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The Effects of Salinity, Temperature and Feed Ratio on Growth Performance of European Sea Bass (*Dicentrarchus labrax* L., 1758) in the Water Obtained Through Reverse Osmosis System and a Natural River

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Abstract.- In the present study, the effects of effluent water with varying salinity levels coming from the river and reverse osmosis device, temperature and different feeding ratios on the growth performance of European sea bass (*Dicentrarchus labrax*, L., 1758) fry was studied. This study was conducted in two different periods (winter and summer seasons). European sea bass fries were obtained from local hatchery with a mean weight of 5.95 (±1.18) g for the first period (November 2010-January 2011) and 5.74 (±1.25) g for the second period (May 2011-July 2011). Fish were progressively exposed to three experimental salinities (10-18-24‰). These experiments were accomplished simultaneously by two repetitions and were performed between November 2010 – January 2011 (14.91±3.49°C) and May – July 2011 (25.23±4.82°C) for 60 days in both periods. In each experiment, 600 European sea bass fries were used. Trail fish were placed into 640 L polyester tanks, which are at three different salinity levels (10-18-24‰) and fed by two different feeding ratios (*ad libitum* and 2.5% of the body weight). As a result of the two different experiments performed in two different periods, it was found that while *ad libitum* feeding ratio and temperature had positive effect on the growth performance of the fish, among three different levels of the salinity, the optimum positive effect on the growth of the fish were found for the 18‰ salinity concentration.

Keywords: Salinity, aquaculture, feeding ratio, relative condition, FCR, SGR.

INTRODUCTION

Aquaculture of European sea bass, *Dicentrarchus labrax* and Gilthead sea bream, *Sparus aurata*, which is performed in cages and earthen ponds, has an important role in Muğla Province, Turkey. In the province, the total production of marine fishes in net cages is 67.000 tones/year and the total marine fish production in earthen ponds using brackish water (2-38‰) is 8.800 tones/year (Tüik, 2012).

Seven villages in Milas District of the province have underground waters having a stable temperature throughout a year and they are brackish waters where salinity range changes from 2‰ to 38‰. Before the aquaculture activities in these areas, farmers used to grow cotton cultivation and agriculture but the soil was too salty for agricultural activities because of the brackish underground waters. Since 1986, these areas have been used for marine fish farming, which compose a great economic value. There are 168 fish farms located in the region with the capacity varying from 30 to 2000 tons/year. All these farms utilize underground water by use of electrical pumps. These farmers take the fish fry 2-5 g from hatcheries and grow-out the fish over kilos. Grow-out period is between 18 and 24 months by using electric powered pumps. As a result of intense discharge of underground water sources and pressure of seawater, the salinity of the underground water has gone up and accordingly, the collapsing risk of sinkholes in the region having carstic structure has increased. Proper use of the rivers for fish farming in the region is important for the sustainability of the fish farming (Köymenoğlu, 2013; Ercan *et al.*, 2012; Köymenoğlu and Ercan, 2013; Ercan and Tarkan, 2014). There are free flow brackish water rivers in this area that have about 198.48x10^6 m^3 total annual flow rate (Barut and Gürpınar, 2005). These rivers were not used for aquaculture activities. Ekinanbarı River is one of the useful rivers in this riverine system having no pollution. Its salinity is the same ratio as in underground water, which used to use for fish farming in this region.

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Gradual decrease in the water sources of the world has naturally resulted in greater interest in the purification and reuse of wastewaters. Purification of wastewaters can be done through various methods. One of them is reverse osmosis, which is the most advanced purification mechanism (Morton *et al.,* 1996). Reverse osmosis membrane serves the function of a barrier for all the dissolved salts, inorganic molecules and organic molecules with a molecule weight bigger than 100u. On the other hand, water molecules freely run through the membrane and make up the non-mineral water called product water (Salt and Dinçer, 2006). The discharge ratio of salt in reverse osmosis system is between 95 and 99% (Kitiş and Yiğit, 2009). The main application areas of reverse osmosis are water (raw water) purification, purification of domestic and industrial wastewaters, softening and removal of organics, obtaining industrial process water, and removal of salt from slightly salty waters and seawaters to obtain drinking water (Kitiş and Yiğit, 2009; Akgül, 2006; Güler, 2011). With this process, drainage of intensely saline water is performed and as an outcome usable water is created for marine aquaculture.

