COMPARATIVE EFFICIENCY OF KISSPEPTIN, GnRH ANALOGUE AND PITUITARY EXTRACT ON GONADOSOMATIC INDEX, FECUNDITY AND MEAN OVA DIAMETER OF STRIPED MURREL, CHANNA STRIATUS (BLOCH)

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ABSTRACT

In the present study, the effect of three reproductive hormones (Kisspeptin, GnRH analogue, Pituitary extract) on the GSI, fecundity and mean ova diameter in captive Striped Murrel (Channa striatus Bloch) was carried out. Among the three hormones injected, Kisspeptin injected fish showed significant increase in the GSI, fecundity and mean ova diameter. The GSI, fecundity and mean ova diameter values were highest in Kisspeptin injected fish. During the experiment, the GSI obtained in male fish was maximum (above 1.1) in Kisspeptin injected fish. Compared to Kisspeptin injected fish, low values of GSI, fecundity and mean ova diameter were observed from GnRH analogue and Pituitary extract injected fish. The maximum GSI value of 6.932 was obtained in female Kisspeptin injected fish, compared to GnRH analogue (4.678) and Pituitary extract injected fish (3.625). The highest fecundity of 10,028 eggs was obtained from Kisspeptin injected fish during May, followed by GnRH analogue and Pituitary extract. Mean ova diameter of Kisspeptin injected fish showed highest level when compared with the other two hormones. Maximum level of ova diameter was attained during March (1.1713) and May (1.1734). The current study shows that, Kisspeptin injected fish, showed positive impact on increase in the level of male and female GSI, fecundity and mean ova diameter in C. striatus.

Key words: Reproductive Hormones, GSI, Fecundity, Ova Diameter, Striped Murrel

INTRODUCTION

Murrels are important tasty freshwater fish which have great demand all over the world. Murrels are commercially cultured in Thailand, Philippines, Vietnam and Cambodia. Murrel culture in India is still not commonly practiced due to lack of seed supply, non-availability of quality seeds from wild and poor seed recovery from natural collection. The murrel seed production is one of the major constraints in murrel culture. According to various research reports, the breeding season varies from region to region in India itself for murrels. Alikunhi (1953) reported that Channa striatus breeds from November to January in Southern India. In Northern India, breeding season is from June to October with the peak from July to August (Qasim and Qayyum, 1961). Murrels spawn naturally during southwest monsoon (June

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to August) and also during northeast monsoon (October to December) in flooded rivers and ponds in India (Alikunhi, 1957). Hormonal treatment is very important for inducing the gonadal maturity especially in C. striatus. These species sometimes fail to breed under captive conditions even after hormonal treatment. The fecundity of striped murrel varies from a few hundreds to a few thousands (2,997 to 20,070) depending on the size of the fish (Alikunhi, 1953). The sexes can be distinguished during breeding season. The abdomen in female is slightly bulged which is not observed in male fishes. Vent is pale and slit like in male, whereas round in shape and reddish in colour in female fish. Recent progress in the development of induced ovulation and spermiation technology has facilitated the reproduction of many commercial aquaculture species. In the present study, an attempt has been made to study the effect of Pituitary extract, GnRH analogue and Kisspeptin-10 on reproductive parameters such as male and female GSI, fecundity and mean ova diameter of Striped murrel C. striatus.

MATERIALS AND METHOD

The mature males and females were collected from Pazhayakayal region of Thoothukudi District. The specimens were transported to the field laboratory in plastic crates of 50 liters capacity. The fish were acclimatized for 5 days in FRP tanks of 500 liters capacity. The average initial length and body weight of the fish were 41.04 ± 0.6 cm and 659 ± 32 g respectively. During the period of acclimatization for 5 days, cooked chicken intestine at 5% of the body weight was given as daily feed ration. After acclimatization, both male and female fish were segregated based on the urogenital opening (Dehadrai et al., 1973), which is circular in female and much elongated in male. After segregation of sexes, fishes were transferred to rectangular cement tanks of size 3 m diameter and 1 m height having 0.5 water depth.

