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RESEARCH ARTICLE

Comparison of Hatching Performances and Yolk Sac Absorptions of Black Sea Trout (*Salmo trutta labrax* Pallas, 1811), Brook Trout (*Salvelinus fontinalis* Mitchill, 1814) and Their Hybrid

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Summary

The aims of this study were to determine hatching performance, body and yolk weight at hatching, the growth rate during the yolk absorption and yolk conversion efficiency of Black Sea trout, brook trout and their hybrid larvae, and to compare the relationships between length, total wet weight, dry yolk body weights of alevins and degree-days. The Black Sea trout egg has shorter incubation duration than brook trout and their hybrid. Survival from fertilization to swim-up of brook trout, Black Sea trout, and hybrid were 45.8%, 54.3% and 30.7%, respectively. Length, total wet weight, dry larva weights, and dry yolk weights of brook trout, Black Sea trout and hybrid exhibited a linear relationship with degree-days; dry yolk weight decreased, while length, total wet weight and dry body weight increased with degree-days. The linear regression parameters excluding length slope were significantly different. Length and weight increase as well as growth rates, daily yolk sac consumption and yolk sac efficiency values were calculated as 0.21 mm/day, 0.63 mg/day, 0.30 mg/day, and 0.61 for brook trout; 0.19 mm/day, 0.87 mg/day, 0.60 mg/day, and 0.76 for Black Sea trout, and 0.20 mm/day, 0.45 mg/day, 0.28 mg/day, and 0.46 for hybrid, respectively.

Keywords: *Brook trout, Black Sea trout, Hybrid, Hatching performances, Yolk sac absorption*

Karadeniz Alabalığı (*Salmo trutta labrax* Pallas, 1811), Kaynak Alabalığı (*Salvelinus fontinalis* Mitchill, 1814) ve Hibridlerinin Kuluçka Performansı ve Besin Kesesi Absorpsiyonlarının Karşılaştırılması

Özet

Bu araştırmanın amacı, Karadeniz alabalığı, kaynak alabalığı ve Hibridlerinin kuluçka performansı ve besin kesesi absorpsiyonu ve değerlendirme randımanlarının ortaya konması ve çıkış süresi (gün-derece) ile boy, toplam yaş ağırlık, kuru vücut ve kese ağırlıkları arasındaki ilişkileri karşılaştırmaktır. Araştırma sonucunda Karadeniz alabalığının, kaynak alabalığı ve hibrid bireylere göre daha kısa bir kuluçka süresine sahip olduğu, yumurtaların yaşama oranının Karadeniz alabalığında %45.8, kaynak alabalığında %54,3 ve hybrid bireylerde ise %30.7 olduğu belirlendi. Karadeniz alabalığı, kaynak alabalığı ve hibrid bireylerde, gün-derece ile boy, toplam yaş ağırlık, kuru vücut ve kese ağırlıkları arasındaki lineer ilişkiler, besin kesesi için azalan, diğerlerinde ise artan olarak ortaya konmuştur. Boy ilişkisinin eğimi hariç, diğer ilişkilerin regresyon parametrelerinde önemli farklılıklar belirlendi. Boy ve ağırlıkça büyümedeki artış, günlük besin kesesi tüketimi ve besin kesesi değerlendirme randımanları, sırasıyla Karadeniz alabalığında 0.19 mm/gün, 0,87 mg/gün, 0.60 mg/gün ve 0.76; kaynak alabalığında 0.21 mm/gün, 0.63 mg/gün, 0.30 mg/gün ve 0.61 ve hibrid bireylerde ise 0.20 mm/gün, 0.45 mg/gün, 0.28 mg/gün ve 0.46 olarak hesaplandı.

Anahtar sözcükler: *Karadeniz alabalığı, Kaynak alabalığı, Hibrid, Kuluçka performansı, Besin kesesi tüketimi*

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INTRODUCTION

Hybridization is the mating of different individuals or crosses between separate species. The desired aim is to produce individuals that are sterile and with better growth performance, flesh quality, disease resistance, etc [1].

