



## Determination of Optimum Weaning Time of Shirbot (*Barbus grypus* Heckel, 1843) Larvae

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### Abstract

The present study was conducted to determine the optimum weaning time of shirbot, *Barbus grypus* larvae with formulated diet. In this regard, shirbot larvae ( $8.98 \pm 0.06$  mg) were fed with 8 experimental diet including A: (3 days rotifer+18 days formulated diet), B: (6 days rotifer+15 days formulated diet), C: (9 days rotifer+12 days formulated diet), D: (12 days rotifer+9 days formulated diet), E: (15 days rotifer+6 days formulated diet), F: (18 days rotifer+3 days formulated diet), G: (21 days rotifer), H: (21 days formulated diet). All experiments were carried as random pattern with three replicates. According to the obtained results, there were no significant differences between treatments, A and B and also F and G in terms of weight gain (WG) values ( $P > 0.05$ ). The values of specific growth rate (SGR) were not significant between treatments, A and C and also E, F and G ( $P > 0.05$ ). Also, there were no significant differences between treatments, A and B; D and E; E and G; F and G in terms of length increment (LI) values ( $P > 0.05$ ). The treatment F showed more WG ( $33.51 \pm 0.17$  mg), SGR ( $7.4 \pm 0.01$  %) and LI ( $6.4 \pm 0.05$  mm) compared to other experimental groups after 21 days experiment ( $P < 0.05$ ). The lowest values of WG ( $13.35 \pm 0.14$  mg), SGR ( $4.33 \pm 0.03$  %) and LI ( $2.56 \pm 0.27$  mm) was found in treatment H after 21 days experiment. Also, the maximum SGR was observed in treatment E ( $98.5 \pm 0.3$  %). The fish of treatment H ( $87.5 \pm 2.36$  %) showed the lowest survival rate (SR) compared to other experimental treatments. Results suggest that a 18 days use of live food is essential for larviculture of shirbot to obtain larvae with highly growth and survival.

**Keywords:** Rotifer, weaning time, formulated diet, shirbot, growth.

### Introduction

The shirbot, *Barbus grypus* is one of the freshwater fish species in Forat and Dejles river basins. This species is nutritionally omnivorous and ecologically euryhaline and eurytherme and widely distributed in Iran, Turkey, Syria and Iraq (Nikpei, 1996). Also, this fish was distributed widely in the rivers and their branches of the west and southwest of Iran, especially in khozestan province (Nikpei, 1996; Banaee, & Naderi, 2014). Shirbot is favorable among indigenous residents of south western provinces of Iran, especially Khuzestan Province (Banaee & Naderi, 2014). During last decade, the artificial reproduction and rearing of barbel fish has been conducted to restock its depleting populations in nature and also to meet market demands (Marammazi & Kahkesh, 2011; Sikorska, Wolnicki, Kamiński & Stolovich., 2012; Nowosad *et al.*, 2016). Similar to other fish species, the successful larviculture of shirbot depends strongly on appropriate nutrition of larvae especially during weaning. Weaning of fish is

considered as transition from live food to formulated feed. It is referred as the most critical step in larviculture of many species, with special regard on the piscivorous fish (Kestemont *et al.*, 2003). The live food utilization is essential for fish larviculture due to the more digestibility, high nutritional value and stimulating behavior of them in water (Kolkovski, Arieli, & Tandler, 1997; New, 1998; Kestemont, Xueliang, Hamza, Maboudou, & Toko, 2007). However, live food is expensive and requires manpower and expensive equipments which this highlights the importance of formulated feeds (as alternative) and thus weaning period (Abowei & Ekubo, 2011). Many studies have concluded that a successful weaning depends mainly on transfer time of fish larvae from live food to formulated feed in addition to the quality of artificial feed (Chau & Zambonini Infant., 2001). Łączyńska *et al.*, (2016) showed that an effective direct transfer from live food to prepared diets (with no gradual transfer) cannot be performed with Crucian carp larvae before 30 days after hatching. In *Barbus barbus* Larval rearing was

started from the beginning of exogenous nutrition (13 days post-hatch = day 1) until day 21, when metamorphosis was completed (Polcar *et al.*, 2011). In kutum, *Rutilus frisii kutum* larvae, the highest specific growth rate were obtained when fish feed with formulated diet 3 days post hatching for 21 days. Above studies clearly indicate that weaning time of fish larvae with artificial diet is essential to obtain healthy larvae with high growth. Therefore, the aim of the present study was to determine the appropriate weaning time for larvae of shirbot.

