

## Effects of different starter diets on growth indices of Caspian Kutum, *Rutilus frisii kutum* larvae

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### Abstract

Six different diets including two rainbow trout starters, *Gammarus* dry powder, shrimp dry powder, egg yolk dry powder and newly hatched *Artemia* were used in feeding of Caspian Kutum larvae. During a 4-week trial, larvae with a mean weight of  $17.5 \pm 4.7$  mg were fed with three replicates and growth parameters were measured at the end of the experiment. Thirty fish in each experimental unit with 3 L volume were fed 6 times daily according to their satiation. Results showed that there were significant differences in the final weight, final length, body weight increase, voluntary feed intake and specific growth rate for weight and length among the treatments ( $P < 0.05$ ), but not in the condition factor ( $P > 0.05$ ). The best performance was observed in fish fed *Artemia* nauplii. The survival rate was significantly different among the treatment ( $P < 0.05$ ) which the highest ( $75.6 \pm 3.8\%$ ) and lowest ( $22.2 \pm 11.7\%$ ) were observed in fish fed *Artemia* nauplii and Coppens, respectively. According to obtained results, it appears that except *Artemia*, the most suitable and inexpensive diet is egg yolk powder, which can result in high growth performance and survival rates. The results of this study suggested that using dry non-live diets with good composition could be replaced with *Artemia* that had suitable growth and survival rate in Caspian Kutum during larval.

**Keywords:** Live food, practical diet, growth, survival, larvae, Caspian Kutum

## Introduction

Caspian Kutum is one of the most economically important bony fish of the southern Caspian Sea (Abdoli, 1999). The main distribution of this species belongs to the southern part of the Caspian Sea from Kura River in the western part to Hossien Gholi bay and Atrak River in the south-east region (Razavi Sayyad, 1984). This fish is locally known as Mahi-Sefid and commonly called as Kutum; it belongs to the Cyprinids family and has a high commercial value (Abdoli, 1999; Abbasi et al., 1999). Several factors such as environmental pollution of the sea and rivers, construction of dams, destruction of spawning grounds in the rivers, over-fishing and reduction of natural spawning cause decreasing of fish stocks in recent decades (Razavi Sayyad, 1984; Abdolmaleki, 2006). Hence, there is special consideration for Kutum artificial breeding and raising them up to one gram size for stocking rehabilitation.

Limited data are available in biology, nutritional requirements and necessary techniques for larval rearing in culture systems. The main food source of Caspian Kutum in the first 2-week age is phytoplankton; from the third week when the larva reach the size of 15 to 17 mm, zooplankton is the main food for Caspian Kutum. After six weeks, when the larvae metamorphosed to advanced-fry of 2 g size, the benthic animals such as Gammarids, small shrimps and other benthic organisms become the main food sources for this species (Razavi Sayyad, 1984; Efatpanah, 1993). The Kutum main food items in the Caspian Sea preferably consist of mollusks and also crustaceans.

High abundance of Caspian Kutum is found particularly in areas where dense mollusca are available (Razavi Sayyad, 1984). Each dietary regime or culturing technique which is able to reduce the natural food dependence is considered as an economical rearing technique (Canavate and Fernandez-Diaz, 1999; Akbari et al., 2011). According to studies done on Caspian Kutum nutrition in the earthen ponds, this fish in addition to using natural foods and wet diets, is also able to utilize the formulated diets and showed good growth and survival rate (Noveirian et al., 2005, 2007, 2008; Haghghi et al., 2009). Several researches have been carried out on Caspian Kutum and its abilities, showing that it is a suitable candidate for aquaculture purposes, but for the achievement of this aim, having the knowledge of the principles of breeding and raising techniques of Kutum is required. Appropriate feed formulation of Caspian Kutum requirements in the pond culture is an essential criterion for rearing them in captivity and it needs more studies in this field. One of the most steps for fish rearing is feeding larvae and shifting its diet from live foods to formulated diets. Therefore, the purpose of this study was to find out the effects of live foods and formulated diets on growth parameters of Caspian Kutum at larval stage.

