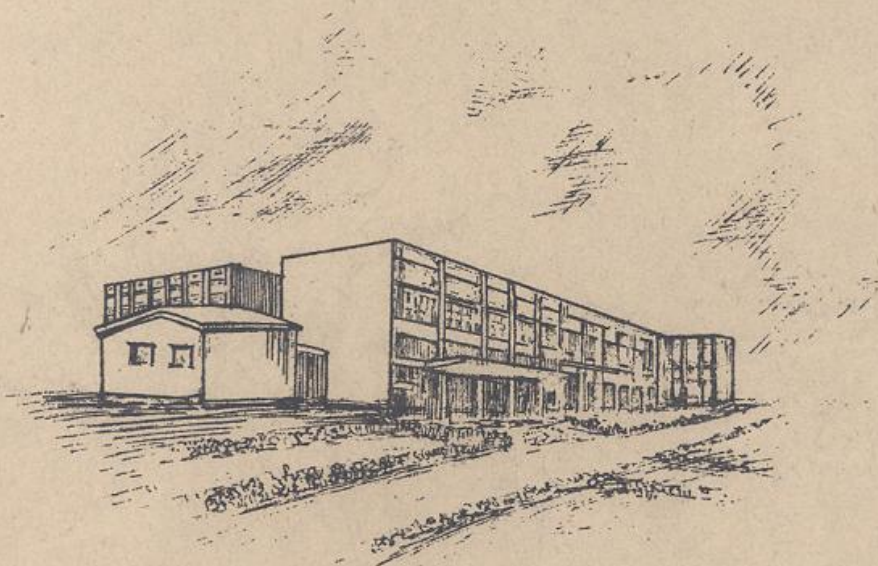


PRELIMINARY OBSERVATIONS ON COMMERCIAL BREEDING OF
INDIAN CARPS UNDER CONTROLLED TEMPERATURE
IN THE LABORATORY.

By

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INTRODUCTION

Successful breeding of Indian carps by injection of pituitary hormones has been reported since 1957 (Chaudhuri & Alikunhi '57; Alikunhi et al, 1960; Chaudhuri 1960; Vijayalakshmanan et al, 1961 and Chaudhuri et al 1961) and this method is now being adopted in various parts of the country for commercial production of quality fish seed for stocking. It has however, been found that fertilization and hatching of eggs are at a considerably low level when warm, sultry weather conditions prevail, with the water temperature exceeding 28-29°C. In several parts in the plains of India temperature of water in ponds ranges from 30 to 34°C during June-August when the carp breeders are gravid and ready to breed. Administering pituitary hormones under such conditions often results in ovulation, but in the majority of such cases the eggs are generally not fertilized.

Gravid breeders when injected breed successfully if weather conditions are favourable. If owing to unfavourable weather conditions gravid breeders are not injected they pass the prime stage of maturity, resorption of eggs starts in the ovaries and thereafter such specimens respond poorly to administration of hormones. Thus under field conditions, even with a large stock of good breeders, the number that could be handled during the season becomes limited because of the limited number of days with favourable weather conditions during the season.

In Assam and Punjab, fully gravid specimens of Catla, Rohu and Mrigal become available in ponds from the last week of May. This being the hottest part of the year and in the almost total absence of rains during May, conditions are quite unfavourable for successful breeding of these carps. By the time the monsoon sets in and water temperature comes down some of the early breeders would have passed the prime stage of maturity. This happens in other places also where the monsoon breaks during the first week of July but the fishes attain full maturity even by the middle of June. The obvious way to overcome the above difficulty is to control water temperature and administer pituitary hormone injections to breeders as and when they become fully gravid. Under field conditions however, this is hardly feasible and would be expensive as a commercial proposition. As Indian carps, after receiving pituitary hormone injections are found to breed successfully in small hapas and cisterns it was felt that they could be induced to breed in similar containers in the laboratory also.

The Director of Fisheries, Orissa got one of the laboratory rooms at the Directorate of Fisheries, Cuttack, air-conditioned and

requested the Central Inland Fisheries Research Sub-station to conduct experiments on inducing commercial breeding of Indian carps in that laboratory. Accordingly a series of experiments were carried out in this laboratory during July-August, 1962, using Rohu, Mrigal and Calbasu breeders and though in several cases there was ovulation the eggs were not fertilized and there was no production of spawn. A few experiments carried out early in July, 1963 were also failures. From the last week of July, 1963 further experiments were planned and by 6th September, 1963 a series of 18 experiments were successfully carried out in the laboratory producing about 22.18 lakhs of fertilized eggs of Catla, Rohu and Mrigal and about 8.54 lakhs of spawn therefrom. This being the first successful breeding of Indian major carps under controlled conditions in the laboratory irrespective of weather conditions prevailing outside the experiments are discussed this paper in some detail.

The authors wish to express their grateful thanks to Shri G.N. Mitra, Director of Fisheries, Cuttack for kindly making the air-conditioned laboratory room available to them for experiments and for his genuine interest in the work. Thanks are also due to Sarvaswari K.P. Biswas, R.B. Dey and P. Jena of the Directorate of Fisheries, Cuttack for ungrudging help in setting up the experiments and to our colleagues D.S. Murthy and B. Dutta for valuable assistance during the course of these experiments. We are deeply indebted to Dr. B.S. Bhimachar for his sustained interest in this work and for valuable suggestions in finalising the paper.

MATERIAL AND METHODS

One of the laboratory rooms was provided with an air cooler. A galvanised iron sheet tub, 9 ft x 4 ft, with gently sloping sides was made as the breeding cistern. The capacity of this cistern ranged from 1600 to 1900 litres at depths ranging from 54 cm to 64 cm. Though there is provision to supply tubewell water into this cistern it was not used, as in earlier experiments positive results were not obtained with this water. The cistern was thoroughly cleaned and freshly painted dry. A cloth hapa of exact dimensions as the cistern was fixed inside the latter so as to facilitate catching the breeders for injection and for taking out the eggs after breeding. Fresh pond water was pumped into the cistern immediately before introducing breeders. Gravid females and oozing males of Catla, Rohu and Mrigal were selected from stock ponds at the Killa farm and transported in tin carriers to the laboratory - a distance of about 2 miles. The first injection to the female was administered at the farm, just before transporting the breeders to the laboratory. The injected female and the uninjected male were together introduced into the cistern which was promptly covered by a close meshed netting

piece to prevent the fish from jumping out. The air cooler was started and within the next 3-4 hours when water temperature came down to about 28°C the cooler was stopped. Six hours after administering the first injection to the female, the breeders were taken out in hand-nets and both male as well as the female were given the second injection and were released back. Because of the small size of the cistern and the limited volume of water in it, the sex ratio of breeders introduced was ordinarily 1:1; generally matching the weight, though often the male weighed less than the female. When the female was not too big, two males were introduced. Once the water temperature came down to about 28°C and the cooler was stopped, there was generally no need to start it again as the temperature did not appreciably rise till the fish spawned and the eggs were taken out from the cistern. Fertilised developing eggs were taken out, measured in beakers for enumeration, percentage of fertilization determined by sample counts and were then transferred to hatching hapas fixed in near by ponds.

