

The effect of salinity on spotting features of *Salmo trutta abanticus*, *S. trutta fario* and *S. trutta labrax* of cultured brown trout

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Salmo trutta is one of the most important fish species due to its aquaculture potential, economic value and wide consumer demand and skin color is an important commercial trait in fish farming, given that this phenotype influences consumer acceptance, thereby determining the commercial value that fish can reach (Colihueque, 2010). Morphological differentiation can be due to two causes, genetic differences or environmental factors, or interaction between them. Environmental factors can produce phenotypic plasticity, which produce different phenotypes for the capacity of a genotype in different environmental conditions (Stearns, 1989; Scheiner, 1993). Salinity is one of the most relevant environmental parameters that effective in aquaculture (Brett, 1979). Several studies have been performed relation with phenotypic traits of salmonid species

(*Thymallus thymallus*, *Salmo salar* m. *sebago*, *Salmo trutta* m. *lacustris* and *Salvenilus alpines*, Pakkasmaa et al., 1998; *Salmo trutta*, Oadri, 1959; İslam et al., 1973; Blanc et al., 1982, 1994; Skaala and Jørstad, 1988; Mezzera et al., 1997; Dyness et al., 1999; Pakkasmaa and Piironen, 2001; Agapova et al., 2002; Alexander and Adams, 2004; Aparicio et al., 2005; Keeley et al., 2005; Keeley et al., 2006; Bronte and Moore, 2007; Bud et al., 2009) although the knowledge is lack of information in the literature related to effect of salinity on spotting of brown trout.

Salmo trutta abanticus (n=33; 17.16±3.75 cm, 67.294±28.92 g), *Salmo trutta fario* (n=43; 21.05±2.07 cm, 124.554±39.16 g) and *S. t. labrax* (n=40; 18.36±0.79 cm, 83.73±15.04 g) were captured monthly from small streams (Bolu, Trabzon and Rize), Turkey, by

electro fishing (SAMUS 725G, 650 W, 5-60 A and 12 V DC) between October 2005 and February 2006 and transferred to the laboratory in a aerated container and stocked in the adaptation pool in Black Sea Technical University Scientific Research Foundation, Trabzon. Each fish was anesthetized (Benzocaine mg/l), and body weight (BW; to 1 g), and fork length (FL; to 1 mm) recorded and they were marked (Visible Implant Fluorescent Elastomer). Incubation of eggs and maintenance of larvae were carried out in fiberglass tanks (40 x 50 x 50 cm). The fish were equally allotted to 3 groups with three replicates for each treatment and fed for 154 days. Each set consisting of one tank with running water from the Black Sea and one with running fresh water from nearby River Çamburnu. Salinity was held at ± 0.5 ppt of the set-point by adjusting the make-up rate of freshwater and seawater. All groups were fed the same daily ration of commercial food (Scretting, 1 mm) with automatic feeders. All the fish in each tank received the same feed treatment. Aerated water in the batches was recirculated and daily water changing was 20%. Temperature was measured with a digital thermometer two times a day (8:00-9:00, 16:00-17:00). The temperature of the incoming water was 10.2 ± 1.08 . Dissolved oxygen value in the groups was 8.30 ± 0.16 mg/l.

Spotting patterns was examined when the larvae swim-up. Eleven quantitative variables were used. The quantitative variables were: diameters of

the (1) spot (2) the number of red spots located at lateral line; (3) number of red black spots on operculum; number of (4) black and (5) red spots above the lateral line; number of (6) black and (7) red spots below the lateral line; number of (8) black spot and (9) red spot on the adipose fin; number of (10) black spot and (11) red spot on the dorsal fin (Aparico *et al.* 2005). The diameters of spots were measured in the field with calipers on the left side of the specimens and rounded to the nearest ± 0.1 mm. It was investigated that differences spotting of three ecotypes of cultured brown trout (*Salmo trutta*) at salinities 0, 9 or 18 ppt and it was also monitored on spotting when three ecotypes were transferred from sea water to fresh water. Differences number of red and black spots of ecotypes at salinities 0, 9 or 18 ppt were tested by one-way one-way ANOVA and Tukey test. Statistical analyses were performed with SPSS 14.0 software package and a significant level of 0.05 was accepted.

