*Ann. Bangladesh Agric. (2021) 25 (2) : 13-25*
www.doi.org/10.3329/aba.v25i2.62409

*ISSN 1025-482X (Print)*
*2521-5477 (Online)*

# GROWTH AND BREEDING PERFORMANCE OF BROODFISH AND LARVAL GROWTH OF *Heteropneustes fossilis* WITH FEEDS

**A. C. Sinha[1], M. A. Salam[1], S. K. Mazumder[1], M. S. Alam[1] and M. L. Rahman[1]***

## Abstract

A 63-day long indoor experiment was carried out to determine the effects of four different diets of: live tubificid worms ($T_1$), live tubificid worms with formulated feed ($T_2$), shrimp with formulated feed ($T_3$) and formulated feed ($T_4$) on brood development and larval growth of *Heteropneustes fossilis*. Broodfish were fed twice a day with experimental diets. Reproductive performance of *H. fossilis* was evaluated based on growth parameters and fry production of broodstock. The result showed that while the highest (16.37 ± 0.90g) and the lowest (7.03 ± 0.85g) weight gain was obtained in $T_2$ and $T_4$ respectively, however, there was no significant difference between treatments $T_1$ and $T_3$. The FCR of $T_2$ was found to be the lowest followed by $T_3$, $T_1$ and $T_4$, respectively. The highest fecundity was found in $T_2$ (4245.48 ± 347.38) followed by $T_3$ (3747.10 ± 317.99), $T_1$ (3583.96 ± 327.27) and $T_4$ (3191.95 ± 444.55). The rate of hatching and their survival was significantly higher in $T_2$ followed by $T_3$, whereas $T_1$ and $T_4$ showed significantly lower hatching and survival rate. After 28 days of rearing, larvae obtained from $T_2$ treatment showed the highest growth performance compared to other treatments. The findings of the current research suggested that live feed (tubificid worms) supplemented with formulated feed may enhance the breeding performance and larval growth of *H. fossilis* and could be considered an affordable option for small-scale hatcheries.

**Keywords:** Catfish, diets, *Heteropneustes fossilis*, fecundity, broodstock, reproduction.

## Introduction

Among various catfish species, *H. fossilis* popularly known as "Shing" is an important air-breathing aquaculture catfish species in many Asian countries (Akand *et al*., 1991). Due to the presence of accessory respiratory organs, they can survive in water with low oxygen levels. Shing is a popular fish in Bangladesh and generally inhabits ponds, ditches, swamps, and marshlands, but sometimes inhabits muddy rivers (Froese and Pauly, 2018). In Bangladesh, the aquaculture interest of shing among fish farmers is increasing day by day due to its high market values, profitable culture and hardy nature for the presence of accessory respiratory organs that enable fishermen to sell them live in the market (Ali *et al*., 2014). Despite the popularity of shing culture in Bangladesh and considerable research efforts have been put into its culture, aquaculture production has been low. This is perhaps due to poor management practices, particularly nutritional management and the scarcity of the fingerlings for culture.

[1]Department of Genetics and Fish Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. *Corresponding author: mlrahman@bsmrau.edu.bd

Therefore, the development in broodstock nutrition is predictable to contribute towards improvement in the production.

Diet plays a significant role in aquaculture production. Different commercial feeds are available in the market such as nursery, and grower feed of various sizes. Those feeds are manufactured based on the nutrient requirement of fry or young fish that may not fulfill the requirement of broodfish. Moreover, very limited feed is available in the market particularly manufactured for broodfish of aquaculture species including shing. Nutrient requirement for broodfish hence may not be fulfilled by the formulated feed as various vitamin and minerals play a vital role during reproduction that may not be present in the formulated feed (Palace and Werner, 2006). In this context, live feed such as tubificid worms, and small fish that contain various minerals along with the protein (Herawati *et al*., 2016) may help fill up the gap in broodstock nutrition during breeding. So, it is necessary to determine which diet is suitable for broodfish either formulated or live feed or a combination of them.

The importance of fish broodstock nutrition has been reviewed by some authors (Izquierdo *et al*., 2001; Hardy *et al*., 1984; Luquet and Watanabe, 1986). Few studies have, however, been conducted on nutritional requirements for gonadal development and egg/larval quality (Palace and Werner, 2006; Washburn *et al*., 1990). Knowledge of the effects of broodstock nutrition on broodstock growth and gonadal maturation is important because a good broodfish feeding regime not only leads to successful spawning but also confers a superior health and growth potential on the progenies (Takeuchi *et al*., 1978). A previous comparative study between live and formulated feed shows that live feed is more suitable than formulated feed for fish (Onura *et al*., 2018). A meta-analysis indicated that larvae fed on artificial diets have a 2.5 times higher chance to die than those fed on live feed (Sales, 2011). However, a lot of attention has been paid to the care of the juvenile stages of catfish (Onura *et al*., 2018; Srichanun *et al*., 2012; Rahman *et al*., 1997), while the pre-spawning treatment of the broodstock and the effect of dietary changes on oocyte development have attracted limited attention. Therefore, the present study was designed to determine the effects of different diets on the growth and reproductive performance of broodfish as well as the larval growth of stinging catfish *H. fossilis*.

