Caspian whitefish, *Rutilus frisii kutum* Kamensky, 1901 a potential aquaculture candidate: study on the cumulative effects of salinity and temperature on culture performance

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Abstract

In this study, growth and survival of the Caspian whitefish, *Rutilus frisii kutum,* (mean weight 0.59±0.06g) have been estimated at different rearing salinities (0, 5 and 10‰) and temperatures (24 and 28˚C). Maximum fish weight gain (493.65±14.07%) and specific growth rate (SGR) (2.74±0.15%), and minimum feed conversion ratio (FCR) (1.66±0.06) were obtained at salinity of 5‰ and temperature of 24˚C. There were significant differences in fish weight and length gain between the two rearing temperatures at salinity of 10‰ and between salinities of 0 and 5‰ at 24˚C ($p<0.05$). However, salinity and temperature did not significantly affect fish survival. Factorial analysis of variance showed significant cumulative effects of salinity and temperature on fish weight and length gain, final weight and SGR ($p<0.05$). This study confirmed that optimization of salinity and temperature is crucial in rearing a new fish species. Caspian whitefish fingerlings can be grown well at 5‰ and 24˚C; although, it is recommended that the effects of a broader range of environmental variables as well as feed items must also be examined.

Keywords: Caspian whitefish, *Rutilus frisii kutum*, Salinity, Temperature, Growth indices, Survival

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Introduction

Domestication of commercial fish species and their mass culture in controlled conditions is perhaps a major concern in world aquaculture trends. The Caspian whitefish, *R. frisii kutum* (Cyprinidae), is one of the economically-important fishes of the Caspian Sea (Abdolhay *et al*., 2010), constituting over 50% of annual bony fish catch in the southern part of this sea (Afraei Bandpei *et al*., 2010). For many decades, it has been regarded as one of the most desired food fish by the native consumers. However, due to overexploitation and environmental pressures, natural stocks of this fish have experienced a drastic decline in the recent years (Jafari *et al*., 2011). This together with increased demand for the fish has drawn attention toward its artificial production. Semi-artificial propagation and larval rearing of Caspian whitefish in earthen ponds have being practiced for many years in Iran and currently, some 180 million fingerlings are annually released into the major rivers entering the Caspian Sea for conservation purposes (Amini *et al*., 2007). However, despite its popularity and the fact that its maximum weight may reach up to 5kg in nature (Afraei Bandpei *et al*., 2010), the fish has not yet been cultured successfully to a marketable size in Iran, and its water quality requirements are less well known. The failure in the commercial rearing of the fish can be attributed to its susceptibility to culture conditions, inadequate knowledge of its environmental preferences at different growth stages and lack of appropriate food items.

Successful farming of any new fish species depends, to a great extent, on optimization of its culture conditions. Several biotic and abiotic factors influence growth and survival of fish, and environmental requirements of various fish species have been evaluated for development of land-based rearing systems (Ruyet *et al*., 2004). The aims of this study were: 1) assessing the effects of different rearing salinities and temperatures on the growth and survival of the Caspian whitefish, and 2) contributing to the knowledge of optimum culture conditions of this potent species.

Materials and methods

Caspian whitefish, *R. frisii kutum*, fry (mean weight 0.59±0.06 g) were obtained from Shahid Ansari Fish Culture Center (Bandar Anzali, Iran). The fish were transferred in plastic bags, containing a mixture of pond water and oxygen in a ratio of 1:3, to the Laboratory of Aquaculture of Mirza Koochak Khan Higher Education Center. The experimental fish were acclimatized to the new culture conditions in 2000 L glass fiber tanks containing freshwater at temperatures of 22±0.5ºC for a week. The treatments comprised culturing the fish at three salinities of 0, 5 and 10‰ and at two temperatures of 24 and 28ºC each in triplicate. These temperatures were found to be best fit with the fish requirements among the others tested (unpublished data). Sixty-nine fish (23 fish per aquarium) were randomly assigned to each of the treatments and transferred to the glass aquaria with dimensions of 50×40×35cm containing 40 L water of the designated salinities and temperatures. Experimental salinities were made by diluting the Caspian Sea brackish water with fresh well water. Required temperatures were provided by using aquarium heaters and remained stable throughout the experiment. Prior to the initiation of the study, the fish were adapted to each temperature and salinity for 5 days. Aeration was provided for the aquaria via a central air generator.

