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Reproductive Performance of a Newly Described Salmonid Fish, Alakir Trout (*Salmo Kottelati*), a Candidate Species for Aquaculture

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A B S T R A C T

The present study aimed to determine reproductive performance of a newly described *Salmo* species, *Salmo kottelati* (Alakir trout), under culture conditions. With this purpose, hatching performance of 10 selected females was evaluated by fertilizing with pooled sperm from 10 males. For determination of sperm characteristics, 10 males were randomly selected, stripped and their milt samples were individually taken. Average fertilization rate, eyed-egg rate and hatching rate were found as 88.3%, 81.9% and 65.2% respectively. Sperm characteristics such as volume per fish (mL), pH, concentration (per mL), motility (%) and duration of motility (second) were 2.60, 7.6, 21.2×10^9, 78.2 and 56.6 respectively. The study results and scientific observations regarding reproductive performance suggest that *S. kottelati* can be considered a candidate species for aquaculture.

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INTRODUCTION

Aquaculture sector is the fastest developing among the food production sector (Yıldırım *et al*., 2014) and supplies an important part of the nutritional requirements of the world population. It is expected that this contribution will increase as a result of further growth of the sector (Saygı *et al*., 2011). However, with the increase in production, there is a decline in profit which compels the farmers to seek alternative species with high economic value and tolerance to culture conditions (Gökçek and Tepe, 2009). So far several locally available and high valued brown trout species have attracted attention of farmers and researchers as an alternate to rainbow trout (Alp *et al.*, 2010; Çakmak *et al*., 2004; Hajirezaee *et al.*, 2010; Hatef *et al.*, 2009; Hatipoğlu and Akçay, 2010; Kocabaş, 2009).

Salmo kottellati is a newly described endemic trout species inhabiting Alakir stream, Altinyaka, Antalya, Turkey (Turan *et al.*, 2014) and is known as stream/brook fish or red-spotted fish in the region. Since these names already exist in the literature, it was named as Alakir trout. The fish was spontaneously introduced in a rainbow trout farm in the beginning of the 2000's through its water intake route, adapted themselves to culture conditions and grew up to about four years when they were spawned by the farmer using a similar method to that in rainbow trout (M. Yavuz, Farm Owner, personal communication). The farmer grew the fry up to a total length of about 60 cm, a size which is almost that of maximum (21 cm) reported by Turan *et al*. (2014) from the wild. Moreover, the trout farm owner claimed that the grown fish was sold with a much higher price compared with rainbow trout. These factors suggested that Alakir trout can be cultured in the current farm conditions as an alternative to rainbow trout. However, for successful rearing activity several aspects culture conditions such as reproductive characteristics, optimum rearing methods and nutritional requirements need to be determined.

During the last decades, many of the fish species have faced a serious extinction threat in nature due to increasing human population and settlements, irrigation, recreational fishing, tourism and construction of hydroelectric power plants (Aydın and Yandı, 2002; Doğan, 2009; Gülle *et al*., 2009). These threats are also there for *S. kottelati* since several hydroelectric power plants are being constructed on Alakir Stream, which discharges into the Mediterranean Sea. Moreover, *S. kottelati* fishing is banned, but illegal fishing by anglers in the region are known

Therefore, the present study was planned to determine reproductive performance of Alakir trout as a potential species under culture conditions to provide preliminary scientific information and evaluation.

MATERIALS AND METHODS

Rearing and selection of breeders

The study was conducted using broodstock of Alakir trout which had been adapted and grown up to maturation size in farm (Mustafa Yavuz Rainbow Trout

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Farm, Alakir, Antalya, Turkey). During the grow-out period, the fish were kept together with rainbow trout and fed with commercial rainbow trout diets (45% crude protein, 18% crude lipid) used in the farm. Once matured, trout were separated by sexes and kept in two rectangular ponds (3×15×1.6m). The fish were weekly checked for ovulation and milt production from mid-December onwards. First ovulation in female was observed at 8th of January 2012, and then 3, 3, 4, 4, 6 and 4 fish were ovulated at 15th, 22nd, 29th of January, 5th, 12th and 15th of February, respectively. Only last 10 females were used in determination of reproductive performance. Male fish matured after third week of December, but only those males randomly selected on 15th of February 2012 were used in determination of sperm characteristics.

