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TAILORING WESTERN BLACK SEA AQUACULTURE TO IMPENDING CLIMATE CHANGE: LABORATORY TESTING OF GILTHEAD SEABREAM *Sparus aurata* (Linnaeus, 1758) AS A POTENTIAL CANDIDATE

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Abstract

Climate change calls for the diversification of aquaculture species, seeking alternatives tolerating warmer summer temperatures. In this context, the research on the adaptability of gilthead seabream Sparus aurata (Linnaeus, 1758) for Romanian marine aquaculture was performed. The experiment demonstrated the possibility of transferring three months old juveniles from a 36‰ salinity into Black Sea water (mean salinity 15‰), without any mortality. The evolution of the stress induced by the difference in salinity was carefully monitored. Glycemic levels between 68-82 mg/dl before, 157-171 mg/dl one hour after the sudden change in salinity and 115-124 mg/dl at 24 hours were documented, respectively. Corroborated with the color changes and the resumption of active feeding and schooling behavior, it can be inferred that the use of the species is feasible in Romania. An average biomass increase comparable to relevant literature was documented: from seven grams initially to 300 grams. The biochemical analysis of the meat also revealed a balanced composition. Overall, S. aurata proved a viable candidate for Western Black Sea cage aquaculture in a rotational system, complementary to colder water species.

Key words: *aquaculture, adaptability, Black Sea, rotation, seabream.*

INTRODUCTION

The Romanian Black Sea coastline is limited to 245 km, with the Danube Delta accounting for more than half of it. Consequently, traditional Romanian aquaculture has focused primarily on freshwater fish species. However, in recent years, more emphasis has been placed on the potential of mariculture, and research activities have been carried out to stimulate the development of the field, both for finfish (Zaharia et al., 2017; Niță et al., 2018) and shellfish culture (Niță & Nenciu, 2020). Despite environmental and administrative concerns, cold-season cage farming for rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) proved feasible (Nenciu et al., 2022). However, impending climate change requires diversification in order to find alternative species tolerating increasing summer temperatures. Climate change is one of the most severe threats to the environment and society in general. The warming of the climate system is an undeniable reality, according to the Intergovernmental Panel on Climate Change (IPCC, 2019).

Observations indicate increases in global average temperatures of the World Ocean, extensive ice cap melting, and global average sea level rise. Global warming can largely be attributed to greenhouse gas emissions from human activities (Cochrane et al., 2019). The World Ocean is warming, registering, however, some geographical differences and some decadal variability. At least two seas in subtropical latitudes (the Mediterranean Sea and the East China Sea) are continuously warming (Rosenzweig et al., 2007). Salinity is generally increasing in surface marine waters in regions of higher evaporation, while there is a decreasing trend at high latitudes (Antonov et al., 2002). The combined effect of temperature and salinity changes due to climate warming is expected to reduce the surface density of the World Ocean, increase vertical stratification, and alter surface mixing (Sarmiento et al., 2004). Globally, sea levels have risen 21 - 24 cm since 1880. This rise is largely due to a combination of melting glaciers and the ice cap with the thermal expansion of seawater as the ocean warms. In 2021, the global mean sea level was 97 mm above 1993 levels, the highest annual average on record since 1993 to date (Lindsey, 2022). The greatest losses expected to be caused by sea level rise are estimated on the Atlantic and Gulf of Mexico coasts, in the Mediterranean, Baltic Sea and small island regions (Nicholls et al., 2007).

Climate change has also caused seawater acidification and the modification of other chemical properties. Continued absorption of atmospheric CO_2 has lowered surface seawater pH by 0.1 units over the past two hundred years (Caldeira & Wickett, 2005). Changes in pH can also affect marine species in ways other than decalcification. Havenhand et al. (2008), found that low pH reduces sperm motility and fertilization success in the sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846) and point out that other broadcast spawning marine organisms may be at a similar risk. Other chemical properties subject to trends driven by climate change include oxygen and inorganic nutrients. Oxygen concentration in the oxic thermocline (from about 100 m to 1,000 m) has decreased in most ocean basins since 1970 (Emerson et al., 2004).

