

## INDUCED BREEDING OF FRESHWATER MENODA CATFISH (*Hemibagrus menoda*) USING OVUPIN HORMONE

Islam, N., M. F. Islam<sup>1</sup>, M. S. Islam<sup>1</sup> and M. I. Miah

Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh;

<sup>1</sup>Department of Fisheries, Bangamata Sheikh Fojilatunnesa Mujib Science and Technology University, Melandah, Jamalpur, Bangladesh

### Abstract

An experiment on induced breeding of menoda catfish, *Hemibagrus menoda* was conducted during the period from May 2017 to July 2018 by using commercial Ovupin hormone (GnRH<sub>a</sub>, Gonadotropin Releasing Hormone Analogues) to attain its optimum dose. The study consists of three treatments, each with three replications. The objective of the experiment was to find out the effective dose for induced breeding. The breeding parameters were determined in terms of ovulation, fertilization, hatching, and survival rate. A total of 36 males and 18 females lived brood fish were kept in the ratio of 2♂:1♀ for breeding purpose. The commercial Ovupin hormone were injected at the doses of 4 (T<sub>1</sub>), 6 (T<sub>2</sub>), 7 (T<sub>3</sub>) mL Ovupin·kg<sup>-1</sup>body weight for female and 1.5(T<sub>1</sub>), 2 (T<sub>2</sub>), 3(T<sub>3</sub>) mL Ovupin·kg<sup>-1</sup> body weight for male in a triplicate replication. In this study, it was found that treatment T<sub>2</sub> demonstrated the best spawning performance for both male and female and were statistically significant (p<0.05). The investigation indicated that the dose of Ovupin hormone 6mL Ovupin·kg<sup>-1</sup>body weight for female and 2 mL Ovupin·kg<sup>-1</sup> body weight for male determines the highest spawning performance of menoda catfish which should be recommended for high quality eggs and larvae.

**Key words:** *Hemibagrus menoda*; Induced breeding; Menoda catfish; Ovupin.

### INTRODUCTION

Induced breeding of catfish is the most potential and dependable technique for ensuring the availability of good quality fish seeds all year round. Catfishes hardly reproduce in the captive water body so artificial propagation is the only way for an enormous source of superior seeds and culture in the different water bodies which will increase the total fish production as well as the GDP of a country. In the freshwaters of Bangladesh, almost 55 species of catfish belonging to 35 genera have been reported to date (Rahman 2005). In recent years, the production of these catfishes has increased, but the appearance of many species of catfishes is falling day by day from natural waters (Hoque *et al.* 1998).

The production of fish seeds all year round has been made possible due to induced breeding techniques (Ayinla 1988). Various types of commercial hormones such as Pituitary Gland (PG), Deoxycorticosterone Acetate (DOCA), Ovaprim, Ovulin, Human Chorionic Gonadotropin (HCG), Ovopel, Dagin and Aquaspawn etc. are used for artificial propagation of different catfishes and these hormones are most familiar in the markets (Cheah and Lee 1980, Brzuska and Adamek 1999, Zohar and Mylonas 2001, Adebayo and Popoola 2008). The effectiveness of using different doses of synthetic hormones have been recorded for artificial propagation of different catfishes (Olubiyi *et al.* 2005, Sahoo *et al.* 2005, Achionye-Nzeh and Obaroh 2012, Shinkafi and Ilesanmi 2014, Marimuthu *et al.* 2015); but very little is known about the doses of Ovupin in catfish breeding. Moreover, it is probably the first study of induced breeding of *Hemibagrus menoda* (Hamilton 1822) by using Ovupin hormone.