The fishes were divided into four groups. Each group consisted of male and female fish stocked in separate tanks. From the first batch, male and female were injected with Pituitary extract at the rate of 2 mg/kg of fish (Shrestha et al., 1990). Second group was injected with GnRH analogue at the rate of 5 µg/kg of fish (Eenennaam et al., 2008). Third group was injected with Kisspeptin at the rate of 0.1 mg/kg of fish. Fourth group was injected with distilled water as control. After the start of experiment, the fishes were fed alternatively with cooked chicken intestine twice a day at ad libitum till the end of the experiment. Monthly samplings of gonad from the above groups were carried out to study the effect of different hormones (Pituitary extract, GnRH analogue and Kisspeptin - 10) on gonadal maturity in Channa striatus. Hormone injections were given every month within the total study period of 6 months. Before injection, the fish were anaesthetized individually in 0.1% Benzocaine solution. Following anaesthesia, the male and female fishes were injected intramuscularly with different hormones. After first injection monthly sampling of gonad were carried out. Sampling of gonad was continued till the end of experiment. Monthly sampling of gonad from hormone injected and control fishes were carried out to assess the maturation using various reproductive parameters such as Gonadosomatic Index (GSI), fecundity and ova diameter.

Each male and female fish sampled from control and hormone injected fish were weighed separately and the ovary and testis of dissected fish were also weighed to calculate the Gonadosomatic Index. The GSI value was calculated using the following formula:

One gram of ovary was weighed, fixed in neutral buffered formalin and counted individually on the same day of sampling. Fecundity was estimated using the following formula:

F = nV/v (n = Number of eggs in the sub sample,

V = Total weight of the sample, v = Weight of the sub sample).

Ova from anterior, middle and posterior portion of ovary (100 each) were collected and fixed in Gilson's fluid. The diameters of collected ova were measured using ocular micrometry on the same day. The diameters of 300 ova were classified into five ranges to assess the monthly changes in ova diameter of control and hormone injected fish. The mean diameters of 300 ova represent the mean ova diameter of each fish. Monthly mean values of GSI, fecundity and ova diameter of control and hormones injected fish were compared by Analysis of Variance (ANOVA).

RESULTS & DISCUSSION

Changes in GSI, fecundity and ova diameter were studied to assess the maturation of Channa striatus. The GSI values of control and Kisspeptin, GnRH analogue and Pituitary extract injected male and female fish are given in Tables 1 and 2. During the period of experiment, testis and ovary were collected from control and different hormones (Kisspeptin, GnRH analogue and Pituitary extract) injected fishes for calculating Gonadosomatic Index (GSI). The calculated GSI for the control and different hormone injected male fish are given in Table 1. During the whole experiment, the GSI was above 1.1 in Kisspeptin injected fish except December. Compared to Kisspeptin injected fish, low GSI was observed in GnRH analogue and Pituitary extract injected fish. Fluctuating levels of GSI were observed in GnRH analogue and Pituitary injected fishes. The GSI was observed less than 0.1561 in control fishes. The treatment values were significant at 1% level. GSI observed from control and hormone injected male fish are depicted in Fig.1. In the month of February, all the three hormones (Kisspeptin, GnRH analogue and Pituitary extract) depicted almost the same level of GSI compared to control murrel male fishes.

GSI calculated from the control and different hormone injected female fishes are given in Table 2. Maximum GSI of 6.932 was observed from Kisspeptin injected fishes, compared to GnRH analogue injected (4.678) and Pituitary extract injected fish (3.625). But, in the control fish, the maximum GSI value, 1.0948 was observed during May. Compared to GnRH analogue and Pituitary injected fish, Kisspeptin injected female fish showed increasing level of GSI upto the month of May. The treatment values were significant at 1% level. GSI observed from control and hormone injected female fish are depicted in Fig.2. Maximum level of GSI was observed for Kisspeptin injected fish during the month of May.