## Materials and Methods

The experiment was carried out in the wet laboratory under the Department of Genetics and Fish Breeding of the Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) for 63 days. Two types of tanks were used for rearing of the broodfish (500L) and for spawning purposes (300L). The spawning tanks were with a continuous flow of water. Hatching trays were used to study the fertilization rate. For rearing of newly hatched spawn, five trays were used and the mortality and deformities were observed and counted.

### Broodfish collection

*H. fossilis* (matured) broodfish were collected from the fish farm (Reliance Aqua Farms) located in trishal, Mymensingh district and were kept in 500L plastic tanks. The fish were

selected carefully for further use and stocked in the wet lab of the Faculty of Fisheries, BSMRAU. Only healthy and uninjured fish were selected for induced breeding. The male and female were determined by eye observation based on the sex determination criteria. The plastic tanks were covered by a clean cloth to protect the fish from escaping and becoming injured.

**Experimental Design**

Four types of feed were used in the study under four different treatments: Live tubificid worms ($T_1$), tubificid worms with formulated feed (1:2) ($T_2$), frozen chopped shrimp (small indigenous) with formulated feed (1:2) ($T_3$) and formulated feed (Quality Feeds Limited) ($T_4$). Each treatment had three replicates making a total of 12 tanks of 500L capacity each containing 300L water. Every day 30% of water was changed and added new water to maintain a suitable environment in tanks.

**Feeding trial of fish**

After the completion of the preparation of experimental tanks, 30 broodfish (50:50 male-female ratio) were released in each treatment tanks. According to the fish size and weight, a required amount of feed was given two times a day (in the morning and evening) into experimental tanks. Before releasing broods in tanks the weight and length data were measured individually and applied formulated feed of 3% and live feed of 1.5% of their body weight. Combined diets (formulated feed and live feed) were applied following 1:1 ratio based on the respective body weight percentages. Proximate composition (PC) of tubificid worms and shrimp was analyzed (Table 1) following the standard protocol (AOAC, 1995) and PC of formulated feed was collected from the company level on a feed sack.

**Fish sampling procedure**

Fish were sampled after the completion of the experimental period for estimating different growth parameters. The final length (cm) and weight (g) of the individual fish were carefully recorded after 63 days of rearing. The final body weight of individual fish was determined by an electronic balance and body length of individual fish was measured by measuring scale.

**Average daily gain (ADG, g/day)**

$$ADG = \frac{\text{Mean final fish weight - Mean initial fish weight}}{\text{Time (T2 - T1)}}$$

Where, $T_2$= Final time; $T_1$= Initial time

**Specific growth rate (SGR, %/day)**

$$SGR = \frac{(LnWT - LnWt)}{(T - t)} \times 100$$

Where, In Wt. = Natural log of weight at time T; T= Final time
In W1= Natural log of initial weight; t= Initial time.

**Feed conversion ratio (FCR)**

$$FCR = \frac{\text{Feed (g) consumed by the fish}}{\text{Weight (g) gain by the fish (W2 - W1)}}$$

Where, W2= Final weight; W1=Initial weight

**Protein efficiency ratio**

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

**Table 1. Proximate composition of different experimental feed on dry basis (%)**

| Parameter | Tubificid worms | Shrimp | Formulated Feed |
|---|---|---|---|
| Protein (%) | 69.8 | 55 | 35 |
| Carbohydrate (%) | 3.8 | 9 | 32 |
| Fat (%) | 12.1 | 19 | 8 |
| Ash (%) | 8.3 | 10 | 14 |
| Moisture (%) | 6 | 7 | 11 |

**Measurement of oocyte diameter**

For the measurement of oocyte diameter, about 20 ova were collected randomly from each ovary of each fish. The diameter of the oocyte was measured by an oculometer. The widest part of the oocyte was measured to determine the oocyte diameter.

**Estimation of fecundity**

For fecundity estimation, the gravimetric method was applied in the present study using the following formula:

$$\text{Fecundity (F)} = N \times \frac{\text{Gonad weight}}{\text{Sample weight}}$$

Where N is the number of eggs in the sample.

**Spawning and fertilization**

Ovaprim, an inducing hormone, was administered at 0.3 ml/kg and 0.1 ml/kg body weight of females and males of *H. fossilis* broodfish respectively (Rahman *et al*., 2013). In this experiment, fish spawning was done by stripping.

**Total number of eggs and fertilization rate**

All the brooders ovulated after a period of 10-15 hrs after hormone injection in the experiment. The brooders were then transferred from the holding tanks after the completion of ovulation. Whereas, the fertilized eggs were transferred into hatching trays with taking precaution to avoid damage and fungal/bacterial contamination during the egg collection. The number of eggs released into each tray was estimated using gravimetric methods adapted from Legender (1986). Afterwards, a continuous flow of water was maintained for aeration to guarantee the environmental conditions were optimal for the hatching process. The total number of eggs and rate of fertilization (transparent eggs were considered as fertilized eggs) were calculated

by direct counting method based on the following formula:

$$\text{Fertilization Rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

**Incubation and hatching**

After 17-20 h of incubation, the eggs were hatched and the hatchlings came out from the eggs. Incubation temperature was maintained at 28-31°C. The hatchlings were counted and the rate of hatching was calculated by the following formula:

$$\text{Hatching Rate (\%)} = \frac{\text{Number of hatching}}{\text{Number of fertilized eggs}} \times 100$$

