# USE OF FISH PITUITARIES TO INDUCE SPAWNING IN CHANNEL CATFISH



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by

Kermit E. Sneed
University of Oklahoma Research Institute
Norman, Oklahoma

and

Howard P. Clemens
Zoology Department, University of Oklahoma
Norman, Oklahoma

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## ABSTRACT

Seventy-two pairs of channel catfish (Ictalurus punctatus) were induced to spawn in glass aquaria by injecting fish pituitary into the females. Control of peritoneal lesions, infections, and adhesions, was anticipated by including 10,000 units of crystalline penicillin-G with each injection.

Spawning was induced by injecting acetone-dried pituitaries from carp, buffalofish, flathead and channel catfish, and gar. Pituitaries from these various species differed little, regardless of time of collection. The amount of pituitary material required to induce spawning varied from 3 to 32 milligrams per pound of fish weight, given in from 1 to 28 injections, with an average of 3. Most females required 3 injections at 2 milligrams per pound of body weight every 24 hours. The period of time from the last injection to spawning varied from 2 1/2 hours to 72 hours, but averaged 16 to 24 hours.

In a recent paper (Clemens and Sneed, 1957) we pointed out the value of the channel catfish (Ictalurus puntatus) to the warm-water fisheries of the United States and described the behavior of this species when induced to spawn by the injection of fish pituitaries. Little information was given concerning the methods employed. Now, however, the accumulated data of 2 years' work[1/] warrant a report on the method, which appears to be suitable for large-scale commercial operations.

James W. Atz in a recent book (Pickford and Atz, 1957) reviewed the literature on induced spawning in fishes and stated that the pituitary method of inducing fish to spawn had been used on a commercial scale in Russia and South America, but only experimentally in the United States.

Recent interest in the catfishes as a commercial food fish to be grown in the ricefield reservoirs of Arkansas, Louisiana, Mississippi, and Texas has increased the need for more information concerning the spawning of these fish in order to produce sufficient numbers of fingerlings for stocking. Since it is very difficult to induce channel catfish to spawn in some hatcheries, we have tried to find a simple method that would ensure a sufficient supply of spawn when needed to stock the hatchery.

There are several advantages to induced spawning in hatchery operations: (1) The spawn can be obtained at a time convenient and desirable to the fish-culturist, thus allowing him to plan his work and to stock his waters according to a rigid schedule. The injections to a great degree eliminate the variables in the environment, such as spawning areas, temperature, light and other climatic conditions. (2) The spawning period can be altered within reasonable limits; i.e., earlier or later seasonal spawning, or all spawn may be taken in a shorter period. (3) Fish that will not spawn naturally sometimes can be induced to spawn. (4) Culture ponds can be stocked with eggs and fry that are more uniform in age and size. (5) The transmission of disease from brood stock to

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Note.--This paper was coauthored by Kermit E. Sneed, Research Institute, and Howard P. Clemens, Zoology Department, University of Oklahoma, Norman. Mr. Sneed's present address is U. S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Southeastern Laboratory, Marion, Alabama.

1/ This investigation was conducted under the Saltonstall-Kennedy Act for commercial fishery research. The contracts are held by the University of Oklahoma Research Institute.

offspring, and predation by adults can be minimized or eliminated.

Figure 1.--One of the more reliable characteristics of an imminent spawner is the raised portion of the body wall behind the pelvics.

Males of a size comparable to our smallest gravid females (12.6 inches, total length) contained motile sperms (as proved by many autopsies). Such males when paired with females of similar size successfully fertilized the eggs. Therefore, less attention was given to the characters of the males than the females, but males with prominent secondary sexual characteristics--a head wider than the body, darkly pigmented underjaw and belly, and large, protruded genital papilla--usually possessed better-developed testes (fig. 3). Such males were sometimes used successfully a second time, whereas those with poor secondary sexual characters did not completely fertilize a second spawn, although they participated in the second spawning act.

## COLLECTION AND CARE OF BROOD STOCK

Some of the brood fish used for pituitary-induced spawning were collected from the natural waters of Oklahoma in 1955 and 1956 and kept in hatchery ponds until used in 1957. Other catfish were collected from natural waters shortly before spawning time in 1957. The brook stock kept in the hatchery was fed a diet of one-third liver and two-thirds dry pellets which were about 36 percent protein. Early experiments showed that fish fed only pellets did not exhibit good gonadal development and were more than normally susceptible to gill diseases and parasites.

