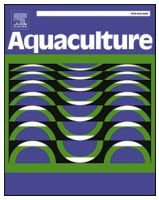
Aquaculture 595 (2025) 741537

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



# Effects of water temperature on growth, health and digestive processes of the thicklip grey mullet *Chelon labrosus*

Markel Sanz-Latorre [a,b,*], Manu Soto [a,b], Urtzi Izagirre [a,b], Xabier Lekube [a,b]

[a] Research Centre for Experimental Marine Biology and Biotechnology PIE, University of the Basque Country UPV/EHU, Areatza 47, Plentzia, Basque Country, Spain
[b] CBET Research Group, Department of Zoology and Animal Cell Biology, Faculty of Science and Technology, Faculty of Science and Technology, University of the Basque Country UPV/EHU, Sarriena z/g, Leioa, Basque Country, Spain

ARTICLE INFO

*Keywords:*
Aquaculture diversification
Water temperature
Metabolism
Digestive enzymes
Mugilidae

ABSTRACT

This study aimed to identify the effects of water temperature on the rearing of the omnivorous teleost *Chelon labrosus* and to determine the optimal temperature for the culture of this interesting candidate for the diversification of European aquaculture. The fish (initial weight 26.81 ± 6.2 g) were held at four water temperatures (18, 22, 26 and 30 °C) in triplicate for 90 days in 100 L recirculating tanks ($n$ = 14 / tank). The effects of said temperatures were assessed on growth, intestinal health and the digestive processes of the fish. At 18 °C, energy consumption was low, leading to a high accumulation of reserves and slow growth, a typical overwintering strategy. The best growth results were obtained at 22 °C, followed closely by 26 °C, which resulted in low energy reserves. The fact that the fish at 26 °C grew less than the ones at 22 °C while having similar energy consumption, suggested that this temperature was close to the end of the optimal range, but no signs of stress were detected. However, at 30 °C the fish had lower energy reserves but grew significantly less, which can be considered indicative of stress. Health impairment at this temperature was confirmed by the epithelial lesions found in this group. The growth and energy availability of the different groups is discussed in the light of the activities of α-amylase, pepsin and alkaline proteases. As an integration of all these results, a quadratic regression model resulting from growth values (SGR) in relation to water temperature was performed, and it allowed predicting an actual optimal water temperature of 22.8 °C for growth of *C. labrosus*. This is the first assessment of the optimal water temperature for the culture of *C. labrosus*, and it will be highly valuable for the development of its intensive culture.

## 1. Introduction

Ambient temperature is considered the most important abiotic factor influencing the metabolism and bioenergetics of poikilothermic animals, such as fish (Hawkins, 1995; Nytrø et al., 2014; Sun and Chen, 2014). Temperature modulates the rates of biochemical reactions that occur within an animal, increasing the oxygen and energy consumption as body temperature increases (Schulte, 2015). This raise of energy consumption in relation to temperature, though, plateaus and eventually declines rapidly when reaching a critical temperature. This phenomenon is mostly explained by conformational changes in the enzymes, although other processes are also important, as reviewed by Schulte (2015). Another factor playing a pivotal role in the determination of the "energy budget" is the minimum amount of energy an animal has to expend in order to maintain essential biological functions, which increases at higher temperatures (Clarke and Fraser, 2004; Farrell, 2009; Schulte, 2015). The difference between these two variables determines the available amount of energy to direct towards processes such as locomotion, growth, immune response or reproduction, creating bell-shaped performance curves in relation to water temperature in fishes (Farrell, 2009).

In the case of cultured fishes, identifying the optimal water temperature range for maximum growth is essential to optimize production and ensure the well-being of the animals, as suboptimal environmental conditions could eventually lead to stress, having a negative impact on fish health (Wang et al., 2019; Islam et al., 2021; Dawood et al., 2022).

* Corresponding author at: Research Centre for Experimental Marine Biology and Biotechnology PIE, University of the Basque Country UPV/EHU, Areatza 47, Plentzia, Basque Country, Spain.
*E-mail addresses:* markel.sanz@ehu.eus (M. Sanz-Latorre), manu.soto@ehu.eus (M. Soto), urtzi.izagirre@ehu.eus (U. Izagirre), xabier.lecube@ehu.eus (X. Lekube).

https://doi.org/10.1016/j.aquaculture.2024.741537
Received 13 May 2024; Received in revised form 6 August 2024; Accepted 28 August 2024
Available online 30 August 2024

Therefore, effects of water temperature and optimal culturing conditions have been successfully identified for commercially important aquaculture species such as Atlantic salmon (Jonsson et al., 2001), European sea bass (Person-Le Ruyet et al., 2004), gilthead sea bream (Seginer, 2016), turbot (Aydın et al., 2021, 2022a), Black Sea flounder (Aydın et al., 2012) or yellowtail kingfish (Bowyer et al., 2014).

Temperature can alter digestive processes such as ingestion, gut passage time of food (Bendiksen et al., 2002; Handeland et al., 2008), as well as the activities of digestive enzymes (Papoutsoglou and Lyndon, 2005; Ahmad et al., 2014; Hani et al., 2018), which directly influence feed efficiency. The fatty acid profile of the edible part of the fish is another relevant aspect that can be influenced by ambient temperature. Due to the homeoviscous adaptation of cell membrane fluidity, at lower temperatures organisms tend to synthesize more unsaturated fatty acids with lower melting points in order to compensate the loss of fluidity caused by cold temperature (Hazel, 1995). As a result, water temperature can directly affect fish quality from the point of view of the consumer, considering the importance unsaturated fatty acids have in human diet.

The thicklip grey mullet *Chelon labrosus* has been identified as an interesting fish for the diversification of aquaculture (Ben Khemis et al., 2006; Zouiten et al., 2008; García-Márquez et al., 2021; Garcia-Marquez et al., 2022). Due to its low-trophic nature, it has great potential for the usage of alternative feed ingredients, helping in the development of the aquaculture industry without increasing its consumption of fishmeal (Naylor et al., 2000). Grey mullets have been cultivated in the Mediterranean region in traditional extensive systems since the antiquity and they are highly valued for human consumption in several countries of said area (Crosetti, 2016). However, there are several knowledge gaps about *C. labrosus* that pose a challenge to the development of its intensive culture. Very little is known about the growth rates of grey mullet in the wild, but it has been described that they alternate a slow-growing phase in autumn-winter and a faster growing one in spring-summer, which lead to the conclusion that the species was not competitive for aquaculture in the early 90s (Arruda et al., 1991). However, conclusions from newer studies are more promising, although the reported data vary greatly depending on the size of the fish considered. Specific Growth Rates (SGR) from 0.4 to 1.15 have been obtained in nutrition researches performed with juveniles in the range of 46 to 100 g (Vílchez-Gómez et al., 2017; Garcia-Marquez et al., 2022; García-Márquez et al., 2023), proving the potential of the species when reared under controlled conditions. The establishment of optimal culturing conditions is of upmost importance in order to further improve the aforementioned results. Many efforts are being directed towards increasing the knowledge about the nutrition of the species (Ojaveer et al., 1996; Davies et al., 1997; Pujante et al., 2015; Pujante et al., 2017; Altunok and Özden, 2017; Garcia-Marquez et al., 2022, García-Márquez et al., 2023; Quirós-Pozo et al., 2023). However, little research has been conducted about optimising other aspects of culturing, apart from the effects of salinity and stocking density (De las Heras et al., 2015; Pujante et al., 2018). Even though water temperature is one of the most important factors for fish growth (Nytrø et al., 2014; Sun and Chen, 2014), no research has been published on *C. labrosus* to our knowledge, aside from a paper focusing on the effects of temperature on osmoregulation (Lasserre and Gallis, 1975) and the aforementioned article by Arruda et al. (1991), where growth in wild conditions was roughly estimated.

In our previous experience working with this species, we observed the best growth results were obtained during summer months at water temperature close to 22 °C (unpublished data), and therefore we could expect this to be the optimal temperature for the rearing of *C. labrosus*. Hence, the objective of the present research was to determine the effects of different water temperatures on growth, energy metabolism (plasma, liver and muscle metabolites), intestinal health (histopathological analysis), digestive processes (digestive enzyme activities) and product quality (fatty acid profile of muscle) of the thicklip grey mullet *C. labrosus* in order to establish the optimal temperature conditions for its culture, always ensuring animal welfare.

## 2. Materials and methods

### 2.1. Experimental design

The fish used in this experiment (thicklip grey mullet, *C. labrosus*) were wild specimens caught at the Urola estuary (Zumaia, Basque Country, Spain) and acclimated to laboratory conditions for eight months in three 300 L open-flow tanks, without temperature control, ensuring natural thermal conditions for the acclimation phase. The fish ($n = 168$, initial fork length 13.35 ± 0.9 cm, initial weight 26.81 ± 6.2 g) were randomly separated into 12 tanks (14 individuals per tank, $n = 3$) 30 days prior to the beginning of the experiment and maintained at room temperature (19 °C). During that period and the entire experiment, fish were fed a commercial diet (Gemma diamond 1 mm; Skretting, Stavanger, Norway) with 57 % of protein and 15 % lipid contents. The pellet size chosen (1 mm) was smaller than the typical one for fish of this size. It was decided based on the experience of the research team with this species, as the sediment filtering feeding behaviour of mugilids does not allow the application of traditional aquaculture feed size tables (Cardona, 2016). Fish were fed manually twice a day, with a total amount that accounted for 2 % of the total biomass of each tank. At the end of the acclimation period (0 D), water temperatures were adjusted to the desired experimental treatments (± 0.5 °C), which were 18, 22, 26 and 30 °C (groups 18, 22, 26 and 30). Each temperature treatment was performed in triplicate. The experimental period lasted 90 days. The initial average stocking density was 4.48 ± 0.28 kg / $m^3$. The stocking density of each particular tank can be found in the Appendix (Table A1).

The experiment was performed in 12,100 L cylindrical-conical tanks. The tanks were recirculating systems, each equipped with a Sun Sun HW - 302 canister filter (WilTec, Eschweiler, Germany). The water flow of the filters was of 540 L / h. Each canister contained a mechanical filter consisting on three foam layers, and a biological filter consisting of ceramic and plastic substrate for nitrifying bacteria. Each tank was equipped with a water cooler or heater, depending on the experimental treatment. The tanks were provided constant aeration to keep adequate oxygen levels of around 6 mg / L. After careful examination of the feeding behaviour of *C. labrosus*, it was decided to close the filters for 15 min at every feeding time, in order to ensure sufficient time for an adequate feeding. After said time, a 10 L water change was performed, and then filters were re-opened. This procedure allowed recovering much of the leftover feed, but not all. This accounted for a daily change of 20 % of the water. At every sampling time, all the water was renewed. Ammonia, nitrite and nitrate levels were checked weekly using commercial kits (SERA, Heinsberg, Germany). Ammonia levels were kept at 0–0.5 mg / L, nitrite at 0–2 mg / L, and nitrate at 0–50 mg / L.