**Larvae rearing and evaluation of growth performance**

The larvae (9 days post hatching) were reared for 28 days to observe growth performance (weight). Larvae were released in 300L tanks obtaining approximately 200 larvae from four treatments in each following three replications. Larvae were fed with commercial feed (starter) at 15% body weight ratio for first 14 days in the morning and evening daily and next 14 days at 10% body weight ratio. Continuous aeration was used to supply oxygen in all tanks. Uneaten feed particles in all tanks were cleaned through syphoning and 30% water exchange was done in every day morning. In every 7 days, weight (mg) of 20 individuals were carefully recorded up to 28 days of rearing. The body weight of individual fish was determined by an electronic balance.

**Estimation of larvae survival rate**

The survival rate of *H. fossilis* larvae for each treatment was estimated at the sampling time at 7-day interval. The survival rate was calculated using the following this formula.

$$\text{Survival Rate(\%)} = \frac{\text{No. of the survived}}{\text{No. of larvae stocked}} \times 100$$

**Statistical Analysis**

Growth performance and all reproductive data of H. fossilis at each treatment were analyzed by one-way analysis of variance (ANOVA) after confirmation of homogeneity of variance. Tukey (HSD) mean separation test were used to determine the differences among the means. Significant differences were stated at (p<0.05) level. All statistical analyses were performed using statistical software SPSS 16.5.0 for Windows (SPSS Inc. Chicago, IL USA).

**Results**

A significantly higher growth rate and lower feed conversion ratio and higher breeding performance were observed among the fish of treatment fed on diets supplemented with tubificid worms and shrimp. The poorer

growth rate, feed conversion and breeding performance were observed among the treatments fed on only tubificid worms or formulated feed.

**Growth performance of broodfish**

The final weight of *H. fossilis* broods after the experimental period of 63 days significantly varied from the initial weight and significant variation was also observed among the treatments (Table 2). Significantly higher weight gain (16.37 ± 0.90g), specific growth rate (0.86 ± 0.04g), protein efficiency ratio (1.64 ± 0.41) and lowest FCR (1.40 ± 0.61) of shingi broods were observed in $T_2$ fish fed with mixed feed (live tubificid worms and formulated feed) than those of fish in $T_1$ and $T_4$. In addition, fish in $T_3$ also showed higher weight gain, SGR, PER, and lower FCR fed with another mixed feed (shrimp and formulated feed) than $T_1$ and $T_4$ but not greater than $T_2$. Significantly lower weight gain (7.03 ± 0.85g), specific growth rate (0.43 ± 0.04g), and higher FCR (2.45 ± 0.53) of shingi broods were found in $T_4$ fed with only formulated feed but the lowest protein efficiency ratio was observed in $T_1$.

**Fecundity and egg size of *H. fossilis***

The biometric parameters and their corresponding fecundity and egg diameter are shown in Table 3. Although the highest mean fecundity (4245.48 ± 347.38) and mean egg diameter (1.21 ± 0.04) were found in $T_2$, higher fecundity (3747.10 ± 317.99) and egg diameter (1.02 ± 0.02) was also found in $T_3$ compared to other treatments where the lowest fecundity (3191.95 ± 444.55) was found in $T_4$.

**Breeding Performance**

**Ovulation rate**

The ovulation rate found in four treatments is shown in Fig. 1. The ovulation rates were 65.95%, 87.84%, 76.87% and 66.56% in treatments $T_1$, $T_2$, $T_3$ and $T_4$, respectively. The highest ovulation rate (87.84%) was found in $T_2$ whereas the lowest ovulation rate (65.95%) was found in $T_1$.

**Fertilization rate**

The fertilization rate found in four treatments is shown in Fig. 2. The fertilization rates were 75.72%, 89.29%, 83.30%, and 73.75% in treatment $T_1$, $T_2$, $T_3$ and $T_4$, respectively. Fertilization rate estimation showed that the rate was similar in $T_1$ and $T_4$ treatment. The

**Table 2. Initial and final weight, weight gain, SGR, FCR and PER of broodfish (g) (Mean ± SEM) of stinging catfish broods at 63 days rearing period**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Initial weight(g) | $22.79 \pm 0.10^{a}$ | $22.87 \pm 0.11^{a}$ | $22.77 \pm 0.14^{a}$ | $22.71 \pm 0.09^{a}$ |
| Final weight(g) | $32.25 \pm 0.15^{b}$ | $39.24 \pm 0.92^{a}$ | $33.33 \pm 0.20^{b}$ | $29.74 \pm 0.81^{c}$ |
| Weight gain(g) | $9.46 \pm 0.14^{c}$ | $16.37 \pm 0.90^{a}$ | $10.57 \pm 0.34^{b}$ | $7.03 \pm 0.85^{d}$ |
| SGR | $0.55 \pm 0.01^{c}$ | $0.86 \pm 0.04^{a}$ | $0.61 \pm 0.02^{b}$ | $0.43 \pm 0.04^{d}$ |
| FCR | $2.27 \pm 0.75^{b}$ | $1.40 \pm 0.61^{a}$ | $1.88 \pm 0.47^{a}$ | $2.45 \pm 0.53^{b}$ |
| PER | $0.97 \pm 0.25^{bc}$ | $1.64 \pm 0.41^{a}$ | $1.40 \pm 0.50^{a}$ | $1.23 \pm 0.55^{b}$ |

