Global Veterinaria 6 (2): 118-125, 2011
ISSN 1992-6197

Effects of Tank Color on Growth, Stress Response and Skin Color of Juvenile Caspian Kutum *Rtilus frisii Kutum*

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Abstract: The Caspian Kutum (*Rtilus frisii Kutum*) is a European and western Asian species that has traditionally been consumed in Iran. As an emerging species for aquaculture in this area, the knowledge of its culture conditions and stress resistance is still very scarce, but urgently required. The aim of the present study was to evaluate the effects of tank color on growth, stress response, body composition and skin color of juvenile Caspian Kutum. Therefore, the juvenile Caspian Kutum (0.21g±0.01) was reared for 6 weeks in black, blue, red, yellow and white tanks. Final body weight (FBW) of the Caspian Kutum was markedly affected by tank color and the FBW of juveniles varied from 1.08 to 1.25g at different treatments but there was no significant ($P<0.05$) difference between experimental groups while the FBW tended to be higher at yellow color. No significant difference in Specific growth rate and (SGR) and feed conversion efficiency (FCE) was observed in the different tank color, but feeding rate (FR) in the yellow color was significantly ($P<0.05$) lower than the black color while there was no significant difference between other groups. Hematocrit was significantly ($P<0.05$) higher at the black tank and lower in yellow. The tank color significantly affected on all body color parameters. Final body lipid content in the yellow tank treatments was significantly ($P<0.05$) higher than black but body moisture was inverse. It was concluded that the tank color is an important factor for this species welfare and it should be considered for optimizing performance of Caspian Kutum in culture.

Key words: Caspian Kutum · Tank color · Growth · Stress response · Skin color

INTRODUCTION

The Caspian Kutum is a European and western Asian species that has traditionally been consumed in Iran. As an emerging species for aquaculture in this area, the knowledge of its culture conditions and stress resistance is still very scarce, but urgently required.

Artificial environments that are very different from the natural habitats of fish may negatively affect fish feeding activity, health, welfare and growth, especially if conditions are stressful to the fish [1, 2]. One environmental characteristic that affects fish physiology is the background or light color. Some environmental color effects on fish have been shown to modulate several physiological and behavioral responses, such as feeding [3], growth [4], reproduction [5], sex determination [6] aggression [7], larval jaw malformation [8] and stress response [9]. Several fish species prefer dark tank walls [10] as they promote a suitable contrast between the prey and the background color [11]. However, some studies have shown a preference by some fish species for light tank colors [12-14].

It is clear therefore, that rearing in different background colors may be of interest in aquaculture practice, not only for the effect it might exert, through related hormone interference, on fish growth, but also on fish response under the effect of acute or chronic stressors [12, 15-17] which may affect their behavior, for instance, by altering swimming performance, activity levels and habitat utilization [18, 19]. Stress may also cause an increase in metabolic rates of fish. [1, 20, 21]. Consequently, both behavioural and physiological stress responses are energy draining processes that may increase the energy expenditure of fish in culture and reduce growth rates and feeding efficiency [2].

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Skin coloration in teleosts is under multi-parametric control and a number of external or internal factors (physical, nutritional, genetic, neuro-hormonal) have been known to influence the chromatic state of fish [22]. In addition, fish can alter their coloration in response to environmental conditions, physiological challenges, stressful stimuli [23] and cultural condition (in some fish such as red porgy, *Pagrus pagrus*) [16]. However fish could adapt to the background color by changing the skin color [24, 25] and this phenomenon causes commercial problems in areas where this species is traditionally fished [16] and leads to a reduced marketability of the cultured fish [25]. Wild Caspian Kutum have a pale skin color and this is a typical characteristic for this fish so that its Persian name is Mahisefid that its meaning is white fish and this character is very important to its marketability. Therefore we have tried to find the best color for maintaining its wild color.

In contrast to larval fishes, comparatively few studies have examined the impact of tank color on the performance of juvenile and adult fishes [17, 26] and there is no information about the effect of tank color on the Caspian Kutum, therefore the objectives of the present study were to determine: (1) the effects of the tank color on the growth and feed efficiency on Caspian Kutum; (2) investigating the impacts of tank color on its body composition and stress response; (3) whether this fish would change its skin color under different tank colors.