As far as biological and ecological changes are concerned, decades of research document that climate variables are the primary factors influencing the distribution and dynamics of marine plankton and fish assemblages (Roessig et al., 2004). Climate change is predicted to displace most species to the poles, expanding the range of marine thermophilic species and reducing that of cryophilic species (Parmesan & Yohe, 2003). Climate-driven changes in species composition and abundance will obviously also alter species diversity, with implications for ecosystem functions such as productivity (Duffy, 2003) and resistance to invasive species (Stachowicz et al., 2002).

Climate change thus affects marine aquaculture through acidification, changes in seawater temperature, salinity and circulation, frequency and severity of extreme events, sea level rise and ecological changes associated with all the phenomena described above. And, moreover, climate change also impacts greatly on the four dimensions of food security (Cochrane et al., 2019): the availability of seafood will vary as a consequence of changes in habitats, stocks and species distribution; the stability of supply will be affected by seasonal changes, increased variation in ecosystem productivity and high production variability and risks; the access to seafood will be affected by changes in livelihoods and opportunities for harvest/fishing and/or farming; and, finally, the use aquatic products will also be affected (Nicolae et al., 2016; Nicolae et al., 2017). For example, some communities will have to adapt to species that are not traditionally consumed, the social acceptability of novel species and/or products being crucial in this adaptation process (Nenciu et al., 2021).

Gilthead seabream *Sparus aurata* (Linnaeus, 1758) is one of the most cultured fish species in the Mediterranean Basin, with an estimated annual production volume of 258,754 tons/year (Mhalhel et al., 2023). In its natural habitat, gilthead seabream lives in environments where temperatures range from 11°C in winter to 26°C in summer (Ibarz et al., 2010). However, aquaculture practices documented that temperatures below 18°C cause changes in the feeding behaviour, while a drastic reduction in food intake has been observed at temperatures below 13°C, greatly affecting fish production (Ibarz et al., 2003; Sánchez-Nuño et al., 2018). In terms of salinity, seabream is more tolerant and thus frequents estuaries and coastal waters, thus it is likely to be cultured in brackish environments (Pavlidis & Mylonas, 2011). In the Black Sea, seabream has only been cultured at the Southern coast, in Türkiye (Öztürk et al., 2020; Öztürk, 2022).

In this broader context, this research aimed at testing the adaptability of gilthead seabream, a widely cultured species all around the Mediterranean Basin, to Western Black Sea conditions, with the view to proposing a rotational farming system in floating cages together with rainbow trout.

MATERIALS AND METHODS

Fish Supply, Adaptation and Transfer

The gilthead seabream fingerlings (N = 218 individuals, aged approximately two months) were purchased in May 2023 from an aquaculture farm and hatchery from Italy (Adriatic Sea) and transported to NIMRD's aquaculture laboratory in oxygen suprasaturated (over 110% dissolved oxygen) plastic bags. Temperature in the transport water was 20°C and the salinity 36‰, similar to the conditions in the hatchery of origin. Prior to the reception of the fish, salinity in the stocking tank was adjusted to 36‰ using Instant Ocean sea salt and kept in a recirculating regime. After a careful temperature check, the fish were gradually released into the stocking tank.

Additional aeration pumps were used to increase the oxygen content of the water. No mortalities were recorded during transportation and/at transfer. The fingerlings were kept in a 900-L stocking tank for 14 days, in order to overcome any stress caused by transport and to guarantee a healthy batch. Throughout adaptation, the fish were fed ad libitum with Skretting Optibream 1P (2 mm) pellets. All food was consumed and faces were removed by syphoning. During the two-week quarantine period, the mean temperature of water in the stocking tank was 20°C. Before performing the transfer to Black Sea water, 2 fish were randomly extracted for blood glucose analysis.

After the initial two-week quarantine period, the gilthead seabream fingerlings were divided in four batches (replicates), each containing 54 individuals, and transferred to the 500-L experimental tanks (Figure 1).

