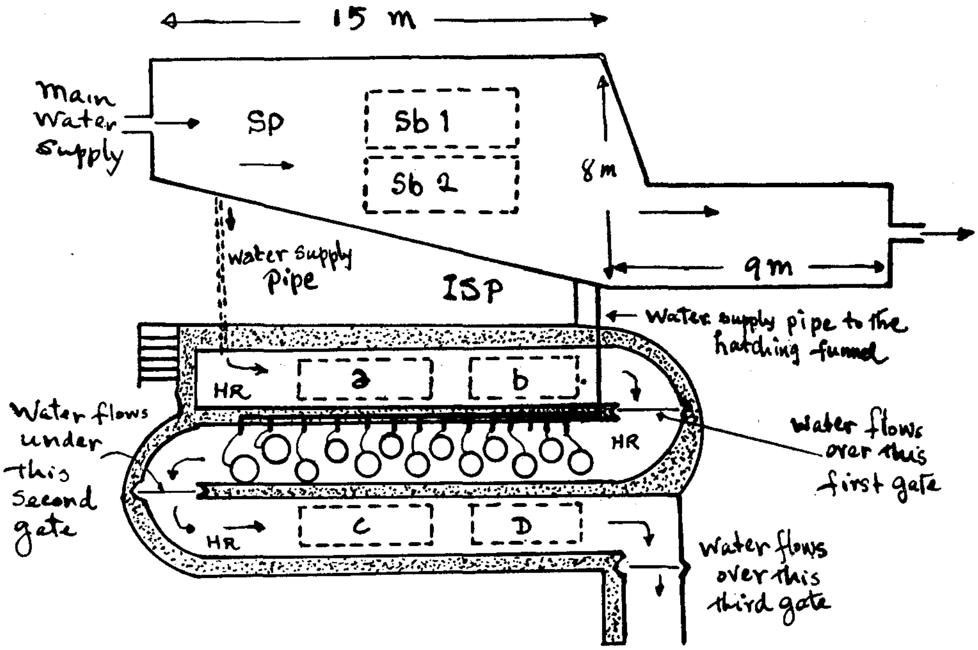

Figure 1. Schematic plan of the spawning pond and hatching-rearing tanks of the Wu-Shan-Tou Hathery. See text for dimension not indicated in this diagram. The circles in the middle section show the positions of the hatching funnels. *a-d,* fish fry rearing troughs; *HR,* Hatching-rearing tank; *ISP,* Injection platform; *Sb I* and *Sb 2,* Spawning boxes; *SP,* Spawning pond.

Figure 2 shows the monthly average surface temperature of Wu-Shan-Tou Reservoir, the water supply from which appears to be suitable for the establishment of a Chinese carp hatchery.

The favourable temperature for spawning and hatching of the Chinese carps ranges from 24° to 28°C in Taiwan, and as a stimulus for the parent fish to develop eggs and sperm, a cold spell in the winter is important. Instances are known however that grass carp, silver carp and big head can spawn at 20°C or even slightly lower, though hatching and survival rate of the fry even at 22°C is low. While the grass carp, silver carp and big head can survive conditions under ice, the mud carp suffers at temperature lower than 12°C.

Table 1. Water Analysis of Wu-Shan-Tou Reservoir made by Prof. Y. P. Hsu of National Taiwan University, 1963. All in mg/1 except pH and conductivity

1.	pH	8.0–8.5
2.	Conductivity Ecx10^6 at 25°C (Micromhos)	270–475
3.	Total dissolved solids	204–339
4.	Calcium	20.2–34.9
5.	Magnesium	10.7–18.8
6.	Sodium	7.6–44.4
7.	Potassium	1.6–3.5
8.	Carbonates (CO_3)	0–9.6
9.	Bicarbonates (HCO_3)	97.0–161.7
10.	Sulfates (SO_4)	36.4–59.0
11.	Chloride (Cl)	3.6–84.5
12.	Nitrates (NO_3)	0–0.25
13.	Borons	0.02–0.13
14.	Silica (SiO_2)	8.7–14.6
15.	Phosphorus	0–0.03

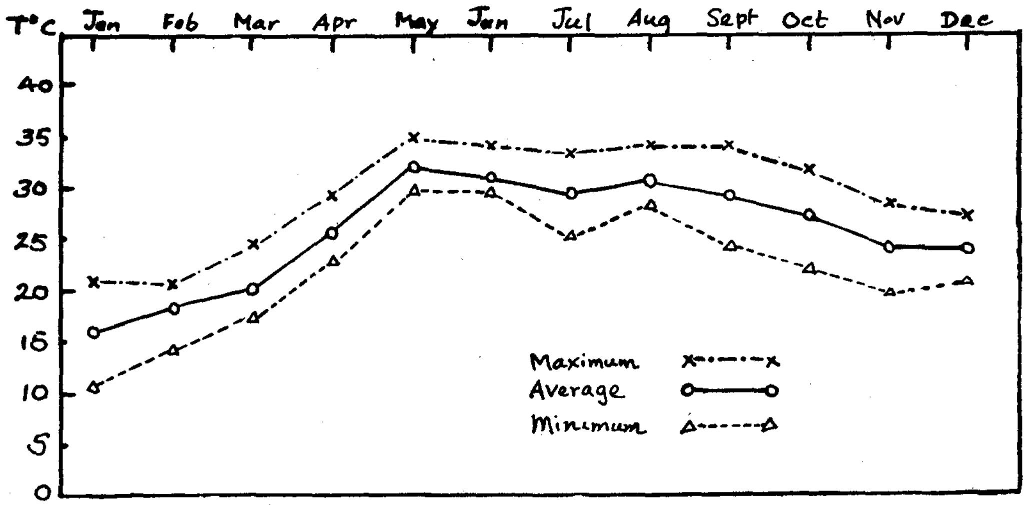

Figure 2. Monthly average surface temperature of Wu-Shan-Tou Reservoir in centigrabe as registered in 1963

Eggs of the Chinese carps are formed during and after the hot summer and as soon as they become fully mature early in the winter they remain dormant during the winter and early spring. Then in April and May when temperature is rising (figure 2) together with other seasonal changes of conditions such as flood, water current and food, the fully mature eggs are rapidly transformed through endocrine autocatalytic action from adhesive lump into free, separate ova ready for expulsion.

Natural spawning of grass carp, mud carp and big head was discovered, since 1962, in Wu-Shan-Tou Reservoir.

It is evident that seasonal temperature variation forms one of the stimuli for adequate egg formation, but in addition to rise of temperature there must be present other factors in combination with temperature for any successful ovulation. Such combination of natural factors commonly found in rivers, lakes and reservoirs is absent in ordinary fish ponds.

In a congested fish pond the presence of ammonia nitrogen in high concentration is most likely to be one of the factors responsible for inhibiting the emission of gonadotropins by the pituitary gland and in the absence of these gonadotrophic hormones no autocatalytic reaction of estrogen can develop in the ovary. Consequently ovulation fails to happen despite the presence of fully mature eggs.

The success of induced reproduction by pituitary injection to take the place of natural stimuli supports such postulation.

Apart from a small laboratory in which chemicals and apparatus for water analysis and biological work, and instruments for pituitary injection, egg collecting, fry examination, pond management and so forth, the following are considered as important installations and equipment with which every Chinese carp hatchery should be provided:

1. Spawner Holding and Recuperation Ponds

Four of the existing 14 ponds (2,000 to 4,000 m^2) are devoted to the culture of the spawners, two for holding and two for recuperation. The holding ponds are used for storing the spawners selected from commercial fish ponds and the recuperation ponds used for holding the spent fish immediately after stripping. In this recuperation pond plenty of food, particularly tender grass for the grass carp, are supplied for quick recovery of the spawners.

2. Common Carp or Donor Pond

A pond 1,000 to 2,000 m^2 is used for the culture of 400 to 700 common carp weighing 300 to 1,000 grams each which would serve as donors of fresh pituitary glands.