MATERIALS AND METHODS

Fish and Rearing Conditions: The experimental juveniles Caspian kutum were obtained from Sijoval teleostian fish Hatchery Farm, Golestan, Gorgan, Iran. Before the experiment, the juveniles were acclimated in rearing tanks for 2 weeks. Fish were fed twice daily during the acclimation and experiment with the increasingly larger commercial dry pellet (Biomar, 0.5 and 0.8 mm).

The experiment was carried out in a semi-recirculation system consisting of 15 polythene tanks (water volume: 100 l). Flow rate of water to each tank was 100 l/day. During the experiment, water temperature and pH were measured daily and dissolved oxygen and ammonia-N measured weekly. Water temperature was maintained at 23°C. The dissolved oxygen content was kept above 7 mg O2/ l, pH between 7.0 and 8 and ammonia-N was less than 0.1 mg/l.

Experimental Design: The experiment began in late June 2009 and was repeated for three consecutive rounds, each lasting 2 weeks. The initial average weight (±SD) of the fish over all experimental rounds was 0.21 g (±0.01). Five different tank colors (white, red, blue, yellow and black), were used as experimental treatments. Triplicate tanks were used for each treatment. The light regime used was 16L: 8D (h), with light supplied between 06:00 and 22:00. Light was turned on and off abruptly. Before the experiment, fish were deprived of feed for 1 day. Twenty fish (about 5 g/fish) were randomly transferred into each tank. During the experiment, the fish were hand-fed 10% of their body mass per day, twice a day (0800, 1800 h). During the first week, dead fish were weighed and replaced. Later, dead fish were removed and weighed. Ten fish were randomly sampled from each of tanks at during the experiment at 14, 28 and were anesthetized in 0.01% Clove powder and wet weight of individuals were measured on an analytical balance and recorded to the nearest 0.01 g. At the end of the experiment, all fish individual wet weight in each tank after 1-day food deprivation was measured.

Sampling: Fifteen fish samples were randomly taken (5 fish/ each tank) at the end of the trial for the chemical analysis of final body composition and blood plasma. At the end of the experiment, two fish from each tank were randomly sampled for measuring skin color.

Color Measurement: Fish skin color was measured in three fish of each tank using a chromameter WSC-S equipped with a D65 light source and a 108 observing angle (SPSIC Inc., Shanghai, P.R. China) calibrated to black and white standards. The value of L* represents lightness (0 for black and 100 for white), the a* value represents the red/green dimension with positive values for red and negative ones for green and the value of b* represents the yellow/blue dimension with positive values for yellow and negative ones for blue. Colorimetric values of skin color were performed on two sides of each fish body [25].

Chemical Analysis: For the experimental fish body moisture, crude protein, lipid and ash were analyzed. Dry matter content was determined by drying to constant weight at 105°C. Nitrogen content was analyzed by the Kjeldahl method. Crude lipid was determined by chloroform–methanol extraction, ash by combustion at 550°C in muffle furnace [27].

Blood Analysis: Blood was collected from the caudal peduncle of fish. Blood samples were centrifuged in heparinized test tubes for 5 min at 3000 rev to sediment blood cells and plasma stored at –20°C until later analysis. Plasma glucose, total protein were determined spectrophotometrically with commercial reagents kits (Pars Azmoon., Iran). Hematocrit was measured as packed cells volume by using a Haemofuge microcentrifuge (Heraeus-Christ, Osterode, Germany).

Statistics: Results are presented as mean± S.E.M. and were analysed via SPSS statistical package. Separate one-way ANOVA were used to test the effects of tank color on the Caspian Kutum juveniles' growth (IBW, FBW, FCR, FR and SGR), blood parameters (hematocrit, glucose and total protein), color parameters and body composition.

RESULTS

Growth Performance: The final body weight (FBW) of Caspian Kutum was markedly affected by the tank color and the FBW of juveniles varied from 1.08 to 1.25 (Fig 1) in different treatments but there was no significant difference between experimental groups while the FBW tended to be higher at yellow color. No significant difference in Specific growth rate (SGR) and feed conversion efficiency (FCE) was observed in the different tank colors, but feeding rate (FR) in the yellow color was significantly ($P<0.05$) lower than that in the black color while there was no significant ($P<0.05$) difference between other groups (Table 1).

Stress Response: Fig. 2 shows that the hematocrit was significantly ($P<0.05$) higher in the black tank and lower at yellow. No significant difference in glucose (Fig. 3) and total protein (Fig. 4) was observed at different tank colors.