Figure 1. Transfer of gilthead seabream juveniles from the stocking tank (salinity 36‰) to the experimental tanks (salinity 15‰) (*Original photos*)

All fish were weighed and measured individually before transfer. The initial supply of Black Sea water was provided by NIMRD's pump-ashore system (PAS). The water entering the PAS is pumped directly from the Black Sea and is stored in a covered settlement tank for sedimentation and suspended solids reduction before entering the tanks (Niță & Nenciu, 2021). The experimental tanks were fitted with an efficient aquaculture recirculation system (RAS), containing mechanical and biological filtration, UV sterilization, oxygen pumps and a protein skimmer, to ensure proper water quality. Water temperature was not adjusted during the experiment. Upon transfer, water temperature in the experimental tanks was 21°C. The entire experimental period covered seven months, from late May to early December 2023.

Monitoring Environmental Parameters

Temperature (°C), salinity (‰), pH and dissolved oxygen (DO) (%) both in the initial stocking tank and in experimental tanks were measured daily, using a Mettler Toledo Seven Excellence Multiparameter. The experimental RAS tanks were equipped with real-time transmission sensors of the above-mentioned parameters and a trigger for the back-up aeration pump in case of values dropping below the set 75% dissolved oxygen threshold.

Blood Glucose Measurements

In order to investigate blood glucose as a stress indicator, measurements were performed in two replicates/fish. A baseline glucoses reading was made by extracting two fish from the stocking tank before transfer. One hour and 24 hours after transfer into Black Sea water, respectively, two fish were randomly extracted from each experimental tank (total number of extracted fish = 16, remaining fish in each experimental tank = 50) and euthanized by immersion in a 500 mg/ml buffered one third tricaine methanesulfonate (MS-22) and two thirds sodium bicarbonate solution (AVMA, 2013). After cessation of breathing activity, the caudal fin was severed (Witeska et al., 2022) and blood was drawn for reading blood glucose values using an OK Meter Match II automatic glucose reader (Figure 2).

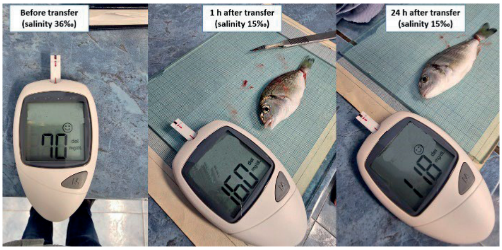

Figure 2. Blood glucose readings on gilthead seabream before, one hour and 24 hours after transfer from 36‰ to 15‰ salinity (*Original photos*)

No treatment was applied to the blood drawn, as it was analysed immediately.

Feeding Protocol

The gilthead seabream juveniles were fed with dedicated Skretting Optibream pellets: Optibream 1P (2 mm) (crude protein 48.5%, crude fat 18%, ash 6.2%, cellulose 2.8%) during the first three months and Optibream 2P (4 mm) (crude protein 44%, crude fat 20%, ash 6.5%, cellulose 3.3%) until completion of the experiment (Ayala et al., 2023). The calculated daily feed ratio was 2% of the biomass (Zaharia et al., 2017), fed in two equal doses during the day (in the morning and in the afternoon). Monthly biomass measurements were performed to adjust the feeding ratio.

Calculating Growth Parameters

Weight and length measurements were performed monthly on all 50 fish from each experimental tank. The total length (TL) of the specimens was measured on millimetric paper to the nearest 0.5 millimetre. The total weight (TW) of the fish was taken on a Kern EW top loading balance. Using the biometric and gravimetric data collected during the 7 months of study, Feed Conversion Ratios (FCR), Specific Growth Rates (SGR%/day), Fulton's Condition Factor (K) were determined for the four batches. The initial and final Length/Weight relationship for the entire lot was also determined (Froese, 2006).

The equations used for calculating the Feed Conversion Ratio (FCR) and the Specific Growth Rate (SGR%/day) are detailed below (Hopkins, 1992):

$$\mathrm{FCR} = \Sigma_{\mathrm{fk}}/\mathrm{W_t}\text{-}\mathrm{W_0} \qquad (1)$$

$$\mathrm{SGR} = 100[(\ln W_t\text{-}\ln W_0)/\mathrm{t}] \qquad (2)$$

Where: t = feeding days; W_0 = initial live weight of fish (g); W_t = final live weight of fish (g); L = total length (cm), and fk = weight of feed consumed by fish at each feeding (feed intake) (g).

