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RESEARCH PAPER

Alteration of Growth and Temperature Tolerance of European Sea Bass (*Dicentrarchus labrax* Linnaeus 1758) in Different Temperature and Salinity Combinations

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Abstract

This study was performed to assess the combined effects of three salinities (0, 20 and 38 ppt) and three acclimation temperatures (15, 20 and 25°C) on growth performance, proximate composition and thermal tolerance of European Sea Bass (32 g) inhabiting southern parts of the Mediterranean Sea. Highest fish final weight (83.8 g) was achieved at 25°C in freshwater (FW). Specific growth rate (SGR) at 25°C was about twice higher than that at 15°C, while 20°C promoted an intermediate growth. The fish cultured in FW at 25°C displayed consistently lower FCR and better SGR than those in full strength seawater (SW). Salinity had strong influence on fillet protein and lipid compositions. Highest lipid content (14.9%) was found in fish held in brackish water (20 ppt), and the fish held in SW had the lowest lipid content. CTMin and CTMax values ranged from 3.26°C to 7.33°C and from 31.81°C to 36.68°C, respectively. The thermal tolerance results indicated that European sea bass is more sensitive to high rather than low temperatures. These results suggested that this species can be farmed in stagnant SW deep ponds with high ambient temperatures during hot summer months in southern parts of the Mediterranean. During cold winter months, however, this fish can be successfully cultured in low salinities or even in FW.

Introduction

Temperature and salinity are two of the most important environmental factors effecting survival and growth performance of marine organisms (Lutterschmidt & Hutchison, 1997). Temperature tolerance in fish varies according to species, acclimation temperature and acclimation time (Das, et al., 2004; Manush, Pal, Chatterjee, Das, & Mukherjee, 2004) and salinity (Jian, Cheng, & Chen, 2003; Kumlu, Kumlu & Türkmen, 2010). Therefore, knowing the effects of acclimation temperature and salinity on critical temperatures is important to understand how a species' biology can respond to spatial or temporal temperature (Herrera, Uribe, Ramirez, & Mora, 1998) during cold winter or dry hot summer months particularly in sub-tropical areas.

The European Sea Bass (*Dicentrarchus labrax* Linnaeus, 1758) belonging to the family Moronidae, is a euryhaline and eurythermic species, which reproduce in marine environment but inhabit mostly coastal waters and lagunar areas with extreme salinity and temperature changes during their juvenile stages (Vargas-Chacoff et al., 2009). It is a commercially valuable species in the Mediterranean countries, where generally cultured in cages or ponds, in which the fish might face great fluctuations in temperatures and salinity throughout the production period, especially in shallow ponds. Although widely used in many countries, there is very limited information on the minimum and / or maximum temperature levels that this species can tolerate under various types of salinity conditions. The sea bass is able to tolerate a wide range of temperatures from 5 to 28 °C and extreme salinity levels from 5 to 50 ppt. Moreover, its optimum temperature is considered to be between 22 and 24 °C (Claridge & Potter, 1983) or even 27 °C (Lanari, D'Agora, & Ballestrazzi, 2002). Juvenile sea bass grow fast at 22-25°C and lethal limits can be 2-3 °C and 30-32 °C. Yet, Russell, Fish & Wootton. (1996) reported that the lethal limit range for the growth of juvenile sea bass in in British waters (at the northern Mediterranean Sea) was high at even 18 °C.

There may also be intra-specific differences in growth–temperature relations among populations from different geographical regions (Imsland & Jonassen, 2001; Dülger et al., 2012). For maximum juvenile growth, optimal temperature and salinity values was estimated as 26 °C and 34-35 ppt in a Western Mediterranean population (Person- Le Ruyet, Mahe, Le Bayon & Le Delliou, 2004). In another study, Eroldoğan & Kumlu (2002) found that European sea bass grew significantly better in freshwater (FW) as compared to either full strength seawater (SW, 39 ppt) or brackish water (BW, 20 ppt). The researchers were related this better growth in FW to osmoregulation and suggested that fish increased feed consumption in order to meet their ionic requirements. Lanari et al. (2002) investigated the effects of seven different temperatures (between 13 and 31 °C) on various sizes European sea bass from different geographical location. They concluded that growth and food consumption elevation starts at 22 °C and this climb continues up to 27 °C in SW. Alliot, Pastoureaud & Thebault, (1983) reported that sea bass at 22 °C grew significantly better and consumed more food than those at 15 °C. More recently, Dülger et al., (2012) investigated thermal tolerance of European sea bass at ambient salinity condition of the eastern Mediterranean Sea (38 ppt) and reported that CTMin ranged from 4.10 °C to 6.77 °C and CTMax from 33.23 °C to 35.95 °C in three acclimation temperatures between 15 °C and 25 °C. Also, Kır & Demirci (2018) reported that dynamic and static thermal tolerance zones of juvenile European sea bass (1.2 g) acclimated to four different temperatures were 861 °C^2 and 613 °C^2, respectively and their study showed that European sea bass has a low acclimatization capacity to survive in aquatic systems characterized by wide temperature fluctuations. Nevertheless, it is still unclear if suboptimal salinities or complete freshwater might alter the thermal tolerance, growth performance and food consumption of this species.