Number of black and red spots of *Salmo trutta abanticus* (N=30), *Salmo trutta fario* (N=30) and *S. t. labrax* (N=30) in varied salinities are presented in Tables 1, 2 and 3. In *Salmo trutta abanticus*, red spots and black spot in the adipose fin and red spots in dorsal fin were not observed at 9 ppt and 18 ppt salinity. At 0 ppt salinity, there were no red spots in dorsal fin. In *Salmo trutta abanticus* with increasing of salinity, red spots were disappeared and red and diameters of black spot were decreased ($p=0.0$).

Table 1: Spotting features in *Salmo trutta abanticus* reared at three salinities (0, 9, 18 ppt) (N=30).

Specification	0‰ mean±SD (min-max)	9‰ mean± SD (min-max)	18‰ mean± SD (min-max)	F	P
Operculum surface black spot	4±3 (0–11)	3±2 (0–9)	2±1 (0–5)	7.45	0.01
Dorsal fin black spot	7±4 (0–17)	3±2 (0–10)	4±2 (0–14)	8.68	0.01
Dorsal fin red spot	0	0	1±0 (0–1)	20.45	0.0
Adipose fin black spot	1±1 (0–3)	0	0	6.98	0.02
Adipose fin red spot	1±1 (1–2)	0	0	6.65	0.02
Above lateral line red spot	5±2 (0–15)	5±3 (0–18)	2±1 (0–8)	4.21	0.02
Above lateral line black spot	44±28 (11–113)	22±9 (10–47)	29±19 (0–67)	6.71	0.02
Below lateral line red spot	4±2 (0–11)	6±3 (0–25)	0±1 (0–5)	4.02	0.02
Below lateral line black spot	7±5 (0–17)	3±2 (0–9)	5±3 (0–21)	2.60	0.08
Diameter of red spot	4.11±1.03 (3.29–5.82)	3.33±0.95 (2.62–4.78)	0	1181.41	0.0
Diameter of black spot	3.99±1.16 (2.35–5.58)	3.32±0.77 (2.09–4.25)	3.08±0.72 (2.12–4.58)	30.09	0.0

a,b,c indicate the differences among the same rows ($P<0.05$).

In *Salmo trutta fario* with increasing of salinity, number of red spots above and below lateral line and diameter of red spots were decreased while number and diameter of black spots were increased ($p=0.0$).

Table 2: Spotting features in *Salmo trutta fario* reared at three salinities (0, 9, 18 parts per thousand) (N=30).

	0‰	9‰	18‰		
Specification	mean±SD (min-max)	mean± SD (min-max)	mean± SD (min-max)	F	P
Operculum surface black spot	6±3 (0–11)	6±2 (2–10)	6±2 (4–10)	0.61	0.55
Dorsal fin black spot	6±7 (0–24)	9±4 (3–13)	11±6 (3–18)	3.12	0.05
Dorsal fin red spot	0	0	0		
Adipose fin black spot	1±1 (0–3)	3±2 (1–6)	1±1 (1–3)	22.64	0.01
Adipose fin red spot	1±1 (1–2)	1±0 (1–1)	1±0 (1–1)	0.0	1.00
Above lateral line red spot	19±13 (0–65)	18±8 (10–42)	14±5 (6–21)	0.86	0.43
Above lateral line black spot	54±38 (0–145)	65±29 (26–117)	83±44 (22–163)	4.10	0.02
Below lateral line red spot	10±8 (0–34)	11±5 (2–19)	8±4 (2–16)	0.57	0.57
Below lateral line black spot	7±5 (0–34)	10±5 (2–18)	13±8 (4–31)	0.57	0.57
Diameter of red spot	3.27±0.75 (2.07–4.93)	3.69±0.55 (2.65–4.78)	3.47±0.37 (2.72–4.13)	10.48	0.0
Diameter of black spot	2.99±0.61 (1.90–4.13)	3.16±0.55 (2.37–4.54)	3.45±0.47 (2.77–4.40)	14.44	0.0

a,b,c indicate the differences among the same rows (P<0.05).