Fulton's Condition Factor (K) was calculated using the equation below (Reis & Ateș, 2019):

$$\mathrm{K} = (\mathrm{W}/\mathrm{L}^3) * 100 \qquad (3)$$

Where: W = total weight (g), L = total length (cm).

Statistical Analysis

For statistical interpretation, data from each group's replicates were pooled for one-way ANOVA analysis, and differences at the 5% level ($p < 0.05$) were considered significant (using the Tukey's significant difference test) (Akbulut et al., 2002).

Biochemical Analysis

Following the completion of the experimental period, a comparative analysis of the proximate composition of the gilthead seabream meat was performed using four replicates of one sample reared in Black Sea water for seven months and one sample reared in an aquaculture farm in the Aegean Sea. The analysis was performed by an accredited laboratory (Biosanivet Ltd.), covering the following parameters: crude fat (%, as per SR ISO 1444:2008), crude protein (%, as per SR ISO 937:2007), total ash (%, as per SR ISO 936:2009), moisture (%, as per SR ISO 1442:2010), carbohydrates (%, as per PA-L-34) and energy values (Kcal/100 g and KJ/100 g, as per PA-L-34).

RESULTS AND DISCUSSIONS

The gilthead seabream lot was carefully observed immediately after reaching NIMRD's aquaculture laboratory. After the transfer into the stocking tank (36‰ salinity), all fish behaved normally, with no visible sign of stress. No mortality was recorded during the entire experimental period. Feeding was started 24 hours after transfer, observing the complete consumption of the pellets. The 2% body mass feeding ratio was applied during the seven-month experimental period.

After transfer into the Black Sea water experimental tanks, temperature, salinity, dissolved oxygen and pH were constantly monitored, showing no significant variations among the two experimental batches ($p \geq 0.05$). During the 7-month period, temperature ranged from 24°C in August to 20°C in November-December, salinity was rather constant, with a maximum of 17‰ in December and a minimum little under 14‰ in peak summer months, dissolved oxygen values ranged between a maximum of 93% in June and a minimum of 79% in August, and pH recorded only small variations throughout the entire experimental period, with a mean around 7.8.

Blood glucose mean values measured as a proxy for indicating stress in gilthead seabream juveniles as a consequence of the sudden change in salinity are shown in Table 1.

Glycaemic levels between 68-82 mg/dl before, 157-171 mg/dl one hour after the sudden change in salinity and 115-124 mg/dl at 24 hours were documented, respectively.

A total number of 18 fish were sacrificed for blood glucose analyses: two from the initial stocking tank, eight from the experimental tanks (two fish from each tank) one hour after transfer and eight from the experimental tanks (two fish from each tank) 24 hours after transfer.

Glucose readings were made in two replicates for each fish and are detailed in Table 1 below.

Table 1. Comparative blood glucose values of *S. aurata* juveniles before, one hour after and 24 hours after transfer to Black Sea water, respectively

Specification	Blood glucose (mg/dl)								
	Before Transfer	1 h after transfer				24 h after transfer			
	Stocking Tank	Tank 1	Tank 2	Tank 3	Tank 4	Tank 1	Tank 2	Tank 3	Tank 4
Mean ± SD	72.25 ±5.67	163.75 ±5.53	163.75 ±4.02	162.25 ±4.96	175.75 ±11.75	119.25 ±1.47	119 ±2.54	119.5 ±3.35	119 ±2.73
	72.25±5.67	166.37±5.44				119.18±0.20			

The growth and biomass gain of gilthead seabream juveniles reared in Black Sea water are summarized in Table 2. In terms of mean fish length evolution, the juveniles experienced a normal linear increase, from an initial length of 7 cm to a final length of 23 cm (Figure 3). No significant differences between the four replicates were recorded. As far as weight gain during the experimental rearing is concerned, all fish grew steadily, from an initial weight around 7 g to a final weight of 300 g (Figure 4, and Figure 5). Similarly, to length evolution, there were no statistically significant differences between the replicates ($p \geq 0.05$).

When referenced to the initial values, monthly percentual increases of both length and weight were high (an increase by more than 200% in length and by more than 4000% in weight, respectively, after seven months compared to the initial value at the beginning of the experiment).