Determination of sperm quality

Milt samples were obtained from 10 fish randomly selected from the farm. The male fish were first anesthetized with 0.3 mL/L phenoxyethanol (Velíšek and Svobodová, 2004) and then weights (MaW) and total lengths (Alp *et al*., 2010) were measured. Special care was taken to avoid any contamination of milt with urine, feces, mucus and also water. The milt samples were collected in 10 mL tubes, then placed in an insulated ice-cooled container, transported to the laboratory and analyzed within 2 h. For sperm motility analysis, a two-step dilution method was used as suggested for salmonid fish by Billard and Cosson (1992). For this purpose, semen was diluted with 10× volume of artificial seminal plasma (130 mM NaCl, 40 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgCl_2$, 2 mM $NaHCO_3$) (Kurokura and Moo, 2008), and then 0.2 µL of the diluted semen was mixed with 20 µL of activation medium (farm water) on a glass slide under a microscope (×200, Olympus BX 51, Tokyo, Japan). Motility (MO) was determined using a camera (Olympus DP 72, Tokyo, Japan) and time (duration of MO, DMO) until die of the last sperm was recorded via a chronometer. MO was analyzed by a semi-quantitative method. In this method, the video recordings were reviewed and MO was expressed as the percentage. Only forward-moving sperm were judged motile while those simply vibrating or turning on their axes was considered immotile. Sperm MO was calculated as the percentage of motile sperm to the total number of sperm (Aas *et al.*, 1991). Sperm concentration (SC) was measured under a microscope using Neaubeaur's hemocytometer and calculated as number of sperm/mL (Tekin *et al.*, 2003). Semen pH was determined with a hand pH meter (HI 8424 Hanna Instruments, USA).

Female reproductive performance

As mentioned above, 10 mature females were sampled from broodstock at 2nd week of February in the same farm and live weights (FeW) and total lengths (FeL) were measured after anesthetization with 0.3 mL/L phenoxyethanol (Velíšek and Svobodová, 2004). After drying with towel, their eggs were obtained by stripping. Live weights of stripped fish and eggs were determined to calculate the absolute (AF) and relative fecundities (RF). Egg weights of each female were determined weighing 100 eggs to the nearest 0.01 g and egg diameters were measured to the nearest 0.01 mm. After then, balanced saline solution (7.5 g of NaCl, 0.2 g of KCl, 0.2 g of $CaCl_2$ $2H_2O$, 0.02 g of $NaHCO_3$, in 1000 mL distilled water) was added over the eggs (Kobayashi *et al.*, 2004) followed by addition of pooled sperm from 10 males. The eggs were then gently mixed for fertilization and left for five minutes. The fertilized eggs were rinsed several times with incubation water to remove sperm remnants as well as dead and broken eggs. The eggs were left for further 25 minutes to facilitate egg hardening by water absorption and disinfected with 100 ppm iodine during 10 minutes. After then they were transferred to rainbow trout incubation trays placed in a vertical hatching system. Water flow rate to each vertical incubator was 10 L/min. One hundred eggs were randomly sampled at day 10 after fertilization to determine fertilization rate (FR) in the clarifying solution (Water, methanol, glacial acetic acid, 1:1:1) (Contreras-Sanchez *et al.*, 1998). At eyed-egg stage, 100 eggs were randomly sampled and the percentages of eyed eggs (EE) were calculated. Hatching rate (HR) was determined by counting all of hatched fry.

Water quality

Water temperature and dissolved oxygen levels of the farms were measured at one to two-week intervals using an oxygen meter (YSI 55-12 FT, Yellow Springs Instrument, Yellow Springs, OH, USA) from September to end of June while pH, electrical conductivity and salinity using a pH meter (YSI 63-12 FT, Yellow Springs Instrument, Yellow Springs, OH, USA). Other water quality parameters of the farm were not analyzed, but they had been already determined by governmental laboratories for official registration of the farm.

Statistical analysis

Results were given as means ± SEM. Multivariate analysis was performed using statistical software; JMP v.8.0 (SAS Institute, Inc., Cary, NC, USA, 2008) for Windows to elucidate correlations among the variables. In multivariate analysis, significant correlations were considered at $P<0.05$ whereas significant trends or tendencies were discussed between $P<0.05$ and $P<0.10$.

RESULTS

Water temperature and dissolved oxygen were 8.8±0.04°C and 10.3±0.1 mg/L, respectively. Other chemical and physical waters quality parameters of the farm (data not shown) were within the range of optimum limits for rainbow trout culture reported by Piper *et al.* (1989).

Descriptive measurements and reproductive performance of female Alakir trout are given in Table I. Fifty percent of eggs reached eyed egg stage at 32^{th} days (280 day-degree in °C), and hatched at 55^{th} days (480 day-degree in °C). Fertilization rate varied between 81.2 and 97.7% whereas hatching rate was between 52.1 and 90.3%.

