EXPERIMENTAL USE OF CARP PITUITARY IN THE PRODUCTION OF FISH

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EXPERIMENTAL USE OF CARP PITUITARY IN THE PRODUCTION OF FISH

By

EDWARD HARLAND BACON

A THESIS

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INTRODUCTION

A serious shortage of bait minnows exists in Michigan and is becoming more critical as the number of anglers on lakes and streams increases. The main sources of bait minnows at the present time are the natural creeks, ponds, and lakes where they are collected by individual and commercial bait dealers and sold to the anglers. This supply is often short of the demand, especially during the summer months when the fishing intensity is the greatest.

To alleviate this situation, the Fish Division of the Michigan Department of Conservation has undertaken to propagate desirable bait minnows artificially, with varying degrees of success. Minnows that are used for bait, such as the creek chub (Semotilus atromaculatus), fathead minnow (Pimephales promelas), golden shiner (Notemigonus chrysoleucas), redbelly dace (Chrosomus eos), and brassy minnow (Hybognathus hankinsoni), have been reared in ponds under experimental conditions to determine possibilities of pond culture of these species by commercial bait dealers. As the younger fish of the white sucker (Catostomus commersonii) furnish a desirable "minnow," adult fish have been collected at spawning time, held until ripe, then stripped and the fertilized eggs

placed in hatching jars to continue development. Eggs of the sucker have also been collected from the spawning grounds.

Since the northern creek chub is one of the most desirable bait species, the Fish Division undertook a study of methods of propagation of fry for distribution to bait dealers. Washburn's method (1945) of propagating the creek chub has received the greatest attention to date. Raceways were constructed for propagation of this species at Almena and Drayton Plains State Fish Hatcheries and the fry obtained from these distributed to bait propagators.

Carp pituitary has been used to induce spawning in certain fish (Hassler, Meyer, and Field, 1939, 1940) and it was believed that if injections of the hormone would be effective on the creek chub it would be possible to bring all fish into a ripe condition at one time. This would greatly facilitate production since the eggs could all be handled at one time, rather than over an extended period, thus effecting a considerable saving of time and effort. Mortality of the eggs would be lessened considerably and as a result production increased. By inducing the fish to spawn earlier, it would be possible to deliver the fry to the bait dealers earlier in the season and thus lengthen the growing season in the pond phase of their propagation.

To test the practical potentialities of carp hormone injections in bait fish production, laboratory and field experiments were planned and carried out during the spring and summer of 1950, using the facilities of Michigan State College and the Wolf Lake Fish Hatchery of the Michigan Department of Conservation.

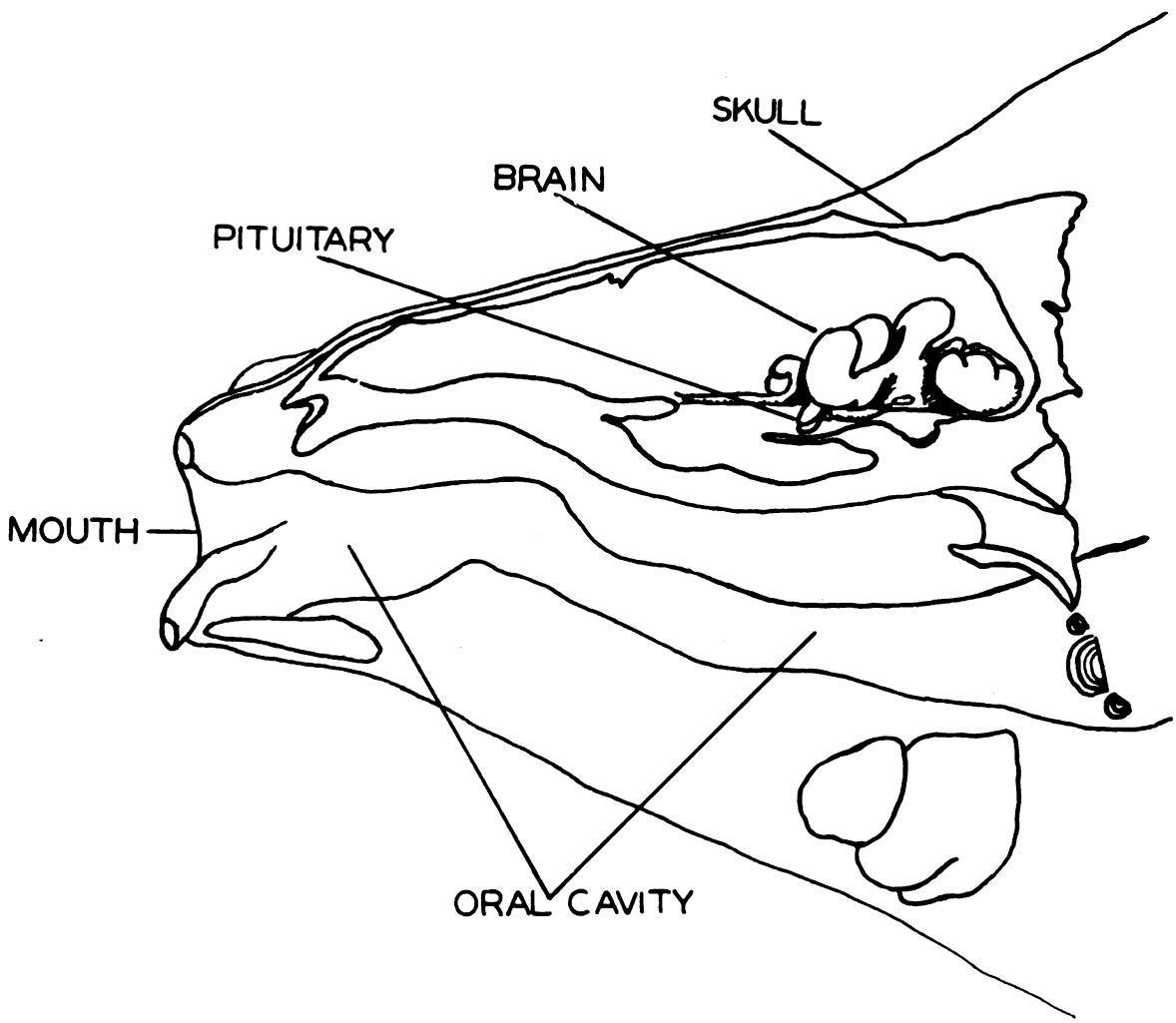
ANATOMY AND PHYSIOLOGY OF PITUITARY GLAND

Harold M. Evans, in his book "The Brain and Body of the Fish" (1940), describes the pituitary body of the fish as a small body (Plate I) resting just in front of the lobi inferiores and attached to the ventral walls of the infundibulum either directly or by a stalk. This gland consists of two lobes, anterior and posterior; entirely different both in structure and development. The posterior lobe is developed as a hollow downgrowth of the part of the embryonic brain which afterwards becomes the third ventricle. The posterior lobe consists of two parts, closely related, the pars intermedia and pars nervosa. In comparison with a mammalian gland, the pars nervosa takes a very small share in the make-up. The anterior lobe is developed as a tubular prolongation from the epiblast of the lining epithelium of the cavity of the mouth with which it is originally in connection.

Relationship between the gonads and the anterior pituitary is based on actual observation. The secretions of the ovary are dependent on the pituitary and occur in cyclic fashion. These functions of the gonads are not automatic, for removal of the pituitary in immature animals prevents their appearance, and in mature animals leads to their arrest. Implantation of anterior pituitary

PLATE I SAGITTAL SECTION OF CARP HEAD

SHOWING RELATIONSHIP OF PITUITARY TO BRAIN AND SKULL



substance, or the injection of suitable extracts before puberty, causes precocious sexual maturity.

A noticeable enlargement of the anterior pituitary has been observed in the eel about to migrate. The source of the gonadotropic hormones is in doubt, but the anterior pituitary is increased in nearly all species during pregnancy. Evan's observations, which extend over a period of two years, led him to believe that the enlargement of the anterior pituitary in the eel is due to a great increase of acidophile cells.

Evans subscribes to the belief that the immediate stimulus for a breeding migration, especially in the eel, came from the periodic increase of the internal secretions, elaborated by the gonads and that the impulse to migrate is induced by changes in the functional activity of certain organs included in the reproductive system. By assuming that the anterior pituitary hormone is responsible at different times for stimulation of growth and reproduction, its physiological effect is explained by the transfer from one function to the other in the process of individual life. In common with a great variety of other animals, and especially of fish, the development of the reproductive glands and cells in the eel is due to a switching off of energy which was previously engaged in those metabolic processes which result in growth.

EARLY INVESTIGATIONS

Injections of pituitary hormones, extracted from mammalian glands, have proved valueless in inducing premature spawning in fish. Only an unphysiologically high dose can produce an effect in the eel as pointed out by Van Oordt and Bretschneider (1941). Secondary sex characteristics, such as change in shape of body and size of cloaca, were brought about in the western brook lamprey (Lampetra planeri) with injections of anterior pituitary extracts of the ox by Young and Bellerby (1935).

The pars intermedia lobe of the mammalian pituitary manufactures a hormone (Harrow, 1946) which can be recognized by its effect on the pigment cells of the skin of the lower invertebrates. The injections of this hormone extract into a minnow (Phomixus laevis) causes the development of red color at the point of attachment of the thoracic, abdominal, and anal fins. Beyond its exerting an influence on the chromatophores of cold-blooded animals, the significance of this hormone, called intermedian, is not clear.

Biological supply houses offer an extract of the posterior lobe of the mammalian pituitary which, when injected into redbelly dace in small doses, stimulates the minnow to produce an intense pigmentation.

Suspensions of carp pituitary have been used by Hassler, Meyer, and Field (1939, 1940) to induce premature spawning in trout and in the propagation of muskellunge. Their results closely resembled the findings of von Iherings and De Azevedo (1935, 1937) who caused releases of eggs in some Brazilian fishes upon injections of pituitary glands of fish of the same species. Hassler and Meyer (1942) noted spawning behavior in goldfish nine months before normal breeding season following injections of carp pituitary.