3. Spawning Pond and Spawning Boxes

The spawning pond built of concrete near the laboratory is widest in the middle (8 m). At the water supply end it measures 4 m and at the drainage end 3.5 m (figure 1). The sluice gate measures 2 m from top to bottom and the depth at the water supply end is 1.5 m, thus providing an excellont slope for drainage. The length from the water inlet to the widest part is 15 m. The inflow of water is 30 to 45 1/second. In the main section of the pond where a uniform current with well-oxygenated water passes, two floating spawning boxes are placed (figure 1, *Sb1* and *Sb2*) in which the hormonated female and male spawners are held together for 10 to 20 hours until ovulation.

This spawning box made of 1 cm-bar-mesh cotton or nylon netting, 3m × 5m × 1m, is suspended from a wooden frame to keep the top wide open and floating a few centimetres above water surface, while the bottom is extended downwards by sinkers tied to the four corners. The top opening is then covered with a piece of netting to prevent the fish from jumping out.

As soon as the female is observed to be chased by the male (usually 10-14 hours after the first pituitary injection) indicating that ovulation may take place at any moment they can be taken out for stripping but if they are not removed for artificial insemination, a large piece of fine-mesh nylon netting should be placed under the spawning box to collect the eggs.

4. Spawner Carrying Cradle

This is an indispensible equipment to protect the soft and delicate spawners from injuries caused by handling. As the spawaners are caught in a fine-mesh netting from the holding pond, they must be transferred to the spawner cradle, one fish at a time, for carrying, weighing and hormonation. This signifies, in short, that during all the manipulations for the purpose of hormonation from netting in the holding pond to the last step of releasing it in the spawning pond, the spawner must be held in the cradle all the time and should never be held in the hands or any other types of unsuitable containers. The cradle is made of cloth, sewed to a wooden frame of convenient dimension (40 cm × 80 cm for example) to suit the size of the spawner, leaving the cloth hanging down to form a basin about 15 cm deep. Another piece of cloth is sewed to only one side of the frame, leaving three sides free to serve as cover. In the basin the spawner could lie comfortably covered, swinging back and forth slightly while it is carried with both hands grasping the frame.

For weighing the spawner, the cradle is hung to a spring balance by means of two loops put on the frame at both ends. When pituitary injection is made, the cradle is supported by a stand about 80 cm high. The head of the spawner is covered with a wet towel and gently kept in place by hand as the part of the body behind the head is exposed for insertion of the needle.

For any long duration of manipulation water should be poured over the fish in the cradle occasionally. Since the cloth becomes fairly thick when it is wet, water is retained for the fish to breathe. In this manner the application of anaesthetics is not necessary.

A cradle of this type is also useful for removal of the spawners from the spawning pond for examination and for stripping and later on for carrying them to release in the recuperation pond.

5. The Hatching and Rearing Tank

This is a S-shaped concrete tank divided into three sections (figure 1, HR). The ground for the construction of this tank should be lower than the spawning pond if the water of the latter is also utilized for circulation in the former. Each section of the hatching and rearing tank measures 14 m long, 2 m wide and 1.5 m deep. The middle section is used for hatching of eggs and both the upper and lower sections are usually reserved for the suspension of fry rearing troughs. Over these troughs water flows out from small holes on pipes to spray over the fry. The water entering at the upper end passes through three gates, the first of which is closed from bottom to about two-thirds deep, the second open at the bottom and the third closed like the first so as to let water firstly pass over, secondly under and lastly over again. In this manner a circulation of well-oxygenated water through all the sections is obtained.

This S-shaped concrete tank of Wu-Shan-Tou Hatchery is designed for the employment of the funnel-like hanging vessels for hatching of eggs.

In another hatchery elsewhere, long hatching troughs made of wood are used. Each trough is divided into three or four compartments by baffle plates to force water to flow up from a hole at the bottom through the hatching baskets (figure 3 and 4).

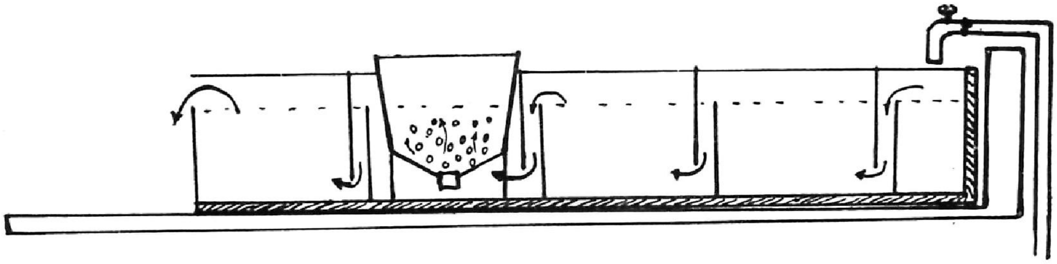

Figure 3. A longitudinal section of the hatching trough with baffle plates to force the flow of water through hatching baskets, one of which is shown in position.

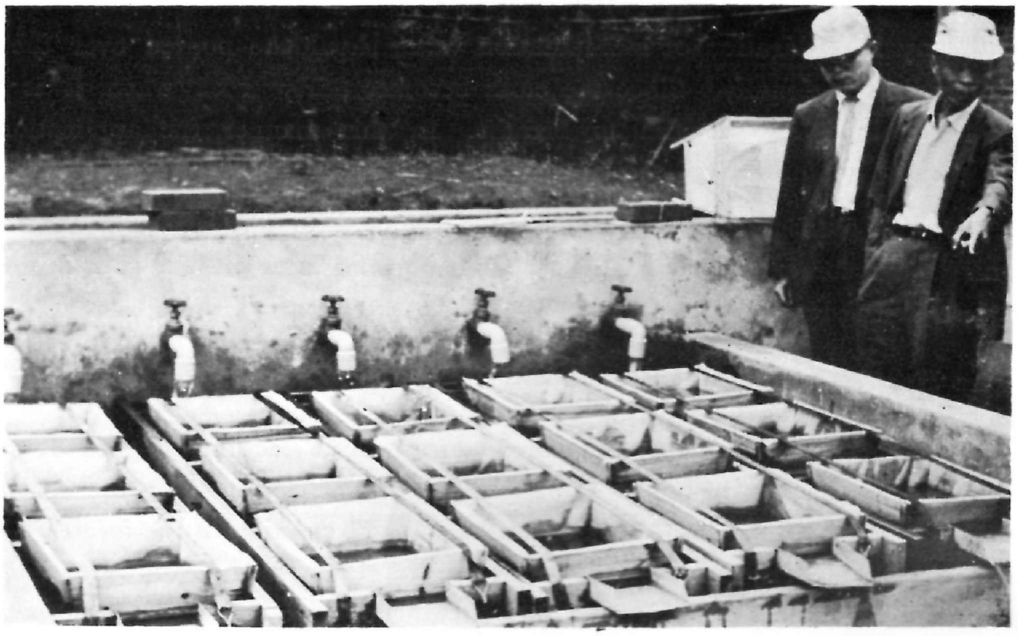

Figure 4. Arrangement of hatching baskets in five troughs, the two baskets of larger-mesh netting at the inflow end each holding 15,000 to 22,000 eggs and the one at the outflowend of fine-mesh netting collecting the newly hatched larvae.

6. Hatching Funnel and Baskets

Two types of hatching devices have been developed in Taiwan and are considered very efficient. Hatching tray is still in use in some hatcheries, but has the drawback of difficulty to bring all the eggs uniformly in contact with the circulating water.

The construction of both the funnel and basket-type of hatching device is simple and, in principle, not new, having been employed in many trout hatcheries, but their application to the hatching of the eggs of the Chinese carps is so perfect that high rate of hatching and of survival of the fry has been achieved.

