# The Influence of Sex Hormones on the Reproductive Organ of a Sôgyo "*Ctenopharyngodon idellus* (*C. and V.*")*

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## Introduction

Sôgyo, *Ctenopharyngodon idellus* (*C. & V.*) is a fresh-water fish which belongs to *Cyprinidae*. Although this fish stands above all other raised fishes, because of its good taste, its rapid growth and its food mainly consisting of weeds, there has been no report that the fish propagated in a fish farm. The author is now in a position to report the result of his nearly nine-year research starting in 1940, to promote the growth of the sexual glands of *C. idellus* with hormones.

In spite of the fact that *C. idellus* attains maturity at the age of four in an average condition in Chinese rivers, each sexual gland of four five-year-old *C. idellus* raised in the fish farm of my experiment in Japan remained so immature that it was hardly recognizable by the author.

Here the questions were raised whether the immaturity of the sexual glands of *C. idellus* in a farm was caused by food, by the water temperature or by any other conditions in the farm. Moreover, the spawning season of this fish in Japan was quite unknown at that time. Accordingly, the author met many unexpected difficulties in the course of this research.

## Fish Farm Employed and Materials Used

a. Pond.

In April, 1940, the Nakamura Fish Farm was designated as the experimental fish farm. In this farm, more than 150 fishes were raised during five years after the delivery in 1935 which were imported from China by the Shiga Prefectural Fisheries Experimental Station. Mr. Tôji Nakamura, owner of the farm, willingly allowed the experiment to be performed on his fish in the pond. At first, a 18-square-metre pond was established in the green house. That was for the purpose of adjusting the water temperature. However, the water-temperature in the green house was higher by 1–2°C than that in the outside and besides, the pond was too small to raise the fish. So after a half month, a mud-bottomed pond which was 600 square-meters and 0.6–1.0 m. in depth, was used.

*Contribution from the Ichthyological Research Institute, near Shimoda, Shizuoka Prefecture, Japan.

The water-temperatures in this new pond were 17° to 18°C in spring, 25°C at maximum in summer and approximately 5°C in winter and ice never formed. This test pond had already been used for raising *C. idellus* since 1935. On the other hand, a 1000-square-meter pond was adopted as a pond for the control experiment.

b. Fishes used.

Of the 150 fishes previously mentioned, 70 were set free into the test pond, while others were put into the control pond. In the experiment of 1940, the fishes were five years old ranging from 1.0 to 1.2 kg. in weight, and from 50 to 60 cm. in length. At the end of the experiment in 1949, the differences ranged from 3, 5 to 5, 9 kg. and from 65 to 85 cm. It was impossible to distinguish between a male and a female at a glance. Vegetables, weeds, chrysalis of silk worms, Asari (*Paphia philippinarum*) and fu (light cake made of wheat gluten) were supplied as food materials.

c. Hormones.

The hormones used were produced carefully by the author himself and determined its unit correctly by means of biological test and also according to the measurements of the melting point of crystals in the Nippon Science Pharmacy. The hormones were dispersed into distilled water (*Hydrosol*) and contain 15 c. u. of Androsteron and 1,000 m. u. of Estron in 1.0 cc. of the water respectively. The quantity of the hormones applied to the fish per kg. in weight were 0,75 c. u. of Androsteron and 25 m. u. of Estron. After being transfered to the larger pond, the fish were supplied with these sex hormones, prepared into fu (food material) when they were hungary.

Therefore, the hormones were supplied effectively through their mouths.

On the other hand, the control fish were supplied with the same food but without hormones. And besides, at the time of maturity the females were injected with the emulsion of the anterior lobe of the hypophysis. About this matter, the author will explain later.

## Method of Investigation

It being difficult to gather the fish for injection every day from such a large pond as 600 square meters, the hormones were supplied by the method above mentioned. This supply of the hormones with food materials was carried out almost every day once or twice a day from 1940 to 1944 except when the fish did not take food in winter. Since 1945, the supply was continued during the period from March to July. Thus gonads were remarkably developed, but not more than about 200 eggs came from the abdomen of each female when it was pressed, while males ejected the sperm with ease when it was pressed. Then the author tried to promote the growth of the ovary through giving 100 m. u. of Estron only for the fishes per kg. from 1944.