METHODS AND EQUIPMENT

Source of Pituitary Glands

Adult carp (Cyprinus carpio) in breeding condition, and ranging in weight from four to twenty pounds, were obtained for this experiment from Saginaw Bay and inland lakes of southwestern Michigan during the latter part of March and early April. The fish were received alive at the fisheries laboratory at Michigan State College and kept in holding tanks for a short interval prior to removal of the pituitary gland.

It is important that the carp be in breeding condition for best results. Investigators in the field of endocrinology have shown that the pituitary gland is very active during this period and therefore more "potent."

Dissection of Carp

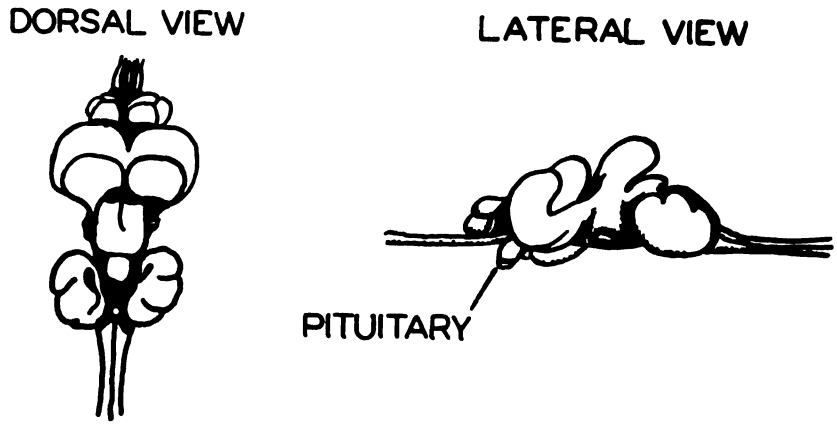
During the process of obtaining the glands it was found advisable to have the carp inactive before cutting off their heads. This was best accomplished by having the carp out of the water for at least twenty minutes prior to processing.

The head is removed from the body by cutting behind the pectoral fins and through the internal organs, then breaking the vertebral column at the base of the head by bending the head back towards the tail. The head, cut surface down, is placed on a wooded base and the top of the brain case is removed with a heavy knife, care being taken not to cut the brain proper. The oily globular fluid surrounding the brain is removed to facilitate locating the pituitary. With tweezers, the broad white ophthalmic nerve attached to the front of the brain (Plate II, top view) is picked up and by it, the whole brain lifted up and ~~layed~~ laid back, in an upside-down position. The pituitary gland may or may not stay attached to the brain. The gland is usually removed attached to the brain, but should it remain on the floor of the brain case (Plate II, lower view) it is advisable to remove the remaining fluids carefully so as to expose the gland. The pituitary, a small organ resembling an acorn (Plate III) on the ventral surface of the brain approximately underneath the largest rounded portion of the brain, is removed from this position by inserting the tips of the tweezers well below the gland, rather than trying to remove it by taking a hold of the gland proper, for the posterior lobe is readily detached from the anterior lobe.

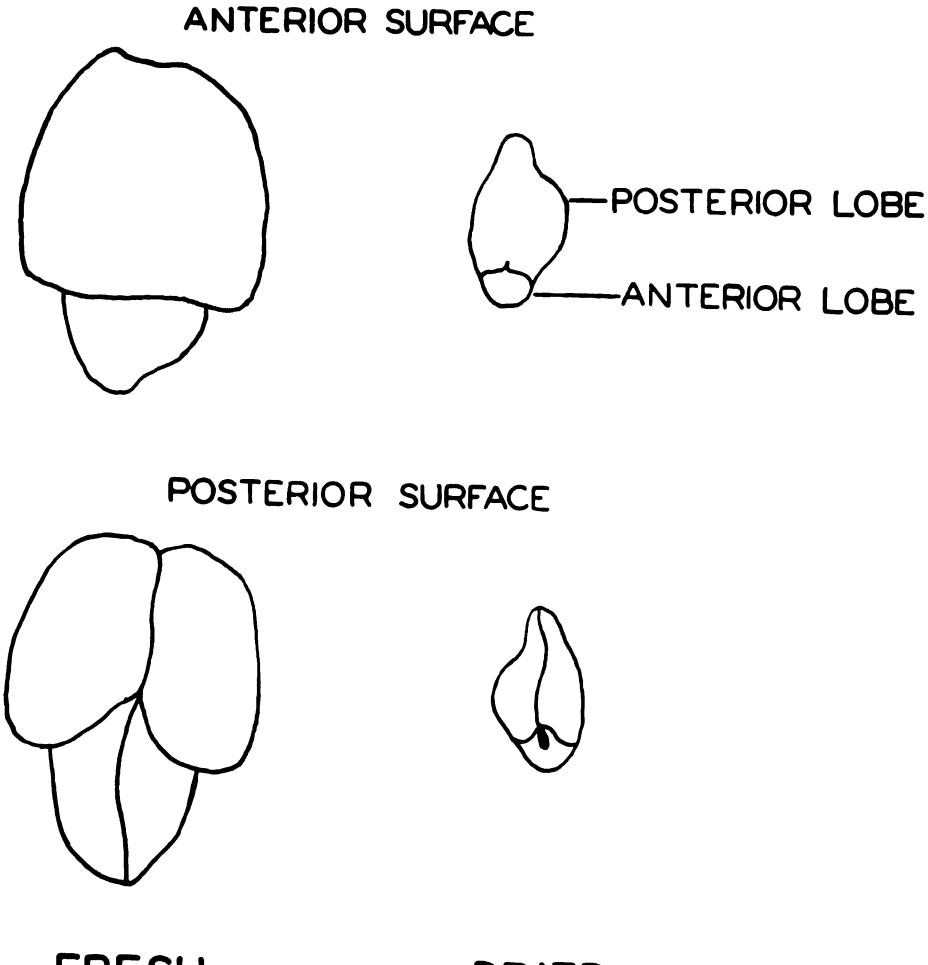
Plate II. Dissection of carp head to obtain pituitary gland. Top view: Brain case removed and brain exposed. Bottom view: Brain removed with pituitary gland remaining on floor of brain case.

Plate III. Carp brain and pituitary gland.

CARP BRAIN



CARP PITUITARY



Treatment of Glands

Pituitaries were frozen or placed in acetone upon removal from the brain. Those placed in acetone were left for three days, during which time they were kept in a refrigerator at approximately 40° F. They were then removed from the liquid, placed on a paper towel and allowed to dry at room temperature, 72° F., for 24 hours, following which they were placed in a sterile vial and returned to the refrigerator until needed. Acetone acts as a dehydrating and defatting agent and the glands shrink considerably during the treatment.

Pituitaries were put into suspension immediately prior to injections and the whole gland was utilized, with no attempt being made to segregate the two lobes. Acetone-dried pituitaries were pulverized in a mortar and the resulting powder mixed with distilled water. Frozen pituitaries were thawed as needed, and macerated to form a thick suspension in as little distilled water as possible.

The ease with which the acetone-dried glands were prepared for injections made them ideal to handle. There is no apparent difference between dried or frozen gland injections as far as the reactions of the fish are concerned.

LIFE HISTORY AND PROPAGATION OF BAIT FISH

Creek Chub

The creek chub, one of the important bait minnows, is sold as crappie and perch bait when small, as bass bait when larger, and used as a decoy minnow when reaching maximum size of eight to twelve inches. Chubs are commonly found in creeks in north, south, and central parts of the United States where it spawns in moving water during April, May and June. During the spawning season the male develops horny tubercles (Plate IV) on the dorsal surface of the head. The male prepares the nest, a depression in the gravel, with the excavated stones placed on the upstream side of the depression. The eggs are deposited when the water temperatures reach 55° F. The female mates with the male and drops her eggs in the depression after which the male proceeds to cover them up with gravel from the downstream side of the hollow. The female does not drop all of her eggs at once and as each mating session is accomplished the nest is lengthened. Completed nests are approximately eight inches wide and two to six feet long, built with the long axis paralleling the current and are found at the base of pools or at the head of riffles. They may contain an

Plate IV. Male creek chub in breeding condition.
Note "horns" on head.

average of 2,500 eggs per foot. During the active period of spawning the male guards the nest.

In the raceways, free swimming fry appear approximately twenty days after spawning. In the hatchery the hatching period is 10 days, 6½ hours at a mean temperature of 55° F. The yolk sac is absorbed and the fry are ready to take food 16 days later. Young fish make an excellent growth in the first year, reaching, in Michigan, a length of 3½ inches by September. Depending on the rate of growth, creek chubs may reach maturity as early as the spring of their second growing season, or as late as the fifth growing season. Males grow faster, become larger, and mature at a later age than females.

Washburn's method (1945) of propagation of creek chubs, with several modifications, is used by the Fish Division of the Michigan Department of Conservation to produce fry to distribute to bait dealers.

Basic plan consists of an artificial raceway, 5 feet wide and 300 feet long, filled with screened gravel, that empties into a pool. The gravel is of one-quarter to three-quarter inch size, free from sand and other fine material, and at least four inches deep. Refuge zones are installed at intervals along the raceway as well as splash boards. The flow of water, which must be clear, should have a velocity of at least 1-1/4 feet per second and be from four to six inches deep. Covers are placed

over the refuge pits and the whole raceway is covered with screen or netting (Plate V) to protect the spawning fish from predaceous birds.

The brood stock is admitted when the water temperatures have risen sufficiently to induce spawning activity. Then one of two procedures can be followed: either the brood stock is allowed to spawn and then removed from the raceway to allow natural incubation to occur, or they are allowed continued access to the raceway and the eggs gathered as the nests are made. After the nests are made the eggs are collected by screening (Plate VI). The screen is a triangular-shaped scoop with copper screening on all but two sides. This screen is placed open side up at the lowest or downstream end of each nest which is then gently torn apart so that the current can carry the eggs into the screen. These eggs are transferred to hatching jars (Plate VII) and held until development reaches the eyed-egg stage.