Figure 2.--The opening of the oviduct in female channel catfish is immediately posterior to the anus and separate from the more posterior urinary opening. In the breeding season folds of skin on each side of the uro-genital openings are raised and divided by a groove.

Ordinarily, in regular hatchery procedures, the injection of pituitary materials merely precipitates the spawning act and does not induce spawning if the female is not already in or near a spawning condition; therefore, the care and selection of females is extremely important, as will be pointed out in greater detail later. The best morphological characters indicative of spawning condition in the female are a well-rounded abdomen, the fullness of which should extend posteriorly past the pelvics to the genital orifice (fig. 1). The ovaries should be palpable and soft, and the genitals raised and reddish (fig. 2).

## FACILITIES FOR SPAWNING

Fish were induced to spawn in glass aquaria with capacities ranging from 5 to 50 gallons. Metal trout-rearing troughs, divided into compartments, were also used, but they were considered less satisfactory because the fish jumped against the divider and cover screens and injured themselves much more than did those in aquaria. In addition, only 1 female spawned of 13 females placed in metal troughs and injected with pituitary material that was proved to be potent in other experiments. Successful use of troughs was suggested by another

experiment, however, in which three pairs were provided with small barrels in which they could spawn. Two of the three pairs spawned and each pair used the barrel. Both facilities were in the holding house of the U. S. Fish Cultural Station, Tishomingo, Oklahoma, where workers and visitors frequently passed them. Each facility was supplied with flowing water. Tar-paper mats were placed on the bottom of each aquarium so that the eggs could be easily removed to a mechnical hatching trough.

## COLLECTION, PRESERVATION, AND STORAGE OF PITUITARIES

Pituitaries of carp (Cyprinus carpio), buffalofish (Ictiobus spp.), flathead catfish (Pilodictis olivaris), channel catfish (Ictalurus punctatus), and gar (Lepisosteus spp.) were used to induce spawning in channel catfish. Several other species were used, but the limited data do not permit positive conclusions other than to suggest that the pituitaries of the fresh-water drum (Aplodinotus grunniens) and river carpsucker (Carpiodes carpio) may be inactive or have low gonadotropic activity in channel catfish.

The fish from which the pituitaries were obtained were not classified as to sex and state of sexual maturity, but the month of collection was recorded. Only those fish believed to be large enough to be sexually mature were used as donors. The minimum size of the fish approached the adult size as determined by age and growth studies of fish in Oklahoma. Small fish do not yield a pituitary of sufficient size to make collection worthwhile.

Pituitaries were removed and placed in acetone soon after death of the donor to prevent enzymatic deterioration of the gonadotropic hormones. Four changes of acetone in the ratio of 12 of acetone to 1 of pituitaries 12 hours apart were sufficient for dehydration. After dehydration, the acetone was decanted and the pituitaries were air dried and stored in vials in a cool, dry place in the laboratory. Pituitaries properly collected and dried retained their activity for at least a year (our longest data period) and according to Hasler, Meyer, and Wisby (1950) for as long as 10 years. Presumably they will last much longer, but data are not available at present. For other methods of handling pituitaries, see the review of Pickford and Atz (1957, pp. 264-265).

## USE OF PITUITARIES

### Preparation and Injection

The pituitaries were weighed, finely ground, and mixed with either distilled water or physiological saline. Usually the carrier was distilled water to which penicillin-G was added. Each female was injected with about 1 cc. of water, 10,000 units of penicillin, and the appropriate amount of pituitary material. In the early experiments before we used penicillin, inflammatory abdominal lesions were common. These peritoneal lesions led to intestinal adhesions and often to infections which resulted in the death of the test fish. Similar results have been reported by Pickford (Pickford and Atz, 1957, p. 265) when unfiltered suspensions of acetone-dried pituitaries were used. However, when we included penicillin, no inflammation resulted even after 20 or more injections. Other antibiotics, such as dihydrostreptomycin, S-R-D (a mixture of penicillin and dihydrostreptomycin), and tetracin were occasionally used, but our data are not sufficient to prove that any of these is superior to penicillin-G. The technique otherwise was not sterile. Usually the pituitary material was injected into the body cavity immediately behind a pelvic

Figure 3.--Male channel catfish can be distinguished from the female by the tubular nature of the external genitalia.