The fecundity of control, Kisspeptin, GnRH analogue and Pituitary extract injected fish are given in Table 3. Eggs were collected from different hormone injected and control fishes during the different sampling period to study the effect of these hormones on the fecundity rate of eggs. Among the different hormone injected fish, high rate of fecundity (10,028 eggs) was obtained from Kisspeptin injected fish during the month of May, followed by GnRH analogue and Pituitary extract injected fish. Kisspeptin played a major role in increasing the fecundity. The treatment values were significant at 1% level. Fecundity observed for control and hormone injected fish are depicted in Fig.3. The high level of fecundity was observed from Kisspeptin injected fish.

The mean ova diameter of the control, Kisspeptin, GnRH analogue and Pituitary extract injected fish are given in Table 4. On sampling, maximum ova diameter of 1.171 mm and 1.173 mm were observed in the month of March and May. There is little variation in ova diameter of hormone injected fishes during the different sampling periods of Kisspeptin injected fish. Among the three hormones injected, ova diameter

of Kisspeptin injected fish was higher than the other hormones injected fishes. Mean ova diameter observed from control and hormone injected fish is depicted in Fig.4.

It is interesting to note that the Kisspeptin, GnRH analogue and Pituitary extract injected fish showed high performance than control fish. The present study showed the effect of different hormones (Kisspeptin, GnRH analogue and Pituitary extract) on changes in the GSI, fecundity and mean ova diameter in the Striped murrel C. striatus. The essential role of Kisspeptin has been reported to enhance maturation of aquatic animals (de Roux et al., 2003; Parhar et al., 2004). Apart from Kisspeptin, Pituitary extract (Khan and Mollah, 2004; Mollah et al., 2008) and GnRH analogue (Afzal et al., 2008; More et al., 2010) have been used for maturation and breeding in fishes. This study clearly states that Kisspeptin injected fish showed highest role of increase the male and female GSI, fecundity and ova diameter. The hormones injected made greater influence on the GSI, fecundity and ova diameter of fish. Among the different hormone injected fish, high rate of fecundity (10,028 eggs) were obtained from Kisspeptin injected fish during the month of May, followed by GnRH analogue and Pituitary extract injected fish. Like fecundity, male GSI was above 1.1 in Kisspeptin injected fish, from January onwards. Compared to Kisspeptin injected fish, low GSI was observed from GnRH analogue and Pituitary extract injected male Murrels. The increase in GSI value may be due to the relatively slow growth of fish leading to the accumulation of fat and protein in the gonad as suggested by Basavaraja et al. (1989) in common carp, Cyprinus carpio.

Mean Ova diameter of different hormone injected fishes are given in Table 4. Maximum ova diameter of 1.171 and 1.173 mm were observed during the month of March and May. Among the three hormones injected, ova diameter of Kisspeptin injected fish is higher than other two hormone injected fishes. In most of the experimental periods, the mean ova diameter of Kisspeptin injected fish were more than 1 mm. In control fish, mean ova diameter were always less than 1 mm during the study period. Statistically, a highly significant (at 1% level) difference was observed between the mean ova diameter of Kisspeptin injected and control fish. Lee et al. (1996) made similar observation in Striped Mullet, Mugil cephalus treated with 17α-methyl testosterone whereas the mean oocyte diameter was significantly higher in hormone injected fish as observed in the present study.

Table 1

GSI of Control, Kisspeptin, GnRH analogue and Pituitary Extract injected male fish during different sampling period

Sampling period	GSI			
	Control	Kisspeptin injected fish	GnRH analogue injected Fish	Pituitary Extract injected fish
December	0.0869	0.0869	0.0869	0.0869
January	0.0654	1.1132	0.0889	0.0990
February	0.0972	1.1167	1.1038	1.1042
March	0.0842	1.1389	0.0938	0.0972
April	0.0625	1.1313	1.1086	0.0858
May	0.1561	1.1209	1.0999	0.1481