The method adopted for the artificial fertilization was the dry method. However, when the eggs met with the sperm which was diluted with physiological salt solution or buffer solution, the secretion of mucus was too much and besides that was coagulated, so this method has proved to be unsuccessful. Then it was discontinued.

On May 28, 1944 in an attempt to promote the growth of the gonads of the fish to accelarate their spawning, the essential solution extracted three times out of a fine-cut, dried anterior lobe of the hypohysis of the bull in 10 cc. of Ringer's solution was used. One cc. of the first extracted solution was injected into five fishes and then two cc. of both the second and the third extracted solutions were injected in the same way.

On July 28, 1946, the emulsion of the anterior lobe of the hypophysis taken out of 12 carps, ranging from 1,0 to 1,2 kg. in weight, by using 12 cc. of physiological salt solution was injected into five female fishes.

In July 1948, the essential solution extracted out of the anterior lobe of the hypophysis of 20 sharks (*Squalus sucklii*) by 16 cc. of Estron solution (1 cc. 1000 m. u.) was injected in the fully-grown fishes, four of them were male and four females.

## Experimental Process

The first experiment was begun on April 19, 1940 by dissecting four fishes (Experimental numbers 1–4, Table 1). It being very difficult to find their immature sexual glands at that time, the author hardly realized that semi-transparent strings of 2 to 3 mm. in diameter running along their air bladders were the sexual glands. Only the histological section could distinguish the male gonads from the female ones. In the first year, eight fishes (No. 1–8) were dissected.

Although some (No. 3) had perceptable eggs, most of their sexual glands remained semi-transparent and string-like as before, showing no effect of the hormones. (see Table 1 and 2) in the first year.

In 1941, the fishes (No. 9–20 in Table 1) had grown fully and their sexual glands were recognized very clearly when dissected, but immature eggs in various shapes were found to be numerous. So the author came to the conclusion that the growth of the fishes did not necessarily go along with that of the sexual gland. The ejection of the sperm through the pressure on the abdomen told the effect of hormones. In 1942, on July 21, five fishes out of 24 ejected sperm by pressure on the abdomen. Dissecting two fishes (No. 21–22), the author realized that their sexual glands had grown as long as 1 cm. in the long way (c. f. The control fishes in Table 2).

Considering the fact that in 1943 the effect of the hormones become remarkable in the males and the ejection of the sperm was good, only Estron solution was decided to be given from May. By July, the males (No. 23–28) did not eject

sperm and the females gave out few eggs when pressure was borne upon their abdomens. Their sexual glands, both male and female, had grown as long as 1, 5 to 2 cm in the long way.

**Table 1.** Results of experiments.

Conditions of reproductive organs of the fish are explained by the signes as follows:

a……fully matured. b……matured c……developed
d……undeveloped. ***……much ejected. **……ejected
*……a few ejected.

| Date | No. exper. | Body weight g. | ♂ | ♀ | uncer-tain | Remarks |
|---|---|---|---|---|---|---|
| 1940 IV 10 | 1–8 | 1,205–1,337 | d. d. | d, c, d, | 3 | |
| 1941 IV 10 | 9–20 | 1,620–3,650 | b*,b*,b*,b*, | b, c, c, c, c, b, d, | 1 | |
| 1942 VI | 21–22 | 2,359–2,505 | b* | 0 | | |
| 1943 V | 23–26 | 2,490–2,615 | a**, a** | b*, b* | | |
| VII | 27–28 | 2,665–2,915 | a*** | b*, | | |
| VIII | 29–30 | 2,870–4,120 | a*, | b*, | | Pearl organ ? |
| IX | 31–32 | 3,107–3,235 | a, | b* | | P. o. disapp. |
| 1944 V | 33–34 | 2,970–3,107 | a*** | a | | Pearl organ |
| VI | 35–38 | 2,887–3,255 | a***, a** | a**, a* | | Inject hypo. of bull. |
| VII–VIII | 39–40 | 2,865–3,055 | a** | a** | | Arti. fertil. Arti. fertil. |
| 1945 V–VI | 41–44 | 3,025–3,462 | a***, a*** | a*, a* | | |
| 1946 V–VI | 45–48 | | a***, a* | a*, a* | | Inject hypo. of carp. |
| 1947 IV–VI | 49–53 | 2,888–3,655 | a**, a* | a*, a*, a*, | | Arti. fertil. |
| 1948 IV | 54–55 | | a*** | a* | | Inject. hypo. of shark. |
| 1949 VII | five fishes set into Lake Biwa (See Table 4). | | | | | |

One of the males (No. 28 in Table 1) ejected a lot of sperm with a slight touch on the thorax and the spermatozoa were very active under the microscope.