In some of the western parts of Turkey, sea bass is farmed in shallow earthen ponds, in which water is mostly pumped from deep-wells, and osmoregulatory and metabolic parameters of the fish might be altered with seasonal changes in temperature and salinity. In such areas, with limited water exchange rate, salinity and temperature of pond water may drop to 5 ppt/5-6 °C during winter or increase up to over 40 ppt/34 °C in the summer (Dülger, 2011). Thus, it is possible that interaction in between temperature and salinity can be expected to alter metabolic responses, growth performance and feed intake of sea bass under such farming conditions. Vargas-Chacoff et al. (2009) have already demonstrated that not only temperature, but also interactive effects of temperature and salinity could alter some metabolic responses of gilthead sea bream (*Sparus aurata*) cultured for 7 weeks in three different salinities (5, 38 and 55 ppt) and temperatures (12, 19 and 26 °C). Nathanailides, Paschos, Tsoumani, Perdikaris & Kapareliotis, (2010) reported that the growth rate of European sea bass juveniles (~7 g) were similar both in FW ponds and marine cages at ambient temperatures, but mass mortalities occurred when temperatures dropped below to 5 °C in ponds compared to those in cages, in which water temperatures always stayed above 15 °C. Understanding of the effects of temperature and salinity or their interaction on physiology, long-term growth performance and food consumption of European sea bass is important for the success of their aquaculture. Therefore, the main aim of the present study was to assess growth, food consumption as well as temperature tolerance of European sea bass juveniles at three different acclimation salinities (0, 20 and 38 ppt) and temperatures (15, 20 and 25 °C).

Materials and Methods

Experimental Fish and Acclimation Procedures

The European sea bass juveniles (initial body weight = 32 g) reared in Yumurtalık Marine Research Station of Cukurova University (Adana, Turkey) were divided into three groups and cultured in SW (38 ppt), BW (20 ppt) and FW (0 ppt) for 2 weeks in 3 different fiberglass tanks (diameter = 2 m) for salinity acclimation. Dechlorinated tapwater was used to reduce the salinity at a rate of 2 ppt day^{-1} until the desired test salinities were reached.

For each temperature/salinity group, a total of 90 fish were taken from the acclimation tanks and randomly stocked into 0.6 m diametered 6 round fiberglass tanks (250 L) at a density of 30 fish per replicate tank. Each salinity group was then acclimated to three temperatures (15, 20 and 25 °C) at a rate of 2 °C day^{-1} by using 300 or 600-watt thermostatically controlled aquarium heaters. After allowing a week for temperature acclimation, a total of 810 fish were separately cultured in their respective water temperatures and salinity combinations (3 x 3 x 3 = temperature, salinity, replicates) for a further 35 days, before the CTM tests to stabilize their internal mediums and allow metabolic compensation (Castille & Lawrence, 1981) or for a 60-day experimental period to investigate growth and food consumption as well.

Growth Trial and Sample Collection

The fish were fed a commercial sea bass diet (45% crude protein, 18% crude lipid) produced by Çamlı Yem Ltd. (Izmir, Turkey) three times a day (09.00, 12:00 and 18.00) until satiation. Throughout the culture period (60 days), temperature was regularly measured twice daily with a thermometer during feeding times. Salinity levels were checked using a YSI salinometer (Model Y30, Yellow Springs Instrument, OH, USA). The photoperiod was maintained at 12 h light/12 h dark. Light intensity was calibrated to be 100 lux at water surface and was kept the same during the entire experimental period. Throughout the experiment, water flow rate was maintained approximately 2 L min^{-1} and rearing water of each tank was permanently saturated with oxygen using air-stones. Oxygen measurements were taken twice daily with an oxygen meter (OxyGuard®, Birkerød, Denmark) and the tanks were siphoned off once every day. At the end of the 60-day, all the fish was sampled and anaesthetized with 0.3 mL per litre 2-phenoxyethanol (Sigma, St. Louis, MO, USA), and individually weighted to the nearest 0.01 g with a digital scale. Feeds were weighted every 10 day in order to estimate weekly feed consumption. Performance indices were calculated using the following formulas;