*H. menoda*, a catfish having the common name Menoda catfish, is locally known as Golsa-tengra, Arwari, Gang magur, Kounemagur in Bangladesh. The habitats of *H. menoda* range from the Ganges, Brahmaputra, Mahanadi and Godavari River drainages in Bangladesh to northern India. *H. menoda* has been utilized as experimental animal and is a valuable food fish because of its large size (450 mm or 17.7" SL, but can attain up to 800 mm), tasty flesh and high market value. But it is less frequently encountered in markets compared to other genera of large Bagrid catfishes such as Rita and Sperata

(Hoque *et al.* 1998, Ng and Ferraris 2000). *H. menoda* was categorized as Near Threatened (IUCN-Bangladesh 2015) because of its rapid disappearance from the rivers, haors and beels of Bangladesh. With a view to conserving this fish, this study attempts to develop conservation strategies through constructing an induced breeding protocol because *H. menoda* does not spawn naturally in captive conditions. In addition to conservation, successful induced breeding of *H. menoda* may lead to successful culture of this fish. Considering the value and significance of *H. menoda*, the present experiment was undertaken to estimate the effective dose of hormone for the induced breeding of the species.

## METERIAL AND METHODS

### *Brood fish collection and rearing*

The research activities were carried out in the Field Laboratory Complex of Fisheries Faculty in Bangladesh Agricultural University, Mymensingh during May 2017 to July 2018. The brood fishes of Gang magur, *Hemibagrus menoda* fish (Fig. 2a) were collected from Jhanjail point-1, Kongsho River of the Netrokona district in May 2017 (Fig. 1). They were reared in earthen rectangular ponds of the Field Laboratory complex. During the rearing period, SIS (small indigenous species of fish) and protein and vitamin-E enriched feed was fed to the fish at a rate of 3.5-5 % body weight. Fertilization, manuring, and liming were performed regularly whenever necessary.

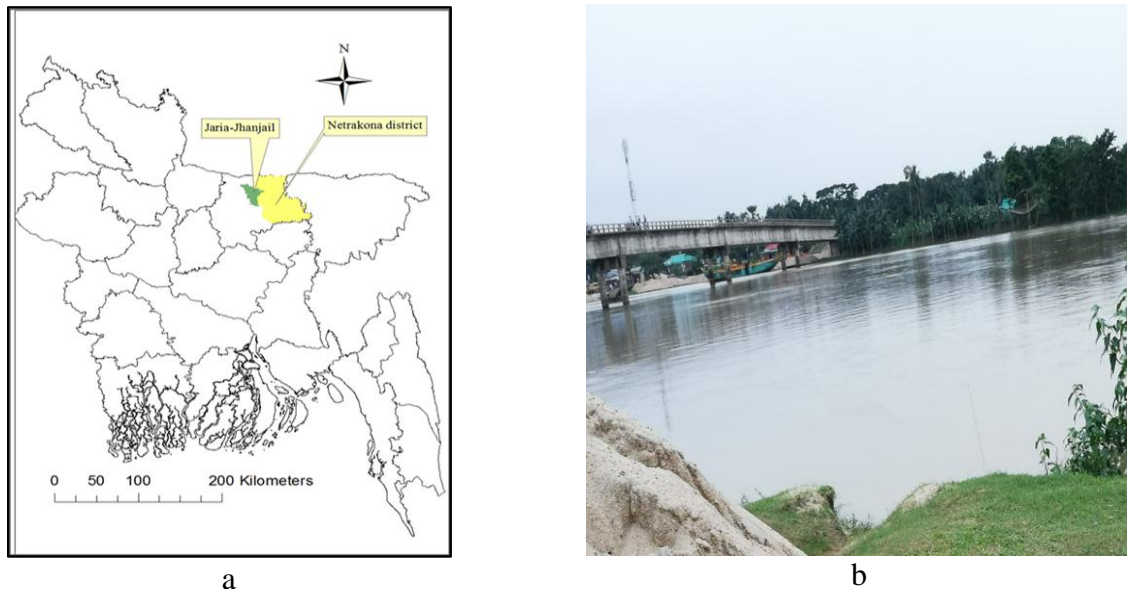


Fig. 1. Pictorial view: **a.** site map; and **b.** study area.

### *Brood selection and conditioning*

The average weight of the males and females were  $354 \pm 42$  g and  $738 \pm 67$ g respectively. Males were relatively smaller, elongated and slender in shape than females. The gravid males and females were selected based on the following criteria: a) males have swollen and reddish urogenital papillae (Fig. 2b); b) females have rounded and protruding abdomen which is soft when touched with fingers and swollen genital opening sometimes reddish in color (Fig. 2c). The selected broods were shifted to the circular cemented tank with continuous water showering for about five hours prior to administering hormones. During conditioning, males and females were kept off-feed.

