

Biological and Ethological Response of Black Sea Golden Grey Mullet (*Chelon auratus* Risso, 1810) Fries to Different Salinities and Temperatures

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Abstract

The aim of this paper was to investigate the biological and ethological response of golden grey mullet *Chelon auratus* (Risso, 1810) fries collected from Romanian Black Sea shallow waters to different salinities and temperatures, in order to document the optimal conditions for controlled rearing. The species' potential for aquaculture is enhanced by its eurihaline and eurithermal adaptability, allowing it to grow in a variety of ecosystems, including the Romanian Black Sea area, with its highly variable salinity and temperature specificities. Three experimental set-ups were designed: a salinity tolerance test, with 5 salinity regimes (0.3‰, 5‰, 10‰, 15‰ and 20‰), a temperature tolerance experiment, involving both temperature decrease (down to 8°C) and increase (up to 34°C), and an extreme salinity test, reaching a maximum value of 95‰. The aggregated results obtained from the three experiments indicated that golden grey mullet fries can indeed tolerate a wide range of temperatures (8°C - 36°C) and salinities (5‰-70‰), with the optimal range between 10‰ and 30‰ salinity, at temperatures of 10°C - 25°C. The practical purpose of this investigation was supporting the diversification of local mariculture species, as mullets can be excellent candidates for the enhancement of aquaculture in the area

Introduction

Along with other members of the Mugilidae family, the golden grey mullet *Chelon auratus* (Risso, 1810) inhabits coastal lagoons and estuaries, where they constitute target species for artisanal fisheries (Katselis et al., 2007). They also play a crucial ecological role as biotic vectors of organic matter between littoral habitats and the open sea (Lefeuvre et al., 1999). For a long time, mullets have been considered among the most interesting coastal species for aquaculture (Pillia, 1975; Mansour, 2013). Approximately 20 mullet species have been cultured in many regions of the world (Lee, 1997).

Golden grey mullet is spread in the Mediterranean, Black and Caspian Seas, as well as along the Atlantic coast, and culture in extensive and semi-intensive enclosures has been practiced worldwide for centuries,

especially in the Far East and the Mediterranean (Crosetti & Cataudella, 1995), reaching a global production of 134,329 tons in 2010 (Mylonas et al., 2019). The species' potential for aquaculture is enhanced by its eurihaline and eurithermal adaptability, allowing it to grow in a variety of ecosystems (Crosetti & Cataudella, 1995). Moreover, fry production is high in certain seasons, and their capture almost entirely supports seed supply for mullet aquaculture (Crosetti & Cataudella, 1995). Golden grey mullets are consumers of the low trophic layers and can therefore be used in most economic and efficient way by culturing them extensively (Crosetti & Cataudella, 1995). They also possess osmoregulation abilities which appear early during the development (Nordlie et al., 1982) and allow them to maintain elevated growth rates also under hyposaline conditions (Cardona & Castello-Orvay, 1997). For this reason, mullets are widely

cultivated in freshwater and brackish ponds (Allen, 1991; Olukolajo & Omolara, 2013).

Because of being euryhaline and eurytherm, mullets can tolerate salinity degrees between 0‰ and 60-70‰ and survive at water temperature between 0°C and 38°C and they can also survive for a long time at dissolved oxygen levels as low as 0.32 ppm (Cardona, 2000; Bozkurt & Secer, 2001). Thus, their production may be carried out in a variety of eco-systems like coastal lagoons with brackish to hyper saline waters (Hotos & Vlahos, 1998), reservoirs and ponds with fresh waters (Losse et al., 1991) or even as supplementary crops in carp ponds (Crosetti & Cataudella, 1995). Moreover, grey mullet aquaculture has the advantage of providing not only affordable whole fish and fillets, but also fish roe, a high value product (>100 €/kg), whose market is expanding around the Mediterranean, for instance. *C. auratus* can reach 4-6 €/kg on the Greek market (Hotos & Katselis, 2011). Therefore, mullets have a great biological and economical potential for fish species and product diversification, and development of value-added products.