### 2.2. Sample collection

Biometrical data (fork length and body weight) were collected at day 0 (0 D), 30 (30 D), 60 (60 D) and 90 (90 D). For handling (weighing and measuring), fish were caught with a net and introduced in a water bath containing 100 mg / L Tricaine methanesulfonate MS 222 (Sigma-Aldrich, Saint Louis, USA) anaesthetic. For sacrifice, the fish were introduced at a water bath of the same compound at a concentration of 300 mg / L. Four individuals of each tank were sacrificed and dissected at 30 D ($n = 48$), and the rest at 90 D ($n = 120$). Blood was collected from the caudal vein with a heparinized 1 mL syringe with a 25 G needle, and centrifuged for 15 min at 10000 rpm. The plasma was collected, flash frozen in liquid nitrogen and stored at −80 °C for metabolite analysis. Whole muscle of fishes were flash frozen at −80 °C for metabolite and fatty acid profile analyses. Liver and gastrointestinal tracts were extracted, weighed and flash frozen at −80 °C for the measurement of metabolites (liver) and digestive enzyme activities (gastrointestinal tracts). A section of proximal intestine was fixed in formalin for further

histo(patho)logical analysis (See Section 2.7).

### 2.3. Calculations

The following calculations were made according to Garcia-Marquez et al. (2022):

Weight gain (WG) = Final weight (g) – initial weight (g).

$$\text{Specific growth rate\%(SGR)} = 100 \text{ x } \frac{\text{Ln (Final body weight)} - \text{Ln (Initial body weight)}}{\text{Feeding days}} \text{ (\%/day)}.$$

$$\text{Viscerosomatic index (VSI)} = 100 \text{ x } \frac{\text{Visceral weight (g)}}{\text{Total weight (g)}} \text{ (\%)}.$$

$$\text{Hepatosomatic index (HSI)} = 100 \text{ x } \frac{\text{Liver weight (g)}}{\text{Total weight (g)}} \text{ (\%)}.$$

$$\text{Fulton condition factor (K)} = 100 \text{ x } \frac{\text{Fish weight (g)}}{\text{Length}^3 \text{ (cm)}}.$$

### 2.4. Metabolite analyses

Metabolite analyses were performed in pooled samples of each tank ($n = 3$). Plasma glucose (Amplex Red Glucose / Glucose Oxidase Assay Kit, Invitrogen, Carlsbad, USA), liver and muscle glycogen (ab65620 – Glycogen Assay Kit, Abcam, Waltham, USA) and plasma, liver and muscle triglycerides (ab65336 – Triglyceride Assay Kit, Abcam, Waltham, USA) were measured (Pujante et al., 2015) with commercial kits, following the instructions provided by the manufacturers.

### 2.5. Digestive enzyme activities

The activities α-amylase (ab102523 Amylase Assay Kit, Abcam, Waltham, USA), lipase (ab102524 Lipase Activity Assay Kit, Abcam, Waltham, USA), pepsin and alkaline proteases (ab112153 Protease Activity Assay Kit, Abcam, Waltham, USA) were measured (Pujante et al., 2017, 2018) using commercial kits. In the case of pepsin, the same kit employed for alkaline proteases was used by changing the provided reaction buffer with 10 mM HCl (pH 2.0), as recommended by the manufacturer.

The samples were prepared for analysis by homogenizing the stomachs and intestines in liquid $N_2$ conditions, using a SPEX Sample Prep 6770 freezer / mill (SPEX Sample Prep, Metuchen, USA). Enzymes were extracted from the resulting homogenates using a Precellis 24 homogenizer (Montigny-Le-Bretonneux, France) with three 60 s passes at 6500 rpm at 4 °C, using the buffer recommended by the manufacturer for each enzyme. The analyses were performed in triplicate in pooled samples of each tank ($n = 3$). The protein content of each pool was measured using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, USA).

Each enzymatic activity was measured twice, as follows. First, all the pools were analysed using the same incubation temperature (25 °C for α-amylase, 37 °C for lipase), as indicated by the manufacturer. For proteases, no incubation temperature was indicated on the protocol, so the optimum temperature was the one described by Pujante et al. (2017), that is, 50 °C for alkaline proteases and 40 °C for pepsin. This measurement was called "absolute activity". Further, a second analysis was performed by incubating every group at its respective experimental temperature. This measurement was called "actual temperature". The enzymatic activities were defined according to the manufacturers. 1 Unit Amylase = amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 μmol of nitrophenol per min at pH 7.20 at 25 °C. 1 Unit Lipase = amount of lipase that hydrolyzes triglyceride to generate 1.0 μmol of glycerol per minute at 37 °C. Proteases: alkaline proteases and pepsin activities were compared to units of trypsin (unit not specified).

### 2.6. Fatty acid profile of muscle

The analysis of fatty acids was performed by ACOI Coimbra Collection of Algae. The characterization of the fatty acid profile of muscle was performed in pooled samples of each tank (n = 3). Muscle samples were homogenized in liquid $N_2$, using a SPEX Sample Prep 6770 freezer / mill (SPEX Sample Prep, Metuchen, USA). The extraction of lipids from the resulting homogenate was performed by the MTBE method (Matyash et al., 2008).

For the characterization of fatty acid profile, dried samples were resuspended in 1 mL of hexane and 0.5 mL of methanol. After vortexing, 400 μL of sodium methoxide were added. The top layer was filtered with a nylon membrane and 150 μL of the filtered solution was placed in a vial, 100 μL of the internal standard methyl nonadecanoate (C19:0) (Sigma-Aldrich, Saint Louis, USA) with a final concentration of 0.3 mg / mL were added. The gas chromatography was performed in a NEXIS GC-2030 (Shimadzu, Kyoto, Japan) chromatograph equipped with a flame ionization detector and a TR-CN 100 capillary column (60 m × 0.25 mm × 0.20 μm). Helium was used as carrier gas at a pressure of 150 kPa at the top of the column. The temperature of the injector and detector was 260 °C and the split ratio was 1:25. The initial temperature of the column was maintained at 90 °C for 7 min after the injection, increasing 5 °C / min to 220 °C and held for more 15 min. Data were acquired and analysed using Lab Solutions data analysis software. Fatty acids (FA) were identified by comparing the relative retention times with an authentic external standard, Supelco 37 component FAME mix (Sigma-Aldrich, Saint Louis, USA). The quantification of FA was based on the internal standard method (Assunção et al., 2017). The results were expressed in percentage of the total FAME (%).

### 2.7. Histological analysis

Intestine samples were prepared for histological analysis following standard histological procedures. Briefly, proximal intestine samples were fixed in a 10 % formalin solution buffered with seawater for 24 h (Martoja et al., 1970). Once fixed, formalin was replaced by 70 % ethanol until the processing. Samples were processed with an automated tissue processor (LEICA ASP 300S; Leica Microsystems Nussloch GmbH, Germany) and embedded in paraffin wax blocks. Five μm thick sections were obtained with a microtome (Leica RM2125RTS; Leica Microsystems Nussloch GmbH, Germany), stained with haematoxylin-eosin (Martoja et al., 1970)using an Autostainer XL (Leica Microsystems Nussloch GmbH, Germany), and mounted with coverslip. The samples were observed under an Olympus BX61 light microscope (Olympus-Lifescience, Tokyo, Japan).

### 2.8. Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics 27 software (IBM Corp. Released, 2020). Normality of the data was

tested using the Shapiro Wilk test. Homogeneity of variances was tested using the Levene's test. In the case of data with normal distributions and homogeneous variances, Student's *t*-test was performed to compare the different variables such as weight, length, SGR, HSI, VSI, K, metabolite levels, enzymatic activities, fatty acids or histological lesions (dependent variables) across water temperature treatments or sampling times (independent variables). When data was not normal and variances not homogeneous, the non-parametric Shapiro-Wilk's test was used. Different linear regression models were performed among SGR data from 0 D to 90 D (dependent) and water temperature (independent), and the one with the best fit was selected. The significance for every statistical test was set at $p < 0.05$. All data are presented as mean ± standard deviation.

### 2.9. Ethical statement

All experimental procedures complied with the Guidelines of the European Union (2010/63/UE) and the Spanish legislation (RD53/2013 and law 32/2007) for the handling and use of laboratory animals under the supervision and acceptance of the Ethics for experimentation and animal welfare committee of the University of the Basque Country (UPV/EHU) and provincial authorities (CEEA: M20_2021_108-Diputación Foral de Bizkaia).

**Table 1**
Specific Growth Rate (SGR) of *C. labrosus* reared at different temperatures for 90 days. 30 D: day 30; 60 D: day 60; 90 D: day 90. Groups with different superscript letters show statistically significant ($p < 0.05$) differences. Capital letter superscripts: comparisons between temperature treatments inside a given time period (row); lower letter superscripts: comparisons between time periods inside a given temperature treatment (column).

| | | 18 °C | 22 °C | 26 °C | 30 °C |
|---|---|---|---|---|---|
| SGR (%/day) | 0 D - 30 D | $0.92 \pm 0.03^{Aa}$ | $1.02 \pm 0.09^{Aab}$ | $1.01 \pm 0.15^{A}$ | $0.51 \pm 0.14^{Bb}$ |
| | 30 D - 60 D | $0.72 \pm 0.14^{Bb}$ | $0.96 \pm 0.06^{Ab}$ | $1.01 \pm 0.16^{AB}$ | $0.87 \pm 0.43^{ABab}$ |
| | 60 D - 90 D | $0.57 \pm 0.17^{Bb}$ | $1.16 \pm 0.10^{Aa}$ | $1.01 \pm 0.21^{A}$ | $0.79 \pm 0.20^{Ba}$ |
| | 0 D - 90 D | $0.80 \pm 0.09^{BC}$ | $1.01 \pm 0.02^{A}$ | $0.95 \pm 0.09^{AB}$ | $0.79 \pm 0.06^{C}$ |

## 3. Results

At the end of the experiment, group 22 exhibited higher length and weight values than the others. This difference was statistically