Table 2. Mean values of *S. aurata* growth parameters recorded during the experiment

Parameter	Tank 1	Tank 2	Tank 3	Tank 4	ANOVA
Initial TL (cm)	7.20±0.03	7.18±0.02	7.19±0.03	7.21±0.03	The values among tanks (replicates) were not significantly different ($p \geq 0.05$)
Final TL (cm)	23.23±0.53	23.61±0.36	23.25±0.55	23.43±0.43	
Initial weight (g)	7.07±0.03	7.01±0.02	7.05±0.03	7.06±0.03	
Final weight (g)	301.52±2.93	303.92±2.53	303.54±2.46	302.43±2.72	
K	2.51±0.59	2.55±0.56	2.54±0.58	2.58±0.50	
FCR	1.52±1.40	1.45±1.29	1.42±1.25	1.46±1.29	
SGR (%/day)	1.90±1.11	1.91±1.12	1.90±1.11	1.90±1.11	

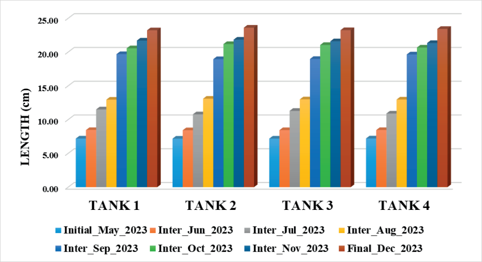

Figure 3. Evolution of fish total length (mean monthly values)

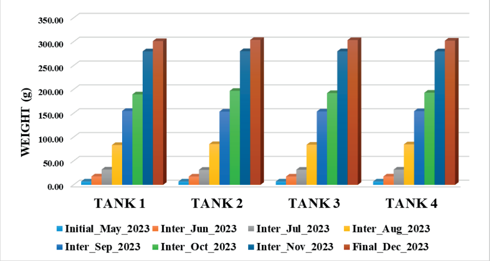

Figure 4. Evolution of fish weight (mean monthly values)

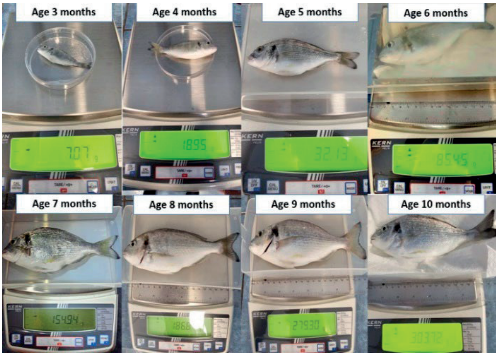

Figure 5. Evolution of gilthead seabream weight during the seven-month experiment in relation to fish age (monthly gravimetric measurements) (*Original photos*)

The calculation of Fulton's Condition Factor (K) for the gilthead seabream juveniles reared in Black Sea water recorded a mean value of 2.54 (Figure 6), while the evolution of the initial (Figure 7) to the final (Figure 8). Length-Weight Relationship indicated a positive allometry (b > 3), the fish increasing in weight faster than in length, corresponding to the rounded shape of adult gilthead seabream.

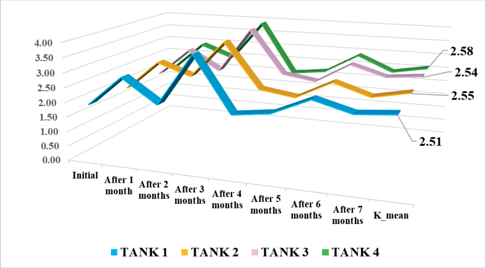

Figure 6. Monthly evolution of the Condition Factor (K)

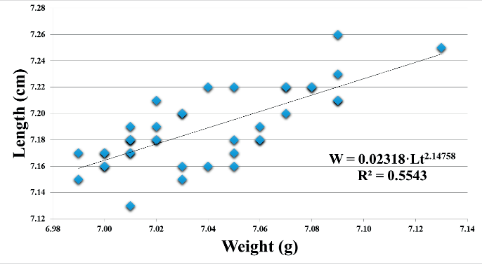

Figure 7. Initial Length-Weight Relationship of gilthead seabream reared in Black Sea water