Note: Values are Mean ± SEM of four groups of 20 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

**Table 3. Mean of total length (TL), body weight (BW), mean fecundity (MF) and egg diameter (MED) of female *H. fossilis***

| Treatments | (MTL) (cm) | (MBW) (g) | MF | (MED) (mm) |
|---|---|---|---|---|
| $T_1$ | $6.68 \pm 0.23^{b}$ | $23.20 \pm 1.10^{c}$ | $3583.96 \pm 327.27^{ab}$ | $0.91 \pm 0.05^{c}$ |
| $T_2$ | $7.07 \pm 0.26^{a}$ | $26.22 \pm 1.95^{a}$ | $4245.48 \pm 347.38^{a}$ | $1.21 \pm 0.04^{a}$ |
| $T_3$ | $7.00 \pm 0.18^{a}$ | $24.31 \pm 0.46^{b}$ | $3747.10 \pm 317.99^{ab}$ | $1.02 \pm 0.02^{b}$ |
| $T_4$ | $6.56 \pm 0.30^{b}$ | $22.87 \pm 1.22^{c}$ | $3191.95 \pm 444.55^{b}$ | $0.91 \pm 0.05^{c}$ |

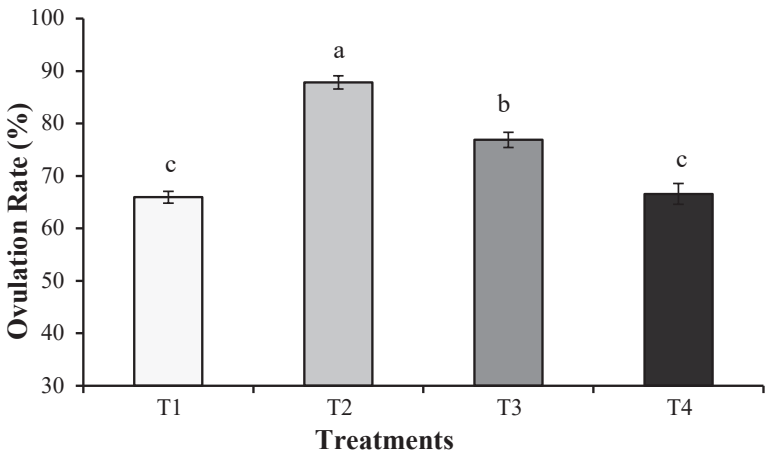


**Fig. 1. Ovulation rate (%) of *H. fossilis* fed on four different types of feed under different treatments (In $T_1$= Live tubificid worms, $T_2$= Live tubificid worms and formulated feed, $T_3$= Shrimp and formulated feed, $T_4$= Formulated Feed).**

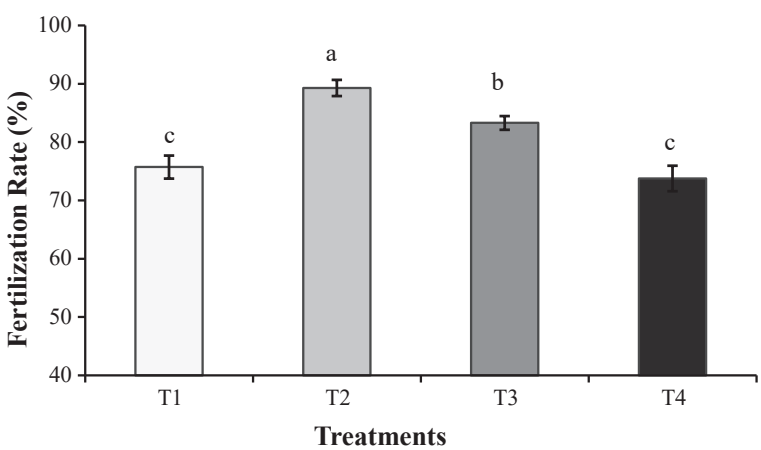


**Fig. 2. Fertilization rate (%) of *H. fossilis* fed on four different types of feed ($T_1$= Live tubificid worms, $T_2$= Live tubificid worms and formulated feed, $T_3$= Shrimp and formulated feed, $T_4$= Formulated Feed).**

highest fertilization rate (89.29%) was found in $T_2$ whereas the lowest fertilization rate (73.75%) was found in $T_4$.

**Hatching rate**

Like fertilization rates, hatching rates showed a similar pattern among different treatments where the highest hatching rate (68.94%) was found in fish reared under $T_2$ compared to other treatments (Fig. 3). Although $T_3$ also showed a higher hatching rate than $T_1$ and $T_4$ but was significantly lower than $T_2$. Moreover, the lowest hatching rate (56.68%) was found in $T_1$ compared to other treatments (Fig. 3).