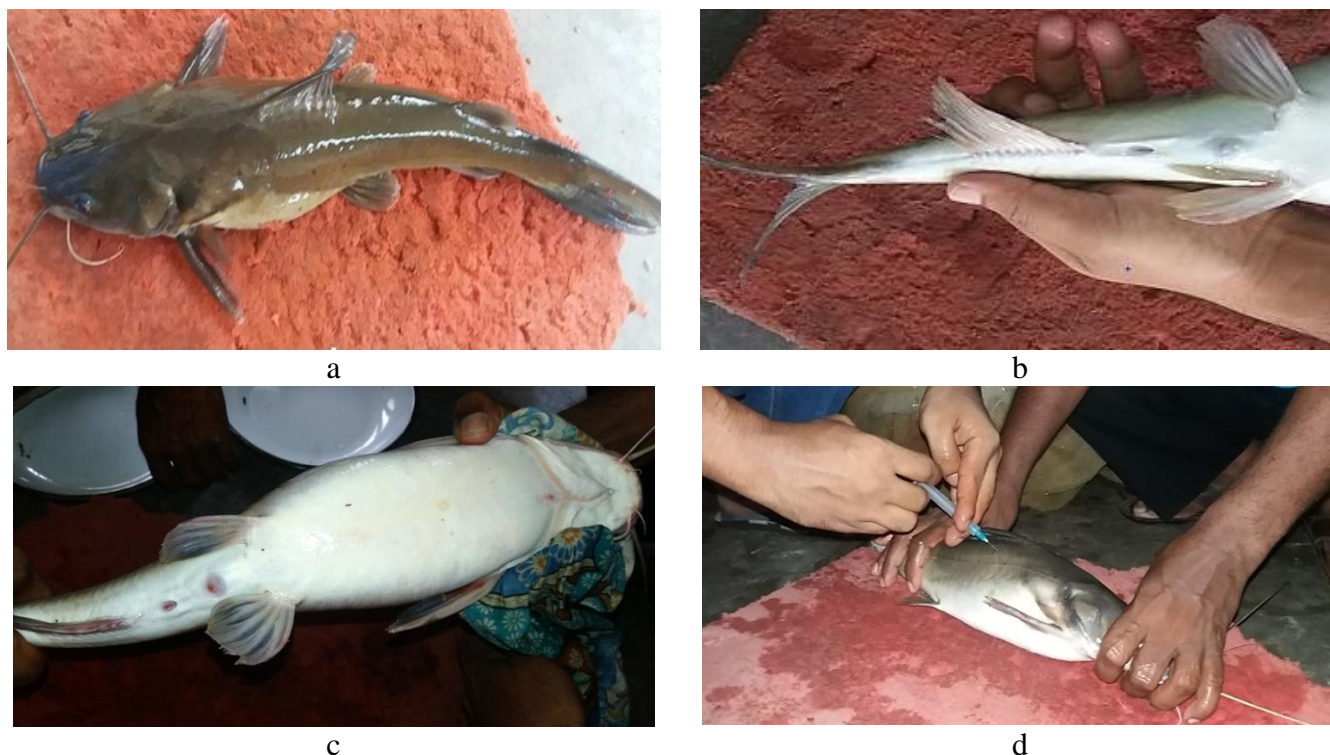


Fig. 2: Pictorial view of Gang magur, *Hemibagrus menoda*: **a.** Mature state; **b.** Ventral view of mature male; **c.** Ventral view of mature female showing swollen abdomen; and **d.** Injection of ovupin hormone to the brood fish.

#### *Collection of Ovupin hormone and preparation of its solution*

For induced breeding purpose, Gonadotropin Releasing Hormone analogues (GnRH<sub>a</sub>) commercially known as Ovupin (100 mg Domperidone + 0.2 mg S-GnRH<sub>a</sub>) were used in this study. Ovupin hormone vial was bought from a reputable shop in Mymensingh town. The proper doses of Ovupin hormone were calculated based on the recommended dose and body weight of the brood fish using this formula:

$$\text{Amount of ovupin (ml)} = \frac{Wt \times Pt}{1000}$$

Where,

Wt = total body weight of the fish injected (g);

Pt = the rate in mL Ovupin injected·kg<sup>-1</sup> body weight for a particular treatment.