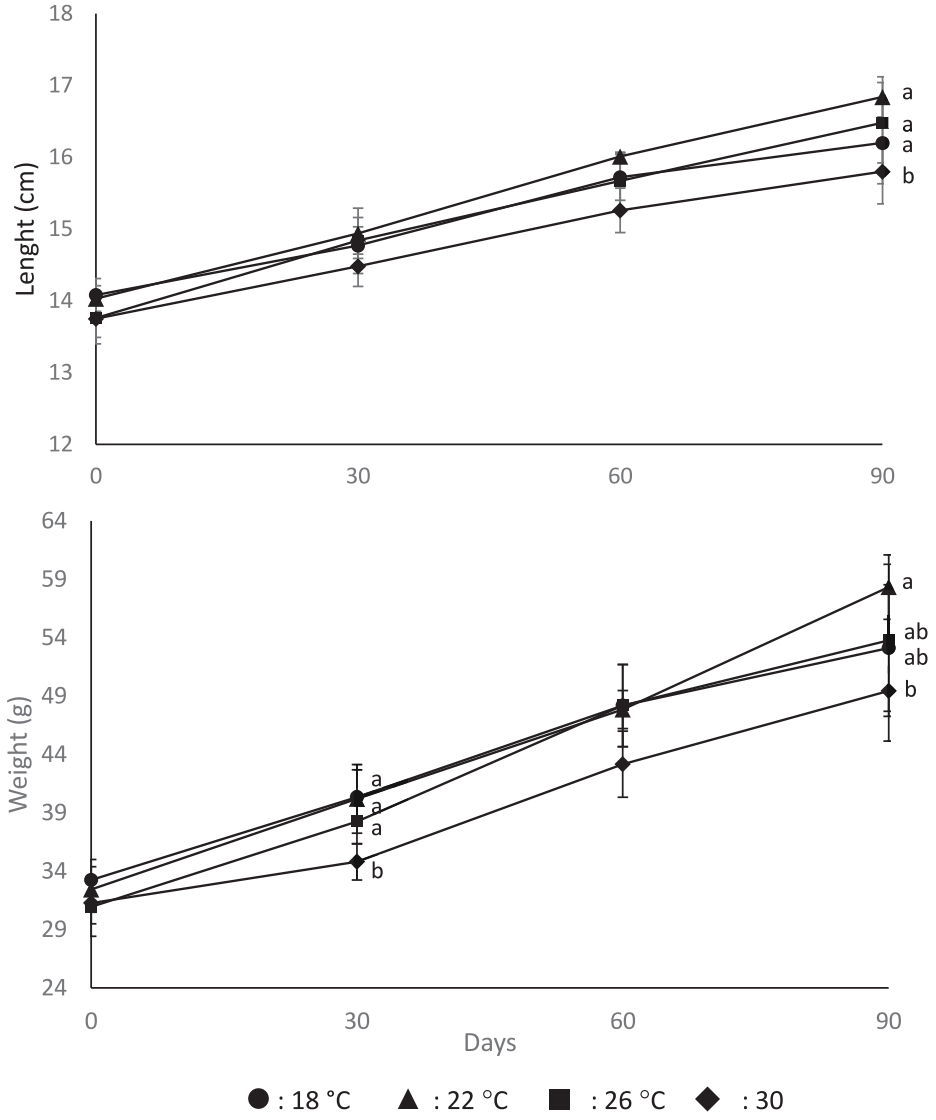



**Fig. 1.** length (cm) and weight (g) of *C. labrosus* reared at different temperatures for 90 days. Groups with different superscript letters show statistically significant ($p < 0.05$) differences.

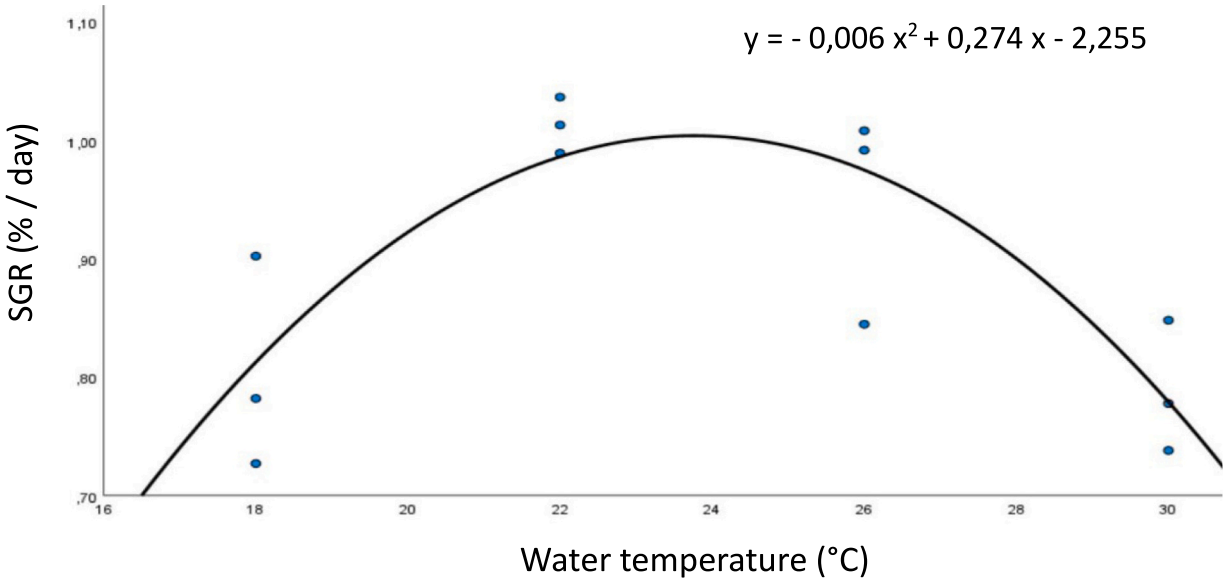



**Fig. 2.** quadratic regression between SGR (beginning to end of the experiment; 0 D – 90 D) and water temperature of *C. labrosus* reared at different temperatures for 90 days.

**Table 2**
weight (W), fork length (Fl), hepatosomatic (HSI) and viscerosomatic (VSI) indexes, and Fulton condition factor (K) of *C. labrosus* reared at different temperatures for 90 days. 30 D: day 30; 90 D: day 90. Capital letter superscripts: comparisons between temperature treatments at 30 D; lower letter superscripts: comparisons between temperature treatments inside 90 D. Significant differences ($p < 0.05$) inside a given treatment over time (30 D vs 90 D) are highlighted in bold.

| | 18 °C | | 22 °C | | 26 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|
| | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D |
| W (g) | **40.33 ± 7.85** $^{A}$ | **52.59 ± 9.56**$^{AB}$ | **40.25 ± 8.77**$^{a}$ | **57.74 ± 11.86** $^{A}$ | **38.41 ± 7.05**$^{a}$ | **55.70 ± 10.28**$^{AB}$ | **34.86 ± 7.36**$^{b}$ | **49.13 ± 8.73**$^{B}$ |
| Fl (cm) | **14.77 ± 0.91** | **16.15 ± 0.93** $^{A}$ | **14.96 ± 1.04** | **16.78 ± 0.99** $^{A}$ | **14.89 ± 0.88** | **16.65 ± 0.97** $^{A}$ | **14.49 ± 0.91** | **15.76 ± 0.86**$^{B}$ |
| HSI | **1.16 ± 0.04**$^{a}$ | **1.03 ± 0.06** $^{A}$ | 0.80 ± 0.12$^{b}$ | 0.78 ± 0.05$^{B}$ | 0.65 ± 0.08$^{bc}$ | 0.65 ± 0.03$^{C}$ | 0.63 ± 0.09$^{c}$ | 0.69 ± 0.04B$^{C}$ |
| VSI | 6.79 ± 0.47 | 7.00 ± 0.33 $^{A}$ | 6.47 ± 0.11 | 6.19 ± 0.22$^{B}$ | 6.10 ± 0.74 | 5.15 ± 0.37$^{C}$ | 6.29 ± 0.74 | 5.84 ± 0.18$^{B}$ |
| K | 1.24 ± 0.03$^{a}$ | 1.24 ± 0.02 $^{A}$ | 1.19 ± 0.02$^{ab}$ | 1.20 ± 0.04$^{AB}$ | 1.15 ± 0.02$^{b}$ | 1.19 ± 0.02$^{B}$ | **1.13 ± 0.03**$^{b}$ | **1.24 ± 0.02** $^{A}$ |

significant ($p < 0.05$) between group 22 and group 30 in both cases (Fig. 1).

At the end of the experiment, group 22 showed the highest SGR values, followed by group 26, the other two having significantly lower values (Table 1). During the experimental period, the SGR values of group 18 significantly decreased, while the opposite happened to group 30.

The regression model between SGR from the beginning to the end of the experiment (0 D – 90 D;dependent variable) and water temperature (independent variable) with the best fit ($R^2 = 0.701$) was the quadratic regression (Fig. 2). The maximum of the curve was found at 22.8 °C.

HSI showed the same pattern at both samplings (Table 2), group 18 having the highest values, followed by group 22, while 26 and 30 groups had the lowest. There were no significant differences in VSI at 30 D, but at 90 D, a very similar result to HSI differences was obtained. Regarding K value, it was also higher in group 18 than in the 26 and 30 groups at 30

**Table 3**
plasma, liver and muscle biochemical data of *C. labrosus* reared at different temperatures for 90 days. 30 D: day 30; 90 D: day 90; Glu: glucose; Tg: triglycerides; Gly: glycogen. Groups with different superscript letters show statistically significant ($p < 0.05$) differences. Capital letter superscripts: comparisons between temperature treatments at 30 D; lower letter superscripts: comparisons between temperature treatments inside 90 D. Significant differences ($p < 0.05$) inside a given treatment over time (30 D vs 90 D) are highlighted in bold.

| | | 18 °C | | 22 °C | | 26 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D |
| Plasma (mg/dL) | Glu | 63.56 ± 35.37 | 84.63 ± 16.49 | 58.58 ± 23.59 | 81.39 ± 18.03 | **54.10 ± 14.72** | **91.45 ± 9.12** | 64.78 ± 28.23 | 80.68 ± 14.89 |
| | Tg | **178. 80 ± 20.89**$^{a}$ | **243.34 ± 38.50** $^{A}$ | **119.25 ± 12.80**$^{b}$ | **175.04 ± 17.60**$^{B}$ | 34.97 ± 28.71$^{c}$ | 97.45 ± 41.28$^{C}$ | **38.13 ± 23.29**$^{c}$ | **96.72 ± 7.62**$^{C}$ |
| Liver (mg/g) | Gly | 13.23 ± 3.37 | 15.41 ± 2.03 $^{A}$ | 7.52 ± 3.33 | 6.72 ± 4.04$^{B}$ | 10.67 ± 2.09 | 12.69 ± 3.42$^{AB}$ | 8.16 ± 2.91 | 10.77 ± 2.24$^{AB}$ |
| | Tg | 86.01 ± 9.05$^{a}$ | 101.35 ± 25.62 $^{A}$ | 55.54 ± 14.22$^{b}$ | 61.56 ± 7.18$^{AB}$ | 63.93 ± 26.17$^{ab}$ | 49.41 ± 10.16$^{B}$ | **47.46 ± 22.25**$^{b}$ | **86.42 ± 15.61** $^{A}$ |
| Muscle (mg/g) | Gly | **2.35 ± 0.31**$^{a}$ | **1.53 ± 0.20** $^{A}$ | 1.33 ± 0.18$^{b}$ | 1.30 ± 0.08$^{AB}$ | 1.45 ± 0.47$^{b}$ | 0.91 ± 0.20$^{C}$ | 1.51 ± 0.26$^{ab}$ | 1.12 ± 0.12$^{BC}$ |
| | Tg | 5.49 ± 1.95 | 8.61 ± 5.87 | 11.79 ± 5.92 | 10.64 ± 3.87 | 9.29 ± 6.31 | 5.34 ± 2.62 | **3.45 ± 0.52** | **9.93 ± 1.26** |

D, but at 90 D, group 26 showed the lowest value.