The hatching funnel (figure 6) developed by M. C. Lin measures 40 to 60 cm in diameter at the open top and 50 to 70 cm from the lower end of the funnel to the rim of the top. The body is made of nylon netting with mesh fine enough to retain the eggs, larvae and fry and yet large enough to let water pass. A constant

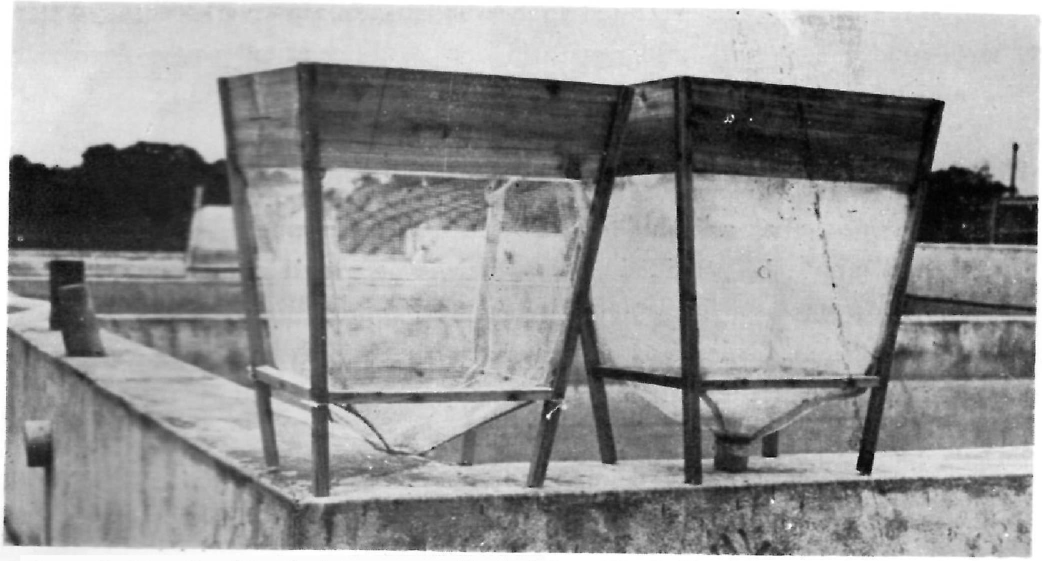

Figure 5. Two hatching baskets: one on the left is made of thin nylon netting through which the newly hatched larvae pass but not the swelled eggs; the right one of fine-mesh nylon retains all the larvae and fry.

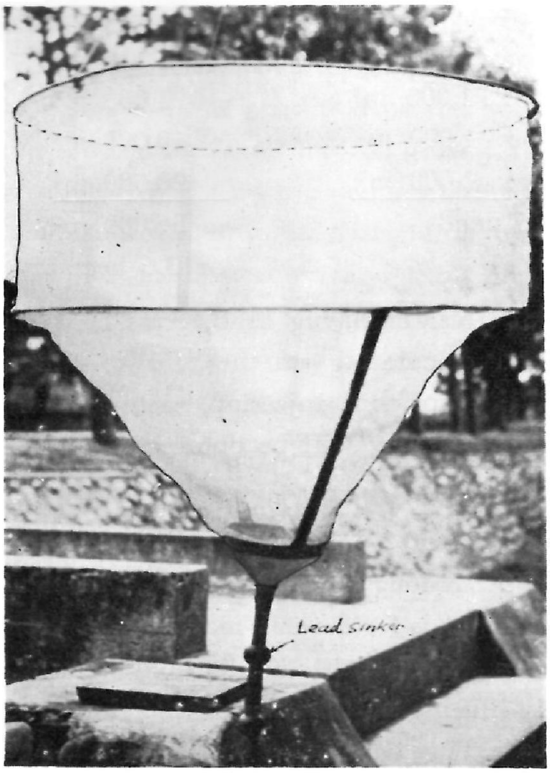

Figure 6. A hatching funnel

flow of water at adequate speed generates from a small plastic funnel attached to the bottom of the hatching funnel. This plastic funnel is connected by means of a piece of rubber tubing to a tap and is kept in position by lead sinkers. The water flow must be regulated so that it is strong enough to cause constant turning over of the eggs, but not too strong to break the egg membrane. In operation the hatching funnel is hung from a pole, keeping the funnel rim about 10 cm above water.

One hatching funnel holds 15,000 to 50,000 eggs dependent on its size.

A hatching basket (figure 5) is made either of large-mesh or fine-mesh nylon netting, fixed to a wooden frame measuring 40 cm × 40 cm × 50 cm, the open top being slightly wider than the bottom. At the bottom a short bomboo or metal tube is attached and a stopper is provided for easy removal of the fry. In operation three baskets are arranged in a wooden trough, two in head water flow with large-mesh netting to hold eggs and one at the end of water flow to collect the fry which escape from the two previous baskets after hatching.

7. Pond Arrangement in Commercial Hatcheries

It is important that a commercial hatchery of silver carp, big head, grass carp and mud carp should be provided with sufficient area and appropriate arrangement of ponds to accommodate both the spawners and fry of various sizes. As there are hatcheries of different scales and purposes existing in Taiwan no one can be defined as a model; a large establishment may occupy an area of more than five hectares while some small ones each less than a hectare in total area for fry rearing. One also finds some hatcheries devoted only to the propagation of either one or two of the four species and others produce all kinds of fry.

However, the following should be the minimum area and designation of ponds for a hatchery whose destination is to propagate all the four kinds of fry by hormone induction.

Spawners holding ponds	5 × 1,000 m^2	5,000 m^2
Common carp donor pond	1 × 1,000 m^2	1,000 m^2
Fry nursery pond	10 × 1,000 m^2	10,000 m^2
Fry nursery pond	10 × 2,000 m^2	20,000 m^2
Total	26 ponds	36,000 m^2
		or 3.6 hectares

With these ponds available 72 pairs of silver carp, big head, grass carp and mud carp of full maturity can be raised and calculating on the basis of half of them being capable to reproduce successfully by hormone injection, each spawning 300,000 eggs or 200,000 fry (about 60%), 7,200,000 fry will be obtained.

From these 7,200,000 young fry it is possible to have 4,000,000 of 1-2 cm long available for sale without much rearing, 2,000,000 of 3-4 cm which must be reared in the ponds for a month or two, 1,000,000 of 5 cm long for a few months in ponds and finally 200,000 of 10 cm long being kept in ponds for several months in a years. Thus the hatchery would have fry of all sizes and ages for distribution the whole year round and by properly and opportunely shifting of the occupants at the appropriate time and size, the same pond can be arranged to rear fry of various ages at different time.

So it follows that provided there is market for fry of different ages and sizes at various seasons these 26 ponds of 1,000-2,000 m^2 each should be sufficient to meet the need.

Culture of Spawners

This is the primary and most important step in the whole work of reproduction by hormone induction, for the success of ovulation and milting depends basically on the well-being of the spawners. The pituitary hormones or gonadotropins can only accelerate the transformation of those fully mature eggs into the final stage for expulsion. The process of complete egg formation takes time, requiring several months for building up the yolk and other vital parts of the ovum. Therefore, immature and abnormal eggs do not show clear response to any pituitary injection and even if they do fertilization and hatching are either very poor or nil. For this reason alone it is to be emphasized that an induction work well done should begin with the culture of spawners.

From the stock of the Chinese carps cultivated in ordinary commercial ponds, spawners can be selected for use in induced reproduction. The stock of this kind in Taiwan usually consists of 1,000 to 1,500 silver carp, 300 to 500 big head, 200 to 300 grass carp 1,0000 grey mullet, and 100 to 200 *Lateolabrax japonica* per hectare dependent on the condition of the pond, water supply and availability of aquatic and land grass (food for the grass carp). The mud carp are occasionally stocked in some rich freshwater ponds and the black carp are uncommon. The best spawners come, however, from the irrigation reservoir type of ponds, each of which has an area of 3 to 28 hectares and a depth of several metres when they are filled to the highest level. Such a pond because of its comparatively favourable physical, chemical and biological conditions produces healthier fish which are less subject to the attack of diseases and parasites than ponds of smaller size.

Grass carp destined for induced reproduction must be given plenty of feeds such as tender land grass, manioc leaves, sweet potato leaves, vegetables, aquatic plant, peanut meal, soybean meal, and rice bran. Care must be taken not to overfeed the fish with fatty food stuff or too much proteinous food to foul the water. In a rich pond the fish tend to grow fat with adipose tissues accumulated on the gonads thus hindering egg and sperm development which in turn causes impotency.