European sea bass is one of the most economical euryhaline fish in Turkey. This fish species is widely cultured in Turkey, as it can live in fresh water and at various salinity levels (Eroldoğan *et al.,* 2004; Memiş, 2010; Sanchez-Vazquez and Munoz-Cueto, 2014). Because of the high tolerance to salinity, this fish species is widely cultured in earthen ponds filled with underground waters of Milas district of Muğla province. Most of these ponds are bigger than thousand m^2 area and the water instauration time is over 2 days. Every pond is oxygenated by pedal-wheel aerators. These aerators are electric powered machines that work on the surface of the water and oxygenating by flapping the surface water by pedals. The water temperature is changed by this equipment due to effect of the air temperature. In the wintertime, the lowest pond water temperature is 10ºC and in the summer time the pond water temperature increases up to 30ºC (Ercan *et al.,* 2011). There are some studies on the effects of the energy uses for osmoregulation on the growth performance of marine fishes (Gatlin *et al.,* 1992; Claireaux and Lagardere, 1999; Boeuf and Payan, 2001; Rubio *et al.,* 2005; Marshall and Grosell, 2006; Conides and Glamuzina, 2006; Deane and Woo, 2009). Many attempts have been done to minimize this energy loss of European sea bass (Boge *et al.,* 1983; Hepher, 1988; Eroldoğan *et al.,* 2004; Rubio *et al.,* 2005; Appelbaum *et al.,* 2008; Cnaani *et al.,* 2009). There are also some studies in the literature investigating the metabolisms and feeding methods of European sea bass in aquatic environments with different temperatures and salinities (Russell *et al.,* 1996; Azzaydi *et al.,* 1998, 1999, 2000; Paspatis *et al.,* 1999; Peres and Oliva-Teles, 1999; Claireaux and Lagardere, 1999; Ayala *et al.,* 2001; Saillant *et al.,* 2003; Ruyet *et al.,* 2004; Eroldoğan *et al.,* 2004; Güroy *et al.,* 2006; Dikel, 2009; Dülger *et al.,* 2012).

The aim of this study was to examine effects of a natural river water with ‰10 salinity and discharged desalination waters of the river with different levels of salinity (18‰ and 24‰) obtained by reverse osmosis system, different feeding ratios and temperature in two different seasons on the growth performance of European sea bass at prior growing period (5-20 g). Also, this study aims to reveal potential use of surface waters in European sea bass rearing and help to converse underground waters by utilizing salty water provided by reverse osmos.

MATERIALS AND METHODS

All experiments were performed in the form of two repetitions in two periods. First period was between November 2010 and January 2011 (14.91±3.49°C) and second period was between May and July 2011 (25.23±4.82°C) for 60 days in each period.

Experimental fishes

For the study, totally 1200 sea bass fries with a mean weight of 5.95±1.18 g for the first period and 5.74±1.25g for the second period were used. For the fish specimens obtained from the hatchery with 30‰ salinity level, gradual adaptation to experimental waters with the salinity levels of 10, 18, and 24‰, respectively were carried out. All fish samples were weighed with a scale (±0.01 g) at the first and last period of sampling and their total length was measured with a ruler. Throughout the experiment, monthly measurements were performed on each fish in all different salinity groups. During the measurements, the fishes were anesthetized by using phenoxyethanol (0.5 ml L^{-1}) (Mylonas *et al.*, 2005).

