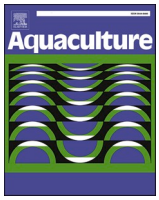
Aquaculture 575 (2023) 739806

Contents lists available at ScienceDirect

Aquaculture

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



The impact of egg thermal regimes on the response to food deprivation and refeeding in juvenile European Sea bass (*Dicentrarchus labrax*)

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ARTICLE INFO

Keywords:
Thermal imprinting
Phenotypic plasticity
Growth axis
Lipid metabolism
Gluconeogenesis
Lactate

ABSTRACT

Fish are ectotherms and this means they are highly vulnerable to changes in ambient temperature, particularly during early developmental stages when temperature can induce persistent effects on phenotypic traits. In this study, the effect of egg incubation temperature on the response of juvenile European sea bass (*Dicentrarchus labrax*) to food deprivation and recovery after refeeding was assessed. Eggs were incubated at 11, 13.5 and 16 °C until hatching and then were reared at a common temperature until 9 months when fish were deprived of food for one week. The recovery from food deprivation was evaluated at 10 h and 2 days post-refeeding. Food deprivation in fish from eggs incubated at the highest temperature (16 °C) compared to 11 and 13.5 °C exhibited the most morphological and metabolic changes in the liver and foregut. Liver metabolism was changed as revealed by the significant reduction in lipid area and the increased number of hepatocyte nuclei. Foregut atrophy was coupled to a significant up-regulation of transcripts associated with gluconeogenesis (*pck1*) and peptide absorption (*pept1*). A modified metabolic response to the fast-refeed regime was revealed by the significantly decreased levels of plasma lactate, which may result from up-regulation of transcripts of the thyroid axis, deiodinase 2 (*dio2*) in the foregut. Fish incubated as eggs at a lower temperature (11 °C) exhibited less changes following the fast-refeed regime. Food deprivation did not significantly modify the morphology of the foregut and the liver parenchyma recovered sooner in fish from the 11 °C egg thermal regime compared to fish from the other thermal regimes following refeeding. The latter group of fish had a temporary stimulation of the GH-IGF axis with significant up-regulation of liver insulin-like growth factor I and II (*igf-1* and *igf-2*) after fasting. The liver parenchyma of fish from the 13.5 °C egg thermal regime (the standard temperature of the hatchery stage) did not recover by the end of the experiment and transcripts of catalase (*cat*), encoding an antioxidant enzyme, were significantly downregulated compared to fish from the other egg thermal regimes. Our results suggest that thermal imprinting at the egg stage in European sea bass modified the juvenile metabolic response to food deprivation and the recovery response when feeding was resumed.

1. Introduction

Food deprivation for varying periods of time prior to manipulation is a common practice in aquaculture since it reduces physiological stress and improves sanitary conditions by minimizing faecal contamination (Farming, 2018). Contrary to what occurs during fish aquaculture where food is frequently supplied, periods of fasting are a normal occurrence in the wild, as a consequence of prey availability or reduced water temperatures (Bar and Volkoff, 2012; Ibarz et al., 2010; Navarro and Gutiérrez, 1995). In the context of climate change, sea surface temperatures are increasing and modifying global marine ecosystems, and changing the distribution of marine organisms (including

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https://doi.org/10.1016/j.aquaculture.2023.739806
Received 28 November 2022; Received in revised form 20 May 2023; Accepted 12 June 2023
Available online 16 June 2023

phytoplankton) and food availability (EEA, 2021; FAO, 2008; IPCC, 2014). Marine heatwaves are becoming more frequent and lengthier and are endangering marine life (Vasseur et al., 2014). As ectotherms, fish are highly susceptible to temperature shifts (Alfonso et al., 2021; Vagner et al., 2019), and the influence of temperature on early developmental stages can have long lasting effects since epigenetic marks that influence the phenotypic plasticity of adult fish may be modified (Anastasiadi et al., 2017; Jonsson and Jonsson, 2019; Kaitetzidou et al., 2015; Metzger and Schulte, 2016; Moghadam et al., 2017; Pittman et al., 2013; Sarropoulou et al., 2019).