## Material and Methods

The study was conducted at laboratory of aquaculture, Islamic Azad University, Ahvaz, Iran. A number of 4800 shirbot larvae were purchased from Propagation and Rearing Center of Barbus Fish (PRCB), Susangerd, Iran and then transported by car to Ahvaz Islamic Azad University Fisheries Laboratory in aerated plastic bags containing 2/3 pure O<sub>2</sub> and 1/3 water. After thermal adaptation in laboratory during 12 hours, larvae delivered to a 25 m<sup>2</sup> pond which had disinfected previously with salt water. The onset of the experiment was 6 days post hatching (dph) with larvae of 8.98± 0.06 mg average weight and 12.7±0.04 mm length and lasted for 21 days. The experiment was designed in the form of 8 experimental treatments with three replicates per treatments, totally twenty-four 25 L tanks with stocking rate of 200 fish per tank. Fish fed with: A: (3 days rotifer+18 days formulated diet), B: (6 days rotifer+15 days formulated diet), C: (9 days rotifer+12 days formulated diet), D: (12 days rotifer+9 days formulated diet), E: (15 days rotifer+6 days formulated diet), F: (18 days rotifer+3 days formulated diet), G: (21 days rotifer), H: (21 days formulated diet: SFC-1, Chineh Company, Iran). All experiments were carried as random pattern with three replicates. 2 h after each feeding operation, wastes (uneaten food and fecal materials) were siphoned out from the tanks. Also, after siphoning, two-thirds of water volume in each tank was renewed with fresh water. Throughout the experiment, water pH (by pH meter), dissolved (mg/l) and temperature were monitored daily which had an average of 6-7 mg/L<sup>-1</sup> and 25 ± 0.5° C respectively. larvae were kept in 12:12 hour dark-light (LD). Aeration was conducted by the aquarium pump. Salt water rotifer, *Brachionus*

*plicatilis* were collected from the culture tanks of rotifer by net with 100 µm mesh. Then, rotifers were delivered to 50l tanks by plastic bags and aerated continually by an aerating pump. Also, a commercial diet (SFC1) was used for feeding shirbot larvae. The composition of formulated diet was presented in Table 1. All groups of larvae were fed *ad libitum* four times (7:30, 11:30, 15:30 and 18:30 h) daily. The physical and swim behaviors of larvae were monitored daily. To investigate growth parameters, 10 shirbot larvae were sampled randomly once every 5 days. The total length was measured to the nearest 0.1 mm using a dial caliper and individual wet weight was determined by precision balance (0.1 mg sensitivity). Before the manipulations, the shirbot were anaesthetized with 99% 2-phenoxyethanol (ethylene glycol monophenyl ether, MERCK-Schuchardt) at a dose of 0.4 ml L<sup>-1</sup>. The fish were then returned to their respective tanks. The growth parameters were determined according to following formula:

$$\text{Mean weight gain (WG)} = \text{Mean final weight} - \text{Mean initial weight}$$

$$\text{Length increment (LI)} = \text{Final length} - \text{Initial length}$$

$$\text{Specific growth rate (\%/day) (SGR)} = 100 \times \frac{(\ln \text{ final weight} - \ln \text{ initial weight})}{t}$$

$$\text{Survival rate (\%)} (\text{SR}) = 100 \times \frac{\text{Final number of fry}}{\text{Initial number of fry}}$$

## Data Analysis

The SPSS software (version 16) was used for data analysis. The data normality was investigated by Kolmogorov–Smirnov test. Analysis of one way variance with the Duncan ( $\alpha = 0.05$ ) multiple range test was applied to find the significant differences among means of data.