For Ovupin preparation, the powder form of the synthetic hormone was diluted with distilled water to dissolve it in the contained vial and shook for a few minutes. Then, the prepared solution was taken in a 5mL disposable syringe for injection.

#### *Experimental design*

This experiment was carried out using commercial Ovupin hormone consisting of three treatments- T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>, each with three replications. The total 54 lived brood fish which were taken and kept them at a ratio of 2 male: 1 female in the tanks. Semi artificial (or induced natural) propagation method was adopted which involved synchronized spawning in breeding hapa whereby the injected brood fish (male and female spawners) were placed into a breeding hapa fixed in circular and rectangular tanks (dimensions: 1.22×2.74×0.37m). Double hapa (upper and lower) were used. After ovulation and fertilization, the upper hapa was removed along with the spent spawners while the fertilized eggs settled in the lower hapa which served as a shelter during the incubation period. Some larvae also hatched in the hapa.

### *Hormone injection*

The weights of selected brood fish and amount of needed Ovupin hormone solution were measured. Then a disposable graduated syringe of 5 mL was used for injecting the hormone to brood fish. The hormone solution was administered intramuscularly to the female (4, 6, 7 mL Ovupin·kg<sup>-1</sup>body weight) and male (1.5, 2, 3 mL Ovupin·kg<sup>-1</sup>body weight) between the dorsal fin and lateral line maintaining about 45° angular position with the body (Fig. 2d). The injected fish were then kept into breeding hapas placed in tanks for synchronized spawning at the ratio of 2♂:1♀ (temperature 26°C; pH 7.5; dissolved oxygen 6.5 ppm) and their reproductive behaviour were closely monitored. It was observed that the males were in ceaseless pursuance of the females though most often they remained together very closely under the shower.

### *Ovulation, determination of fertilization rate and incubation of eggs*

After 24 h of injection, all of fish were found ovulated. Ovulation and fertilization occurred in the spawning hapa. After ovulation the upper hapa was removed along with the spent spawners leaving the fertilized eggs. Approximately 100 eggs were placed in plastic bowls to determine fertilization rate with three replications of each and water flow was ensured using porous PVC pipe and outlet facility. Fertilized eggs were identified using a magnifying glass and then counted with the help of a soft thin brush. The ovulation and fertilization rate were determined by using the following formula (Mollah *et al.* 2008):

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

### *Determination of hatching rate*

The larvae were separated from unhatched eggs by siphoning with a 1.5mm rubber hose. Then the larvae were closely observed to see the time of yolk sac absorption for first feeding of larvae. Aerators were used through the systems for aeration. After completion of hatching, about 6 h post hatching; the number of larvae/hatchlings in each bowl was counted by siphoning them out. The hatching rate was determined by using the following formula (Islam *et al.* 2011):

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

### *Determination of survival rate*

For determination of survival rate, 300 hatchlings were randomly collected from each replication under a particular treatment and stocked in the trays for 10 days (without feeding). During the experimentation, all other conditions were maintained same. The number of total live larvae in the tray was counted at 10<sup>th</sup> day of the experiment for calculation of survival rate.

### *Physico-chemical parameters analysis*

The temperature, dissolved oxygen (DO) and pH value of water were ranged between 26 °C to 29 °C, 5.5 to 6.5 ppm and 6.7 to 7.5 respectively under different treatments.

*Statistical analyses:* The data were subjected to a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a significance level of p<0.05. The computer software SPSS version 20 was used for statistical analysis.

## RESULTS AND DISCUSSION

### Spawning responses of *Hemibagrus menoda* injected with Ovupin hormone

The doses of Ovupin given, latency period, incubation temperature and spawning responses of the fish are showed in Table 1.