EXPERIMENTS CONDUCTED

During the period 26th July - 6th September, 1963, eighteen experiments with 14 controls were carried out, 2 with Catla, 14 with Rohu, 1 with Mrigal and 1 with Silver carp. Out of the fishes kept in the laboratory only one Catla and two Rohu did not spawn. All the other 15 fishes spawned. In 12 of these the eggs were fertilized and spawn was obtained for stocking in ponds. In 2 sets of Rohu though the eggs were fertilised, the development was abnormal with irregular gastrulation. The Silver carp also spawned and though early cleavage stages were seen in the eggs stripped and fertilized, no embryos were formed.

Details of the fishes injected and transferred to the laboratory, together with doses of injections administered and particulars of eggs laid are given in Table I.

In all these experiments, controls were kept by keeping comparable size breeders in hapas in the pond and administering to them identical doses of injections at the same time as the fish in the laboratory cistern. Though 9 out of 14 of these control fishes also spawned the results in terms of fertilisation and number of eggs or spawn obtained were generally very much poorer than in the case of fishes that spawned in the laboratory cistern. As breeding environments, the laboratory cisterns and the hapa in the pond are quite different from each other, the physico-chemical conditions of water that prevailed in these two situations during some of the experiments are given in Table II.

DISCUSSION

Doses of injections Administered and Spawning

As is shown in Table I, in most cases the female was given an initial dose of 2 mg/kg homoplastic gland, followed by a higher dose of 5 to 6 mg/kg, six hours after. Only in one case an initial high dose was tried. Ordinarily the freely oozing males were given only a single injection of 3 mg/kg at the time of the second injection to the female. When the male is not freely oozing an initial 1.0 mg/kg dose was administered at the same time as the first injection to the females. In most cases spawning commenced 4 to 6 hours after the second injection to the female. In a few cases the female started spawning, about 6 hours after receiving the initial low dose injection and before receiving the second injection. Such spawning took place in the presence of uninjected males which, however, responded and fertilised the eggs laid. The dose of injection administered, time of spawning, percentage of fertilisation, etc. in the experimental cistern and in the control hapa are summarised in Table III for easy comparison.

86% of the specimens injected and kept in the laboratory cistern spawned; while in the field (control) only 75% of them responded. Amongst these, eggs were fertilised in 90% of the spawning in the laboratory; while in the field only in 75% the eggs were fertilised. In the control as well as in the laboratory those specimens which showed positive response behaved similarly as no difference in the time of commencement of spawning after the second injection was evident.

The dose of hormones required for inducing successful spawning varied from a single low dose of 2 mg/kg to the female to two doses amounting to 2 to 3+5 to 6 mg/kg. In the former cases the males had not received any injection of hormones, but, in spite of that the laid eggs were duly fertilised. However, the response of these uninjected males was limited as spawning was only partial and the number of eggs laid and spawn obtained were usually very small. Alikunhi et al (1963) discussing induced spawning of the Chinese Silver carp and Grass carp in ponds have stated that an appreciable percentage of specimens of Rohu and Catla spawn as a result of a single injection at 2 mg/kg of pituitary hormones in the presence of injected as well as uninjected males. In those instances also spawning has been observed to be partial and the yield of spawn limited. Ordinarily the 2 mg/kg dose is not considered sufficient to induce spawning; and as a routine, 6 hours after receiving the above dose the females are disturbed for administering the second dose. This disturbance, just at the time when spawning had commenced, probably

accounts to some extent for the incomplete spawning mentioned above.

The dose of hormone administered, 7 to 9 mg/kg per female, is not markedly varying to permit any inferences on the quantity of hormone administered and the extent or nature of spawning, or fertilisation of eggs. The t a b l e IV summarises the position with particular reference to water temperature, pH, percentage of fertilisation and quantity of spawn obtained.

It would be seen from the table that at all the four different doses of injections tried all the specimens spawned when the temperature was controlled. In the control experiments however, only 75% of the specimens spawned.

While spawn production was appreciable from specimens that spawned in the laboratory, spawning in the majority of the controls was only partial and the bulk of the eggs soon disintegrated and disappeared. Soon after commencement of spawning fairly good percentage of developing eggs could be seen in the control hapas but at the time of taking them out after completion of spawning ordinarily very few eggs would be seen in the hapa and as such spawn production was extremely limited.

Though at the dose of 2.5 and 3 mg/kg respectively for the female and male spawning, fertilisation and spawn production appear to be better than at higher doses, it is premature to draw such a conclusion in view of the very small number of experiments conducted.

As hatching of the eggs was done in hapas fixed in ponds the hatching environment was entirely different from the spawning environment in the case of experimental fishes. The differences in water temperature of the order of 2-4°C during transfer of eggs from the laboratory to the hapa in the pond, could affect development and hatching. It could be seen from table I that except for two instances where hatching was over 80%, in most other cases of successful spawning in the laboratory, even when the fertilisation was over 80%, the hatching rate did not exceed 30%.

Shell hardening of eggs

In the first two experiments a gentle continuous spray of tube well water was provided in the cistern after the breeders were injected and released into it. In both these cases, though the spawning was complete and fertilisation excellent, the egg membrane was not properly water hardened and even at the time of collecting and measuring the eggs for keeping for hatching several were breaking

off. This also would partly account for the lower hatching rates observed.

Samples of eggs taken from the cistern within half an hour after commencement of spawning and kept in trays in fresh water, showed in most cases high percentage of fertilisation. Ordinarily, eggs were taken out from the spawning cistern only 3-4 hours after commencement of spawning. Samples taken at that time in several cases showed appreciably lower percentage of fertilisation than the earlier samples. For instance, in the Rohu that spawned in the laboratory on 29/30-7-1963 (vide Table I) at 3-30 hours 88% of the eggs appeared fertilised and developing, while the sample taken two hours after, at 5-30 hours, showed only 59% fertilisation.

Water conditions

A critical study of Table II shows that in the experimental cistern, except when the continuing spray was given, the water conditions such as temperature, pH and dissolved Oxygen show distinct decreasing trends, whereas free carbondioxide shows an increasing trend. In the control hapas when the experiments were taken up during day time, temperature, pH and dissolved oxygen showed the opposite trend, distinct and continuing rise from the time of first injection to the time of spawning. Free Carbon-dioxide recorded a decreasing trend in these controls. Prevailing water conditions are thus diametrically opposite in the experimental and in the control environments. It is interesting to record in this connection that spawning, fertilisation of eggs and production of spawn are markedly superior in the experimental cistern than in the control hapas.

Timing of injections and spawning

When the initial dose of injection was given in the evenings between 16.00 to 19.00 hours and the second injection at night, between 22.00 and 01.00 hours, spawning occurred during the morning hours, from 03.00 to 07.00 hours. In this case, water temperature in the pond will be steadily decreasing till the time of spawning. The timings are, however, inconvenient, since the fishes are to be injected late in the night, and keeping the eggs for hatching would be at a time when the water temperature is rising. A more convenient timing would be the initial injection at noon and the second injection late in the evening, so that spawning will be at night, after 22.00 hours. In this case, however, when the eggs are kept for hatching at about 10.00 hours, water temperature would be on the increasing trend.