Characteristics of male fish and examined sperm samples are given in Table II. Average male live weight was 684.5±74.1 g. Alakir torut males were found slightly bigger than females. In maturation stage dark body coloration was observed for all male fish as a seconder sexual characteristic of other salmonids. Sperm color was milk white. When evaluated subjectively, sperm viscosity was lower compared with rainbow trout held in the same farm.

According to multivariate analysis shown in Table III there were significant negative relationships between RF and FeL, RF and EW, and EDI and ED, whereas significant positive correlations were detected between EW and FeL, FR and EER, HR and FR, and HR and EER (P<0.05).

Table I.- Female size, egg characteristic and hatchery performance of *Salmo kottelati* (Mean ± SE)

	Variables	Range (Min-Max)
Fish weight (g)	567 ± 17	486 - 641
Fish length (cm)	33.7 ± 1.26	31.5 - 35.5
Absolute fecundity (egg/fish)	1324 ± 65	1035 - 1632
Relative fecundity (egg/kg fish)	2334 ± 91	1913 - 2762
Egg diameter (mm)	4.5 ± 0.09	4.26 - 5.04
Hardened egg diameter (mm)	5.2 ± 0.08	5.0 - 5.68
Egg diameter increase (%)	16.2 ± 2.07	5.3 - 27.1
Egg weight (mg)	81.4 ± 0.91	78 - 87
Hardened egg weight (mg)	100.1 ± 3.14	83 - 116
Egg weight increase (%)	23.2 ± 3.24	6.1 - 40.9
Fertilization rate (%)	88.3 ± 2.88	81.3 - 98.5
Eyed egg rate (%)	81.9 ± 4.58	69.2 - 97.7
Hatching rate (%)	65.2 ± 7.02	52.1 - 90.3
Hatching weight (mg)	97 ± 1.16	94 - 103

Table II.- Male size and sperm characteristics of *Salmo kottelati* (Mean ± SE).

	Variables	Range (Min-Max)
Weight (g)	685 ± 74.1	500 - 1007
Length (cm)	37.1 ± 1.3	33.0 - 42.1
Semen volume (mL)	2.6 ± 0.56	1.5 - 5
Semen pH	7.6 ± 0.03	7.5 - 7.7
Motility (%)	78.2 ± 1.04	75 - 80.9
Sperm concentration (10^9/mL)	21.2 ± 1.15	17.0 - 25.6
Duration of motility (sec)	56.6 ± 3.4	45.1 - 67.0

DISCUSSION

Spawning period of Alakir trout started in early January and continued till the final check at 15^{th} of February in our study. However, we further observed that the farmer was still able to collect egg and sperm by the end of May, suggesting that spawning period was between January and May. This period is longer than those reported for *Salmo trutta* by Alp *et al.* (2003) and Aslan and Aras (2007), who recorded as December and April in Turkey. Duration from fertilization to eyed egg stage was at 32 days with 280 day-degree and hatching took place in 55 days with 480 day-degree. Durations of eye pigmentation and hatching of various trout species are reported in Table IV. When findings are compared with other *Salmo* species, especially both eyed-egg stage and hatching are longer in Alakir trout except *S. t. abanticus*. In other words, Alakir trout has species-specific characteristics regarding lengths of eyed-egg and hatching stages.

AFs of Alakir trout were between 1035 and 1632 egg/fish and RFs were between 1913 and 2762 egg/kg. Even if egg productivity per fish can give a general information about the fecundity, egg production per kg fish (RF) is thought to be more informative. RFs values of Alakir trout were consistent with those of *Salmo trutta* (1600 to 4000 egg/kg fish) (Alp *et al*., 2010; Aslan and Aras, 2007; Demir *et al.*, 2010; Estay *et al.*, 2004; Firidin *et al*., 2009; Kocabaş, 2009). In well agreement with the literature (Estay *et al*., 2004; Heinimaa and Heinimaa, 2004), a negative correlation (r^2 = -0.52) was recorded between RF and fish size in the current study (Table III).

