

## The Study of Growth Performance, Body Composition and Some Blood Parameters of *Rutilus frisii kutum* (Kamenskii, 1901) Fingerlings at Different Salinities

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### ABSTRACT

This study was carried out to investigate the effects of salinity levels (0, 2, 4, 7 and 10 ppt) on growth indices, blood biochemical parameters, and body composition in *Rutilus frisii kutum* fingerlings (initial weight  $1.33 \pm 0.02$  g) during 60 days. Results indicated that the highest rates of daily growth, specific growth and weight gain per fish were obtained at the levels of 4 and 2 ppt ( $P < 0.05$ ). The lowest rates of these indices were observed at 10 ppt ( $P < 0.05$ ). FCR (food conversion ratio) and CF (condition factor) showed no significant difference among various treatments ( $P > 0.05$ ). Findings of blood biochemical factors at the end of the study (hematocrit, total protein, glucose, cholesterol, calcium, sodium, potassium and magnesium) revealed no significant variation in different salinities ( $P > 0.05$ ). Changes in protein, moisture, fat and ash content were not significant at the end of the experimental period ( $P > 0.05$ ).

**Keywords:** Blood parameter, Body composition, Growth indices, Kutum fingerlings, Salinity.

### INTRODUCTION

Kutum, *Rutilus frisii kutum* (Kamenskii, 1901), is an endemic fish to the Caspian Sea. The Iranian Fisheries Organization (Shilat) produces up to 200 million fry (1–2 g b.w.) to restock the Caspian Sea population. These fish are produced by artificial breeding using carp pituitary extract (CPE) [26]. Kutum lives near the coast, from the Terek River in the north to the southern part of the Sea. Due to the great demand on the market, its good taste and culinary customs of the local people, it is consumed all year round in the southern part of the Sea ([www.shilat.com](http://www.shilat.com)).

Energy values can also be expressed as a ratio of absorbed or assimilated energy, so as to help us understand how the energy is channeled to growth and which forms of

energy are mainly consumed in a particular condition. Energy allocations are always determined by ontogenesis (developmental stages, body weight and reproduction) and extrinsic factors (salinity, temperature, and pH etc.) [42]. Salinity is one of the most important abiotic factors in aquaculture and its optimal levels are species-specific for growth, survival and production efficiency [29]. Furthermore, osmotic stress has been reported to elicit physiological responses such as increased dissolved oxygen consumption [8] and ammonia excretion [31] that may substantially alter the culture environment in a closed culture system. The capacity of fish for osmoregulation in the face of changing environmental salinity has been thoroughly investigated in species that migrate between fresh water and sea water during their life cycle, or species that live in estuaries [23].

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Regarding to the economic importance and commercial value of kutum (*Rutilus frisii kutum*) in aquaculture of the country and lack of information on optimum salinity for growth in cultivation and breeding centers of this species on one side, and the necessity to determine this crucial factor for the successful rearing of the fingerlings destined to release in the sea on the other side, we intended to investigate effects of salinity levels on growth rate, physiological and biochemical characteristics of kutum fingerlings in this research.

## MATERIALS AND METHODS

### Fish Stock and Rearing Conditions

In June 2008, fingerlings ( $n = 3 \cdot$ ) were obtained from the Breeding and Cultivation Center of Sijaval (in Bandar Torkman) and transported to the rearing laboratory of the Natural Resources and Agricultural Sciences University of Gorgan. To acclimatize the fingerlings were placed in fiberglass tanks at  $24 \pm 1$  °C and pH of  $7 \pm 0.2$  and were acclimated to these conditions for 2 weeks prior to experiment. The fish showed a normal behavioral pattern during this period. Fish were held at 0ppt prior to treatment exposure. The experiment was conducted in 15 aquaria, each with a volume of 70 L, stocked with 20 fish ( $1.33 \pm 0.02$  g in average weight) per tank and the initial weight of fingerlings showed no significant ( $P > 0.05$ ) difference among treatments. Salinity levels were monitored using the salinometer (Horiba – U10, Japan). Once every week, water samples were taken for pH measurement using a pH-meter (Model 713 Metrohm, Switzerland).