Plasma glucose (Table 3) did not show any significant difference between treatments at any of the sampling times, but it did have an upwards trend from 30 D to 90 D in every group, this difference being significant in group 26. Plasma triglyceride changed between groups and sampling times. At both samplings, group 18 had the highest values, followed by group 22, while the other groups had the lowest values. Similar to what happened with glucose, plasma triglyceride also increased over time, but in this case the differences were always significant between 30 D and 90 D, except at group 26. Liver glycogen only showed a significant difference at 90 D, when group 18 had the highest values and group 22 the lowest. At 30 D, group 18 showed a significantly higher liver triglyceride content than the other groups, but at 90 D, group 30 had the highest values alongside group 18. In fact, group 30 showed the only significant increase in liver triglycerides over time. Muscle glycogen values were the highest in group 18 at both samplings. Muscle triglyceride levels did not show any significant difference between temperature treatments at any sampling, but they did increase over time at group 30.

When measuring the amylase activity of every group at the same incubation temperature (absolute activity), group 18 showed the highest value at 30 D, but at 90 D, group 22 had the highest activity (Table 4). Group 18 was the only one where absolute amylase activity did not increase over time. When measuring the amylase activity at the experimental temperatures (actual activity), groups 26 and 30 showed the highest values at 30 D, but at 90 D, there were no differences between 22, 26 and 30 groups. Lipase activity was below detection limit at every temperature tested.

At 90 D group 18 had the lowest actual alkaline protease activity, despite showing the highest absolute activity. In the case of pepsin, groups 18 and 22 had the highest absolute activities at 90 D, while 22, 26 and 30 groups demonstrated significantly higher actual activities than the group 18.

Fatty acid content of fish muscle (Table 5) did not differ among temperature treatments in terms of total SFA content, but there were slight changes in MUFA and PUFA. The fish with the lowest MUFA content at the end of the experiment were the ones held at 22 °C, while they had the highest content of PUFA. ω-3 and ω-6 PUFA content were also equal across all experimental treatments. Full information about fatty acids can be found at the Appendix (Table A2).

Most of the analysed intestinal samples presented the expected structure (Fig. 3A). This consists of the serosa layer in the outermost part, muscular layer, submucosae and the mucosa, comprised of the epithelium and lamina propria (Rašković et al., 2011). Interestingly, a disruption of the mucosal barrier characterised by the loss of enterocytes (Fig. 3B) was detected in certain samples. Aside from the gaps in the epithelium caused by the enterocyte loss, some debris could be seen coming out from these lesions. These anomalies were most prevalent at the end of the experiment at group 30 (Fig. 3C).

## 4. Discussion

In the present research, *Chelon labrosus* were maintained under four water temperatures (18, 22, 26 and 30 °C) to assess the one rendering the best yields in terms of growth and animal welfare. The highest length increase, weight gain and SGR values of *C. labrosus* juveniles were achieved at 22 °C, closely followed by the fish reared at 26 °C. The other two groups (18 and 30 °C) showed significantly lower values on all the aforementioned parameters at the end of the experiment. The SGR values obtained herein are within the range reported for *C. labrosus* individuals of similar sizes in previous research, as SGRs from 0.89 to 1.15 were obtained in juveniles weighing 46 g in a nutritional research (Vílchez-Gómez et al., 2017). These results suggest that the optimal temperature range for a healthy growth is between 22 and 26 °C for *C. labrosus*, 26 °C being close to the upper limit. The other temperatures tested are sub-optimal for thicklip grey mullet culture. In order to summarize all these results, absolute SGR was used as a general representation of fish performance during the experiment, and it was correlated to water temperature. The regression model with the best fit was found to be the quadratic regression, which accurately reflected the dome-shaped growth curves usually caused by water temperature on fishes (Nytrø et al., 2014). The resulting model allowed predicting the actual optimal temperature for the rearing of *C. labrosus* juveniles, which was 22.8 °C, a very similar temperature to the originally expected one (22 °C). In a research focusing on fatty acids of *C. labrosus* under different culturing conditions, the authors mention "good growth rates" at temperatures from 26 to 30 °C (Rabeh et al., 2022), but further detail is lacking, as this is not the focus of said paper. The accurate interpretation of these results, though, requires considering the origin of the fish used. The experimental fish used herein were original from the Cantabrian Sea, which rarely reaches temperatures above 22 °C even in summer months (Borja et al., 2019). Moreover, it is known that optimal water temperature for fish growth is typically somewhat higher than the temperature normally encountered at their natural habitat (Handeland et al., 2008), which supports our observations. However, this species is widely distributed across the North-East Atlantic Ocean from Mauritania to Norway, Mediterranean and Black Seas (Turan, 2016). Although the existence of genetically differentiated subpopulations has not been proven (Nzioka et al., 2023), it is conceivable for populations inhabiting warmer areas, such as the Mediterranean, to have adaptations to cope with the thermal characteristics of their habitat, which ultimately could affect growth. For this reason, the provenance of the fish has to be taken into account when farming *C. labrosus*, and optimal temperature re-evaluated if necessary. Other fish species of temperate waters show an

**Table 4**
α-amylase (Amy), alkaline proteases (Alk Prot) and pepsin (Pep) activities of *C. labrosus* digestive tract reared at different temperatures for 90 days. 30 D: day 30; 90 D: day 90; Abs: absolute activity measured as recommended by the manufacturer; Act: actual activities measured at the experimental temperatures. Groups with different superscript letters show statistically significant ($p < 0.05$) differences. Capital letter superscripts: comparisons between temperature treatments at 30 D; lower letter superscripts: comparisons between temperature treatments inside 90 D. Significant differences ($p < 0.05$) inside a given treatment over time (30 D vs 90 D) are highlighted in bold.

| | | 18 °C | | 22 °C | | 26 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D |
| Amy (U / μg protein) | Abs | 100.40 ± 11.13$^{a}$ | 81.35 ± 5.35$^{B}$ | **71.20 ± 10.84$^{b}$** | **104.29 ± 9.50 $^{A}$** | **73.20 ± 6.57$^{b}$** | **88.18 ± 3.00$^{B}$** | **71.46 ± 4.34$^{b}$** | **94.12 ± 10.89$^{B}$** |
| | Act | 68.82 ± 10.74$^{ab}$ | 53.18 ± 7.16$^{B}$ | **57.62 ± 11.24$^{b}$** | **83.98 ± 6.14 $^{A}$** | 74.24 ± 9.49$^{a}$ | 85.38 ± 5.62 $^{A}$ | **78.84 ± 3.58$^{a}$** | **119.49 ± 28.39 $^{A}$** |
| Alk Prot (mU/μg protein) | Abs | 1.74 ± 0.24 | 1.78 ± 0.19 $^{A}$ | 1.80 ± 0.16 | 1.64 ± 0.22$^{AB}$ | **1.60 ± 0.14** | **1.21 ± 0.20$^{B}$** | 1.34 ± 0.40 | 1.30 ± 0.27$^{AB}$ |
| | Act | 0.93 ± 0.12 | 0.73 ± 0.10$^{B}$ | 1.08 ± 0.08 | 1.16 ± 0.06 $^{A}$ | 1.29 ± 0.25 | 1.26 ± 0.19 $^{A}$ | 0.91 ± 0.29 | 1.13 ± 0.31$^{AB}$ |
| Pep (U / g protein) | Abs | 54.37 ± 1.52$^{a}$ | 55.11 ± 7.84 $^{A}$ | 52.72 ± 3.62$^{a}$ | 49.56 ± 0.97 $^{A}$ | **45.91 ± 7.81$^{a}$** | **11.44 ± 2.11$^{B}$** | 13.70 ± 7.94$^{b}$ | 10.57 ± 3.74$^{B}$ |
| | Act | 10.63 ± 2.44$^{b}$ | 7.42 ± 3.31$^{B}$ | 14.27 ± 3.81$^{ab}$ | 16.01 ± 3.03 $^{A}$ | 21.37 ± 3.38$^{a}$ | 16.16 ± 2.28 $^{A}$ | 11.42 ± 4.18$^{b}$ | 14.74 ± 2.48 $^{A}$ |

**Table 5**
summary of fatty acid contents of *C. labrosus* muscle reared at different temperatures for 90 days (expressed as % of the total fatty acid methyl esters). 30 D: day 30; 90 D: day 90; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ω-3 PUFA: omega-3 polyunsaturated fatty acids; ω-6 PUFA: omega-6 polyunsaturated fatty acids. Groups with different superscript letters show statistically significant ($p < 0.05$) differences. Capital letter superscripts: comparisons between temperature treatments at 30 D; lower letter superscripts: comparisons between temperature treatments inside 90 D. Significant differences ($p < 0.05$) inside a given treatment over time (30 D vs 90 D) are highlighted in bold. Full information about fatty acids can be found at the Appendix (Table A2).