EDs and EWs obtained here varied between 4.26 and 5.04 mm and 78 and 87 mg respectively, being consistent with those reported in the literature for *S. t. labrax* (Firidin *et al*., 2009; Kocabaş, 2009; Serezli *et al.*, 2010). At the end of the hardening process, increases in

Table III.- Interactions of variables obtained from *Salmo kottelati.*

	FeW	FeL	AF	RF	EW	HEW	EWI	ED	HED	EDI	FR	EER	HR	HW	MaW	MaL	pH	SV	SC	MO
FeL	**0.939***																			
AF	0.399	0.119																		
RF	-0.264	**-0.515***	0.779																	
EW	0.310	**0.573***	-0.647	**-0.897***																
HEW	0.773	0.861	0.166	-0.343	0.327															
EWI	0.707	0.714	0.396	-0.053	0.009	**0.948***														
ED	-0.540	-0.462	-0.449	-0.089	-0.281	-0.110	-0.028													
HED	-0.151	-0.064	0.267	0.393	-0.234	0.344	0.461	0.096												
EDI	0.223	0.242	0.517	0.390	-0.021	0.355	0.402	**-0.564***	0.768											
FR	0.126	0.270	-0.620	-0.751	0.822	-0.162	-0.454	-0.341	-0.675	-0.347										
EER	0.027	0.185	-0.731	-0.798	0.793	-0.204	-0.490	-0.157	-0.710	-0.494	**0.982***									
HR	0.170	0.291	-0.456	-0.612	0.796	-0.168	-0.447	-0.556	-0.564	-0.116	**0.964***	0.899								
HW	-0.531	-0.626	-0.138	0.228	-0.607	-0.389	-0.218	0.857	-0.170	-0.689	-0.403	-0.237	-0.590							
MaW	0.128	0.446	-0.690	-0.815	0.945	0.340	0.047	-0.148	0.060	0.137	0.650	0.632	0.636	-0.581						
MaL	0.265	0.573	-0.576	-0.788	0.922	0.506	0.231	-0.183	0.153	0.237	0.549	0.519	0.547	-0.634	**0.982***					
pH	-0.818	-0.965	0.089	0.651	-0.733	-0.848	-0.649	0.401	-0.015	-0.267	-0.367	-0.293	-0.380	0.680	-0.658	-0.766				
SV	-0.109	-0.079	0.391	0.497	-0.398	0.365	0.536	0.167	0.977	0.705	-0.813	-0.836	-0.719	-0.034	-0.123	-0.014	0.047			
SC	-0.471	-0.467	0.235	0.561	-0.202	-0.426	-0.363	-0.363	0.537	0.680	-0.187	-0.280	0.023	-0.345	-0.020	-0.061	0.392	0.439		
MO	0.423	0.342	0.325	0.035	0.312	-0.083	-0.189	-0.971	-0.281	0.391	0.491	0.324	0.684	-0.780	0.145	0.138	-0.289	-0.362	0.347	
DMO	0.457	0.411	0.229	-0.089	0.427	-0.029	-0.171	-0.961	-0.310	0.359	0.576	0.414	0.754	-0.817	0.256	0.247	-0.378	-0.406	0.286	**0.992***

Statistically important at $P < 0.05$ (*), tendency $0.05 < p < 0.1$ (underlined)
FeW, Female fish weight; FeL, Female fish length; AF, Absolute fecundity; RF, Relative fecundity; EW, Egg weight; HEW, Hardened egg weight; EWI, Egg weight increase; ED, Egg Diameter; Hardened egg diameter; HDI, Hardened egg diameter increase; FR, Fertilization rate; EER, Eyed egg rate; HCR, Hatching rate; HcW, Hatching weight; MaW, Male fish weight; MaL, Male fish length; SV, Semen volume; SC, Sperm concentration; MO, Motility; DMO, Duration of motility.

egg weight and diameter were 23.3 and 16.2% respectively. Lahnsteiner and Patzner (2002) found egg weight increse after the hardening, linearly correlated with viability of eggs in rainbow trout, but this was not the case in Alakir trout.

Table IV.- Summary of eyed and hatching stage durations of *S. kottelati*, respect to those reported in *S. trutta* and *Salvelinus fontinalis*.

Species	Eyed stage (day-degree)	Hatching stage (day-degree)	Reference
S. kottelati	280	480	This study
S. t. macrostigma	-	370 - 380	Demir *et al.* (2010)
S. t. macrostigma	244 - 261	387 - 413	Alp *et al.* (2010)
S. t. macrostigma	268	354	Kocabaş (2009)
S. t. abanticus	330	589	Kocabaş (2009)
S. t. labrax	220 - 269	432 - 490	Firidin *et al.* (2009)
S. t. labrax	233	-	Kocabaş (2009)
S. t. labrax	-	472 - 499	Çakmak *et al.* (2004)
S. t. caspius	242	415	Kocabaş (2009)
S. t. fario	221	394	Kocabaş (2009)
Salvenilus fontinalis	245	415	Başçınar and Okumuş (2004)

Findings of FRs of *S. kottelati* were higher than those of *S. trutta caspius* and *S. trutta fario* by Kocabaş (2009) and *Salvelinus fontinalis* by Başçınar and Okumuş (2004), but lower than other *Salmo* species (Alp *et al*., 2010; Estay *et al*., 2004; Firidin *et al*., 2009; Kocabaş, 2009). Apart from species difference, one of the reasons of this discrepancy could be method chosen for FR determination. We determined the FR using a more reliable method than the subjective one which is based on enumeration of apparently fertilized and dead eggs as suggested by Contreras-Sanchez *et al.* (1998). As the method we followed relies on determination of the notochord and until this stage some fertilized egg may die, the resulting FR expectedly can be lower than those of the literature that were based on the subjective or other methods.