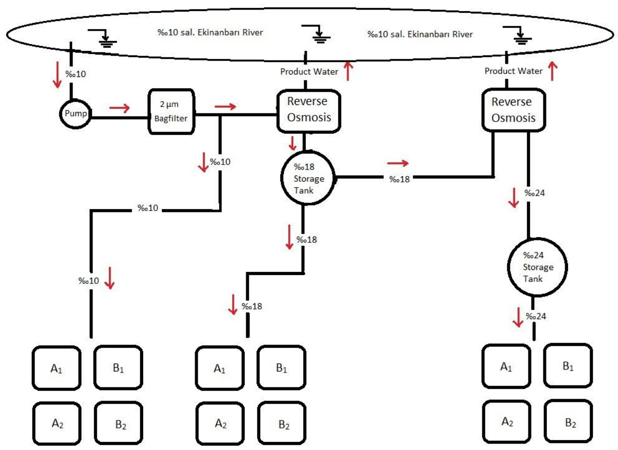

Fig. 1. Schematic view of produced water with different salinities from Ekinanbarı River generated by reverse osmosis system (A, *ad libitum* group; B, 2.5% BW feeding group).

Experimental water

The experiments were performed with the water taken from Ekinanbarı River having 10‰ salinity. This water was mechanically filtered through bag filters (2µm) and then used for the generation of discharge waters having differing salinity levels (18‰ and 24‰) by reverse osmosis (Fig. 1). The river water was first processed with reverse osmosis to have 18‰ discharge water and through the second reverse osmosis processing of this water, 24‰ discharge water was obtained. These waters were then used as the experimental waters. Throughout the study, dissolved oxygen, pH, salinity and temperature parameters were measured with multi-parameter (Oxyguard Polaris oxygen meter and WTW pH meter) twice each day, one in the morning and the other in the evening. All data collected were evaluated at the end of experiment.

Experimental set-up

In the study, the fishes were placed in groups of 50 fry/tank into 12 polyethylene tanks (640 L, 80 cm x 80 cm x 100 cm) with three different levels of salinity. All the experimental tanks were fed by atmospheric air for oxygenation. Each tank had different water inlet and outlet and the water was added to the system in the form of water renewal. Throughout the study, cleaning of the tanks was regularly performed. Water was exchanged twice a day and the system was open flow system.

Feeding rations

In the study, two different feeding rates were applied to the experimental groups. Feeding was 2.5% of the body weight and *ad libitum. Ad libitum* ratios were changed according to the season (*i.e.*, 3.87% of BW in winter; 6.90% of BW in summer). Differences in growth performance was calculated and statically analyzed at the end of experiments. Same commercial feed was used in both periods (KILIÇ fish feed manufacturer, 2mm European Sea Bass fish feed with 49%Crude Protein, 19% Crude Fat).

Analysis of the growth performance

Throughout the study, live weight and length measurements were monthly taken. Records of the dead fishes were kept daily and the percentages of survival were calculated at the end of the experiment. Comparing body condition of the samples, relative condition factor (RCF) was used (*e.g.*. Gaygusuz *et al.,* 2013). In order to be able to use this index, all groups were sampled at the same time. RCF = W/W', W is measured weight of the fish, W' is weight value obtained from the relationship between the length and weight of the fish, the relationship between the length and weight of the fish was calculated with the formula $W' = aL^b$. In this equation, a = intercept, b = slope of the line. The results higher than 1 indicate that the mean condition is better than that of the compared fishes at the same length and the results lower than 1 indicate that the mean condition is worse. Through the equations given below the growth of the fishes were calculated based on their weights and lengths (Dahl, 1909; Carlander, 1945; Ricker, 1979).

$$\text{Weight gain (\%)} = (A_2 - A_1)/A_1)*100 \quad (1)$$

A_1, mean initial weight; A_2, mean final weight.

Specific growth rate (SGR) = $[(lnB_2\text{-}lnB_1)/t]$ ×100 (2)

B_1, individual mean initial weight; B_2, individual mean final weight; t, days of periods.

Feed conversion rate (FCR) = $[C/(B_2\text{-}B_1)]+M$ (3)

B_1, individual mean initial weight; B_2, individual mean final weight; C, amount of feed.

Survival rate (%M) = (N_2/N_1)×100 (4)

M, mortality; N_1, initial number of fish; N_2, final number of fish

Statistical analysis

Differences of growth variables between the seasonal periods, feeding ratios at each salinity level, and temperature differences between two examined seasonal periods for all salinity levels and sampling dates were tested by Student's *t*-test, whereas differences in growth variables and relative condition values among different salinity levels were statistically tested by analysis of variance (ANOVA). When significant differences among the variables were detected, Tukey's honestly significant difference (HSD) test was used to determine which feeding ratios and environmental variables (temperature and salinity) were different. Significance levels were set at $P < 0.05$ (Zar, 1984).

