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Eggs Incubation, Early Development and growth in Frys of Brown Trout (*Salmo trutta macrostigma*) and Black Sea Trout (*Salmo trutta labrax*)

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Abstract

Some of the embryonic development stages and growth of the frys of brown trout (*Salmo trutta macrostigma*) and Black Sea trout (*Salmo trutta labrax*) were examined. The first eye pigmentation in the brown trout eggs were realised on the 35 days (244 day-degree) at 7.23°C and 31 days (260 day-degree) at 8.21°C post fertilisation. First eye pigmentation of the Black Sea trout eggs were seen on day 25 (215 day-degree) at 8.21°C. Brown trout larvae were hatched on day 56 (387 day-degree) at 7.23°C and on day 50 (413 day-degree) at 8.21°C post fertilisation. Black Sea trout larvae were hatched on day 53 (440 day-degree). Survival rates in the eyed stages were 84.50% (7.23°C) and 86.65% (8.21°C) and 81.59%. Survival rates in the hatching were 82.27% (7.23°C) and 80.96% (8.21°C) in brown trout and 78.30% in Black Sea trout. Alevins were initially fed with only *Artemia* naupliii and then were acclimated to the commercially dry feed. The brown trout frys reached from 76.8±6.1 mg to 3527.4±250.3 mg in the period of 111 days. Growth in the frys was the best described using an exponential model and it was $W(t)=18.741 \times \exp^{(0.0358xAge)}$ ($r^2= 0.989$) for brown trout. The Black Sea trout frys reached form 76.6±3.2 mg to 3,680.2±390.5 mg in the period of 111 days. The exponential growth equation of Black Sea trout frys was $W(t)=15.742 \times \exp^{(0.0368xAge)}$ ($r^2= 0.989$). Growth in length was fitted to the exponential model and the equations were formed as $L(t)=11.240 \times \exp^{(0.0124xAge)}$ ($r^2=0.991$) for brown trout frys and $L(t)=10.673 \times \exp^{(0.0128xAge)}$ ($r^2= 0.987$) for Black Sea trout frys. Incremental growth rates were not different between brown trout and Black Sea trout frys ($P>0.05$).

Keywords: *Salmo trutta macrostigma, Salmo trutta labrax*, eggs, embryo, fry, growth.

Kahverengi alabalık (*Salmo trutta macrostigma*) ve Karadeniz Alabalığı (*Salmo trutta labrax*)'nda Yumurta İnkübasyonu, Erken Gelişim ve Yavrularda Büyüme

Özet

Kahverengi alabalık (*S. t. macrostigma*) ve Karadeniz alabalığında (*S. t. labrax*) bazı embryonic gelişim safhaları ve yavrularda büyüme incelenmiştir. Yumurtalarda ilk gözlenme kahverengi alabalıklarda 7,23°C de 35. günde (244 gün-derece) ve 8,21°C'de ise 31. günde (260 gün-derece) tespit edilmiştir. Karadeniz alabalığı yumurtalarında gözlenme ise 8,21°C'de 25. günde (215 gün-derece) olmuştur. Larva çıkışları kahverengi alabalıklarda 7.23°C 'de 56. günde (387 gün-derece), 8,21°C'de ise 50. günde (413 gün-derece) görülmüştür. Karadeniz alabalıklarında larva çıkışları ise 53. günde (440 gün-derece) olmuştur. Gözlü safhada hayatta kalma oranları %84,50, %86,65 (7,23°C) ve %81,59 (8,21°C) olmuştur. Larva çıkış oranları kahverengi alabalıklarda %82,27 (7,23°C) ve %80,96 (8,21°C), Karadeniz alabalığında ise %78.30 olmuştur. Yavrular başlangıçta *Artemia* naupliii ile beslenmiş ve daha sonra ise ticari toz yemlere alıştırılmıştır. Kahverengi alabalık yavrularının ağırlıkları 111 günde 76,8±6,1 mg'dan 3.527,4±250,3 mg'a yükselmişlerdir. Kahverengi alabalıklarda büyüme; $W(t)=18.741 \times \exp^{(0.0358xAge)}$ ($r^2= 0.989$) şeklinde ifade edilmiştir. Karadeniz alabalığı yavruları 111 günde 76,6±3,2 mg'dan 3.680,2±390,5 mg'a çıkmışlardır. Karadeniz alabalıklarında büyüme $W(t)=15.742 \times \exp^{(0,0368xAge)}$ ($r^2= 0,989$) şeklindedir. Boyca büyüme ise kahverengi alabalıklarda $L(t)=11.240 \times \exp^{(0,0124xAge)}$ ($r^2=0.991$) ve Karadeniz alabalıklarında ise $L(t)=10.673 \times \exp^{(0,0128xAge)}$ ($r^2= 0,987$) şeklinde bulunmuştur. Kahverengi alabalık ve Karadeniz alabalığı yavrularında büyüme oranları birbirlerinden farksız bulunmuştur ($P>0,05$)