The eggs are removed from the hatching jar at the eyed-stage and placed in hatching screens since the sac-fry are too heavy to swim over the overflow of the hatching jar. The fry are ready for distribution four days after reaching the sac-fry stage and can be shipped a considerable distance without appreciable loss.

A certain amount of debris which cannot be separated from screened eggs is always present and may be sufficient

Plate VI. Screening raceways for creek chub eggs.

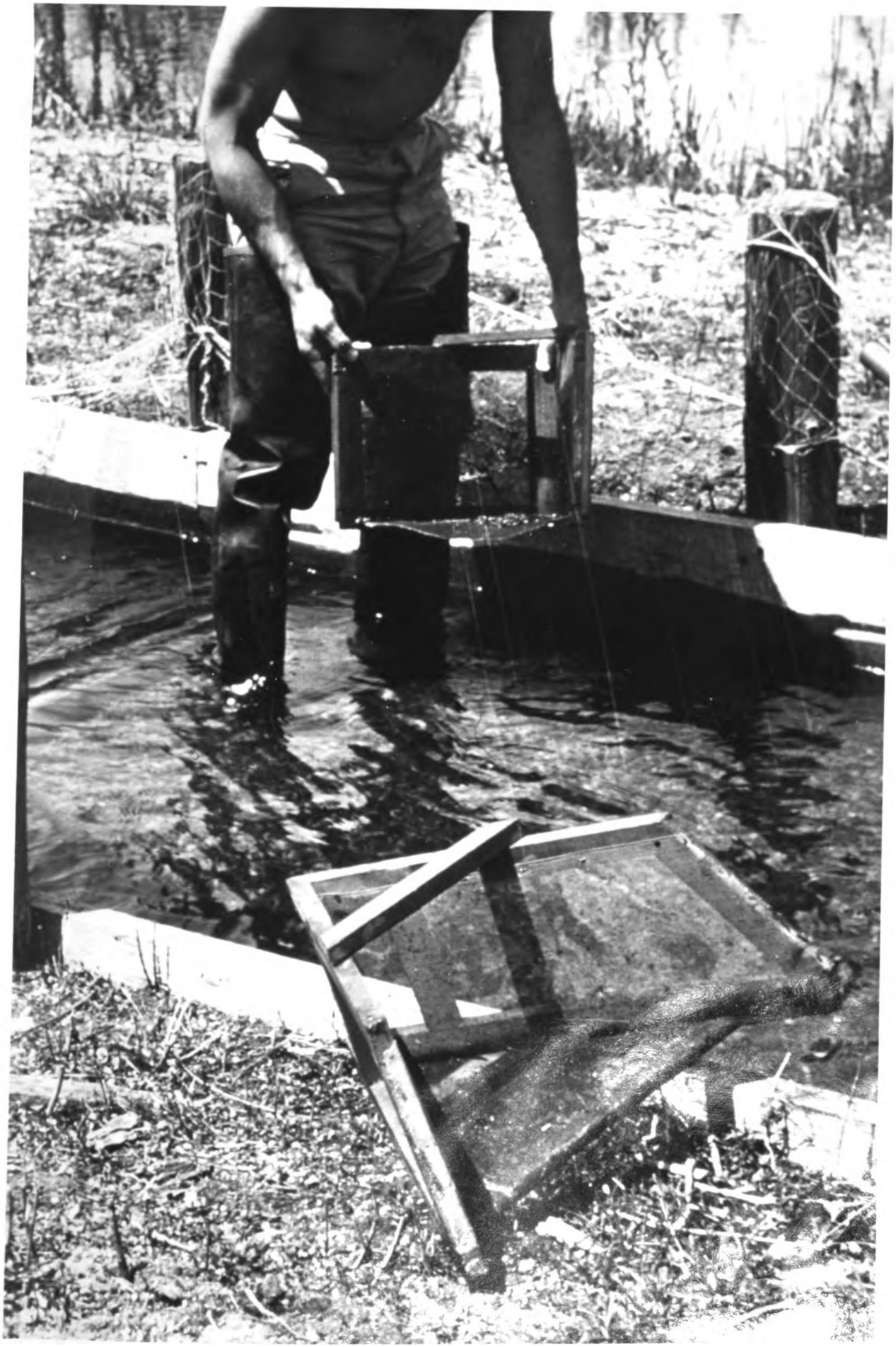


Plate VII. Screened creek chub eggs in hatching jars. Note debris.

to interfere with movement of the eggs within the hatching jar. Loss of eggs gathered in the manner described amounts to approximately 25 percent. All fish do not ripen at the same time and consequently the nests have to be screened several times over a period of several days in order to obtain all the eggs. This is costly in labor and time.

Chubs can also be held in ponds until ripe and then stripped, a procedure which has proved unsatisfactory because of the number of times the fish must be handled to obtain all the eggs and milt, and also because of the possibility of injuring the fish.

White Sucker

The common sucker is a desirable bait "minnow" for pan fish when small, for larger game fish when of medium size and serves as a decoy or pike bait when nearly grown. It is commonly found east of the great plains in northeastern and north central United States and Canada. Suckers generally spawn in swift running water during the latter part of April or early May in southern Michigan, or if inhabiting lakes they will seek wind-swept shoals on which to deposit their eggs. During the breeding season the male fish develops pearl-organs on its anal fin, both surfaces of the pelvic fins and the rays of the dorsal

fin bear small organs which aid the fish (Reighard, 1920) in its spawning habits. Two or more males accompany the female when she is ready to spawn, signified by her entering the main part of the rapids where the males wait in attendance. After several "coy" refusals of the attentions of the males, the female settles to the bottom of the rapids where the males press against her sides and actual spawning takes place. Eggs and milt are scattered freely in the current during this process which lasts only a few seconds. The female then moves upstream to spawn with other males while the males she has just mated with settle back to their former positions to await another female.

Artificial production of sucker fry for distribution to bait dealers centers around the gathering of adult fish making their seasonal migration to spawning grounds or those actually spawning. After the fish are obtained they are held in ponds until ripe, then stripped and the fertilized eggs placed in hatching jars to continue development. When stripping methods are used to obtain eggs, the fish must be handled several times as all the females do not ripen at one time. Eggs can also be collected from the rapids in which they spawn by stretching a fine mesh net (Dobie, Meehean, and Washburn, 1948) across the stream below the nesting area. Since some of the suckers spawn on wind-swept shoals of a lake, eggs

tend to congregate in shallow waters as a result of wave action and can be readily picked up with a scap net and transferred to hatching jars.

Other Species

The majority of the minnows commonly reared for sale as bait will breed in ponds and thus there is little or no advantage of resorting to artificial means to obtain the eggs.

GENERAL EXPERIMENTAL PROCEDURE

Injecting and Stripping

Experiments recorded in this report were conducted in two places: the fisheries laboratory at Michigan State College, and at Wolf Lake State Fish Hatchery, located in southwestern Michigan in Van Buren County. The artificial raceway constructed for creek chub propagation previously mentioned is located at Almena, Michigan, a short distance from the main hatchery.

Because of the nature of the tests only a few fish could be treated at a time in the laboratory but the results of the tests established the strength of injections necessary and length of time before effective stripping could take place. Conditions at the hatchery permitted the use of many more fish.

All creek chubs used were in breeding condition. Males averaged 9.3 inches and females averaged 6.4 inches in length.

Procedures for all experiments centered around the injection of a pituitary product into a fish and then stripping it later at intervals of several hours. All carp pituitary injections were measured and recorded as percentages of a single pituitary. Injections of mammalian

posterior pituitary extract were recorded as an actual measurement of liquid volume.

A two cubic centimeter glass hypodermic syringe, such as used in insulin treatments, with a size 19 needle was used to make injections of carp pituitary suspensions. A size 24 needle was used to make the mammalian extract injections. Injections were made intraperitoneally from the ventral surface of the fish at a point anterior to insertion of pelvic fins (Plate VIII). The needle was placed under a scale, pushed just through the wall of tissue over the body cavity, and then directed forward, parallel to the ventral surface. This insured the retention of the injection. All injections were made at a downward angle to facilitate equal applications of the suspension which quickly settled when undisturbed.

Ether was used in the first experiments to inactivate the fish for injections and stripping according to standard hatchery practices in handling fish for tagging. There was no mortality of the anesthetized fish during any of the experiments and recovery occurred when the fish were returned to untreated water. In some instances ether was not available and urethane, or ethyl carbamate, (Gerking, 1949) was substituted. Under hatchery practices it proved more satisfactory to have two men working on the process of injecting the fish (Plate VIII), one to hold the fish and one to make the injections. The

Plate VIII. Injecting creek chub with carp pituitary suspension.

ether treatment was dispensed with and as a result the whole process was greatly accelerated.

Following the injection of pituitary the fish were returned to holding tanks until they were in a condition to release the eggs or milt, or it was evident that the treatment was ineffective.