A market for grey mullet is well established, though a niche one, in the Mediterranean. Even without any marketing effort by the aquaculture industry, the European market demand for grey mullet is likely to increase in the coming years, due to the demand from established and newly immigrant families originating from North Africa, Middle East and Asia. Currently, the industry is a capture-based aquaculture, relying exclusively on capture of wild fry (ca 1,000,000,000) that are subsequently grown out to market weight (600-1,200 g) in captivity, in lagoons or earthen ponds (Mylonas et al., 2019).

In this context, in addition to performing research experiments on rearing highly valuable finfish species, such as sturgeons (Russian sturgeon *Acipenser gueldenstaedti* and Siberian sturgeon *Acipenser baerii*), turbot (*Scophthalmus maximus*) (Niță & Nenciu, 2017), the golden-grey mullet's potential for culture was tested in a Recirculating Aquaculture System within NIMRD (Niță et al., 2018).

The aim of this complex research was to investigate the biological and ethological response of golden grey mullet fries collected from Romanian Black Sea shallow waters to different salinities and temperatures, in order to document the optimal conditions for controlled rearing. The practical purpose of this investigation was supporting the diversification of local mariculture species.

Materials and Methods

Fish Collection and Acclimation

The collecting procedure of *C. auratus* fries was conducted in a way to minimize habitat disturbance and unnecessary mortality of fish. Transport plastic tanks were disinfected before use (EPA, 2002; American

Fisheries Society, 2004). Thereby, the *C. auratus* fries used for this experiment were collected using appropriate mesh size hand nets from the Mamaia Bay area, located close to NIMRD's premises, placed in a 40-liter plastic barrel and transported to the laboratory. The fish were gently transferred from the barrels to the acclimation tank, making sure that temperature difference between the barrels and the tank was not higher than 3°C. The fish were acclimated in a 900 l fibre glass tank in a flow-through system (UV sterilized water) at a flow of approximately 100 l/hour and properly aerated, in order to obtain around 80 - 90% dissolved oxygen (DO) saturation. The fish were maintained for 14 days in the acclimation tank (EPA, 2016) and fed with JBL Novo Grano Mix mini pellets, at an approximate ration of 2% of total fish biomass in the tank (Zaharia et al., 2017). Subsequently, the same feeding ration was given to all fish involved in the three experiments. During the acclimation period, fish health was monitored daily, in order to observe any signs of illness that may cause a change in the fish' natural behaviour.

Salinity Tolerance Experiment

The *C. auratus* fries, previously acclimated for 14 days, were divided into 5 experimental batches. 5 glass aquaria provided with air flow, with a total volume of 7 l of water/aquarium, were used. The 5 tanks were filled with water at different salinity levels, as follows: 0.3‰ (dechlorinated tap water), 5‰, 10‰, 15‰ and 20‰. For higher salinities, standard *Instant Ocean* sea salt was added, in order to obtain the required salt concentration. After the experimental aquaria were prepared, fish were extracted from the acclimation tank and distributed using a randomization procedure. Because it is generally extremely difficult for experimenters to eliminate bias using only their expert judgment, the use of randomization in experiments is common practice (Suresh, 2011). The fish were carefully extracted from the acclimation tank individually using a fish net. Following a randomization block scheme (<https://www.randomlists.com>), the fish were placed one by one in 5 plastic buckets filled with seawater. When reaching the desired number (10) of individuals per bucket, the contents of each bucket was strained through a net and subsequently fish were placed in the matching labelled experimental aquarium (Figure 1).