Anahtar Kelimeler: *Salmo trutta macrostigma, Salmo trutta labrax*, yumurta, embriyo, fry, büyüme.

Introduction

Brown trout is one of the most important fish species for aquaculture, commercial fisheries and sport fishing. Therefore they have been introduced at least 24 countries outside Europe, including USA, Canada, Australia, as well as several countries in South America, Africa and Asia (Elliot, 1994). There is a considerable confusion regarding the taxonomy of the *Salmo* and 20 species name were reported in Europe (Kottelat, 1997). In the previous studies, *Salmo trutta macrostigma*, *Salmo trutta labrax*, *Salmo trutta abanticus* subspecies and *Salmo platycephalus* were reported in Turkish waters (Behnke, 1968; Balık, 1984; Geldiay and Balık, 1988; Çetinkaya, 1996; Tabak *et al*., 2001; Alp *et al*., 2003; Alp and Kara, 2004; Kuru, 2004). However, the last molecular studies claimed that trout in Turkey were divided into three phylogenetic assemblages, namely Danubian, Adriatic and Tigris lineages including one species of *Salmo trutta* (Bernatchez, 2001; Bardakçı *et al*., 2006). Since the present study was not related with that taxonomic confusion, research materials were admitted as *Salmo trutta macrostigma* and *Salmo trutta labrax* resting on the above researcher.

S. t. labrax is a migratory species and inhabits in the Black Sea and in the streams connected to the Black Sea (Tabak *et al*., 2001). Therefore, it is also called as Black Sea trout. However *S. t. macrostigma* forms local populations in the upper parts of the rivers in Turkish waters. In combination with illegal methods and heavy fishing pressure, reduced spawning success caused by pollution of streams, degradation of spawning habitats, river damming and interspecific competition with introduced rainbow trout has caused a decline in the stocks or extinction of native trout populations in Turkey (Alp *et al*., 2005).

In addition to conservation of the stocks, treatment of the habitats and regulation of the fishing, stocking is generally regarded as a main tool in conservation and enhancement of the fish populations (Welcomme, 2001). Aquaculture techniques can be used to aid conservationists. Conservation aquaculture has been used to enhance current stocks and reintroduce. Information about the embryological and early development of the fish would assist in this process. Further, this would be directly applicable to culture facilities of the related species, too.

Embryonic development and early life history of the different trout species were previously well described (Grande and Andersen, 1990; Killeen *et al*., 1999; Halacka, 1995; Başçınar and Okumuş, 2004). The early development and rearing conditions of *S. t. labrax* and *S. t. abanticus* were investigated (Çakmak *et al*., 2004; Uysal and Alpbaz, 2002a). However, information regarding the early development of *S. t. macrostigma* is limited.

The aim of the present study is to determine some of the embryonic development stages such as eyed and hatching time, survival of the embryos etc. at the two different temperature, and to acclimatize to the commercial feed of the frys and to examine growth characteristics of brown trout and Black Sea trout frys.