Table 1: Effect of tank color on growth and feed utilization for Caspian Kutum (means± S.E.M.)[a]

Tank color	Blue	White	Black	Yellow	Red
IBW[1]	0.20±0.06	0.21±0.04	0.21±0.05	0.21±0.05	0.22±0.08
FBW[2]	1.08±0.27	1.09±0.24	1.14±0.32	1.25±0.33	1.07±0.29
FR[3]	4.97±0.40[ab]	5.29±0.55[ab]	5.34±0.37[b]	4.50±0.23[a]	4.69±0.29[ab]
SGR[4]	3.91±0.35	3.89±0.19	3.90±0.37	4.17±0.26	3.89±0.40
FCE[5]	155.23±20.20	165.24±21.89	167.62±23.51	147.73±21.39	147.26±17.06

a: Means with different superscripts are significantly different (P<0.05)

1: IBW: initial body weight.

2: FBW: final body weight.

3: FR: feeding rate (%/day)=100*feed intake / (((initial body weight+final body weight) /2)*days).

4: SGR: specific growth rate in wet weight (%/day)=100*(ln (FBW)_ln (IBW)) / day.

5: FCE: feed conversion efficiency in wet weight (%)=100*wet weight gain / total feed intake.

Table 2: Instrumental color analyses of Caspian Kutum under different tank colors and compare with wild specimens (means±S.E.M.)[a]

Tank color	L*	a*	b*	w*	c*
wild	44.05±2.76[a]	5.90±0.71[a]	-1.06±0.78[a]	43.73±2.80[a]	6.02±0.84[a]
blue	41.83±0.67[ab]	5.36±0.41[ab]	-0.93±0.96[ab]	41.57±0.71[ab]	5.51±0.49[ab]
white	42.98±1.14[abc]	5.63±0.65[abc]	-0.80±0.83[abc]	42.69±1.45[abc]	5.73±0.73[abc]
black	33.96±2.37[d]	7.02±0.71[acd]	-1.04±1.27[abcd]	33.56±2.36[d]	7.18±0.70[acd]
yellow	43.70±1.09[abc]	7.85±0.38[de]	1.06±0.78[ad]	43.13±1.08[abc]	7.95±0.28[de]
red	38.08±0.08[d]	8.22±0.53[de]	1.34±1.08[acd]	37.51±0.23[d]	8.37±0.60[de]

a: Means with different superscripts are significantly different (P<0.05).

L*: Lightness.

a*: Redness.

b*: Yellowness.

w*: whiteness= $100-\sqrt{((100-L^*)^2+a^{*2}+b^{*2})}$

c*:saturation= $\sqrt{(a^{*2}+b^{*2})}$.

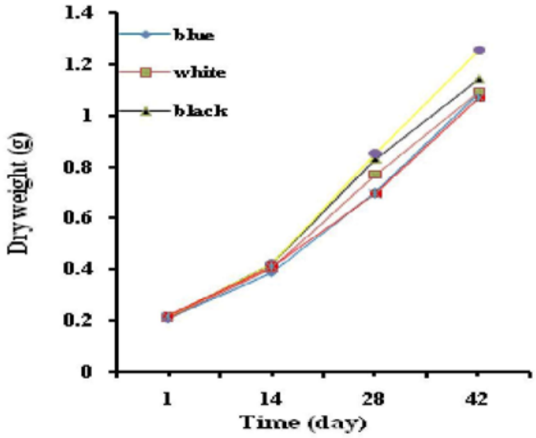

Fig. 1: Increase in dry weight (gram) of Caspian Kutum grown in tanks with different colors with Time (day). Values are mean±SD.

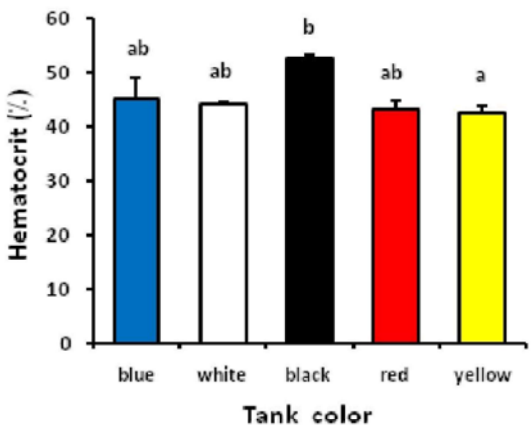

Fig. 2: Effect of tank color on hematocerit values are means±S.E.M. Means not sharing a common letter differ significantly (P<0.05).