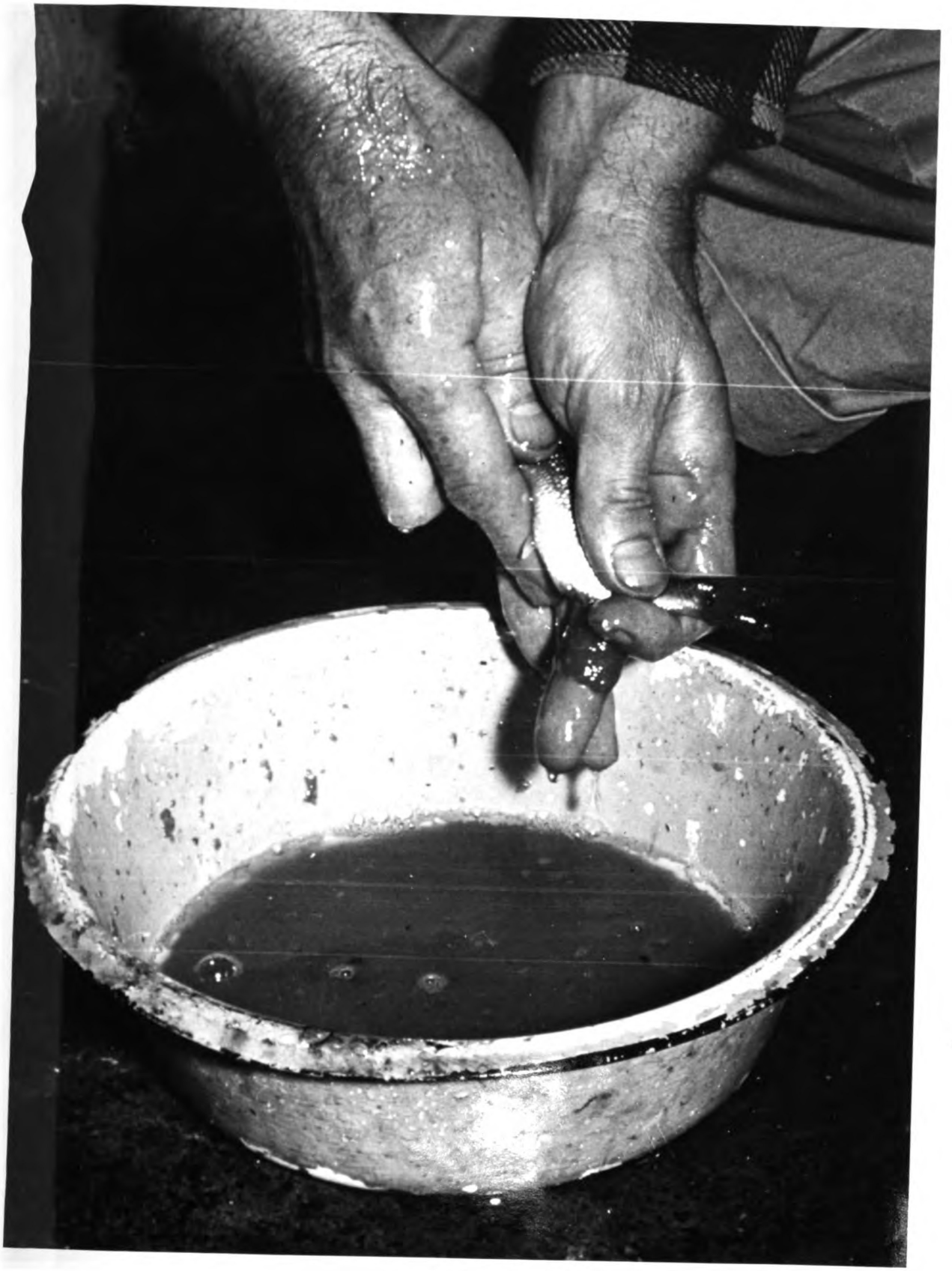
In the first stripping operations ether was used to relax the fish and avoid injury to them. However, the final stripping of fish in the field test at Wolf Lake Hatchery was done without aid of the ether, since it was believed that the small amount of ether which drips off the fish into the egg pan during stripping operations might cause some mortality among the fertilized eggs.

In stripping the fish, a smooth, even, down-and-forward pressure applied with the forefinger and thumb in the region of the pelvic fins (Plate IX) produced the best results.

Reactions to Mammalian Pituitary

Although investigators in the field of endocrinology have shown repeatedly that the use of pituitary which is non-specific to the species experimented with generally yields negative results, a series of experiments using mammalian posterior pituitary extract were conducted in the fisheries laboratory. Biological supply houses offer this extract for use in demonstrations in the classroom,

Plate IX. Method of stripping creek chubs. Note eggs in pan and on finger of operator.



for, when injected into redbelly dace it promotes an intense pigmentation, a secondary sexual characteristic. The extract used was a biologically standardized sterile solution of posterior pituitary, obstetrical, intended for subcutaneous injections. The United States Pharmaceutical average potency represents 10 International Units per cubic centimeter. Chlorobutanol (chloroform derivative), 0.5 percent, is added as a preservative.

A number of tests were conducted prior to actual experiments to ascertain the problems in handling fish and injection equipment and preparing pituitaries for injection. Distilled water, in varying amounts, was injected into a number of redbelly dace. All fish survived the treatment and showed no ill effects of handling. Mammalian pituitary, posterior extract, was then injected into several fish. Reactions to this injection were rapid, with breeding colors appearing in one fish twenty minutes after injection. During the normal breeding season, the abdomen of the male fish turns a bright red and the fins are highly colored with red and yellow. The abdomen of the female acquires a yellowish tinge. Two dace receiving 0.3 cubic centimeters each of the solution died within the hour following injections. One fish receiving 0.2 cubic centimeters of the solution showed definite yellow coloring on ventral surfaces twenty minutes after injection and of the three fish receiving 0.1 cubic

centimeter each of the solution, two had yellow coloring on ventral surfaces twenty minutes after injection.

The data collected (Table 1) from a group of dace, each of which received similar injections of mammalian posterior pituitary extract five days apart, showed that they had a tendency to become refractive with additional injections of the same material. The control group remained normal during the test period. The temperature remained constant at 19° C. throughout the experiment.

TABLE 1

REACTION OF REDBELLY DACE TO INJECTIONS OF MAMMALIAN POSTERIOR PITUITARY EXTRACT

Number of Fish	Amount of Extract Injected	Date	Remarks
4	0.1 cc	16 March	No coloration.
	0.1 cc	21 March	No coloration.
4	1 minim	16 March	One fish showed red coloration on ventral surface 45 minutes after injection.
	1 minim	21 March	One fish showed yellow coloring on ventral surface before injections. No new reactions.
4	2 minims	16 March	One fish showed yellow coloring 45 minutes after injection.
	2 minims	21 March	No new reactions.
1	1 minim	16 March	No coloration.
	1 minim	21 March	No coloration.

To further test the material, especially to determine its possibilities in "forced spawning" procedures, injections were made into several kinds of fish. All experiments (Table 2), with the exception of a trial on two suckers at Wolf Lake, were carried out in the fisheries laboratory. Water temperatures ranged from 66° to 70° F. during the trials. None of the creek chubs showed a positive reaction to the injections. Of five white suckers injected, two showed a positive reaction. Those treated at Wolf Lake were negative in reactions. Seven carp received injections of the extract and only two male fish had a positive reaction. One female carp, receiving two similar injections of the same extract, four days apart, emitted eight eggs 15 hours after the second injection. All later strippings were fruitless.

The influence of the posterior pituitary extract in affecting coloration was quite noticeable in the carp. Overall coloring was generally lightened and they were quite distinguishable from fish not treated.

TABLE 2

REACTIONS OF CREEK CHUBS, WHITE SUCKERS, AND CARP TO INJECTIONS OF MAMMALIAN POSTERIOR PITUITARY EXTRACT

Kind of Fish	No. of Fish	Amt. of Extract Inject.	Temp. (°C.)	Date of Inject.	Time Before Positive Stripping (hours)	Fish Not Responding
Creek chub	2	.2 cc	20	30 March	--	2
	1	.2 cc	20	31 March	--	1
	1	.2 cc	25	1 April	--	1
	1	.2 cc	25	5 April	--	1
White Sucker	3	.3 cc	19	6 April	13	2
					24	2
					40	1
	2	.4 cc	11	8 May	--	2
Carp	2	.3 cc	17	30 March	--	1
	1	.5 cc	17	30 March	12	0
	1	.4 cc	19	4 April	--	1
	1	.1 cc	17	21 April	--	1
	1	.25 cc	17	21 April	--	1
	1	.5 cc	17	21 April	24	0
	1*	.4 cc	18	31 March	--	1
		.4 cc	19	5 April	14	0

* This fish received two injections.

Location of Site of Injection

Previous workers have injected pituitary extracts and suspensions into the body cavity of fish to facilitate the absorption by the gonads.

The results of an experiment conducted to determine the reactions of creek chubs to pituitary injections made into the various parts of the body is shown in Plate X and Table 3. Only frozen, male, carp pituitary was used. Temperatures during the test ranged from 66° to 70° F.

Injections made into the body musculature were difficult to deposit and invariably caused a swelling to develop. It was noted that although the fish were completely anesthetized, the pressure of the needle entering the flesh in the area of the lateral line caused a vigorous muscular reaction. It is also noteworthy that those fish receiving injections in the lateral line or near it were the first to die.

The experiment was terminated at the end of 14 days and the remaining fish were killed and preserved in formalin. Dissections showed that only one male fish, injected in the area of the liver, had responded to the injections of carp pituitary. The results clearly indicate that injections of pituitary are not effective if deposited in the muscle tissue and generally resulted in a swelling at the point of injection and eventually death.

