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An investigation on the effects of juvenile rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) monoculture and duo-culture farming in freshwater and seawater on growth performance

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Abstract

The objective of this study was to compare growth performance and feed conversion ratios of rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) juveniles in monoculture and duo-culture in freshwater and seawater under aquarium conditions. The fish were about 2-months old hatchery-reared brook and rainbow trout with initial weights of 0.934 ± 0.033 (n=360) and 1.014 ± 0.019 (n=360) g, respectively. The juveniles were kept in 10 L aquaria. Each aquarium contained 40 fish: 40 fish from each species for monoculture, 20+20 (rainbow trout+brook trout) fish from each species for duo-culture. The fish were in 3 groups with three replicates in freshwater and seawater and 18 aquaria were used. At the end of the 45 day study; mean live weights of brook trout were 2.735±0.153 g, rainbow trout 2.925±0.262 g in monoculture and brook trout 2.354±0.186 g, rainbow trout 3.882±0.494 g in duoculture in seawater, brook trout 3.088±0.085, rainbow trout 3.364±0.093 g in monoculture and brook trout 2.164±0.169, rainbow trout 3.948±0.124 g in duo-culture in fresh water. While there were similarities between brook trout and rainbow trout in monoculture groups in sea water and fresh water, some differences were realized in duo-culture (p < 0.001).

Keywords: Rainbow trout, *Oncorhynchus mykiss*, Brook trout, *Salvelinus fontinalis*, Polyculture, Growth Performance, Feed conversion ratio

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Introduction

Salmon family members have a special importance among economic fish species most of which are anadromous. They are the first fish species cultivated intensively and today rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar) and several Pacific salmon species (Oncorhynchus sp.) are cultivated widely. Apart from these, other salmons (e.g., Salmo trutta, fontinalis, Salvelinus Salvelinus alpinus) are cultivated in European and North American countries under similar climatic conditions to that in our country with the purpose of fish release, stock supplement, and sporting in general (Jobling, 1995; Okumuş et al. 1999). One of the salmon species cultivated in Turkey, rainbow trout is the most cultured species in our country with over 80.000 tones of annual production (approximately 51% of total cultivation) (TUIK, 2010). Brook trout (Salvelinus fontinalis) and Black Sea trout (Salmo trutta labrax) are reared at a few facilities in the Eastern Black Sea but these two species are not included in official statistics.

Polyculture is the technique of cultivating two or more species in the same culture environment. In most cases, two or more animals are grown together with polyculture technique (Yabanlı, 2009). At the same time, polyculture is used to familiarize wild species to commercial feeds. Of the species in polyculture environments, those with good adaptation to the environment, high food consumption, and high social hierarchy have advantage in growth rates. Brook trout which has fresh water and anadromous varieties in original spill areas (Cihar, 1986) prefers similar foods as rainbow trout (Bristow, 1992). If brook trout is reared in the same environment with rainbow trout or other salmon species, little is known about the outcome of the competition (Jobling, 1995; Başçınar *et al.*, 2010).

Each species has its own limits to salinity change to sustain normal physiological activities. Optimum values vary depending on the stage of life as well as species. In addition, different salinity levels may be preferred for growth, reproduction, and juvenile development. Rainbow trout among freshwater species grows better in sea water (Celikkale et al., 1997), but trout needs brook 20% salinity (Başçınar, 2010).

In the previous polyculture studies done on salmon, Atlantic salmon (Salmo salar) (77.7 mm-5.6 g) and Alpine salmon (Salvelinus alpinus) (79.1 mm- 4.9 g) (Cihar, 1986), Atlantic salmon (Salmo salar) (72.1 mm- 3.87 g) and crayfish (Astacus astacus) (32.7 g) (Çelikkale, 1994), the Baltic salmon (Salmo salar) and Brown trout (Salmo trutta) (2+ years) (Tveiten et al., 1996), three salmon species (Salmo salar, Salmo trutta, Salmo gairdneri) (8-14 g) (Stevenson, 1987), rainbow trout $(27.2\pm6.22 \text{ g})$ and brook trout (17.6±6.97 g) (Jobling, 1995) fingerlings of the above size and weight were used. Therefore, the individuals used in this study were much smaller than the ones used in previous studies, and also two different media (freshwater and sea water) were used.