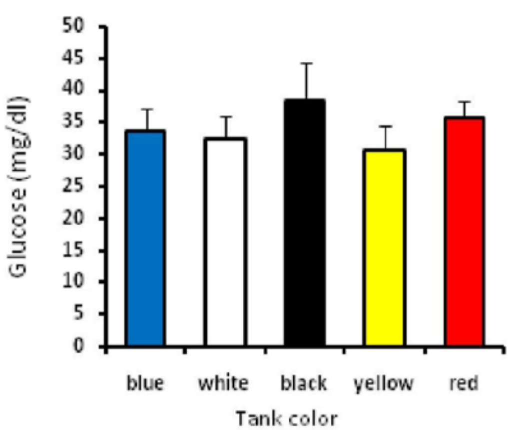

Fig. 3: Effect of tank color on glucose. Values are means±S.E.M.

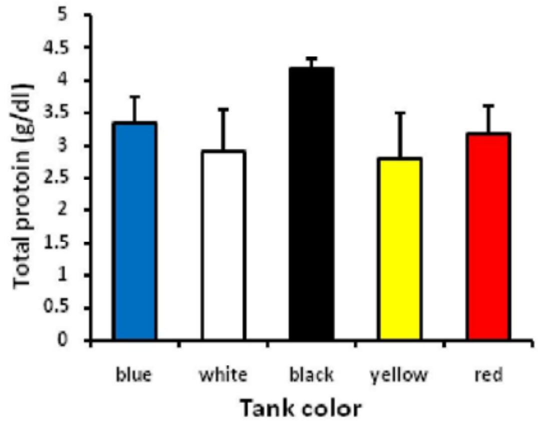

Fig. 4: Effect of tank color on total protein. Values are means±S.E.M.

Table 3: Effect of light intensity on body composition (in wet weight) of Caspian Kutum (means±S.E.M.)

Tank color	Moisture (%)	Ash (%)
Blue	63.27±0.14[ab]	7.11±0.11
white	63.67±0.90[ab]	6.79±0.27
black	65.81±0.46[b]	7.60±0.56
yellow	60.99±1.44[a]	8.25±0.55
Red	61.06±0.95[a]	7.72 ±0.72

a: Means with different superscripts are significantly different (P < 0.05).

Skin Color: The tank color significantly affected on all body color parameters (Table 2). The values of L* (lightness) and W* (whiteness) were significantly (P<0.05) lower in black and red tank color than other treatments and wild specimens. a* (redness), b* (yellowness) and c*(saturation) were respectively higher in red, yellow and red tank color and were different from the wild specimens significantly (P<0.05). However the blue and white colors did not significantly affect on the color parameters in compare with the wild specimens.

Body Composition: The body compositions of final fish were presented in Table 3. The final body lipid content in the yellow tank treatments was significantly (P<0.05) higher than black but the body moisture was inverse (Table 3). There were no significant difference between body ash and protein content in different tank colors (Table 3).

DISCUSSION

Early studies illustrated that tank color, in the case of various shades of grey and white and black, might affect growth, survival and resistance to disease.

Fishes maintained in the black tanks appeared more susceptible to disease [28]. In the present study, no significant disparities were observed in the growth performance for any five colors and this was similar to results were reported for other species. For example [14] reported that seahorses (*Hippocampus abdominalis*) held in clear, white, yellow, orange or green tanks expressed no differences in feeding strike rates, growth, or survival. In Atlantic salmon (*Salmo salar*) there were no differences in growth when fish were maintained in either green or grey colored tanks, irrespective of the spectral composition of light, although smoltification appeared to proceed in grey–colored tanks [29]. No significant differences were observed in the growth or feed conversion efficiencies of common carp reared in white, black or green tanks [12]. Significantly lower feeding rate has been observed in the yellow tank, in comparison with the black tank may be explained by the higher visibility of feed in the light tanks, resulting from higher contrast between the feed and the tank's background [2]. It could also be related to a chronic stressful condition resulting in elevated levels of stress hormones, e.g. cortisol (this factor has not been determined in our survey) [12, 17].