## Materials and methods

### *Eggs source and incubation*

In March 2008, ripen and matured fish (4 males with average weight 750 g; and 5 females with average weight 1200 g) were collected by seine net from Ghaziyan area

(Anzali, Gilan, Iran) and transferred to aquaculture facilities at Faculty of Natural Resources, University of Guilan in Sowmeh Sara, Guilan, Iran. All fish were carefully stocked at a fiberglass tank of 5 m<sup>3</sup> capacity which was filled by 3 m<sup>3</sup> of fresh and filtered water. After 3 days, all fish were spawned at the same tank. Broods were exited from spawning tanks and eggs were incubated at the same place until they were hatched. Eggs were eyed after 4 days and all were hatched out to larvae after 7 days. The temperature, dissolved oxygen and pH of the incubation tank (well-water supply) were  $17 \pm 1.3^{\circ}\text{C}$ ,  $7.7\text{-}8 \text{ mg L}^{-1}$  and 7.7, respectively.

#### *Larval rearing and adapting with artificial food*

All larvae in tank were adapted with egg yolk and liquid soybean as artificial food for one week; then 540 healthy larvae were selected and transferred to experimental containers with 3.5 L capacity which was filled with 3 L of fresh and filtered water. In each container, 30 larvae with an average weight of 17.5 mg and 13.8 mm total length were randomly distributed in 18 tanks. All larvae in containers were held for acclimation 3 days prior to the experiment. During the experiment, photoperiod was set as 12L: 12D.

#### *Experimental design*

Six treatments with triplicate groups were conducted for six diets including *Artemia* nauplii as live food; rainbow trout starter foods (Coppens<sup>®</sup> and Roshd Daneh<sup>®</sup>), *Gammarus* powder, shrimp powder and yolk egg powder were considered as larval diets. Larvae were fed six times daily at 8, 10, 12, 14, 16 and 18 h for 4 weeks at satiation. During the experimental period,

temperature was measured 3 times daily at 8, 12 and 16 h and the average was  $19 \pm 2^{\circ}\text{C}$ . The water was siphoned from the bottom of fish container every morning to remove wastes and fifty percent of the water was changed with fresh and filtered water. Probable mortalities were recorded and removed from the container every day.

#### *Calculation of growth indices*

Due to the delicacy and tiny size of larvae and their mortalities all samples were measured (weight to nearest 0.01 g and total length at 1 mm) at the end of 4 weeks. Condition factor (CF), body weight increase (BWI), specific growth rate for weight (SGR<sub>w</sub>) and length (SGR<sub>L</sub>), voluntary feed intake (VFI) and survival rate (SR) were calculated according to the following formulas (Huang et al., 2003; Ai et al., 2004; Falahatkar et al., 2009):

$$\text{CF} = \text{body weight} / \text{fork length}^3 \times 100$$

$$\text{BWI} = (\text{final body weight} - \text{initial body weight}) / \text{initial body weight} \times 100$$

$$\text{SGR}_w = (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days} \times 100$$

$$\text{SGR}_L = (\ln \text{ final length} - \ln \text{ initial length}) / \text{days} \times 100$$

$$\text{VFI} = [(\text{feed consumed} / \text{days}) \times (2 / \text{final biomass} + \text{initial biomass})] \times 100$$

$$\text{SR} = (\text{number of fish at final} / \text{number of fish at start}) \times 100$$

#### *Statistical analysis*

Homogeneity of data was checked with Kolmogorov-Smirnov test. Data were then analyzed by one-way analysis of variance (ANOVA). When the difference was observed, Tukey's test as *post hoc* was used for mean comparison of treatments at a level of 5% ( $P < 0.05$ ). All performance was done using SPSS software (version, 13. Chicago, IL). Data are presented as mean  $\pm$  standard deviation (SD).