The salinity tolerance experiment covered a period of 56 days (8 weeks) and the parameters monitored in each experimental aquarium were: temperature (°C), salinity (‰), pH and dissolved oxygen (DO) (%), twice a week, using a Mettler Toledo SevenExcellence Multiparameter. Moreover, initial standard fish length (mm) and weight (mg) were measured. In order to determine the growth performance in the different salinity regimes, a final measurement (after 8 weeks) was performed. The maximum standard length (SL) of the specimens was measured on a measuring board to the nearest 1 millimetre. The total weight (W) of the fish

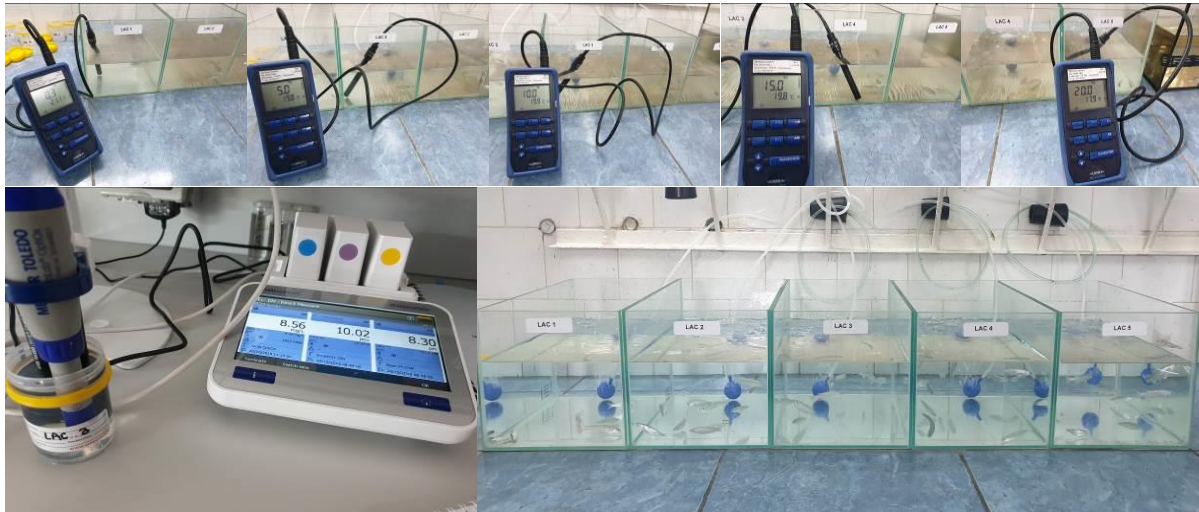


Figure 1. Salinity tolerance experimental set-up: individual tanks salinity measurements (up); pH and DO (%) measurements (down left); overview of test aquaria (down right) (*original photos*).

was taken on a Kern EW top loading balance.

Fulton's Condition Factor (K) was calculated using the equation: $K = (W/L^3) * 100$, where W is the total weight (mg), L is the maximum standard length (mm) (Reis & Ateş, 2019).

Mortalities were recorded daily in each experimental aquarium.

Temperature Tolerance Experiment

Two batches of *C. auratus* fries (30 individuals each), previously acclimated for 14 days in the fiber glass acclimation tank, were introduced in two glass aquaria, carefully avoiding extracting fish having signs of distress/abnormal behavior (Barton & Iwama, 1991).

The temperature tolerance experiment was designed in two directions: increasing temperature in one aquarium and decreasing water temperature in the other, starting from a 20°C baseline. For water cooling, the test aquarium was placed in a temperature-controlled room, while for water heating a professional JBL Pro Temp S300 heater was used. Both experimental batches were progressively adapted by modifying the controlled room/water heater temperature with 1-1.5°C each day, starting from an initial temperature of 20°C. Thus, after 10 days of experiment, fish were brought to 8°C in the temperature-controlled room, while in the heated aquarium the maximum water temperature was 34°C. Proper aeration was provided in both experimental aquaria - an air flow of about 50l/hour, in order to obtain around 80-90% DO saturation (depending on conditions - temperature, number of fish, etc.) and water recirculation/filtration/protein skimming.