Table 1.--Female channel catfish of comparable gonadal development injected with different amounts of carp pituitaries.

[I = Injected; S = Spawn; D = Died; Ⓘ = Altered frequency or incorrect dose; O = Ovlutating; H = Handstripped]

| Milligrams per pound body weight | Dates of injections made in June | | | | | | | | | | | Number of injections | Total milligrams pituitaries per pound body weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | | |
| Group I | I | | S | | | | | | | | | 1 | 8 |
| (8 mg/lb/96 hrs) | I | | S | | | | | | | | | 1 | 8 |
| | I | | | | I | | S | | | | | 2 | 16 |
| | I | | | | I | | Ⓘ | | Ⓘ | | S | 4 | 32 |
| Group II | I | | | I | | | ID | | | | | 3 | 18 |
| (6 mg/lb/72 hrs) | I | | | I | | S | | | | | | 2 | 12 |
| | I | | | I | | D | | | | | | 2 | 12 |
| | I | | D | | | | | | | | | 1 | 6 |
| Group III | I | | I | Ⓘ | S | | | | | | | 3 | 12 |
| (4 mg/lb/48 hrs) | I | | I | Ⓘ | S | | | | | | | 3 | 12 |
| | I | S | | | | | | | | | | 1 | 4 |
| | I | 1/4Ⓘ | I | S | | | | | | | | 3 | 9 |
| Group IV | I | I | I | I | S | | | | | | | 4 | 8 |
| (2 mg/lb/24 hrs) | I | I | I | O | H | | | | | | | 3 | 6 |
| | I | I | I | I | I | I | D | | | | | 6 | 12 |
| | I | D | | | | | | | | | | 1 | 2 |
| Group V | I | II | II | S | | | | | | | | 5 | 5 |
| (1 mg/lb/12 hrs) | I | II | II | S | | | | | | | | 5 | 5 |
| | I | II | II | S | | | | | | | | 5 | 5 |
| | I | II | S | | | | | | | | | 3 | 3 |

fin, but sometimes it was injected through the abdomen or into the muscles of the back. We preferred the area just behind the pelvic fin (fig. 4). The muscles of this region closed the needle wound and prevented the loss of materials more effectively than did other areas, and early experience indicated that inflammatory ulcers were less likely to occur with intraperitoneal injections.

Figure 4.--Female is held in a plastic screen net while pituitary injections with penicillin-G is being administered by glass hypodermic syringe and a No. 20 needle into the body cavity near the base of the pelvic fin.

## Frequency, Number, and Size of Injection

To determine the relation between frequency, size, and number of injections required to induce spawning in channel catfish, 20 females that were considered equal in gonadal development were selected and divided into 5 groups. 2/ The test fish were injected with carp pituitaries at one of the following dosages: Group I, 8 milligrams per pound of body weight each 96 hours (8 mg/lb/96 hrs); Group II, 6 mg/lb/72 hrs; Group III, 4 mg/lb/48 hrs; Group IV, 2 mg/lb/24 hrs; and Group V, 1 mg/lb/12 hrs (table 1). If these dosages are prorated on a 24-hour basis, it is obvious that each female in the experiment received 2 milligrams per pound of weight each 24 hours, even though the dosage and interval of injection was different in the 5 groups.

The death of 4 fish from various injuries (male bites, handling) and 5 instances of altered frequencies due to experimental errors limit evaluation of the data, but these conclusions may be drawn: (1) Fish in every dosage category spawned; therefore, the frequency and size of the dosage were probably not critical within the limits of this experiment. (2) The total amount of pituitary materials used to induce spawning in a fish varied from 3 milligrams to 32 milligrams per pound of body weight. (3) With the exception of 1 fish, which spawned with 1 injection of 4 milligrams per pound of body weight, the fish that received the greatest number of injections (5) at the lowest dosage (1 mg/lb) spawned with the least amount of pituitary material. These data suggest that the gonadotropic level necessary to produce spawning was maintained more effectively by low dosages frequently injected than by higher dosages less frequently given. The fish in other categories had received at least 6 milligrams per pound by the time these fish had received 5 milligrams, and only 3 had spawned. Low doses frequently injected may save pituitary materials, but the saving is not very significant. On the other hand, high doses require less frequent injections and may be more convenient for the fish-culturist.