Materials and Methods

This current study was carried out in the two experiments. The first experiment was conducted in a private trout hatchery located at Nurhak town in Kahramanmaraş. The eggs of *S. t. macrostigma* were incubated at 7.23±0.86°C (5.0-8.8°C) and durations of the eying, hatching and survival of the embryos were described. Second experiment was conducted in an another trout hatchery located at Tekir in Kahramanmraş. In the second experiment, the eggs of *S. t. macrostigma* and *S. t. labrax* were incubated at 8.21±0.74°C (7.0-9.7°C) and growth of the frys were examined in addition to duration of the embryological developments as in the first experiment.

Brown trout broods were caught from the wild stocks in the streams of Fırnız and Egemen in Kahramanmaraş and Black Sea trout broods were obtained from a private trout farm in Güneysu town of Rize. Broods were stocked into the concrete ponds in two private trout farms in Nurhak and Tekir, Kahramanmaraş.

In the first trial, 14 female brown trout broods were spawned and their mean weight was 540±212.6 g (154-945 g). The obtained eggs from each female were fertilised with milk from 2 males and incubated in the trout hatchery in Nurhak on 20 December 2003. Totally 10,842 eggs were incubated in 4 trays in a vertical trout incubator at the temperature of 7.23°C (5.0-8.8°C) and hatching was conducted on the trays in fiberglass incubation troughs (200 cm x 50 cm x 40 cm).

In the second trial, the eggs were collected from 6 female brown trout broods with a mean weight of 758±395.9 g (465-1,540 g) and 5 female Black Sea trout broods with a mean weight of 641±98.6 g (515-780 g). Eggs from each female were fertilised with milk from 2 males and incubated in the trout hatchery in Tekir, Kahramanmraş on 07 December 2004. A total of 7,930 eggs spawned form 6 brown trout were incubated in 3 trays in a vertical trout incubator and 5,045 eggs spawned from 5 Black Sea trouts were incubated in 2 trays at the temperature of 8.21°C (7.0-9.7°C). However, hatching was conducted on the trays in fibreglass incubation troughs (200 cm x 50 cm x 40 cm). After hatching and the absorption of the yolk sacs, the trays were removed.

Water temperatures in the hatcheries were measured daily during 100 days and death or unfertilised eggs were removed by a piped and counted in the every day during the incubation period. The first eye pigmentation of the eggs were defined as when the eyes were clearly visible as black spots and the hatching time was defined as when 50% of the

embryos were swim-up larvae (Halacka, 1995).

In the second trial, 12 days old brown trout alevins and 15 days old Black Sea trout alevins (62 days for brown trout alevins and 65 days for Black sea trout alevins post fertilisation) were initially started to feed with only concentrated *Artemia* nauplii (168,000 *Artemia* nauplii/200 cc) three times a day (for 12 days) and then were fed with enriched feed with *Artemia* nauplii+commercially dry feed (for 24 days) and finally feeding with *Artemia* nauplii was weaned and they were fed only commercially dry feed. These feeding activities were continued in the fibreglass troughs in the hatchery.

Twenty-four days after the alevins were acclimated to the commercially dry feed (120 days post fertilization), brown trout frys (36 days old) and Black Sea trout frys (39 days old) were transferred into the 3 fibreglass tanks (115 cm x 115 cm x 50 cm). The weight and length of the frys stocked were taken from 150 individuals on the first (29 March), 35 (03 May), 42 (10 May), 49 (17 May), 56 (24 May), 63 (01 June), 70 (08 June) 97 (05 July) and 111 (19 July) days.

Growth in weight and in length of the frys were investigated by exponential equation as;

$$y = a \times \exp(b * day)$$

where "*a*" and "*b*" are the constant coefficients. The differences between observed and estimated weight or length were tested by t-test and correlation coefficient (r^2). Exponential modelling efficiency (EF) was also defined as follows (Mayer and Butler, 1993);

$$EF = 1 - \frac{\sum (yi - ye)^2}{\sum (yi - \overline{yi})^2}$$

Where *yi* is the observed, *ye* the calculated value and $\overline{yi}$ is the mean of the observed value. Modelling efficiency (EF) gives an indication of goodness of fit, with value of 1 describing a perfect fit and values approaching 0 indicating poor model performances.