1. Feed conversation ratio (FCR) = (total feed consumed in g) / (weight gain in g).
2. Specific growth rate (SGR, % day^{-1}) = [Ln (final weight in g) – Ln (initial weight in g)] / (experimental period in days) × 100.
3. Daily feed intake (DFI, g) = (total feed consumed in g) / (experimental period in days)
4. Protein efficiency ratio (PER) = (final weight – initial weight) / (protein consumed during the entire experimental period).
5. Lipid efficiency ratio (LER) = (final weight – initial weight) / (lipid consumed during the entire experimental period).

A total of 6 fish from each salinity/temperature group were sampled for proximate analyses and the fillet of these fish were kept at -20 °C until analyses. All the fish handling procedures used in the present study were fully compliant with the Turkish guidelines for animal care (No. 28141), as set by the Ministry of Food, Agriculture and Livestock.

Chemical Analyses

The samples were thawed at +4 °C and fillet of 6 fish from each group were homogenized with a blender. The homogenats were dried at 103 °C for 12 hours and then, burnt at 450 °C for 6 hours to determine dry matter and crude ash, respectively. Crude protein content of the homogenats was analyzed via Kjeldal method (Matissek, Schnepel, & Steiner, 1989) and their crude lipid content was determined according to Bligh & Dyer (1959).

CTM Tests

All the culture procedures before the CTM tests were identical to those used in the growth-trial. Following the 35-day acclimation period, the fish were fasted for one day before performing the thermal tolerance tests, which were carried out in rectangular plastic containers (0.8 m x 0.35 m x 0.5 m), placed in thermostatically controlled water baths. One container was allocated for each replicate of the respective acclimation group and a total of 15 fish (5 from each replicate) were used in each assay. During the assay period, dissolved oxygen levels in the containers were maintained above 5 ppm by means of continuous aeration. The tests were separately conducted for CTMin and CTMax at cooling or heating rates of 0.3 °C min^{-1} as recommended by Beitinger, Bennett & McCouley (2000), Das et al. (2004; 2005), and Dalvi, Pal, Tiwari, Das & Baruah (2009).

The CTMin and CTMax values were calculated as the arithmetic mean of the collective thermal point at which endpoint was reached by individual random sample of fish. The endpoint is defined as the temperature, at which point the fish lost equilibrium (Beitinger et al. 2000; Paladino, Spotila, Schubauer & Kowalski 1980). Subsequently, all the fish were immediately transferred to their respective acclimation temperatures after the CTM tests to determine recovery rate for a duration of 24 h. The acclimation response ratio (ARR) was calculated by dividing the tolerance change to the total change for the 3 acclimation temperatures as suggested by Claussen (1977). The CTM data of the SW group was not included into the current study, because they were published earlier in Dülger et al. (2012).

Statistical Analysis

Growth performance and proximate composition data were given as mean ± standard deviation (n = 3). After checking for normality by Shapiro–Wilk's test and homogeneity of variances by Levene's test, two-way ANOVA was used to compare the groups with the help of SPSS software for Mac (version 21.0; IBM Corporation, Armonk, NY, USA). Independent samples t-test was used to identify significant differences between the two salinity levels of each rearing temperature. Tukey's pair-wise comparison test was used to localize differences in CTM results at the three-acclimation temperatures.

Results

Environmental Parameters

Actual average temperature and salinity levels measured throughout the study were 15.0±1.0 °C, 20.2±1.3 °C, 24.9±1.81 °C and 0.2±0.01, 20.0±1.20 and 38.0±0.60 ppt, respectively. Total ammonia, nitrite and nitrate were observed to be lower than critical levels (NH_3<0.1 mg L^{-1}, NO_2<0.1 mg L^{-1}, NO_3< 0.5 mg L^{-1}), while water pH levels ranged between 8.0 and 8.2.