**Growth performance of larvae**

The mean initial weights of the larvae obtained from different broodstock treatments $T_1$, $T_2$, $T_3$ and $T_4$ were 16.26 ± 4.20mg, 16.58 ± 5.76mg, 15.63 ± 5.36mg and 14.39 ± 3.47mg, respectively while after 28 days of rearing the average final weights were found 341.63 ± 48.29mg, 428.00 ± 11.71mg, 399.75 ± 10.09mg and 289.41 ± 18.17mg under different treatments (Table 4). Weight gain in every week sampling for four weeks (Fig. 4) showed that larvae from $T_2$ gained the best weight compared to other treatments. The highest fry survival (%) and specific growth rate (%SGR) were also observed 60.94 ± 2.02 and 7.29 ± 0.04 in treatment $T_2$ followed by treatment $T_1$, treatment $T_3$ and treatment $T_4$. But the highest deformed fry (%) were observed 3.59 ± 0.72 in $T_1$ followed by others. The weight gain, specific growth rate (% SGR) and fry survival (%) were significantly ($P<0.05$) higher in treatment $T_2$ compared to $T_1$, and $T_4$ (Table 4).

**Discussion**

Acceptance of live food (LF) than formulated feed (FF) to fish is undoubtedly better in terms of nutritional and other factors (Mandal *et al*.,

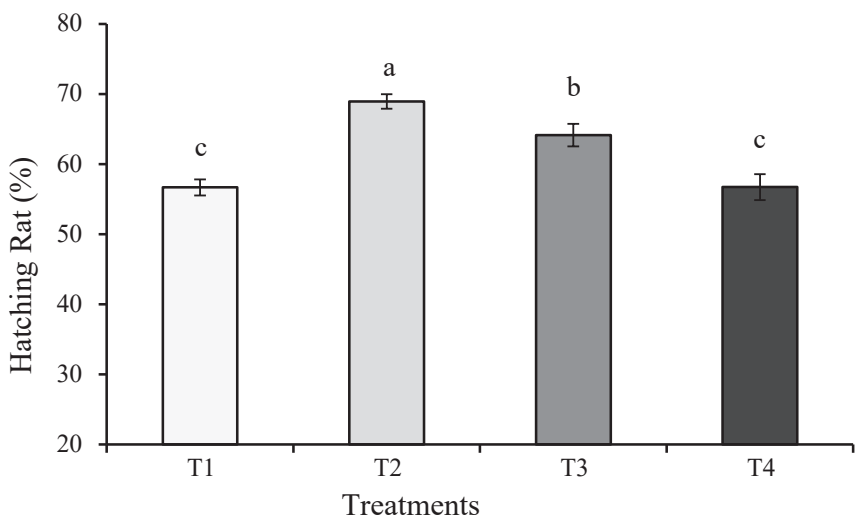


**Fig. 3. Hatching rate (%) of *H. fossilis* female eggs on four different types of feed under different treatments (In $T_1$= Live tubificid worms, $T_2$= Live tubificid worms and formulated feed, $T_3$= Shrimp and formulated feed, $T_4$= Formulated feed).**

**Table 4. Initial and Final weight, weight gain (mg), SGR, Fry survival (%), deformed fry (%) of larvae (Mean ± SEM) of stinging catfish at 28 days rearing period**

| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Initial Wt (mg) | 16.26 ± 4.20[a] | 16.58 ± 5.76[a] | 15.63 ± 5.36[a] | 14.39 ± 3.47[a] |
| Final Wt (mg) | 341.63 ± 48.29[b] | 428.00 ± 11.71[a] | 399.75 ± 10.09[a] | 289.41 ± 18.17[c] |
| Weight Gain | 325.37 ± 51.06[b] | 411.41 ±17.39[a] | 384.12 ± 4.74[a] | 275.01 ± 19.59[b] |
| SGR | 7.05 ± 0.16[b] | 7.29 ± 0.04[a] | 7.22 ± 0.01[a] | 6.89 ± 0.07[b] |
| Fry survival (%) | 40.92 ± 1.96[d] | 60.94 ± 1.85[a] | 52.67 ± 1.50[b] | 44.52 ± 2.61[c] |
| Deformed fry (%) | 3.59 ± 0.72[a] | 1.15 ± 0.46[d] | 1.62 ± 0.58[c] | 2.37 ± 1.02[b] |

