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Extensive study and data collection on the pituitary gland: A promising prospect revealed by surveying the fish market during the monsoon season

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Abstract: Because of India's large population, the fish supply demand is increasing daily. Among all fishes, the Indian major carp (*Labeo rohita*, *Catla catla*, etc.) is one of the most demanded fishes in India. Indian major carps breed once a year during the early monsoon season (June and July). At that time, the collection of spawns from natural sources is insufficient for our population. To overcome this situation, induced breeding is the most effective process to increase carp culture. IMC bred twice in a season using this method. The first step in this process is collecting and preserving pituitary glands from donor fish. This gland-pituitary extract is later produced and injected into spawn to facilitate spawning. So, in our survey, we know how many pituitary glands are collected daily as well as weekly averages from the survey site and where they are transferred.

Introduction

Induced breeding is a technique that uses pituitary extract as an artificial stimulant to breed economically important fish (Paul & Chanda,2014). This pituitary extract helps transform their physiological activity, such as the maturation of the ovary (Pawar et al., 2019). Due to the huge population in India, spawn collection from natural bodies is insufficient, and many natural seeds are impure and defective. So, hypophyzation or "induced breeding" is an effective way to collect pure seeds in sufficient amounts (Surnar et al., 2015; Rokade et al., 2006; Mishra et al., 2001). Punjab also imports pituitary glands from West Bengal, and farmers are seeing better outcomes (Shanthanagouda et al., 2018). In India, the breeding season of Indian Major Carp is from May to July, so during that time, the collection of pituitary glands reaches its maximum number and is transported to different hatcheries (Pawar et al., 2019; Mammen and Sulochanan, 1962; Dhara and Saha, 2014; Bhowmick et al., 1977; Hollander-Cohen et al., 2018). But this technique becomes dependent on the collection of pituitary glands at different times of the year (Surnar et al., 2015; Rokade et al., 2006). This survey work at Nabadwip fish market in Nadia is illuminating the technique of pituitary extraction, which is part of induce breeding, as well as observing the amount of isolated pituitary from Rohu (*Labeo rohita*), Catla (*Catla catla*), and Rita (*Rita rita*) during the monsoon season (June to August).

Figure 1. Nabadwip fish market.

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# These authors have contributed equally to this work.

History of Induced breeding

Houssay pioneered inducement breeding in 1930. This research is being conducted in Argentina on viviparous fish using pituitary extract to induce premature birth. In 1934, the Brazilians used the pituitary extract to induce breeding, and other countries such as America and Russia also used this technique (Paul and Chanda, 2014). In India, Choudhury and Alihunhi first successfully induced breeding in Indian major carp *Chirrhinus mrigala*, *C. reba*, and *Labeo rohita* in 1957 (Paul and Chanda, 2014; Rokade et al., 2006; Varghese et al., 1975). From then on, this technique spread all over India and improved (Varghese et al., 1975).

Hormonal regulation of induce breeding:

Pituitary glands secrete gonadotropin hormones, i.e., FSH (follicle-stimulating hormone) and LH (Luteinizing hormone). Both hormones are secreted throughout the year. FSH helps ovarian development and maturation in female fish and spermatogenesis in the testes of male fish. LH hormones stimulate luteinization in females and increase testosterone production in men (Surnar et al., 2015; Rokade et al., 2006; Mishra et al., 2001; Shanthanagouda et al., 2018). Beside the pituitary extract, some other hormones are also used to induce breeding, such as GnRH, HCG, etc. Synthetic GnRH and dopamine receptor blockers, such as ovaprim, dagin, and ovopel, are now widely used to induce breeding (Peter et al., 1988; Nandeesha et al., 1990; Brzuska et al., 1999; Sharma et al., 2010). GnRH activates the reproductive gonadal endocrine axis and plays a key role in FSH and LH secretion from the pituitary, while GnRH ultimately stimulates gametogenesis (Kah et al., 2007; Counis et al., 2005). In Japanese eels (*Anguilla japonica*), mullet (Mugil cephalus), bream (*Abramis brama*), and *Anguilla*, HCG is used for the induction of spermiation (Elakkanai et al., 2015).

Pituitary extract preparation is subdivided into the following phases:

Isolation of pituitary

Pituitary gland is always separated from a pregnant fish for better results. In the months of May to July, it is most effective to collect pituitary in India. Pituitary isolation is accomplished through two methods. The first is the dorsal side of the skull, in which the head of the fish is dissected and the pituitary gland is collected; however, this method is not commercially used because the dissected fish is unfit for human consumption. The second one is a very popular method that is basically used in many fish markets. In this method, the posterior cavity of the brain is opened through the foramen magnum, and the pituitary gland is collected very carefully by a flat, curved probe (Paul & Chanda, 2014; Pawar et al., 2019; Surnar et al., 2015). In our survey site, pituitary isolation occurred by this second method, and we also observed the technique of pituitary gland isolation mainly from Rohu (*Labeo rohita*), Catla (*Catla catla*), and Rita (*Rita rita*), which is shown in Figure 3. The average weight of donor fish is about 6-8 kg at Nabadwip Fish Market.