### Experimental Design

The range of salinities selected in these trials was based on the environmental salinities which Kutum encounters in the Caspian Sea and surrounding rivers. Water

supplied from the Caspian Sea and fresh well water was used to regulate the salinity level. The aquaria were covered with net screens to prevent fish from jumping out, and continuous aeration was provided to maintain dissolved oxygen near saturation levels. In each aquarium, 75% of water volume was renewed daily with water of the salinity selected for the treatment. The fish were gradually transferred from the holding salinity (0ppt) to the five experimental salinities. During the acclimation period, salinity was raised up to 2ppt per 24 h for all treatments by adding salt water (from the Caspian Sea) to reach the following salinity groups: 0, 2, 4, 7 and 10 ppt [22]. Kutum fingerlings were exposed to these salinities for 60 days. Salinity in each tank was measured once daily. These five treatments were tested in three replicates each.

### Biometric Indexes and Growth

To begin the experiment, the fish were measured and those with the nearest weight of 1.33 g and length of 2.5 cm were captured from the holding tank. Initial mean weights did not differ significantly ( $P > 0.05$ ) among treatments. Fish mortality was checked every day. No mortality occurred during the 60 days of exposure to any of the tested salinities except for the salinity of 10 ppt during the first 24 hr of acclimation period.

Body weight gain (WG, g) was calculated as the increase in total biomass at the end of the experimental period,  $WG = W_f - W_i$ , where  $W_f$  and  $W_i$  are the final and initial body weights, respectively [22]. The specific growth rate (SGR,  $\% \text{day}^{-1}$ ) was measured according to the formula  $(\ln W_f - \ln W_i) \times 100 / \Delta t$ ; where  $\Delta t$  is the time interval (in days) between  $W_i$  and  $W_f$  measurements [38]. The food conversion ratio (FCR) was assessed as total food given (g)  $\times$  weight gain<sup>-1</sup> (g) over the experimental period (60 days) [22]. Condition factor (CF, %) =  $100 \times W \times L^{-3}$ , where  $W$  is the final weight (g), and  $L$  is total final length (cm) [15]. For the first 30 days of experimental period, the fish

were fed with Biomar fish food on 10% of body weight (average diameter of 0.5 mm). Considering the lower demand of food in fish fingerlings increasing in size, they were fed on 7% of their body weight (diameter of 0.8 mm in average) for the latter experimental period. The fish were hand-fed, twice a day at 12 hour intervals [34]. The mean fish weight and final length in each treatment were measured once every two weeks and at the end of the experiment, respectively.

### Hematology and Proximate Composition Analysis

At the end of the experimental period, blood from each fish was taken using heparinized capillary tubes after caudal severance. Plasma samples obtained by centrifugation were immediately frozen and stored at  $-80^{\circ}\text{C}$  for analyses [22]. The fish were beheaded, eviscerated and muscle tissue samples were taken from both sides below the dorsal fin and were stored at  $-80^{\circ}\text{C}$  for the determination of moisture, ash, crude protein and lipid. Protein content was assessed by converting the nitrogen content as described by the method of Kjeldahl [3] ( $\text{N} \times 6.25$ ) after acid digestion. Moisture content was calculated by drying the sample in an oven at  $70^{\circ}\text{C}$  for 48 h and comparing dry matter after drying to the mass of the fresh weight; fat determination was conducted by ether extraction method [3] with Soxhlet System, and ash content was measured by dry-ashing in a furnace at  $500^{\circ}\text{C}$  for 8 h [3]. For hematocrit determination, a microhematocrit reader (I.E.C.CAT micro-capillary 2201, USA) was used and the values were expressed as the percentage of erythrocytes. Photometric methods were applied for plasma glucose, total protein, calcium, magnesium and cholesterol assays using a lightwave-S2000 UV/VIS diode array spectrophotometer. Sodium and potassium concentrations of plasma were measured using a flame

photometer and ion-dedicated electrodes (Coring Flame Photometer 410, England).

### Statistical Analysis

In the present trial, a completely randomized design with 3 replicates was used. Average weight and food given to each tank was calculated to determine growth indices, proximate composition and blood parameters at different salinity levels. Data were tested for the homogeneity of variance and then statistically analyzed using one-way ANOVA, followed by comparison of means by Duncan's multiple range test ( $\alpha=0.05$ ). The correlation between salinity and WG, DGR, SGR, FCR, and CF was investigated using a linear regression method. The statistical analysis was conducted using SPSS11.0 for Windows. Values are expressed as the mean  $\pm$  standard deviation.

## RESULTS

### Effects of Salinity on Growth

In this research, the average final weight of kutum fingerlings was  $2.6 \pm 0.04\text{g}$ . Weight gain (WG), daily growth rate (DGR) and specific growth rate (SGR) were significantly ( $P < 0.05$ ) influenced by water salinity, but food conversion ratio (FCR) and condition factor (CF) did not significantly differ among treatments at the end of the experimental period ( $P > 0.05$ ).