For silver carp, big head, mud carp and black carp, peanut cake and soybean cake are good feeds, and in additson the pond should be fertilized with inorganic fertilizers and barn manures. Green manure and compost are beneficial for all kinds of ponds provided that not too much is given to foul the water.

There have been no standards clearly defined for the selection of spawners of different species. In general the fish to be selected as spawners must be fast growing, free from diseases and parasites, the largest and strongest among all the individuals of the same age, bear the largest number of eggs and mature normally. In order to realize such standardization for quality breeding it is necessary, first of all, to keep the spawners alive for induced reproductions in successive years, and the best spawners up to a certain standard must be marked either by electric device, fin cutting or the use of tags. Genetic characteristic development of each spawner, either male and female, must be observed and recorded.

Under the climatic conditions in Taiwan, the silver carp becomes fully mature in two to three years, big head three to four years, grass carp four to five years and the mud carp three to four years.

Accordingly the age and weight of the spawners to be selected during the regular harvest season from November to February are as follows:

1. Silver carp—2 years of age—2 kg or more.
2. Big head—3 years old—5 kg or more.
3. Grass carp—4 years old—3 kg or more.
4. Mud carp—3 years old weighing one kg or more.
5. Black carp—Occasionally found in ponds in Taiwan; no attempt has been made to spawn this fish by induction method maturing at 5 years weighing more than 10 kg.

As a rule the male becomes mature one year earlier than the female. Premature fish of high quality are also selected for culture under special care as spawners.

The selected spawners are placed in the holding ponds for keeping or for further growing. Combination stocking is recommended because of different feeding habits of different species. Different species of the same age are stocked in the same pond but never fish of different ages. The size of such holding pond measures 1,000 to 4,000 sq. m. and in each pond the following system of association is adopted:

Grass carp—100 fish per hectare
Silver carp—140 fish per hectare
Big head—60 fish per hectare
Mud carp—100 (to 200) fish per hectare.

This holding system provides approximately one fish per 25 sq. m. of water superficies. Male and female are in the proportion of 1:1. Therefore a holding pond of 1,000 sq. m. can hold five pairs of grass carp, five pairs silver carp, three pairs big head and five pairs mud carp. Calculating on the basis that each pair can give rise to 200,000 fry, facilities must be provided to handle 4,000,000 fry per annum.

Satisfactory, however, this association system of holding appears to be, there are numerous problems the solution of which has not as yet been found. The first difficulty experienced by most hatchery workers involves the declining of potency and general health of the spawners by too many fishings done at short intervals. The grass carp are particularly known to be sensitive to fishing disturbance; usually they stop eating for two or three days after vigorous struggle in a fishing net and rough handling. This creates a serious problem for the hatchery which can only handle the injection of two or three pairs of spawners at a time and once this is done, it requires four to six days to complete the work of hormonation, insemination, hatching and the first phase of fry nursing. So from a holding pond of 1,000 m^2 with 18 pairs of spawners, six to nine fishings have to be made within a period of 24 to 54 days. It has been observed that the spawners netted from the same pond for the first two or three injections always gave satisfactory ovulation and milting, but those caught later often declined to respond.

The questions arising in this connection are: (1) Should spawners be segregated by sex and species before the administration of hormonation and if so for how long? (2) What dimension would a segregation pond be suitable? Would small ponds be equally healthy for spawners? How about depth? (3) How important is the question of economy of space and time? etc.

Along this line of thinking a great deal of experiments have to be done for unless the production of potent spawners is secured no induced reproduction can be considered a success.

For a hatchery of moderate magnitude it has been suggested to make the following arrangement: A minimum of five holding ponds are allocated to accommodate five associations of different ages of maturity. For example, while one pond is to hold an association of fishes of the first year of maturity, a second must be made available for the second year of maturity, the third for the third year, the fourth for the fourth year and the fifth for the immature fish. When a spawner has offered four years of satisfactory service, it can be used for other purpose or discarded. Thus once a year the first pond must be replaced with new recruits of first year maturity and those of fourth year maturity discarded after spawning. This means that the holding period for each association of fishes through the four ponds varies from several months to a maximum of four years during which the fishes are expected to reproduce by induction once a year.

It is further expected that at most the first half of the spawners caught from a holding pond are capable to spawn by induction successfully and the second half, as mentioned before, are likely to suffer from gonad regression due to too much disturbance by fishing.

For better management each spawner holding pond should be paired with a recuperation pond. When the spawners from a holding pond have finished their turn of induced reproduction, they are immediately released into their respective recuperation pond where adequate feeding and well-oxygenated water are administered for quick recovery.

When a holding pond becomes empty after the spawners have been induced to spawn and released in the recuperation pond, it can be used for fry rearing until next year to serve as a recuperation or holding pond again. Thus the function of a pond of this sort is three-fold: holding, fry nursing and recuperation or *vice versa* as needed in different times of the year.

Maturity and Secondary Sexual Characters

Despite similar age and conditions of the holding pond, spawners even of the same species are found, for reasons unknown, to vary in degrees of maturity, and in some extreme cases the gonads never develop at all as compared with other fish of the same age with normal sexual maturity. This is particularly true of the grass carp which in small rich ponds are commonly found to reach maturity at different rates. The silver carp, big head and mud carp, however, appears to be hardier, not so much influenced by environments as the grass carp. Perhaps density dependent factors are responsible for the abnormal development of the grass carp gonads, but such postulation is not within the scope of the present discussion.

The important task is how to recognize the full maturity of the spawners and this can be accomplished in many ways.

For the male of all the Chinese carps, a few drops of milting by application of light pressure to the abdomen is sufficient to indicate ripeness. When this fails search should be made for the existence of secondary sexual characters. During the spawning season from late spring to summer and again in early autumn there usually appear deciduous finely serrated ridges, rough to the touch, each along each ray of the pectoral fins, which fall away after spawning or by rubbing. But if such secondary sexual character is found in full growth, it is often convincing that the male is ripe for hormonation.

For the female it is a good sign to see a distended belly caused by full growth of the ripe ovary. A healthy mature female always exhibits her fullness in appearance with shiny hue silvery below and greenish above. There are no secondary sexual characters on the pectoral fins, but the swelling cloaca, pinkish in front can easily be recognized by experienced workers.

Some workers recommend the use of catheter to examine the eggs. It is indeed a sure way to tell the exact state of ripening of the eggs, but this requires skill in manipulation of the catheter to remove some eggs and not to hurt the fish.

Induction Technique and Procedure

Fully mature common carp, either male or female, but not yet spent, are selected as the donor of pituitary hormone, whose weight should be, in principle, equal to that of the recipient, though in practice variation is found from 80% to two or three times the latter depending very much on the physiological conditions of the spawner as a result of culture and care. In case a spawner is fully mature and appears to be in perfect condition, a small dose of pituitary hormone would be effective for adequate ovulation; otherwise a heavier dose is needed.

As one kg of common carp supplies approximately 3 mg of pituitary in dried weight, a spawner weighing 5 kg, for example, would require 15 mg of dried pituitary gland from 5 kg of common carp culculated on equal weight basis between donor and recipient. This 15 mg of pituitary is divided into two equal doses for injections at an interval of 6 hours.

The actual procedure is that a spawner is first selected, weighed and placed in a small fine-mesh netting in a spawning pond for temporary keeping. Suppose the spawner is known to be 5 kg, then one or several common carp of approximately 2.5 kg, which have been caught and held handy for use, are selected and weighed (figure 7). And as soon as the total weight is summed up and recorded the carp pituitary glands are immediately removed, finely ground in a tissue grinder and suspended in 1.5 ml of normal saline for the first injection (fig. 8-10). A similar dose is administered a second time after an elapse of 6 hours. In this hormonation common carp is the only donor of the pituitary and no other kind of hormone is added (tables 2-3).