The Black Sea trout, *Salmo trutta labrax,* is a native finfish species of the Eastern Black Sea coast and rivers, is a new species for intensive aquaculture [2] while the brook trout *Salvelinus fontinalis,* is not a very popular culture species. However, it has been cultured commercially in the North America, Europe and in the Eastern Black Sea region of Turkey in some trout farms. A few studies were done about hatching [2,3] and yolk sac absorption on the Black Sea trout [4], and brook trout [5].

The first external feeding activities of the fish larvae start when the larvae swim-up. The observations demonstrated that, larva has a remnant of yolk at the swimming up stage. In trout hatcheries the first external feeding starts when over 30% percent larvae begin swimming up. As in other finfish these development and transition stages are very critical due to susceptibility to diseases and pathogens, environmental fluctuations and starvation. It is nevertheless possible to increase survival and growth rates through proper hatchery management skills during these critical stages. Although several studies have been performed on larval development of salmonid species, namely, sea trout [6], Black Sea trout [4], Atlantic salmon [7,8]; rainbow trout [9], brook trout [5], brook trout and Arctic charr and their hybrids [10], this study is the first on Black Sea trout and brook trout hybrid.

Aims of this study are to determine hatching performance, body and yolk weight at hatching, the growth rate during the yolk absorption and yolk conversion efficiency of Black Sea trout, brook trout and their hybrid larvae, and to compare the relationships between length, total wet weight, dry yolk and body weights of alevins and degree-days under pilot hatchery conditions in the Eastern Black Sea in Turkey.

MATERIAL and METHODS

The eggs were collected from eight brook trout and five Black Sea trout broods, 3-year-old with a mean weight of 432.6±99.69 g, 639.2±212.34 g, respectively, and they are fertilized with milt obtained from two males at trout hatchery in Faculty of Marine Sciences, Karadeniz Technical University. Four cross-types were produced: brook trout (Sf), Black Sea trout (St), and the hybrids between brook trout and Black Sea trout (Sf x St, St x Sf; the first letter indicates the female parent species). The all Sf x St hybrid eggs could not survive in eyed-stage, so this group are not included in this study. The eggs (2143 brook trout, 2164 Black Sea trout and 2220 hybrid) were divided into three parts and placed in an egg tray of 5 liter in vertical incubator, and hatched in well spring water at a temperature of 9.9±1.3°C.

Observations of the eggs and alevins were made together with the temperature readings. The eyed-egg stage was defined as when the eyes were clearly visible as black spots. The 50% hatch time was defined as when 50% of the embryos were swim-up larvae. The swim-up stage was considered to begin when approximately 50% of the alevins were starting to feed and were actively swimming up in the water for food intake. The dead eggs and larvae were removed and counted periodically.

The larvae sampling was commenced at 5-day intervals from hatching to 764.8 degree-days post-hatch. The first larvae were sampled at 443.5 degree-days (50% eggs hatched) and then at 489.0, 537.0, 583.5, 628.8, 670.5, 717.8 and 764.8 degree-days (after swim-up stage started), respectively. Ten larvae were randomly sampled at each sampling period (8 times), i.e. a total of 80 larvae were used during the study at each cross-types. They were anesthetized in 20 ppm benzocaine, and then preserved in 10% formalin until further analysis. After a minimum interval of 3 weeks, fixed larvae were dissected to separate the yolk from the body. The body and yolk were dried separately at 60°C for 48 h and weighed (±0.1 mg) after 48 h [6].

Egg and larvae survival rates ($S=(N_2/N_1)x100$), growth performance (increase in weight) and growth ($G=[(Wt_2-Wt_1)/t]$) were determined periodically (N_1 and N_2: number of eggs or larvae time 1 and time 2; W: weight, mg; t: time, day). Yolk conversion efficiency (E) was calculated from the formula; $E=(L_t-L_0)/(Y_0-Y_t)$, where, L is larval weight at any time t and time zero, and Y is yolk weight at any time *t* and time zero [9].