Growth Performance and Feed Intake

At the end of the 60-day experimental period, temperature and temperature × salinity interaction were found to significantly affect the final weights of the groups (P<0.001), while no significant effects of salinity was observed in this respect (P>0.05). Regardless of the salinity, the groups cultured at 25 °C grew better than that of the other groups and average final weights of fish at this temperature ranged between 74.5 g at SW, 79.3 g at BW and 83.8 g at FW (Table 1). The lowest final weights (48.9 and 52.6 g) were observed in the groups reared at 15 °C. FCR values ranged between 1.1 and 1.4 (P>0.01) and both temperature and salinity had not significant effects (P>0.05) on this parameter. Specific growth rates (SGR) were significantly affected by temperature (P<0.001), whereas salinity or temperature × salinity interaction were insignificant (P<0.05). Highest and lowest SGRs were calculated as 1.6% day^{-1} and 0.7% day^{-1} in FW at 25 °C and 15 °C, respectively. Daily feed intake significantly increased with increasing temperature (P<0.001). No effects of salinity, temperature or temperature × salinity interaction (P>0.05) were detected on protein or lipid efficiency ratios (PER and LER). Neither salinity nor interactive effect with temperature altered SGR or feed intake parameters (FCR and DFI) among the experimental groups (P>0.05, Table 1).

Proximate Composition

Salinity was observed to be significantly important effects on dry matter content of the fish (P<0.001), while temperature had less pronounced effects on this parameter (P<0.05, Table 2). The highest dry matter was measured in fish held in SW at 25 °C, while the fish held FW at 15 °C had the lowest dry matter content (23.7%). There was no interactive effect of the two factors on dry matter contents of the fish. Temperature had significant effects on protein content, while salinity or interaction of the two factors had no impact. Temperature, salinity or temperature × salinity interaction significantly affected lipid composition of the fillet (P<0.001). Highest lipid content (14.9%) was found in fish held in BW, and lowest lipid content (9.1%) was found in fish held at SW (Table 2). Average lipid contents of the fillet, regardless of temperature, were calculated as 11.4%, 13.3% and 11.2% in FW, BW and SW, respectively. Regardless of the salinity, average lipid content were 11.1%, 12.9% and 11.9% in 15, 2 and 25 °C groups, respectively. The effects of temperature or temperature × salinity interaction on ash content of fillet were insignificant, while salinity had effects on the ash content significantly (P<0.05). The highest and lowest ash contents were found in SW at 25 °C (1.6%) and in FW at 20 °C (1.2%), respectively.

CTM tests

Overall, temperature (P<0.001), salinity (P<0.05) and the interaction of these two parameters significantly affected the CTMin and CTMax values of the fish (Table 3). However, our results have clearly demonstrated that the acclimation temperatures had clearly more profound effects on thermal tolerance of juveniles than salinity. The CTMax values rose from 31.81 to 34.46 °C in FW while from 32.25 to 36.68 °C in BW over the 10 °C temperature range used in this study. The CTMin ranged from 3.26 to 7.33 °C in FW and from 3.70 to 7.17 °C in BW in the three acclimation temperatures. In general, tolerance towards cold increased with decreasing acclimation temperatures (Table 3). The fish cultured at the lowest temperature (15 °C) displayed significantly higher tolerance (P<0.001) to cold than those held either at 20 °C or 25 °C.

Yet, no clear pattern of salinity effect on cold tolerance was observed within a given rearing temperature. At 15 °C, the fish reared in FW had higher tolerance (P<0.05) to cold than those held in BW, while this was just opposite for fish cultured at 20 °C (Table 4). Tolerance to cold did not differ (P>0.05) between FW and BW-groups when the juveniles were held at 25 °C. In general, the overall ARR values within the temperature range of 15 and 25 °C were calculated as 0.23-0.41 in FW and 0.34-0.44 in BW (Table 4). Thermal tolerance polygons of juvenile sea bass in between 15 and 25°C was calculated as 272.69 and 291.95°C^2 for FW and BW, respectively (Figure 1).

Following the CTMin tests, when the fish were returned to their respective experimental conditions, all of them successfully recovered and returned to normal state, whereas a few mortalities occurred after the CTMax tests (3 fish at 15 °C and 1 fish at 25 °C in FW).