| | 18 °C | | 22 °C | | 26 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|
| | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D |
| ΣSFA | 43.00 ± 1.65 | 43.33 ± 1.86$^{AB}$ | **41.95 ± 0.33** | **44.26 ± 0.85** $^{A}$ | 43.12 ± 2.23 | 42.99 ± 0.83 $^{AB}$ | 40.23 ± 3.32 | 42.17 ± 1.07$^{B}$ |
| ΣMUFA | 30.76 ± 0.97 | 31.33 ± 0.97 | 30.39 ± 1.73 | 27.54 ± 2.02 | 30.49 ± 1.00 | 29.31 ± 1.71 | 32.12 ± 2.86 | 31.59 ± 1.73 |
| ΣPUFA | 26.24 ± 2.61 | 25.34 ± 0.92$^{B}$ | 27.81 ± 1.68 | 28.20 ± 1.20 $^{A}$ | 26.38 ± 1.23 | 27.70 ± 0.99 $^{A}$ | **27.62 ± 0.47** | **26.24 ± 0.66**$^{AB}$ |
| Σ ω-3 PUFA | 14.26 ± 2.63 | 15.91 ± 1.29 | 16.57 ± 1.99 | 19.63 ± 2.45 | 15.64 ± 0.24 | 18.36 ± 2.33 | 14.78 ± 2.53 | 16.12 ± 1.64 |
| Σ ω-6 PUFA | 10.61 ± 1.76 | 8.12 ± 0.82 | **9.87 ± 0.31** | **7.35 ± 0.83** | 9.40 ± 1.13 | 7.76 ± 0.92 | 11.04 ± 2.41 | 8.37 ± 0.81 |

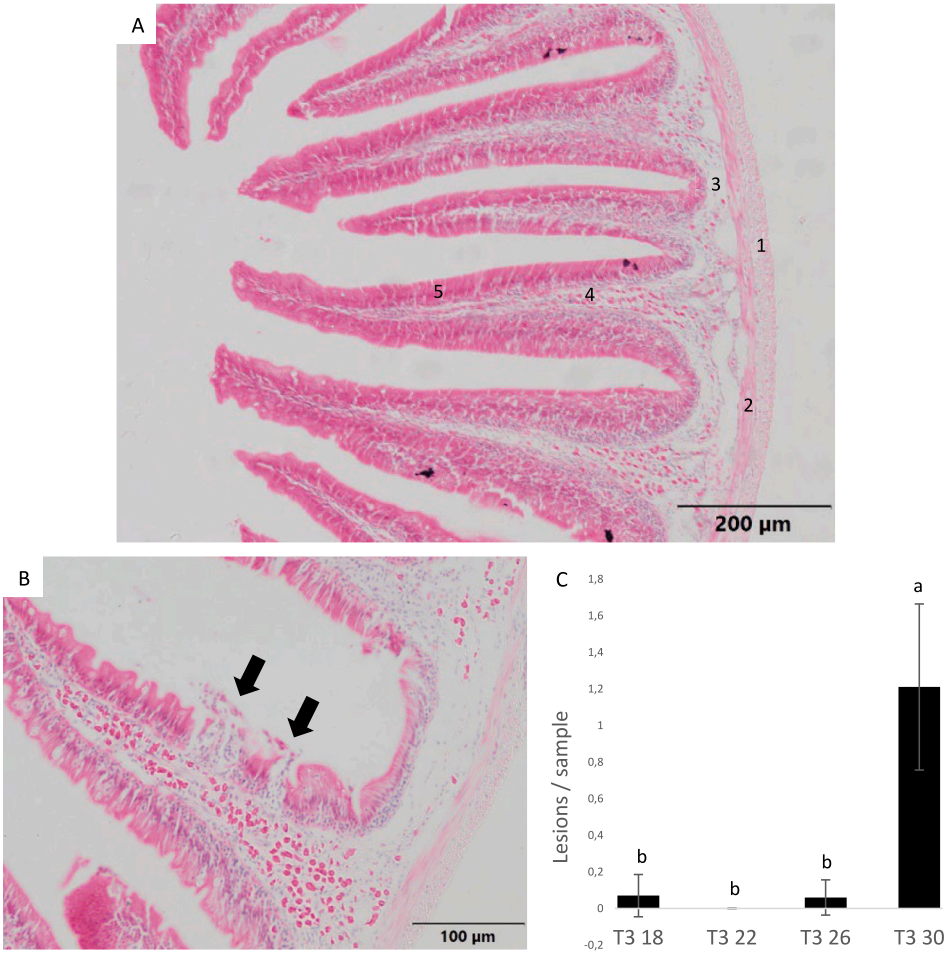



**Fig. 3.** A) normal structure of intestinal villi. 1: serosa layer. 2: muscular layer. 3: submucosae. 4: lamina propria. 5: epithelium; B) example of intestinal villi with epithelial lesions (highlighted with arrows). Note the gap caused by enterocyte loss and the debris coming out of the lesion; C) incidence (lesions per sample) of epithelial lesions in *C. labrosus* intestine reared at different temperatures at day 90.

optimal thermal range similar to *C. labrosus,* although species-specific variations exist. Yellowtail kingfish exhibits a very narrow optimal temperature range peaking at 24 °C, very similar to *C. labrosus* (Bowyer et al., 2014). The performance curve of the European sea bass is slightly displaced towards higher temperatures, as it performs better at temperatures close to 26 °C, and growth starts to be depressed at 29 °C (Person-Le Ruyet et al., 2004). Gilthead sea bream also exhibits higher optimal temperatures, close to 25 °C (Seginer, 2016). The opposite happens in the case of turbot, as its optimum temperature is close to 18 °C (Aydın et al., 2021), which has been identified as sub-optimal for *C. labrosus*. Compensatory growth is a phenomenon that could make the interpretation of these results more difficult. It consists of accelerated growth appearing when recovering optimal conditions after a period of adversity like food deprivation or sub-optimal temperature (Py et al., 2022). Water temperature during the acclimation phase was around 19 °C, close to the 18 °C identified as sub-optimal for *C. labrosus* rearing. Therefore, increasing water temperature to 22 and 26 °C, in the optimal range of the species, could trigger compensatory growth, and the effects observed would be a product of the temperature change, not of the experimental temperature itself. However, compensatory growth is known to be stronger after periods of severe adverse conditions, when energy reserves of the fish have been strongly exploited (Py et al., 2022). As seen in the present study, fish at 18 °C had the highest energy reserves, so the sub-optimal conditions of the acclimation period probably

were not severe enough to induce a compensatory growth response.

It is worth noting that while the performance of groups 22 and 26 was constant across the entire experimental period, the other two groups showed remarkable alterations over time, as evidenced by the changes in SGR across the different sampling times. In the case of group 30, the performance improvement observed over time might be a result of acclimation after a sharp increase in water temperature. A wide range of mechanisms for thermal acclimation exist in teleost fishes, some of them being almost instantaneous while others can take several weeks to activate (Johnston and Dunn, 1987). However, despite the ability showed by *C. labrosus* to acclimate to temperatures up to 30 °C and improve their performance over time, their compensatory mechanisms are not enough to overcome the challenge posed by this extreme ambient temperature completely. Feed intake data could allow for a better understanding of the decrease of growth observed at group 18 from the beginning to the end of the experiment, but unfortunately, it was not recorded. It is known that feed intake can vary depending on several factors, including water temperature, although the understanding of appetite regulation on fish is still a "work in progress" (Volkoff and Rønnestad, 2020). In general, food intake in fishes increases alongside water temperature until reaching a maximum, usually at a temperature a bit higher than the optimal one for growth (Burel et al., 1996). A decrease in feed intake when lowering water temperature has been observed in fishes such as the yellowtail kingfish *Seriola lalandi* (Miegel et al., 2010), Asian catfish *Clarias batracus* (Ahmad et al., 2014), turbot *Scophtalmus maximus* (Guerreiro et al., 2016), cobia *Rachycentron canadum* (Sun and Chen, 2014) or Atlantic salmon *Salmo salar* (Bendiksen et al., 2002). This hypothetical low feed intake of the fish at 18 °C does not completely explain the decrease in the growth rate observed after the first 30 days. Excessive fat reserve accumulation can hinder feed intake by providing negative feedback on the food intake regulation centres (Jobling and Johansen, 1999) and cause retardation of growth (Jobling et al., 2002). In this experiment, perivisceral fat was extracted and weighed alongside the digestive tract, so VSI could be used as an approximation of perivisceral fat reserves in this particular case. This index was higher on group 18, and this high fat accumulation could lead to lower feed intake and consequently, a decrease in the performance of this group over time. However, there is not enough evidence in order to support this hypothesis.

As expected, water temperature also had an impact on the bioenergetics of the fish. The fish held at 18 °C had the highest levels of triglycerides, in both plasma and liver, as well as glycogen in liver and muscle. Therefore, the slow growth observed on this group cannot be attributed to low energy availability. High energy storage was also reflected on HSI, as this group had the highest values of said index, and on VSI, as previously mentioned. In winter, food availability might be compromised, and some fishes do exhibit a reserve accumulation behaviour at cold temperatures (Schultz and Conover, 1997), coupled with a depressed metabolic activity (Reeve et al., 2022). On a research conducted on juvenile roach *Rutilus rutilus* it was seen that growth was completely stopped at 12 °C, and that the energy reserves were higher in winter than in summer, especially in fish acclimated at 4 °C (Van Dijk et al., 2005). The extent of this behaviour and the temperature at which it takes place is variable among species and responds to the environmental factors they encounter in their natural habitat (Schultz and Conover, 1997). In the case of our experimental fish, 18 °C seems to be too high to trigger an overwintering behaviour, because this temperature is high for winter in their natural habitat at the Cantabrian Sea (Borja et al., 2019). In a research about wild grey mullet populations of the Atlantic coast of Portugal, it was seen that most of the growth experienced by *C. labrosus* happened during spring and summer, when water temperature was above 14 °C (Arruda et al., 1991). Perhaps, in natural conditions, 18 °C could be close to the threshold at which this fish starts to reorganize energy partitioning, withdrawing energy from growth towards reserve accumulation, as a preparation for winter dormancy. The results of plasma, liver and muscle metabolites, as well as HSI and VSI suggest that fish of group 26 had a higher energy expenditure than the fish of group 22, which was not reflected as a growth increase. This could imply that at this temperature, the fish are starting to deviate energy from growth in order to fuel the mechanisms for thermal stress compensation (Alfonso et al., 2021), a strategy that could be considered successful at this temperature, as it did not compromise growth. A similar but more pronounced response was seen at 30 °C, were higher energy expenditure did not cause growth improvement, this being the group with the lowest SGR. Interestingly, after 30 D, an increase in lipid reserves was noted, as seen by the high liver triglyceride levels and VSI, coinciding with the moment where the growth in this group started to raise.