Table 2

GSI of Control, Kisspeptin, GnRH analogue and Pituitary Extract injected female fish during different sampling period

Sampling period	GSI			
	Control	Kisspeptin injected fish	GnRH analogue injected Fish	Pituitary Extract injected fish
December	0.9824	0.9824	0.9824	0.9824
January	0.9924	3.9880	1.4732	1.6252
February	1.0620	4.3319	2.8103	2.0920
March	1.0783	4.3314	3.4151	2.8253
April	0.9989	4.6213	3.6778	3.5141
May	1.0948	6.9322	4.6782	3.6252

Table 3

Fecundity of Control, Kisspeptin, GnRH analogue and Pituitary Extract injected female fish during different sampling period

Sampling period	Fecundity (Numbers)			
	Control	Kisspeptin injected fish	GnRH analogue injected Fish	Pituitary Extract injected fish
December	2730	2730	2730	2730
January	2978	3287	2978	2962
February	2554	3878	3160	2821
March	2714	4802	3839	3180
April	4319	7066	4852	4289
May	5210	10028	5415	4812

Table 4

Mean ova diameter of Control, Kisspeptin, GnRH analogue and Pituitary Extract injected female fish during different sampling period

Sampling period	Fecundity (Numbers)			
	Control	Kisspeptin injected fish	GnRH analogue injected Fish	Pituitary Extract injected fish
December	0.6867	0.6867	0.6867	0.6867
January	0.4353	1.0572	0.8697	0.6982
February	0.3291	1.0722	0.8452	0.8754
March	0.3041	1.1713	1.0512	0.8928
April	0.3777	1.1293	1.0628	0.9672
May	0.4353	1.1734	1.1214	1.0572

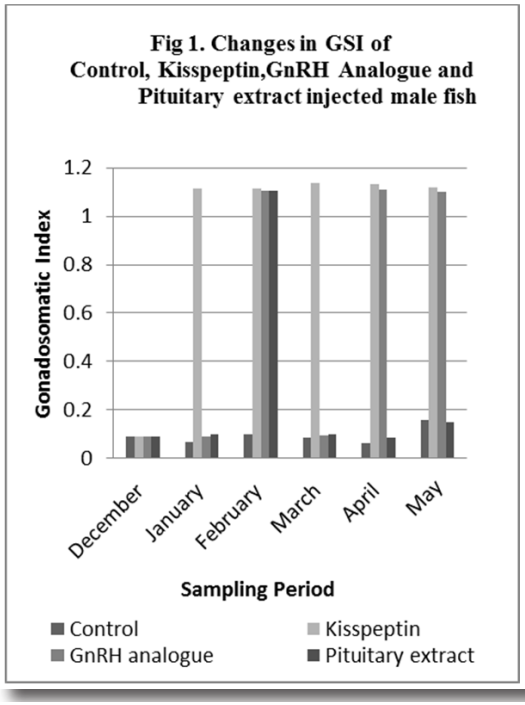
Fig 1. Changes in GSI of Control, Kisspeptin,GnRH Analogue and Pituitary extract injected male fish
Gonadosomatic Index
1.2
1
0.8
0.6
0.4
0.2
0
December
January
February
March
April
May
Sampling Period
Control
Kisspeptin
GnRH analogue
Pituitary extract