Ordinarily, under field conditions breeders are not injected in the morning, as the water temperature would be steadily increasing

during day time and satisfactory spawning is not expected under such conditions. As the temperature of water in the laboratory could be controlled, injections were tried in the morning. Breeders were netted from the pond and selected in the afternoon and were carefully kept in hapas over-night in the pond. Early in the morning, by about 05.00 - 06.00 hours the female was given the first injection and the set soon after was transferred to the cistern. Water temperature was lowered to about 28°C and the second injection was administered at about 11.00 - 12.00 hours. The fishes spawned at about 15.00 - 16.00 hours and the eggs could be kept for hatching at about 19.00 - 20.00 hours. In this case when the hatching is to be done in ponds, the water temperature would be on the decreasing trend for the next 10-12 hours.

The majority of the experiments after 5th August, 1963 were carried out according to the above time schedule. A study of Table II would show that while the temperature of water in the cistern at the time of spawning ranged from 28.0°C to 29.0°C, in the control hapas in the pond, the range of temperature at spawning was 31.6°C to 34.6°C - a difference of 3.1°C to 5.6°C. The fact that majority of the control fishes also spawned in spite of the high water temperature probably indicates that while the correct dose of hormones might induce spawning irrespective of environmental conditions, fertilisation and hatching of eggs may not be satisfactory under such conditions.

Experiments carried out upto 5th August '63 were on a different time schedule - the injections being administered in the afternoon and at night. Table II shows that in these experiments, in the cistern as well as in the control hapas, water temperature, pH and dissolved oxygen showed a decreasing trend. Water temperature at the time of spawning was only 1.8 to 2.3°C higher in the controls than in the experimental cistern. It is significant to record here that spawn production from the control specimens was appreciable during this phase. In the subsequent experiments when injections were given in the morning and noon and water temperature was steadily increasing up to the time of spawning, rising to 2.8-5.5°C over the laboratory cistern, though several control specimens spawned, spawn production was practically nil. Thus by controlling water temperature it is possible to successfully breed the fish in the laboratory even during day time, which is ordinarily not feasible in the field.

Sex ratio

Ordinarily in induced breeding two males to one female are recommended in order to ensure high percentage of fertilisation of eggs. In the present case as the volume of water in the experimental

cistern is limited the number and weight of fish that could be successfully kept in the cistern for spawning had to be carefully ascertained. In the majority of the experiments therefore the injected female was mated with only one male which almost invariably was lower in weight than the female (Vide Table I.). It is interesting to observe that in the majority of these cases the percentage of fertilization of eggs was quite high, the highest being 96%. In the control hapa also when there was successful spawning, fertilization was high. When the male and female breeders are in good condition a single male is adequate to fertilize the eggs laid by a female and it would appear that ordinarily there is no need to introduce a second male with the pair.

Number and weight of breeders

In the cistern holding 1600 to 1900 litres of water all the three species of major carps Catla, Rohu and Mrigal were successfully bred. In the very first experiment on 26.7.1963 two female and two male Mrigals, together weighing 2.9 kg were successfully bred in the cistern. On 23.8.1963 eight small Rohu breeders (4 + 4) together weighing 5.9 kg were successfully bred in the cistern, producing about 5.7 lakhs of eggs. The maximum weight of breeders attempted amounted to 6.5 kg with the female weight 3.5 kg. It is thus seen that even in the limited volume of water in the cistern small and medium size specimens of all the three species of Indian major carps could be successfully bred. After spawning, however, when several lakhs of eggs are present in the cistern, the water conditions change rapidly (Vide Table II). On 20.8.1963 when a pair of Rohu, together weighing 3.15 kg spawned, the dissolved oxygen in water came down from 6.8 to 2.4 ppm in about 10 hours from the time of first injection to the time of spawning. During the same period carbon dioxide increased from 0.9 to 4.2 ppm. On 23.8.1963 when 4 sets of Rohu simultaneously spawned in the cistern, dissolved oxygen decreased from 4.8 to 3.6 ppm. Carbon dioxide increased from 2.6 to 5.2 ppm in the course of 10 hours when spawning commenced. With 5.7 lakhs of eggs spawned in the cistern oxygen fell rapidly to 1.0 ppm and carbon dioxide rose to 6.1 ppm in the course of the next 3 hours when the eggs were taken out for keeping for hatching. Similar fluctuations are recorded during other experiments also (Table II). In the control, however, the fluctuations in water conditions are quite different and not so marked or sharp as in the cistern.

The sudden, sharp fall in dissolved oxygen in the cistern necessitates removal of eggs when they are ordinarily in the morula or early gastrula stages. In the case of hapa in the pond, the eggs are removed to hatching, hapas only after the embryos are formed and have started movement inside the eggs membrane. It is to be

ascertained whether the earlier removal of eggs from the cistern would in any way affect the percentage of hatching.

Suggestions for Induced Breeding Under Controlled Temperature

Mid-May to end of August is the breeding season for Indian carps in different parts of the country. Rainfall is scanty and temperature high in most places during May-June; while, during July-August, even though rainfall is generally heavy and temperature lower, these are widely fluctuating. Cool, rainy days, with relatively low temperature are preferred for successful spawning of fish. Such optimum conditions become available for only very few days during May-August and this drastically curtails the scope for induced breeding of fish even when an adequate stock of excellent breeders are available. Dependable weather conditions have therefore to be provided to ensure enhanced production of seed by induced breeding. When such conditions are provided breeding could be attempted everyday of the season if adequate stock of breeders is provided.

Experiments carried out under controlled temperature during 1963 have shown that while over 80% of the fishes spawned, hatching of the developing eggs under field conditions was not as satisfactory. It is therefore necessary, when spawning and production of millions of developing eggs are ensured, to provide for satisfactory hatching of these eggs, if necessary under controlled temperature, at least during that phase of the season when temperature is high and conditions of summer stagnation are predominant. The laboratory should therefore be equipped for breeding the fish and hatching the eggs so that production from the laboratory would be spawn ready for stocking in the nursery pond.

As the techniques of breeding the fish and hatching eggs are quite different from each other, it would be preferable to have two separate rooms, one for breeding and one for hatching. Both the rooms should be air-conditioned so that temperature of water in the rooms could be controlled. While the size of the rooms would depend on the targets the following details may be helpful.

Breeding Laboratory (Fig.I)

Inside dimensions of the room may be 20'x16'. This can accommodate three cement cisterns, each 10'x5'x3', leaving about 1.5' space between them. Inlet pipes with control valves should be so provided that the cisterns could be filled with water from an overhead supply source. Outlets may lead to a common drain, built in the floor and leading outside. Brass rings provided at bottom and top

corners would facilitate fixing hapas in the cisterns. Net covers fixed on to wooden frames would keep the cisterns covered when the fishes are introduced in them.