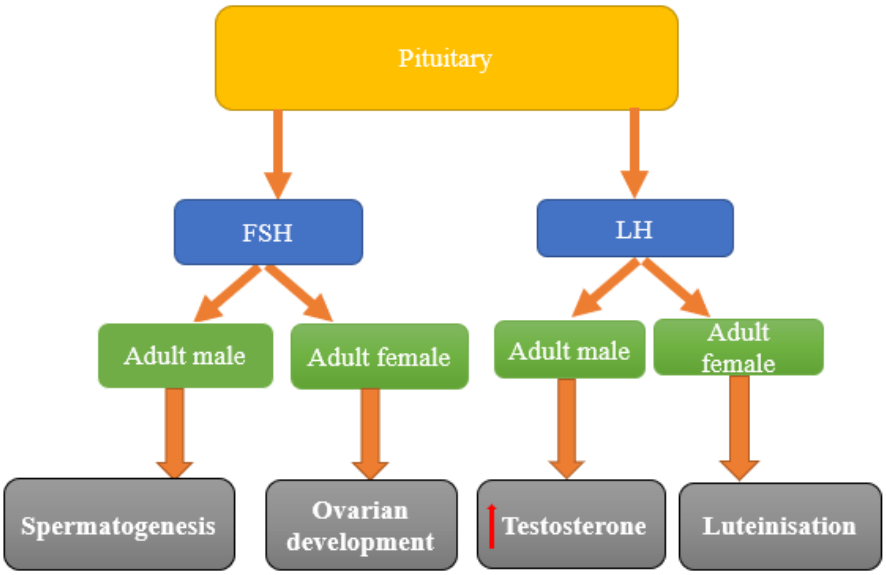

Figure 2. Hormonal regulation of pituitary extract

Preservation of pituitary

Preservation of the pituitary is a very important phase in pituitary extract preparation. In Nabadwip fish market (Figure 5), the isolated pituitary transports many regions of Nadia district (Jagulia, Ranaghat) and Burdwan district (Kalna) by train, so the proper preservation of the pituitary is necessary for a fruitful result. Pituitary preservation occurs with either alcohol or acetone. In alcoholic preservation, an isolated pituitary is

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immediately poured into absolute alcohol for dehydration, then washed with alcohol and poured into absolute fresh alcohol after one day. In the case of acetone, the pituitary gland is kept at -20°C for two days with chilled fresh acetone, and the acetone solution is changed every 8-12 hours (Surnar et al., 2015).

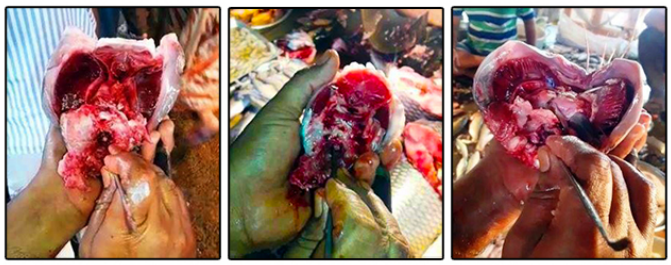

Figure 3(a) Figure 3(b) Figure 3(c)

Figure 3. Isolation of pituitary from Catla (3a), Rohu (3b), Rita (3c) at Nabadwip fish market

Figure 4. Pituitary isolation from fishes by knife and probe

Figure 5. Preserved pituitary gland in absolute alcohol

Extract formation

Isolated pituitary gland kept in a 2 ml beaker with 0.9% normal saline solution. Now the prepared solution goes for centrifugation, and after completion, the supernatant is collected for injection. Extract preparation occurs immediately before the injection (Surnar et al., 2015).

Dosage of pituitary extract

In the case of coelomic injection, it is injected below the pectoral fin in a scale-less area. This procedure is difficult because the heart is located near the pectoral fin, where it can be injured (Surnar et al., 2015; Khan and Ali, 2021). Muscular injection, on the other hand, is injected at a 45° angle between the dorsal and lateral lines of the scale (Surnar et al., 2015; Khan and Ali, 2021).