## SUCCESS OF SPAWNING FOLLOWING INJECTIONS

During the 1957 season (May through July), 40 percent of the females that spawned had received 6 milligrams or less of pituitaries per pound of body weight, and 80 percent had received 12 milligrams or less. To some extent these figures reflect the procedure used, since most of the fish were given 2 milligrams of pituitary per pound of body weight in each injection, and most fish responded positively after 3 injections (table 2).

Twenty females were selected for hormone spawning from adults collected from the Red River below Lake Texoma. Thirteen females were injected with carp pituitaries (Nos. 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 20; table 2), 2 with flathead pituitaries (Nos. 44, 45), 2 with channel catfish

2/ Control groups were usually not set up at this period in our investigations, since many previous, controlled experiments had indicated that the environmental requirements of the channel catfish are so strict that they will not spawn in small aquaria without pituitary injections. We have never had uninjected fish spawn in aquaria.

Table 2.--Spawning of female channel catfish following pituitary injections, according to donor species, 1957.

[ I = Incomplete spawning; C = Complete spawning ]

| Serial number and weight of test fish | Date of pituitary collection | Injections | | | | Interval between last injection and spawning (hours) | Spawning | |
|---|---|---|---|---|---|---|---|---|
| | | Initial | Number | Time between (hours) | Quantity (mg/lb) | | Date | Completeness |
| Carp: | | | | | | | | |
| 1 - 1.96 | Nov. | July 8 | 3 | 24 | 2 | 18 | July 11 | I |
| 2 - 1.3 | Apr. | June 28 | 2 | 48 | 2 | 28 1/2 | July 1 | C |
| 3 - 0.7 | Dec. | July 6 | 2 | 24 | 2 | 20 | July 8 | I |
| 4 - 1.3 | Dec. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 5 - 2.4 | Nov. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 6 - 2.2 | Nov. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 7 - 1.8 | Nov. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 8 - 2.37 | Nov. | July 5 | 3 | 24 | 2 | 20 | July 8 | I |
| 9 - 1.8 | Nov. | July 5 | 3 | 24 | 2 | 24 | July 8 | C |
| 10 - 2.0 | Apr. | July 5 | 3 | 24 | 2 | 24 | July 8 | C |
| 11 - 0.5 | Nov. | July 6 | 4 | 24 | 2 | 16 | July 10 | C |
| 12 - 2.3 | Nov. | July 8 | 3 | 24 | 2 | 16 | July 11 | C |
| 13 - 1.5 | Nov. | July 8 | 3 | 24 | 2 | 16 | July 11 | C |
| 14 - 1.2 | July | July 22 | 3 | 24 | 2 | 3 | July 25 | C |
| 15 - 1.5 | July | July 22 | 3 | 24 | 2 | 3 | July 25 | C |
| 16 - 1.5 | July | July 22 | 3 | 24 | 2 | 16 | July 25 | C |
| 17 - 1.2 | July | July 2 | 3 | 24 | 2 | 16 | July 25 | C |
| 18 - 2.0 | July | July 22 | 3 | 24 | 2 | 16 | July 25 | C |
| 19 - 1.2 | July | July 22 | 3 | 24 | 2 | 16 | July 25 | C |
| 20 - 1.6 | July | July 8 | 3 | 24 | 2 | 16 | July 11 | C |
| 21 - 2.2 | ---- | June 25 | 3 | 24 | 2 | 16 | June 29 | C |
| 22 - 1.0 | ---- | June 13 | 5 | 12 | 1 | 13 | June 15 | C |
| 23 - 1.0 | Apr. | June 10 | 1 | | 3 | 18 | June 11 | C |
| 24 - 1.4 | Apr. | June 10 | 2 | 96 | 1/2 | 17 | June 15 | C |
| 25 - 1.5 | ---- | June 13 | 4 | 24 | 2 | 13 | June 17 | C |
| 26 - 1.4 | ---- | June 13 | 2 | 48-24 | 4 | 12 | June 17 | C |
| 27 - 2.8 | ---- | June 13 | 3 | 48-24 | 4 | 12 | June 18 | C |