Water temperature was measured three times daily and pH and dissolved oxygen once a day by digital instruments. One-fourth of the water in each aquarium was refreshed on each day, and every 5 days 100% of the water was replaced by new medium.

The fish were hand-fed four times daily (8 am and 1, 6 and 10 pm) with a commercial trout feed (Biomar, France) with a pellet size of 0.5 mm containing 58% protein, 15% fat, 15% ash and 1.5% fiber. The amount of feed offered was 5% of the fish biomass in each aquarium per day in the beginning. This increased to 8% when fish adapted to the feed up to the time when they reached a body weight of about 2g and then, fixed at 6% of the biomass up to the end of the experiment. The feeding rates were estimated by considering the apparent satiation of the fish in successive culture periods.

The fish were cultured for 6 weeks. In the beginning of the experiment and then, at the end of every week, weight and length of the fish from each treatment were measured by using a precise digital balance and biometric ruler. A number of 10 fish from each replicate were randomly used for weight and length measurements. Mortalities were removed daily and percent survival was estimated for the treatments. Weight gain (WG), length increment (LI), SGR, condition factor (K), and FCR were calculated by the following formulae:

$WG (\%) = (W_f - W_0)100/W_0$

Where: W_f and W_0 were final and initial fish body weights, respectively

$\text{Length increment} (\%) = (L_f - L_0)100/L_0$

Where: L_f and L_0 were final and initial fish body lengths, respectively.

$K = 100 (W / TL^3)$

Where: W= mean fish weight (g) and TL= fish total length (cm)

$SGR = 100\% (\ln W_F - \ln W_0)\, t^{-1}$

$FCR = P (W_F - W_0)^{-1}$

Where: P= weight of the feed distributed (kg); W_F= final stock biomass (kg); W_0= initial stock biomass (kg); t= duration of culture (in days).

The data were analyzed by employing SPSS ver. 15 software. Analysis of variance (ANOVA) followed by a Tukey's test to assess the effects of salinity and temperature on each of the estimated parameters. A between-subjects factor (factorial) analysis was applied to explore the cumulative effects of salinity and temperature on the parameters. Significance level was set at $p<0.05$ in all the comparisons.

Results

The examined fish had minimum final weight at temperature of 24˚C and salinity of 10‰ (mean±standard deviation=3.2± 0.28g), while their maximum final weight (3.74±0.16g) was recorded at 24˚C and 5‰. Weight gain was lowest in the fish reared at 24˚C and 10 ‰ (331.84±18.83%) and highest at 24˚C and 5 ‰ (493.65± 14.07%). Minimum and maximum length increments were, at 10 ‰ (48.08±8.89%) and 5 ‰ (67.86±4.12 %), respectively both at 24˚C.

Minimum SGR (2.25±0.07%) was calculated for the fish cultured at 24˚C and 10 ‰, while the SGR was maximum (2.74±0.15%) at 24˚C and 5 ‰. The SGR of all the treatments had an overall declining trend from the initiation toward the termination of the experiment (Fig. 1). There were significant differences in fish weight and length gains between salinity of 10 ‰ and the two other salinities at 24˚C ($p<0.05$) (Table 1). Minimum K (0.92±0.08) was for the fish reared at 28˚C and 5‰, while maximum K (0.94±0.09) was obtained at 28˚C and 0‰. The lowest and highest FCR values were 1.66±0.06 and 2.03±0.21 at 24˚C and salinities of 5 ‰ and 10 ‰, respectively. Trends of the FCRs of different treatments during the study period are shown in Fig. 2. Minimum survival (91.3±8.69%) was observed in the fish cultured at 24˚C and 5‰, while maximum survival (98.55±2.5%) was at 28˚C and 0 ‰. However, survival was not significantly different among the salinity and temperature treatments ($p>0.05$). Survival showed sharp decrease with time in some treatments, while remained almost steady in the others during the course of the experiment (Fig. 3).

