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Effects of Different Diets on Growth, Survival and Body Composition of *Rutilus frisii kutum* Larvae

[1]M. Jafari, [1]M.S. Kamarudin, [1]C.R. Saad, [2]A. Arshad, [3]S. Oryan and [4]M.H.T. Guilani
[1]Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia, P.O. Box 43400, Malaysia
[2]Faculty of Bioscience, University Putra Malaysia, P.O. Box 43400, Malaysia
[3]Department Physiology, Faculty of Biology, Teacher Training University, P.O. Box 14115-175, Karaj, Iran
[4]Shahid Dr. Beheshti Sturgeon Propagation and Rearing Complex, Rasht, Guilan, Iran

Corresponding Author: M.S. Kamarudin, Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia, P.O. Box 43400, Malaysia Tel: +603-89464171 Fax: +603-8946 4146

ABSTRACT

The main objective of this study was to investigate the effect of different diets on Caspian kutum larval growth, survival and body composition, thus the Caspian kutum was examined in diets, Starved (S), Egg yolk (E), *Artemia nauplii* (A) and *Artemia nauplii* plus egg yolk (A+E). Totally four dietary treatments were tested in triplicate for 30 days. A significant growth difference between fry fed was observed in *Artemia* plus egg yolk ($p<0.05$). Final mean lengths and weights for each treatment (S, A, E, A+E), respectively were 7.0±0.07, 26±0.91, 23.5±0.91, 28.6±0.18 mm (Mean±SE, n = 12) and 4.9±0.08, 57±2.14, 51±2.18, 74±3.64 mg (Mean±SE, n = 12). The larvae accumulated increasing protein in Treatment A+E and lipid in treatment E ($p<0.05$). Also, the highest survival rate, 70.9±2.1%was in the treatment A+E and was significantly higher compared to 59.5±1.45% and 56.6±0.98%and in the other group ($p<0.05$). During culture period some other factors such as DGC (Daily Growth Coefficient), DWG (Daily Weight Gain), DLG (Daily Length Gain) and SGR (Specific Growth factor Rate) were measured. The obtained result showed that diet *Artemia* plus egg yolk is promising for use in Caspian kutum culture for in early stages of life cycle.

Key words: Stress test, starvation, growth rate, body composition, *Caspian kutum*

INTRODUCTION

Rutilus frisii kutum is known as one of the most important commercial species in the Caspian Sea. Some reasons such as illegal fishing of brood stocks by beach-seine especially during spawning season, decrease of water flow of rivers, water river pollution increase, gravel and sand removals in rivers which broodstocks coming to river for spawning caused decrease in Caspian kutum stocks in the Caspian Sea (Salehi, 2002; Kapoorchali *et al*., 2009).

Caspian kutum spring spawners arrive on the spawning ground in March to May. Due to sharp decrease in stocking of *R. frisii kutum* in Caspian Sea attempted made in artificial propagation of this fish for enhancing and restocking (Yousefian and Mosavi, 2008).

Rutilus frisii kutum culture is aimed at restocking the natural environment (southern Caspian Sea) and every year up to 200 million of one gram fry are released to improve the stocks into the Caspian Sea (Abdolhay and Tahori, 2006) this resulted in significant increase of catches of Iran in recent years (Kavan *et al*., 2009).

Caspian kutum broodstocks migrate to rivers for spawning, where broodstocks are caught, eggs and sperm are stripped by artificial propagation fertilized and transferred to the hatcheries to develop to fingerling larvae (Abdolhay and Tahori, 2006).

The onset of exogenous feeding is an important time in larviculture. In many marine fish larvae in this stage accrued massive mortality both in nature and in hatchery populations and high percentages of larvae are killed before reaching the fingerling stage. The source of nutrient and energy necessary to continue the larval development changes from is the main point of this stage (Yufera and Darias, 2007). Low abundant of appropriate prey organisms or lack of suitable feed are assumed to be responsible for the mortalities, but criteria to ascertain the suitability of the feed are not available (Hans, 1989). The larvae are sensitive to prey density during the first week of exogenous feeding (Ljunggren, 2002).