The observations performed during the temperature tolerance experiments focused on general fish behavior and adaptation to cold/warm water (swimming, feeding etc.). Mortalities were recorded daily in each experimental aquarium.

Extreme Salinity Tolerance Test

After completion of the salinity and temperature tests, an extreme conditions experiment was performed. Using the same aquarium for the temperature tolerance test, standard *Instant Ocean* sea salt was added, progressively increasing salinity over a period of 36 days, starting from 20‰ and reaching a maximum value of 95‰. A professional JBL Pro Temp S300 heater was used, water temperature being kept very high, with peak of 36°C.

Euthanasia and Disposal

At the conclusion of the salinity and temperature tolerance tests, the surviving fish from each aquarium were strained through a net and placed in a glass jar (1 l) filled with iced water. When observing complete immobility, the fish were individually extracted, and the spinal cord was rapidly sectioned using a sharp scalpel. The euthanized fish were then placed in labelled zip-lock bag and stored in a freezer until collection for disposal by a specialized company (AVMA, 2013).

Results and Discussion

Salinity Tolerance Experiment

The salinity tolerance experiment was designed to cover a rather long period of 56 days (8 weeks), in order to allow quantifying the changes in fish length and weight. The results of measurements performed are summarized in Table 1 below.

It was noticed that increases in fish length were recorded in all salinity regimes, with the highest in the 15‰ and 0.3‰ aquaria (by 8.82% and 8.24%, respectively), the latter results being statistically influenced by the small number of fish for the final measurement (3 surviving fish out of 10). The lowest fish

length increase was recorded, however, in the 20‰ regime, as fish grew only by 4.10% (Figure 2). Overall, except for the 20‰ salinity, all fish grew more in length than in weight, regardless of the salinity regime (Figure 2).

As far as the evolution of fish weight is concerned, it was notable that the highest increase (by 12.04%) was recorded in the 20‰ salinity regime, while in the freshwater aquarium the weight increase was negative (-2.05), yet this results were statistically influenced by the small number of fish for the final measurement (3 surviving fish out of 10) (Table 1, Figure 2). For the remaining salinities, weight gains were positive, slightly increasing in correlation with salinity (by 0.43%, 3.04% and 3.22%, corresponding to 5‰, 10‰ and 15‰ salinities, respectively) (Table 1, Figure 2).

Fulton's Condition Factor (K) is another measure of an individual fish's health that uses standard weight. Thus, in order to better assess the state of *C. auratus* at the different salinities tested, it was calculated both after the initial and final measurements. The values were calculated using the length and weight from the final measurements and show a correlation between the Condition Factor and salinity. The results obtained

indicate an overall good state of the fries, with K values ranging between 1.35 and 1.72, with the best condition being reported for fish kept at a 20‰ salinity (Figure 3). Our results are consistent with other findings for mullet species (Verdiell-Cubedo, 2006; Guino-o, 2012).

During the entire experimental period, water parameters in the 5 test aquaria were monitored. Temperature was kept constant, ranging between 19.5°C and 19.7°C, in order not to influence in any way the state and behavior of fish. pH values were normal in all experimental aquaria (8.22-8.55), while dissolved oxygen (DO) percentages increased with salinity (from 94.4% at 0.3 ‰ to 102.5 at 20‰).

The survival percentage of fish in all salinity regimes was 100%, except for the 0.3‰ aquarium, where mortalities occurred in the first week (first and second day) of the test (2 and 5 dead fish, respectively)

Regarding the ethology of *C. auratus* during the experimental period, the swimming and feeding behavior were carefully observed. After the initial stress of being handled for length and weight measurements, the fish placed in the test aquaria started swimming and feeding normally, with slight differences between the different salinities. Thus, the fish kept at 0.3‰ salinity

Table 1. Summary of *C. auratus* length and weight in the different salinity regimes.