RESULTS

Water quality

In both experimental periods, the average temperature was 14.91°C ± 3.49 between November 2010 - January 2011 and 25.23°C ± 4.82 between June - July 2011 being first period significantly colder than second period at all salinity levels and differ ent sampling times (Student's *t*-test, $P < 0.001$) (Table 1). There were no significant differences observed among the oxygen and pH values among the salinity levels and times (ANOVA, $P > 0.05$); mean values were measured to be 8.31 ± 0.32 and 8.86 ± 0.30, respectively. Periodical changes among the water quality parameters are presented in Table I.

Growth performance of the fishes

Growth performances were studied by evaluating the data obtained from the measurements done periodically in the groups subjected to varying salinity levels. All growth variables obtained in the first period conducted in November 2010 – January 2011 period when the weather was relatively colder were significantly lower (Student's *t*-test, $P < 0.001$) than those obtained in the second period conducted in May and July 2011 period (Table II).

In the first period, the highest percentage live weight gain was found to be 84.03% in the group fed with *ad libitum* in the tank with 10‰ salinity being significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$). The lowest live weight gain was found to be 53.28% in the group fed with 2.5% of its weight in the tank with 24‰ salinity. In the second period conducted in April-July period, the highest percentage weight gain was calculated to be 300.52% in the group fed with *ad libitum* in the tank with 18‰ salinity being significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$) and the lowest percentage weight gain was calculated to be 209.40% in the group fed with 2.5% of its weight in the tank with 24‰ salinity (Table II).

As regards the specific growth ratio (SGR), in the first experiment conducted in November-January period, the highest value was calculated to be 1.02 in the group fed *ad libitum* at 10‰ salinity, which was significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$). The lowest value was calculated to be 0.71 in the group fed 2.5% of its body weight at 24‰ salinity. In the second period, the specific growth rate was calculated to be highest as 2.31 in the group fed *ad libitum* at 18‰ salinity (ANOVA, Tukey HSD, $P < 0.05$) being significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$). Here, the lowest value was calculated to be 1.88 in the group fed 2.5% of its weight at 24‰ salinity (Table II).

The highest value of food consumption ratio (FCR) was calculated to be 3.67 in the first period for the group fed *ad libitum* at 24‰ salinity in November-January period, which was significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$). The lowest value was calculated to be 2.21 for the group fed 2.5% of its weight at 18‰ salinity.

Table I.- **Water quality parameters during the experiment.**

Salinity	Day	Dis.O_2 (mg L^{-1})	pH	Salinity (ppt)	1st Period temp. (°C)	2nd Period temp. (°C)
	1	7.92±0.34	8.60±0.29	10.17±0.24	18.13±3.24	20.13±4.82
‰10	30	8.52±0.34	8.90±0.29	10.28±0.24	15.84±3.24	25.84±4.82
	60	8.50±0.34	9.18±0.29	10.63±0.24	11.72±3.24	29.72±4.82
	1	7.94±0.32	8.54±0.30	18.27±0.09	18.15±3.59	20.13±4.82
‰18	30	8.51±0.32	8.84±0.30	18.11±0.09	15.10±3.59	25.84±4.82
	60	8.50±0.32	9.15±0.30	18.28±0.09	10.98±3.59	29.72±4.82
	1	7.94±0.32	8.55±0.30	23.46±0.43	18.24±3.65	20.13±4.82
‰24	30	8.50±0.32	8.85±0.30	23.97±0.43	15.14±3.65	25.84±4.82
	60	8.50±0.32	9.16±0.30	24.33±0.43	10.96±3.65	29.72±4.82

Table II.- **The mean value of periodically growth parameters and comparisons within the groups.**