Dose for Rohu (*Labeo rohita*)

The first dose in females is 2-4 mg/kg body weight, and the second dose is 6-8 mg/kg body weight, given 5-6 hours after the first dose. Males receive the first dose during the second dose of females at a rate of 2-3 mg/kg of body weight (Surnar et al., 2015; Tiwana and Raman, 2012; Hossain et al., 2012; Brzuska and Adamek, 1999). The dose also depends on the fish's maturation, sex, age, and environmental condition (Tiwana and Raman, 2012; Hossain et al., 2012; Brzuska and Adamek, 1999).

Methodology

In this survey, we collected data on pituitary collection for 84 consecutive days at the Nabadwip fish market during the monsoon season (June to August). We observed that the maximum level of pituitary collection occurs in July. We notice the process of pituitary isolation and count the number of isolated glands between 8:00 a.m. and 11:00 a.m. We discussed with some skilled persons who are experts in the pituitary collection, and we observed the process of the pituitary collection as well as preservation procedures.

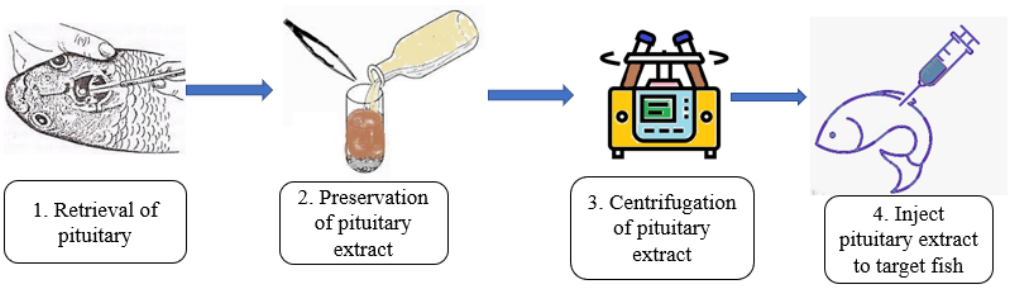

Figure 6. Phases of pituitary extract preparation

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Table 1. Pituitary collection report for three months based on three different species of fish for damaged and undamaged glands.

Week	Date	No. of total pituitary gland	Undamaged pituitary gland	Damaged pituitary gland	No. of fishes (from which pituitary glands were collected)			Average weight (Kg) of fishes (from which pituitary glands were collected)			Time of collection	Transfer to	Transport media
					Rohu	*Catla*	*Rita*	*Rohu*	*Catla*	*Rita*			
1	01.06.2022 to 07.06.2022	1012	940	72	499	453	60	7.3	8.2	5.7	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
2	08.06.2022 to 14.06.2022	630	601	29	256	313	61	7.1	8.1	6.5	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
3	15.06.2022 to 21.06.2022	759	702	57	357	303	42	7.4	7.8	5.8	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
4	22.06.2022 to 28.06.2022	812	744	68	412	274	58	7.5	7.9	6.4	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
5	01.07.2022 to 07.07.2022	924	870	54	455	366	49	7.2	8.4	6.9	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
6	08.07.2022 to 14.07.2022	857	802	52	419	317	66	7.9	8.2	6.7	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
7	15.07.2022 to 21.07.2022	1035	948	87	495	416	37	8.3	8.1	6.9	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
8	22.07.2022 to 28.07.2022	924	857	67	424	387	46	7.8	8.4	6.3	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
9	01.08.2022 to 07.08.2022	989	911	78	415	437	59	7.9	8.6	6.2	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
10	08.08.2022 to 14.08.2022	878	811	67	437	313	61	8.3	8.7	5.3	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
11	15.08.2022 to 21.08.2022	792	705	87	358	292	55	7.6	8.4	6.1	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus
12	22.08.2022 to 28.08.2022	824	722	102	347	329	46	7.8	8.2	5.8	7.30 am to 11.30 am	Kalna, Burdwan, West Bengal	Train & Bus

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In this chart, we consecutively collected 84 days of data from June 2022 to August 2022. We calculate that the total number of isolated pituitaries during these 84 days is about 10,436 out of these 9,613 undamaged pituitaries, which were ready to use, and 823 damaged pituitaries. Figure 6a depicts the highest number of pituitary extractions (3,740) in July and the highest number of damaged pituitaries (334) in August. Figure 7b shows that during the survey period, the maximum number of undamaged pituitaries isolated from Rohu (*Labeo rohita*) was 4874 and the least number of undamaged pituitaries collected from Rita (*Rita rita*) was 640. The maximum number of Rohu and *Catla* sacrificed the pituitary gland in July, but in the case of *Rita, the* maximum number was isolated during the months of June and August (Figure 7c).