When EER and HR values compared with other *Salmo* species, EER and HR levels are higher than those of Demir *et al*. (2010), Kocabaş (2009), and Başçınar and Okumuş (2004). EERs in Alakir trout are compatible with those by Firidin *et al*. (2009) and Alp *et al.* (2010), but lower than those by Niksirat *et al.* (2007) (*S. t. caspius*) while HRs are lower than those by Niksirat *et al.* (2007) and Alp *et al.* (2010). There were strong positive correlations with FR and EER ($r^2 = 0.98$), FR and HR (r^2

= 0.96), and EER and HR (r^2 = 0.90) (Table III). Small eggs tended to have lower viability when judged from the negative relationship between RF and EER (r^2 = -0.8) (Table III). This was highly likely due to a lower energy depot of these eggs for embryonic development as underlined by Vassallo-Agius *et al.* (2001).

Fish eggs are significantly affected by genetic and environmental factors in otogenesis (Grande and Andersen, 1990). But there is no information about the effects of such kinds of factors on embryonic development of Alakir trout. There are also further information requirements concerning hatchery management of Alakir trout such as photoperiod application, stocking density, feeding program, and nutritional requirements of broodstock. During the adaptation period in the present instance, Alakir trout were fed with rainbow trout ongrowing feed at the farm, which may not be fully suitable for this species even though being a related species. A necessity of requirements of a balanced feed meeting nutritional requirements of species being cultured (Ahmadi *et al.*, 2006; Bobe and Labbé, 2010; Vassallo-Agius *et al.*, 2001) and application of a proper feeding program during the ovarian development (Bromage *et al.* (1992) have been emphasized. The later reported that diet should be offered to rainbow trout broodstock at %1 of body weight until 3 months to spawning, than reduced to 0.4% of body weight to get high quality gamete. These issues in Alakir trout are yet to be elucidated.

Average SVs determined in Alakir trout are higher than those in *Salvenilus alpinus* (1.64 to 2.01 ml) (Mansour *et al.*, 2011), but lower than those reported for *S. t. abanticus* (7.4 ml) (Hatipoğlu and Akçay, 2010), *S. t. caspius* (44 ml) (Hajirezaee *et al.*, 2010), *S. t. macrostigma* (13.9) Bozkurt *et al.* (2011) and rainbow trout (Secer *et al.*, 2004; Tekin *et al.*, 2003). Despite lower SV yields compared with most of the other Salmo species, SCs are higher than those reported in the literature (Bozkurt *et al.*, 2011; Hajirezaee *et al.*, 2010; Hatipoğlu and Akçay, 2010; Mansour *et al.*, 2011). Although not histologically analyzed here, this may be due to a lower testicular hydration that semen stays viscose (Clemens *et al.*, 1964)

Semen pH values are consistent with those of other *Salmo* species (Bozkurt *et al.*, 2011; Hajirezaee *et al.*, 2010; Hatef *et al.*, 2009; Hatipoğlu and Akçay, 2010). MO and DMO are higher than those in *S.t. caspius* by Hatef *et al.* (2009) and Hajirezaee *et al.* (2010), but compatible with *S. t. abanticus* by Hatipoğlu and Akçay (2010). Sperms of Alakir trout had an effective fertilizing ability according to correlation analysis which did not detect any significant relationship between FR and sperm parameters. But there were positive relationship between MO and DMO (r^2=0.99), which is consistent with Bozkurt *et al.* (2011). We did not investigate the ionic composition of the semen but this phenomenon could be related to the ionic composition of semen which has a significant influence on MO (Bozkurt *et al.*, 2011) and DMO (Hajirezaee *et al.*, 2009).

CONCLUSIONS

In conclusion reproductive performance of Alakir trout under culture conditions are within the range of available data of other *Salmo* groups. Considering a successful seed production (and possibly juvenile growing) was possible using the same methods as in rainbow trout, Alakir trout can be an alternative species for rainbow trout for aquaculture. However, further studies are clearly required to determine several aspects of this fish under culture conditions.

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