Figure 7. Weighing of the common carp donor

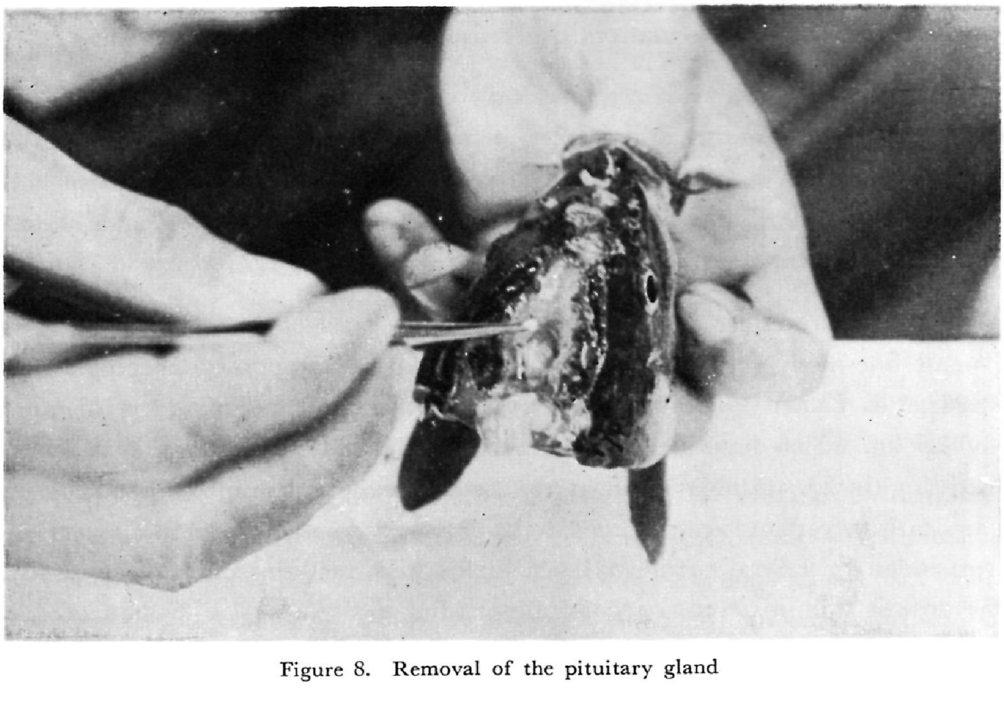

Figure 8. Removal of the pituitary gland

With some workers, the first dose may be heavier or lighter than the second and even a third or fourth injection be given, but unless the spawner is definitely underdosed or wrongly dosed with a type of pituitary hormone to which no response occurs, further administration after the second would only weaken the spawner and cause expulsion of immature ova which can hardly be fertilized to produce healthy young. Sick spawners always fail to spawn, no matter how heavy the dose or doses of pituitary are given.

Pituitaries dehydrated by absolute alcohol and acetone process and kept at low temperature are also used and prove to be effective (Tang *et al*, 1964), but fresh pituitary is preferred and claimed to be more potent as it is now practiced in Taiwan. It is also reported that pituitary from fish dead less than two or three hours is still functional.

Injection is made intramuscularly somewhere between the dorsal fin and the lateral line, care being given in inserting the needle in a soft space between scales. It is still better to insert the needle a short distance behind either the anal or the dorsal fin where scales are small and few. Intra-abdominal injection is also practiced in Taiwan with similar results as intramuscular, but it requires more care and skill not to hurt the fully developed ovary and other parts of the viscera by the needle.

Either one of the following two routines (i.e. day or night schedule) is followed dependent on the convenience and preference of the workers:

Day schedule:

1. Female and male spawners (1:1) are caught late in the afternoon from the holding or rearing pond after well oxygenated freshwater has been admitted for several hours, and they are then placed together in a spawning pond which is provided with moderately running water. Some workers consider that acclimatization of the spawners in the spawning pond over-night before hormonation is necessary, but many others keep them in the spawning pond only one or two hours, especially in the case of a night schedule being practiced.

2. First injection is given to the female alone in the next morning at 0500 hours. Never the male receives any injection at this time, because if this is done milting will occur far ahead of ovulation.

3. The hormonated female is returned to the same spawning pond with the male, running water being maintained all the time.

4. At 1100 hours, i.e. six hours after the first injection, the second dose

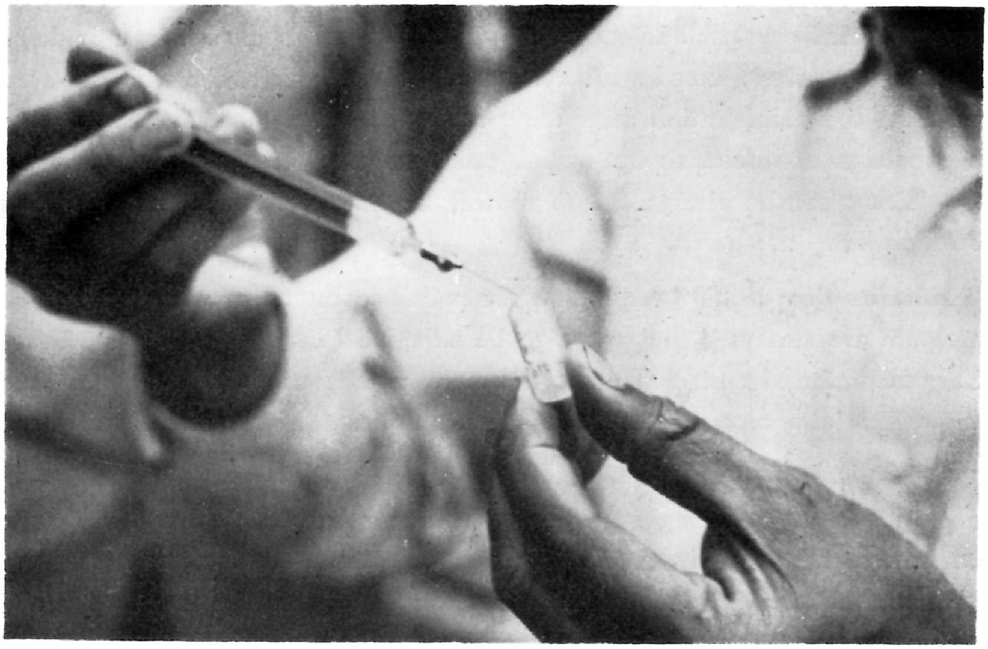

Figure 9. Addition of synahorin to the common carp pituitary suspension in normal saline

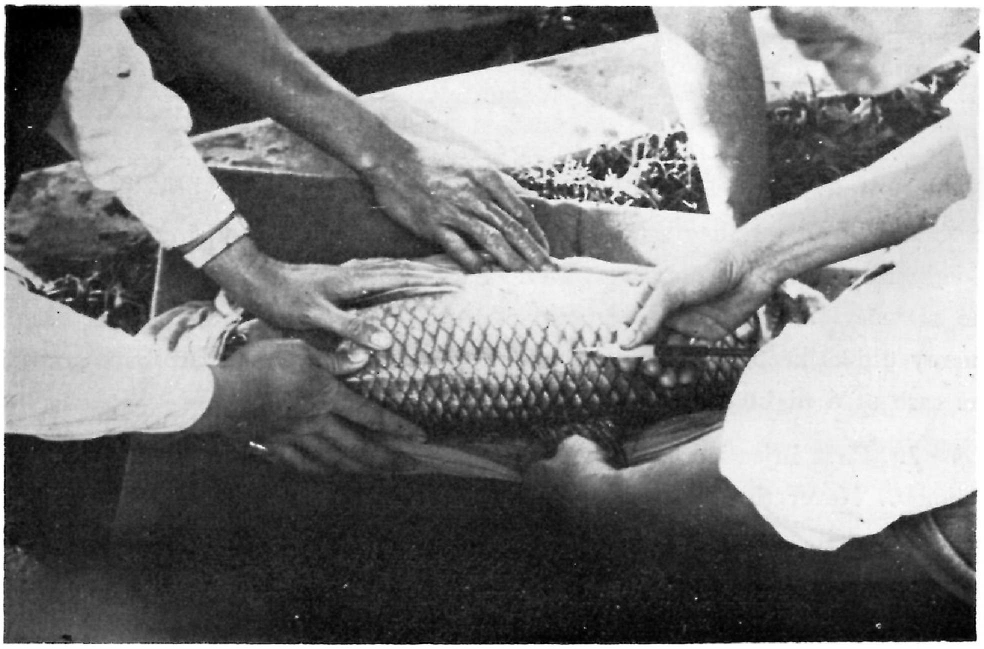

Figure 10. Injection given to a female grass carp of 5.2 kg.

is administered to the female exactly in the same manner as the first. The male spawner may receive an injection of similar dosage at this time if full milting is desired, but usually a male without artificial hormonation can supply sufficient free sperm to fertilize all the eggs from one female of more or less similar size.