At this time, the atmospherical and the water temperature of the pond were 33°C and 24°–25°C high respectively. Regarding the fact that the females gave out few eggs, the artificial fertilization was not attempted.

In view of the fact that some fishes (No. 29–32 in Table 1) gave out less genital substance at the time of the experiment on August 7 and September 13,

**Table 2.** Results of control experiments.

The signes of conditions of reproductive organs of the fish are the same as in Table 1.

| Date | Sex | Conditions |
|---|---|---|
| 1940 IV 19 | — | d |
| " | — | d |
| 1941 IV 27 | — | d |
| 1942 VI 21 | ♀ | c |
| " | — | d |
| 1943 VII " 29 | ♂ | c* |
| | ♀ | c |
| VIII 7 | — | Pearl organ could not be seen |
| 1944 V 29 | — | ♂* (three fishes) ♀* (none) |
| VIII | — | all fishes were d (in the fish farm of Shiga Figh. Experimental Station) |
| 1947 V 14 | ♂ | ♂* (two fishes) |

the author supposed that the spawning season had ended. But one fish (No. 30) swelled up to the thorax, gave out more than ten eggs with pressure on the abdomen, so the author dissected it and was surprised at its fully-grown ovary. (See Table 3).

**Table 3**

| No. | Age | Body length | Body weight | Weight of ovary | Eggs |
|---|---|---|---|---|---|
| 30 | 8 years old | 59 cm. | 4,120 g. | 520 g. | mostly inseparable |

On the occasion of the experiment in August, some parts of the surfaces of fins of the grown-up male *C. idellus* were rough, when the author touched them with his finger-tips, but in September they were not rough any more. For this reason, pearl organs were supposed to appear on the fins. On July 28, the tags (No. 472, 474, 477, 479) were fastened to five matured males. But on August 7 four signs had fallen off and both males and females were carrying numerous wounds on their bodies. These wounds were perfectly healed by September 13. Gathering these facts, the author concluded that males and females must have fiercely touched each other towards the end of July. Attributing this conduct to their spawning, the author searched the test pond very carefully in September expecting to find two-month-old fish larva but it was in vain.

In 1944, dissecting 8 fishes (No. 33–40), the author found that they had such body weight as ranging from 2,865 kg. to 3,255 kg.

About half of them could ejected sperm with ease but the rest did not give out many eggs. Then, the author injected them with emulsion of the phypophysis of a bulls but effect could not be recognized. On July 10, about 200 eggs were gathered, each from three fishes and on June 18, and July 11, two fishes were dissected and about 2,000 eggs were obtained out of the ovaries each time. Artificial fertilization was tried on these eggs. A few hours after the experiment, the fertilized eggs began to swell and trebled in diameter, but in 12 hours, all eggs lost shapo.

At the experiment on May 28, the author found the appearance of pearl organs on the surface of the spines and some part of fin rays next to those spines of pectral, dorsal, and caudal fins. Receiving the report of Mr. Seizô. Suzuki, head of the Shiga Prefectural Fisheries Experimental Station, stating that in the farms of the Station any of *C. idellus* which had been imported with these test fishes, failed to give out sexual substance, the author became more confident of the effect of hormones (see Table 2).

In 1945 some male fishes (No. 41–44 in Table 1), ejected sperm easily, but females gave out only a few eggs. The trial on the artificial fertilization to the eggs of No. 43 fish was in vain just like that of the year before.

In 1946, a female (No. 47 in Table 1) together with four other females were injected with the anterior lobes of the hypophysis of carps, but the discharge of eggs was not satisfactory. The artificial fertilization failed, too.

In 1947, of the fishes (No. 49–53 in Table 1) the males matured well but the females did not yet give out enough eggs, though the sexual glands were found by dissection to become fully grown.