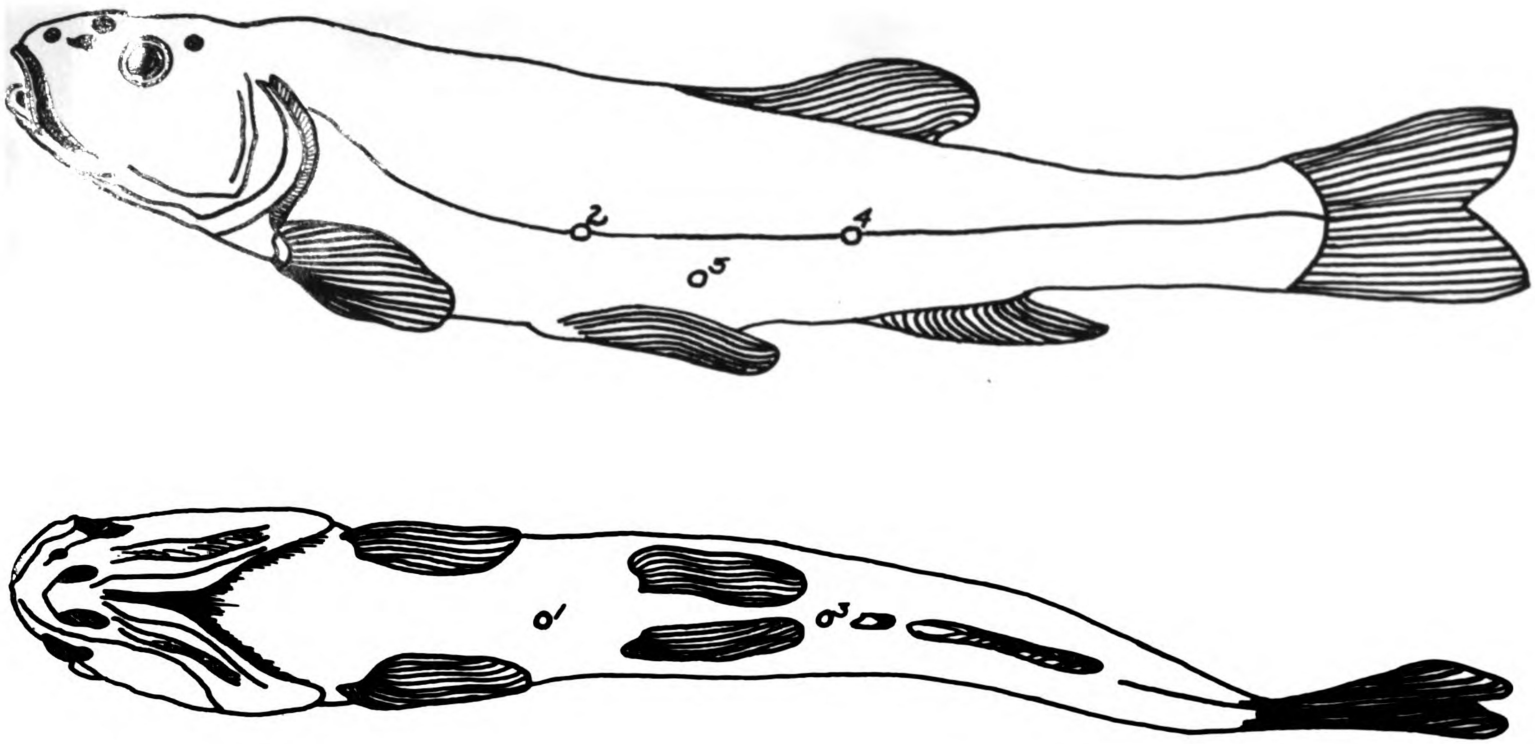


PLATE X LOCATION OF PITUITARY INJECTIONS

TABLE 3

SUMMARY OF EXPERIMENTS CONCERNING PLACEMENT OF INJECTIONS

Sex of Fish	Area of Injection*	Amount of Pituitary Injected	Reaction	Condition of Gonads
M	(5). Halfway between lateral line and ventral surface on left side, in area between pelvic fins and anus.	0.85	Vigorous reaction to injection. Swelling apparent 48 hours later; scales standing on end. Swelling increasing until death. Died May 4.	Normal; testes not swollen.
F	(4). In lateral line at a point perpendicular to anus, on left side.	1.00	Vigorous reaction to injection. Area of injection greatly swollen 48 hours later; increasing in size until death. Died May 4.	Normal; ovaries not swollen.
M	(2). In lateral line on left side, mid-body section.	1.00	Vigorous reaction. Normal at all times. Died May 9.	Normal; testes not swollen.
F	(3). Perpendicular to ventral surface, just anterior to anus.	1.00	Actions were normal at all times, stomach region greatly shrunken 72 hours later. Killed on May 12.	Ovaries small, only a few large eggs apparent.
M	(1). Perpendicular to body surface (ventral) in area of liver, needle inserted 1/2 inch.	1.00	Normal at all times. Killed on May 12.	Testes well developed.

* Numbers in parentheses indicate the area of the injection as shown on the diagram on the facing page (Plate X).

Results of Injections of Carp Pituitary

Creek Chubs

Laboratory tests. Adult creek chubs, in breeding condition, from Wolf Lake Hatchery were received at the fisheries laboratories on March 29. A number of these fish were placed in the constant-temperature room (50° F.), the rest being kept in the laboratory where the temperature ranged from 66° to 70° F.

In order to determine the earliest possible date that viable eggs of this species could be obtained, a group of four fish was selected three days after their arrival from those that appeared to be acclimated to the new environment.

Injection of the first carp pituitary into this group was done without the aid of ether. The frozen, female gland was macerated and mixed with distilled water to form a suspension so that each fish received 0.25 of the pituitary. Stripping at various lengths of time after injections (Table 4) was attempted with only one male fish showing positive reactions 44 hours later. Identical injections were given to the same group of fish sixty hours after the first injection and three fish, two males and one female, showed positive reactions to this injection fifteen hours later. The temperature during this period ranged from 66° to 70° F.

Eggs and milt obtained from these fish on April 5 were mixed and prepared for incubation. Hatching was complete four days later. This was approximately six weeks before the normal spawning period in southern Michigan, which, in 1950, reached its height during the period of May 17-25.

TABLE 4

DATA ON FIRST EXPERIMENT WITH PITUITARY INJECTED CREEK CHUBS

Sex of Fish	Amount of Pituitary Injected	Date	Time Before Positive Stripping (hours)	Fish Not Responding
Female	0.25	1 April	--	1
	0.25	4 April	15	0
Female	0.25	1 April	--	1
	0.25	4 April	23	0
Male	0.25	1 April	44	0
	0.25	4 April	15	0
Male	0.25*	1 April	--	1
	0.25	4 April	15	0

* Some of this suspension was lost during injection.

After the initial trial of injections of pituitary material, experiments were designed to determine the effects of various concentrations of the pituitary, the most effective length of elapsed time before stripping was successful, the comparative effectiveness of male or

female carp pituitary and the effect of different types of pituitary preparations. With the exception of the initial attempts at injections, all fish had been acclimatized to either laboratory or constant-temperature room conditions for at least a six-day period prior to experimentation.

Data collected from a group of creek chubs at the College laboratories which received a single injection of either frozen or acetone-dried pituitary are shown in Table 5. The amounts of pituitary injected in all tests were calculated as parts of a whole gland. During the period of the test, April 6-13, the temperature ranged from 66° to 70° F. Of the 22 fish treated, only nine showed positive reactions. Positive reactions indicate any appearance of eggs or milt resulting from stripping. There was no apparent difference in the reactions caused by either frozen or acetone-dried glands. Although the table indicates that frozen glands accounted for seven of the nine positive cases, it is noted that those were also the cases where the higher concentrations of pituitary were used. Female carp pituitary, though used more extensively than the male, showed more positive results in both male and female fish. Male carp pituitary produced positive results only in male fish. The most effective length of elapsed

TABLE 5

RESULTS OF INJECTIONS OF CARP PITUITARY ON CREEK CHUBS

No. of Fish	Type of Pituitary		Amount Injected	Injection Date-1950	Time Before Positive Stripping (hours)	Fish Not Responding
	Frozen	Dried				
1		F*	0.016	13 April	--	1
1		F	0.027	13 April	24	0
2		F	0.050	10 April	--	2
1		F	0.055	13 April	24	0
3		F	0.10	10 April	--	3
2	F		0.16	10 April	31	0
1		F	0.16	13 April	--	1
1		F	0.25	13 April	--	1
2	F		0.50	6 April	--	2
2	F		0.50	7 April	15	1
2	F		0.50	9 April	24	0
2	M**		0.50	7 April	15	1
1		F	0.50	10 April	--	1
1	M		1.00	13 April	24	0

* Pituitary from female carp.
** Pituitary from male carp.

time before stripping produced positive results in this experiment was approximately 24 hours.

Since some creek chubs did not respond positively to single injections of carp pituitary in the first trial, an experiment was conducted to determine the effect of two or more injections. A number of pituitary preparations

of various concentrations were used in this test during the period of April 4-17. Water temperatures in the laboratory ranged from 66° to 70° F.

The data collected from this experiment are shown in Table 6. The hours recorded for effective length of elapsed time before stripping occurred showed some interesting results. In all tests where the first injection was a carp pituitary preparation and stripping showed positive results, a second injection of a carp pituitary preparation produced positive results in a considerably shorter length of time. The two fish receiving an initial injection of mammalian posterior pituitary extract showed negative reactions; a second injection with a carp pituitary preparation produced positive reactions 25 hours later; the third injection, with mammalian posterior pituitary extract, was positive in one case, negative in the other.

In the only instance where reactions to a carp pituitary preparation were negative after one injection, they were also negative after a second injection. In the two tests where a carp pituitary preparation was the first injection and stripping showed positive results, the second injection of mammalian posterior pituitary extract produced positive reactions in only one test.

Reactions from injections of mammalian posterior pituitary extract in this experiment, as well as in all

TABLE 6

REACTIONS OF CREEK CHUBS TO TWO OR MORE INJECTIONS OF VARIOUS TYPES OF PITUITARY
[Carp pituitary is designated as (F)-female or (M)-male.]
[Mammalian pituitary is a posterior extract.]