5. In a typical case ovulation occurs 10 to 12 hours after the first injection at temperature 25°-28°C; 12 to 13 hours at 24°C and more than 14 hours at temperature below 22°C. So the whole process from first injection to ovulation takes place almost totally in day time. It is interesting to note that the increare of one degree above 22°C shortens the time for ovulation one hour for as in the case of grass carp, ovulation occurs 15 hours after the first injection at 24°C, it requires only 12 hours at 27°C.

6. But before actual ovulation, it should be observed that the female is always chased by the male for 15 to 30 minutes in the case of silver carp, and big head, and 30 minutes to one hour for the grass carp. So when courting begins the worker should watch closely and wait 15 minutes to one hour for the spawners to enter a state of ecstasy as being ready for ovulation and there at this exact moment the female is first removed for stripping and next the male. If stripping is done before this moment of ecstasy, ovulation is bound to fail. Alternatively some workers prefer to let the fish spawn naturally in the spawning pond.

If a night schedule is chosen, the spawners are netted at noon or early in the afternoon, first injection is carried out at 1700 to 1800 hour, second injection at 2300 to 2400, and ovulation may be expected early in the next morning between 0500 and 0800 or 0900 hours dependent on the temperature. The advantage of the night schedule is that hatching will take place in the day time when the water temperature is usually slightly higher, thus accelerating hatching and reducing mortality, whilst in the day schedule, it is convenient for the workers but as hatching takes place during the night a sudden change of temperature may cause high mortality of the eggs.

The dosage of carp pituitary is 2-3 mg dried weight per kg of the silver carp or big head spawners, but for grass carp the dosage should better be 3-4 mg/kg.

The effect by administration of synahorin, gonagen-forte or other kind of pharmaceutical product of chorionic gonadotropins prepared from mammal pituitary and extracts of placenta and pregnant mammal urea, alone is negative with the Chinese carps as indicated in all experiments known in Taiwan and Japan, but it proves to be positive as booster for the carp pituitary (table 4 and 7). It is observed that injection with carp pituitary plus synahorin results in ovulation almost in every case, whereas that with carp pituitary alone more often fails. Table 7 shows convincingly the effect of synahorin as a booster to carp pituitary in ovulation. All those four silver carp which received synahorin in the first and second injections ovulated eight hours after a third injection with carp pituitary alone which was given 15 hours after the first injection. This means a total of 23 hours from the first injection to the time of ovulation. The other two fish receiving no synahorin at all in the first and second injections failed completely to ovulate even by a third injection of carp pituitary plus synahorin, a failure interpretted to mean that synahorin requires time to prepare the way in the ovary for the action of the carp pituitary hormone and that a late administration of synahorin to a tired fish does not result in any successful ovulation.

On the basis of this finding the common practice in Taiwan now is to use a minimum dose of 40 rabbit units of synahorin in combination with fresh carp pituitary for any silver carp and big head spawners less than 4-5 kg and 60 to 100 rabbit units for fish larger than 5 kg. But for grass carp, the synahorin administration should be heavier for sharp result.

Two instances show precisely the significance of this technique. On 20 May, 1965 a female grass carp of 8 kg was given fresh pituitary of common carp weighing totally 3 kg plus 60 rabbit units of synahorin in the first injection and 3 kg common carp pituitary plus synahorin 40 rabbit units in the second injection. The result proved a success in ovulation. In the same day another grass carp female of 3.5 kg was treated with the fresh pituitary of a 2-kg common carp plus 20 units of synahorin in the first injection and the pituitary of a 900-gram carp plus 20 units of synahorin in the second injection. Satisfactory ovulation also resulted. Under similar treatments but double the synahorin dosage in the second injection ten (10) other grass carp females also ovulated successfully.

But a few days later, a female grass carp of 5.7 kg was given a first injection with a dose of fresh pituitary from 2.6 kg of common carp (7.8 mg) plus 20 rabbit units of synahorin and a second injection with similar dosage of pituitary and synahorin resulting in partial ovulation which occurred only when pressure was applied to the abdomen. Bleeding was found during stripping. Whether bleeding be responsible for the unsatisfactory ovulation could not be ascertained but the light dosage of synahorin appears to be a cause of difficult spawning.

Further in June, 1965 two grass carp (3.2 and 2.9 kg) were treated with fresh pituitary from common carp of equal weight plus each 60 rabbit uuits of synahorin allocating 20 ratbits units for the first injection and 40 units for the second.

The result was complete ovulation 14 hours after the first injection at temperature 24°C.

Dr. C. F. Hickling while visiting here in May, 1965 carried out an experiment in Chupei, Taiwan where he treated three silver carp females (1.5 to 1.6 kg) with lyophilized pituitary of *Perca fluviatilis* mixed with or without synahorin. The result was negative in an elapse of 15 hours after the first injection (table 6).

In another series of 3 silver carp females (1.5-1.8kg) using *Perca fluviatilis* pituitary pressrved in absolute alcohol, one female (1.82 kg) was injected with 6 mg per kg plus 40 units of synahorin, one (1.5 kg) with 4 mg per kg alone and the third fish with synahorin (40 units) alone. After an elapse of 15 hours no ovulation occurred at all (table 6).

The seventh silver carp (1.4 kg) was injected with fresh carp (1.4 kg) pituitary plus synahorin 40 rabbit units resulting in satisfactory ovulation 12 hours after the first injection. The water temperature was 25°C.

When Hickling's experiment was considered finished (15 hours after the first injection) each of the first 6 silver carp was injected wiht 1.5 ml of fresh pituitary suspension (equivalent to about 2 mg dried weight) from 4.5 kg of common carp and 20 rabbit units of synahorin were added to those fish which had not received synahorin before. The results are shown in table 7.

It is interesting to know that in 1965 as a result of the past three years experience and experimental knowledge the induced reproduction of hundreds of fully mature silver carp, big head, grass carp and mud carp by injection witn fresh common carp pituitary plus synahorin came out almost 100 per cent successful in Taiwan.

However it should be noted that successful ovulation can be obtained by induction almost invariably in every month from March to October (table 2-5) but the condition of the eggs, the rate of fertilization and hatching vary considerably with species and time.

The mud carp ovulates easily by induction and produces healthy eggs from June to September. The grass carp nevertheless is most delicate; not only female and male with healthy gonads are few, but also satisfactory spawning by induction resulting in high rate of fertilization and hatching appears to be confined only to the months of May, June and early July.

The silver carp and big head are hardier than grass carp, producing more uniformly high quality eggs and sperm in March to June, but the eggs begin to deteriorate in July becoming only slightly better again in early September.

While general observations of this nature need verification, investigation and further research it is likely that the deterioration of the eggs in July and August is attributed to (1) too must disturbance by fishing because it is generally known that fish, particularly the grass carp, stop feeding and development for two to a few days after a heavy shock from fishing and handling and (2) rhythmic change of gonads due to seasonal fluctuation in temperature and other conditions of water.