Periods	Salinity	Feeding rate	Day 1 (Initial)		Day 60 (Final)		%M	%W.G.	S.G.R.	FCR
			Weight (g)	Length (cm)	Weight (g)	Length (cm)				
1st	‰10	Ad Lib.	5.95±1.18	8.12±0.55	10.95±2.28	9.75±0.67	1	84.03^a	1.02^a	3.24^a
		%2.5	5.95±1.18	8.12±0.55	10.48±2.28	9.64±0.67	1	76.13^a	0.94^b	2.82^b
2nd	‰10	Ad Lib.	5.74±1.25	7.94±0.57	20.56±4.96	11.75±0.91	0	258.18^b	2.13^c	1.33^c
		%2.5	5.74±1.25	7.94±0.57	19.89±4.96	11.52±0.91	1	246.51^b	2.07^c	1.25^c
1st	‰18	Ad Lib.	5.95±1.18	8.12±0.55	10.44±2.28	9.42±0.67	4	75.46^b	0.94^b	2.59^d
		%2.5	5.95±1.18	8.12±0.55	10.25±2.28	9.38±0.67	9	72.27^b	0.91^b	2.21^d
2nd	‰18	Ad Lib.	5.74±1.25	7.94±0.57	22.99±4.96	12.03±0.91	0	300.52^d	2.31^c	1.07^e
		%2.5	5.74±1.25	7.94±0.57	22.02±4.96	11.77±0.91	0	283.62^d	2.24^c	1.05^e
1st	‰24	Ad Lib.	5.95±1.18	8.12±0.55	9.73±2.28	9.96±0.67	7	63.53e	0.82b	3.67a
		%2.5	5.95±1.18	8.12±0.55	9.12±2.28	9.19±0.67	8	53.28e	0.71b	2.41f
2nd	‰24	Ad Lib.	5.74±1.25	7.94±0.57	18.34±4.96	11.41±0.91	1	219.51f	1.94e	2.63f
		%2.5	5.74±1.25	7.94±0.57	17.76±4.96	11.68±0.91	1	209.40f	1.88e	2.53f

% W.G., S.G.R. and FCR values that do not share the same letter show significant differences (P<0.05). %M, percentage of mortality; %W.G., percentage of weight gain; FCR, feed conversion rate; SGR, specific growth rate

In the second period conducted in April-July period, the lowest value of FCR was calculated to be 1.05 for the group fed with 2.5% of its weight at 18‰ salinity and the highest value was calculated to be 2.63 for the group fed *ad libitum* with 24‰ salinity, which was significantly higher than other groups (ANOVA, Tukey HSD, $P < 0.05$) (Table II).

At 10‰ salinity level in November-January period, both SGR and FCR fed by *ad libitum* had significantly higher values than those fed 2.5% of its weight (Student's *t*-test, $P < 0.05$) (Table 2). That was the same only for FCR at 24‰ salinity level in November-January period (Student's *t*-test, $P < 0.05$) (Table II).

When these results are evaluated for the first experiment, it is suggested that temperature has direct influence on the sea bass. While percentage dead ratio was calculated to be 5% in the first experiment, it was found to be 1% in the second experiment and it is assumed that the fishes died because of the manipulation of the fishes after periodic measurements (Table II).

Regression lines for the relationship between body weight and length were expressed separately for two experiments, three salinity levels and two feeding ratios, and all results showed that length and weight were highly correlated ($r^2 > 0.85$) (Fig. 2). Relative condition factor was found to be the highest in all groups at 18‰ salinity level (ANOVA, $P < 0.05$) (Table III).

Table III.- The relative condition factor.