Final weight (FW), weight gain (WG), daily growth rate (DGR) and specific growth rate (SGR) were the highest at 4ppt and lowest at 10ppt.

### Effects of Salinity on Blood Parameters

Table 2 summarizes the hematological and biochemical parameters in Kutum (*Rutilus frisii kutum*) fingerlings. The plasma glucose, total protein, cholesterol,



calcium, magnesium, sodium, potassium and hematocrit levels did not significantly change in salinity-exposed fingerlings ( $P>0.05$ ). The highest plasma glucose content was measured at 0 ppt and similar trends were observed for cholesterol, total protein and calcium (Table 2).

### Effects of Salinity on Body Composition

In this research, body composition (crude protein, fat, moisture and ash) of fingerlings was investigated in dry matter (1 g) at the end of the experiment. Results revealed that these factors did not significantly ( $P>0.05$ ) differ among treatments at the end of the experiment (Table 3).

## DISCUSSION

Among ectotherms, aquatic organisms represent a large number of species that are directly and acutely exposed to environmental changes. Environmental factors such as salinity influence fish growth and several authors have studied the influence of water salinity on fish growth with salinity affecting growth frequently [5 and 17].

In our study, maximum and minimum growths were observed at 10 and 4 ppt, respectively, indicating that high levels of salinity detrimentally affected metabolic rate, and implying that energy expenditure for osmoregulation which is considerably dependent on this environmental factor may have occurred at the expense of growth (Table 1). Growth enhancement has been

observed at lower salinity levels (0–9 ppt), in striped bass (*Morone saxatilis*) larvae [27], and milkfish (*Chanos chanos*) fry [1]. Conversely, in a previous report by Amiri *et al.* (2008), Kutum fingerlings exposed to salinity levels for 60 days, had the highest final weight, SGR, CF and minimum FCR at 10 ppt with the lowest values of the growth indices and maximum FCR being obtained at 0ppt. In this case, higher salinity levels (20–55 ppt) have also shown to improve growth in other species such as black bream (*Acanthopagrus butcheri*) juveniles [25], European sea bass (*Dicentrarchus labrax*) fingerlings [13], and milkfish juveniles [32].

In these species, better growth rates were attributed to a preference by younger fish to environments of lower salinity levels found in estuaries or fresh water environments during their natural development [1], or a lower energetic requirement for the maintenance of the osmotic and ionic equilibrium in lower salinities (from low to intermediate levels) compared to salt water. As stated by Garcia *et al.* (1999) on the acclimation ability of big head fry (*Aristichthis nobilis*) under a wide range of salinity (0-16 ppt), 15 and 18 day-old fry could adapt better to higher salinities of 6 and 7 ppt than 11 day-old fry.

Better growth rates have also been obtained at intermediate salinities (10–19 ppt) in golden-line seabream (*Sparus sarba*) (150–250 g) [38], and young grey mullet (*Mugil spp.*) [27]. Gaumet *et al.* (1995) reported that on a long term schedule, growth conditions in seawater adapted juvenile of turbot (*Scaphthalmus maximus*) could be improved by their adaptation to brackish waters (salinities between 10 and

**Table 1.** Growth indices of kutum fingerlings reared at five salinities (0, 2, 4, 7 and 10 ppt) for 60 days

Treatment	WG (g)	DGR (mg)	SGR(% day <sup>-1</sup> )	FCR	CF
0 PPT	1.3±0.15 <sup>bc</sup>	21.66± 1.2 <sup>bc</sup>	1.15 ±0.09 <sup>b</sup>	1.3 ±0.15 <sup>a</sup>	1.29 ±0.04 <sup>a</sup>
2 PPT	1.37±0.1 <sup>ab</sup>	22.83 ±2.7 <sup>ab</sup>	1.22 ±0.02 <sup>ab</sup>	1.1 ±0.08 <sup>a</sup>	1.45 ±0.15 <sup>a</sup>
4 PPT	1.44±0.14 <sup>a</sup>	24± 5.03 <sup>a</sup>	1.38 ± 0.02 <sup>a</sup>	1.2 ±0.1 <sup>a</sup>	1.41 ±0.12 <sup>a</sup>
7 PPT	1.29± 0.11 <sup>c</sup>	21.5 ±4.7 <sup>c</sup>	1.14 ± 0.04 <sup>b</sup>	1.3 ±0.09 <sup>a</sup>	1.4 ±0.19 <sup>a</sup>
10 PPT	1.17± 0.11 <sup>d</sup>	19.5 ±3.4 <sup>d</sup>	0.95 ±0.07 <sup>c</sup>	1.4 ±0.01 <sup>a</sup>	1.19 ±0.04 <sup>a</sup>

**Table 2.** Blood biochemical parameters in Kutum reared at different salinities (0, 2, 4, 7 and 10 ppt) during 60 days.