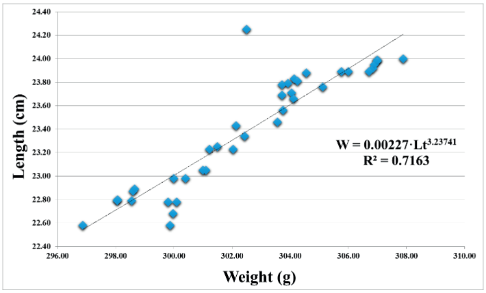

Figure 8. Final Length-Weight Relationship of gilthead seabream reared in Black Sea water (after seven months)

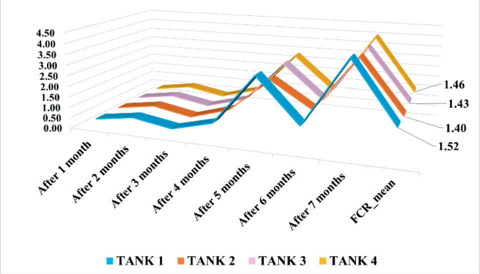

Figure 9. Monthly evolution of the Food Conversion Ratio (FCR)

With reference to feed efficiency and growth, the Food Conversion Ratio (FCR) recorded a mean value of 1.45 (Figure 9), with no significant difference among replicates, while the average Specific Growth Rate SGR (%/day) was 1.90 (Figure 10). Regarding the biochemical parameters investigated, the results of the comparative analysis of the proximate composition of seabream meat (reared in Black Sea water vs. reared in an aquaculture farm in the Aegean Sea) are summarized in Table 3 below (where FW = fresh weight).

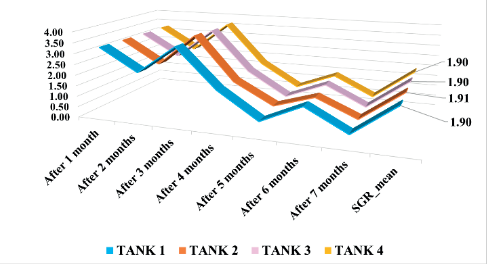

Figure 10. Monthly evolution of the Specific Growth Rates (SGR%/day)

Overall, the values of the two samples were comparable, with only one statistically significant difference in lipid content, which was higher in the Aegean seabream: 10.18% FW compared to 6.14% FW. In the same time, Black Sea reared seabream showed a higher protein content of the flesh, namely 21.15% FW compared to 19.63% FW.

Ash (1.32% FW and 1.41% FW, respectively) and moisture content (68.87 % FW and 71.30% FW, respectively) of the two samples were very similar, while the energy value of the Aegean reared seabream was a little higher, due to the higher fat content. No carbohydrates were detected in any of the samples.

The overall results of the experiment (in terms of growth rhythm, food conversion ratio, condition factor, biochemical composition) suggest that gilthead seabream reared in Black Sea water is comparable in quality to its Mediterranean counterpart.

Table 3. Meat composition of gilthead seabream (reared in Black Sea water vs. in an aquaculture farm in the Aegean)

Culture Environment	Total lipid content (% FW)	Protein content (% FW)	Carbohydrate content (% FW)	Total ash (% FW)	Moisture (% FW)	Energy	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Kcal/100 g	KJ/100 g
Aegean	10.18±0.02	19.63±0.10	0.00±0.00	1.32±0.005	68.87±0.16	170	710
Black Sea	6.14±0.15	21.15±0.06	0.00±0.00	1.41±0.01	71.30±0.15	140	587
ANOVA	$p < 0.05$	NS	NS	NS	NS	$p < 0.05$	$p < 0.05$

The adaptability of a species to the local environment is of utmost importance when proposing aquaculture developments (Nenciu et al., 2022). Gilthead seabream is a sedentary eurythermal and euryhaline fish that can tolerate wide ranges of temperatures and salinities and thus frequents estuaries and coastal waters (Ibarz et al., 2010; Mhalhel et al., 2023). Moreover, *S. aurata* is documented as occurring in the Black Sea, mostly on the southern coast and only as isolated specimens in the north-western part (Bănărescu, 1964; Aydin & Sözer, 2016). Its tolerance to brackish water (Öztürk et al., 2020) and the continuous warming of seawater at the Romanian coast, with a 2022 average temperature of 14.36°C (2.24°C higher compared to the 1953-2021 annual means) (Vlăsceanu-Mateescu & Lazăr, 2023) suggests that seabream could gradually adapt to this environment after penetration through the Bosphorus.