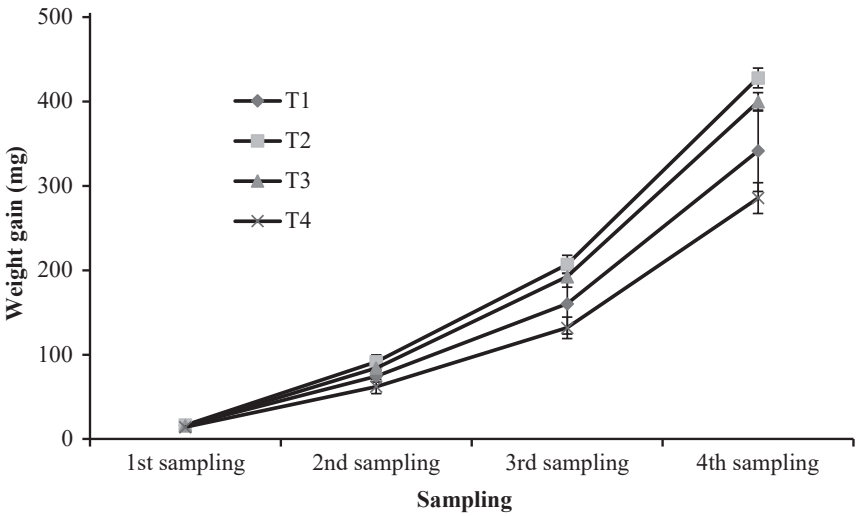


**Fig. 4. Weight gain of H. fossilis larvae produced from broodfish reared under different diets in every 7 days interval sampling (vertical bars = ±SD).**

2010), which contain all the essential nutrients such as proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1999). Therefore, in the present study, an attempt was made to evaluate the effects of different diet combinations of formulated feed with live food (Tubificid worms, shrimp) on the growth, survival and spawning performance of *H. fossilis*. After experimental periods the highest final weight (39.24 ± 0.92), weight gain (16.37 ± 0.90) and specific growth rate (0.86 ± 0.04) were observed in $T_2$ and the lowest final weight (29.74 ± 0.81), weight gain (7.03 ± 0.85) and specific growth rate (0.43 ± 0.04) was observed in $T_4$. In $T_4$ the broods were fed with only the formulated feed which probably contains limited nutrients that is not sufficient for broodfish but in $T_2$ the broods were fed with mixed feed (live tubificid worms and formulated feed) which might stimulated appetite and probably contain balanced nutrition to increase the growth.

Hashim and Abdullah (1993) conducted experiments supplementing artificial diets for catfish *(Clarias macrocephalus)* with tubificid worms and found the highest growth rate and breeding performance. In this study, $T_1$ showed lower growth performance than $T_2$ although live feed is always preferable for fish. This is because only live feed may not fulfill all the nutritional requirement that is supported by Soundarapandian *et al*. (2002) who reported the highest FCR and significantly lower survival in *Macrobrachium malcomsonnii* fed with adult *Artemia* in comparison with artificial feed. Interestingly, the lowest protein efficiency ratio (0.97 0.25b) was observed in $T_1$ (only tubificid worms) instead of being higher protein content (Table 2) but in $T_2$ (1.64 0.41) which indicates that *H. fossilis* could have efficiently utilized the low protein in Formulated Feed (FF) compared with Live Feed (LF), which is in agreement with the results observed by Abbas *et al*. (2005). So, it can be presumed that a mixed diet of LF and FF is preferable to LF or FF alone for *H. fossilis* broods and this presumption can be stronger from the positive result of the combined effect of *Artemia* and formulated feed on African catfish (Onura *et al*., 2018).

The quantity and quality of feeds are important factors affecting the reproduction in fish (Degani *and* Yehuda, 1996). Therefore, the breeding performance of *H. fossilis* was observed after the rearing period in the present study. The highest breeding performance like fecundity, ovulation rate, fertilization rate, and hatching rate was found in $T_2$ and the lowest breeding performance was found in $T_4$ shown in Fig. 2 to 4 and in Table 4. These results reveal that the composition and palatability of diets significantly affected the reproduction in *H. fossilis*. Previous findings revealed that fish fed with a mixed diet of LF and FF exhibited a comparatively better result than only FF in the case of breeding performance (Kumaraguru *et al*., 2007; Mandal *et al*., 2010) which is in agreement with the present study. Similarly, *Xiphophorus helleri* fry production was found to increase 30% more when the daily FF was supplemented with LF Daphnia sp. (Kruger *et al*., 2001). In addition, mean fertilization and hatching rates were also found higher in the zebrafish fed with a mixed diet of LF and FF (Rabbane and Rahman, 2017). In the present study, $T_3$ also showed better performance where fish fed with mixed fed (shrimp 2% BW and formulated feed 3% BW) which was proximate to $T_2$ and contain more nutrients than other treatments except $T_2$. But the breeding performance was lower in $T_3$ than $T_2$ because freezing of shrimp may reduce the nutritive quality of some natural feeds and hence are unsuitable for the fish (Chinavenmeni and Natesan, 2007). And $T_1$ and $T_3$ showed lower breeding performance because probably only tubificid worms or formulated feed contained insufficient nutrients. However, Degani and Yehuda (1996) did not find any significant difference in breeding performances in angelfish, *Pterophyllum scalare* fed with different types of feeds. Moreover, most of the above research was performed on the ornamental fish which are mostly short spawners. Since catfish is a yearly asynchronous spawner, need more research to know the detailed effects of a mixed diet on *H. fossilis* breeding.