## Results

This study showed that a significant differences between treatments after 21 days experiment ( $P < 0.05$ ). In this regard, the highest and lowest SGR, LI and WG (Figure 1,  $P < 0.05$ ) were found in

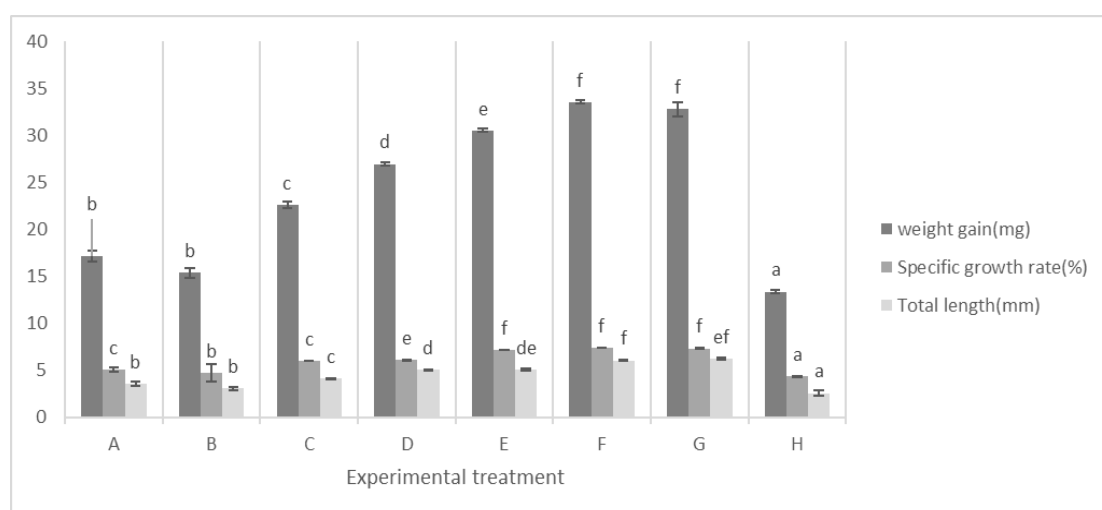
**Table 1.** The composition of formulated diet and rotifer used in the present study(%)

Diet composition	Formulated diet	Rotifer
Moisture	4.85	88.7
Total protein (Dry Matter)	45.48	67.2
Total fat (Dry Matter)	14.5	21.5
Ash (Dry Matter)	12.53	4.9

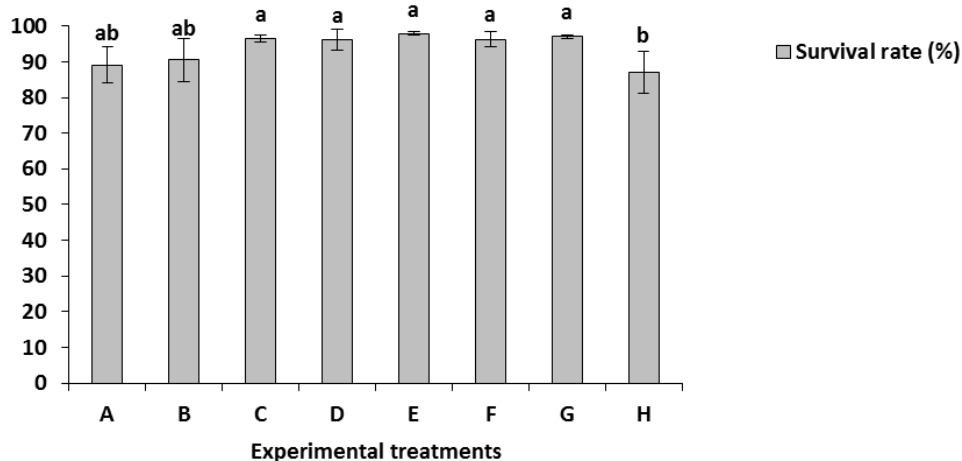
treatment F (SGR:  $7.40 \pm 0.01$ ; LI:  $6.40 \pm 0.05$ ; WG:  $33.51 \pm 0.17$ ) and H (SGR:  $13.35 \pm 0.14$ ; Li:  $2.56 \pm 0.27$ ; WG:  $13.35 \pm 0.14$ ) respectively. There were no significant differences between treatment F and G in terms of SGR, LI and WG (Figure 1,  $P > 0.05$ ). Also, the maximum SR was observed in treatment E ( $98.5 \pm 0.3$  %) (Figure 2,  $P < 0.05$ ). The fish of treatment H ( $87.5 \pm 2.4$  %) showed the lowest SR compared to other experimental groups (Figure 2,  $P < 0.05$ ). SR in treatment E had no significant differences with treatments C, D, F and G (Figure 2,  $P > 0.05$ ). During 21 days experiment, the mean weight and length of larvae increased continually (Figure 3, Figure 4,  $P < 0.05$ ). But, the significant elevating trend of SGR was found more till day 10 (Figure 5,  $P < 0.05$ ).