Discussion

Temperature is well known factor to have effects on biochemical and physiological activities of aquatic animals. Its long-term effects lead to some enzymatic changes in fish metabolism (Hazel & Prosser, 1974). Rising temperature up to a certain limit favours aquaculture production, but beyond optimum limit, the metabolic stress caused by temperature adversely affects growth, food consumption and/or health of the fish under culture (Wedemeyer, Meyer, & Smith, 1976; Samaras, Papandroulakis, Lika, & Pavlidis, 2018). Studies conducted, so far, have been demonstrated that thermal tolerance of European sea bass ranges between 4 and 34°C (Dendrinos & Thorpe, 1985; Venturini et al., 1992; Allegrucci, Fortunato, Cataudella, & Sbordoni, 1994; Jensen, Madesen, & Kristiansen, 1998; Lanari et al., 2002; Person-Le Ruyet et al., 2004; Samaras et al., 2018). The optimal temperatures for the best growth lies in between 22°C and 28°C (Eroldoğan & Kumlu, 2002; Eroldoğan, Kumlu & Aktaş, 2004; Eroldoğan, Kumlu, Aktaş & Kiriş, 2005; Nebel et al., 2005;

Table 1. Growth performance of juvenile European sea bass (*Dicentrarchus labrax*) reared in different salinity (0, 20 and 38ppt) and temperature levels (15, 20 and 25 °C) for 60 days

		Growth Parameters						
Salinity (ppt)	Temperature (°C)	Initial Weight (g)	Final Weight (g)	SGR(% day^{-1})	FCR	DFI (g day^{-1})	PER	LER
0	15	31.5±1.0	^{C}48.9±1.9	^{C}0.7±0.1	1.4±0.1	^{C}5.8±1.1	1.5±0.2	3.9±0.5
	20	32.1±0.8	^{B}62.7±3.7	^{B}1.1±0.1	1.2±0.2	^{B}8.3±0.9	1.8±0.2	4.7±0.6
	25	31.9±0.5	^{A}83.8±5.7	^{A}1.6±0.1	1.2±0.1	^{A}14.1±1.1	1.6±0.5	4.2±1.1
20	15	31.9±0.4	^{C}52.6±1.4	^{C}0.8±0.1	1.2±0.2	^{C}5.7±0.3	1.8±0.2	4.7±0.6
	20	31.4±1.0	^{B}66.9±4.6	^{B}1.3±0.1	1.1±0.1	^{B}9.8±1.1	1.9±0.1	5.1±0.1
	25	32.3±0.5	^{A}79.3±4.6	^{A}1.5±0.1	1.2±0.1	^{A}14.2±0.5	1.7±0.1	4.4±0.3
38	15	33.6±0.7	^{C}55.1±4.4	^{B}0.8±0.1	1.3±0.2	^{C}6.4±0.5	1.6±0.5	4.3±0.8
	20	32.9±0.2	^{B}66.1±4.6	^{A}1.2±0.1	1.3±0.1	^{B}10.5±0.4	1.8±0.1	4.8±0.4
	25	32.1±0.4	^{A}74.5±5.9	^{A}1.1±0.2	1.4±0.2	^{A}13.6±1.1	1.6±0.2	4.2±0.8

Capital superscript letters indicate significant differences between the temperature levels within a salinity level. All values were presented as mean ± SD (n=3).

Table 2. Fillet proximate composition of juvenile European sea bass (*Dicentrarchus labrax*) reared in different salinity (0, 20 and 38ppt) and temperature levels (15, 20 and 25 °C) for 60 days

		Proximate Composition			
Salinity (ppt)	Temperature (°C)	Dry Matter (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
0	15	^{A}23.7±1.3^{b}	^{C}19.8±0.4^{c}	^{A}11.9±0.3^{a}	^{A}1.3±0.1^{b}
	20	^{A}24.1±1.3^{b}	^{C}20.4±0.1^{b}	^{A}11.7±0.6^{b}	^{A}1.6±0.1^{a}
	25	^{A}23.9±1.2^{b}	^{C}20.6±0.6^{b}	^{B}10.7±0.3^{b}	^{A}1.5±0.1^{a}
20	15	^{A}24.2±0.3^{b}	^{C}20.4±0.2^{b}	^{B}12.3±0.1^{a}	^{A}1.4±0.2^{a}
	20	^{A}24.9±1.1^{b}	^{C}19.2±0.2^{c}	^{A}14.9±1.4^{a}	^{A}1.4±0.1^{a}
	25	^{A}24.3±0.8^{b}	^{C}19.5±0.3^{c}	^{B}12.7±0.5^{a}	^{A}1.4±0.1^{a}
38	15	^{A}24.5±0.1^{b}	^{C}21.2±0.3^{a}	^{B}9.1±0.1^{b}	^{A}1.4±0.1^{a}
	20	^{A}26.8±0.9^{a}	^{B}22.5±0.3^{a}	^{A}12.2±0.8^{b}	^{A}1.3±0.1^{b}
	25	^{A}27.9±1.7^{a}	^{A}23.1±0.2^{a}	^{A}12.3±0.5^{a}	^{A}1.2±0.1^{b}

Capital superscript letters indicate significant differences between the temperature levels within a salinity level. Lowercase superscript letters indicate significant differences between the salinity levels within a temperature level. All values were presented as mean ± SD (n=3).