Table 2. Results of 1962 Experiments on Induced Reproduction of Chiness Carps in Taiwan by Fish Pituitary Injection alone (Tang *et al* 1963)

Month	Recipient wt. (kg)	Number of female treated	Wt. of donor (kg.)	Result: Success in ovulation
July	Grass carp (9)	1	Big head (2)	No
	Grass carp (15)	6	Common carp (25)	2 fish
	Silver carp (14)	7	Common carp (4)	4 fish
			Big head (24)	
	Big head (108)	16	Big head (114)	5 fish
August	Big head (15)	3	Big head (52)	No
Total:		33		11 or 33%

Table 3. Result of Induced Reproduction of Mud Carp by Hormone Injection (Lin, 1964)

Date	Donor	Recipient (mud carp)	Result
1962 July	Prehormone (500 units) plus toad pituitary	1 fish (1.0 kg)	Ovulation
1963 July	Common carp pituitary	3 fish (0.801.2 kg)	2 ovulation
Aug.	Common carp pituitary	21 fish	19 ovulation
Sept.	Common carp pituitary	20 fish	8 successes
Total:		45 fish	30 successes
Percentage of success			77%

Table 4. Results of Some 1963 Experiments on Induced Reproduction of Chinese Carp by Common Carp Pituitary and Synahorin Injection (Liu, 1964)

Month	Recipient wt (kg)	Number of ♀ treated	Donor wt (kg)	Synahorin (Rabbit units)	Result. (success in ovulation)
1963 July	Grass carp (2.8)	1	Common carp (2.8)	nil	Ovulation
	(3.0)	1	(1.5)	nil	No ovulation
	(2.0)	1	(2.2)	nil	Ovulation
	(2.8)	1	(1.8)	nil	No ovulation
	(3.1)	1	(3.0)	nil	No ovulation
	(4.0)	1	(3.4)	nil	Partial ovulation
	(2.8)	1	(2.6)	nil	No ovulation
	(3.6)	1	(3.6)	20	Ovulation
	(2.5)	1	(2.4)	40	Ovulation
	Silver carp (0.7)	1	nil	40	No ovulation
	(0.8)	1	(2.4)	Prehormone (1000 units)	Ovulation
	(0.8)	1	(3.0)	Synahorin 40	Ovulation
	(1.3)	1	(5.0)	40	Ovulation
	(1.2)	1	(2.5)	40	Ovulation
Total:		14			9 or 64%

Table 5. The Percentage of Success of Ovulation by Fresh Common Carp Pituitary plus Synahorin Injection in Chupei Working Station, 1964 (unpublished data)

Month	Silver carp		Big head		Grass carp	
	No. treated	Success	No. treated	Success	No. treated	Success
1964						
March	1	1				
April	8	8				
May	4	2	1	1		
June	2	1				
July	7	3	1	1		
August	2	2	2	1	3	1
September	13	8	4	3	5	1
October	5	2	1	0		
Total:	42	27	9	6	8	2
Percentage of success		64%		66%		25%

Table 6. Result of Experiments on the Effect of Injection with Pituitaries of *Perca fluviatilis*, and *Cyprinus carpio* and Synahorin in the Ovulation of Silver Carp (1965).

Treatment	Result
A. Lyophilized *Perca* pituitary	
1 fish—6 mg/kg+synahorin 40 Rabbit units	No ovulation
1 fish—4 mg/kg+synahorin 40 Rabbit units	No ovulation
1 fish—4 mg/kg only	No ovulation
B. Alcohol preserved *Perca* pituitary	
1 fish—6 mg/kg+synahorin 40 Rabbit units	No ovulation
1 fish—4 mg/kg only	No ovulation
1 fish—synahorin 40 Rabbit units only	No ovulation
C. Fresh common carp pituitary	
1 fish—fresh pituitary of equal wt. of carp +synahorin 40 Rabbit units	Ovulation 12 hours after first injection

Table 7. Result of Further Treatment of the Silver Carp of Previous Experiment as Shown in Table 6

Previous treatment	Further treatment	Result
A. Lyophilised pituitary		
1 fish—6 mg+synahorin	Carp pituitary only	Ovulation 8 hours after further treatment
1 fish—4 mg+synahorin	Carp pituitary only	Ovulation 8 hours after further treatment
1 fish—4 mg	Carp pituitary+ synahorin	No ovulation at all after further treatment
B. Alcohol pituitary		
1 fish—6 mg+synahorin	Carp pituitary only	Ovulation 8 hours later
1 fish—4 mg	Carp pituitary+ synahorin	No ovulation at all after further treatment
1 fish—synahorin	Carp pituitary only	Ovulation 8 hours later

Evolution of technique and problems

In reviewing literature it appears that the idea of artificial propagation of the important domesticated Chinese carps, apart from the common carp and gold fish which have been bred in captivity since time immemorial, started from the discovery of the natural spawning of the silver carp, big head and grass carp in Ah Kung Tien Reservoir in 1959. The intensive investigation of the population, reproduction and hatching of fry by Tang *et al* in 1960 led to the success of the induced spawning of the Chinese carps in 1962 (Tang 1963). Following this, many experiments were carried out by different fish culture stations and fish farmers with ever incresing percentage of success through techniqne improvemont. The Lin's experiment (Lin 1964) to induce mud carp reproduction by common carp pituitary injection alone with 77% success and Liu's introduction of synahorin (Liu, 1964) into the technique resulted in almost 100% success with silver and big head are remarkable. However there is not as yet any standardized method developed so far to insure 100% success with the grass carp probably due to the special ecological nature of the fish and insufficient care for the spawners.

Another drawback in the current technique employed in Taiwan is the high mortality of the spawners after induced spawning. This is probably due to the rough handling during and after injection, overdose of hormone, and no provision of recuperation ponds for the spent fish.

In term of technique improvement a lot of thinking and discussion are required. First of all the dosage of pituitary must be standardized, at least in units of dried weight, and it would be still better to extract the gonadotropins in pure crystal form from the common carp pituitary, tested, analysed and assayed to determine its biological units of potency and then to prepare them by pharmaceutical process for commercial distribution. Of course tedious work is further required to evaluate the effectiveness of such gonadotropin extracts in comparison with the fresh pituitary. As fish pituitary is very small it may be extremely difficult, if not impossible, to extract sufficient quantity of gonadotropin from it even for test, not to mention commercial use.

It is now well recognized that common carp pituitary is abundant and most potent for various Chinese carp injection, but there may be pituitary from other species of fish, which is equally or even more abundant in supply and higher in potency. Continuous experiments are needed along this line. Hickling's experiments in Chupei gave hint of the impotency of *Perca fluviatilis* pituitary for the Chinese carps, although fresh pituitary was not available for comparison at that time.

It is reported that the spermatozoa of the Chinese carps can live in normal saline for 20-35 minutes, but in freshwater for only 1 minute (Fukushima, 1965). Experiments should be carried out in this direction to determine how long the potency of the milt of male can be maintained if it is kept in normal saline under subzero temperature. Although it has been known that the spermatozoa of many warm water fish die when they are subject to tempenature below 5°C, some kind of experiment along this line is still worth while to be carried out. The result of these experiments may lead to the elimination of the inconvenience of providing the services of the male for milting following each injection by making available cold-stored milt for use all the time.

However, despite many snags and problems to be solved, induced reproduction of the Chinese carps has been commercialized since 1964 and in 1965 an estimate of more than 30 fish farms in Taiwan are established to propagate particularly grass carp, big head and mud carp by hormone induction, some of which are capable to handle several millions of fish fry. Though it seems too early to say, it would not be surprising to witness in Taiwan a production of Chinese carp fry in a quantity not only sufficient to meet the domestic demand but also with large surplus for export in the next few years. The demand of grass carp, silver carp, bis head and mud carp fingerlings for stocking ponds in Taiwan amounts to 20-30 millions a year.

Artifical Insemination

The dry method is adopted in Taiwan. It takes only a few seconds to complete the fertilization process during which the milt from the male mixed with the eggs

Figure 11. Washing away the sperm fluid, mucous and faeces from the fertilised eggs.