To determine statistical significance ($P<0.05$) of differences in length, wet weight, dry body and yolk sac weights among the group, analysis of variance and Tukey tests were used. The significance of all slopes (Ho: b=0) and regressions were tested at the 0.05 probability level [9,11], and statistical significance of differences among regression evaluations and slopes values were determined with analysis of covariance [12].

RESULTS

Egg size of brook trout and Black Sea trout were 4.6±0.03 mm (4.3-4.9) and 4.6±0.02 mm (4.5-4.7), respectively. Incubation water temperature was varied

Table 1. *Duration (day) of eyed-egg, hatch and swim-up phases (degree-days values in parenthesis, letters showed differences)*

Tablo 1. *Gözlenme, çıkış ve serbest yüzme süresi (gün) (parantez içleri gün-derece, harfler istatistiksel farklılıkları göstermektedir)*

Parameter	Brook Trout	Black Sea Trout	Hybrid	ANOVA
Eyed-egg Stage	18-24 (212-283)	17-26 (201-306)	18-25 (212-295)	P>0.05
Hatching	38-48 [a] (410-518)	35-41 [b] (388-443)	38-48 [a] (410-518)	P<0.05
Swim-up	75-80 [a] (743-792)	71-76 [b] (703-752)	76-81 [a] (752-802)	P<0.05

between 7.5 and 12.6°C (9.89±1.26). The Black Sea trout egg has shorter incubation duration than brook trout and hybrid. Survival from fertilization to swim-up of brook trout, Black Sea trout, and hybrid were determined as 45.8%, 54.3% and 30.7%, respectively *(Table 1)*.

Length *(Fig. 1)*, total wet weight *(Fig. 2)*, dry larva weights *(Fig. 3)*, and dry yolk weights *(Fig. 4)* of brook trout (Sf), Black Sea trout (St) and hybrid (StxSf) exhibited linear relationship with degree-days (ranged 443.5-764.8); dry yolk weight decreased, while length, total wet weight and dry body weight increased. Among the linear regression parameters were significantly

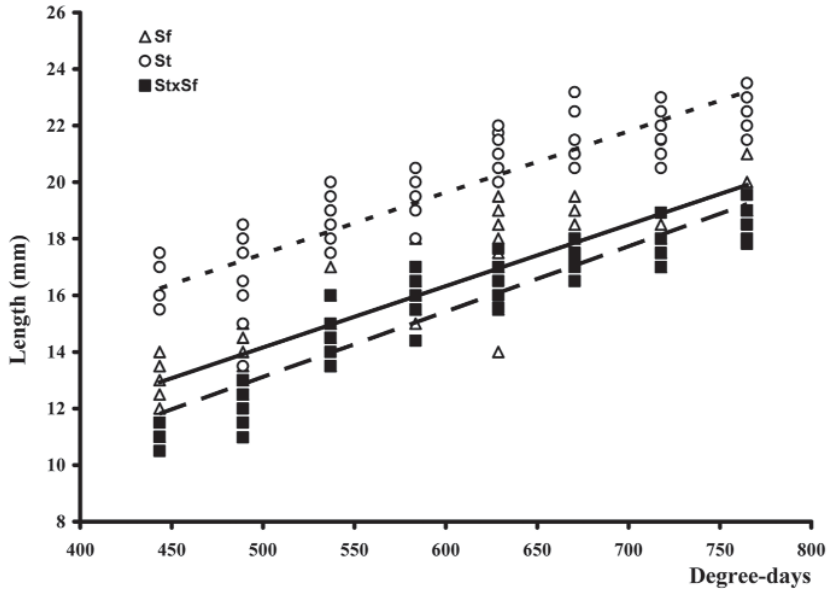

Fig 1. Relationship between total length (y) and degree-days (x) [in brook trout (Sf; Δ, —): y = 3.3072 + 0.0217 x, r^2 = 0.82; Black Sea trout (St; ○, ...) : y= 6.5831 + 0.0218 x, r^2 = 0.79; and hybrid (StxSf; ■ , --) : y= 2.0300 + 0.0223 x, r^2 = 0.82]. Among the groups, constant (a) showed similar, and slope (b) differed significantly (P<0.001)