Table 3. CTMin and CTMax values of juvenile European Sea Bass (*Dicentrarchus labrax*) cultured in three acclimation temperatures and two salinities. Each value is mean ± SD (n = 15)

Temperature(°C)	CTM	Salinity	
		0 ppt	20 ppt
15	CTMin	^{C}3.26 ± 0.53^{b}	^{C}3.79 ± 0.14^{a}
20		^{B}6.75 ± 0.57^{b}	^{B}5.90 ± 0.16^{a}
25		^{A}7.33 ± 0.33^{a}	^{A}7.15 ± 0.32^{a}
15	CTMax	^{B}31.81 ± 0.8	^{C}32.25 ± 0.9^{b}
20		^{A}33.23 ± 1.6^{b}	^{B}35.22 ± 0.8^{a}
25		^{A}34.46 ± 1.7^{b}	^{A}36.68 ± 1.0^{a}

Capital superscript letters represent significant differences among the temperatures levels within a salinity level. Lowercase superscript letters indicate significant differences between the salinity levels within a temperature level.

Nathanailides et al., 2010; Hunt, Özkan, Engin & Tekelioğlu, 2011). Our results have demonstrated that temperature as well as the interaction of temperature x salinity had significant influences on growth of juvenile sea bass. At the end of the 60-day culture period, the SGR at 25 °C was about twice higher than that of at 15 °C, while 20 °C promoting an intermediate growth. DFI at 25 °C were almost two to three-fold higher than that of at 15 °C (P<0.05). Person le Ruyet et al. (2004) concluded that 26 °C optimized growth performance in the Western Mediterranean population of European sea bass. In our study, 25 °C (the highest temperature tested) promoted much faster growth than the lower rearing of 15 and 20 °C. In another study, Saillant, Fostier, Haffray, Menu & Chatain (2003), observed that the Western Mediterranean population of sea bass grew well at 29 °C, but only for a short period (2 months) and after which the growth rate drop significantly compared to those fish reared at 25 °C. These authors stated that their population had the capability to adapt high

Table 4. Regression equations and acclimation response ratio (ARR) of juvenile European Sea Bass (*Dicentrarchus labrax*) cultured in three different acclimation temperatures and two salinities

			ARR values for CTmin	
Salinity (ppt)	Regression Equations	15 – 20 °C	20 – 25 °C	15 – 25 °C
0	Y = 2.04x + 1.67 (R = 0.87)	0.68	0.14	0.41
20	Y = 1.68x + 2.26 (R = 0.98)	0.43	0.25	0.34
			ARR values for CTMax	
Salinity (ppt)	Regression Equations	15 – 20 °C	20 – 25 °C	15 – 25 °C
0	Y = 1.14x + 30.93 (R = 0.99)	0.26	0.19	0.23
20	Y = 2.20x + 30.36 (R = 0.96)	0.60	0.28	0.44