Measurements	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Length	Weight
Salinity	0.3‰		5‰		10‰		15‰		20‰	
Initial 21.10.2019	43.7	1460	42.5	1392	43.4	1514	43.1	1582	44.4	1520
Final 16.12.2019	47.3	1430	44.7	1398	45.9	1560	46.9	1633	46.2	1703
Increase by %	8.24	-2.05	5.18	0.43	5.76	3.04	8.82	3.22	4.10	12.04

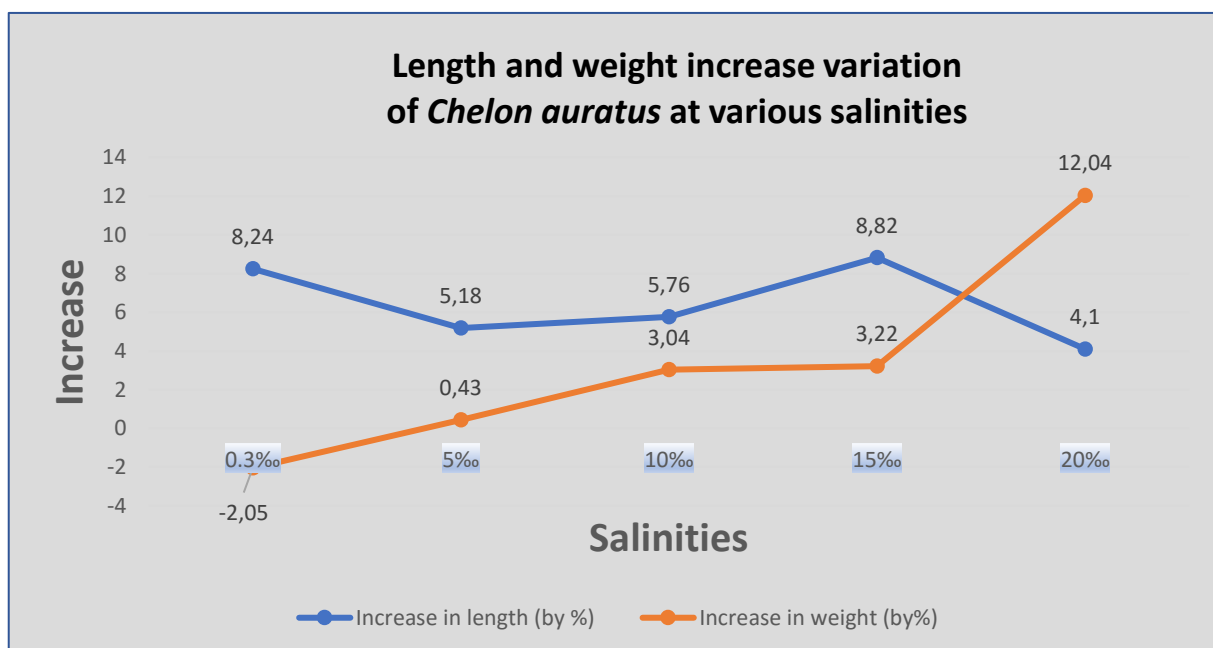


Figure 2. Comparative length and weight increase variation of *C. auratus* at various salinities.