Şekil 1. Toplam boy (y) ile gün-derece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): y = 3.3072 + 0.0217 x, r^2 = 0.82; Karadeniz alabalığı (St; ○, ...) : y= 6.5831 + 0.0218 x, r^2 = 0.79; hibrid (StxSf; ■ , --) : y= 2.0300 + 0.0223 x, r^2 = 0.82]. Gruplar arasında sabit değer (a) benzer, eğim (b) ise istatistiksel olarak farklı (P<0.001) bulunmuştur

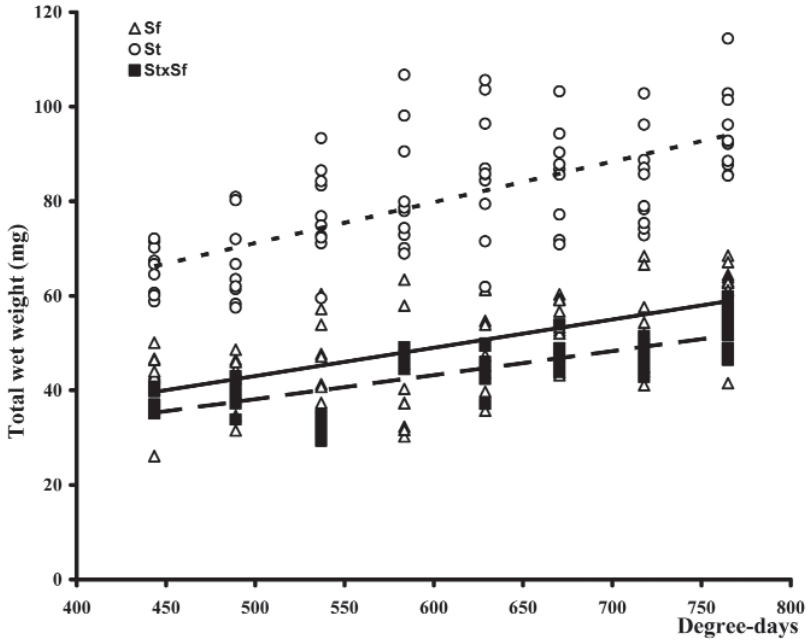

Fig 2. Relationship between total wet weights (y) and degree-days (x) [in brook trout (Sf; Δ, —): y = 13.1346 + 0.0598 x, r^2 = 0.33; Black Sea trout (St; ○, ...) : y= 28.8957 + 0.0854 x, r^2 = 0.39; and hybrid (StxSf; ■ , --) : y= 12.7089 + 0.0509 x, r^2 = 0.57]. Constant (a) and slope (b) values of St differed significantly than the others (P<0.001)

Şekil 2. Toplam yaş ağırlık (y) ile gün-derece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): y = 13.1346 + 0.0598 x, r^2 = 0.33; Karadeniz alabalığı (St; ○, ...) : y= 28.8957 + 0.0854 x, r^2 = 0.39; hibrid (StxSf; ■ , --) : y= 12.7089 + 0.0509 x, r^2 = 0.57]. Karadeniz alabalığına ait sabit değer (a) ve eğim (b) diğer gruplardan istatistiksel olarak farklı (P<0.001) bulunmuştur