Results

Spawning and Egg Incubation

In the first trial, total and relative fecundities of the brown trout broods were 775±438 eggs/fish and 1,350±550 eggs/kg, respectively. The mean egg weight of the brown trouts was 73±6 mg (64-84 mg). The mean weight of the broods in the first trial was significantly different (P<0.05) from the brown trouts in the second trial but the mean eggs size was not different (P>0.05).

During the spawning period in the second experiment, a total of 7,930 eggs from 6 *S. t. macrostigma*, and 5,045 eggs from 5 *S. t. labrax* were spawned. *S. t. macrostigma* broods have a total and relative fecundities of 1,322±233 eggs/brood and 1,990±440 eggs/kg , while *S. t. labrax* broods have 1,009±90 eggs/brood and 1,600±170 eggs/kg. Individual egg size varied from 69 to 75 mg/egg (mean 73±1 mg/egg) for *S. t. macrostigma* and from 60 to 74 mg/egg (mean 72±5 mg/egg) for *S. t. labrax*. Total fecundity was significantly different (P<0.05) between *S. t. macrostigma* and *S. t. labrax* broods while egg size was not different (P>0.05).

Water temperature in the incubation period varied from 5.0 to 8.8°C (mean 7.23±0.86) in the first trial, while it varied from 7.0 to 9.7°C (mean 8.21±0.74°C) in the second. Daily variations in the water temperature and duration of eyed-egg, hatching days and feeding days of the alevins were given in Figure 1A and Figure 1B.

One day after the eggs were incubated, in the first trial, 9,787 eggs (90.26%) from 10,842 were survived. The eggs were eyed at the 35 days (244 day-degree) post fertilisation and survival rate from fertilisation to eyed stage was 84.50%. Larvae were hatched at the 56 days (387 day-degree) and survival rate was 82.27% (Table 1).

In the second experiment, 7,543 brown trout eggs (95.12%) from the total incubated eggs were fertilised (Table 1). The eyed egg stage was determined at 31 days (260 day-degree) post fertilization and survival form fertilization to eyed stage was 86.65% (6871 eggs). Brown trout larvae were hatched at 50 days post fertilization (413 day-degree) and survival rate was 80.96% (6420 eggs).

Fertilization rate in the 5,045 eggs of Black Sea trout was defined as 90.17% (4,549 eggs) (Table 1). They were eyed at 25 days post fertilization (215 day-degree) and survival rate was 81.59% (4,161 eggs) in that period. Black Sea trout larvae were hatched at 53 days post fertilization (440 day-degree) and survival rate from fertilization to hatching was 78.30% (3950 eggs).

Growth in Frys

The population of 4,227 brown trout frys, 36 days old, stocked into fibreglass tanks had an average weight of 76.8±6.1 mg. These fish exhibited an exponential increase in body weight over the experimental period of 111 days (147 days old) and reached to 3,527.4±250.3 mg (Figure 2A). The exponentially growth is represented by the equation of $W(t)=18.741 \times \exp^{(0.0358 \times Age)}$. Correlation coefficients and modelling efficiency (EF) showed that there were strong relationship between predicted and calculated weights of the frys (r^2= 0.991, P>0.05 and EF= 0.999). During the first 35 days, fish grew at a rate of 2.91 mg/day to a final weight of 175.0±10.8 mg at the age of 70 days. They grew at an approximately constant rate with an average incremental growth rate of 16 mg/day between the 71-77 and 85-91 days old.