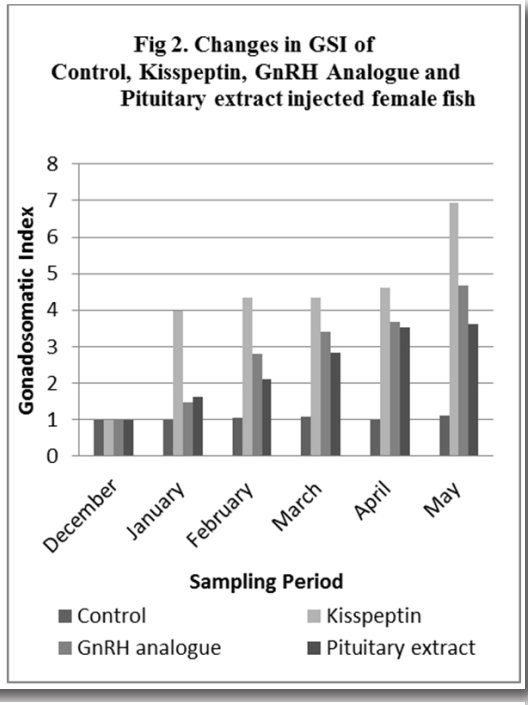
Fig 2. Changes in GSI of Control, Kisspeptin, GnRH Analogue and Pituitary extract injected female fish
Gonadosomatic Index
8
7
6
5
4
3
2
1
0
December
January
February
March
April
May
Sampling Period
Control
Kisspeptin
GnRH analogue
Pituitary extract

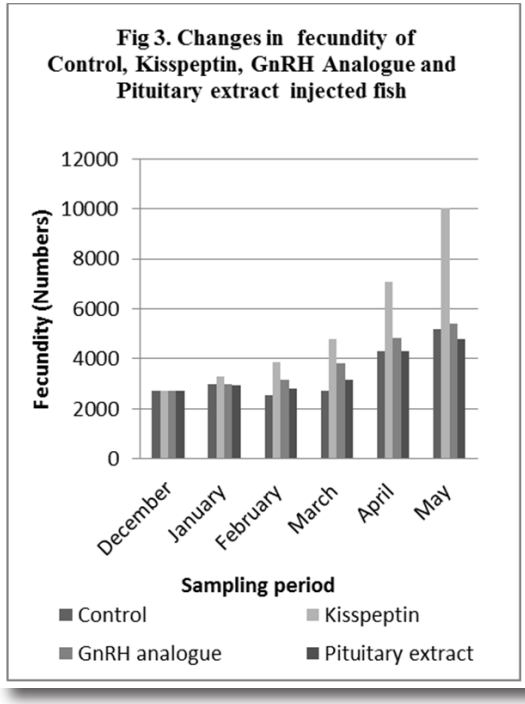
Fig 3. Changes in fecundity of Control, Kisspeptin, GnRH Analogue and Pituitary extract injected fish
Fecundity (Numbers)
12000
10000
8000
6000
4000
2000
0
December
January
February
March
April
May
Sampling period
Control
Kisspeptin
GnRH analogue
Pituitary extract

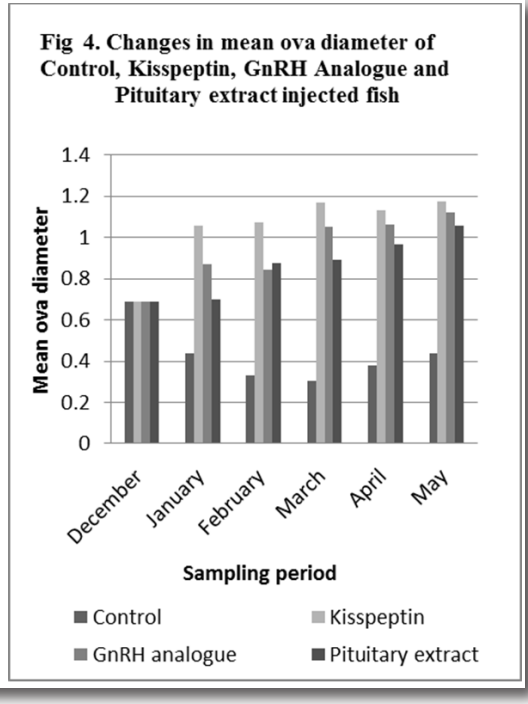
Fig 4. Changes in mean ova diameter of Control, Kisspeptin, GnRH Analogue and Pituitary extract injected fish
Mean ova diameter
1.4
1.2
1
0.8
0.6
0.4
0.2
0
December
January
February
March
April
May
Sampling period
Control
Kisspeptin
GnRH analogue
Pituitary extract

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