The evaluation of forage and feed intake are the most important factors in fish farming and there is a competition between brook trout and rainbow trout in terms of feeding in the same environment (Okumuş *et al.*, 1999). In this study aiming to determine which juveniles of the two species grow faster and whether freshwater and sea water have any effect on their growth performance, the growth performance and feed intakes of the pure and polyculture rainbow and brook trout juveniles in culture conditions were compared in seawater and freshwater.

Materials and methods

The study was conducted in glass aquaria of 15×25×35 cm (10 liters of available water volume) at Karadeniz Technical University, Faculty of Marine Sciences Prof. Dr. İbrahim Okumuş Research and Application Unit for 45 days. . In the study, brook trout juveniles of 0.934±0.033 g and 4.739±0.046 cm (n=360) and rainbow trout juveniles of 1.014±0.019 g and 4.673±0.053 cm (n=360) were used. The study was done with three replications in fresh water and sea water as pure rainbow trout and brook trout culture, polyculture and (rainbow+brook trout), and 40 fish were put in each aquarium. There were 20 brook trout in rainbow trout+20 polyculture aquaria.

Commercial trout fishmeal of 1 mm in diameter including 50% protein was used to feed the juveniles. They were fed by hand three times a day (at 08:00, 12:00, 17:00 hours) until they were full. The aquaria were provided with 0.5 to 1.2 L/min water in varying amounts depending on water temperature and biomass. The water temperature was measured in the morning, afternoon, and evening every day.

In the study, measurements were done every other week to determine specific growth and relative growth rates (length and weight), condition factor (CF), feed conversion ratio (FCR), and thermal growth coefficient (TGC). In every measurement, the individual size (± 1 mm) and weight (± 0.01 g), and total biomass of the juveniles in each aquarium was determined. The following equations were used in the calculations (Akhan, *et al.* 2010; Jafaryan, *et al.* 2011):

Specific Growth Rate (SGR) = ($\ln W_2$ - $\ln W_1$) 100/ Δt ,

Thermal Growth Coefficient (TGC)= $(W_2^{1/3} - W_1^{1/3}) / (T x Day) \times 100$

Relative Length Increase = $((L_2 - L_1)/L_1) \times 100$

Relative Weight Increase = $((W_2 - W_1)/W_1) \times 100$

Condition Factor (CF) = $(W / L^3) \times 100$ FCR = Food consumed / (Last biomass – First biomass)

These refer to; L: length (cm), W: weight (g), t: day, T: mean temperature (°C), 1: first value, 2: last value.

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Analysis of variance (ANOVA) and Tukey test were used for the statistical analysis of the data.

Results

Water temperatures varied between 11.2 and 18.7 °C in sea water and between 11.1 and 17.7 °C in fresh water. The results of the study showed live weights as follows: in pure culture brook sea water trout was in 2.735±0.153 g and rainbow trout was 2.925 ± 0.262 g; in polyculture environment brook trout was 2.354±0.186 g and rainbow trout was 3.882±0.494 g; in pure culture in fresh water brook trout was 3.088±0.085 g, rainbow trout was 3.364±0.093 g, and in polyculture environment brook trout was 2.164±0.169 g, and rainbow trout was 3.948±0.124 g (Fig. 1). There were similarities between brook trout and rainbow trout in the pure groups in fresh water and sea water but there were differences in polyculture groups (p < 0.001).

The rainbow trout in freshwater polyculture environment had the highest growth value in weight thermal growth coefficient and it was similar to the polyculture rainbow trout in sea water. The brook trout in fresh water had the lowest value and was similar to the polyculture brook trout in sea water (p < 0.001).