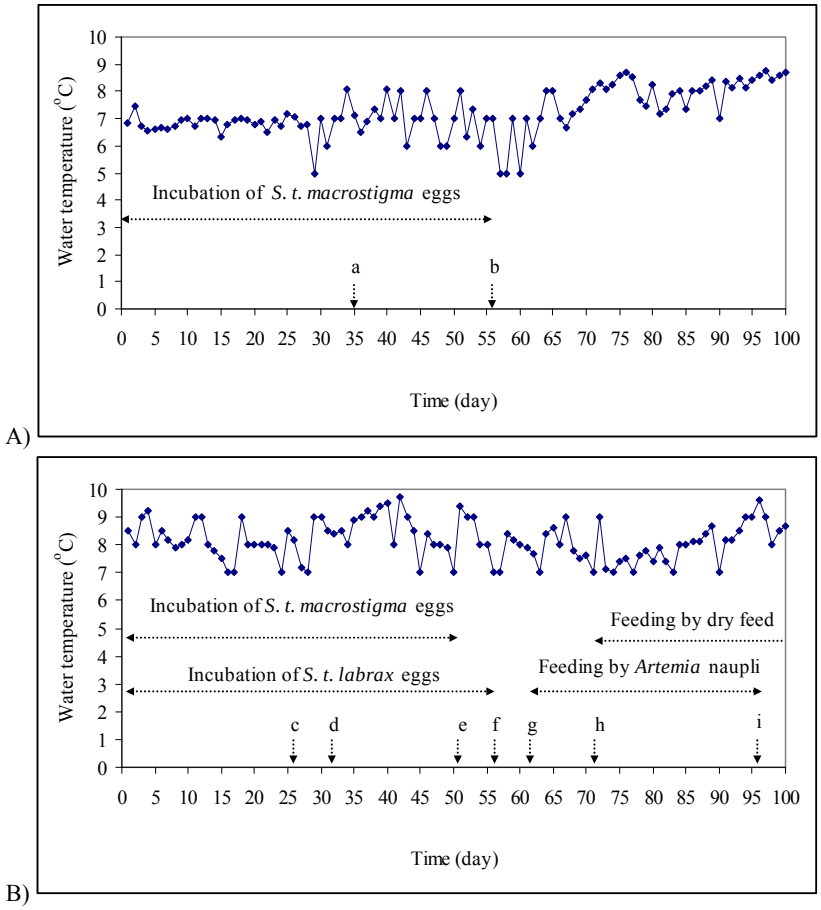

Figure 1. The daily variations in the water temperature and some important stages of the incubation periods and fry feeding in the A) first experiment and B) second experiment.
a) eyed of *S. t. macrostigma* eggs and b) hatching of *S. t. macrostigma* c) eyed of *S. t. labrax* eggs, d) eyed of *S. t. macrostigma* eggs, e) hatching of *S. t. macrostigma*, f) hatching of *S. t. labrax*, g) starting of feeding by *Artemia* naupliii, h) starting of feeding by dry feed and i) acclimation to the dry feed and weaning of feeding by *Artemia* naupliii in the second experiment.

Table 1. Development and survival in the incubated eggs of *S. t. macrostigma* (S.m.) in 8.21°C and 7.23°C and *S. t. labrax* (S.l.) in 8.21°C. (A: 8.21°C and B: 7.23°C).

	Egg number			Day			Day-degree			Survival rate (%)		
	S.m. (A)	S.m. (B)	S.l. (A)	S.m. (A)	S.m (B)	S.l. (A)	S.m. (A)	S.m. (B)	S.l. (A)	S.m. (A)	S.m. (B)	S.l. (A)
Incubated egg	7,930	10,842	5,045	1	1	1	8.5	6.8	8.5	100	100	100
Fertilised egg	7,543	9,787	4,549	2	2	2	17	13.8	17	95.12	90.26	90.17
Eyed-egg	6,871	9,162	4,116	31	35	25	261	244	215	86.65	84.50	81.59
Hatching larva	6,420	8,920	3,950	50	56	53	413	387	440	80.96	82.27	78.30

Incremental growth rate decreased from 23.28 mg/day in 92-98 to 12.43 mg/day in 99-105 days old (Figure 3A). These probably resulted from interrupting of the diet because of turbidity of the water in that season. The incremental growth rate increased progressively during subsequent phases of growth to maximum values of 49.11 and 102.42 mg/day recorded between the 106-132 and 133-146 days old, respectively with fish attaining a final weight of 3,527.4±250.3 mg.

Incremental growth rates showed no differences between brown trout and Black Sea trout frys ($P>0.05$).