The impact of temperature on fish development can be detected before blatant physiological or biochemical modifications occur (Georgakopoulou et al., 2007). Initial clues about the potential effects of exposure to modified thermal regimes during early life-stages comes from observations that fish eggs incubated at higher temperatures hatched earlier than eggs incubated at lower temperatures (Jennings and Pawson, 1991; Saka et al., 2001) and produced bigger larva during subsequent developmental stages (Ayala et al., 2001; Koumoundouros et al., 2001). Modified thermal regimes during development also impact the adult phenotype in fish and modify the incidence of skeletal deformities (Abdel et al., 2004; Boglione and Costa, 2011; Fraser et al., 2015), muscle growth (Garcia de la serrana et al., 2012; Johnston, 2006), bone homeostasis (Mateus et al., 2017a; Riera-Heredia et al., 2018), thermal tolerance (Scott and Johnston, 2012), sex determination (Villamizar et al., 2012), reproduction (Donelson et al., 2014), the stress response (Mateus et al., 2017b; Varsamos et al., 2006), immune function and swimming performance (Kourkouta et al., 2021). Epigenetic marks are identified as one factor responsible for phenotypic plasticity and it was shown that abiotic factors such as temperature induce them (Anastasiadi et al., 2017; Jonsson and Jonsson, 2019; Kaitetzidou et al., 2015; Metzger and Schulte, 2016; Moghadam et al., 2017; Pittman et al., 2013; Sarropoulou et al., 2019). But although there is an increasing number of studies examining how thermal regimes during development affects the adult phenotype of fish, their influence on the response to food availability and more specifically prolonged food deprivation has not been studied.

Since periodic lack of food is part of the natural lifecycle of animals in the wild a number of studies exist characterising the physiological response to food deprivation in fish, reptiles, birds and mammals (Bar and Volkoff, 2012; Furne and Sanz, 2018; Hervant, 2012; McCue, 2010; Navarro and Gutiérrez, 1995; Power et al., 2000; Secor and Lignot, 2010; Wang et al., 2006). One of the first consequences of food deprivation in fish is modified behaviour since swimming is reduced presumably to conserve energy (Blaxter and Ehrlich, 1974; Killen et al., 2011; Rescan et al., 2007; Simpkins et al., 2003; Zheng and Fu, 2021). If food deprivation is prolonged metabolic reserves are mobilized from the liver and muscles, the condition factor of fish falls (Hvas et al., 2021; Pottinger et al., 2003), and the growth rate slows or stops under the modulation of the growth axis (Davis and Gaylord, 2011; Fox et al., 2006; Rescan et al., 2007; Rueda et al., 1998; Siharath et al., 1996). Generally, food deprivation induces a stress response in fish (Bermejo-Poza et al., 2016; Piccinetti et al., 2015; Rodgers et al., 2003), modifies osmoregulation (Alix et al., 2017; Polakof et al., 2006; Wood, 2019), improves the innate immune capacity (Agius and Roberts, 1981; Caruso et al., 2011; Liao et al., 2021; Martin et al., 2010; Vieira et al., 2011; Wang et al., 2019) and enhances antioxidant defence (Antonopoulou et al., 2013; Ensminger et al., 2021; Pascual et al., 2003; Yang et al., 2019).

The intestine and liver are the organs of the digestive system that most rapidly reflect morphological and functional changes in response to food deprivation (Rašković et al., 2011; Wang et al., 2006; Zaldúa and Naya, 2014). In starved fish, there is a decrease in the length of the gut and its wet mass, a reduction in the mucosal fold number and height, a decrease in enterocyte and microvilli height, and a modification (increase or decrease) in goblet cell number (Emadi Shaibani et al., 2013; Hall and Bellwood, 1995; Krogdahl and Bakke-McKellep, 2005; Zaldúa and Naya, 2014; Zeng et al., 2012). Food deprivation not only affects the morphology of the gut but also changes the gut microbiome presumably to strengthen the immune defence as the intestinal mucosa is changed (Butt and Volkoff, 2019; Li et al., 2019; Tran et al., 2018; Xia et al., 2014). Modifications in the liver of starved fish, such as hepatocyte atrophy and modified nuclear size (Hammock et al., 2020; Hur et al., 2006; Mohapatra et al., 2017; Power et al., 2000; Zaldúa and Naya, 2014; Zeng et al., 2012), are presumably part of the homeostatic response in the initial stage of food deprivation and result from increased mobilization of glycogen and lipids mediated by peptides, like glucagon, of the brain-gut-axis (Cardoso et al., 2018; Nadav, 2014; Navarro and Gutiérrez, 1995). Despite the substantial changes in behaviour, physiology, tissue structure and metabolism during short and long-term periods of food deprivation, fish can fully recover once feeding is resumed (Jia et al., 2019; Navarro and Gutiérrez, 1995; Pascual et al., 2003; Power et al., 2000). The mechanisms involved in the recovery response after food deprivation and if early thermal regimes can influence this process remains to be established.