In *S. t. labrax*, number of black spots at 18 ppt salinity was more than 0 ppt and 9 ppt salinity. Diameter of red and black spots were increased at 18 ppt salinity (p=0.0).

Table 3: Spotting features in *S. t. labrax* reared at three salinities (0, 9, 18 ppt) (N=30)

Specification	0‰	9‰	18‰	F	P
	mean±SD (min-max)	mean± SD (min-max)	mean± SD (min-max)		
Operculum surface black spot	5±2 (3–9)	5±4 (1–10)	7±4 (1–21)	2.30	0.11
Dorsal fin black spot	4±5 (0–11)	1±1 (0–5)	8±6 (0–20)	3.03	0.05
Dorsal fin red spot	0	0	0		
Adipose fin black spot	2±1 (0–4)	1±1 (0–5)	1±1 (0–5)	2.14	0.13
Adipose fin red spot	1±0 (1–1)	0±0 (0–1)	1±0 (0–1)	0.74	0.48
Above lateral line red spot	16±3 (13–20)	7±5 (0–17)	7±7 (0–23)	13.16	0.01
Above lateral line black spot	43±17 (25–74)	57±31 (24–130)	84±41 (10–182)	6.87	0.02
Below lateral line red spot	20±7 (14–33)	2±3 (0–10)	8±7 (0–33)	22.52	0.01
Below lateral line black spot	6±5(0–12)	10±7(0–26)	14±10 (0–50)	22.52	0.01
Diameter of red spot	2.27±0.37 (1.75–2.83)	3.49±0.53 (2.83–4.25)	3.23±0.67 (2.29–4.75)	24.18	0.00
Diameter of black spot	2.24±0.17 (2.01–2.43)	3.34±0.51 (2.48–4.24)	3.03±0.57 (1.90–4.45)	34.32	0.00

a,b,c indicate the differences among the same rows ($P<0.05$)

Number of black and red spots of *Salmo trutta abanticus* (N=15), *Salmo trutta fario* (N=15) and *S. t. labrax* (N=15) which were transferred from sea water to fresh water are presented in Table 4, Table 5 and Table 6. After transferred, increasing in the number of black spots on operculum was

determined in *Salmo trutta abanticus*. The spots disappeared in the dorsal fin. The number of black spots below and above lateral line was increased. Diameters of the spots diminished increasing salinity ($p=0.0$). Spots were disappeared at 18 ppt salinity.

Table 4: Phenotypic changes in *S.t.abanticus* transferred from sea water to fresh water (N=15)

Specification	Sea water (18‰)	Fresh water (<1‰)	F	P
	mean±SD	mean± SD		
	(min-max)	(min-max)		
Operculum surface black spot	2±1 (0-4)	2±2 (0-5)	2.18	0.15
Operculum surface red spot	0	0		
Dorsal fin black spot	2±1 (0-6)	0	6.63	0.01
Dorsal fin red spot	0	0		
Adipose fin black spot	0	0		
Adipose fin red spot	0	0		
Above lateral line red spot	0	0		
Above lateral line black spot	32±15 (11-63)	34±14 (10-55)	0.18	0.68
Below lateral line red spot	0	0		
Below lateral line black spot	8±5 (1-27)	12±8 (2-28)	3.10	0.09
Diameter of red spot	0	0		
Diameter of black spot	3.06±0.71 (1.55-4.73)	2.70±0.60 (1.44-4.25)	9.56	0.02

Increasing in the number of black spots on operculum was determined in *Salmo trutta fario*. The number of black spots below (p=0.01) and the above lateral line (p=0.67) were increased. The red spots decreased (p=0.01).

The numbers of black spots on operculum were decreased in *S. t. labrax*.

The number of black spots on dorsal fin were increased (p=0.02). It was determined that the number of black spots above (p=0.16) and below (p=0.21) lateral line were increased, the number of red spot above of lateral line decreased and diameter of spots decreased (p=0.51; p=0.05).