The population of Black Sea trout frys, 39 days old, showed a similar early growth pattern with brown trouts. Body weight increased exponentially from an initial weight of 76.6±3.2 mg to a final weight of 3,680.2±390.5 mg at the age of 149 days (Figure 2B). The frys of the Black Sea trout grew exponentially and their growth is represented as $W(t)=15.742 \times \exp^{(0.0368xAge)}$. The differences between observed and predicted in weight was not significant and the efficiency of the exponential growth model was very high ($r^2=0.989$, $P>0.05$ an EF=0.997). The incremental growth rates showed similar changes with brown trouts. It was decreased from 37.57 mg/day in 95-101 days old to 23.86 mg/day in 102-109 days old

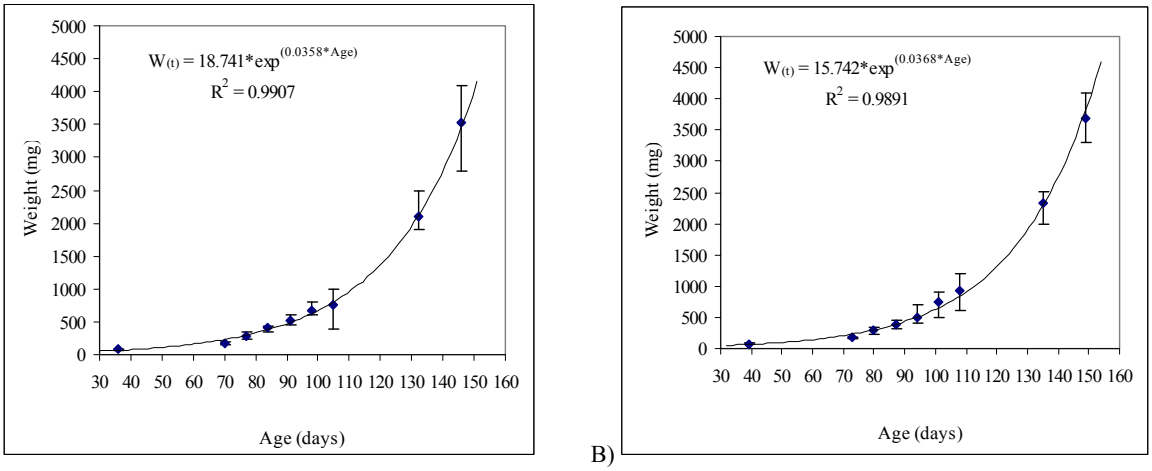

Figure 2. Weight velocity curves of A) brown trout (*S. t. macrostigma*) and B) Black Sea trout (*S. t. labrax*). (The vertical lines shoved minimum and maximum body weight).

because of turbidity and interrupting of the diet. The incremental growth rates increased from 51.86 mg/day in 110-135 days old to 97.14 mg/day in 136-149 days old (Figure 3B).

The total length of brown trout frys showed an exponential increase from an initial length of 25.2±0.50 mm to 68.1±2.78 mm on day 111 (Figure 4A). Exponential growth equation was formed as $L(t)=11.240 \times \exp^{(0.0124 \times Age)}$ and predicted length values were not different from the observed lengths (r^2= 0.991, P>0.05 and EF= 0.995). The incremental growth rate varied from 0.277 mm/day (at the 99-105 days old) to 0.764 mm/day (at the 133-146 days old).

The growth in length of Black Sea trouts showed a similar growth pattern with brown trouts. Their average initial length was 25.2±0.65 mm and increased to 69.4±2.42 mm on day 111. Growth equation of the Black sea trout was formed as; $L(t)=10.673 \times \exp^{(0.0128 \times Age)}$. The efficiency of the equation was very high (r^2= 0.987, P>0.839 and EF=0.990). The incremental growth rates varied from 0.343 mm/day (at the 102-109 days old) to 0.729 mm/day (at the 136-149 days old).