In early life stage Kutum larvae need to foods containing protein and lipid with small-size and slow movements (Kapoorchali *et al*., 2009).

Success of larval rearing depends mainly on the availability of suitable diets that are readily consumed, efficiently digested and that provide the required nutrients to support well growth and health (Giri *et al*., 2002). One of the best life foods are Zooplanktons which the decrease in the cost of production and increase in the growth and survival.

Sustainable aquaculture depends on friendly and economically and socially viable culture. Wang *et al*. (2005) found that the survival was significantly higher in larvae fed live food than in larvae fed the three formulated diets.

Many investigators have shown that *Artemia* represents an interesting food source for marine fish in first life stage (Koueta *et al*., 2002).

Ojutiku (2008) reported live Daphnids is recommended for larvae though Frozen Daphnids can be used as supplement and *Heteroclarias* is recommended for fish farmer for better growth and survival. The results of study on *Coregonus lavaretus* showed that the use of rotifer as live food for fry has provided better survival rate (Shamsaie *et al*., 2007).

Availability of feed significantly affected survival, body size and specific growth rate of fish larvae. In fact, making use of living foods at early stage causes the decrease of mortality and increase of fast growth and survival and recruitment and decrease in production costs (Kapoorchali *et al*., 2009).

The present study has been conducted to evaluate the effects of different diets on the growth, survival, chemical composition of larvae and fingerlings of Caspian kutum larvae.

MATERIALS AND METHODS

Four feeding trial in three replicates were conducted at the Dr. Dadman International Sturgeon Research Institute, in Guilan province in Iran by using twelve 20 L glass aquaria with aeration, according to a Completely Randomized Design (CRD). The period of experiment was among March to April 2006. With the aquaria were filled with sand filtered river water. Experimental kutum larvae (5.1±0.10 mg body weight and 6.1±0.15 mm total body length) were obtained from Rajaii Fish Propagation and Rearing Center in mazandaran province in Iran. Each aquarium was stocked with 500 fish (25 larvae L^{-1}. The experiment was carried out for 30 days under a 14:10 h light-dark photoperiod. The larvae were randomly subjected to four feeding regimes: Egg yolk (E), *Artemia urmiana nauplii* (A), *Artemia urmiana* plus egg yolk (A+E) and without feeding, starved (S). Feeding was given two times daily *ad libitum*. Debris and feed leftovers were siphoned

out before and after each feeding. Depleted water was replaced with river after each cleaning. Water temperature was taken twice daily at 0800 and 1600 h. Dissolved oxygen and pH were determined three times a week. Samplings of fish (20 larvae per aquarium) were done at every two days. The initial total length (mm) and weight (mg) of individual fish was recorded. At the end of the 30-day trial, fish were weighed and samples were taken randomly from ten individuals per treatment. Daily Growth Coefficient (DGC) was calculated following (Dabrowski and Culver, 1991).

Kutum growth was also measured in term of weight increment through Specific Growth Rate (SGR) (Hopkins, 1992) and Weight Gain (WG). Survival was calculated based on the difference between the starting number and the absolute final number of larvae remaining in each tank. Dead fish were removed daily and cumulative mortality rate was recorded as follows:

$$\text{Survival (\%)} = 100 \times \frac{\text{Final fish No.}}{\text{Initial fish No.}}$$

Proximate analysis: The proximate analyses (crude protein, crude lipid and ash) for the whole body composition of kutum fry at the beginning and end of experiment were carried out following the AOAC methods (AOAC, 1995). Samples were analyzed in duplicates. Ash content was determined after incinerating the dried residue in a muffle furnace at 550°C for 12 h. Protein was determined using the micro-Kjeldahl procedure while crude lipid was determined following Soxhlet extraction of dried samples using ether.

Statistical analysis: Statistical analyses were performed using SPSS 12 statistical software package. Effects of different treatments on total length, mean weight, condition factor and specific growth rate were tested by one-way analysis of variance. The mean differences were tested using Tukey's test ($p<0.05$). Results are presented as Mean±Standard Error (SE).