There were similarities between brook trout and rainbow trout in sea water and fresh water, and brook trout and rainbow trout in polyculture environment in sea water and fresh water in terms of specific weight growth rate (p<0.001).

Table 1: Mean (±SD), Growth (Wi: Initial ve Wf: Final Weights, Li: Initial ve Lf: Final Length, TGC: Thermal Growth Coefficient, SGR: Specific Growth Rate, RWI: Relative Weight Increase, RLI: Relative Length Increase, CF: Condition Factor and Feed Conversion Ratio (FCR) parameters.

	Seawater				Freshwater					
	Mono	culture	Polyc	culture	Mono	culture	Polyc	ulture	F	n
	S. fontinalis	O. mykiss	S. fontinalis	O. mykiss	S. fontinalis	O. mykiss	S. fontinalis	O. mykiss	. 1	P
Wi (g)	0.973±0.012 ^b	$0.997{\pm}0.008^{a}$	0.967±0.006 ^b	1.002±0.048ª	0.959±0.035 ^b	1.020±0.002 ^a	$0.967{\pm}0.060^{b}$	1.017±0.026 ^a	4.44	< 0.010
Wf(g)	2.735±0.153 ^{cd}	2.925±0.262 ^{bc}	2.354±0.186 ^{de}	3.882±0.494 ^a	$3.088{\pm}0.085^{bc}$	3.364±0.093 ^b	2.164±0.169e	3.948±0.124 ^a	40.93	<0.001
Li (cm)	4.68±0.005ª	4.64±0.02 ^b	4.79±0.04ª	4.62±0.000 ^b	4.76±0.007ª	4.72±0.015 ^b	4.73±0.138ª	4.18±0.021 ^b	3.56	< 0.050
Lf (cm)	6.44±0.07 ^{cd}	6.49±0.13°	6.16±0.18 ^{de}	7.00±0.243 ^{ab}	6.72±0.03 ^{bc}	$6.88{\pm}0.03^{b}$	6.06±0.10 ^e	7.28±0.150 ^a	41.38	<0.001
TGC (W)	0.096±0.007 ^{de}	$0.101{\pm}0.011^d$	$0.073{\pm}0.010^{ef}$	$0.151{\pm}0.017^{ab}$	$0.118{\pm}0.002^{cd}$	$0.128{\pm}0.003^{bc}$	$0.066{\pm}0.004^{\rm f}$	$0.162{\pm}0.005^{a}$	47.23	<0.001
TGC (L)	0.093±0.006°	0.097±0.006 ^c	$0.072{\pm}0.010^{d}$	$0.126{\pm}0.009^{ab}$	0.109±0.002 ^{bc}	$0.120{\pm}0.001^{b}$	$0.074{\pm}0.002^d$	$0.142{\pm}0.005^{a}$	49.17	<0.001
SGR (W)	2.461±0.120 ^b	$2.388{\pm}0.154^{b}$	1.975±0.220°	3.005±0.127 ^a	2.599±0.056 ^b	2.609±0.041 ^b	1.791±0.082 ^c	3.013±0.036 ^a	39.40	< 0.001
SGR (L)	$0.710{\pm}0.038^{d}$	$0.746{\pm}0.044^{cd}$	0.562±0.074°	$0.928{\pm}0.057^{ab}$	$0.770{\pm}0.008^{cd}$	$0.840{\pm}0.005^{bc}$	0.551±0.028 ^e	$0.962{\pm}0.026^{a}$	39.78	< 0.001
RWI (W)	202.990±16.365 ^b	193.410±20.644 ^b	143.998±23.852°	287.020±22.312ª	222.190±8.113 ^b	223.474±5.967 ^b	124.007±8.214°	288.087±6.364ª	42.52	< 0.001
RLI (L)	37.683±2.371 ^d	39.920±2.751 ^{cd}	28.842±4.269°	51.844±3.897 ^{ab}	41.413±0.481 ^{cd}	45.904±0.319bc	28.154±1.591°	54.197±1.974ª	41.18	< 0.001
CF	1.022±0.018 ^{bc}	$1.065{\pm}0.017^{ab}$	1.001±0.023 ^{bc}	1.125±0.017 ^a	1.015±0.034 ^{bc}	1.030±0.015 ^b	0.965±0.019 ^c	$1.022{\pm}0.031^{bc}$	12.87	< 0.001
FCR	0.699±0.014 ^{ab}	0.702±0.012ª	0.624±0.048 ^{cd}		0.616±0.014 ^d	0.685±0.016 ^{abc}	0.636±0.003 ^{bcd}		8.65	< 0.005