Discussion

Embryonic and larval development stages such as first eye pigmentation, hatching and swim-up larvae as day-degree reveal differences in *Salmonids* (Başçınar and Okumuş, 2004). In the present study, the eggs of the brown trout incubated at 7.23±0.86°C were eyed 4 days later than the egges incubated at 8.21±0.74°C. Similarly, brown trout larvae in the first trial were hatched 6 days later than that of the second. The eggs of *S. t. labrax* were also eyed 6 days earlier than the eggs of *S. t. macrostigma* and hatching of the *S. t. labrax* larvae were 3 days later than that of *S. t. macrostigma* at the same temperature. Başçınar and Okumuş (2004) indicated that in addition to genetic condition, incubation temperature and its variation during these periods were the main factors controlling the duration of the early development stages of fish embryos and larvae. The day-degree value in the embryonic development is lower in cold water than in warmer water (Grande and Andersen, 1990; Başçınar and Okumuş, 2004). The eggs of brown trout were eyed at 244 day degree (35 days) and hatched at 387 day-degree (56 days) at the temperature of 7.23°C, in contrast, they were eyed at 261 day-degree (31 days) and hatched at 413 day-degree (50 days) at the temperature of 8.21°C. The duration of the first eye pigmentation of various trouts were reported as 30-33 days (Killeen *et al*., 1999), 220 day-degree (Gjerdem and Gunnes, 1978), 195 day-degree (Grande and Andersen, 1990) for *Salmo trutta* and 245 day-degree (29 days) (Başçınar and Okumuş, 2004) for *Salvelinus fontinalis*. The duration of the hatching were 490 day-degree (Gjerdem and Gunnes, 1978), 250 day-degree (176 days) (Grande and Andersen, 1990) for *S. trutta*, 415 day-degree (52 days) for *Salvelinus fontinalis* (Başçınar and Okumuş, 2004) and 477.2-488.8 day-degree (33-40 days) (Çakmak *et al*., 2004) for *S. t. labrax*. Survival rates in the present study varied from 78.3% to 82.3%. On the other hand, survival rates form fertilising to hatching were reported as 56.5% for *S. fontinalis* (Başçınar and Okumuş, 2004) and 69.7% for *S. t. labrax* (Çakmak *et al*., 2004).

In the present study, various growth models were tried and the exponential growth model was seen to be the most appropriate model for the growth of trout frys. It was suggested that von Bertelannffy growth model or some modified form adjusted to seasonal change is preferable for older fish. Gompertz or parabolic growth models seem to be more appropriate for the description of young fish growth. The use of the exponential growth model for long periods of time is not recommended. However, in the aquaculture studies required for short periods of time, exponential growth model is relatively common (Gamito, 1998). In the present study, the frys of

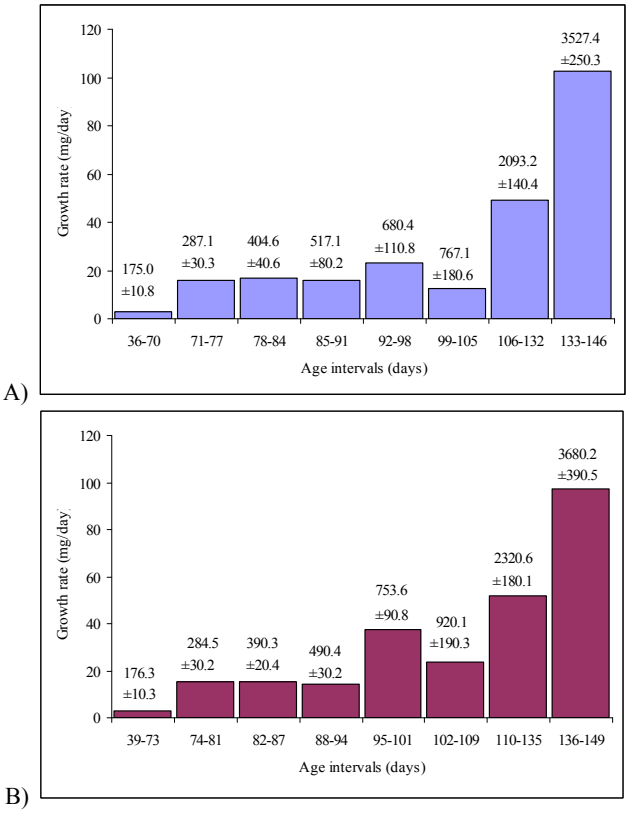

Figure 3. Incremental growth in body weight of A)*S. t. macrostigma* and B) *S. t. labrax*. (Numbers above each column are the final weight (±SD) at the end of the interval of ages).