Sex of Fish	Date of Injections	Type of Pituitary	Amount Injected	Time Before Stripping (hours)	Results
Female	1st - 10 April	Carp:Frozen,(F)	0.16	55	Positive
	2nd - 17 April	Carp:Dried,(F)	1.00	3	Positive
Male	1st - 8 April	Carp:Frozen,(F)	0.50	15	Positive
	2nd - 17 April	Carp:Dried,(M)	0.15	2	Positive
Female	1st - 8 April	Carp:Frozen,(F)	0.50	0	Negative
	2nd - 17 April	Carp:Dried,(F)	0.33	0	Negative
Female	1st - 10 April	Carp:Dried,(F)	0.05	22	Positive
	2nd - 17 April	Carp:Dried,(F)	0.55	2	Positive
Male	1st - 13 April	Carp:Dried,(F)	0.06	24	Positive
	2nd - 17 April	Carp:Dried,(F)	0.50	2	Positive
Male	1st - 6 April	Carp:Frozen,(F)	0.50	6	Positive
	2nd - 17 April	Mammalian	0.40 cc	6	Positive
Female	1st - 6 April	Carp:Frozen,(F)	0.50	168	Positive
	2nd - 17 April	Mammalian	0.40 cc	0	Negative
Male	1st - 4 April	Mammalian	0.20 cc	0	Negative
	2nd - 10 April	Carp:Dried,(F)	0.10	25	Positive
	3rd - 17 April	Mammalian	0.20 cc	6	Positive
Female	1st - 4 April	Mammalian	0.20 cc	0	Negative
	2nd - 10 April	Carp:Dried,(F)	0.10	25	Positive
	3rd - 17 April	Mammalian	0.20	0	Negative

other experiments, were recorded as positive if even small amounts of eggs or milt resulted from stripping after injection.

Field tests. Application of pituitary injections under field conditions was tried on three different occasions (Table 7) at the Wolf Lake Hatchery.

The first field test began on April 25. A group of thirty females were injected with female carp pituitary and a group of twenty males with male carp pituitary. These fish had been put in a holding tank inside the hatchery eight hours prior to injections. The temperature of the water at the time of the injections was 56° F., but was raised, during the following 48 hours, by adding warm tap water at different times during the day, to reach a maximum of 63° F. All of the injected fish were successfully stripped 48 hours later and 69 ounces of viable eggs were obtained. Several control fish which were kept with this group failed to produce either eggs or milt when stripping was attempted.

The second experiment began on May 8 when six male chubs were injected with male carp pituitary, twelve female chubs with female carp pituitary and three female chubs with mammalian pituitary extract. These fish were also confined to the holding tank inside the hatchery where the water temperature ranged from 56° to 62° F. during the experiment. Stripping procedures did not produce

TABLE 7

RESULTS OF DRIED CARP PITUITARY INJECTIONS ON CREEK CHUBS AT WOLF LAKE

No. of Fish	Sex of Gland	Amount Injected	Date of Injection	Temp. Range (°F.)	Time Before Positive Stripping (hours)	Fish Not Responding
12	Female	0.25	8 May	56-62	--	12
210	Female	0.25	15 May	46-64	48	0
14	Male	0.28	9 May	56-62	48	0
15	Female	0.28	9 May	56-62	48	0
30	Female	0.33	25 April	56-63	48	0
20	Male	0.33	25 April	56-63	48	0
6	Male	0.33	8 May	56-62	--	6

any eggs or milt 24, 48, or 72 hours later. It is possible that these fish had not been acclimatized to warmer water a sufficient length of time before injections were made.

On May 9, fourteen male chubs received injections of male carp pituitary and fifteen female chubs were injected with female carp pituitary. The water temperature inside the hatchery ranged from 56° to 62° F. during the experiment. This group of fish was successfully stripped 48 hours later and 27 ounces of viable eggs were obtained. During the 48 hours following injections, two

male and two female fish died. Dissection showed one fish with a blood clot surrounding the liver, which indicated the liver had been punctured during injection, while the other three fish were normal. Control fish did not produce eggs or milt during attempts at stripping.

The third experiment began on May 15 and was conducted entirely out-of-doors. The fish, which were in live-boxes in a raceway, had been sorted out from a group of breeders seined from a near-by hatchery pond the previous day, and contained only the largest males and females. Fish of this group, 101 females and 109 males, were injected with female carp pituitary. The ether treatment to inactivate the fish was dispensed with when it was found that two men could do the work much quicker when one man held the fish while the other did the injecting. All injected fish were returned to the raceway proper. Stripping procedures applied 48 hours later produced 142 ounces of viable eggs. There were no "green" fish and a few females were void of eggs before stripping occurred. Several nests had been constructed by the fish in the raceway, indicating spawning activity had taken place even though the water was three feet deep and the current was sluggish. The daily temperature range was from 46° F. at night to 64° F. during the day. A control group was not set aside for this experiment.

This series of three field tests demonstrated the feasibility of using carp pituitary injections to obtain creek chub eggs. In all tests the eggs were handled in regular hatchery manner, being transferred to hatching jars shortly after they were obtained.

Midseason injections. An experiment was conducted to determine the reactions of creek chubs to pituitary injections six months in advance of their regular breeding season. Fish were obtained from Wolf Lake Hatchery on November 2 and acclimated to the room temperature of the fisheries laboratory 24 hours prior to injections. Temperatures during the experiment, which began on November 3 and ended December 3, ranged from 64° to 72° F., with a mean of 69° F. In Table 8 are recorded the results of the various tests conducted in this experiment. Only male or female carp pituitary, acetone-dried, was used for the injection. The pituitary was of the same lot as used in prior experiments. Strength of the injections ranged from 0.06 of a pituitary to four whole pituitaries, each fish receiving at least two injections. Of 23 fish injected, only one showed a positive reaction. Stripping procedures were applied every 24 hours after injection for a period of fifteen days. Control fish did not respond to stripping at any time during this experiment. Of the five fish making up this group, two died 48 hours after experiments started and a third died

seven days later.

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seven days later. The two remaining fish were killed on December 3. Ethyl carbamate, or urethane, was used to inactivate the fish for injections and stripping. All fish that had died during the experiment and those that were killed on December 3, were dissected and the condition of the ovaries and testes compared with untreated fish. There was no significant difference in the appearance of the gonads between the two groups of fish. The experiment was considered negative as far as the production of eggs and milt six months ahead of normal breeding season was concerned.

A summary of all experiments conducted with the creek chubs and their reactions to various types of pituitary injections is shown in Table 9. This table also shows the evaluation of the comparative differences in the abilities of the male and female carp pituitaries to produce positive results when injected into fish. Cognizance of the fact that female pituitaries were more "potent" than male greatly influenced the type of pituitary selected when injections were made.

Data from laboratory experiments showed that male carp pituitary injections failed to cause female creek chubs to react positively and it appeared that the male gland worked best on male fish. As a consequence no female fish received any male carp pituitary in the field tests at Wolf Lake while male pituitary injections in male

TABLE 9

SUMMARY OF REACTIONS OF CREEK CHUBS TO VARIOUS PITUITARY INJECTIONS

Source of Pituitary	Number of Fish Injected	Positive Results (Percent)	Negative Results (Percent)
Mammalian:			
Posterior Pituitary Extract	8	0	100
Carp:			
Whole, Frozen, Male	7	29	71
Whole, Frozen, Female	12	67	33
Carp:			
Whole, Acetone-dried, Male	54	69	31
Whole, Acetone-dried, Female	297	89	11

fish were successful in 34 of 40 fish treated. On the other hand, female carp pituitary worked equally well on both male and female fish.

Several factors such as temperature, condition of certain fish, standardization of hormone strength and others were not in all cases constant and may have influenced the high proportion of negative results.

White Sucker

Although the principal objective of this report was concerned with the production of eggs from creek chubs, some work was carried out with the white sucker. A group

of fish in breeding condition, from the Wolf Lake hatchery, were received alive at the fisheries laboratory on April 6, and experiments were conducted to determine their reactions to injections of carp pituitary. Data collected from this experiment ~~is~~ shown in Table 9. Only frozen, female, carp pituitary was used in the first trial, to which all the fish showed a positive reaction. Temperatures in the laboratory ranged from 66° to 70° F. during the experiment. Elapsed time, before stripping produced positive results, was less than 24 hours for four fish and well over 24 hours for three fish. An extract of mammalian pituitary was injected into three fish under the same conditions as listed above, with two of the fish showing positive reactions. The pituitary was removed from a female sucker, and injected into another female. Stripping produced a few eggs 48 hours later and a few more 60 hours later. The fish died 64 hours after injection.

Some work was also done on suckers at Wolf Lake (Table 10) on two different occasions. In the first trial, male fish were injected with male carp pituitary and females with female carp pituitary. The fish were kept in a live-box out-of-doors where the water temperature was consistent at 52° F. Stripping was fruitless in both sexes. Two fish receiving 0.4 cubic centimeter

TABLE 10

RESULTS OF INJECTIONS OF CARP PITUITARY IN THE WHITE SUCKER

Number of Fish	Type of Pituitary		Amount Injected	Date of Injection	Temperature (°F.)	Time Before Positive Stripping (hours)	Fish Not Responding
	Frozen	Dried					
Laboratory							
1	F*		0.16	6 April	62-66	10	0
1	F		0.16	6 April	62-66	50	0
3	F		0.20	10 April	62-66	8	0
1	F		0.42	6 April	66-70	29	0
1	F		0.57	6 April	66-70	45	0
Wolf Lake Hatchery							
95		F	0.25	15 May	46-64	48	***
10		M**	0.50	9 May	52	--	10
10		F	0.50	9 May	52	--	10

* F - Pituitary from a female carp.
** M - Pituitary from a male carp.
*** A few males were ripe enough to strip 48 hours after injection, but no females responded. Since an equal number of males were ready to strip prior to injection, the experiment was considered to be negative as far as the use of pituitary was concerned.

each of the mammalian pituitary extract also reacted negatively to stripping procedures.

The second test was conducted entirely out-of-doors. The fish were confined to a raceway, and after injections were returned to another raceway. Some male fish were stripped prior to injections and a few of them yielded milt. This group of 95 suckers ~~were~~ injected with female carp pituitary. The daily range of water temperatures was 46° to 64° F. Stripping was tried 48 hours after injections with apparently negative results. No females responded to stripping procedures and since the number of males that did show positive reactions to the injection about equaled the number that stripped before injections were made, the whole experiment was considered negative as far as the use of carp pituitary was concerned.

It will be noted in Table 11, that those suckers injected with frozen pituitary, carp or sucker, were the only ones that showed positive reactions. Although these injections were made in the laboratory, it is not to be inferred that only frozen pituitary will cause positive reactions. Field tests at Wolf Lake were generally negative in nature, and, while the fish did receive injections of dried carp glands, only a few indicated any positive reaction. It is believed that the reason that these suckers did not respond is that they were held too long beyond their normal breeding period before injections were made.