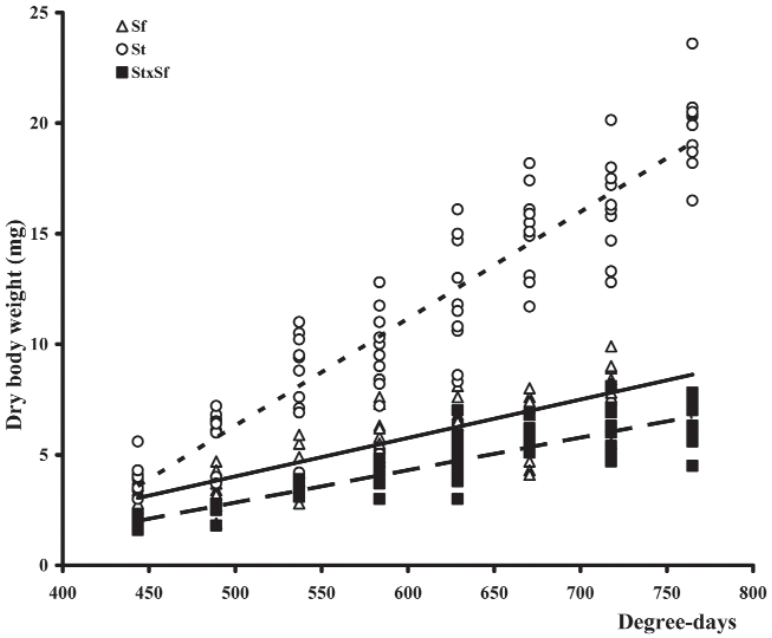

Fig 3. Relationship between dry body weights (y) and degree-days (x) [in brook trout (Sf; Δ, —): y = -6.0905 + 0.0183 x, r^2 = 0.79; Black Sea trout (St; ○, ...) : y= -17.7872 + 1.4737 x, r^2 = 0.84; and hybrid (StxSf; ■ , - -) : y= -4.4839 + 0.4841 x, r^2 = 0.81]. Among the groups, all constant (a) and slope (b) values differed significantly (P<0.001)

Şekil 3. Toplam kuru ağırlık (y) ile günderece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): y = 13.1346 + 0.0598 x, r^2 = 0.33; Karadeniz alabalığı (St; ○, ...) : y= 28.8957 + 0.0854 x, r^2 = 0.39; hibrid (StxSf; ■ , --) : y= 12.7089 + 0.0509 x, r^2 = 0.57]. Tüm gruplarda sabit değer (a) ve eğimler (b) birbirinden istatistiksel olarak farklı (P<0.001) bulunmuştur

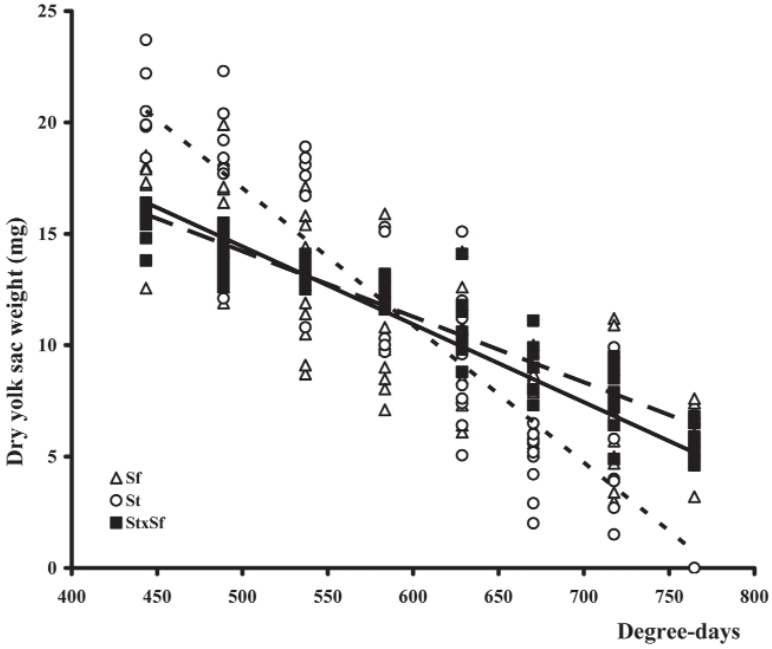

Fig 4. Relationship between dry yolk sac weights (y) and degree-days (x) [in brook trout (Sf; Δ, —): y = 31.8916 - 0.0349 x, r^2 = 0.71; Black Sea trout (St; ○, ...) : y= 47.8207 - 0.0616 x, r^2 = 0.87; and hybrid (StxSf; ■ , --) : y= 28.9151 - 0.0294 x, r^2 = 0.90]. Constant (a) and slope (b) values of St differed significantly than the others (P<0.001)