Treatment	Magnesium (mg dl <sup>-1</sup> )	Calcium (mg dl <sup>-1</sup> )	Sodium (Mm L <sup>-1</sup> )	Potassium (Mm L <sup>-1</sup> )	Total protein (g dl <sup>-1</sup> )	Cholesterol (mg dl <sup>-1</sup> )	Glucose (mg dl <sup>-1</sup> )	Hematocrit (%)
0 PPT	4.9 ± 1.9 <sup>a</sup>	178.4 ± 42.6 <sup>a</sup>	116.8 ± 15.3 <sup>a</sup>	3.86 ± 1.6 <sup>a</sup>	13.1 ± 1.01 <sup>a</sup>	278.7 ± 92.1 <sup>a</sup>	92.7 ± 94.1 <sup>a</sup>	47.8 ± 0.76 <sup>a</sup>
2 PPT	6.5 ± 2.3 <sup>a</sup>	135.9 ± 56.3 <sup>a</sup>	123.5 ± 15.3 <sup>a</sup>	3.83 ± 0.55 <sup>a</sup>	11.4 ± 5.4 <sup>a</sup>	265.2 ± 44.6 <sup>a</sup>	60.7 ± 55.2 <sup>a</sup>	48 ± 2.9 <sup>a</sup>
4 PPT	9.4 ± 6.9 <sup>a</sup>	140.8 ± 74.6 <sup>a</sup>	112.5 ± 2.6 <sup>a</sup>	3.83 ± 1.9 <sup>a</sup>	9.8 ± 6.2 <sup>a</sup>	204.2 ± 96.2 <sup>a</sup>	79.2 ± 23.7 <sup>a</sup>	49.3 ± 1.9 <sup>a</sup>
7 PPT	5.2 ± 3.2 <sup>a</sup>	92.2 ± 61.1 <sup>a</sup>	15.3 ± 133.5	4.13 ± 1.6 <sup>a</sup>	10.2 ± 4.5 <sup>a</sup>	194.6 ± 56.8 <sup>a</sup>	58.6 ± 38.2 <sup>a</sup>	51.7 ± 0.02 <sup>a</sup>
10 PPT	8.6 ± 3 <sup>a</sup>	144.6 ± 79.9 <sup>a</sup>	9.9 ± 140.1	3.77 ± 0.49 <sup>a</sup>	10.6 ± 1.03 <sup>a</sup>	178 ± 74 <sup>a</sup>	90.3 ± 48.3 <sup>a</sup>	47.8 ± 1.2 <sup>a</sup>

Results are given as mean ± standard deviation. The same letters represent no significantly different (P>0.05).

19 ppt). Additionally, Imsland *et al.* (2003) working on turbot suggested that the lower cost of ion regulation at 15ppt may contribute to enhanced growth at that salinity. These studies support the hypothesis that the energetic cost for osmoregulation is lower at an isosmotic medium, in which gradients between the blood and water are minimal, and the energy saved is directed to increase growth [21].

Erythropoietic activity, as reflected by changes in hematological parameters such as hematocrit, hemoglobin concentration and erythrocyte counts is modulated in fish by several factors such as hypoxia [37], exercise [20], management-induced stress [24], reproductive stage [6] and seasonal variations closely related to thermal cycles [39].

In contrast to the findings of Zeitoun *et al.* (1974), who determined significant increase of hematocrit values (P<0.05) with an increase in salinity from 10ppt to 20ppt in rainbow trout [39], there was no significant effect (P>0.05) of salinity on hematocrit in the present trial.

Plasma cortisol levels and alterations in carbohydrate metabolism, such as plasma glucose concentrations, can be used as general stress indicators in fish [30]. As detected in our study, changes in plasma glucose and cholesterol of kutum fingerlings exposed to different salinities were not significant (P>0.05).

Total plasma protein (TPP) concentration relative to a reference interval is used as a broad clinical indicator of health, stress, and well being of aquatic organisms [28]. As shown in Table 2, no significant difference (P>0.05) was found among plasma protein concentrations of kutum fingerlings.

In fish, potassium plays additional critical roles in osmo- and ionoregulation and acid/base balance [14]. Analysis of plasma potassium, calcium, magnesium and sodium concentration revealed no significant difference (P>0.05) in various treatments.