TABLE 11

SUMMARY OF REACTIONS OF THE WHITE SUCKER TO INJECTIONS OF VARIOUS TYPES OF PITUITARY

Source of Pituitary	Number of Fish Injected	Positive Results (Percent)	Negative Results (Percent)
Mammalian:			
Posterior Pituitary Extract	5	20	80
Carp:			
Whole, Frozen, Female	7	100	
Carp:			
Whole, Acetone-dried, Male	10		100
Whole, Acetone-dried, Female	10		100
	95		*
Sucker:			
Whole, Frozen, Male	1	100	

* A few males were ripe enough to strip 48 hours after injection, but no females responded. Since an equal number of males were ready to strip prior to injection, the experiment was considered to be negative as far as the use of pituitary was concerned.

Carp

Several carp were retained from earlier shipments to be used in testing the effects of various pituitary injections. An account of testing with mammalian pituitary was discussed earlier and tabulated in Table 2. Of the fish injected with carp pituitary, the one fish receiving female, dried carp pituitary, did not show

positive reactions. A male and a female carp, each receiving injections of frozen, female carp pituitary were stripped 22 hours later with only the male showing a positive reaction. He continued to show milt in small quantities at 41 and 48 hours but was non-reactive 72 hours later. The female did not react positive to the injection and died 93 hours later. Autopsy showed nothing unusual.

A summary is presented of all experiments (Table 12) conducted with the carp, and shows the results, in percentage, of its reaction to various types of pituitary injections.

TABLE 12

SUMMARY OF REACTIONS OF CARP TO INJECTIONS OF VARIOUS TYPES OF PITUITARY

Source of Pituitary	Number of Fish Injected	Positive Results (Percent)	Negative Results (Percent)
Mammalian: Posterior Pituitary Extract	8	37*	63
Carp: Whole, Female, Fresh	2	50	50
Carp: Whole, Female, Acetone Dried	1	0	100

* Very few eggs.

Lake Chubsucker

The lake chubsucker (Erimyzon sucetta) is a bait species of some importance. As they are a difficult species to propagate, even under favorable conditions, an attempt was made to induce forced spawning with injections of carp pituitary.

A group of 79 fish, 58 males and 21 females, were each injected with 0.25 acetone-dried female carp pituitary on May 17. These fish had been transported from the Hastings State Fish Hatchery to Wolf Lake Hatchery the same day of the injection. Water temperatures were maintained as near as possible to that from which the fish were taken. Of a total of 87 fish captured, eight died before injections were made.

Injections were appreciably more difficult to make in the chubsuckers than in the creek chubs. Later investigations showed that the flesh that covered the ventral area between the pelvic and pectoral fins was three to five times thicker than that of the creek chub. The temperature of the water was gradually raised during the ensuing 48 hours after injections by removing some of the water from the tank and replacing it with warmer tap water. At the end of 24 hours, five male and one female fish were dead and fungus (Saprolegnia sp.) was prevalent on the heads of a number of remaining fish. Stripping was attempted 48 hours after injections and

approximately two ounces of eggs were obtained. Very little milt was procured. Nearly all fish had fungus present on their heads. Most of the fish developed an inflamed, hard, swelling in the area of the injection with the scales standing on end. A general decrease in activity was noted. Most of the fish were dead within 72 hours.

Failure to get the pituitary injection wholly within the body cavity is probably responsible for the high mortality and caused the strong reaction described above. It is probable that moving the fish and injecting them the same day were also contributing factors.

Largemouth Black Bass and Pumpkinseeds

Although largemouth black bass (Micropterus salmoides) and pumpkinseeds (Lepomis gibbosus) are not considered bait minnows, the feasibility of procuring eggs of these species by injections of carp pituitary was considered.

A group of four bass and five pumpkinseeds were brought to the fisheries laboratories on May 12. Each fish was injected with one whole female carp pituitary, acetone-dried, twelve hours later. The temperature of the water had been increased slowly from 52° F. to 72° F. before injections. There were no positive results from any of the injections. Stripping was attempted 24, 48, and 120 hours after injections.

Two bass were dead 120 hours after injections and the remaining two were killed and preserved 17 days later. Dissection showed that two males and two females had been treated. The ovaries of the females were noticeably full and tough and the testes of the males were very firm. The formalin solution may account for the apparent toughness of the ovary sac.

A heavy growth of fungus appeared on one pumpkinseed 24 hours after injections and may have hastened its death, which occurred 57 hours later. The four remaining fish started to show fungus 48 hours after injections and all were dead 120 hours later. Dissections showed four males and one female had been injected. The ovary was full of eggs but the sac was very firm, testes of the males well expanded.

Failure to achieve positive reactions with these fish is not to infer that pituitary will not in some instances produce a reaction. These fish may not have been acclimated to warmer water temperatures a sufficient length of time before injections were made or perhaps were tested too far in advance of their normal breeding season. Whether these fish will ever lend themselves to stripping practices remains for other experimenters to determine.

Redbelly Dace

On March 20, a pituitary was removed from the head of a large lake trout (Cristivomer namaycush), macerated and mixed with distilled water to form a suspension and injected into five dace, each fish receiving 0.20 percent of the pituitary. These fish did not develop breeding colors as a result of the injection. Temperatures for the five days following injections ranged from 66° to 70° F.

Since lake trout spawn in the fall, the seasonal swelling of the pituitary, which accompanies breeding activities, was not evident and perhaps indicated a lessened degree of potency.

A group of ten dace were injected with frozen, female carp pituitary on April 30. None of the fish developed breeding colors or responded to stripping methods as applied to the other species.

A summary of all experiments conducted with the redbelly dace is presented in Table 13, showing the reactions of the fish to various types of pituitary injections. Positive results in this table indicates an attainment of breeding colors.

A summary of all experiments conducted with the several kinds of fish (Table 14) shows their reactions to injections of various types of pituitary.

TABLE 13

SUMMARY OF REACTIONS OF REDBELLY DACE TO VARIOUS PITUITARY INJECTIONS

Source of Pituitary	Number of Fish Injected	Positive Results (Percent)	Negative Results (Percent)
Mammalian: Posterior Pituitary Extract	13*	23	77
Lake Trout: Whole, Frozen	5		100
Carp: Whole, Frozen, Female	10		100

* This group of fish received two injections, five days apart, with only three fish showing positive reactions.

Reactions to Pituitary Injections

General

A general reaction of all fish to a pituitary injection was an immediate lightening in overall coloration which might persist for only a few minutes or for several hours. Pupils of the eyes were dilated and a noticeable nervousness, accounted for by excessive fin movement, were also noted. Some fish appeared to be trying to rid themselves of some trailing object or seeking a hiding place by the manner in which they moved about upon being returned to the tank after injections.

TABLE 14

SUMMARY OF EXPERIMENTS USING PITUITARY HORMONES ON SEVERAL SPECIES OF FISH

Species	Source of Pituitary	Results	Temperature (°F.)	Side Results
Redbelly dace	Mammalian	Breeding colors. Fish refractive to further injections	66-70	Some fish died of strong injections. Stripping fruitless.
	Carp:Frozen	Negative	66-70	Stripping fruitless.
	Lake Trout: Frozen	Negative	66-70	Stripping fruitless.
Creek chub	Mammalian	Negative	66-70	Stripping fruitless. Repeat injections proved negative.
	Carp:Frozen and Dried	Positive-Negative	66-70 lab 46-64 field	Viable eggs six weeks early. Field tests successful.
White sucker	Mammalian	Positive-Negative	66-70	A few eggs from one female.
	Carp:Frozen and Dried	Positive-Negative	66-70 lab 46-64 field	Positive in laboratory. Negative in field test.
	Sucker: Frozen	Positive	66-70	A very few eggs.
Carp	Mammalian	Positive-Negative	66-70	Traces of milt from a male.
	Carp:Frozen and Dried	Positive-Negative	66-70	Some milt from one male. No eggs from female.
Lake chubsucker	Carp:Dried	Positive-Negative	56-60	Fish were difficult to inject. 2 oz. eggs from 20 females.
Largemouth black bass	Carp:Dried	Negative	66-70	Stripping fruitless.
Pumpkinseed	Carp:Dried	Negative	66-70	Stripping fruitless.

Other fish remained quiescent on the bottom of the tank. Active characteristics mentioned above were displayed in direct proportion to the strength of the pituitary injection received, and were especially noted after injections of the mammalian posterior pituitary extract.

The following reaction following a pituitary injection was noted. Due to the lack of space in which to segregate the fish for experiments, a pair of white suckers and a pair of creek chubs were kept in the same tank. The suckers had received injections of mammalian pituitary and the chubs had been injected with carp pituitary. A half hour after the chubs had received injection, the male chub became aggressive, nudging the female in the area of the vent and pursuing her around the tank. The male chub appeared to have assumed "territorial rights" as he attempted to dislodge the suckers from where they were resting by grabbing hold of one of their pectoral fins and pulling backwards. This would go on for several minutes at a time, ending when the sucker broke away from the chub to move to a different location in the tank.

The chubs and suckers were the only fish to show positive results from the injections of pituitary glands, the other fish generally showed a negative reaction to the injections. Some viable eggs and milt were obtained from a few which would seem to indicate that with more work, better results could be obtained.

While there were negative reactions of both sexes of fish to injections of both male and female carp pituitary, it is believed that the female gland ~~is~~ more potent in its effect upon the fish in this experiment.

The greatest dilution of carp pituitary that produced positive results in laboratory work was 0.027 of a pituitary. Results were also obtained in higher concentrations, up to one whole gland. Field work at Wolf Lake, with a greater number of fish, was very successful with injections of 0.33 of a gland one month prior to breeding season, 0.28 of a gland two weeks before, and 0.25 of a gland during the beginning of the normal breeding season.