European sea bass (*Dicentrarchus labrax,* Linnaeus, 1758) is an economically important Mediterranean aquaculture species, which in the wild spends its embryonic and larval development in a marine environment before migrating as juveniles to coastal lagoons and estuaries (Bagni, 2021; Jennings and Pawson, 1991). The lifecycle of the wild species, particularly the early stages that cannot migrate to cooler waters, are likely to be particularly vulnerable in the future to rising seawater temperatures as they inhabit regions that are likely to register the greatest increase in sea surface temperatures (Azzurro et al., 2019; EEA, 2021; Pinto et al., 2021). Under aquaculture conditions thermal regimes are controlled and recently there has been increased interest in using early thermal regimes to exploit the inherent phenotypic plasticity of fish and enhance beneficial traits or overcome less desirable traits such as male bias and their early sexual maturation during production (Gavery and Roberts, 2017; Moghadam et al., 2015; Vandeputte et al., 2020; Vandeputte and Piferrer, 2018). The objective of the present study was to evaluate if modified temperatures during European sea bass egg incubation, when the digestive system is developing (e.g. gut, liver and accessory glands) (Cucchi et al., 2012; Zambonino Infante and Cahu, 2001), modifies the response of juveniles to food deprivation and the recovery response on refeeding. Two key organs, the liver and foregut, were selected for evaluation by histology and to determine the molecular response to fasting and refeeding, using qPCR and targeting genes associated with previously reported responses linked to the growth axis, metabolism and the antioxidant response.

2. Materials and methods

All experiments were performed at Ifremer, Palavas-Les-Flots, France. Experiments were authorized by the ethics committee agreement APAFIS#10745 and all procedures involving animals were in accordance with the ethical standards of the institution and followed the recommendations of Directive 2010/63/EU.

2.1. Temperature conditions during early development

European sea bass eggs (*Dicentrarchus labrax*) from a West Mediterranean population were obtained by combining eggs from 10 females and frozen sperm from 13 males by in vitro fertilization using a full factorial crossing design, in October 2016. Eggs were distributed into 9 different tanks and incubated at 11 °C, 13.5 °C or 16 °C until hatching (within the optimal temperature range for European sea bass, Fig. 1A). Larvae from all temperature regimes (3 tanks per temperature) were then reared following previously optimized procedures for European sea bass (Chatain, 1994). Hatching temperatures were gradually changed at a rate of 1 °C/day to 15 °C until December 29th, and then gradually increased at the same rate to 25 °C by January 3rd of 2017. On February 15th, 600 fish per experimental tank were randomly transferred into