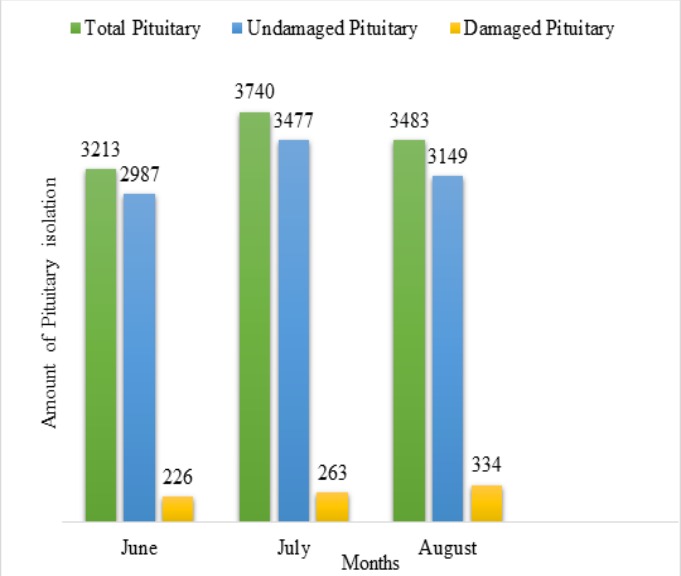

Figure 7a

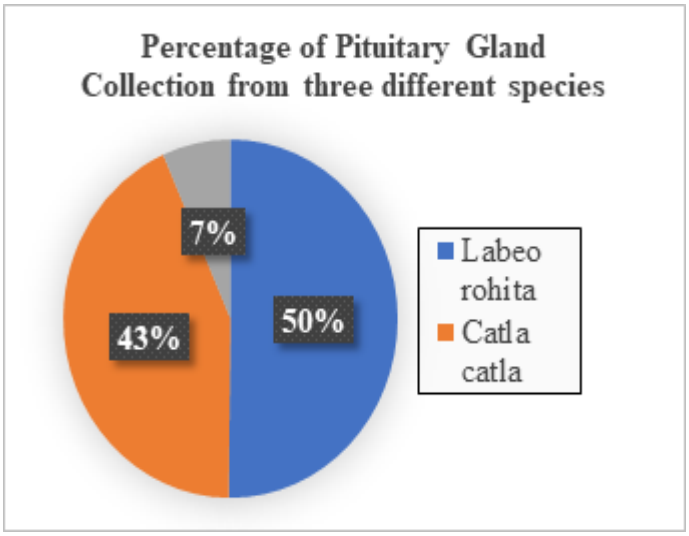

Figure 7b

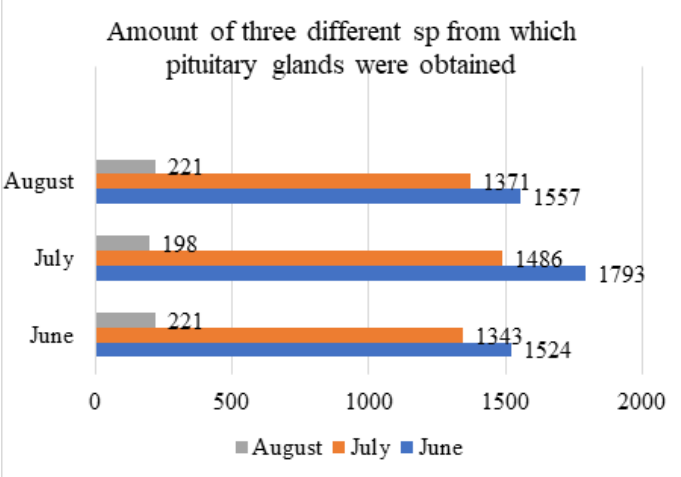

Figure 7c

Figure 7. Figure 7a shows the graphical representation of the pituitary collection from June to August in Nabadwip fish market (Nabadwip, Nadia, West Bengal). Figure 7b indicates that out of 9,613 isolated pituitary samples, Rohu, Catla, and Rita contribute the respective amounts of undamaged glands: 4874 (50%), Catla 4200 (43%), and Rita 640 (7%); Figure 7c shows the number of glands extracted from Rohu, Catla, and Rita at different months of the monsoon.

Conclusion

Pituitary glands are collected commercially by many fishermen from the market during the early monsoon season (May-June) for induced breeding. According to the survey data, the total number of pituitary glands that have been collected on a weekly average basis is 821 and are from Rohu (*Labeo rohita*), Catla (*Catla catla*), and Rita (*Rita rita*). In this survey, we found about 823 damaged pituitaries. Some research work requires using this damaged pituitary for aquaculture and some other endocrinological activities. Some workshops and other courses are organised to help those entrusted with this noble profession improve their skills.

Conflict of interest

None

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