Time Lapse

The time required for effective reaction of the pituitary gland injections was determined from data collected on the several laboratory studies in this experiment. Positive results were obtained not less than six hours after injections for creek chubs, and eight for the white sucker, with increasingly better results after a period of 24 hours. There were no noticeable results for the first, second, or third hour following injections. Field tests were successful with an elapsed period of 48 hours after injections.

A three-hour check over a period of 24 hours did not furnish enough evidence to warrant stripping any earlier than 24 hours after injection. A few eggs and traces of milt were obtained, but, not to the degree that would be termed successful by fish culturists. Failure to obtain larger quantities of eggs and milt might be attributed to the physiological reaction of the ether treatment.

Effect of Water Temperatures

The temperature of the water appeared to be a controlling factor in the ripening progress of pituitary-injected gravid fish. To determine the influence that different periods of increased temperature had on the fish before they were injected, fish were held at several temperatures and time periods prior to and following injections of pituitary gland material.

Fish in water of consistently low temperatures, 50° F., did not respond to the injections as well as those fish which had been acclimated to higher temperatures for at least 24 hours prior to treatment, with increasingly better results as the period acclimation to higher temperatures was lengthened. Positive results from pituitary injected fish in the laboratory were more consistent when the temperature was approximately 68° F. Several fish which had shown positive reactions to

injections while in the constant temperature room (50° F.) failed to show further development when brought into the warmer (68° F.) temperatures of the laboratory.

Tests at the hatchery were quite variable as conditions of temperature could not be controlled. Temperatures in the raceways fluctuated daily as much as 18° F. with the limits being between 46° and 64° F. Good results occurred from one group of fish that had been acclimated to temperatures of 53° to 63° F. for 24 hours prior to injections. Another group of fish acclimated under similar conditions failed to respond to the injections. Excellent results were obtained when 210 fish were taken from an outdoor raceway, injected, and returned to the raceway. Injections were made on May 15, stripping occurring 48 hours later with nearly one hundred percent positive results. Those that did not, obviously had spawned in the raceway. This presumably could have been avoided by the separation of the sexes after injections.

It would appear that the closer the natural conditions of spawning can be approximated, the better the results will be. Fluctuating temperatures, in the trials at Wolf Lake, seem to have added impetus to the stimulus that induced spawning activity.

Although the temperature of the fisheries laboratory was generally much higher than that found at Wolf Lake Hatchery, the reaction of fish to pituitary injections

of similar concentrations was often positive in both places.

Number of Eggs Produced by Female Creek Chubs

There is little agreement in the literature as to the number of eggs produced by adult female creek chubs or the number per ounce. In order to determine the number of eggs obtained by pituitary-injected fish (Table 15) a count of fertilized eggs that had been in hatchery jars 24 hours was made which indicated that there were 88 eggs per cubic centimeter, or 2,605 per ounce.

The eggs, of variable size, came from a group of females which averaged 6.3 inches in length. It is known that eggs of trout vary directly in accordance with the size of the fish and since there was little similarity of egg counts of the creek chub in reference material it is assumed that data presented were based on the eggs produced by fish of different sizes.

Though the average number of eggs per female is higher for the fish stripped on April 27 (Table 15), the females that were stripped May 17 were much larger fish. The fact that the former group of fish were treated inside the hatchery as opposed to the latter group which were in outdoor raceways with male fish - where nests were apparent before stripping began - does account for the difference.

TABLE 15

EGGS OBTAINED FROM CARP PITUITARY INJECTED FEMALE CHUBS

Date	Number of Female Chubs	Ounces of Eggs Obtained	Average Ounces per Fish	Number of Eggs Obtained	Average Number of Eggs per Fish
April 27	30	69	2.3	179,745	5,991
May 11	15	27	1.8	70,635	4,709
May 17	101	142	1.4	369,910	3,662
Total	146	238	1.6	620,290	4,248

The eggs obtained on April 27 were placed in hatching jars thirty minutes after stripped from the fish. The water temperature was adjusted slowly to conform to that of the water supply at the hatchery. The drop in temperature, from 63° F. to 52° F., had a deleterious effect on the eggs for less than 25 percent hatched over an extended period of time.

The eggs from the May 11 stripping were put in two places: two ounces were kept inside at room temperatures (72° F.) and hatched two days later; 25 ounces were put in a hatching shed, where daily water temperatures ranged from 46° to 66° F. and the eggs developed normally in 10 days. The fry hatched inside were exceedingly weak.

The 142 ounces of eggs stripped from chubs on May 17 were placed in a hatching shed where water temperatures

fluctuated daily from 58° to 68° F. Development was normal, hatching occurred in 5 days.

The last two lots of eggs, 27 and 142 ounces, furnished the bulk of the fry that was distributed to bait dealers in 1950.

DISCUSSION

The data that have been presented are from experiments conducted to determine the feasibility of using carp pituitary injections in the production of fish. Hasler, Meyer, and Field (1939, 1940) have shown that trout and muskellunge injected with carp pituitary prior to normal spawning, could be successfully stripped.

Since the Fish Division of the Michigan Department of Conservation is seeking improved methods of bait minnow production in order to supplement the rapidly diminishing wild stock from which bait dealers are obtaining their stock, emphasis was placed on creek chub propagation during these experiments.

Present methods of creek chub propagation involve considerable expenditure of time and effort since artificial raceways must be constructed and the process of obtaining the naturally spawned eggs is laborious. With carp pituitary injections it is possible to expedite the acquisition of eggs for production of fry. As the loss of eggs due to natural causes is greatly reduced, production is increased.

Procedures for the experiments were based on injecting fish with a pituitary product and then stripping them later at intervals of several hours. Live carp were

processed for removal of the glands which were either frozen or acetone-dried. Pituitaries were macerated and mixed with distilled water immediately prior to injections.

Although this work was primarily concerned with the creek chub, some work was also done on the white sucker, carp, lake chubsucker, largemouth black bass, pumpkinseeds, and redbelly dace.

Initial work on the creek chub produced viable eggs six weeks in advance of the normal breeding season. Field tests at Wolf Lake Hatchery were successful, the last two tests furnishing the majority of fry distributed to bait dealers in 1950. Tests conducted six months in advance of breeding season were considered negative as far as the production of viable sex products were concerned.

Reactions of the white sucker were positive in the laboratory and generally negative at Wolf Lake. It is believed that the differences in water temperature were responsible. Experiments with other fish were considered negative as far as the production of eggs and milt were concerned.

SUMMARY

1. Carp pituitary injections can be used advantageously to propagate the creek chub. An elimination of artificial raceways and screening process for eggs and a reduction of the number of times that fish must be handled are major features which results in a greatly reduced expenditure of time and money.

2. It is very probable that carp pituitary injections can be used in production of the white sucker and lake chubsucker, although initial attempts were not totally successful. Largemouth black bass, pumpkinseeds, and carp did not respond to pituitary injections under the conditions of these experiments.

3. Adult carp, in breeding condition, were used to obtain the most "potent" pituitaries.

4. There is no apparent difference in the reaction of the fish to either frozen or acetone-dried glands. The ease with which the dried glands are prepared for injections makes them ideal to handle.

5. The gland of the female carp appears to be most effective in producing positive reactions in both sexes of fish.

6. Mammalian posterior pituitary extract proved ineffective in producing viable eggs. Investigators in the field of endocrinology have shown that using a pituitary preparation which is non-specific to the species upon which the experiment is conducted, is generally inefficacious.

7. The smallest concentration of pituitary that will produce desirable results is 0.25 of a gland.

8. Ether or ethyl carbamate (Urethane) can be used to inactivate the fish for injections and stripping when only one man is doing the work.

9. The most effective length of elapsed time before stripping should take place after injections is 24 hours.

10. Water temperatures are important, but are not the single controlling factor in determination of time of release of sex products in the experimental fish.

11. A volumetric count of eggs of the creek chub showed 88 eggs per cubic centimeter, or approximately 2,605 per ounce.

LITERATURE CITED

Bretschneider, L. H. and J. J. Duyvene De Wit
1947 Sexual endocrinology of non-mammalian vertebrates. Elsevier Pub. Co., Inc., New York, N. Y. 146 pp.

Dobie, J. R., O. L. Meehan, and G. N. Washburn
1948 Propagation of minnows and other bait species. U. S. Fish and Wildlife Service Circular 12, 1948. 113 pp.

Evans, Harold Muir
1940 Brain and body of the fish. Blakiston Co., Philadelphia, Penn. 164 pp.

Gerking, Shelby D.
1949 Urethane (ethyl carbamate) in some fishery procedures. U. S. Fish and Wildlife Service, Prog. Fish Cult. Jan. 1949, p. 73.

Harrow, Benjamin
1946 Textbook of biochemistry, 4th edition. W. B. Saunders, Co., Philadelphia, Penn. 564 pp.

Hasler, A. D., R. K. Meyer and H. M. Field
1939 Spawning induced prematurely in trout with the aid of pituitary glands of the carp. Endocrinology, Vol. 25, No. 6, Dec. 1939: 978-983.

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1940 The use of hormones for the conservation of muskellunge, Esox masquinongy immaculatus Garrad. Copeia, March 1940, No. 1: 43-46.

Hassler, A. D. and R. K. Meyer
1942 Respiratory responses of normal and castrated goldfish to telost and mammalian hormones. Jour. Exper. Zoo., Vol. 91: 391-404.

von Iherings, R. and eP. De Azevedo
1934 The curimata of the artificial lakes of the northeast (Brazil). Bio. Abst. 10, Pt. 2. (Aug.-Sept., Vol. 10, No. 7) 1936.

Reighard, Jacob
1920 The breeding behavior of the suckers and minnows. Part 1. Bio. Bull. Vol. XXXVII, No. 1, Jan. 1920.

Washburn, G. N.
1945 Propagation of creek chubs. Trans. Am. Fish Soc. 1945, Vol. 75: 336-350.

Young, J. Z., and C. W. Bellerby
1935 The response of the lamprey to injection of anterior lobe pituitary extracts. Jour. Exp. Bio. 12: 246-253.