The primary aim of this research, however, was to test whether a rapid transfer to Black Sea water of *S. aurata* juveniles for aquaculture purposes is feasible. When stocking aquaculture farms (marine cages) with fingerlings coming from hatcheries with higher salinities, there is no time for gradual adaptation and a quick osmoregulation is essential (Mancera et al., 1993; Tandler et al., 1995). The swift adaptation was demonstrated by using blood glucose as stress level indicator. The basic stress response in fish involves catecholamine release and activation of the hypothalamic-pituitary-internal axis. Hypothalamic-pituitary-internal activation causes energy source mobilization, glycogen depletion, and an increase in plasma glucose levels, as well as excessive muscular activity, anaerobic glycolysis, and an increase in plasma lactate (Arends et al., 1999). As a result, the level of glucose in plasma is frequently used to determine stress levels (Fazio et al., 2015). The values measured prior to transfer into brackish water (in the 68-82 mg/dl range) are in line with normal blood glucose levels reported in fish (40-90 mg/dl) (Malini et al., 2018). A sudden increase (157-171 mg/dl) was documented 1 hour after the rapid change of salinity, along with a change of colour and altered swimming behaviour: the fish became darker, swam individually and refused to eat. However, just 12 hours after the transfer, the resumption of active feeding and schooling behaviour was observed, along with the return of the normal colour. The blood glucose measurement performed 24 hours after transfer (115-124 mg/dl) indicated the reduction of stress, which was confirmed by the fact that no mortalities were recorded throughout the entire experimental period.

The second objective of this research pursuit was to assess the growth rate of seabream under local Black Sea water conditions, aiming at scientifically substantiating the species' potential for commercial exploitation in the area. During the seven months of experimental rearing in Black Sea water, the seabream juveniles grew constantly and reached the first commercial size of 300 g, this showing a normal growth rhythm compared to the Mediterranean (Gjije et al., 2022). The mean condition factor (K = 2.54) indicated an excellent condition of the fish (Kop et al., 2019), which displayed a positive allometric growth (b > 3), consistent with the regular growth pattern of seabream Mediterranean (Gjije et al., 2022). The tested seabream lot proved a good feed conversion efficiency, the mean FCR of 1.45 suggesting an optimal intake of the pellets provided (Sadek et al., 2004), which was also confirmed by the high average value of the Specific Growth Rate (SGR = 1.90%/day) (Kraljević et al., 2004).

Upon completion of the experiment, the biochemical analysis revealed a good quality of the meat, with a high protein content (21.15 %FW), in line with documented values of Black Sea reared seabream (Öztürk, 2022). The slightly lower fat content compared to the Aegean seabream (6.14 %FW vs. 10.18 %FW) could be explained by the fact that the commercial counter-sample contained older and larger individuals.

CONCLUSIONS

The research endeavour aiming at laboratory testing of the adaptability of gilthead seabream to Western Black Sea conditions proved to be successful, with good growth parameters and no mortalities, indicating *S. aurata* as a feasible candidate for aquaculture in the region. Moreover, the rapid transfer to brackish water (from 36‰ to 15‰ salinity), mimicking a commercial stocking from a Mediterranean hatchery directly into sea cages, did not cause irreversible stress to the juveniles, which adapted quickly to the new salinity regime.

Despite the documented raise of seawater temperature at the Romanian coast, in the case of open sea farming there are still limitations during the winter. Given the successful testing of rainbow trout during the cold season, the suggested solution is a rotational farming system in floating cages.

This approach would foster the development of Romanian marine aquaculture by maximizing economic profit and using the production facilities all year long. For an efficient outcome, gilthead seabream fingerlings transfer into Black Sea conditions should be made at a larger size (minimum 50 g), to guarantee a higher final commercial value of the harvested fish.

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