## Discussion

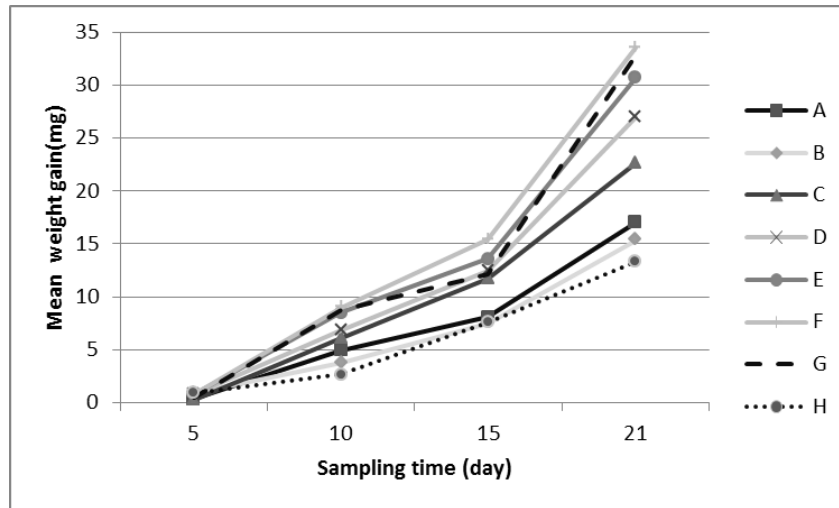
The present study was carried out to determine appropriate weaning time of shirbot larvae. The higher WG, SGR and LI obtained when fish of this study were fed with combination of rotifer (for 18 days) and formulated diet (for 3 days) (treatment F) or only rotifer (treatment G). Generally, the efficient consumption and digestion of formulated dry feed is highly depending on physical and physiological ability of fish larvae to capture, digest and metabolize the feed (reviewed by Cahu & Zambonino Infante, 2001). The results of treatment F clearly show that 24 days post hatching is most appropriate transfer time of shirbot larvae from live food to formulated dry feed. Likely, the physical and physiological ability of shirbot larvae to digest and metabolize formulated dry