With 3 cisterns operating, 3 sets of fish could be kept for breeding at a time in the laboratory. For successful spawning a set of fish will remain in the cistern only for about 12 hours. It is therefore possible to breed three sets of fish a day in the laboratory room. Allowing for all possible difficulties and inconveniences, if 20 experiments could be conducted in a month, 60 sets of fish would be handled. If the female breeders average 2 kg in weight, with 80% spawning and 70% fertilisation of eggs, the production capacity of the laboratory would be about 140 lakhs of fertilized eggs a month. In a season of about 70 days the total production of fertilised developing eggs from the laboratory should exceed 320 lakhs.

In order to get about 130 sets of good breeders there should be a stock of at least 400 breeders. Depending on the stock of breeders available and the targets of production, the size of the laboratory room can be conveniently decided.

Hatching Room (Figs 2 & 3)

Carp eggs are demersal and settle down in still water. In the present traditional methods of hatching of carp eggs in India, developing eggs are kept in a round-meshed mosquito netting hapa, fixed inside a larger markin cloth hapa. The hapa may be in a pond or river. In the former case the eggs settle down at the bottom of the inner hapa; in the latter they may crowd at one end of the hapa, because of current. Hatching results depend on the water conditions and the percentage, of fertilization (or the number of dead eggs present in the lot). In a 5'x2.5x1.5' inner hapa, fixed inside a 6'x3'x3' outer hapa in a pond, upto 1 lakh of eggs could be kept and if the fertilisation is over 80%, successful hatching, ranging from 70-80% may be expected.

For successful hatching of a large number of eggs in a limited space the eggs should be continuously kept in gentle motion, without allowing them to settle down at the bottom. This can be done in specially devised hatching trays arranged in series of batteries and provided with running water from an overhead source as are being used for successful hatching of the eggs of the Chinese Silver carp and Grass carp in Japan (Suzuki et al, 1958). Each tray is about 1.5' square at the open top, gently sloping towards the bottom and about 1.5' in height. On to a fairly strong soft wood frame round mesh mosquito netting is fixed. The bottom, provided with a central metal ring, fixed on to the four corners by metal rods is closed by netting cloth.

The tray fits inside a wooden box with a false bottom. The metal ring of the tray fits over a round hole in the false bottom. Water from the overhead tank falls into a narrow side compartment, fills the false bottom, rushes up through the hole into the tray and on filling it, over-flows into the side compartment of the false bottom of the adjoining tray. A series of 3-4 trays can thus be arranged. To direct the current straight up from the bottom a metal cylinder may be kept inside the tray against the metal ring.

By the flow of this water, the eggs are kept in constant motion. Upto 50,000 eggs can be kept in a tray for hatching. When the eggs hatch the hatchlings will pass out through the mesh and can be collected in a suitably devised floating tray in the cistern at the end of each battery of trays (Fig.3).

In a laboratory room 20'x16', a convenient arrangement of hatching trays would be as shown in Fig.3. The trays can be arranged on concrete frames, about 2 ft from ground level. Pipes from overhead tank should be arranged to supply each battery of trays. With adequate water supply, the set of 44 trays would be able to handle over 20 lakhs of eggs at a time. If a batch of eggs are kept in the trays for hatching, they would be removed only about 24 hours after, when all the eggs are hatched. The hatchlings, collected in trays in the cisterns would have to be kept for another two days when they would be ready for stocking in nursery ponds. When the hatchlings from a batch of eggs are being kept in the cisterns, the hatching trays can be reset for fresh batches of eggs.

The source of water for the hatching trays should be dependable and to provide continuous supply an automatic electric pump would be required to feed the overhead tank.

Location of the laboratory is a very important consideration. As transport of the gravid breeders from the pond is to be avoided as much as possible it is essential that the laboratory rooms are located within the farm premises where stock of breeders is available. Water supply to the breeding cisterns as well as hatching trays may be from a large natural pond, into which the water will flow back after circulation.

S U M M A R Y.

The first successful experiments in inducing the major Indian carps, Catla, Rohu and Mrigal to breed in the laboratory by administering pituitary hormone injections and controlling water temperature, carried out at Cuttack during July-September, 1963 are reported in detail.

In a rectangular metal container of 1,600 - 1,900 litres capacity large females weighing upto 3.7 kg could be induced to breed in still water.

Sex ratio was generally kept 1:1 and the maximum weight of fish kept for breeding was 6.9 kg.

More than one female could be simultaneously bred in the cistern as was demonstrated in a few experiments.

With single male, percentage of fertilisation of eggs ranged as high as 96 in certain cases.

86% of the specimens injected and kept in the laboratory spawned; while, in the cisterns only 75% spawned.

Spawn production from the laboratory bred fish was far superior to the controls.

In 18 experiments conducted in the laboratory 22 lakhs of fertilised developing eggs were obtained. Hatched in hapas in ponds over 8 lakhs of spawn were obtained out of these for stocking. Corresponding to this in 14 controls the spawn production was only just over 3 lakhs.

Fishes injected early in the morning and at noon spawned at about 4 p.m.

Water temperature in the laboratory was maintained at about 28°C while in the controls, spawning temperature was 3.1-5.6°C higher.

Water conditions prevailing in the cisterns and in the control hapas during various phases of spawning have been studied and discussed in detail.

It is now demonstrated that if the laboratory room is air-conditioned and the water temperature controlled, fish could be successfully bred every day of the season without depending on weather conditions outside.

Detailed suggestions for setting up breeding and hatching laboratories with controlled temperature are included.

REFERENCES

- Alikunhi, K.H., Vijayalakshmanan, M.A. & Ibrahim, K.H. (1960) Preliminary observations on the spawning of Indian carps, induced by injection of pituitary hormones. Indian J. Fish., 7(1): 1-19.
- Alikunhi, K.H., Sukumaran, K.K. & Parameswaran, S. (1963) Induced spawning of the Chinese carps Ctenopharyngodon idellus (C & V) and Hypophthalmichthys molitrix (C & V) in ponds at Cuttack, India, Curr. Sci. March, 1963.
- _____ (1963) Induced spawning of the Chinese Grass carp Ctenopharyngodon idellus (C & V) and the Silver carp Hypophthalmichthys molitrix in pond at Cuttack, India. Proc. I.P.T.C. 10th Session, Second.
- Chaudhuri, H. (1960) Experiments on induced spawning of Indian carps with pituitary injections. Ind. Jour. Fish. 7(1): 20-49.
- Chaudhuri, H. & Alikunhi, K.H. (1957) Observations on the spawning of Indian Carps by hormone injection. Curr. Sci. 26(12): 381-82.
- Chaudhuri, et. al. (1961) Induced breeding of carps during 1960 season at Cuttack. Proc. 40th Ind. Sci. Congr. Cuttack.
- Suzuki et al. (1958) Study on the artificial rearing of Grass carp and Silver carp (in Japanese) Saitamaken Suisen Shiken jo Gyoma Hokoku, No.9.
- Vijayalakshmanan, M.A. et. al. (1961) Induced breeding of carps during 1959 season at Cuttack. Proc. 40th Ind. Sci. Congr., Cuttack.

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Table I. Details of fishes injected and eggs and spawn obtained by breeding major carps in the laboratory under controlled temperature.