RESULTS

At day 0 of the experiment, the larvae were at 3 DAH. The growth and survival of kutum at the end of the feeding trial are shown in Table 1.

The initial mean weigh and initial mean length of experimental Caspian kutum larvae were (5.1±0.01, 6.1±0.02) (Table 1) these variation in initial weight and led to significant ($p<0.05$) difference in final weight and length. The better growth in weight and length observed in larvae fed with egg yolk and *Artemia nauplii* with 74±0.01g in weight and 28.6±1.18 mm in length, respectively and lowest growth observed in starved group (4.9±0.08 g, 7±0.07 mm).

For specific growth rate, comparison between different diets, *Artemia* diet with 8.04±0.1 and egg yolk and *Artemia nauplii* with 8.91±0.7 indicated maximum growth rate. SGR was -0.19±0.01 in starved group that was less than others.

The combination of egg yolk and *Artemia nauplii* gave the highest survival and gains in length and weight. In terms of main daily growth rate and SGR, those fed on a combination of egg yolk and *Artemia nauplii* had a significantly higher growth ($p<0.05$) than those of other treatments. The performance of egg yolk was at par with *Artemia nauplii*. No growth was observed among the starved larvae. By 13 DAH, more than 50% larvae had died. The starved larvae failed to survive beyond 21 DAH. The initial and final whole body compositions of kutum larvae are presented in

Table 1: Growth parameters and survival of kutum larvae fed on different diets

	Treatment			
Growth parameters	S	A	E	A+E
Initial mean weight (mg)	5.2±0.01^{a}	5.1±0.01^{a}	5.1±0.01^{a}	5.1±0.01^{a}
Final mean weight (mg)	4.9±0.08^{c}	57±2.41^{ab}	51±2.18^{b}	74±0.01^{a}
Mean weight gain (%)	-5.76±0.02^{c}	91.05±2.21^{ab}	90±2.16^{b}	93.10±1.7^{a}
Mean daily growth rate (mg day^{-1})	0.01±0.001^{c}	1.73±0.03^{b}	1.53±0.02^{b}	2.29±0.01^{a}
Specific growth rate (% day^{-1})	-0.19±0.01^{c}	8.04±0.1^{b}	7.67±0.3^{b}	8.91±0.7^{a}
Initial mean length (mm)	6.3±0.01^{a}	6±0.01^{a}	6.3±0.01^{a}	6.1±0.02^{a}
Final mean length (mm)	7±0.07^{c}	26±0.91^{ab}	23.5±0.91^{b}	28.6±1.18^{a}
Mean length gain (mm)	0.7±0.01^{c}	20±0.12^{ab}	17.2±0.15^{b}	22.5±0.14^{a}
Mean length gain (%)	11.11±0.9^{c}	333.3±4.2^{a}	273±3.65^{b}	368±4.4^{a}
Mean daily length rate (mm day^{-1})	0.02±0.001^{c}	0.66±0.01^{ab}	0.57±0.02^{b}	0.75±0.03^{a}
Survival (%)	0±0.00^{c}	61.3±1.45^{ab}	56.6±0.98^{b}	70.9±2.1^{a}

Mean±SD. Values are as Same superscript letters for the same element, notes no statistical differences within the prey ($p>0.05$). Within rows values with different superscripts are significantly different ($p<0.05$)

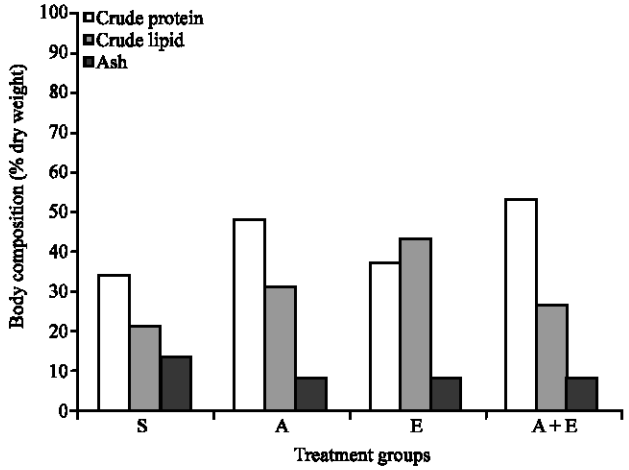

Fig. 1: Body compositions of kutum larvae fed different diets

Fig. 1. Crude protein, crude lipid and ash content of whole body were affected by feeding regimes. All compositions were significantly differences ($p<0.05$) between treatments.