The lowest condition factor was seen in polyculture brook trout in fresh water and it was similar to polyculture rainbow trout in fresh water, pure brook trout, and pure and polyculture brook trout in sea water. The highest condition factor was seen in polyculture rainbow trout in sea water and it was similar to pure rainbow trout in sea water and it was similar to pure rainbow trout in sea water (p < 0.001).

Feed conversation ratio of brook trout and rainbow trout was similar in polyculture groups in sea water and fresh water, different in pure groups in fresh water but similar in sea water (p < 0.05).

Proportional weight increase was similar in pure groups in sea water and fresh water, and in polyculture brook trout and rainbow trout (p < 0.001).

Discussion

Good feed intake, feed conversation and growth rates, ideal (4 pieces/ m^3) stock density, water quality and other characteristics of the test environment, indicate that, the fish have a very positive performance in test conditions, and thus the study was acceptable. It is known that the most suitable growth temperature for rainbow trout is 15 to 17 °C (Stevenson, 1987), and 12 to 14 °C, slightly lower, for brook trout (Huet, 1970; TUIK, 2010). While water temperature was optimal at the beginning of the study, it reached slightly higher values, especially for brook trout, towards the end of the study. However, it did not fall below 4°C at which point the growth becomes slow, (Stevenson, 1987; Okumuş et al., 1999) and it did not reach sublethal levels (>20°C). The results indicated that both species of salmon showed variations in feed intake, growth (thermal growth coefficient (length, specific weight)), growth (length, weight), relative growth (length, weight), and condition factor (Table 1, Figs. 1, 2, 3, 4, 5 and 6). Although there was not a similar study done on juveniles before, this variation was considered to be due to the significant change in water temperature. It was found out that when water temperature kept constant, some species is especially those living in high altitudes (i.e. Salvelinus alpinus) show seasonal variations in growth (Séther et al., 1996; Tveiten et al., 1996). In addition, feed consumption and growth are affected by some factors other than water temperature. Fish size is the most important of them (Austreng et al., 1987: Storebakken and Austreng. 1987). Feed consumption and growth rates continued on a regular basis until the end of the experiment, but towards the end of the study fish growth by weight declined. The reason for this is the reduction in feeding depending on the increase in water. Among other factors affecting growth are forage quality, feeding activity of the species, stock density, oxygen content of water, and genetic line of the fish (Smith et al., 1985).



Figure 1: Increments in live weight (W; g) of the experimental fish during the trial.



Figure 2: Variations of specific growth rates (SGR; %; Weight) of the experimental fish during the trial.



Figure 3: Variations of specific growth rates (SGR; %; Length) of the experimental fish during the trial .



Figure 4: Variations of condition factors (CF) of the experimental fish during the trial .



Figure 5: Variations of thermal growth coefficient (TGC; Weight) of the experimental fish during the trial



Figure 6: Variations of thermal growth coefficient (TGC; Length) of the experimental fish during the trial.