Expt No.	Date	Species injected	Size of Breeders Sex Length (mm)	Weight (kg)	Donor species of glands	Time and Dose of		Water Temp. (°C) (at introduction 2nd injection & spawning)	Time of spawning (hrs/date)	No. of eggs laid.	Percentage of Fertn. eggs.	No. of developing eggs.	No. of spawn obtained			
						First injection Time (hrs)	Second injection Time (hrs)									
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
1.	26.7.63	C.mrigala	F	415	0.90	C.mrigala	16.00	2.0	22.00	5.0	30.0-	02.30/ 27.7.63	1,75,000	90	1,57,500	86,000
			F	414	0.90		2.0	5.0	29.2-							
			M	387	0.65	nil	nil	3.0	28.2							
			M	393	0.65	nil	nil	3.0								
2.	29.7.63	L.rohita	F	460	1.40	L.rohita	16.30	2.0	22.30	5.0	30.0-	03.00/ 30.7.63	2,96,000	83.0	2,45,680	64,500
			M	449	1.25		nil	3.0	28.6- 28.0							
3.	30.7.63	C.catla	F	519	2.40	C.catla	18.30	2.0	00.30	5.0	30.5-	06.30/ 31.7.63	Eggs were stripped; and mixed with milt. Fertilized	1,000	30,000	
			M	432	1.55		1.0	31.7.63	2.0	27.9- 28.0						
4.	31.7.63	C.catla	F	503	2.00	C.catla	19.00	2.0	24.00	5.0	30.5-	Fish did not spawn even after a third injection.				
			M	473	1.50		1.0	2.0	28.0- 28.3							
5.	5.8.63	L.rohita	F	491	1.90	L.rohita	16.50	2.0	22.50	5.0	30.5-	03.10/ 6.8.63	1,53,660	84.0	1,29,074	32,500
			M	472	1.40		nil	3.0	29.6- 28.5							
6.	8.8.63	L.rohita	F	510	1.50	L.rohita	06.45	2.0	12.45	5.0	30.9-	18.00	3,09,502	96	2,97,120	64,500
			M	451	1.20		nil	3.0	29.0							
7.	17.8.63	L.rohita	F	474	1.50	L.rohita	05.15	2.0	11.15	6.0	28.0-	15.30	3,75,000	96.0	3,60,000	3,54,750
			M	469	1.30		nil	3.0	28.0							

contd.....

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
8.	20.8.63	L.rohita	F	478	1.75			2.0	11.30	6.0	30.0- 28.0-	15.30	4.30,000	30.0	1.29,000	32.0
9.	21.9.63	H.molitorix	F	463	1.25	H.molitorix	19.30	3.0	00.30	7.0	30.1-	07.00/	Eggs were not fertilised.			
			M	521	1.45			nil	22.8.63	4.0	29.3-	22.8.63	Artificial fertilization also			
			M	381	0.55			nil		4.0	28.4		did not succeed.			
10.	28.8.63	L.rohita	F	390	3.20	L.rohita	05.30	2.0	11.30	6.0	30.1-28.5	16.15	5.70,680	65	3.70,942	3.0
			F	397				2.0		6.0	29.9					
			F	405				2.0		6.0						
			F	393				2.0		6.0						
			M	405						3.0						
			M	395						3.0						
			M	378				nil		3.0						
			M	386						3.0						
11.	25.8.63	L.rohita	F	607	3.50	L.rohita	05.30	2.0	11.30	6.0	31.3-	15.30	1.71,000	Development appeared		
			M	600	3.00			nil		3.0	29.5			abnormal.		
											29.0			51,300	50	
12.	27.8.63	L.rohita	F	505	1.60	L.rohita		3.0		6.0	30.5-27.9	15.45	5.56,875	40.0	2.22,750	1.38
			F	473	1.50		05.30	3.0	11.30	6.0	28.0					
			M	463	1.10			nil		3.0						
			M	432	0.90			nil		3.0						
			M	433	1.00			nil		3.0						
13.	28.8.63	L.rohita	F	501	1.70	L.rohita	05.15	2.0	11.30	6.0	30.1-28.5	16.30	2.50,500	90.0	2.25,450	32.5
			M	513	1.50			nil		3.0	28.5					
14.	29.8.63	L.rohita	F	497	1.95	L.rohita	05.15	2.0			29.8-27.6					
			M	474	1.25			nil			28.5					
			M	464	1.20			nil								
												11.15				
15.	29.8.63	L.rohita	F	497	1.95			6.0	07.00	3.0	30.1-28.5					
			M	474	1.35		21.50	4.0	30.8.63	nil						
			M	467	1.30			4.0		nil						

Fish did not spawn, even after the second infection.

Started spawning before second injection. Males were injected and female stripped an hour after. Eggs fertilized.

Eggs fertilized low. Eggs not for hatch.

(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
L.rohita	F	466	1.50	L.rohita	05.20	2.0			30.7-29.7	11.30	Started spawning before second injection, stripped, eggs fertilized. Low			
	M	449	1.35			nil					Eggs kept for hatching.			
	M	432	1.15			nil								
L.rohita	F		1.40	L.rohita	19.00	2.0	01.00	6.0	31.3-29.0	07.00	2,96,000	Spawning partial. Laid eggs fertilized; development abnormal.		
	M		1.40	L.rohita	19.00	2.0	3.9.63	2.0	29.0			Eggs not kept for hatching.		
	M		1.00			nil		4.0						
L.rohita	F		1.10	L.rohita	19.30	2.0	02.00/	6.0	29.4-26.6		Did not spawn.			
	M		1.00			nil	6.9.63	2.0	26.5					

F = Female

M = Male

Table II.

Physico-Chemical conditions of water in the experimental cistern in the laboratory and in the control pond and details of fish introduced during induced breeding experiments at controlled temperature.

Date	Water conditions at	Temperature (°C)		pH		Dissolved Oxygen (ppm)		Free CO ₂ (ppm)		Weight and No. of fish introduced (kg) E	No. of eggs Laid (lakhs)
		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)		
26.7.63	First injection	30.0	31.7	7.5	8.2	3.2	10.4	3.1	Nil	2.9/4	1.75
	Second injection	28.2	30.4	7.4	8.4	4.4	8.2	3.7	Nil		
	Spawning	28.2	30.0	7.2	7.6	4.8	5.2	3.5	1.0		
29.7.63	First injection	30.0	30.2	7.3	7.9	6.4	10.4	4.0	Trace	2.65/2	2.96
	Second injection	28.6	30.9	7.4	7.7	5.2	5.2	3.5	2.6		
	Spawning	28.0	30.2	7.4	7.5	5.2	5.1	4.0	2.6		
30.7.63	First injection	30.5	31.1	7.5	8.4	6.4	8.8	2.6	Nil	3.95/2	-
	Second injection	27.8	30.1	7.3	7.5	4.9	6.0	4.4	1.3		
	Spawning	26.0	29.8	7.4	8.4	4.8	5.2	6.1	1.8		
31.7.63	First injection	30.0	34.1	7.7	7.9	4.6	14.0	4.4	Nil	3.50/2	-
	Second injection	29.6	31.1	7.5	8.4	5.8	8.0	3.5	Nil		
	Spawning	29.6	30.5	7.4	7.5	5.6	5.2	3.5	1.8		
20.8.63	First injection	29.0	31.6	7.7	7.4	6.8	5.2	0.9	3.5	3.15/2	4.3
	Second injection	28.0	32.5	7.8	8.2	4.4	6.4	1.8	0.4		
	Spawning	28.0		7.6		2.4		4.2			
23.8.63	First injection	30.1	30.4	7.9	7.4	4.8	4.4	2.6	5.2	5.90/8	5.7
	Second injection	28.5	34.8	7.6	7.9	3.9	8.0	2.4	0.9		
	Spawning	28.8	34.6	7.5	8.2	3.6	9.6	5.2	Nil		
	Spawn lifting	29.0	33.2	7.4	8.1	1.0	9.2	6.1	0.6		

contd.....