Table 1: Values (mean±standard deviation) of the measured growth indices in the Caspian whitefish at different salinities and temperatures.

Parameter	Salinity (‰)	24°C	28°C	Sig.
FW (g)	0	3.70 (±0.21)[a]	3.34 (±0.04)[a]	0.090
	5	3.74 (±0.16)[a]	3.26 (±0.12)[a]	0.015
	10	3.21 (±0.28)[a]	3.49 (±0.27)[a]	0.276
		F=5.288 Sig.=0.047	*F*=1.471 Sig.=0.302	
WG (%)	0	484.70 (±6.95)[a]	474.99 (±63.35)[a]	0.805
	5	493.65 (±14.07)[a]	446.74 (±28.93)[a]	0.418
	10	331.84 (±18.83)[b]	489.40 (±41.17)[a]	0.004
		F=20.282 Sig.=0.002	*F*=0.648 Sig.=0.556	
LI (%)	0	67.80 (±5.64)[a]	63.02 (±10.90)[a]	0.537
	5	67.86 (±4.12)[a]	61.37 (±6.88)[a]	0.233
	10	48.08 (±8.89)[b]	66.57 (±2.47)[a]	0.026
		F=9.158 Sig.=0.015	*F*=0.369 Sig.=0.706	
K	0	0.93 (±0.09)[a]	0.95 (±0.09)[a]	0. 865
	5	0.94 (±0.02)[a]	0.92 (±0.08)[a]	0.746
	10	0.93 (±0.08)[a]	0.93 (±0.04)[a]	0. 951
		F=0.007 Sig.=0.993	*F*=0.111 Sig.=0.896	
SGR	0	2.72 (±0.02)[a]	2.69 (±0.17)[a]	0.780
	5	2.74 (±0.15)[a]	2.61 (±0.08)[a]	0.447
	10	2.25 (±0.07)[b]	2.73 (±0.11)[a]	0.003
		F=22.978 Sig.=0.002	*F*=0.616 Sig.=0.571	
FCR	0	1.67 (±0.18)[a]	1.72 (±0.29)[a]	0.837
	5	1.66 (±0.06)[a]	1.94 (±0.12)[a]	0.022
	10	2.03 (±0.21)[a]	1.75 (±0.15)[a]	0.133
		F=4.831 Sig.=0.056	*F*=1.127 Sig.=0.384	
SR (%)	0	97.10 (±2.51)[a]	98.55 (±2.51)[a]	0.518
	5	91.30 (±8.70)[a]	92.75 (±2.51)[a]	0.795
	10	95.65 (±4.35)[a]	94.20 (±5.02)[a]	0.725
		F =0.812 Sig.=0.487	*F*=2.167 Sig.=0.196	

FW–final weight; WG– weight gain; LI– length increment; SGR– specific growth rate; FCR–feed conversion ratio; K– condition factor; SR– survival

At each temperature, values of every parameter with different alphabetic letters are significantly different between the salinities ($p<0.05$). The last column compares the values between two temperatures (t-test significant levels).

Table 2: Results of factorial analysis of variance (ANOVA) showing the effect of salinity and temperature and their cumulative effects on the estimated growth indices in Caspian whitefish.

Parameter		Temperature	Salinity	Tem.×Salinity
FW	*F*	4.055	1.253	6.475
	Sig.	0.067	0.320	0.012
WG	*F*	4.083	4.741	9.785
	Sig.	0.066	0.030	0.003
LI	*F*	0.522	2.386	5.844
	Sig.	0.484	0.134	0.017
K	*F*	0.004	0.053	0.066
	Sig.	0.949	0.949	0.937
SGR	*F*	5.241	5.992	11.377
	Sig.	0.041	0.016	0.002
FCR	*F*	0.024	1.670	3.558
	Sig.	0.880	0.229	0.061
SR	*F*	0.046	2.181	0.182
	Sig.	0.834	0.156	0.836

FW–final weight; WG– weight gain; LI– length increment; SGR– specific growth rate; FCR–feed conversion ratio; K–condition factor; SR– survival, Tem.: Temperature