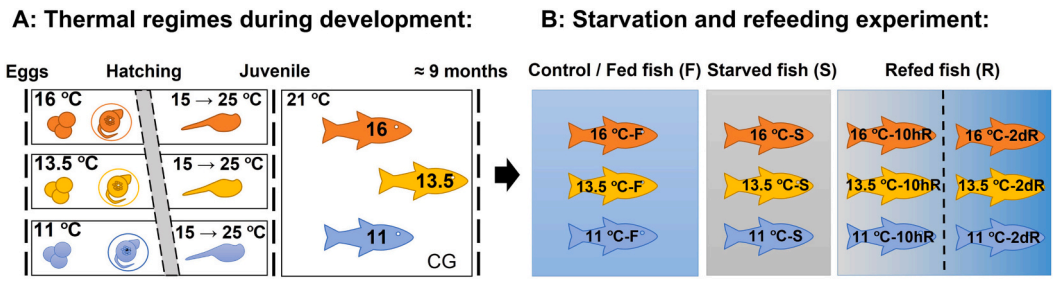

Fig. 1. Experimental design. A: Thermal regimes during early development of European sea bass. Eggs were incubated until hatching at different temperatures: 11 °C, 13.5 °C (control) and 16 °C. After hatching, larvae from each batch of eggs (thermal regime groups) were reared in the same conditions: larvae were initially reared at 15 °C and then the temperature was gradually increased until 25 °C. Juvenile sea bass were PIT tagged for identification of egg thermal regime and then randomly distributed into 3 common garden tanks until the onset of the next experiment (~9 months old sea bass). B: Starvation and refeeding experiment. Juvenile fish from different egg thermal regimes (n = 8/thermal regime) were sampled without subjecting them to food deprivation (Control/Fed group = F). The remaining fish were deprived of food for one week (Starved group = S) and then sampled (n = 8/thermal regime). Fish remaining in the common garden tanks were refed and then sampled at 10 h (10hR) and at 2 days (2dR) after feeding was resumed (n = 8/thermal regime/experimental group).

larger tanks (1.5m^3) and reared at 21 °C throughout the rest of the experiment (3 tanks per thermal regime). At 175 dpf, 225 fish per thermal regime were randomly selected from all rearing tanks and distributed into 3 replicate tanks (common garden design; $n = 675$ fish per common garden). All fish were PIT tagged and then an equal number of fish from each thermal regime was mixed in a common tank (a.k.a. common garden). The PIT tag permitted the fish from the different thermal regimes during the egg phase to be identified during the fast/refeed experiment. Fish were fed using self-feeders until the food deprivation and refeeding experiment was run (≈ 9 months old fish). The egg temperature programming experiment produced a large stock of fish that were used for several independent experiments and provide complimentary information about the impact of thermal imprinting on several different physiological traits (Mateus et al., 2023; Sadoul et al., 2021; Sadoul et al., 2022).

2.2. Food deprivation and refeeding experiment

To evaluate if egg incubation temperature impacted the general physiology of juveniles and their response to food deprivation and refeeding, ~9 months old fish from the common gardens were randomly distributed between 3 further tanks, which also had a common garden design ($n = 16$/thermal regime/common garden). During fish distribution, the intramuscular PIT tag in each fish was read to identify the thermal background and to ensure an equal distribution of fish from each thermal regime in the common garden tanks. Fish were then acclimated for 3 weeks prior to the start of the food deprivation and refeeding experiment. Four experimental groups were established with 9 month old thermally imprinted fish from eggs incubated at 11 °C, 13.5 °C or 16 °C: the Fed/Control group (F), were fed 2.5% their body mass per day using a rotating belt feeder; the Starved group (S), fish subjected to one week of food deprivation; and two Refed groups (R), fish deprived of food for one week and then refed using the same conditions as for the Fed group and sampled at 10 h (10hR) and 2 days after refeeding (2dR, Fig. 1B).

Fish from the fed/control group were sampled from one common garden ($n = 8$/thermal regime) while fish from starved and refed groups were sampled from the other 2 common gardens ($n = 4$/thermal regime/experimental group/common garden). Fish from each experimental group (n = 8/thermal regime) were euthanized with 225 mg.L^{-1} benzocaine (E1501, Sigma, USA), and fish from each egg thermal regime identified by reading the PIT tag code with a handheld device were weighed and measured (Table 1). Blood was collected by caudal puncture using a heparinized 1 mL syringe, transferred to a 1.5 ml microcentrifuge tube and centrifuged (10,000 rpm for 5 min) and the plasma stored at −20 °C. The liver and the proximal portion of the intestine (hereafter named the "foregut") were sampled and stored in RNA*later*® (Sigma-Aldrich) at −20 °C until subsequent molecular analysis or fixed in 4% paraformaldehyde (PFA) for histology.

2.3. Plasma analysis

The physiological condition and response of juvenile European sea bass to food deprivation and refeeding was evaluated by determining the concentration of cortisol, glucose and lactate in plasma samples ($n = 8$/thermal regime/experimental group). A validated radioimmunoassay was used to determine the cortisol concentrations in plasma samples (Rotllant et al., 2005a; Rotllant et al., 2005b). Glucose and lactate were quantified in duplicate reactions in 96 well plates using 2.5 μL of non-diluted plasma and a commercial colorimetric kit (Ref. 1,001,192 and

Table 1
Summary of biometric parameters of juvenile sea bass incubated as eggs under different thermal regimes (11 °C, 13.5 °C and 16 °C) and subjected to food deprivation and then refeeding (F, S, 10hR and 2dR).