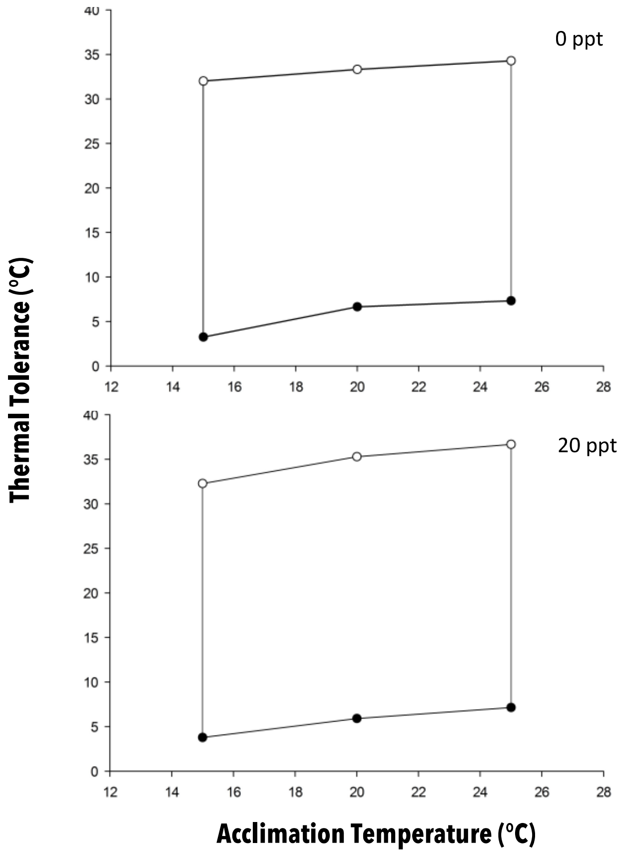

Figure 1. CTM based thermal tolerance polygons of juvenile European sea bass (*Dicentrarchus labrax*) reared in three acclimation temperatures (15, 20 and 25 °C) and two salinities (0 and 20 ppt). The area of thermal tolerance polygons were calculated as 272.69 °C^2 for freshwater and 291.95 °C^2 for 20 ppt salinity level.

temperatures, but the upper limit for good growth was close to 29 °C. Lanari et al. (2002) investigated the effects of seven different temperatures between 13 and 31 °C on various sized European sea bass and concluded that growth and food consumption rates start to increase at 22 °C and this trend continues up to 27 °C. We know from the results of our previous studies that the Eastern Mediterranean population of sea bass perform well at or even above 30 °C (Eroldoğan et al., 2004; 2005). Nevertheless, it still remains unknown whether the Eastern strain is able to sustain this adaptability for prolonged periods.

It is known that temperature is the key element influencing growth by metabolic regulations in poikilothermic fish in general (Lanari, 2002; Jun, Pao, Haizhen, Raiwei & Hui, 2012) and our data show that sea bass is not an exception of this. Besides, salinity can also contribute to growth by lowering energetic cost for osmoregulation, thus; this energy can be saved for other metabolic functions such as growth. Our study showed that salinity had considerably little effect on growth parameters of European sea bass but its influence was much clearer on fillet protein and lipid compositions of fish. Salinity also affects the transport of nutrient (Nordrum, Bakke-McKellep, Krogdahl & Buddington, 2000), which might cause selective accumulation of some macronutrients. Also, fish size or age, reproductive stage, and other seasonal variables rather than salinity are known to have effect on fat content and body composition of fish fillet (Alasalvar, Taylor, Zubcov, Shahidi & Alexis, 2002; Eroldoğan et al., 2004). The significance of previous thermal exposure history or acclimation temperatures on thermal tolerance have previously been well acknowledged in various fish species (Chatterjee, Pal, Manush, Das, & Mukherjee, 2004; Das et al., 2004 and 2005; Diaz et al., 2007). Our current results also confirmed that acclimation temperature had great impacts on thermal tolerance of the European sea bass juveniles. In general, tolerance towards cold increased when acclimation temperature lowered to 15 °C. The fish cultured at this lowest temperature displayed higher cold tolerance, whereas those acclimated to the highest rearing temperature of 25 °C had greater tolerance to high temperatures. Regardless of the acclimation temperatures, the CTMax values rose from 32.01 to 34.29 °C (in FW) or from 32.29 to 36.68 °C (at BW) over the 10 °C temperature range evaluated in this study. In an agreement with our results, Rajaguru (2002) reported a rise in CTMax from 39.75 °C to 43.5 °C in *Etroplus suratensis* and from 40.75 °C to 43.15 °C in *Therapon jarbua* upon a 15 °C increase in acclimation temperature. It is also suggested that the typical seasonal acclimation in subtropical climates allows fish to be more tolerant to higher temperatures in summer or lower temperatures in winter (Bevelhimer & Bennett, 2000).

In general, tropical marine fish species have been reported to have CTMax values approaching or even exceeding 40 °C (Mora & Ospina, 2001; Rajaguru & Ramachandran, 2001; Eme & Bennet, 2009). Extremely high CTMax values (44.5 °C) for Asian sea bass (*Lates calcarifer)* and for a mullet species (*Liza dussumeri)* have been reported in the study of Rajaguru (2002). It can be postulated from our present data that our European sea bass strain is rather more of a temperate species compared to the above mentioned tropical fish species. Although our observations and results show that the Eastern Mediterranean strain of European sea bass are tolerant and perform well at temperatures as high or even higher than 30 °C, we are still yet to know if European sea bass strains inhabiting a wide range of geographic regions (i.e. western Mediterranean and/or Atlantic sea bass populations) would have different tolerance to varying temperatures.