**Table 4** *Ctenopharyngodon idellus* fished in Lake Biwa.

| Date | Body weig. | Total length | Sex | Gonad | Place | Remarks |
|---|---|---|---|---|---|---|
| 1945 V | 9,00 g. | — | — | — | River Echi. | Many fishes seen |
| 1146 V 9 | 20,640 | 1,24 m. | ♀ | 2,5×1 cm. | Coast of Maibara 〃 | By net |
| 1948 VI | 24,000 (about) | — | — | — | | Many fishes seen in the riv. Echi. |
| 1949 VI 20 | 24,000 ( 〃 ) | 1,130 | ♀ | — | Coast of Nagahama | By net |
| 1949 X 8 | 11,800 | 0,92 | ♂ | — | Coast of Hikone | By net |

In 1948, fourty hypophysises of sharks were employed to promote the maturity of the ovary, but it was in vain.

In 1949, the author set the five *C. idellus* with tags on them into Lake Biwa. The main reason why decided to make this attempt are as follows:

1. The fact that all efforts to accelerate the spawning of eggs had been of little avail suggested that the test pond might lack some necessary conditions for their spawning. Consequently, if these tested *C. idellus* with fully-grown sexual

glands were set free, it was thought that they would spawn naturally in the lake in the near future.

2. Large *C. idellus* grown in Lake Biwa were frequently captured along the shore at the spawning season. *C. idellus* which line naturally with insufficiently grown sexual glands seemed to approach the estuary for spawniug. But the fishes which were given hormones in the pond, were believed to gain enough ability to spawn in the lake which fulfilled conditions that lacked in the pond (see Table 4 and Table 5).

**Table 5.** *Ctenopharyngodon idellus* set into Lake Biwa, near Takei island on 27 July in 1949.

| Total length | Body length | Body weight | Sex | No of signes |
|---|---|---|---|---|
| 68 cm. | 58 cm. | 3,465 g. | ♀ | 489 |
| 73 | 69 | 4,087 | ♀ | 498 |
| 85 | 74 | 5,868 | — | 453 |
| 69 | 66 | 3,900 | ♂ | 458 |
| 65 | 52 | 3,075 | ♂ | 497 |

## Discussion

According to Messrs. Chen and Lin(1), to Mr. S. Y. Lin(4), and to Mr. Shichiro Ichô (6), it is said that *C. idellus* will spawn nowhere but in a natural river in China. In fact, although the fish had been imported ten times since the 10th year of Meiji, every effort to raise them in Japan has been unsuccessful. The Formosa Fisheries Experimental Station reported that they found only one mature male and two mature females in 1925 and 1928 respectively out of numerous *C. idellus* which were kept in their farms(7). Although Messrs. Lin and Chen (1) reported that the fishes which line natnrally reached maturity at 4 or 5 years of age, the fishes with which this experiment had been begun in 1940 had quite immature sexual glands in spite of the fact that they had been raised in the farm for five years.

Moreover in 1944, four years after the first experiment, considerable development of the sexual glands indicated the great effect of hormones upon them since that time. The artificial fertilization was tried every year but all proved to be in vain.

Considering the above mentioned facts, the author came to the conclusion that the pond lacked some important conditions for *C. idellus* to sparm, which were duly provided in natural rivers.

Furthermore, considering the fact that the *C. idellus* which had grown in Lake Biwa came towards the shore with the intention of spawning with comparatively less grown sexual glands (see Table 4) and that the fish had propagated recently in

Lake Kasumigaura and Lake Taura(2), the above-mentioned plan to set free these fishes with fully-grown sexual gland (see Table 5) into Lake Biwa would be of great help to propagate.

The author found that pearl organs appeared on the surface of the fins of males at the spawning season. Though Messrs. Kimura and Tao(5) reported that pearl organs appeared on the head of male fish, the author could not make sure about this point. The author hopes the further observation will solve this question.

## Summary

Since 1940, the author had given the hydrosol of Androsteron and Esteron to Sôgyo, Ctenopharyngodon *idellus* with undeveloped sexual gland and by 1949, gained the following results:

1. The sexual glands of males and females of *C. idellus* were fully developed by giving hormones through the medium of the mouth with food processed. However the artificial fertilization was not successful.

2. The spawning season of *C. idellus* at Hikone in Shiga Prefecture was found to be from April to July, and during the period pearl organs appeared on the surfaces of the fins of males.

3. Judging from the fact that large *C. idellus* were often captured along the shore of Lake Biwa, they seemed to come towards the shore for spawning. But, their sexual glands were far less developed than those of the fishes which were given hormones.

4. Consequently, the author concluded that it was wise and proper for the propagation of *C. idellus* to set free the fishes after sexual glands had fully grown as the result of hormones given in the test pond.

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