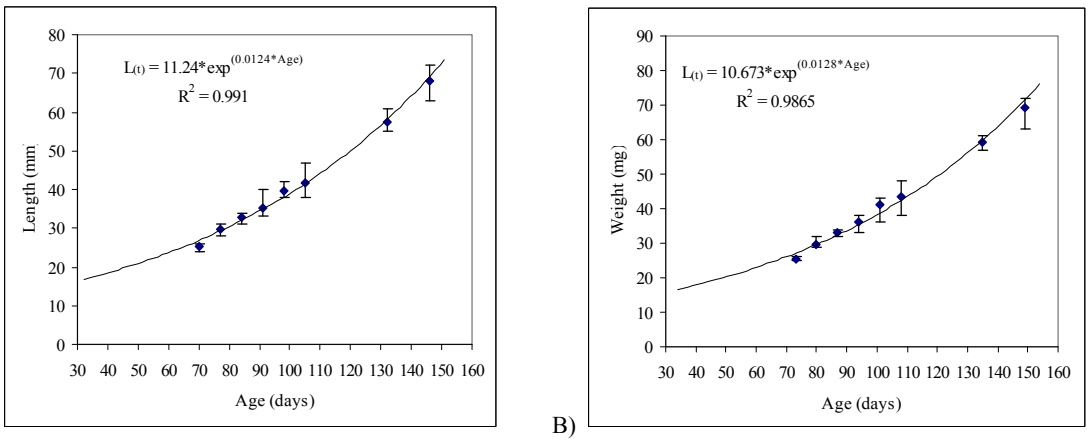

Figure 4. Length velocity curves of A) brown trout (*S. t. macrostigma*) and B) Black Sea trout (*S. t. labrax*). (The vertical lines shoved minimum and maximum length).

brown tout and Black Sea trout reached from 76.8 mg to 3,527 mg and from 76.6 mg to 3680.2 mg, respectively during the period of 111 days in 8.21°C. Black Sea trout frys were reported to be 68.43-107.68 mg to 230-310 mg during the period of 50 days (Çakmak *et al*., 2004). Abant trout (*S. t. abanticus*) frys reached from 80-88 mg to 4,530-5,250 mg in the period of 350 days, in contrast rainbow trout frys reached from 50-90 mg to 173.42-133.96 g at the same period (Uysal and Alpbaz, 2002b). Güner and Tekinay (2002) showed that rainbow trout frys reached from 70 mg (prelarva) to 2300 mg at the period of 120 days at the 10°C. In our study, brown trout frys reached to 2,093.2 mg at the age of 132 days, and Black Sea trout frys reached to 2,320.6 mg at the age of 135 days. Rainbow trout frys also reached to 4-5 g in the 120-130 days post fertilisation, and reached to 20 g in the period of 140 days post fertilisation at the temperature of 10°C and 14°C, respectively (Shepherd and Bromage, 1988). *Salvelinus fontinalis* frys reached from 62.4-93.1 mg to 937.0-1,690.0 mg at the period of 63 days

(Başçınar and Okumuş, 2004). In the present study, brown trout and Black Sea trout frys reached to 600-800 mg and 500-900 mg, respectively, at the same period (63 days). The variations in these studies may be probably resulted from differences in water temperature and feeding of the frys. It is well known that water temperature is the most important environmental factor affecting larval and fry development.

In summary, the results obtained in the current experiments suggest that *S. t. macrostigma* and *S. t. labrax* are promising for aquaculture as an alternative to rainbow trout when compared with the results of Güner and Tekinay (2002) who studied with rainbow trout. However, to be more conclusive, further studies are required for large implication of *S. t. macrostigma* and *S. t. labrax* especially at fingerlings and larger size fish. This study also suggests that it is possible *S. t. macrostigma* and *S. t. labrax* fry can be fed, successfully until fingerling stage in aquaculture media, then stocking or reintroducing to the natural waters, which may result in improvement in survival rate of the *S. t. macrostigma* and *S. t. labrax*.

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