Larvae obtained from broods fed with mixed feed (live tubificid worms and formulated feed) in $T_2$ and Shrimp and formulated feed in $T_3$ fed more actively from the beginning

of the rearing compared with other larvae found from broods fed with diets only i.e. tubificid worms in $T_1$ and formulated feed in $T_4$. A significantly higher growth rate and improved feed conversion were observed in $T_2$, indicating that the comparatively lower growth performance of the fry in $T_1$ and $T_4$ could be improved by supplemental feeding with tubificid worms. And in $T_4$ the poorer growth rate and feed conversion of the fry could be attributed to the poor digestibility and assimilation of the diet (Wilson *et al.,* 1981) or its unattractiveness. The higher feeding activity in $T_2$ in the present study suggests that the tubificid worms not only contains food attractants but it also stimulates the appetite of which ultimately increases the acceptability of formulated feed in the culture system. Several studies have been conducted to improve the acceptability and feed consumption of experimental diets. Previously, Siamese fighting fish (*Betta splendens*) larvae showed the highest weight gain fed with a mixed diet compared to only formulated feed (Mandal *et al*., 2010) and similar findings were observed on catfish (*Ompok bimaculatus*) larvae fed with a mixed diet (Malla and Banik, 2015). In addition, the dominancy of live feed over formulated feed for larval growth has been proven in several studies conducted in different fish species (Khader and Altaff, 2021; Nuswantoro and Rahardjo, 2018; Rabbane and Rahman, 2017; Mohideen *et al*., 2014; Malla and Banik, 2015).

Our results showed a significantly higher larval survival rate (60.94%) in $T_2$ which was in agreement with the results found in the experiment conducted with *Betta splendens* where the highest fry survival (65.54%) was recorded in fish fed with a mixed diet of LF and FF (Mandal *et al*., 2012). Interestingly, the lowest survival and higher *H. fossilis* fry death was observed in the $T_1$ where only tubificid worms was fed to fish. Similar to our results, the lowest survival was found in zebrafish where dried tubificid worms was fed to fish (Rabbane and Rahman, 2017). It is likely to cause that the nutrients present in the only live tubificid worms were not balanced to sustain the fry over the feeding trial period.

In conclusion, this study has demonstrated that *H. fossilis* breeding performance can be improved by the combined diet of 50% tubificid worms and 50% formulated other than the live feed or formulated feed only. Moreover, larval growth and survival can also be increased with that mixed diet. The present results are very promising in terms of providing higher fry production as well as raising of *H. fossilis.*

## References

Abbas, G., K. Jamil, R. Akhtar and L. Hong. 2005. Effects of dietary protein level on growth and utilization of protein and energy by juvenile Mangrove Red Snapper (*Lutjanus argentimaculatus*). *J. Ocean Univ. China*. 4: 49-55.

Akand, A. M., M. R. Hasan and M. A. B. Habib. 1991. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, *Heteropneustes fossilis* (Bloch). In *Fish nutria. Res. Asia.* Proceedings of the Fourth Asian Fish Nutrition Workshop. ed. De Silva SS Asian Fisheries Society, Manila, Philippines, Pp. 93-100.

Ali, M. F., M. M. Rahman, K. M. Bashar, R. Rabeya, M. Hadiuzzaman and M. A. Ruhul. 2014. Comparative study on induced breeding of shing, *Heteropneustes fossilis* (Bloch) between HCG and PG with different combination. *J. Fish Aquat. Stud.* 2(2): 104-108.

AOAC (Association of Official Analytical Chemicals). 1995. Official Method of Analysis. 12th ed, Association of official Analytical Chemists, Washington DC 832 P.

Chinavenmeni, S. V. and M. Natesan. 2007. Composition and nutritional efficacy of adult fairy shrimp *Streptocephalus dichotomus* as live feed. *Food Chem.* 100: 1435-1442.

Degani, G. and Y. Yehuda. 1996. Effects of diets on reproduction of angel fish *Pterophyllum scalare* (Cichlidae). *Indian J. Fish.* 43: 121-126.

Froese, R. and D. Pauly. 2018. Fish Base 2018, World Wide Web electronic publication.

Hardy, R. W., K. D. Shearer and I. B. King. 1984. Proximate and elemental composition of developing eggs and maternal soma of pen-reared coho salmon (Oncorhynchus kisutch) fed production and trace element fortified diets. *Aquaculture*. 43(1-3): 147-165.

Herawati, V. E., R. A. Nugroho, J. Hutabarat and O. Karnaradjasa. 2016. Profile of amino acids, fatty acids, proximate composition and growth performance of *Tubifex tubifex* culture with different animal wastes and probiotic bacteria. *AACL Bioflux.* 9(3): 614-622.

Hashim, R. and A. B. Abdullah. 1993. Experimental diets from local resources for catfish *(Clarias macrocephalus)* fry and its effect on growth. In Modernization in Tropical Livestock and Poultry Production. ed. E.A. Ahmed *et al.* Proceeding of the 12th Annual Conference of the Malaysian Society of Animal Production, Dept. of Animal Science, Universiti Pertanian Malaysia, Selangor, Malaysia, Pp. 246 - 250.

Izquierdo, M. S., H. Fernandez-Palacios and A. G. J. Tacon. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*. 197(1-4): 25-42.

Khader, S. M. A. and K. Altaff. 2021. Influence of Different Types of Diets on the Growth Performance of Juvenile Guppies (*Poecilia reticulata* Peters, 1859). *Mangifera Edu*. 5(2): 93-102.