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
25.9.63	First injection	31.3	31.5	7.8	7.4	6.0	4.5	3.5	2.7	6.50/2	1.71
	Second injection	29.5	32.4	7.4	7.7	3.6	7.0	4.4	1.8		
	Spawning	29.0	32.1	7.5	7.8	2.4	7.8	6.1	0.9		
	Spawn lifting	29.3	32.6	7.5	7.9	1.4	9.2	6.4	1.4		
27.8.63	First injection	30.5	30.8	7.5	7.5	4.4	4.8	2.6	2.6	6.10/5	5.56
	Second injection	27.9	32.4	7.6	7.6	4.8	6.4	4.4	1.8		
	Spawning	28.0	32.2	7.3	7.8	2.4	8.0	6.1	0.9		
	Spawn lifting	28.0	32.0	7.3	7.8	1.6	7.2	7.0	2.6		
28.9.63	First injection	30.1	30.4	7.6	7.3	5.6	4.4	1.8	4.4	3.20/2	2.50
	Second injection	28.5	31.8	7.5	7.5	4.4	6.8	2.6	1.4		
	Spawning	28.5	31.6	7.7	7.6	4.4	7.2	3.1	1.4		
	Spawn lifting	28.5	31.3	7.7	7.6	2.8	6.8	4.1	3.5		
29.8.63	First injection	29.8	29.8	7.6	7.0	5.2	4.0	3.5	4.4	4.4/3	-
	Second injection	27.6	32.3	7.4	7.2	3.6	7.2	3.5	1.3		
	Spawning	28.5	34.0	7.3	8.2	1.8	9.2	3.5	N11		
2.9.63	First injection	31.3	-	7.4	-	6.8	-	6.2	-	3.8/3	2.96
	Second injection	29.0	-	7.3	-	3.9	-	7.0	-		
	Spawning	29.0	-	7.1	-	2.8	-	6.1	-		

C = Control hapa

E = Experimental cistern

Table III

-: 19 :-

Response to different doses of pituitary hormones administered to major carp breeders in Experimental cistern in the laboratory and in control hapas in ponds.

	<u>L.rohita</u>		<u>C.catla</u>		<u>C.mritala</u>	
	<u>Control</u>	<u>Laboratory</u>	<u>Control</u>	<u>Laboratory</u>	<u>Control</u>	<u>Laboratory</u>
No.of sets of breeders injected.	14	18	1	2	1	2
No.of sets spawned.	11	16	-	1	1	2
No.of sets in which eggs were fertilised.	8	14	-	1	1	2
No.of sets from which spawn obtained.	2*	14	-	1	1	2
No.of sets spawned after one low dose injection - 2mg/kg/female	1	2	-	-	-	-
No.of sets in which eggs were fertilised by uninjected males.	1	2	-	-	-	-
No.of sets spawned after two injections -						
F. 2+5 mg/kg; M. 3 mg/kg		3			1	2
F. 2+6 mg/kg; M. 3 mg/kg		7				
F. 2+5 mg/kg; M. 1+2 mg/kg				1		
F. 2+6 mg/kg; M. 4 mg/kg		1				
F. 2+6 mg/kg; M. 2+2 mg/kg		1				
F. 3+6 mg/kg; M. 3 mg/kg		2				
Interval between second injection and spawning (in hrs.)	4-6	4-6	-	6	4.5	4.5

* In others very few eggs remained and so were not kept for hatching.

Table IV

Dose of hormones administered, spawning and fertilisation of eggs in relation to prevailing water conditions in the laboratory and in control ponds.

Dose of Pti. glands administered	Avg. weight of female breeders (kg)		No. of Expts. conducted		Water conditions				No. Spawmed			No. in which eggs were fertili- sed.			Percentage of fertilisation.			Average No. of developing eggs obtained			
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	
F 2+5 mg/kg	1.1	1.26	2	5	31.7	30.0	8.2	7.5	2	5	2	5	2	5	94	83-96	1, 65, 500	1, 65, 600			
M 3 mg/kg					30.0	28.0	7.6	7.2								(85.6)					
F 2+6 mg/kg	1.71	1.41	5	7	30.4	30.5	7.3	7.9	3	7	3	7	3	7	-	30-96	very few	1, 43, 956			
M 3 mg/kg					34.6	28.0	8.2	7.3								(68)					
F 2+6 mg/kg	1.75	1.75	4	1	31.6	29.0	7.4	7.7	3	1	2	1	2	1	low	30	nil	1, 29, 000			
M 4 mg/kg					34.0	28.0	8.2	7.6													
F 3+6 mg/kg	1.5	1.55	1	2	30.8	30.5	7.5	7.5	1	2	1	2	1	2	-	40	nil	1, 11, 400			
M 3 mg/kg					32.2	23.0	7.8	7.3													