Verdegem *et al.* (1997) reported that salinity had no significant (P>0.05) influence on hematocrit in hybrid red tilapia (*Oreochromis niloticus* (Linnaeus) × *O. mossambicus* (Peters) [37]. By contrast, Dheer *et al.* (1990) reported

**Table 3.** Body composition (muscle) in kutum held under five salinities (0, 2, 4, 7 and 10 ppt) during 60 days.

Treatment	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
0 PPT	69 ±0.38 <sup>a</sup>	3.5 ±0.14 <sup>a</sup>	18.5 ±1.4 <sup>a</sup>	8.4±0.00 <sup>a</sup>
2 PPT	68.2 ±1.1 <sup>a</sup>	4 ±0.63 <sup>a</sup>	18.8 ±0.7 <sup>a</sup>	8.3 ±0.7 <sup>a</sup>
4 PPT	68.8 ±0.66 <sup>a</sup>	3.8 ±0.35 <sup>a</sup>	18.9 ±0.7 <sup>a</sup>	7.9±0.7 <sup>a</sup>
7 PPT	68.5 ±0.35 <sup>a</sup>	3.9 ±0.21 <sup>a</sup>	19 ±0.7 <sup>a</sup>	8.2 ±0.7 <sup>a</sup>
10 PPT	68.9 ±0.57 <sup>a</sup>	3.9 ±0.92 <sup>a</sup>	18.7 ±1.4 <sup>a</sup>	8 ±0.7 <sup>a</sup>

Results are given as mean ± standard deviation. The same letters represent no significantly different ( $P>0.05$ ).

that blood parameters of *Channa punctata* were affected by changes in salinity.

There was no significant difference ( $P>0.05$ ) in body composition of kutum fingerlings under different salinities as analyzed at the end of the experimental period. As reported by Dendrinis and Thorpe (1985), salinity (0.5-33 ppt) did not affect the proximate composition of the white muscle of the European bass (*Dicentrarchus labrax*). Water content of the catfish (*Mystus vittatus*) was found to decrease with increase in salinity while maximum ash (25.56%) and fat (42.25%) were exhibited by fish reared in 10 ppt salinity [4]. Considering our results, it should be noted that two main factors can contribute to the absence of stress in kutum fingerlings. On the one hand, salinity was gradually increased, which reduces the osmotic stress and facilitates the acclimation to different salinities. On the other hand, cholesterol, glucose and hematological parameters were analyzed after a long period of salt exposure in fingerlings (60 days).

### CONCLUSION

With regard to the aim of the study to determine the optimum salinity to increase the growth performance of kutum fingerlings at this life stage, this study suggested the levels of 2 and 4 ppt as the best salinity range.

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### مطالعه عملکرد رشد، ترکیبات بدن و برخی پارامترهای خونی بچه ماهیان انگشت قد سفید (*Rutilus frisii kutum kamensky, 1901*) در سطوح مختلف شوری

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#### چکیده

مطالعه حاضر جهت بررسی اثرات سطوح شوری (۰، ۲، ۴، ۷ و ۱۰ گرم در لیتر) روی شاخصهای رشد، پارامترهای بیوشیمیایی خون و ترکیب بدن بچه ماهیان انگشت قد سفید (*Rutilus frisii kutum*) (میانگین وزن اولیه  $1/33 \pm 0/02$  گرم) به مدت ۶۰ روز انجام گرفت. نتایج نشان داد که بیشترین میزان نرخ رشد روزانه (DGR)، نرخ رشد ویژه (SGR) و وزن اکتسابی (WG) بچه ماهیان در سطوح شوری ۲ و ۴ گرم در لیتر به دست آمد ( $P < 0/05$ ). پایین ترین میزان این شاخصها در شوری ۱۰ گرم در لیتر به دست آمد ( $P < 0/05$ ). ضریب تبدیل غذایی (FCR) و شاخص وضعیت (CF) تفاوت معنی داری بین تیمارهای مختلف نشان نداد ( $P > 0/05$ ). نتایج فاکتورهای بیوشیمیایی خون (هماتوکریت، پروتئین کل، گلوکز، کلسترول، کلسیم، سدیم، پتاسیم و منیزیم) در پایان دوره آزمایش تغییر معنی داری در سطوح مختلف شوری نشان ندادند ( $P > 0/05$ ). تغییرات پروتئین، رطوبت، چربی و خاکستر در پایان دوره آزمایش معنی دار نبود ( $P > 0/05$ ).