Kruger, D. P., P. J. Britz and J. Sales. 2001. The in£uence of live feed supplementation on growth and reproductive performance of swordtail (*Xiphophorus helleri* Heckel 1848) broodstock. *Aquar. Sci. Conserv*. 3: 265-273.

Kumaraguru, K. P., A. V. Shanmugam and S. Rajagopal. 2007. Dietary e¡ect on fry production and growth performance of sailfin molly, *Poecilia latipinna*, in saltwater. *Acta Ichthyol. Et Piscat.* 37: 29-35.

Legender, M. 1986. Seasonal changes in sexual maturity and fecundity and HCG induced breeding of the catfish *Heteropneustes longifilis* val. (Flarridae) reared in Ebrie lagoon (Ivory coast). *Aquaculture.* 55: 201-213

Luquet, P. and T. Watanabe. 1986. Interaction "nutrition-reproduction" in fish. *Fish Physiol. Biochem.* 2(1): 121-129.

Malla, S. and S. Banik. 2015. Larval rearing of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) with live and artificial diets: A preliminary study in Tripura, India. *Int. J. Fauna Biol. Stud.* 2: 16-21.

Mandal, S. C., N. P. Sahu, M. P. S. Kohli, P. Das, S. K. Gupta and S. Munilkumar. 2010. Replacement of live feed by formulated feed: effect on the growth and spawning performance of Siamese fighting fish (*Betta splendens*, Regan, 1910). *Aquac. Res.* 41(11): 1707-1716.

Mandal, S. C., M. P. Kohli, P. Das, S. K. Singh, S. Munilkumar, K. Sarma and K. Baruah. 2012. Effect of substituting live feed with formulated feed on the reproductive performance and fry survival of Siamese fighting fish, *Betta splendens* (Regan, 1910). *Fish Physiol. Biochem*. 38(2): 573-584.

Mohideen, A. S., M. A. Sheriff and K. Altaff. 2014. Effect of three different feeds on the growth and survival of sailfin molly *Poecilia latipinna* (Lesueur, 1821). *Revel. Sci.* 4(1): 45-48.

New, M. B. 1999. Global aquaculture: current trends and challenges for the 21st century. *World Aquac.* 30: 8-13.

Nuswantoro, S. and S. S. P. Rahardjo. 2018. Effect of Using Silkworms (*Tubificid worms* sp.) Living on the Survival Rate and Growth of the Catfish Larvae (*Clarias* sp.). *IOSR J. Agric. Veteri. Sci.* 1(2): 42-46.

Onura, C. N. A., W. Van den Broeck, N. Nevejan, P. Muendo and G. Van Stappen. 2018. Growth performance and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on live and dry feeds. *Aquaculture*. 489: 70-79.

Palace, V. P. and J. Werner. 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. *Scien. Mar.* 70(S2): 41-57.

Rabbane, M. G. and M. R. Rahman. 2017. Effects of natural and commercial diets on growth, reproductive performances and embryogenesis of zebrafish *Danio rerio*. *Dhaka Univ. J. Biol. Sci.* 26(2): 159-166.

Rahman, M. A., A. Bhadra, N. Begum and M. G. Hussain. 1997. Effects of some selective supplemental feeds on the survival and growth of catfish (*Clarias batrachus* Lin.) fry. *Bangladesh J. Fish. Res.* 1(2): 55-58.

Rahman, M. M., M. Y. Hossain, M. I. Hossain, S. J. Provhat, M. S. Islam and M. B. Hossain. 2013. Induced breeding of the stinging catfish, *Heteropneustes fossilis*: comparison among different inducing agents. *Turkish J. Fish. Aquat. Sci.* 13(3): 523-527.

Sales, J. 2011. First feeding of freshwater fish larvae with live feed versus compound diets: a meta-analysis. *Aquac. Int.* 19(6): 1217-1228.

Soundarapandian, P., S. Ravichandran and T. Kannupandi. 2002. Effect of live and artificial feeds on the growth and survival of *Macrobrachium malcolmsonii* (H. Milne Edwards) larvae and juveniles. *Indian J. Fish.* 49: 79-84.

Srichanun, M., C. Tantikitti, V. Vatanakul and P. Musikarune. 2012. Digestive enzyme activity during ontogenetic development and effect of live feed in green catfish larvae (*Mystus nemurus* Cuv. *and* Val.). *Songklanakarin J. Sci. Technol.* 34(3): 247-254.

Takeuchi, T., T. Watanabe and C. Ogino. 1978. Studies on the nutritive value of dietary lipids in fish. XI. Supplementary effects of lipid in a high protein diet of rainbow trout. *Bull. Jpn. Soc. Sci. Fish*. 44: 677- 686.

Washburn, B. S., D. J. Frye, S. S. Hung, S. I., Doroshov and F. S. Conte. 1990. Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss). Aquaculture.* 90(2): 179-195.

Wilson, R. P., E. H. Robinson and W. E. Poe. 1981. Apparent true availabilty of amino acids from common feed ingredients for channel catfish. *J. Nutr.* 111: 923-929.

Zafar, N. and M. A. Khan. 2019. Growth, feed utilization, mineralization and antioxidant response of stinging catfish *Heteropneustes fossilis* fed diets with different levels of manganese. *Aquaculture*. 509: 120-128.