Period	Feeding rate	Sal. (ppt)	Day 1 (Initial) Mean Value		Day 60 (Final) Mean Value		Relative condition
			Weight (g)	Length (cm)	Weight (g)	Length (cm)	
1st period	Ad Lib.	‰10	5.95±1.18	8.12±0.55	10.95±2.28	9.75±0.67	0.98763^{a}
		‰18	5.95±1.18	8.12±0.55	10.44±2.28	9.42±0.67	1.04508^{b}
		‰24	5.95±1.18	8.12±0.55	9.73±2.28	9.96±0.67	0.99315^{a}
	2.5% BW	‰10	5.95±1.18	8.12±0.55	10.48±2.28	9.64±0.67	0.98429^{a}
		‰18	5.95±1.18	8.12±0.55	10.25±2.28	9.38±0.67	1.02895^{b}
		‰24	5.95±1.18	8.12±0.55	9.12±2.28	9.19±0.67	0.99558^{a}
2nd period	Ad Lib.	‰10	5.74±1.25	7.94±0.57	20.57±4.96	11.76±0.91	1.08654^{b}
		‰18	5.74±1.25	7.94±0.57	19.89±4.96	11.52±0.91	1.12452^{c}
		‰24	5.74±1.25	7.94±0.57	18.34±4.96	11.42±0.91	1.07212^{b}
	2.5% BW	‰10	5.74±1.25	7.94±0.57	19.89±4.96	11.52±0.91	1.11009^{c}
		‰18	5.74±1.25	7.94±0.57	22.03±4.96	11.77±0.91	1.12980^{c}
		‰24	5.74±1.25	7.94±0.57	17.76±4.96	11.70±0.91	0.99340^{a}

Relative conditions values that do not share the same letter show significant differences (*P*<0.05).