In general, daily relative growth rate as percentage of live weight in rainbow trout is reported to vary between 0.2% and 1.0%, and, in some cases, it reaches up to 4.0% (Logan and Johnston, 1992). In this study, brook trout and rainbow trout showed similarities in pure environment in sea water and fresh water. but brook trout showed similarities themselves among in polyculture environment in sea water and rainbow trout among themselves. However, there were differences with the pure environment (Table 1). The rapid growth, being commercially viable, better feed conversion, feed loss kept to a minimum, and the optimum usage of storage volume are the most important criteria in fish farming. The feed meeting the qualitative requirements of the fish needs to be shared equally among group members to ensure fast and uniform growth. Different feed intake between individuals may result from behavior differences due to excessive levels of competition for limited resources, and from the dominant hierarchy in the (Jobling, 1995), and this group increases the difference in size between individuals and decreases the rise in biomass. According to studies (Jobling, 1995; Okumuş et al., 1999), area defense and dominant hierarchy is inevitable in the farming of Salmonidae family members in small groups. In this case, the larger and more active individuals are located at the top of the hierarchical order and require more food, which leads to inequality in feed consumption (Holm et al., 1990). Therefore, in cases when constant feeding is not possible, that is, when daily feed is given in 1 to 3 meals in a short time, competition among differences individuals and size (Jobling. 1995) increases and competition for food becomes an important factor limiting the growth of young individuals. For these reasons, as in this study, dominant hierarchy and different food consumption is inevitable in an environment of two species with behavioral characteristics. different Rainbow trout in polyculture environment in fresh water and sea showed better water growth performance. There were similarities between the two species in pure cultures (Table 1, Fig. 1). In short, rainbow trout which is more active and provides better adaptation to culture conditions is certain to be more advantageous than brook trout in terms of growth. The rainbow trout in polyculture group grows faster than pure bred counterparts, which results from their being more advantageous in competition with brook trout than with each other. The difference between groups can be said to be, largely, due to food consumption. Condition factor was the highest for rainbow trout in polyculture environment in sea water and was similar to the pure environment. However, there were similarities between rainbow trout in the pure environment in sea water and pure and polyculture rainbow trout groups in fresh water. There was no

difference between condition factors for trout. Although increasing brook condition factor is, partially, due to the fullness of stomach and intestines, it is, to a large extent, due to textural growth as the fish were left hungry before weighing (Storebakken and Austreng, 1987). In general, the average rainbow trout condition factor values were relatively high. This is due to relatively deeper body of the rainbow trout, its higher feed consumption and rapid growth. In this study, it was determined that both species can converse the feed very well if conditions are available, which is proved by the feed conversion ratio falling below 1.0 in all groups (Table 1). Because feed conversion ratio is affected by different factors such as biological value of the feed, the ratio of the main components, stocking density, genetic line of the fish, its size and need for life, water temperature, feeding method and frequency (Smith et al., 1985; Austreng et al., 1987; Storebakken and Austreng, 1987; Johnston, 1992), Logan and the comparison of the results of different studies may not be of practical value. However, the values obtained in this study (in sea water, 0.702±0.012 pure rainbow trout, 0.624±0.048 polyculture and 0.699±0.014 pure brook trout, in fresh water, 0.685±0.016 pure rainbow trout, 0.636±0.003 polyculture and 0.616±0.014 pure brook trout) are not similar to the values (1.2-3.0) defined for rainbow trout by different researchers and evaluated by Logan and Johnston (Logan and Johnston, 1992).

Predicted feed conversion values during the study indicated that there is no need for over-feeding to provide rapid growth.

In conclusion, this study aimed to evaluate pure and polyculture farming of rainbow and brook trout in sea water fresh water. Growing faster and compared to other salmon. the dominant species rainbow trout provided significant advantage in terms of growth and feed conversion. Brook trout caused no problems other than slower growth compared to pure culture. Rainbow trout had faster growth than brook trout under pure and polyculture conditions in seawater and freshwater. In addition, although brook trout culture was claimed to be successful in places with no contamination risk which have lower temperature rise even in winter and which are supported with clean spring water (Huet, 1970), it was observed that it could be grown in similar conditions with rainbow trout.

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