In a previous study about the activity of digestive enzymes of fish reared at different water temperatures, the enzymatic extracts of every experimental group at the same reaction temperature were tested to estimate enzyme secretion levels (Navarro-Guillen et al., 2022), called "absolute activity" herein. However, the enzymatic activities measured at the experimental temperatures are more relevant to elucidate the actual digestive capabilities of the fish, as they are poikilotherms and strict temperature conformers (Volkoff and Rønnestad, 2020), with the exception of some species with big body sizes that can produce and keep enough heat so as to increase their temperature above said threshold (Carey et al., 1971). The overall low lipase activity found in the present research is in concordance with the results obtained by Pujante et al. (2017) on the same species, which was almost negligible. This is in agreement with the expected lipase activity of herbivorous fish, which theoretically would have a low intake of dietary lipids in the wild (Opuszynski and Shireman, 2019), and it suggests that *C. labrosus* has a limited ability to digest lipids (Pujante et al., 2017), independently of water temperature. As a result, these species would rely more on carbohydrates as energy source. One of the most important carbohydrases in fishes is α-amylase (Kaushik et al., 2022). When measuring absolute activity, all groups except group 18 showed an increase in α-amylase activity in the gastrointestinal tract over time. It has been reported that α-amylase activity of *C. labrosus* increases alongside the size of the fish (Pujante et al., 2017), and the raise of activity over time found in the present experiment could respond to the same principle. Even though not statistically significant, the α-amylase activity of the 18 °C group decreased over time. This could potentially lower carbohydrate digestibility, and it could partially explain the decrease of SGR experienced over time. Although feed digestibility was not measured, the alteration of the pattern observed on the rest of the groups seems relevant, and the possibility of a decrease on energy digestibility cannot be ruled out. When measured at their respective physiological temperatures, groups 22, 26 and 30 showed similar α-amylase activities, while group 22 showed the highest absolute activity of said enzyme. Enzymatic activities increase with reaction temperatures (Schulte, 2015), so it is plausible for group 22 to be producing a higher amount of α-amylase in order to compensate for the lower activity at said temperature. Even though this mechanism has been observed at some cases (Savoie et al., 2008), it does not seem to be a widespread strategy. Despite being a general trend, the increase of the α-amylase activity at 30 °C from 30 D to 90 D was the most pronounced and may explain the improvement of performance during the experimental period, as well as the increase of lipid reserves. Protease activities showed similar patterns to α-amylase, but there were not any remarkable differences over time. At 18 and 22 °C, higher absolute pepsin and alkaline protease activities were found, suggesting a higher concentration of said enzymes, but when measuring at the physiological temperatures, actual activities were similar at groups 22, 26 and 30, and lower at 18. The higher production of proteases at 18 and 22 °C could respond to the same compensation mechanism previously hypothesized for α-amylase, but with different effectiveness. At 22 °C, the increased enzyme production would be enough to reach the same physiological activity than at warmer temperatures, whereas at 18 °C would not. The pepsin activity measured herein is remarkably low when compared to alkaline protease activity,

contrary to the findings by Pujante et al. (2017), who found that these fish do have a relatively high pepsin activity at early life stages, which is almost lost during development. Therefore, our results align better with the expected protease profile for older fish, as the experimental fish of the present research were even younger/smaller than the fish analysed by Pujante et al. (2017). This discrepancy deserves more attention in future research, and the understanding of the different experimental strategies, protocols, feeding, origin of the fish and sampling would make the comparison between researches easier.

Even though group 30 was not the only one showing epithelial damage in the intestines, the prevalence of such lesions was significantly higher than at other temperatures. It is known that stressful conditions can alter fish digestive epithelial integrity by damaging enterocyte junctions (Olsen et al., 2002). The observed enterocyte damage could be a result of such weakening of the epithelia, and it could lead to a disruption of its barrier function, endangering the osmotic balance and facilitating pathogen infection (Del-Pozo et al., 2010). This condition strongly suggests that 30 °C are stressful for *C. labrosus*, and the health and well-being of the fish could be compromised at this temperature.

Water temperature did not have a profound impact on *C. labrosus* muscle fatty acid profile. Even though cell membranes show the ability to alter their fatty acid composition depending on ambient temperature (Hazel, 1995), other biotic factors such as phylogeny, diet, age, reproductive status or ploidy have been found to be more relevant when explaining the fatty acid profile of a given fish species (Kaushik et al., 2006; Skalli et al., 2006; Senso et al., 2007; Sushchik et al., 2018, Aydın et al., 2022b). Among abiotic factors, water salinity or pH can also have a profound impact on fish fillet fatty acid profile (Rabeh et al., 2022). This would support the lack of differences found among groups in the present research when it comes to muscle fatty acids. In any case, groups 22 and 26 did show a higher content of PUFA, especially of the ω-3 group. Conversely, Rabeh et al. (2022) reported significant changes in *C. labrosus* fatty acid profile depending on water temperature and salinity, but the interaction between those two abiotic variables make comparisons with our results difficult.

## 5. Conclusions

In conclusion, optimal water temperature for rearing *C. labrosus* juveniles was found to be 22.8 °C. Colder temperatures of around 18 °C caused growth delay and high energy reserve accumulation. At 30 °C, fish exhibited a high energy expenditure that did not lead to fast growth. This, coupled with the epithelial lesions observed in intestines of these fish, evidences that 30 °C are stressful for *C. labrosus* juveniles. Even though the exact water temperature were said stress started could not be elucidated, at 26 °C the fish showed similar growth values to the ones reared at 22 °C, but they spent more energy for that, therefore, it is not recommended to increase water temperature above 26 °C. However, fish performance in relation to water temperature and, concomitantly, optimal temperature for growth, is highly variable not only across different species, but also across different life stages of a given one (McCauley and Huggins, 1979). Consequently, the results obtained in this research cannot be extrapolated to the entire life cycle, and conducting similar experiments to the present one is recommended with individuals at different development stages in order to obtain more accurate information about the optimal water temperature for *C. labrosus* across its entire life cycle. The results obtained herein are particularly interesting for the extensive culture of the thicklip grey mullet, as the lack of temperature control mandates a deep understanding of the response of the fish to said variable, in order to adjust farming conditions, feeding protocols or harvesting strategies accordingly.

## Funding sources

This research was funded by the Basque Government [00007-INA2019–33, 00003-INA2022–33, 00010-PIT2020–22, 00005–2,101,022,023, GIC19/IT-1302-19]. Markel Sanz-Latorre had a predoc contract financed by the Basque Government (IKERTALENT grant Programme)

The funding institutions did not have any input in the design, data collection and interpretation, writing or publication of the investigation presented herein.

## CRediT authorship contribution statement

**Markel Sanz-Latorre:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Manu Soto:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Urtzi Izagirre:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Xabier Lekube:** Writing – review & editing, Validation, Supervision, Resources, Methodology.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Markel Sanz-Latorre reports financial support was provided by Basque Government. Manu Soto Lopez reports financial support was provided by Basque Government. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors are thankful for the technical and human support from the ACOI Coimbra Collection of Algae at the fatty acid analysis, and from Kardala LHII at fish capture and delivery.

## Appendix A. Appendix

**Table A1**
initial stocking density of every experimental tank at the beginning of the experiment. T: temperature treatment (°C); SD: stocking density (kg / $m^3$).

| T (°C) | 18 | | | 22 | | | 26 | | | 30 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tank | 18.1 | 18.2 | 18.3 | 22.1 | 22.2 | 22.3 | 26.1 | 26.2 | 26.3 | 30.1 | 30.2 | 30.3 |
| SD (kg / $m^3$) | 4.73 | 4.85 | 4.38 | 4.85 | 4.35 | 4.42 | 4.74 | 4.10 | 4.15 | 4.45 | 4.58 | 4.10 |

**Table A2**
fatty acid contents of *C. labrosus* muscle reared at different temperatures for 90 days (expressed as % of the total fatty acid methyl esters). 30 D: day 30; 90 D: day 90. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ω-3 PUFA: omega-3 polyunsaturated fatty acids; ω-6 PUFA: omega-6 polyunsaturated fatty acids. Groups with different superscript letters show statistically significant ($p < 0.05$) differences. Capital letter superscripts: comparisons between temperature treatments at 30 D; lower letter superscripts: comparisons between temperature treatments inside 90 D. Significant differences ($p < 0.05$) inside a given treatment over time (30 D vs 90 D) are highlighted in bold.