## Results

The growth performance of Caspian Kutum larva fed with different diets for four weeks are shown in table 1. There are significant differences among treatments ( $P < 0.05$ ) in most parameters. The highest final weight was related to the fish which

has been fed with *Artemia* nauplii and the poorest weight belonged to fish fed *Gammarus* powder and Roshd Daneh; but there were no significant differences in CF among treatments ( $P > 0.05$ ).

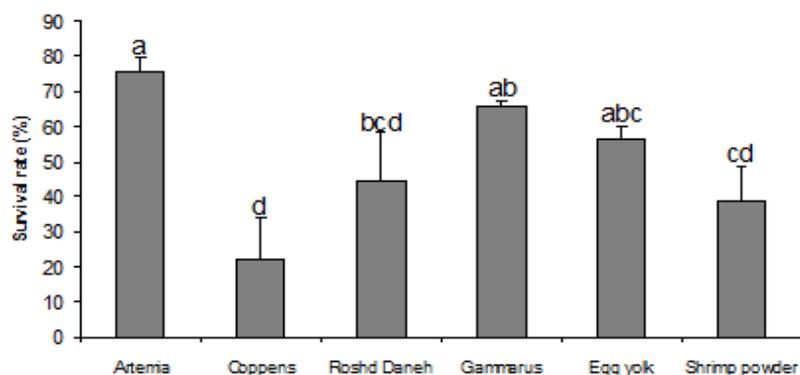
**Table 1: Growth indices of Caspian Kutum larvae fed different starter feeds after 4 weeks of rearing (mean  $\pm$  SD)**

Treatment	Initial weight (mg)	Initial length (mm)	Final weight (mg)	Final length (mm)	CF	BWI (%)	VFI (%wet weight/day)
<i>Artemia</i>	17.4 $\pm$ 3.8	13.7 $\pm$ 1.9	87.1 $\pm$ 10.9 <sup>a</sup>	22.9 $\pm$ 0.9 <sup>a</sup>	0.7 $\pm$ 0.01	398 $\pm$ 62.7 <sup>a</sup>	nd
Coppens	17.4 $\pm$ 4.5	13.8 $\pm$ 1.8	55.6 $\pm$ 6 <sup>b</sup>	19 $\pm$ 0.9 <sup>b</sup>	0.8 $\pm$ 0.1	217.4 $\pm$ 34.2 <sup>b</sup>	59 $\pm$ 0.5 <sup>a</sup>
Roshd Daneh	17.6 $\pm$ 4.9	13.7 $\pm$ 1.5	46.7 $\pm$ 4.8 <sup>b</sup>	17.8 $\pm$ 0.7 <sup>b</sup>	0.8 $\pm$ 0.01	166.8 $\pm$ 27.6 <sup>b</sup>	52.9 $\pm$ 7.8 <sup>a</sup>
<i>Gammarus</i> powder	17.5 $\pm$ 5.1	13.8 $\pm$ 1.4	46 $\pm$ 15.1 <sup>b</sup>	16.8 $\pm$ 0.4 <sup>b</sup>	1 $\pm$ 0.3	163.1 $\pm$ 86.4 <sup>b</sup>	32.7 $\pm$ 3.2 <sup>b</sup>
Egg yolk	17.4 $\pm$ 5.7	13.7 $\pm$ 1.5	70.4 $\pm$ 8.9 <sup>ab</sup>	19.8 $\pm$ 0.3 <sup>ab</sup>	0.9 $\pm$ 0.1	302 $\pm$ 51.1 <sup>ab</sup>	31.1 $\pm$ 2.3
Shrimp powder	17.5 $\pm$ 3.9	13.8 $\pm$ 1.7	61.4 $\pm$ 10.3 <sup>ab</sup>	19.2 $\pm$ 2.3 <sup>b</sup>	0.9 $\pm$ 0.2	250.9 $\pm$ 59 <sup>ab</sup>	41.3 $\pm$ 1.4 <sup>b</sup>