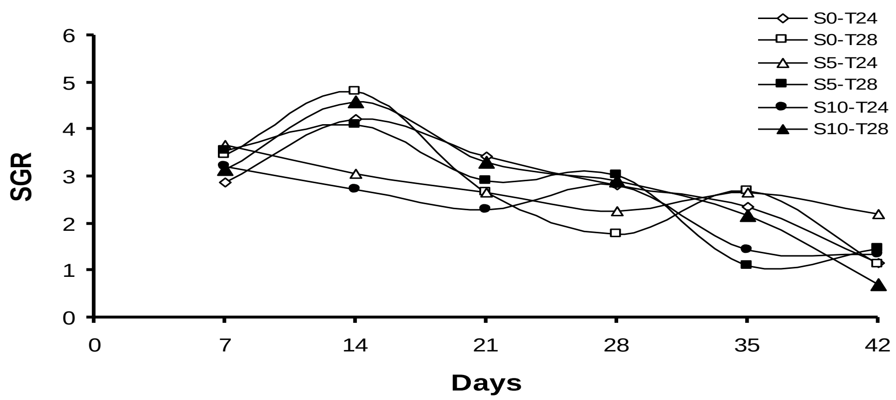

Figure 1: Estimated specific growth rate (SGR, %) of Caspian whitefish at different salinity and temperature treatments during the period of this study. S– salinity; T– temperature.

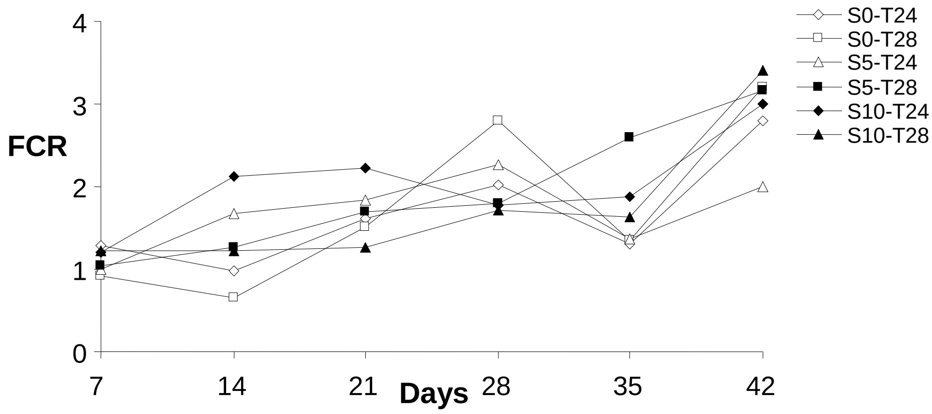

Figure 2: Changes in the feed conversion ratio (FCR) at different salinity and temperature treatments during the period of this study. S– salinity; T– temperature.

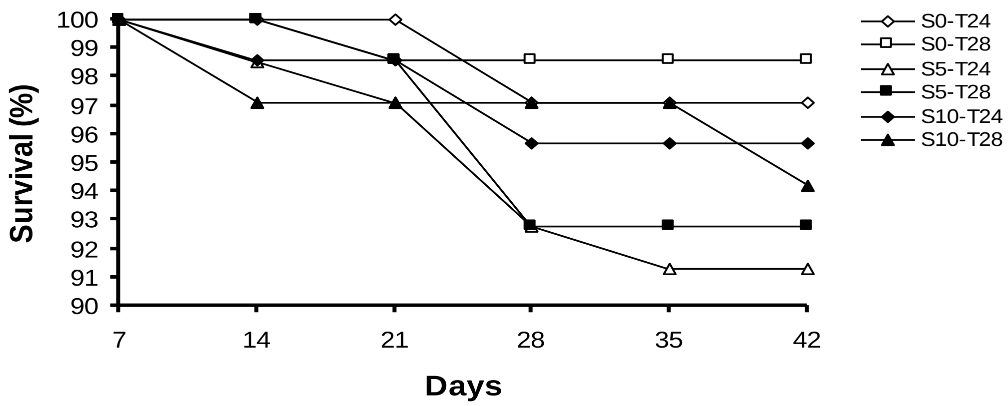

Figure 3: Survival of Caspian whitefish at different salinity and temperature treatments during the period of this study. S– salinity; T–temperature.