C = Control pond

E = Experimental cistern

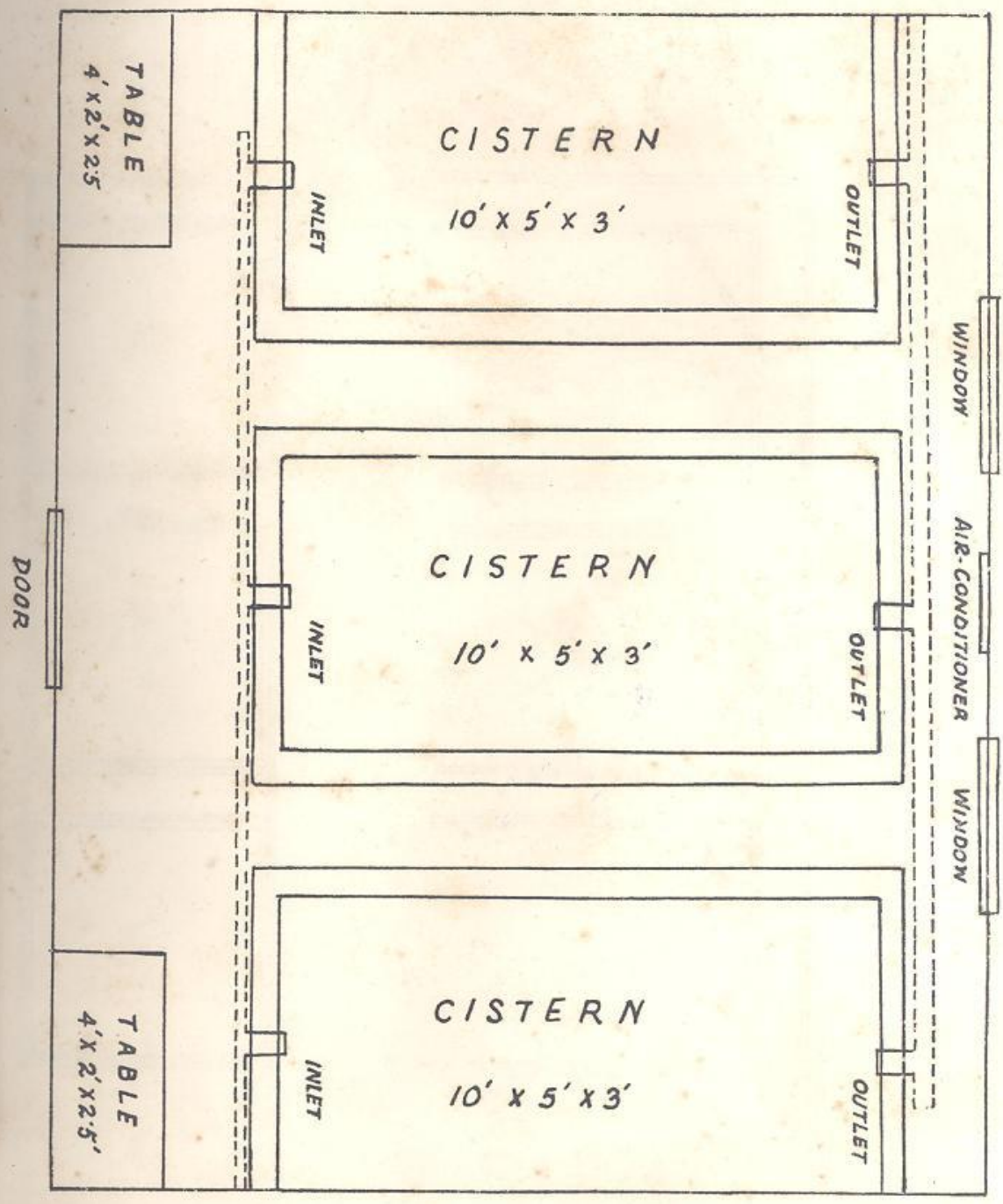


FIG. 1 AIR-CONDITIONED FISH BREEDING ROOM
 (1" = 2'5')