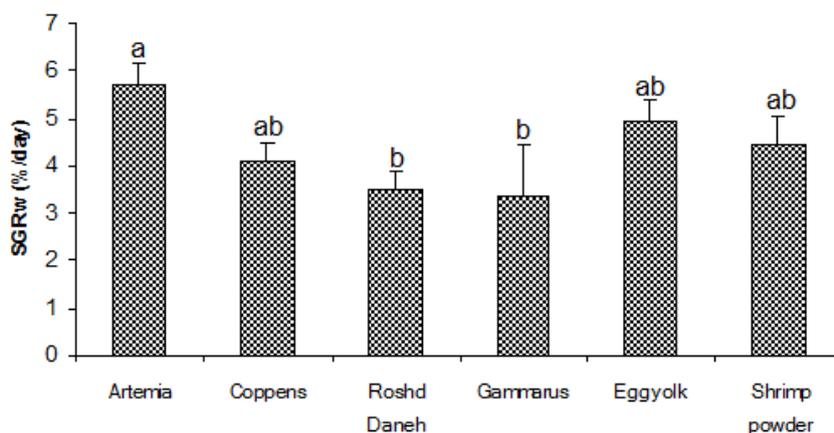
Different letters in each column show significant differences at a level of  $P < 0.05$ . CF (condition factor), BWI (body weight increase), VFI (voluntary feed intake). nd: not determined

After four weeks of experiment, the maximum weight and BWI were found in fish fed *Artemia* nauplii and the minimums were observed in the treatment in which fish were fed *Gammarus* powder ( $P < 0.05$ ).

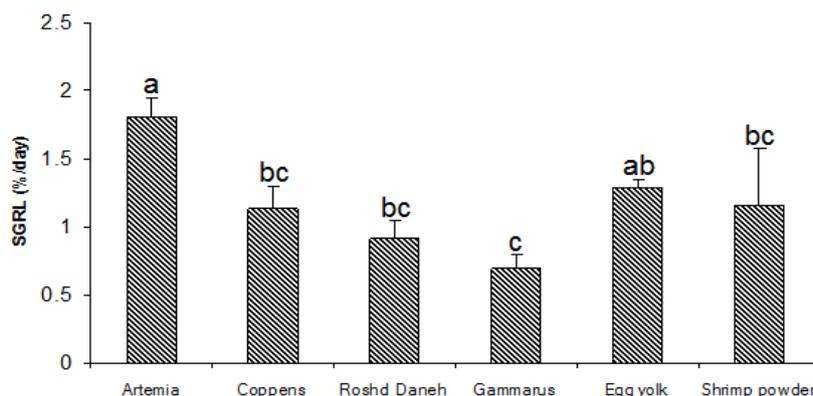
The maximum VFI (59%/day) was found in treatment with a diet of Coppens and the minimum was in fish fed egg yolk ( $P < 0.05$ ).



**Figure 1: Survival rate of Caspian Kutum larva fed different diets after 4 weeks of rearing (mean  $\pm$  SD). Different letters above each column show significant difference at a level of  $P < 0.05$**