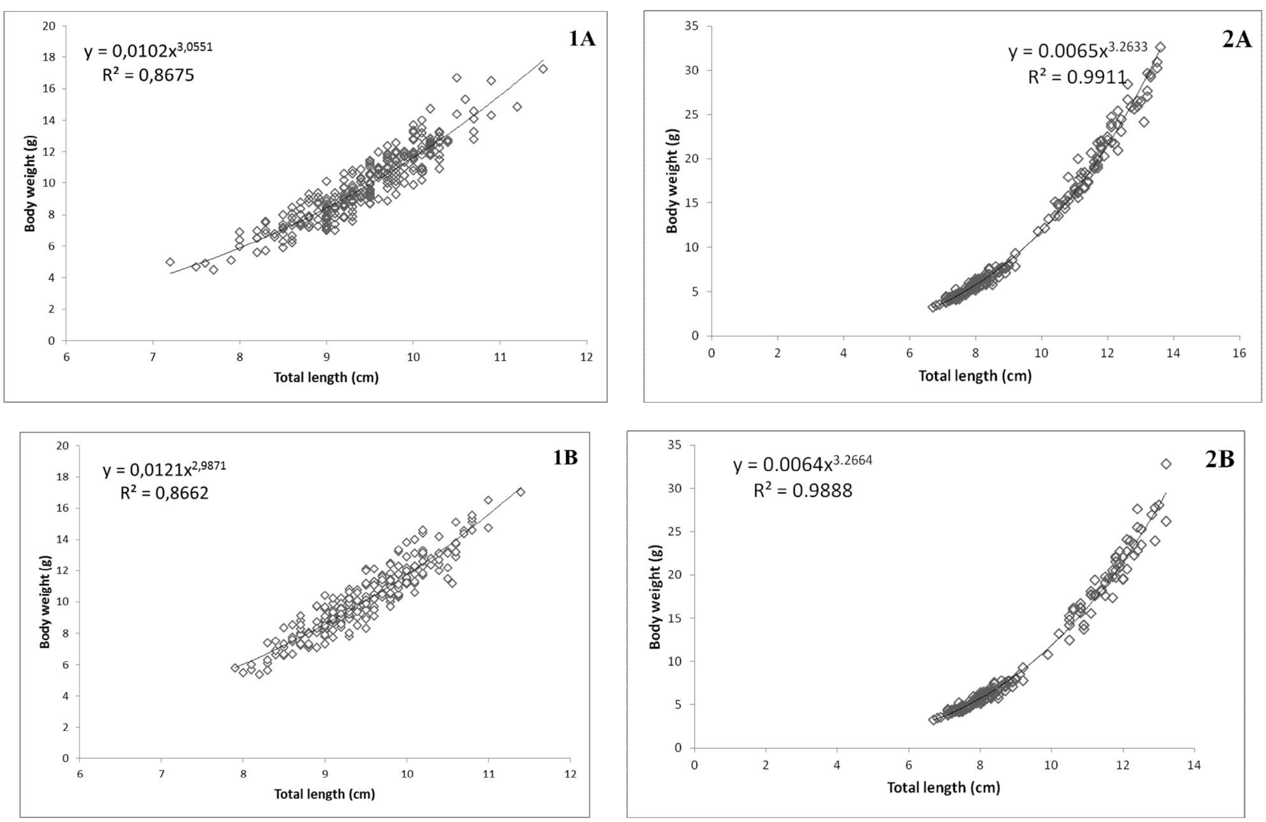

Fig. 2. Length-weight relationships in different experimental groups. 1A group, *ad libitum* group; 1B group: 2.5% BW feeding group; 2A group, *ad libitum* group; 2B group, 2.5% BW feeding group.

DISCUSSION

Reverse osmosis is the most advanced purification technology used in obtaining drinking water from seawater and removal of nutrients from fresh water sources (Morton *et al.*, 1996; Güler, 2011). In the current study, by using reverse osmosis system, water samples with 18‰ and 24‰ salinities were generated from Ekinanbarı River that has 10‰ salinity. This study, which attempts to find the effects of waters with different salinities, two different feeding ratios and growth performance of European sea bass is the first study at the use of desalination effluent water generated from reverse osmosis in marine aquaculture.

Physical and chemical quality of water is of great importance in aquaculture. Throughout the experiments, water temperature, dissolved oxygen, pH and salinity levels were measured and no negative variations in these variables affecting the life of fishes was detected at the end of experiments. When the growth performance of the fishes at the same period is examined, it was observed that there were some statistical differences in the same salinities but different feeding ratios (Table II). When feeding ratios are considered, it seems to be clear that the group fed *ad libitum* exhibited better growth performance than the group fed 2.5% of its weight. Specific growth ratio is the function of water temperature and size of the fish. It was reported that specific growth ratio decreased with decreasing water temperature and increasing fish size (Sumpter, 1992; Jobling, 1985; Karataş, 2005). In line with this, for the specific growth ratio calculated in the current study, the groups in the first experiment conducted in winter months exhibited worse growth performance than the groups in the second period conducted in summer months. Accordingly, live weight gain was also found to be best in the second period. When the effects of three different levels of salinities on growth are compared, best growth performance was in 18‰ salinity level. This growth performance detected in the present study is better than other studies conducted with similar salinity levels (Akbulut and Şahin, 1999; Abdalla, 2009). There were also some other studies examining effects of different salinities on food intake, food conversion ratios and growth rates of European sea bass. According to these studies (Dendrinos and Thorpe, 1985; Conides and Glamuzina, 2006; Rubio *et al.*, 2005) the authors found different results for the optimum environmental conditions, which affect the growth rate, feeding ratio and food conversion ratio. The results showed that salinity levels between 25-30 ‰ were better for the growth rate. They specifically recorded that 18 ‰ salinity level at 19°C provided optimum conditions for food intake and growth performance. These results are closely consistent with the findings found in the present study. All of the aforementioned studies also stated that the fish need more energy for osmoregulatory process at low salinity levels.

As a result, in the present study, it is revealed that under the climatic conditions of Milas province, water temperature is an important factor restricting the culturing of European sea bass. In the months when water temperature is below 10ºC the growth performance of the fishes is slow and their FCR is high. In light of the findings of the present study, it can be argued that fish farms buying fishes from hatcheries to raise them in their earthen ponds in winter months for growing over kilos would have some economic and time loss and consequently, wintering European sea bass fries in earthen ponds becomes useless. As a result of the regulations put into effect in 2007, fishes should be carried to open cages and this leads to great loss, as well (Ercan *et al.*, 2011). Use of earthen ponds to grow the fish up to 20-30 g may have great contribution to the prevention of this losses and this may bring some economic benefits.

In the current study, saline water generated from reverse osmosis system is used in aquaculture for the first time and it was concluded that in proper temperatures, this generated water could be used in aquaculture. Through reverse osmosis, river waters can be reached to the desired salinity levels (found as 18‰ in the present study) and with this way potential water sources in this region can be used effectively and in a sustainable manner.

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