Table 2.--Spawning of female channel catfish following pituitary injections, according to donor species, 1957. Cont'd:

| Serial number and weight of test fish | Date of pituitary collection | Injections | | | | Interval between last injection and spawning (hours) | Spawning | |
|---|---|---|---|---|---|---|---|---|
| | | Initial | Number | Time between (hours) | Quantity (mg/lb) | | Date | Completeness |
| 28 - 2.8 . . . . | Jan. | June 13 | 1 | | 4 | 39 | June 15 | C |
| 29 - 2.9 . . . . | ---- | June 13 | 2 | 72 | 6 | 12 | June 18 | C |
| 30 - 1.6 . . . . | Jan. | June 13 | 1 | | 8 | 39 | June 15 | C |
| 31 - 1.1 . . . . | Jan. | June 13 | 1 | | 8 | 39 | June 15 | C |
| 32 - 2.0 . . . . | ---- | June 4 | 3 | 72-24 | 1 | 15 | June 9 | C |
| 33 - 1.5 . . . . | ---- | June 10 | 1 | | 2 | 36 | June 12 | C |
| 34 - 3.4 . . . . | ---- | June 13 | 5 | 12 | 1 | 12 | June 16 | I |
| 35 - 3.6 . . . . | ---- | June 13 | 3 | 48-24 | 4 | 12 | June 16 | I |
| 36 - 1.4 . . . . | ---- | June 13 | 5 | 12 | 1 | 13 | June 16 | I |
| 37 - 1.9 . . . . | ---- | June 13 | 4 | 96-48-48 | 8 | 48 | June 23 | C |
| 38 - 1.3 . . . . | ---- | June 13 | 2 | 96 | 8 | 48 | June 19 | C |
| 39 - 2.3 . . . . | ---- | May 27 | 3 | 24 | 0.5-1.0-1.5 | 16 | May 31 | C |
| 40 - 1.0 . . . . | ---- | June 13 | 3 | 12 | 1.3 | 13 | June 15 | C |
| 41 - no data . . | ---- | May 25 | 3 | 24 | 1-2-3 | 24 | May 30 | C |
| Flathead catfish: | | | | | | | | |
| 42 - 4.3 . . . . | Dec. | June 25 | 11 | Irregular | 1/ | 3 | July 8 | C |
| 43 - 3.4 . . . . | Dec. | June 19 | 11 | " | 2.3 | 21 | July 5 | C |
| 44 - 3.2 . . . . | July | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 45 - 1.6 . . . . | July | July 8 | 3 | 24 | 2 | 16 | July 11 | C |
| 46 - 3.3 . . . . | ---- | June 19 | 11 | Irregular | 3 | 21 | July 5 | I |
| 47 - 3.7 . . . . | ---- | June 25 | 3 | 48-24 | 4 | 16-7 1/2 | June 28 | C |
| Channel catfish: | | | | | | | | |
| 48 - 2.2 . . . . | Feb. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 49 - 3.4 . . . . | Feb. | July 5 | 4 | 24 | 2 | 18 | July 9 | C |
| 50 - 2.4 . . . . | ---- | June 25 | 2 | 24 | 4 | 17 | June 28 | I |
| 51 - 2.7 . . . . | ---- | June 25 | 3 | 48-24 | 3.2 | 24 | June 28 | C |
| Gar: | | | | | | | | |
| 52 - 2.3 . . . . | July | June 19 | 2 | 48 | 3 | 16 | June 22 | C |

Table 2.--Spawning of female channel catfish following pituitary injections, according to donor species, 1957. Cont'd:

| Serial number and weight of test fish | Date of pituitary collection | Injections | | | | Interval between last injection and spawning (hours) | Spawning | |
|---|---|---|---|---|---|---|---|---|
| | | Initial | Number | Time between (hours) | Quantity (mg/lb) | | Date | Completeness |
| Buffalofish: | | | | | | | | |
| 53 - 2.1 . . . . | Jan. | June 25 | 11 | Irregular | 4 | 12 | July 6 | C |
| 54 - 3.0 . . . . | Dec. | June 19 | 12 | Irregular | 3 | 21 | July 5 | C |
| 55 - 1.4 . . . . | Nov. | July 5 | 3 | 24 | 2 | 20 | July 8 | C |
| 56 - 1.3 . . . . | ---- | July 8 | 3 | 24 | 2 | 15 | July 11 | C |
| 57 - 1.7 . . . . | Dec. | June 19 | 6 | 48-48-48-48-24 | 3 | 16 | June 30 | I |
| 58 - 0.9 . . . . | ---- | June 21 | 4 | 48 | 5 | 2 | June 27 | C |
| Combined species: 2/ | | | | | | | | |
| 59 - 5.0 . . . . | Various | April 9 | 28 | Irregular | Various | 14 | May 12 | C |
| 60 - 2.0 . . . . | ---- | May 29 | 2 | 24 | " | 21 | June 1 | C |
| 61 - 2.6 . . . . | Various | May 11 | 12 | Irregular | " | 21 | May 31 | I |
| 62 - 1.6 . . . . | ---- | May 26 | 8 | 24 | " | 3 days | June 6 | I |
| 63 - 3.5 . . . . | Various | May 26 | 8 | Irregular | " | 48 | June 5 | I |
| 64 - No data . . | ---- | May 27 | 3 | 24 | " | 48 | May 30 | C |
| 65 - 2.5 . . . . | ---- | June 19 | 6 | Irregular | " | 15 | June 29 | C |
| 66 - 1.0 . . . . | ---- | July 13 | 10 | " | " | 24 | July 24 | C |
| 67 - 1.3 . . . . | July | June 19 | 21 | " | " | 28 | July 14 | C |
| 68 - 1.2 . . . . | May | June 19 | 22 | " | " | 12 | July 14 | C |
| 69 - 2.0 . . . . | Nov. | June 25 | 15 | " | " | 18 | July 12 | C |
| 70 - 2.2 . . . . | ---- | June 26 | 6 | " | " | 6 | July 4 | C |
| Combined pituitaries: 3/ | | | | | | | | |
| 71 - 1.8 . . . . | Several | July 13 | 3 | 24 | 2 | 9-16 | July 17 | I |
| 72 - 0.9 . . . . | Several | July 13 | 5 | 24 | 2 | 2 1/2 | July 17 | C |

1/ 1.2/lb for 9; 4/lb for 2.
2/ Pituitary material from two or more species injected on different days.
3/ A mixture of pituitary material from 7 species.

pituitaries (Nos. 48, 49), and 2 with buffalofish pituitaries (Nos. 55, 56). All fish received the same dosage, 2 milligrams per pound of body weight each 24 hours. One female escaped, but all other fish spawned. One female spawned after 2 injections, 17 after 3 injections, and 1 spawned after 4 injections.

A study of these data suggest several possibilities: (1) The recipient females were in similar physiological condition and the pituitaries were about equal in potency. (2) The fish were in similar physiological condition and the pituitaries differed in potency, even though all were active enough to produce spawning. (3) The fish differed in physiological condition and the pituitaries were equal, and therefore the condition of the female is relatively unimportant. (4) The females were not in similar physiological condition, but differences in pituitary potencies compensated for differences in the fish. Actually the accumulated data and experience of 3 years' work on channel catfish and other species lead us to believe that the physiological condition of the recipient is more variable than the gonadotropic activity of the pituitaries of the various donor fish.

Time of Response

The interval of time between the last injection and the spawning act varied from 2 1/2 hours to 48 hours, with the exception of 1 fish which spawned after 72 hours. However, in the cases of short intervals of response the fish might have spawned without the last injection. Three fish spawned 39 hours after a single injection and another spawned 18 hours after 1 injection. Here again the physiological condition of the recipient seemed to be the important factor in early spawning, since the 1-injection fish and the early spawners were usually selected and marked "good" previous to the beginning of the injections. Most of the fish spawned 16 to 24 hours after the last injection. No relation to time of day was evident, as the fish began to spawn at practically all hours. However, more prespawning behavior was noted during the hours of darkness, which suggested to us that in future work the period of response and number of injections might be reduced by keeping the paired fish in darkness. However, in fish-hatchery practice this technique would be inconvenient, but might be of interest from a biological point of view.

Induced Spawning of Catfish Refractive to Usual Hatchery Procedures

In 1956 we attempted to induce spawning without pituitary injections in catfish that were collected in 1955 and reared under hatchery conditions. Although these fish appeared to have well-developed gonads, they failed to spawn. No eggs were obtained from 30 pairs stocked in regular hatchery pens, although 3 pairs obtained from the Red River during late May 1956 did spawn within 2 weeks when placed in hatchery pens. Also, only 5 of 30 comparable pairs responded to pituitary injection in aquaria. Now, however, we believe that part of this failure was due to infections which can be reduced by routine use of penicillin.