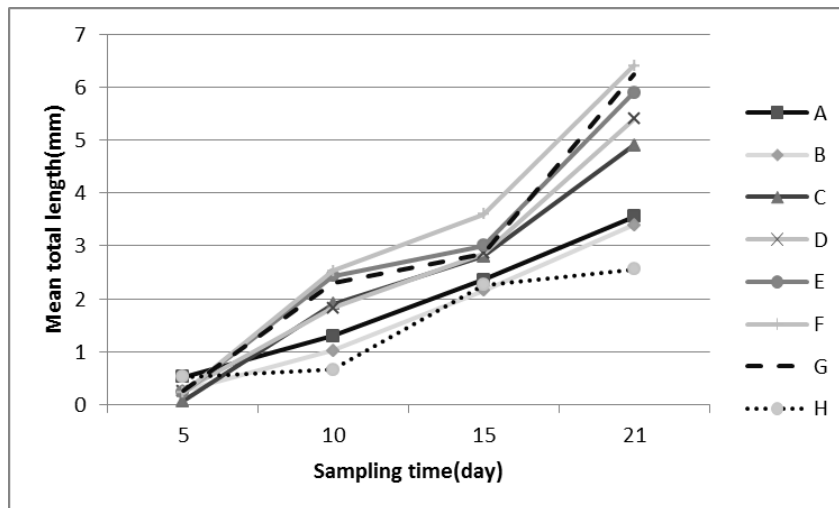
**Figure 1.** Comparison of weight gain, specific growth rate and total length of shirbot, *Barbus grypus* larvae between experimental treatments after 21 days experiment. Bars (mean  $\pm$  SD) with different letters are significantly different ( $P < 0.05$ ).



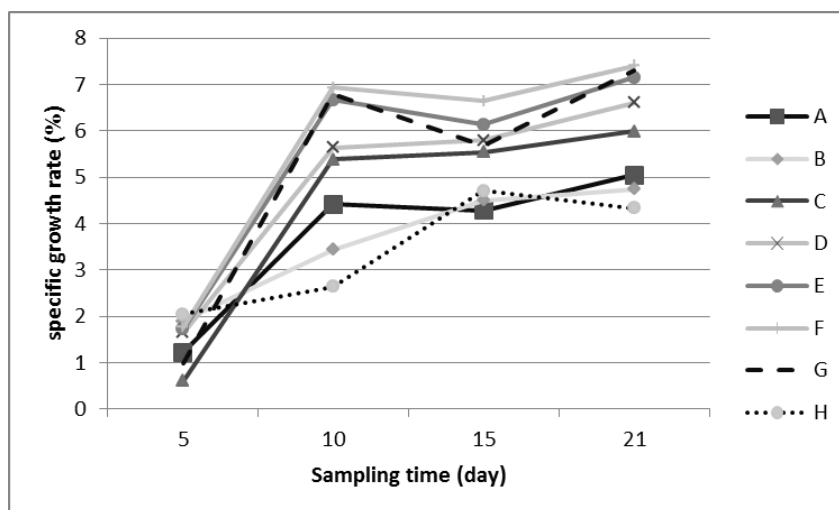
**Figure 2.** Comparison of survival rate of shirbot, *Barbus grypus* larvae between experimental treatments after 21 days experiment. Bars (mean  $\pm$  SD) with different letters are significantly different ( $P < 0.05$ ).



**Figure 3.** Changes of weight gain of shirbot, *Barbus grypus* larvae in experimental treatments during 21 days experiment. Bars (mean ± SD) with different letters are significantly different (P<0.05).



**Figure 4.** Changes of mean length increment of shirbot, *Barbus grypus* larvae in experimental treatments during 21 days experiment. Bars (mean ± SD) with different letters are significantly different (P<0.05).



**Figure 5.** Changes of specific growth rate of shirbot, *Barbus grypus* larvae in experimental treatments during 21 days experiment. Bars (mean ± SD) with different letters are significantly different (P<0.05).

diet elevates after this period of time. Also, the highly growth of the fish of treatment G may be due to the presence of digestive enzymes in live foods that help to digestion capabilities of first-feeding larvae (Verreth, Eding, Rao, Huskens, & Segner, 1993; Kolkovski, Arieli, & Tandler, 1995). In this regard, live foods contribute the enzymes, trypsin, chymotrypsin and amylase into the digestive system of fish larvae. It has been reported that live food contributes significantly 43-60% protease, 78-88% esterase and 89-94% amylase into the digestive system of *Scophthalmus maximus* larvae (Munilla-Moran, Stark, & Barbour, 1990). In addition, some live foods containing high protein levels (such as rotifer: 67.2% protein) can meet the requirements of body protein synthesis for fast-growing larvae of fish during early stages of development (Watanabe, Oowa, Kitajima, & Fujita, 1987a,b; Bengston, Leger, & Sorgeloos, 1991).