For Crud protein between treatments fed with *Artemia* and egg yolk with *Artemia nauplii* no significant difference 48.17±0.38, 53.12±0.31 (% dry weight). Lipid protein rate in body composition in egg diet was higher than other treatments 43.11±0.18. Ash in body composition was higher than in starved group 13.57±0.32 and lower than in treatment fed with egg yolk and *Artemia nauplii* 7.3±0.23.

Values in the same row with the same superscript are not significantly different ($p>0.05$). Statistical analysis for the three treatments indicated significant difference ($p<0.05$) between survival percentage that in treatment fed with egg yolk and *Artemia nauplii* survival rate was higher than other treatments (70.9±2.1).

DISCUSSION

The major morphological changes in the digestive system of kutum larvae happened during the first 3 DAH when the alimentary canal is subdivided into functionally different regions. In nature, kutum larvae feed on rotifers, cladocerans, diatoms, cyclops and copepods in their first development stages, in fact kutum remain planktonphagous in the first year of life. The main objective of developing a successful larval rearing strategy is the establishment of a feeding regime that produces best growth, survival and health of the fish larvae. Zooplanktons generally present in aquaculture ponds, are an essential diet for fish larvae, supplying many nutrients (in the digestive form) required by the larvae. The production of fish larvae and fingerlings of most fish species still relies largely on feeding strategies based on live food. Since food organisms are also needed to be cultured, these strategies pose extra strains mainly on hatchery management are lead to increase fingerling production costs (Carneiro *et al*., 2003). Development of a suitable formulated larval diet could alleviate these problems, increasing the reliability and improving the cost-benefit of the fingerling production in fish hatcheries. Several larval diets have been successfully developed especially for freshwater fish species (Jones *et al*., 1993). Nevertheless, several studies have also shown that co-feeding artificial diet and live food gives better results than artificial feed alone (Fermin and Bolivar, 1991; Petkam and Moodie, 2000). In this study, the performance of live, artificial food and the combination were been evaluated. Live *Artemia nauplii* have long been using as first and primary food for the larviculture of many farm fishes and crustaceans worldwide (IFRO, 2007). Egg yolk has also been used in the larviculture of *Pangasius sutchi* (Potaros and Sitasit, 1967) and *Helostoma temmincki* (Cheah *et al*., 1985). The result of this study showed that kutum larvae have highest survival and growth and stress tolerance when fed with a combination of egg yolk and *Artemia nauplii*. The yolk might have supplement some of nutritional deficiency of *Artemia* (Kolkovski *et al*., 1997; Kolkovski, 2001) while the endogenous enzymes in *Artemia* might help the digestion of egg yolk (Jones *et al*., 1993; Sfakianakis *et al*., 2003).

Starvation causes an immediate cessation of growth and poor larval health. In the absence of prey, the larvae tend to retain ingested food in the gut (Parra and Yufera, 2001). It seemed that starved kutum larvae were able to survive up to 21-22 DAH. Nearing the last days of living, the larvae were in needle form and swam very slowly. Scoliosis was also observed in some larvae showing a serious malnutrition or nutrient deficiency. The point of no return for starved kutum has not been reported and should be studied.

CONCLUSION

In summary the present results indicate that the optimal diet for kutum larvae in early life stage is egg yolk plus *Artemia nauplii* that would be beneficial for larval rearing in terms of survival, growth and normal development. Further investigations are needed on larval nutrition with enriched *Artemia* or other zooplankton with HUFA and vitamin C order to reduce mortality, deformity during this critical period of early development.

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