Verbal reports from workers in both Arkansas and Nebraska indicate that wild catfish brought into the hatchery do not make good brood fish the first year, whereas fish taken from natural waters shortly before spawning time will usually spawn. Marzolf (1957) reported a similar situation in Missouri and assumed that the wild fish needed a period of 1 to 2 years to become acclimated to pond conditions. By 1957, our brood stock had been in the hatchery 2 or more years, and the spawning success in hatchery spawning pens (without pituitary injections) was considerably better than with the same fish in 1956. Of 31 pairs stocked in pens, 22 pairs spawned. The management of this brood stock and the feeding was not significantly different from that practiced in 1955-56.

On June 25, 1957, 8 females were recovered from the outdoor spawning pens and brought into the holding house. These females had been stocked in spawning pens for various lengths of time (10-21 days) but these fish were refractory to the pen treatment and had not spawned, probably because of water temperatures at this time (84° - 86° F.). They were injected with various kinds and combinations of pituitary materials (fish Nos. 21, 42, 47, 50, 51, 53, 69, 70; table 2) and all fish eventually spawned. The lowest number of injections was 2, the highest 15. The number of injections required for spawning is not very significant since the fish were used in different experiments and received various

kinds of pituitaries at different intervals of time. The significant point is that these fish were spawned with pituitary injections after they had proved refractory to regular pen treatment.

On July 5, 1957, a number of unspawned females were obtained from the draw tubes of the power plant at Lake Texoma. Twenty of these were selected for pituitary injections. One female escaped during the experiments, but the other 19 were induced to spawn. Three of the 19 spawned only part of their eggs, but the remaining 16 spawned completely. One female spawned after 2 injections. All fish were injected with the same dosage, 2 milligrams per pound of fish weight each 24 hours, but various donor species were used. It appears that these fish were particularly receptive to pituitary injections, and therefore spawned readily.

Two weeks later, July 18, about 40 unspawned females were obtained from the same source. Autopsies of a few of these fish revealed that deterioration of the eggs had begun in some fish. The eggs had lost their gloss and some were whitish in color. We selected the most promising of these females and injected them with pituitaries. A total of 28 was injected, but only 6 spawned successfully (Nos. 14, 15, 16, 17, 18, 19; table 2), which suggested that in southern Oklahoma the optimum spawning time had passed by mid-July.

Degree of Spawning Success

The degree of spawning success increased as the spawning season progressed and new information was gained. We attempted to induce spawning in 124 females with pituitary materials. Fifty (40 percent) of these females did not spawn. Of these, 10 were abandoned as being in poor reproductive condition, 7 escaped due to improperly constructed aquarium lids, 12 died of injuries inflicted by the male, 5 died with infections (before the routine use of penicillin was adopted), 2 were overinjected with streptomycin, 2 were autopsied for information on gonadal condition, 6 died unaccountably, and 6 died from handling, bruises, and glass cuts (broken aquaria). It is obvious that the unsuccessful attempts may be categorized as incomplete sexual development in the female, infections due to injections and male bites, and human and mechanical faults, all of which appear to be correctible in future work.

BEHAVIOR OF MALES

Many of the females of the late group were severely bitten by the males, which accounted for several deaths. The males appeared to be more belligerent toward the females than in any previous experiment. One possible explanation of the increased aggressiveness of the males was the physiological condition of the females. Most of our observations suggest that when the female is receptive and is easily induced to spawn with pituitary injections the male does not bite her severely. Losses of females due to male bites occurred mostly early in the spawning season (May) and late in the season (late July). Very few died from this cause from June through mid-July.

Only one experiment with one female was conducted to determine the possible reason for abnormal male aggressiveness. This female was paired with several males, all of which vigorously attacked her. She was then placed by herself and injected with pituitaries. After about 20 hours she was similarly tested with several males, with results as before. She was given a second injection and the next day again paired with several males. The males did not attack her and she was placed with the original male that had been selected for her. Normal spawning occurred after the third injection. These observations have led us to believe that the physiological condition of the female, as related to spawning, in a large measure influences the behavior of the male. Perhaps, in future work, the losses due to male bites can be reduced by injecting the female with one or two doses of pituitaries before pairing her with a male.