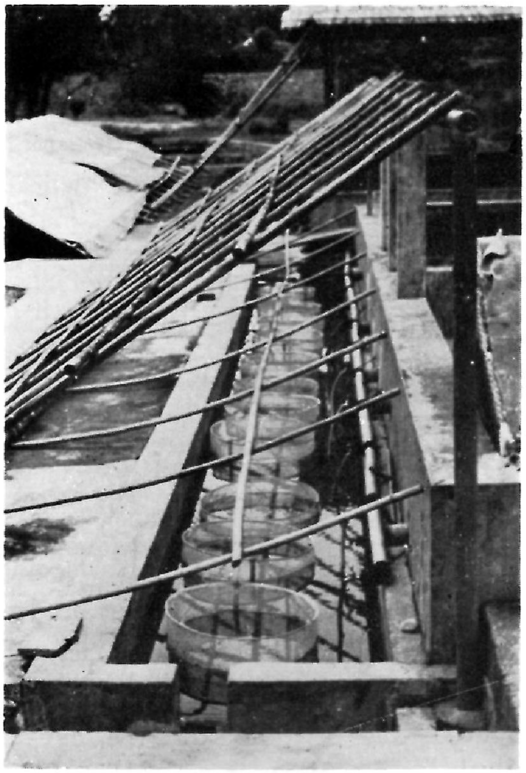

Figure 12. Hatching funnels hnng from poles are arranged in series, each funnel holding 15,000 to 50,000 eggs

with the help of a feather in an enamel vessel of about 25 cm in diameter. Some workers prefer to add normal saline for the reason that the spermatozoa live longer in normal saline solution, but many others omit this addition and still have satisfactory results. Clean water is then admitted into the vessel to wash away the sperm fluid, mucous and faeces from the digestive tract (figure 11). After washing the eggs are transferred to a box of nylon netting with meshes fine enough to retain the eggs in the container, which is suspended in slowly running water. An hour later when the eggs absorb water and swell almost to maximum size they are transferred again to the hatching funnels or baskets which will hold the eggs and the newly hatched larvae or fry for about three and half days (figures 4 and 12).

Hatching

Hatching takes 16 to 30 hours at water temperature 30°C down to 22°C, lower or higher than this range being unfavourable for hatching and survival of the fry. Constant temperature is highly desired for hatching and survival of the fry. Sudden change of temperature over 5°C would cause complete mortality of both eggs and fry. Optimum temperature for hatching and development of the larvae ranges from 24° to 30°C. Such temperature is sufficiently high to stimulate the embryo and newly hatched larvae to attain rapid growth so as to resist the attack of protozoa parasites and diseases.

Very little difference is found among grass carp, silver carp and big head as to duration of incubation and hatching (about 30 hours at 22°C and 24 hours at 25°C) and larval development (48 hours at 22°C and 34 hours at 25°C) the mud carp requires slightly less time (hatching 19 hours at 25°C and absorption of yolk sac 27 hours at 25°C).

The newly hatched larvae with yolk-sac are delicate creatures requiring no feeding and should be left to tranquility in the same hatching box for about two to three days dependent on temperature until the yolk-sac is completely absorbed. Water inflow in the box must be kept constant and in adequate volume to supply sufficient oxygen, but the current must never be too strong to hurt the delicate eggs and the newly hatched larvae which can only move occasionally up and down in shallow water. At temperature from 22° to 25°C the yolk-sac of the larvae will be absorbed in $1^1/_2$ to 3 days and the fry begin to swim horizontally in search of food. The stage during which the yolk sac is being absorbed is known here as larval development.

Rearing of the fry

There has not been much research work done in the question of proper feeding and care of the larvae and fry, and therefore no standardized method can be described. However as the Chinese farmers are fish-culture-minded and adept in making adjustment to special need according to personal experience, considerably high percentage of fry survival has been achieved, and it would not be out of place here to discuss some of the essential points in fish fry feeding.

Generally speaking the fry, three days after hatching, are held first in a rearing trough of fine-mesh nylon netting, 2m x 3m x 50cm or 1m x 5m x 50cm, suspended in a pond, and to each trough 50,000 to 100,000 fry are introduced (figure 1, *a-d*) The fry are then fed with a mixture of hard-boiled egg yolk and soybean milk or wheat flour strained through cloth. Meanwhile the pond water must be in a well advanced eutropic stage containing plenty of plankton. This is achieved by application of inorganic fertilizer, especially superphosphate, night soil, barn manure and some special kinds of land plant with juicy leaves which can easily decay in water. pH may range from 6.8 to 8.5 and dissolved oxygen at a minimum of 4 ppm. Such a nursery pond must be free from any kind of fish and as far as possible harmful insects, amphibians, snakes, turtles and mammals. In preparing such a pond for fish fry rearing, the pond bottom must be kept dry for at least a month, sterilized with quick lime (CaO) or tea seed cake before water is admitted and plankton cultivated.

When fish fry are reared in a nylon trough suspended in the pond, they feed actually on the nannoplankton and net plankton and the supplementary food supplied to them artificially. The quantity of artificial feed given to the fry is adjusted by daily observation of the fry and the colour of the water. Light brownish in the morning and greenish brown in the afternoon is most desirable and if the fry appear plump (full belly) and healthy, artificial feed is given at normal rate, but when the fry are in poor condition with large head and thin body, both the pond water and artificial feed must be readjusted and enriched.

After one week's rearing in this manner any healthy normal fry should reach 2-3 cm in length. At this stage they are released into the same pond in which the nylon trough has been suspended, at the rate of one to $1^{1}/_{2}$ million fry per hectare for 2 to 4 weeks rearing.

In many cases trough rearing is not necessary and the fry, after remaining in the hatching box for three days (with the complete absorption of the yolk-sac) are released into the nursery pond directly. Artificial feed is given in the usual manner but with less efficiency because of large area. This disadvantage is compensated however by the natural food available in the form of nannoplankton and net plankton.

Another kind of fry-feed consisting of yolk of egg, soybean milk, yeast powder and vitamin B complex is also employed, but there is no experimental data to show its nutritional value with respect to fry survival and growth.

Anyway, fish fry rearing is a very complicated matter. It concerns feeds and feeding, water quality, oxygen supply, light, temperature, enemies and diseases, handling, stocking rate and every minute thing in management. For this reason only through long experience, well-planned experiments and keen observations, the best result can be harvested.

Summary

1. The discovery of natural spawning of the silver carp, big head and grass carp in a reservoir of Taiwan led to intensive study of their life histories, population dynamics, hatching of eggs and rearing of fry by Tang in 1960.

2. The above study stimulated experiments on the induced reproduction by pituitary injection with success in 1962.

3. Many more experiments on pituitary injection were made in 1963 and 1964 with ever increasing percentage of success by fish culture stations and fish farmers through technique improvement.

4. The establishment of commercial hatcheries began in 1964 for artificial propagation of silver carp, big head, grass carp and mud carp by induction with

pituitary hormone and consequently in 1965 more than 30 such establishments of various scales sprang up.

5. A careful survey of the techniques and procedures employed by these establishments in Taiwan shows that the following dosages of fresh common carp pituitary boosted with or without synahorin give the best result in ovulation:

Recipient (kg.)	First injection		Second injection		Ovulation after first injection at 24°-25°C
	Live common carp (kg.)	Synahorin (Rabbit units)	Live common carp (kg.)	Synahorin (Rabbit units)	
Silvercarp (2—5)	1—2.5	20	1—2.5	20	12 hours
Big head (5)	2.5	20	2.5	20	13 hours
Grass carp (<5)	<2.5	20	<2.5	40	13 hours
Grass carp (>5)	>2.5	40	>2.5	40-60	13 hours
Mud carp (1—2)	0.5—1	20	0.5—1	20	10 hours
Mud carp (1—2)	0.5—1	0	0.5—1	0	12—14 hours

6. The problems concerning culture of spawners, hormone injection technique, fertilization, hatching and rearing of fry are still numerous.

7. It is suggested that experiments be first carried out by different fish culture research stations in order to determine:

   7.1—The association system of spawner culture in holding ponds and the effect of fishing on gonad regression or reabsorption of yolk and sperms if there is any.

   7.2—Rhythm of gonad development in relation to seasonal change of temperature and light.

   7.3—Feeds and feeding of grass carp spawners.

   7.4—Suitable area and depth of holding pond.

   7.5—Standard for spawner selection.

   7.6—Secondary sexual characters and maturity.

   7.7—Biological units of pituitary dosage.

   7.8—Feeds and feeding of fish fry.

   7.9—Diseases and parasites of spawners and fry and methods of control

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