The acclimated response ratio (ARR) estimates the ability of fish to alter CTM values with changing water temperature. Species with low ARR are expected to be more sensitive to increasing thermal regimes and may not be able to withstand large daily or annual temperature fluctuations. In this study, over the 10 °C range of acclimation temperatures, the ARR values were calculated as 0.23-0.41 in FW and 0.34-0.44 in BW for sea bass juveniles. These values are higher than those reported by Dülger et al. (2012) for the same species reared in full-strength sea water (0.25-0.27) and for some tropical fish species i.e. *E. suratensis* (0.25) and *T. jarbua* (0.20) reported by Rajaguru (2002).

Eme & Bennett (2009) report that overall thermal tolerance polygon area (as °C^2) offers a convenient and useful comparative index of eurythermicity between fish species. In our study, the polygon areas for European sea bass juveniles cultured in FW or BW over the three acclimation temperatures (15, 20, and 25 °C) were calculated to be 272.69 and 291.95 °C^2, respectively. This value was reported to be a little higher for the same size (296.14 °C^2, Dülger et al. 2012) or smaller (301 °C^2, Kır & Demirci 2018) sea bass juveniles when they were reared in full strength seawater. It is known that thermal tolerance zone is dependent on acclimation temperatures that are used during the experiment. When, we compared our present data with the extrapolated data of the red sea bream *Chrysophrys major* (Woo & Fung, 1980), we found out that thermal zone of European sea bass is fairly similar to that of the red sea bream (278 °C^2), which is reported to display poor growth and great mortality during high summer water temperatures. Kır & Demirci (2018) reported that the temperature tolerance area of 861 °C^2 developed for European sea bass as a result of dynamic temperature tests run in their study is 40% larger than the static thermal tolerance zone of 613 °C^2, which reflects both a bigger potential acclimation range and a large acclamatory scope.

The regression slopes for CTMax and CTMin of the European sea bass juveniles revealed that for every 5 °C increase in the acclimation temperature, the CTMax ($y = 1.14x + 30.93$, $R = 0.99$) and CTMin ($y = 2.04x + 1.67$, $R = 0.87$) values of the fish reared in FW increased by 1.14 and 2.04°C, while the CTMax ($y = 2.20x + 30.36$, $R = 0.96$) and CTMin ($y = 1.68x + 2.26$, $R = 0.98$) values of those reared at BW increased by 2.20 and 1.68 °C, respectively. These results signify that European sea bass juveniles have greater tolerance to low temperatures than high temperatures when reared in FW, but this was just the opposite when they were cultured in BW or even higher salinities (Dülger et al., 2012). Therefore, it could be suggested that salinity has some influence on thermal tolerance capability of sea bass juveniles and the species possesses some adaptability towards both cold and warm temperatures. This could be linked to a hydromineral balance in plasma under different saline conditions. Masroor, Farcy, Gros & Lorin-Nebel (2018) stated that sea bass acclimated to FW under warm conditions (24 °C) exhibited a strong capacity to osmoregulate and balance low Na^+ levels through increased Cl^- uptake at low salinity and high temperature. Thus, this allows European sea bass to tolerate contrasted environmental conditions such as lagoons and shallow waters.

Our data indicate that lower and upper temperature tolerance zones attained in this study for this species ranged from 3.26 to 7.33 °C and from 32 to 36.68 °C in FW. In the case of full strength SW, lower and upper thermal tolerance zones were reported to be slightly smaller, ranging from 4.10 to 6.77 °C and from 33.23 to 35.94 °C (Dülger et al., 2012). Culturing the juveniles in FW provides a little advantage towards cold tolerance, but this lowers the upper thermal tolerance limits.

In conclusion, our results have revealed that the interaction between temperature and salinity have significant effects on growth and feed intake of juvenile European sea bass and the Eastern Mediterranean strain are more sensitive to high rather than low temperatures. At the upper temperature range, growth and feed intake could be improved by rearing juveniles at intermediate salinities or even in FW, at least for two months. It is suggested that necessary precautions should be taken when this species is farmed in the southern parts of the Mediterranean in stagnant shallow pond waters with high ambient temperatures (>33-34 °C) during summer months. During cold winter months, however, this fish can be successfully cultured in low salinities or even in FW as long as low temperatures (<15 °C) are avoided by using underground warm waters in a farming system utilising cost-effective greenhouse. Based on our data, we can draw a conclusion that if sea bass is grown in ponds under greenhouse at low salinity and water temperature is maintained at around 25 °C throughout the year by using underground warm-water, SGR and yield could easily be doubled under the Mediterranean climatic conditions.

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