In this study, the lowest growth was observed when fish were fed only with formulated dry feed over the course of the experiment. In whole, in addition to physiological condition of fish larvae, the growth responses of fish return to the quality of feed including palatability, texture and size (Dabrowski, 1984). In the present study, it seems that the quality of feed is not a problem since shirbot larvae would attract to the dry feed and consume it intensively after every time feeding. Thus, the poor growth in shirbot feed only with formulated dry feed may be due to the incomplete development of the digestive system including relatively low level of enzyme activities especially pepsin-like enzymes, resulting poor capability in digestion and assimilation and finally poor growth (Dabrowski, 1984; Govoni, Bochlert, & Watanake, 1986; Segner, Rosch, Schmidt, & Von Poepplinghausen, 1989; Verreth *et al.*, 1992; Kolkovski, Tandler, Kissil, & Gertler, 1993, 1997; Day, Howell, & Jones, 1997). Policer *et al.*, (2011) showed that live feed is not an essential diet for common barbel (*Barbus barbus* L.) larvae at the start of exogenous feeding. This fact is caused by relatively advanced ontogenic development of barbel larvae at the beginning of exogenous nutrition compared to larvae of other cyprinids. The reason for this difference could be related to growth and development of the digestive system.

Similar conclusion was stated in study of Mahmoudzadeh, Ahmadi, and Shamsaei (2009) and Ouraji, Khalili, Ebrahimi, and Jafarpour (2011) when fish larvae were fed with dry formulated feed. In these studies, the poor growth obtained despite use of formulated feed with high quality. In the present study, the SGR increased significantly till day 10 for almost experimental groups, although the rate of SGR increase decreased afterward. The decreases in SGR after day 10 of the experiment may arise from the fact that the rate of metabolism and anabolism is greater in the earlier stage of fish larvae development (Wieser, 1995). The values of SR were stable over the course

of the experiment for all experimental treatments except treatment H in which SR showed a significant decrease after day 21 days experiment. This indicates that SR of shirbot larvae decreases when formulated feed is used for longer period of times. The effects of formulated diets on SR values fish larvae were investigated previously. For example, the poor survival rate of 69.16% was found in Kutum larvae in response to longer period of feeding with formulated feed (Ouraji, Khalili, Ebrahimi, & Jafarpour, 2011). In common carp larvae, *Cyprinus carpio* the SR of less than 20% obtained when fish were fed with diet containing 75 % formulated feed (Bambroo, 2012). The lower SR (63.3% and 56.0%) was obtained in grass carp, *Ctenopharyngodon idella* and bighead carp, *Hypophthalmichthys nobilis* larvae respectively when were fed with formulated diet compared to those fed with live food (Rottmann, WShireman, & Lincoln, 1991). The higher values of obtained SR in this study (87.5±2.4 %) than in above studies may be due to the higher quality of formulated diet used in the present study or more capabilities of shirbot in digestion and assimilation of formulated diet compared to other cyprinid fishes examined in the studies of Ouraji, Khalili, Ebrahimi, and Jafarpour (2011), Bambroo (2012) and Rottmann *et al.*, (1991).

In conclusion, almost 4-10 days dph is suggested for initiation of weaning in cyprinidae (Dabrowski 1984; Wolnicki & Gorny 1995 a,b; Wolnicki 1996). However, our results suggest that the use of live food at least for 18 days dph is essential to obtain shirbot larvae with highly growth and survival which is longer than in other Cyprinidae i.e. 4-10 days dph. Therefore, After 18 days, the formulated feed could be applied for feeding of shirbot to reduce rearing costs and other problems arising from the use of live food.

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