Table 5: Phenotypic changes in *Salmo trutta fario* transferred from sea water to fresh water (N=15)

Specification	Sea water (18‰)	Fresh water (<1‰)		
	mean±SD	mean± SD	F	P
	(min-max)	(min-max)		
Operculum surface black spot	6±3 (0–11)	6±5 (0–15)	0.02	0.89
Operculum surface red spot	1±1 (0–4)	0		
Dorsal fin black spot	8±4 (0–15)	9±5 (0–17)	0.27	0.61
Dorsal fin red spot	1±1 (0–4)	0		
Adipose fin black spot	1±1 (0–5)	1±1 (0–4)	2.10	0.16
Adipose fin red spot	2±1 (0–4)	1±1 (0–3)	4.36	0.05
Above lateral line red spot	18±7 (9–33)	9±7 (0–19)	13.09	0.01
Above lateral line black spot	58±19 (25–85)	61±22 (27–89)	0.19	0.67
Below lateral line red spot	9±6 (1–24)	5±4 (0–17)	7.50	0.01
Below lateral line black spot	6±3 (0–12)	17±14 (1–51)	6.93	0.01
Diameter of red spot	2.27±0.64 (1.08–3.53)	2.30±0.59 (1.31–3.94)	0.04	0.84
Diameter of black spot	2.16±0.58 (0.81–3.68)	2.37±0.73 (1.14–5.15)	3.59	0.60

Table 6: Phenotypic changes in *S.t.labrax* transferred from sea water to fresh water (n=15)

Specification	Sea water (18‰)	Fresh water (<1‰)		
	mean±SD	mean± SD	F	P
	(min-max)	(min-max)		
Operculum surface black spot	11±3 (6–19)	10±3 (6–16)	0.22	0.65
Operculum surface red spot	0	0		
Dorsal fin black spot	9±5 (0–17)	13±4 (7–19)	6.52	0.02
Dorsal fin red spot	0	0		

Adipose fin black spot	2±1 (0–5)	2±1 (1–3)	0.07	0.80
Adipose fin red spot	1±1 (0–2)	2±1 (0–4)	5.72	0.03
Above lateral line red spot	9±7 (0–23)	6±4 (0–14)	1.22	0.28
Above lateral line black spot	114±35 (67–183)	136±35 (87–197)	2.14	0.16
Below lateral line red spot	6±5 (0–19)	5±4 (0–11)	0.63	0.44
Below lateral line black spot	22±14 (3–58)	32±18 (16–58)	1.70	0.21
Diameter of red spot	2.67±0.66 (1.35–4.21)	2.56±0.81 (1.41–4.93)	0.45	0.51
Diameter of black spot	2.59±0.55 (1.35–3.88)	2.32±0.54 (1.19–3.68)	7.98	0.05

Salinity is one of environmental parameters in regards to fish physiology, modifying food intake and growth performance in many fish species; however, it is possible effects on spotting are unknown in brown trout. In a study on the body pigmentation in the brown trout (*S. trutta*), Bud et al. (2009) stated that red spots number and distribution on body surface and on dorsal fin level, as well the black spots number on the operculum and pre-operculum surface, features important and insignificant influenced by the environmental factors. By contrast, our results suggest that the pattern of variation in morphology three ecotypes of brown trout is strongly influenced by salinity which is one of environmental factors. Generally, appreciable decrease in the number of spots and disappear at spots was recorded with increased salinity. In a different study, Kittilsen et al. (2009) suggested that an aquaculture population of Atlantic salmon individuals with more spots showed a reduced physiological and behavioral response to stress. In this study,

disappearing of spots may be depending on stress due to increasing salinity.

Consequently, the results obtained in the present study indicated that salinity is an important factor and affected to spot in three ecotypes. It was observed that black spots and red spots in three ecotypes appeared prominently and trout had a brownish appearance when three ecotypes were transferred from sea water to fresh water. It was also determined that fish have been gained more weight in sea water than fresh water. We suggested that the result would be useful to investigate and they could regain to red spots providing that they are keep in fresh water for a longer time.

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