| | 18 °C | | 22 °C | | 26 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|
| | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D | 30 D | 90 D |
| C14:0 | 3.77 ± 0.28 | 3.52 ± 0.24 | 3.42 ± 0.12 | 3.18 ± 0.38 | 3.48 ± 0.05 | 3.67 ± 0.55 | 3.63 ± 0.48 | 3.80 ± 0.44 |
| C15:0 | 0.40 ± 0.03 | 0.33 ± 0.01 | 0.38 ± 0.02 | 0.35 ± 0.04 | 0.39 ± 0.02 | 0.40 ± 0.03 | 0.41 ± 0.04 | 0.41 ± 0.02 |
| C16:0 | 28.72 ± 2.69 | 26.64 ± 1.58 | 25.98 ± 0.44 | 26.88 ± 0.85 | 26.39 ± 1.78 | 26.10 ± 0.57 | 24.65 ± 2.03 | 25.61 ± 0.95 |
| C17:0 | 0.00 ± 0.00 | 1.10 ± 0.14 | 0.80 ± 0.70 | 0.75 ± 0.66 | 1.16 ± 0.09 | 0.92 ± 0.38 | 1.26 ± 0.12 | 0.83 ± 0.72 |
| C18:0 | 6.43 ± 0.59 | 5.84 ± 0.59 | 6.07 ± 0.24 | 6.97 ± 0.80 | 6.70 ± 0.98 | 6.10 ± 1.01 | 5.68 ± 1.60 | 6.13 ± 1.21 |
| C20:0 | 0.10 ± 0.17 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| C24:0 | 3.57 ± 3.13 | 5.90 ± 0.18 | 5.30 ± 0.15 | 6.14 ± 0.28 | 5.00 ± 0.00 | 5.79 ± 0.30 | 4.62 ± 0.35 | 5.40 ± 0.07 |
| C14:1 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.02 ± 0.03 | 0.00 ± 0.00 |
| C16:1 | 6.96 ± 0.34 | 6.96 ± 0.28 | 6.45 ± 0.54 | 5.24 ± 1.30 | 6.36 ± 0.14 | 5.68 ± 1.30 | 6.77 ± 1.06 | 6.17 ± 1.67 |
| C18:1n9c | 21.17 ± 1.09 | 21.13 ± 0.90 | 20.43 ± 1.15 | 19.33 ± 0.57 | 20.76 ± 0.46 | 20.34 ± 0.62 | 21.47 ± 0.97 | 22.07 ± 0.52 |
| C20:1n9 | 2.63 ± 0.64 | 3.21 ± 0.46 | 3.51 ± 0.25 | 2.96 ± 0.24 | 3.38 ± 0.44 | 3.29 ± 0.43 | 3.89 ± 0.88 | 3.36 ± 0.29 |
| C18:2n6c | 8.87 ± 0.84 | 7.92 ± 0.74 | 9.58 ± 0.31 | 7.15 ± 0.70 | 9.21 ± 1.02 | 7.53 ± 0.75 | 10.86 ± 2.48 | 8.14 ± 0.62 |
| C20:2 | 1.07 ± 0.12 | 1.09 ± 0.20 | 1.09 ± 0.03 | 1.04 ± 0.11 | 1.07 ± 0.14 | 1.15 ± 0.16 | 1.30 ± 0.34 | 1.19 ± 0.18 |
| C20:3n5 | 0.33 ± 0.02 | 0.22 ± 0.21 | 0.28 ± 0.03 | 0.19 ± 0.33 | 0.28 ± 0.01 | 0.43 ± 0.39 | 0.50 ± 0.25 | 0.56 ± 0.04 |
| C20:3n3 | 0.92 ± 0.14 | 1.12 ± 0.06 | 1.04 ± 0.15 | 1.46 ± 0.12 | 1.09 ± 0.08 | 1.37 ± 0.10 | 1.21 ± 0.30 | 1.52 ± 0.30 |
| C22:2n6 | 1.74 ± 2.55 | 0.20 ± 0.17 | 0.30 ± 0.02 | 0.20 ± 0.17 | 0.19 ± 0.16 | 0.22 ± 0.20 | 0.18 ± 0.16 | 0.23 ± 0.20 |
| C20:5n3 EPA | 0.33 ± 0.11 | 0.34 ± 0.04 | 0.24 ± 0.04 | 0.49 ± 0.14 | 0.42 ± 0.17 | 0.57 ± 0.06 | 0.28 ± 0.17 | 0.53 ± 0.08 |
| C22:6n3 DHA | 13.01 ± 2.55 | 14.45 ± 1.24 | 15.29 ± 1.82 | 17.68 ± 2.34 | 14.12 ± 0.36 | 16.42 ± 2.18 | 13.29 ± 2.33 | 14.07 ± 1.27 |
| ΣSFA | 43.00 ± 1.65 | 43.33 ± 1.86$^{AB}$ | **41.95 ± 0.33** | **44.26 ± 0.85** $^{A}$ | 43.12 ± 2.23 | 42.99 ± 0.83$^{AB}$ | 40.23 ± 3.32 | 42.17 ± 1.07$^{B}$ |
| ΣMUFA | 30.76 ± 0.97 | 31.33 ± 0.97 | 30.39 ± 1.73 | 27.54 ± 2.02 | 30.49 ± 1.00 | 29.31 ± 1.71 | 32.12 ± 2.86 | 31.59 ± 1.73 |
| ΣPUFA | 26.24 ± 2.61 | 25.34 ± 0.92$^{B}$ | 27.81 ± 1.68 | 28.20 ± 1.20 $^{A}$ | 26.38 ± 1.23 | 27.70 ± 0.99 $^{A}$ | **27.62 ± 0.47** | **26.24 ± 0.66**$^{AB}$ |
| Σ ω-3 PUFA | 14.26 ± 2.63 | 15.91 ± 1.29 | 16.57 ± 1.99 | 19.63 ± 2.45 | 15.64 ± 0.24 | 18.36 ± 2.33 | 14.78 ± 2.53 | 16.12 ± 1.64 |
| Σ ω-6 PUFA | 10.61 ± 1.76 | 8.12 ± 0.82 | **9.87 ± 0.31** | **7.35 ± 0.83** | 9.40 ± 1.13 | 7.76 ± 0.92 | 11.04 ± 2.41 | 8.37 ± 0.81 |

## References


Ahmad, T., Singh, S.P., Khangembam, B.K., Sharma, J.G., Chakrabarti, R., 2014. Food consumption and digestive enzyme activity of *Clarias batrachus* exposed to various temperatures. Aquac. Nutr. 20 (3), 265–272.

Alfonso, S., Gesto, M., Sadoul, B., 2021. Temperature increase and its effects on fish stress physiology in the context of global warming. J. Fish Biol. 98 (6), 1496–1508.

Altunok, M., Özden, O., 2017. Effect of dietary protein on the growth of mullet, *Chelon labrosus*, reared in sea cages. Fisher. & Aquatic. Life 25 (3), 157–164.

Arruda, L.M., Azevedo, J., Neto, A.I., 1991. Age and Growth of the Grey Mullets (Pisces, Mugilidae) in the ria de Aveiro (Portugal).

Assunção, M.F., Varejão, J.M., Santos, L.M., 2017. Nutritional characterization of the microalga *Ruttnera lamellosa* compared to *Porphyridium purpureum*. Algal Res. 26, 8–14.

Aydın, İ., Ak, O., Küçük, E., Polat, H., Ceylan, B., 2012. Optimum temperature and growth performance of hatchery reared Black Sea flounder (*Platichthys flesus luscus* Pallas, 1814). Turk. J. Vet. Anim. Sci. 36 (2) https://doi.org/10.3906/vet-1005-319.

Aydın, İ., Küçük, E., Polat, H., Öztürk, R.Ç., Terzi, Y., Altınok, İ., 2021. Growth and feed conversion ratio of diploid and triploid induced juvenile turbot reared at different water temperatures. Aquaculture 543, 736981.

Aydın, İ., Öztürk, R.Ç., Küçük, E., Polat, H., Altınok, İ., 2022a. Temperature appeared to be an effective factor on growth performance of diploid and triploid turbot, *Scophthalmus maximus*, during the early life stage. Aquaculture 546, 737316.

Aydın, İ., Terzi, Y., Polat, H., Küçük, E., Öztürk, R.Ç., 2022b. Growth, gonadal development, and fish fillet quality in diploid and triploid turbot, *Scophthalmus maximus*, originated from the Black Sea. Aquaculture 560, 738558.

Bendiksen, E.Å., Jobling, M., Arnesen, A.M., 2002. Feed intake of Atlantic salmon parr *Salmo salar* L. in relation to temperature and feed composition. Aquac. Res. 33 (7), 525–532.

Borja, A., Amouroux, D., Anschutz, P., Gómez-Gesteira, M., Uyarra, M.C., Valdés, L., 2019. The Bay of Biscay. In: World Seas: An Environmental Evaluation. Academic Press, pp. 113–152.

Bowyer, J.N., Booth, M.A., Qin, J.G., D'Antignana, T., Thomson, M.J., Stone, D.A., 2014. Temperature and dissolved oxygen influence growth and digestive enzyme activities of yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833). Aquac. Res. 45 (12), 2010–2020.

Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Severe, A., Boeuf, G., 1996. Effects of temperature on growth and metabolism in juvenile turbot. J. Fish Biol. 49 (4), 678–692.

Cardona, L., 2016. Food and feeding of Mugilidae. In: Blaber, S.J.M., Crosetti, D. (Eds.), Biology, Ecology and Culture of Grey Mullets (Mugilidae), 1st ed. CRC Press, pp. 165–195.

Carey, F.G., Teal, J.M., Kanwisher, J.W., Lawson, K.D., Beckett, J.S., 1971. Warm-bodied fish. Am. Zool. 11 (1), 137–143.

Clarke, A., Fraser, K.P.P., 2004. Why does metabolism scale with temperature? Funct. Ecol. 18 (2), 243–251.

Crosetti, D., 2016. Current state of grey mullet fisheries and culture. Biol. Ecol. Cult. Grey Mullet (Mugilidae) 388–450.

Davies, S.J., Brown, M.T., Camilleri, M., 1997. Preliminary assessment of the seaweed *Porphyra purpurea* in artificial diets for thick-lipped grey mullet (*Chelon labrosus*). Aquaculture 152 (1–4), 249–258.

Dawood, M.A., Noreldin, A.E., Sewilam, H., 2022. Blood biochemical variables, antioxidative status, and histological features of intestinal, gill, and liver tissues of African catfish (*Clarias gariepinus*) exposed to high salinity and high-temperature stress. Environ. Sci. Pollut. Res. 29 (37), 56357–56369.

De las Heras, V., Martos-Sitcha, J.A., Yúfera, M., Mancera, J.M., Martínez-Rodríguez, G., 2015. Influence of stocking density on growth, metabolism and stress of thick-lipped grey mullet (*Chelon labrosus*) juveniles. Aquaculture 448, 29–37.

Del-Pozo, J., Crumlish, M., Turnbull, J.F., Ferguson, H.W., 2010. Histopathology and ultrastructure of segmented filamentous bacteria–associated rainbow trout gastroenteritis. Vet. Pathol. 47 (2), 220–230.

Farrell, A.P., 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J. Exp. Biol. 212 (23), 3771–3780.

García-Márquez, J., Galafat, A., Alarcón, F.J., Figueroa, F.L., Martínez-Manzanares, E., Arijo, S., Abdala-Díaz, R.T., 2021. Cultivated and wild juvenile thick-lipped grey mullet, *Chelon labrosus*: A comparison from a nutritional point of view. Animals 11 (7), 2112.

Garcia-Marquez, J., Galafat, A., Vizcaíno, A.J., Barany, A., Martos-Sitcha, J.A., Mancera, J.M., Abdala-Díaz, R.T., 2022. Dietary use of the microalga *Chlorella fusca* improves growth, metabolism, and digestive functionality in thick-lipped grey mullet (*Chelon labrosus*, Risso 1827) juveniles. Front. Mar. Sci. 9, 902203.

García-Márquez, J., Vizcaíno, A.J., Barany, A., Galafat, A., Acién, G., Figueroa, F.L., Abdala-Díaz, R.T., 2023. Evaluation of the combined administration of *Chlorella fusca* and *vibrio proteolyticus* in diets for *Chelon labrosus*: effects on growth, metabolism, and digestive functionality. Animals 13 (4), 589.

Guerreiro, I., Enes, P., Rodiles, A., Merrifield, D., Oliva-Teles, A., 2016. Effects of rearing temperature and dietary short-chain fructooligosaccharides supplementation on allochthonous gut microbiota, digestive enzymes activities and intestine health of turbot (*Scophthalmus maximus* L.) juveniles. Aquac. Nutr. 22 (3), 631–642.