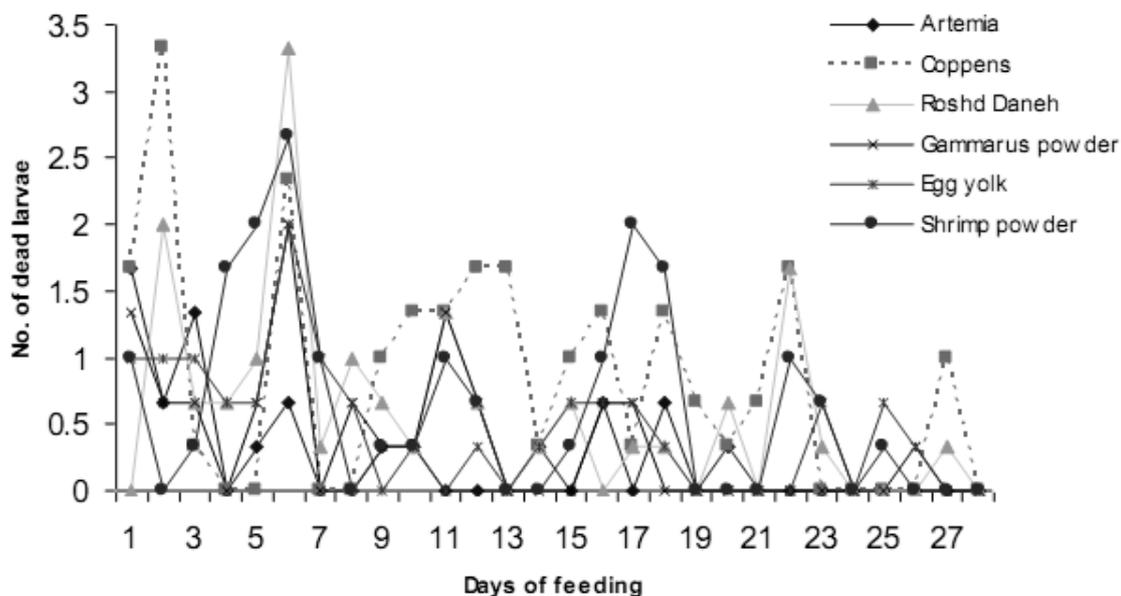
**Figure 2:** Specific growth rate for weight (SGR<sub>w</sub>) of Caspian Kutum larva fed different diets for 4 weeks (mean ± SD). Different letters above each column show significant difference at a level of P<0.05



**Figure 3:** Specific growth rate for length (SGR<sub>L</sub>) of Caspian Kutum larva fed different diets for 4 weeks (mean ± SD). Different letters above each column show significant difference at a level of P<0.05

Specific growth rates (SGR<sub>w</sub>, SGR<sub>L</sub>) were affected by diets and there were significant differences among treatments (P<0.05); the highest performance of SGR<sub>w,L</sub> was observed in fish which were fed *Artemia* nauplii and the lowest was found in those were fed *Gammarus* powder (Figs. 1 & 2). The highest survival rate was observed in

fish fed *Artemia* nauplii and the lowest rate was in fish fed Coppens commercial feed (P<0.05); but there were no significant differences in survival among fish fed shrimp powder and Roshd Daneh diet (P>0.05; Fig. 3). Trend of mortalities of fish fed different diets during the experimental days are presented in Figure 4.



**Figure 4: Trend of mortalities in Caspian Kutum larvae fed different diets during 4 weeks of experimental period**

## Discussion

Using live foods for the nutrition of larva is essential for aquatic organisms. However, some species at larval stage are absolutely dependent on the live foods, while some others can utilize the formulated commercial food from the first feeding. Nowadays, utilizing costly live foods is one of the main obstacles in aquaculture and it is attempted to decrease the live foods as a diet and impel the aquatic animals to the suitable commercial feeds. The results of this research indicated that the fish fed *Artemia* nauplii have the best performances in all growth indices; the difference of weight (>20 mg) of this treatment compared to the poorest diet (*Grammarus* powder) showed the suitability of *Artemia* nauplii. It has also been shown that the survival rate was significantly higher than the other treatments. The results of many studies indicated the suitability of live foods in the diet of larvae compare to combine or the practical diet (Callan et al., 2003). Although, the live foods have positive effects on weight gain and survival rate of larvae, but they

increase the expenses in the hatcheries, because of this, many researchers attempted to reduce or even eliminate the live foods from the diet of larvae or starter stages of aquatic organisms (Hung et al., 2002; Alvez et al., 2006). Of course, the strategy of hatcheries for replacing the live foods with commercial ones must be in such a way that it reduces the stress and diseases and it should also have acceptable growth and survival rate. Feeding of Caspian Kutum larvae with Rotifer demonstrated that survival rate and growth was higher when compared to fish fed the concentrated diet (Fallahi et al., 2004). The increase of growth parameters of larvae which were fed *Artemia* nauplii in this research indicated the suitability of live food in this stage of life; on the other hand this diet fulfills all nutritional requirements of fish. Although the VFI has been increased in two commercial diets, but the other growth indices compared to the live food treatment did not show any improvement.