**Table 1. Spawning responses of *Hemibagrus menoda* to different doses of Ovupin at 1♀:2♂ ratio.**

Treatment	Ovupin dose (mL Ovupin·kg <sup>-1</sup> body weight)		Latency period (h)	Incubation temperature (°C)	Remarks
	♀	♂			
T <sub>1</sub>	4	1.5	24.5	26-27	Complete ovulation, considerable number of larvae hatched but survival rate was poor.
T <sub>2</sub>	6	2	24	26-27	Successful ovulation, considerable number of larvae hatched and survived.
T <sub>3</sub>	7	3	25	27-29	Amount of ovulation was very little, hence a few numbers of larvae hatched and survived

### Ovulation rate

From the experiment the highest average ovulation rate (100%) was found both in T<sub>1</sub> and T<sub>2</sub> whereas the lowest value (66.67%) was found in T<sub>3</sub> (Table 2). The ANOVA test showed that T<sub>3</sub> was significantly ( $p < 0.05$ ) lower than T<sub>1</sub> and T<sub>2</sub>. However, no significant difference was found between T<sub>1</sub> and T<sub>2</sub> (Table 2). The ovulation rate (100.0±0.0%) indicates highly mature brood fish in better brood stock management practice with special diet. Rahdari *et al.* (2014) stated that lack of synchronization was attributed due to long time of latency in the achievement of readiness of spawning by the fish. Hasan *et al.* (2021) have observed that ovulation rate was 100% and 63% when *Hemibagrus menoda* was induced by Ovatide hormone at the rate of 5 & 3 mL Ovatide·kg<sup>-1</sup> body weight for female and 2.5 & 1.5 mL Ovatide·kg<sup>-1</sup> body weight for male which is more or less similar to the present findings. Mollah *et al.* (2008) reported that for riverine catfish (*Rita rita*), the dose of Pituitary Gland (PG) at the rate of 80, 100, 120 and 140mg·kg<sup>-1</sup> body weight resulted 0%, 100%, 100% and 100% ovulation, respectively.

**Table 2. Breeding performance (mean±SD) of *Hemibagrus menoda* with different doses of Ovupin hormone.**

Treatment	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
T <sub>1</sub>	100 <sup>a</sup>	71.00±3.06 <sup>ab</sup>	64.00±2.08 <sup>ab</sup>	43.00±3.79 <sup>ab</sup>
T <sub>2</sub>	100 <sup>a</sup>	84.67±3.17 <sup>b</sup>	83.00±1.53 <sup>b</sup>	59.33±2.33 <sup>b</sup>
T <sub>3</sub>	66.67±0.0 <sup>b</sup>	50.00±2.89 <sup>a</sup>	45.00±0.58 <sup>a</sup>	32.67±1.45 <sup>a</sup>

Values of the parameter in each column with different superscripts (ab, a, and b) differs significantly ( $p < 0.05$ ).

### Fertilization rate

Fertilization rates reveal mention worth differences in the effectiveness among the hormones in the 3 different treatments with Ovupin (Table 2). The fertilization rates were recorded as (71.00±3.06%) in T<sub>1</sub>, while (84.67±3.17%) and (50.00±2.89%) in T<sub>2</sub> and T<sub>3</sub>, respectively (Table 2). ANOVA test results revealed a significant difference among three doses of Ovupin hormone, T<sub>2</sub> was significantly ( $p < 0.05$ ) higher than T<sub>3</sub> and T<sub>1</sub> (Table 2). Hasan *et al.* (2021) have observed that fertilization rate was 97% and 90% when *Hemibagrus menoda* was induced by Ovatide hormone at the rate of 5 & 3 mL Ovatide·kg<sup>-1</sup> body weight for female and 2.5 & 1.5 mL Ovatide·kg<sup>-1</sup> body weight for male which is higher than the present findings. Mollah *et al.* (2008) reported that for riverine catfish (*Rita rita*), fertilization rate was 71.66±7.64% when female treated with 100mg PG·kg<sup>-1</sup> body weight while those treated with 120mg PG·kg<sup>-1</sup> body weight fish showed 12.50±2.50% fertilization. The fertilization rate is lower than the 98% fertilization rate found in *Heteropneustes fossilis* injected with Pituitary Gland (PG) extract at 75 mg/kg

body weight (Ali *et al.*, 2014). Lower fertilization rate (75%) was recorded for *Cirrhina reba* (Verghese 1969). The present findings agree with Haniffa and Sridhar (2002) who observed that irrespective of hormones and fish species, fertilization in *Channa punctatus* was 70% and above.