Şekil 4. Kuru besin kesesi ağırlığı (y) ile günderece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): y = 13.1346 + 0.0598 x, $r2^2$ = 0.33; Karadeniz alabalığı (St; ○, ...) : y= 28.8957 + 0.0854 x, r^2 = 0.39; hibrid (StxSf; ■ , --) : y= 12.7089 + 0.0509 x, r^2 = 0.57]. Karadeniz alabalığına ait sabit değer (a) ve eğim (b) diğer gruplardan istatistiksel olarak farklı (P<0.001) bulunmuştur.

differences, excluding length slope.

Length and weight increasing and growth rates, daily yolk sac consumption and yolk sac efficiency values were calculated as 0.21 mm/day, 0.63 mg/day, 0.30 mg/day, and 0.61 for brook trout; 0.19 mm/day, 0.87 mg/day, 0.60 mg/day, and 0.76 for Black Sea trout, and 0.20 mm/day, 0.45 mg/day, 0.28 mg/day, and 0.46 for hybrid, respectively.

DISCUSSION

Durations of incubation periods differed among the three cross-types, and Black Sea trout has shorter period than the others. Egg survival rate of hybrid was low as expected [13] and survival rates of brook trout [3] and Black Sea trout [2] eggs and larvae were normal. Although Black Sea trout and hybrid have the same egg size, the

variation observed in net body weight at hatching was significantly related to paternal and maternal species [10], Black sea trout showed larger larvae (P<0.01).

Dry yolk weight of brook trout, Black Sea trout, and hybrid larvae at hatching were calculated as 16.43±0.59 mg, 20.83±2.00 mg and 15.56±0.73, respectively in the present study. Brook trout and hybrid values are higher than the value of 12.2 mg reported by Dumas et al.[10], whereas it is smaller than that presented by Hodson and Blunt [9] for rainbow trout which was estimated as 31 mg from figures, and Black Sea trout value is smaller than that presented by Peterson and Martin-Robichoud [8] for Atlantic salmon (27.8 mg). These differences in yolk size within and between species might arise from egg and larva size, incubation temperature and thus period, and nutrition and husbandry of brood fish. There are contrasting views on relation between size of larva and amount of yolk at hatching. For example, as cited by Dumas et al. (1995), Rana (1990) observed an inverse relationship between the size of larva and the yolk. In contrast, Dumas et al.[10] found that hybrids of Arctic charr (female) and brook trout (male) had higher net body weights at hatching than pure Arctic charr larva, but they both had the same amount of yolk. The authors concluded that the amount of yolk hatching is mainly determined by the maternal species and larvae of brook trout females had more yolk reserves than larvae from Arctic charr females.

The observed value of YCE on a dry weight basis approached 0.7, although the theoretical value was 0.82. Blaxter (cited Hodson and Blunt [9]) reviewed estimates of YCE for several species and the range was 0.4-0.8. The values of YCE for *Salmo* species were from 0.41 to 0.70 at 10°C. Dumas et al.[10] and Başçınar et al.[5] reported that YCE values were 0.65 at 8-13°C, and 0.50 for brook trout at 4.5-13°C, respectively, and Hansen [6] reported values ranging from 0.46 to 0.68 at 7.0-8.5°C for *Salmo trutta*. All these values can be compared well with the present findings. A high value of YCE for Black sea trout suggests that yolk sac consumption results the earlier than the others *(Fig. 4)*, moreover brook trout and hybrid larvae start free swimming at about half yolk sac consumption. Regression slope (b values) differed significantly, and it supported that yolk consumption rate of hybrid was affected from maternal species (brook trout).

This study was undertaken to evaluate hatching performance and early development (from hatching to free swimming stage) of brook trout, Black Sea trout and their hybrid larvae and has provided data on the growth rate during the yolk absorption, yolk conversion efficiency and dry weight. These can be used both for further comparative studies and developing efficient hatchery management programs.

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