The factorial ANOVA showed that temperature did not have significant effects on the estimated parameters, except a weak significant effect on the SGR; while salinity significantly affected WG and SGR. According to the analysis, salinity and temperature had significant cumulative effects on the fish weight and length gains, final weight and SGR ($p<0.05$) (Table 2).

Discussion

Introduction of any new fish species for aquaculture will require considering various relevant components including its environmental limits. Reliance of the species on its culture environment should be taken into account from two viewpoints: first, growth performance of the species in certain culture conditions and second, the economic cost of its culture under those conditions. Salinity and temperature are two influential elements in fish culture practices. In this study the fish had higher growth rates at lower salinities (i.e., 0 and 5‰) and temperature of 24°C. This is in accordance with the described ecology of this fish as a fresh to slightly brackish and semi-coldwater species. Being anadromous, the Caspian whitefish spends its early life stages at lower salinities of the rivers and estuaries and subsequently, moves to the Caspian Sea where the average salinity ranges between 12 and 13 (Paavola *et al*., 2005). The migratory behavior of the fish has made it an excellent model for studying the effects of varying environmental conditions on its physiological functions such as growth. However, there is scarce data on the optimum salinity and temperature for its growth at different life stages. Nevertheless, effects of temperature and salinity, on feeding and growth rates, metabolism, activity and survival of fish are well-documented (Wuenschel *et al*., 2004).

Our results showed that salinity had marked negative effects on two main growth indices, i.e., weight gain and SGR. Majority of the estimated parameters had almost similar values at 0 and 5‰, while differed at 10‰. Furthermore, though not significantly, the FCR notably increased at 10‰. Similarity of survival rates at different salinities may be attributed to the euryhaline characteristic of this fish. In contrast to our results, Amiri *et al*. (2008)

obtained higher weight gain and SGR and lower FCR for Caspian whitefish fingerlings (average weight 1g) cultured at 10‰ than at 4, 6 or 0‰. Salinity can have a significant effect on growth of many fish species, and optimal salinity for growth varies among species (Bœuf and Payan, 2001; Suchy, 2009). Different fish species respond differentially to salinity changes even if they inhabit similar natural environments (Saoud *et al*., 2007). Rearing a number of freshwater fish species at low salinity (2‰) improved their feed conversion ratio and growth rate (Bœuf and Payan, 2001), while growth rate of a freshwater cyprinid fish, *Cyprinus carpio*, significantly declined by increasing the salinity to 10‰ (Wang *et al*., 1997). Salinity preference of fish also depends on their size and life stage (Li and Yamada, 1992; Luz *et al*., 2008). For instance, marine fish in their juvenile stages grow better at lower salinities (Wuenschel *et al*., 2004), though there are conflicting results on salinity preferences of both fresh and marine water fish in culture conditions (Bœuf and Payan, 2001; Wuenschel *et al*., 2004; Luz *et al*., 2008). From a physiological point of view, salinity effects are mainly related to the fish energy expenditure for ionic and osmotic regulation (Imsland *et al*., 2001; Imsland and Gunnarsson, 2010; Rhody *et al*., 2010), chiefly through Na^{+}–K^{+}– ATPase (NKA) activity (Saoud *et al*., 2007). The successful establishment of a species in a given habitat depends on the ability of each developmental stage to cope with changes in salinity through osmoregulation (Rhody *et al*., 2010). Fish may spend 20–68% of their total body energy for osmoregulation (Tseng and Hwang, 2008). Thus, fish that are reared at their optimal salinity level have reduced osmoregulatory energy demands, leaving energy available for other activities, such as growth (Suchy, 2009). It has been proposed, however, that growth differences in response to salinity are not necessarily a reflection of changes in the metabolic costs of osmoregulation, and salinity may also affect ingestion, digestion and absorption processes and feed conversion efficiency which may ultimately have net effects on the fish growth (Wuenschel *et al*., 2004; Resley *et al*., 2006; Bernatzeder *et al*., 2010). Effects of rearing salinity on feed conversion efficiency, feed intake, protein digestibility, energy expenditure and growth of several freshwater and marine fish have been proven (Li and Yamada, 1992; Bœuf and Payan, 2001; Tseng and Hwang, 2008; Imsland and Gunnarsson, 2010). Because salinity is an easily-controlled variable, fish growth can be maximized by selecting the salinities which would decrease energy expended maintaining homeostasis (Resley *et al*., 2006).