Handeland, S.O., Imsland, A.K., Stefansson, S.O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture 283 (1–4), 36–42.

Hani, Y.M.I., Marchand, A., Turies, C., Kerambrun, E., Palluel, O., Bado-Nilles, A., Dedourge-Geffard, O., 2018. Digestive enzymes and gut morphometric parameters of threespine stickleback (*Gasterosteus aculeatus*): influence of body size and temperature. PLoS One 13 (4), e0194932.

Hawkins, A.J., 1995. Effects of temperature change on ectotherm metabolism and evolution: metabolic and physiological interrelations underlying the superiority of

multi-locus heterozygotes in heterogeneous environments. J. Therm. Biol. 20 (1–2), 23–33.
Hazel, J.R., 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57 (1), 19–42.
IBM Corp. Released, 2020. IBM SPSS Statistics for Windows, Version 27.0. IBM Corp, Armonk, NY.
Islam, M.J., Slater, M.J., Thiele, R., Kunzmann, A., 2021. Influence of extreme ambient cold stress on growth, hematological, antioxidants, and immune responses in European seabass, *Dicentrarchus labrax* acclimatized at different salinities. Ecol. Indic. 122, 107280.
Jobling, M., Johansen, S.J.S., 1999. The lipostat, hyperphagia and catch-up growth. Aquac. Res. 30 (7), 473–478.
Jobling, M., Larsen, A.V., Andreassen, B., Olsen, R.L., 2002. Adiposity and growth of post-smolt Atlantic salmon *Salmo salar* L. Aquac. Res. 33 (7), 533–541.
Johnston, I.A., Dunn, J.E.F.F., 1987. Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In: Symposia of the Society for Experimental Biology, 41, pp. 67–93.
Jonsson, B., Forseth, T., Jensen, A.J., Næsje, T.F., 2001. Thermal performance of juvenile Atlantic Salmon, *Salmo salar* L. Funct. Ecol. 15 (6), 701–711.
Kaushik, S.J., Corraze, G., Radunz-Neto, J., Larroquet, L., Dumas, J., 2006. Fatty acid profiles of wild brown trout and Atlantic salmon juveniles in the Nivelle basin. J. Fish Biol. 68 (5), 1376–1387.
Kaushik, S.J., Panserat, S., Schrama, J.W., 2022. Carbohydrates. In: Fish Nutrition. Academic Press, pp. 555–591.
Khemis, I.B., Zouiten, D., Besbes, R., Kamoun, F., 2006. Larval rearing and weaning of thick lipped grey mullet (*Chelon labrosus*) in mesocosm with semi-extensive technology. Aquaculture 259 (1–4), 190–201.
Lasserre, P., Gallis, J.L., 1975. Osmoregulation and differential penetration of two grey mullets, *Chelon labrosus* (Risso) and *Liza ramada* (Risso) in estuarine fish ponds. Aquaculture 5 (4), 323–344.
Martoja, R., Martoja-Pierson, M., Mocanut, E., Coll, D.I., 1970. Técnicas de Histología Animal.
Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A., Schwudke, D., 2008. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J. Lipid Res. 49 (5), 1137–1146.
McCauley, R.W., Huggins, N.W., 1979. Ontogenetic and non-thermal seasonal effects on thermal preferenda of fish. Am. Zool. 19 (1), 267–271.
Miegel, R.P., Pain, S.J., Van Wettere, W.H.E.J., Howarth, G.S., Stone, D.A.J., 2010. Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (*Seriola lalandi*). Aquaculture 308 (3–4), 145–151.
Navarro-Guillen, C., Yufera, M., Perera, E., 2022. Biochemical features and modulation of digestive enzymes by environmental temperature in the greater amberjack, *Seriola dumerili*. Front. Mar. Sci. 9, 960746.
Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405 (6790), 1017–1024.
Nytrø, A.V., Vikingstad, E., Foss, A., Hangstad, T.A., Reynolds, P., Eliassen, G., Imsland, A.K., 2014. The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). Aquaculture 434, 296–302.
Nzioka, A., Madeira, M.J., Kokokiris, L., Ortiz-Zarrogoitia, M., de Cerio, O.D., Cancio, I., 2023. Lack of genetic structure in euryhaline *Chelon labrosus* from the estuaries under anthropic pressure in the southern Bay of Biscay to the coastal waters of the Mediterranean Sea. Mar. Environ. Res. 106058.
Ojaveer, H., Morris, P.C., Davies, S.J., Russell, P., 1996. The response of thick-lipped grey mullet, *Chelon labrosus* (Risso), to diets of varied protein-to-energy ratio. Aquaculture Research 27 (8), 603–612.
Olsen, R.E., Sundell, K., Hansen, T., Hemre, G.I., Myklebust, R., Mayhew, T.M., Ringø, E., 2002. Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: an electron microscopical study. Fish Physiol. Biochem. 26, 211–221.
Opuszynski, K., Shireman, J.V., 2019. Digestive mechanisms. In: Herbivorous Fishes: Culture and Use for Weed Management. CRC Press, pp. 21–31.
Papoutsoglou, E.S., Lyndon, A.R., 2005. Effect of incubation temperature on carbohydrate digestion in important teleosts for aquaculture. Aquac. Res. 36 (13), 1252–1264.
Person-Le Ruyet, J., Mahe, K., Le Bayon, N., Le Delliou, H., 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquaculture 237 (1–4), 269–280.
Pujante, I.M., Martos-Sitcha, J.A., Moyano, F.J., Ruiz-Jarabo, I., Martínez-Rodríguez, G., Mancera, J.M., 2015. Starving/re-feeding processes induce metabolic modifications in thick-lipped grey mullet (*Chelon labrosus*, Risso 1827). Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 180, 57–67.
Pujante, I.M., Díaz-López, M., Mancera, J.M., Moyano, F.J., 2017. Characterization of digestive enzymes protease and alpha-amylase activities in the thick-lipped grey mullet (*Chelon labrosus*, Risso 1827). Aquac. Res. 48 (2), 367–376.
Pujante, I.M., Moyano, F.J., Martos-Sitcha, J.A., Mancera, J.M., Martínez-Rodríguez, G., 2018. Effect of different salinities on gene expression and activity of digestive enzymes in the thick-lipped grey mullet (*Chelon labrosus*). Fish Physiol. Biochem. 44, 349–373.
Py, C., Elizondo-González, R., Peña-Rodríguez, A., 2022. Compensatory growth: fitness cost in farmed fish and crustaceans. Rev. Aquac. 14 (3), 1389–1417.
Quirós-Pozo, R., Moyano, F.J., Bainour, K., Ramírez-Bolaños, S., Ventura-Castellano, A., Roo, J., Robaina, L., 2023. Evaluation of the effects of two different feeding frequencies on the digestive biochemistry of two mullet species (*Chelon labrosus* and *Liza aurata*). Animals 13 (2), 287.
Rabeh, I., Telahigue, K., Hajji, T., Kheriji, S., Besbes, A., Besbes, R., El Cafsi, M., 2022. Fatty acid composition of phospholipids and triacylglycerols in the flesh of the thick-lipped grey mullet (*Chelon labrosus*) living in Tunisian geothermal water and seawater: A comparative study. Grasas Aceites 73 (1), e448.
Rašković, B.S., Stanković, M.B., Marković, Z.Z., Poleksić, V.D., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agricul. Sci. Belgrade 56 (1), 87–100.
Reeve, C., Rowsey, L.E., Speers-Roesch, B., 2022. Inactivity and the passive slowing effect of cold on resting metabolism as the primary drivers of energy savings in overwintering fishes. J. Exp. Biol. 225 (8), jeb243407.
Savoie, A., Le François, N.R., Cahu, C., Blier, P.U., 2008. Metabolic and digestive enzyme activity profiles of newly hatched spotted wolffish (*Anarhichas minor* Olafsen): effect of temperature. Aquac. Res. 39 (4), 382–389.
Schulte, P.M., 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J. Exp. Biol. 218 (12), 1856–1866.
Schultz, E.T., Conover, D.O., 1997. Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). Oecologia 109, 516–529.
Seginer, I., 2016. Growth models of gilthead sea bream (*Sparus aurata* L.) for aquaculture: A review. Aquac. Eng. 70, 15–32.
Senso, L., Suárez, M.D., Ruiz-Cara, T., García-Gallego, M., 2007. On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). Food Chemistry 101 (1), 298–307.
Skalli, A., Robin, J.H., Le Bayon, N., Le Delliou, H., Person-Le Ruyet, J., 2006. Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). Aquaculture 255 (1–4), 223–232.
Sun, L., Chen, H., 2014. Effects of water temperature and fish size on growth and bioenergetics of cobia (*Rachycentron canadum*). Aquaculture 426, 172–180.
Sushchik, N.N., Zuev, I.V., Kalachova, G.S., Ageev, A.V., Gladyshev, M.I., 2018. Content of highly unsaturated fatty acids in fish from rivers of contrasting temperature. River Research and Applications 34 (6), 565–574.
Turan, C., 2016. Biogeography and distribution of Mugilidae in the Mediterranean and the Black Sea, and north-East Atlantic. In: Blaber, S.J.M., Crosetti, D. (Eds.), Biology, Ecology and Culture of Grey Mullets (Mugilidae), 1st ed. CRC Press, pp. 116–127.
Van Dijk, P.L.M., Hardewig, I., Hölker, F., 2005. Energy reserves during food deprivation and compensatory growth in juvenile roach: the importance of season and temperature. J. Fish Biol. 66 (1), 167–181.
Vílchez-Gómez, L., Cámara-Ruiz, M., Guardiola, F.A., Esteban, M.Á., October 2017. Inclusión de levaduras de la industria cervecera en dietas para *Chelon labrosus* L. In: XVI Congreso Nacional de Acuicultura, Zaragoza, Spain.
Volkoff, H., Rønnestad, I., 2020. Effects of temperature on feeding and digestive processes in fish. Temperature 7 (4), 307–320.
Wang, Y., Li, C., Pan, C., Liu, E., Zhao, X., Ling, Q., 2019. Alterations to transcriptomic profile, histopathology, and oxidative stress in liver of pikeperch (*Sander lucioperca*) under heat stress. Fish Shellfish Immunol. 95, 659–669.
Zouiten, D., Khemis, I.B., Besbes, R., Cahu, C., 2008. Ontogeny of the digestive tract of thick lipped grey mullet (*Chelon labrosus*) larvae reared in "mesocosms". Aquaculture 279 (1–4), 166–172.