It is not necessary for the male to bite the female to achieve normal spawning. Many of the females that successfully spawned did not show any marks caused by male bites. Some were only slightly marked by the males' teeth. In fact, there appeared to be some correlation between ease of spawning and lack of male bites; i.e., the more susceptible the female was to induced spawning the less severe the bites, whereas in the case of unripe females and females delayed beyond the normal spawning

season, the bites were often serious enough to produce death. Identical behavior occurs in channel catfish that spawn in lakes, creeks, and hatchery ponds; and even here sometimes the male bites are severe enough to cause the death of the female.

## RECOMMENDED PROCEDURES

To induce spawning in channel catfish the following principles and precautions should be observed:

a. Brood stocks, particularly the females, should receive good care and food from one spawning season to the next. If it is necessary to obtain new brood stock from wild waters, it is best to do so immediately before or during the natural spawning season.

b. Only well-developed females nearly ready to spawn should be used. Hold poorly developed or "hard" females in earthen ponds until later in the season. Optimum feeding must be continued in the ponds, but no food is necessary while fish are spawning in aquaria, even though the male will often accept food until the beginning of the spawning act.

c. Acetone-dried pituitaries from carp, flathead and channel catfish, gar, and buffalofish are satisfactory. River carpsucker and fresh-water drum are not recommended, but other fish pituitaries which we have not tested may also be satisfactory.

d. The pituitaries should be finely ground and mixed with 1 cc. of distilled water and 10,000 units of penicillin per dose.

e. The dosage recommended is 2 milligrams of pituitaries per pound of body weight each 24 hours. Dosages may be increased or decreased according to the condition of the fish; i.e., soft, ripe females require less pituitary material. Ripe females will usually respond to the third injection, but ought not to be abandoned as hopeless until after 10 or more injections, and only then if the spawning facilities are needed for more desirable females.

f. Males selected need not be injected, but are approximately the size of the females with which they will be paired.

g. If the male severely bites the female, it is probably best to remove him immediately and keep the pair separated until after the female has received one or two injections, when he can be placed with the female again.

h. Aquaria with at least 2 glass sides appear to be in the most satisfactory spawning containers. No sand or other substrate is necessary, although a tar-paper or plastic mat may be used if the eggs are to be moved to a mechanical hatching device.

i. The male may be left to attend the eggs in the aquarium; or, if spawning facilities are scarce, the eggs can be hatched in a mechanical hatching trough.

## SUMMARY

1. Seventy-four pairs of channel catfish were induced to spawn in glass aquaria with fish pituitary injections. The first injected female spawned when the water temperature was 65° F., 3 weeks before the first spawning in conventional outdoor pens when the temperature was 72° F. The first induced spawning was on May 12 and the last on July 25.

2. Ten-gallon glass aquaria were the most satisfactory containers for spawning fish up to 2 1/2 pounds in weight. Larger fish, of course, required proportionally larger containers.

3. Peritoneal lesions, infections, and adhesions which often accompany intraperitoneal injections were controlled by including 10,000 units of crystal line penicillin-G with each injection. Before the routine use of penicillin, more than 30 percent of the deaths of injected females were attributed to body-cavity infections.

4. Spawning was induced in channel catfish by injecting pituitary material from carp, buffalofish, flathead and channel catfish, and gar into the female. Little difference was apparent in the potency of the pituitaries from these different species, regardless of month of collection.

5. The total amount of acetone-dried pituitary material required to induce spawning in channel catfish varied from 3 to 32 milligrams per pound of body weight of the

fish. Most females required 6 milligrams per pound (i.e., 3 injections at 2 milligrams per pound of body weight).

6. The total number of injections required for spawning varied from 1 to 28 with the average being 3. Five fish spawned with 1 injection, 8 with 2, 35 with 3, 6 with 4, 4 with 5, 3 with 6, and 13 with more than 6 injections.

7. Fish spawned when injected with 1 milligram of pituitary material per pound of body weight each 12 hours, with 2 milligrams per pound each 24 hours, and with increasing doses up to 8 milligrams per pound each 96 hours.

8. The period of time from the last injection to spawning varied from 2 1/2 hours to 72 hours. Most fish began spawning within 16 to 24 hours following the last injection.

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