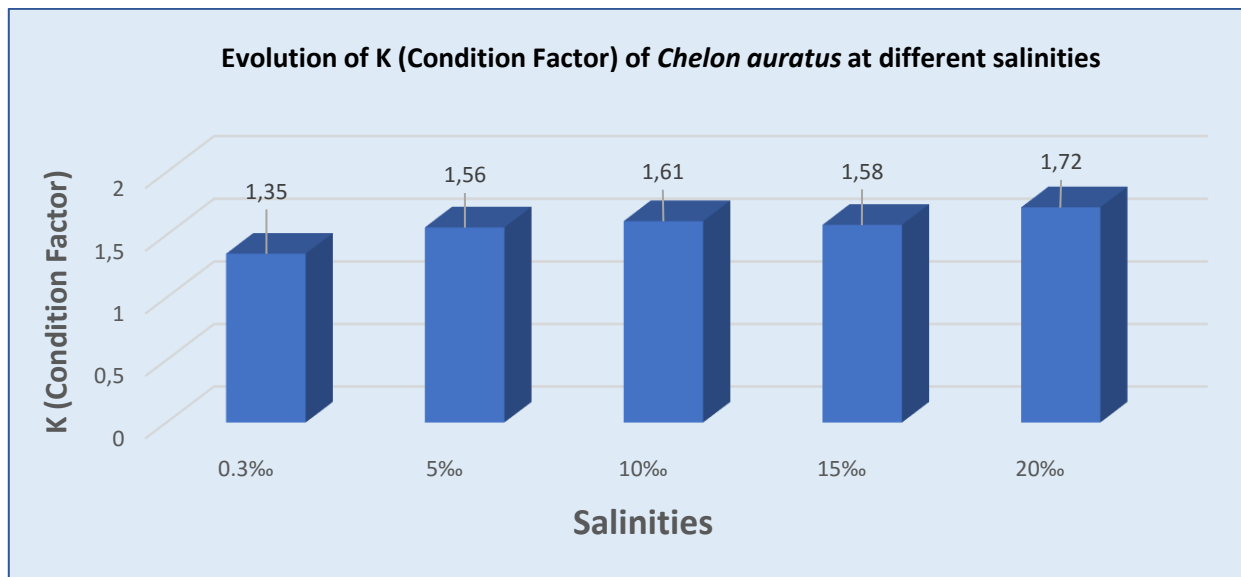


Figure 3. Evolution of K (Condition Factor) of *C. auratus* at various salinities.

were less mobile and less voracious while feeding and significant amounts of pellets were left uneaten on the bottom. In the 5‰ aquarium, swimming was more active, however some of the food was still left not consumed. In the higher salinity tanks, namely at 10‰, 15‰ and 20‰ concentrations, all fish were swimming actively and full consumption of the pellets was observed throughout the experimental period.

These findings are consistent with the evolution of length, weight and condition factor of the tested fish, and indicate that the optimal salinity range for golden grey mullet fries is between 10‰ and 20‰ salinity, which make them very appropriate for Black Sea rearing conditions (with salinities ranging between 13.03‰ - 20.98‰) (Nicolaev et al., 2019). Moreover, *C. auratus* fries can tolerate even lower salinities (5‰), with no major disturbances of growth and overall behaviour, which makes them good candidates for brackish Black Sea water aquaculture.

Temperature Tolerance Experiment

During the temperature tolerance test, salinity was maintained constant around 16‰, yet a slight increase was observed in the warm water aquarium, due to evaporation caused by higher temperatures (around 19‰ maximum).

In the cold-water set-up, no mortalities were recorded during the experimental period. The general behaviour was good and fish were observed feeding and swimming normally down to a 10-11°C water temperature. Below this threshold and down to the final temperature of 8°C, swimming became less active and food consumption reduced, but fish still continued to feed.

Regarding the warm water experiment, progressive temperature increase resulted in an accelerated swimming speed, fish becoming more and

more active along with the temperature rise. Beyond 25°C, the feeding behaviour started to be affected, the fish eating less, yet continuing to feed up to the final temperature of 34°C. No mortalities were recorded in the warm water experimental aquarium.

The temperature tolerance test revealed that golden grey mullet fries can tolerate a wide range of temperature (from 8°C to 34°C), with an optimal range for rearing between 10-25°C. These findings, correlated with the usual Romanian Black Sea water temperatures ranging between 4.4°C and 24°C (Nicolaev et al., 2019), indicate *C. auratus* as a species with good potential for diversifying local aquaculture.

Extreme Salinity Tolerance Test

After completion of the temperature tolerance test, the 30 fish in the warm water aquarium were maintained and a progressive increase of temperature and salinity was applied. The maximum temperature induced using the JBL Pro Temp S300 heater was 36°C. Salinity was increased daily (from 20.5‰), reaching an extreme value of 95‰. Dissolved oxygen values were very high, increasing along with salinity (maximum 176.1 at 95‰).