#### Hatching rate

The hatching rate was observed ( $64.00 \pm 2.08\%$ ), ( $83.00 \pm 1.53\%$ ) and ( $45.00 \pm 0.58\%$ ) in three treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively (Table 2). In T<sub>2</sub>, the highest hatching rate was reported, while T<sub>3</sub> recorded the lowest hatching rate. Further, hatching rate in T<sub>2</sub> was found significantly ( $p < 0.05$ ) higher than that of T<sub>3</sub> and T<sub>1</sub> (Table 2). Islam (2002) have observed that hatching rate of (76.21%) for *Ompok pabda* in a PG dose of 18 mg/kg and lowest (36.59%) at a dose of 20 mg PG/kg body weight of fish. Begum *et al.* (2001) reported that hatching rate was 38% as the highest for Shing, *Heteropneustes fossilis* injected with PG dose of 75 mg/kg body weight. Akhteruzzaman *et al.* (1992) and Kohinoor *et al.* (1990) also observed the same variation during their induced breeding trials. Marimuthu and Haniffa (2010) have observed that induced breeding experiments among three doses of ovaprim showed the best result in the form of hatching rate (96.3%).

#### Survival rate

The survival rate was observed ( $43.00 \pm 3.79\%$ ), ( $59.33 \pm 2.33\%$ ) and ( $32.67 \pm 1.45\%$ ) in three treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively (Table 2). Among the three treatments with Ovupin, the highest survival rate was recorded in T<sub>2</sub> while the lowest survival rate was in T<sub>3</sub>. A significantly ( $p < 0.05$ ) higher survival rate was found in treatment T<sub>2</sub> in comparison with T<sub>3</sub> and T<sub>1</sub> (Table 2). Hasan *et al.* (2021) have observed that survival rate was 85% and 80% when *Hemibagrus menoda* was injected with Ovatide hormone at the rate of 5 & 3 mL Ovatide·kg<sup>-1</sup> body weight for female and 2.5 & 1.5 mL Ovatide·kg<sup>-1</sup> body weight for male which is higher than the present findings. The survival rate of 5-day old hatchling of *Mystus vittatus* varied from 55.5-68% (Sarker *et al.* 2002, Alam *et al.* 2006, Islam *et al.* 2011), which is lower than the present findings. Survival rate noted in this study is remarkably higher than that obtained by Haniffa and Sridhar (2002) who reported that survival rate of 55% in *Channa punctatus* injected 3000 IU HCG/kg body weight.

The time interval between the first hormonal injection of Ovupin and ovulation (latency period) varied between 24 and 25h and occurred within a temperature range of 26–29°C. Tan-Fermin and Emata (1993) reported that latency period of 12–16 hrs when PG and ovaprim were used for induced breeding of *Clarias gariepinus* and *Clarias macrocephalus* which is extremely lower than the present findings. Mollah and Tan (1982) reported that when the temperature was high, the incubation period was short and consequently affects the hatching and survival rate of the eggs of *Clarias macrocephalus*. Okere *et al.* (2015) stated that the reproductive output was better when the latency period was short. Pillay (1996) mentioned that for successful induced breeding, the application of the appropriate dose of hormone is the key factor.

*Hemibagrus menoda* is one of the most valuable catfish in our country which has high demand to the consumers but its biodiversity is undergoing fast declining day by day. Developed protocol of breeding will help the hatchery managers to protect this nearly threatened species from the risks of extinction. On the basis of the highest percentage of ovulation, fertilization, hatching and survival rate, single dose of 6mL Ovupin·kg<sup>-1</sup> body weight for female and 2mL Ovupin·kg<sup>-1</sup> body weight for male could be considered as the effective dose for the induced breeding of *H. menoda* in a 2♂:1♀ ratio. The findings of the study will be helpful in the commercial hatcheries as it is cost savings. It enables to produce the high-quality seeds by dint of proper application of Ovupin hormone dosages in menoda cat fish.

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