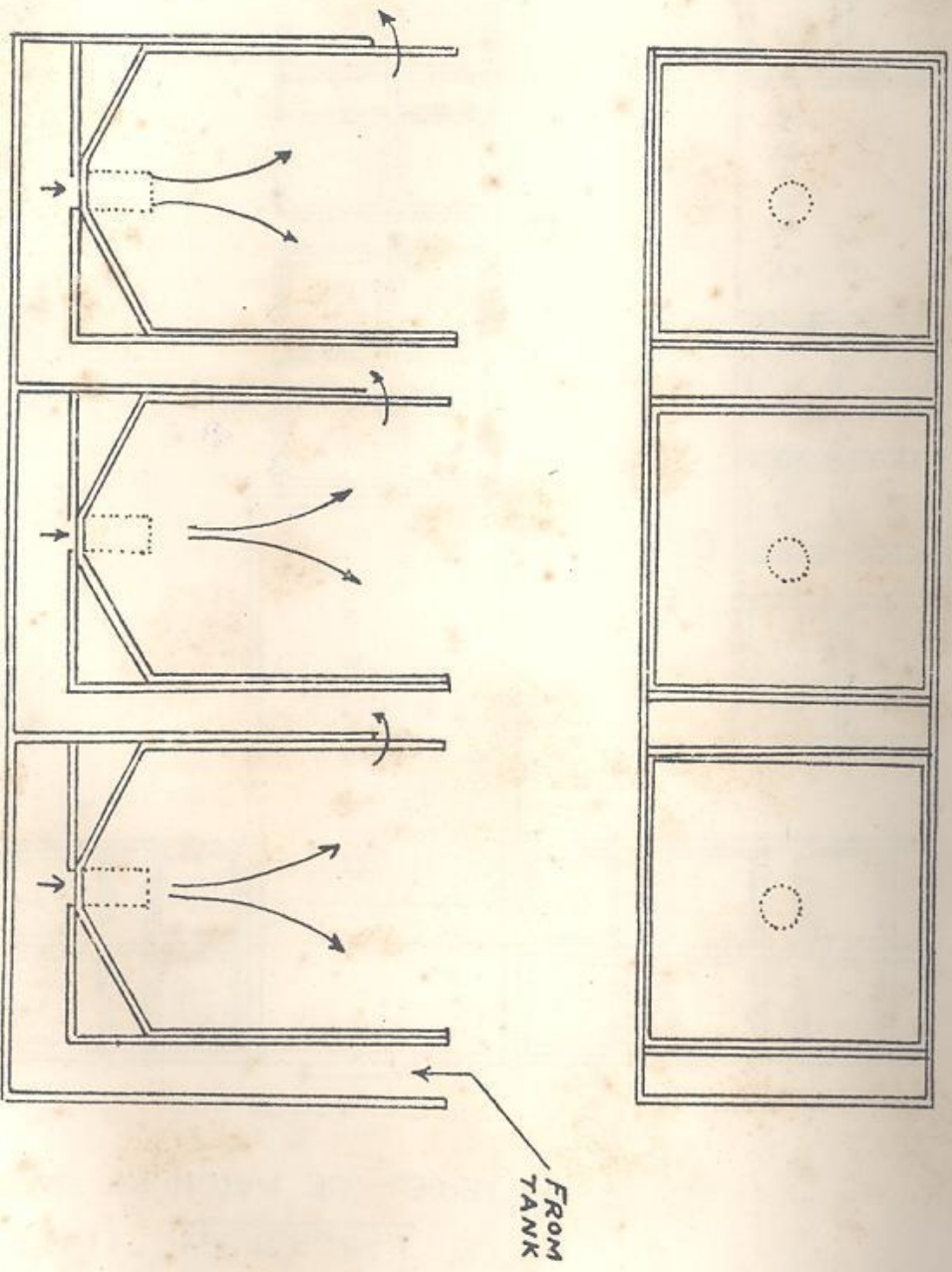


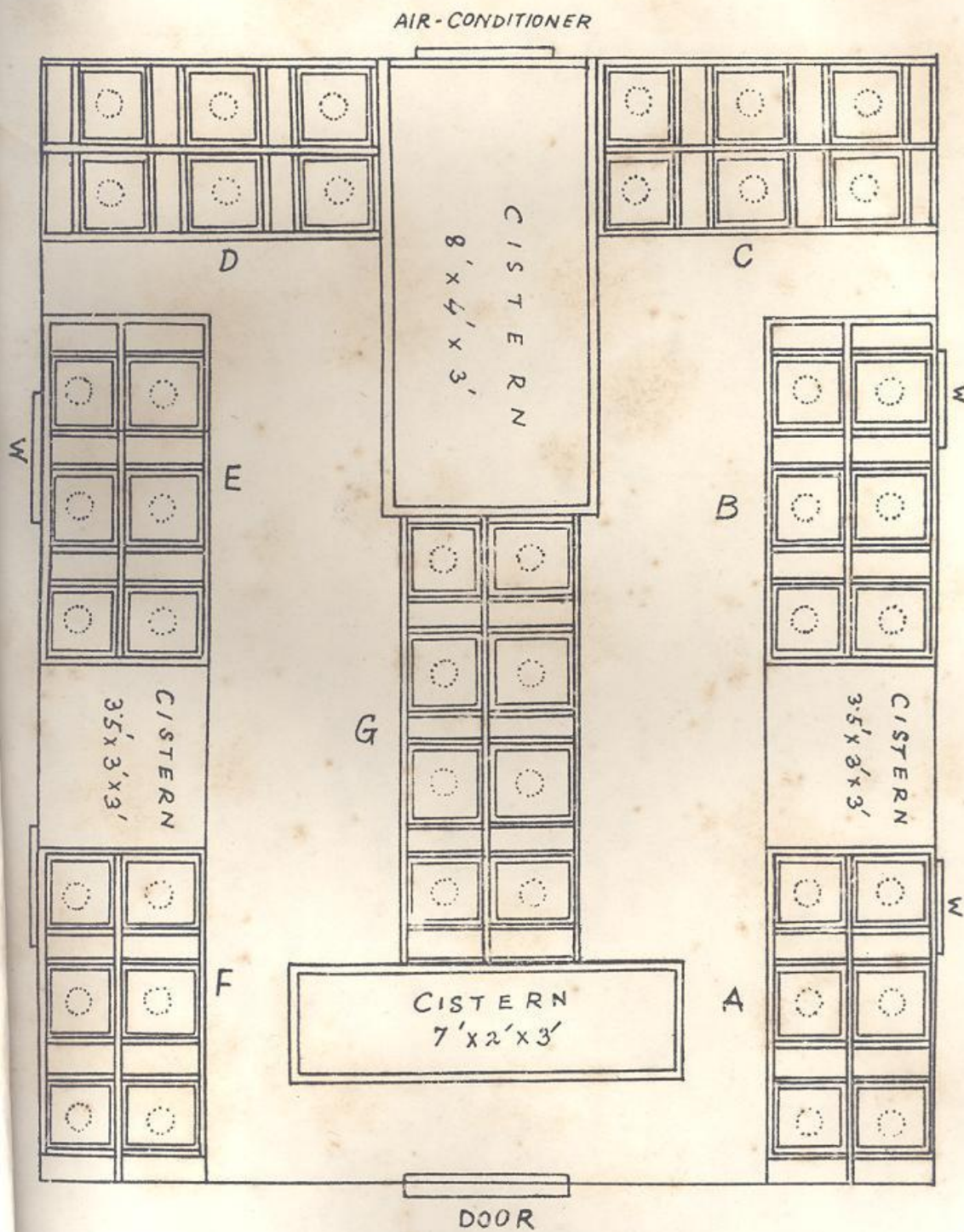
FIG. 2 HATCHING TRAYS FOR CARP EGGS

(AFTER Suzuki et al.)

FIG. 3 AIR-CONDITIONED CARP EGGS HATCHING

LABORATORY (1"=2'5')

A-G. BATTERIES OF HATCHING TRAYS.



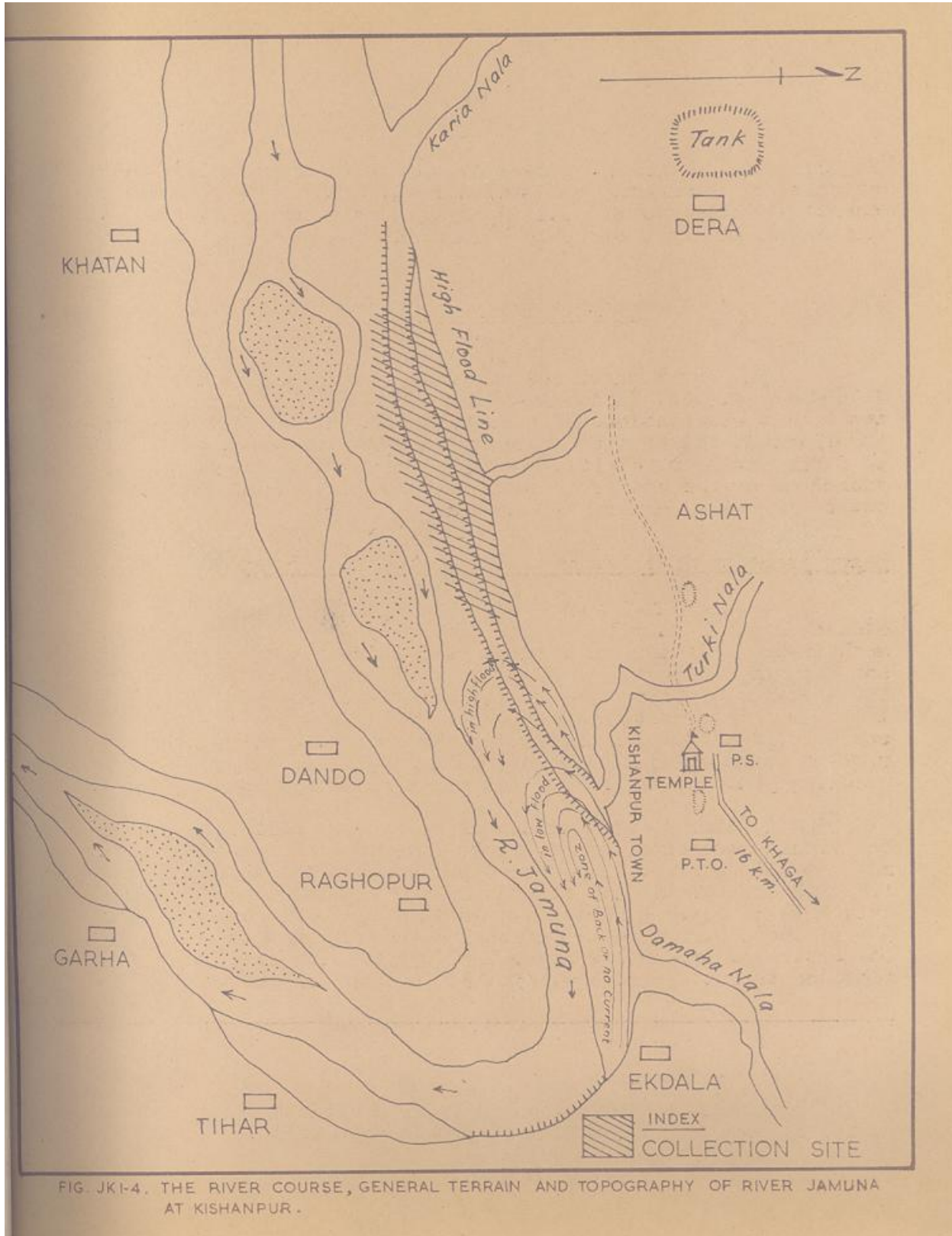


FIG. JK1-4. THE RIVER COURSE, GENERAL TERRAIN AND TOPOGRAPHY OF RIVER JAMUNA AT KISHANPUR.