The most significant observations during the 36 test days concerned behavioral changes (Table 2). As such, at salinities of 20-30‰, all fish performed normal swimming movements and feeding was constant. After overcoming the 30‰ threshold, an accelerated swimming was noted, and fish stopped consuming the pellets offered. When salinity exceeded 50‰, the fish started to display an uncoordinated swimming behavior and no feeding was observed. At salinities between 70‰ and 90‰, partial immobility was observed, as fish rested close to the aquarium bottom and no feeding was documented.

Table 2. Behavioral response of *C. auratus* to extreme salinity.

Extreme Salinity	Ethological response of fish
20-30‰	Normal swimming/feeding
30-50‰	Accelerated swimming/no feeding
50-70‰	Uncoordinated swimming/ no feeding
70-90‰	Partial immobility/no feeding
90-95‰	Total immobility and death

During the last day of the test, when salinity reached the peak value of 95‰, all fish became totally immobile and finally died, due to the impossibility of osmotic regulation. These behavioral effects are all consistent with other findings regarding mullets' response to high temperatures and salinities, which state that the fish became stressed, showing dark coloration, loss of appetite and abnormal swimming behavior, and ultimately death (Hotos & Vlahos, 1998).

Conclusions

The results of our research on golden grey mullet *C. auratus* fries collected from Romanian Black Sea shallow waters indicate that they can tolerate a wide range of temperatures and salinities, which makes them an appropriate species for extensive and semi-intensive aquaculture, in open (flow-through) systems (ponds, lagoons, close to the coast), applicable in the north-western part of the Black Sea, area subject to significant environmental variations, due to the influence of the Danube's freshwater input.

The salinity tolerance experiment revealed that increases in fish length were recorded in all salinity regimes, with the highest in the 15‰ and 0.3‰ aquaria (by 8.82% and 8.24%, respectively). Overall, except for the 20‰ salinity, all fish grew more in length than in weight, regardless of the salinity regime. The highest increase in weight (by 12.04%) was recorded in the 20‰ salinity regime, while in the freshwater aquarium the weight increase was negative (-2.05) (probably influenced by the small number of surviving fish for the final measurement). For the remaining salinities, weight gains were positive, slightly increasing in correlation with salinity (by 0.43%, 3.04% and 3.22%, corresponding to 5‰, 10‰ and 15‰ salinities, respectively). The results obtained for the Condition Factor (K) indicate an overall good state of the fries, with K values ranging between 1.35 and 1.72, with the best condition being reported for fish kept at a 20‰ salinity. These findings indicate that the optimal salinity range for golden grey mullet fries is between 10‰ and 20‰ salinity. Moreover, *C. auratus* fries can tolerate even lower salinities (5‰), with no major disturbances of growth and overall behaviour, which makes them good candidates for brackish Black Sea water aquaculture.

The temperature tolerance test revealed that golden grey mullet fries can tolerate a wide range of temperature (from 8°C to 34°C), with an optimal range

for rearing between 10-25°C, values at which fish swim and feed normally.

The extreme salinity tolerance test showed that at salinities of 20-30‰, all fish still swam and fed normally. After the 30‰ threshold, swimming abnormalities were observed, along with refusal to feed, which culminated, at 90-95‰, with complete immobility and ultimately death of all fish caused by the impossibility of osmoregulation.

The aggregated conclusions of the three experiments are that golden grey mullet fries can tolerate temperatures from 8°C up to 36°C, with an optimal range for rearing between 10-25°C, with no behavioral alterations. Regarding salinity, our findings showed that *C. auratus* fries can indeed tolerate an extremely wide range, from 5‰ up to 70‰, yet the optimal salinity for rearing is between 10‰ and 30‰, even which makes them excellent candidates for the enhancement of aquaculture in the Romanian Black Sea area

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