Although we did not observe a significant effect of temperature on the growth and survival of the studied fish, temperature is considered as a driving force in fish life whose effects are even more than any other single factor (Niklitschek and Secor, 2009). It is predictable that using a broader temperature range will provide deeper insight into its impacts on the fish growth and feeding parameters. Different fish species vary in their tolerance to water temperature and each species has an optimum temperature for growth (McCarthy and Houlihan, 1997). Water temperature affects the rate of food consumption by a fish and consumption increases to a peak at the optimum temperature (Imsland et al., 2008). Such an effect is mainly mediated by altering the standard metabolism and enzyme activity of the fish (McCarthy and Houlihan, 1997; Cui and Xie, 2000). This can consequently be mirrored in fish growth if temperature increases efficiency in which fish both feed and convert food to weight gain (Handeland *et al*., 2008). Imanpoor *et al*., (2011) reported that fish stomach evacuation rate was also highly temperature-dependent. Similar to that for salinity, fish temperature optima for growth and survival may change with age and size, as juveniles of many species prefer warmer temperatures than adults do (Handeland *et al*., 2008; Imsland *et al*., 2008). The water temperatures tested are almost close to the average temperature range recorded for the Caspian Sea (Paavola *et al*., 2005). The temperature can be lower in the sea during autumn and winter. Hence, it can be assumed that this fish can tolerate and grow at lower temperatures as well. Unlike most cyprinids, low temperature preference has been observed in a congeneric fish, *R.rutilus* (Volta and Jepsen, 2008). This together with our findings, suggest that the suitable temperature for the Caspian whitefish must be lower than that for other farmed cyprinid fishes. The Caspian whitefish fry reared in freshwater at 19˚C showed a weight gain of about 200% by feeding on an enriched live food (Gholami, 2010). Fallahi *et al*. (2004) proposed that Caspian whitefish fry actively feed on live foods at 18–22˚C. Nevertheless, Amiri *et al*. (2008) obtained a much lower growth rate and a higher feed conversation ratio than this study by culturing Caspian whitefish fingerlings at a lower temperature (21˚C). Handeland *et al*. (2008) suggested that the optimal temperature for growth is usually higher than the temperatures the species meet in nature.

Though the effect of salinity was found to be more pronounced than temperature, results of this study testify the importance of considering integrated effects of environmental parameters, e.g. salinity and temperature, in aquaculture trails. For instance, while salinity of 10‰ had an adverse effect on the fish weight gain and SGR at 24˚C, these growth factors peaked at the same salinity but at 28˚C. Although several fish species are considered euryhaline, their physiological response to salinity is often temperature-dependent, with greater salinity effects at higher temperatures (Wuenschel *et al*., 2004). This is via the basic influences of temperature on metabolism and a number of related physiological processes including oxygen consumption, and ammonia and urea excretion (Uliano *et al*., 2010). The significant interactive affects of salinity and temperature on growth indices of fish were also observed by Wuenschel *et al*. (2004).

Despite its economic importance, there is a gap in the fundamental knowledge of water quality requirements of the Caspian whitefish under controlled environmental conditions. This fish has demonstrated substantial capability to be farmed in both monoculture and polyculture systems, reaching weights of up to 155g in a 6 month culture period (Khosh-Asl, 1997). It, thus, deserves to be tracked as a new aquaculture candidate in Iran and as the 'food of the future'. The findings of this study can help with accumulating data on the rearing preferences of Caspian whitefish, while further detailed investigations by applying various levels of environmental variables and diverse feeding regimes are to be performed.

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