Several environmental factors such as temperature, salinity, time of day, wavelength of light and even background color of the tanks may influence fish stress response [30, 31] and hematological measures, are useful indicators of sublethal environmental stress in fish [32-34]. The observed differences in the level of hematocrit in present study may have been due to an increased metabolic rate in black-adapted Caspian Kutum compared to that in other color specially the yellow tank and it have been affected from high level of stress [1, 20, 21] in the black tanks. Papoutsoglou *et al*. [12] showed highest level of cortisol as an indicator of stress in black-adapted carp. However, the preference for darker or clear environments is variable within species of fish. Darker environment are preferred by *Lates calcifer* [35], *Perca fluviatis* [36] and clear environment are preferred by *Melanogrammus aeglefinus* and *Pagrus auratus* [37] and *Diplodus sargus* [38]. One explanation for these discrepancies might be given by the different feeding habits and food that is in contrastwith the color of the wall or bottom, as determined for *Perca fluviatis* [36] and for *Paralabrax maculatofasciatus* [39]. These two species showed enhanced growth in darker tanks because of the high prey contrast against the background color. In contrast, *Melanogrammus aeglefinus* showed reduced growth in tanks with black walls because of poor prey contrast [31]. So could be said that a species–specific reaction to background color exists [26].

Although fish adapted to black backgrounds expressed lower lipid levels indicating, likely stress–related, modifications to their metabolism [26]. Support for the latter metabolic effect of background color on fish is lent by studies with Nile tilapia (*Oreochromis niloticus*) in which fish on white backgrounds had higher respiratory frequencies than when held on black, blue, green, yellow or red surroundings [12].

The differences of the colorimetric values of L* (lightness) and W* (whiteness) suggested that the skin color of juveniles turned darker under black and red tank color. In vertebrates, in which the pigmentation of the skin can be changed by hormonal stimulation, the color of the background and the illumination are determining factors for the intensity and/or the pattern of skin pigmentation [3, 12, 16, 40 - 42]. Lower vertebrates (e.g., some teleost and amphibians) adjust the color of their skin in response to changes in background color and/or reflectivity [24, 25]. The long-term hormonal control of color change involves two peptide hormones released from the pituitary, namely a-MSH and MCH (melanin-concentrating hormone). a-MSH is traditionally best known for its melanotropic function. When the animal is placed on a dark background, the MSH cells are activated and a-MSH release into the blood is increased, causing a dispersion of pigments in the dermal melanophores of the skin. Placing animals on a white background results in concentration of the pigment and paling of the skin, because of the inhibition of a-MSH release and an increase of MCH release. Interestingly, results in rainbow trout demonstrate that the sensitivity of the HPI stress response varies between fish kept in black or white tanks, which has been interpreted as evidence for a role of a-MSH in the regulation of the HPI axis [43] and for the involvement of another system, in particular the arginine vasotocine system [44]. Furthermore, skin darkening in fish appears to be related to stress [45]. The main pigmentation controlling hormones α-MSH and MCH are pleiotropic and not only control skin pigmentation but also regulate the response to stressors [15, 46] During stress, the hypothalamus–pituitary–interrenal axis is activated [20], Besides adrenocorticotropic hormone (ACTH), the pituitary gland releases αMSH that induces cortisol release from the interrenal tissue, as has been demonstrated for the tilapia, *Oreochromis mossambicus* [47]. Classically, αMSH is considered the main hormone causing dispersion of the melanin granules in melanophores and the subsequent darkening of the skin. MCH has opposite effects and causes pallor [46]. It is released from the hypothalamus and for a number of fish species, it was shown to inhibit α-MSH release [48]. Moreover, MCH exerts a direct effect on cortisol release in fish [15]. So our result demonstrated that clear colors (white, blue and yellow) are adequate colors for maintaining the natural Caspian Kutum pale color.

In conclusion, the present study shows that rearing in black tanks should be considered stressful for Caspian Kutum juveniles and should be avoided in aquaculture practice. Moreover our results suggest that yellow tank color seems to be the best between these five colors because it create lowest feeding rate, best final body weight, lowest level of stress and its positive effect on the skin color of Caspian Kutum juveniles. Also our results as first survey demonstrate importance of the tank color in intensive culture of Caspian Kutum.

ACKNOWLEDGMENT

We are grateful to Gorgan university aquaculture research center and its central lab incumbents for their helps and Sijoval teleostian fish Hatchery Farm for fish preparing.

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