One reason is that these diets could not complete all nutritional requirements of Caspian Kutum larva. The other reason is that even though the fish took the food, but due to the fish's lack of suitable digestive enzymes they were unable to digest the foods and finally led to poor growth and survival. Therefore, it was always supposed that the younger stages of animals (larvae) have less digestive enzymes for practical diets and these enzymes are provided only through the live foods (Cahu and Zambonino Infante, 2001). Drying of *Gammarus* and shrimp for powdering would destruct many essential nutrients such as vitamins, and other nutrients such as fatty acids during processing which ultimately lead to poor growth and survival rate of larvae. It is probably the enrichment of these diets with vitamins and mineral premixes and highly unsaturated fatty acids which will enhance the growth and survival rate of this fish.

Among the diets used in the present study (except *Artemia* nauplii), the egg yolk due to having some essential nutrients had shown better performance on growth and survival rate; therefore, it is used as a full replacement for the live foods for a short time. Nowadays, many hatcheries use egg yolk as food for rearing early larvae of Cyprinids.

To conclude, the results of the present study showed that there were not many significant differences in final weight, SGR, BWI and survival rate between larvae which were fed *Artemia* nauplii or egg yolk. Therefore, utilization of the egg yolk in contrast to *Artemia* nauplii is inexpensive and more

applicable. It is suggested that the diet with specified combination also having suitable external enzymes be used as food for early stage of larvae. It is also important to study more histological and enzyme activities of larvae for shifting the diet from live foods to practical diets and digestibility and absorption of the practical diets in future researches.

#### Acknowledgements

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## اثر جیره های آغازین بر شاخص های رشد لارو ماهی سفید دریای خزر (*Rutilus kutum*)

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### چکیده

شش نوع غذای مختلف شامل دو غذای آغازین قزل آلا، پودر گاماروس، پودر میگو، پودر زرده تخم مرغ و ناپلیوس تازه هچ شده آرتمیا در تغذیه لارو ماهی سفید دریای خزر مورد استفاده قرار گرفت. لاروهای ماهی سفید با وزن متوسط  $4/7 \pm 17/5$  میلی گرم به مدت ۴ هفته در سه تکرار مورد پرورش قرار گرفته و پارامترهای رشد در انتهای این دوره مورد سنجش قرار گرفت. ۳۰ ماهی در هر ظرف ۳ لیتری با شش بار غذادهی روزانه در حد اشتها برای هر تکرار در نظر گرفته شد. نتایج این آزمایش نشان داد اختلاف معنی داری در وزن نهایی، طول نهایی،  $SGR_L$ ،  $SGR_w$ ،  $VFI$ ،  $BWI$  در تیمارهای مختلف مشاهده شده اما این اختلاف در خصوص  $CF$  معنی دار نبود. درصد بقا نیز در تیمارهای مختلف معنی دار بود بطوری که بالاترین و پایین ترین میزان آن به ترتیب در ماهیان تغذیه شده با ناپلیوس آرتمیا ( $3/8 \pm 75/6$  درصد) و غذای کوپنز ( $11/7 \pm 22/2$  درصد) مشاهده شد. پس از آرتمیا بنظر می رسد مناسب ترین جیره مورد استفاده، پودر زرده تخم مرغ باشد که وزن نهایی مناسبی را در ماهیان ایجاد کرد. نتایج این مطالعه پیشنهاد می کند استفاده از غذاهای دستی دارای ترکیب مناسب بجای آرتیما، می تواند رشد و نرخ بقای مناسبی را در ماهیان سفید در مرحله لاروی ایجاد نماید.

**واژگان کلیدی:** غذای زنده، غذای فرموله شده، رشد، بقا، لارو، ماهی سفید