	Weight (g)				Length (cm)				K			
Time	F	S	10hR	2dR	F	S	10hR	2dR	F	S	10hR	2dR
11 °C	37.6 ± 10.2	34.5 ± 11.5	28.3 ± 7.2	27.1 ± 9.3	14.1 ± 1.2	13.9 ± 1.1	12.9 ± 1.0	12.6 ± 1.3	1.28 ± 0.06	1.38 ± 0.13	1.36 ± 0.15	1.34 ± 0.02
13.5 °C	36.7 ± 7.6	25.9 ± 8.8	32.3 ± 11.0	33.2 ± 7.2	13.8 ± 0.9	12.4 ± 1.2	13.2 ± 1.2	13.2 ± 1.1	1.37 ± 0.05	1.31 ± 0.06	1.31 ± 0.07	1.33 ± 0.02
16 °C	39.6 ± 13.6	32.3 ± 9.1	27.4 ± 4.5	29.5 ± 12.8	14.1 ± 1.2	13.3 ± 1.1	12.7 ± 0.7	13.0 ± 1.8	1.32 ± 0.16	1.38 ± 0.11	1.33 ± 0.15	1.34 ± 0.02
All °C	38.0 ± 2.0^{a}	30.9 ± 2.0^{ab}	29.4 ± 2.0^{b}	29.9 ± 2.0^{b}	14.0 ± 0.2^{a}	13.2 ± 0.2^{ab}	12.9 ± 0.2^{b}	12.9 ± 0.2^{b}	1.34 ± 0.05	1.42 ± 0.10	1.28 ± 0.12	1.35 ± 0.02

Body weight (g). standard length (cm) and condition factor (K) calculated as 100 x (body weight/length^3) of fish exposed to different temperatures during egg incubation that as juveniles (Control/Fed = F) were exposed to 1 week of starvation (S) and sampled 10 h and 2 days after refeeding (10hR and 2dR, respectively). Data is presented as the mean ± standard deviation (s.d., $n = 8$/thermal regime/experimental group). Different letters indicate the groups that were significantly different under food deprivation or refeeding, irrespective of the egg thermal regime. Two-way ANOVA; $p < 0.05$.

1,001,330, respectively; Spinreact, Spain), following the manufacturer's instructions. The results of the glucose and lactate analysis were determined by reading the absorbance at 505 nm using a microplate reader (BioTek Synergy 4; BioTek Instruments, Inc., USA).

2.4. Histology of the liver and foregut

PFA-fixed liver and foregut samples were processed in an automated tissue processor (Leica TP1020). Samples were dehydrated through a graded ethanol series (70%, 96% and 100%), saturated in xylene and impregnated with low melting point paraffin wax (Histosec, Merck), followed by paraffin embedding. Serial sections (5 μm) of the liver and transverse sections of the foregut were mounted on 0.01% Poly-L-Lysine (Sigma-Aldrich) coated glass slides and stained using haematoxylin-eosin and the Alcian Blue-Periodic Acid Schiff technique (AB-PAS) (Myers et al., 2008), respectively. Stained sections were analysed and photographed using a microscope (Leica DM2000) coupled to a digital camera (Leica DFC480) and linked to a computer for digital image analysis with Fiji v1.52p software (Schindelin et al., 2012).

2.4.1. Histomorphometry of the liver and foregut

Histomorphology of the liver and foregut was assessed using an adaptation of the methods reviewed by Rašković et al. (2011). Histomorphometric analysis of the liver and foregut was performed on 3 sections per fish with a space of 15–20 μm between the sections analysed and using tissue from 8 individuals/thermal regime/experimental group. One image was captured of the liver (taken at 400×; with an area of approximately 89 × 10^3 μm^2) per section and was used to analyse nuclei number and area (μm^2) and the total area (μm^2 and %) occupied by lipids. The nuclei and lipid area were analysed using the Otsu method (Papadopulos et al., 2007; Zhang and Hu, 2008) by adjusting the colour threshold according to the targeted parameter. Caution was taken to avoid counting cell nuclei that were not from hepatocytes (e.g., blood cells since fish haemocytes are nucleated).

Analysis of the foregut was carried out on merged images of each section (100× magnification) using the software Adobe Photoshop 19.1.5 release (Adobe Systems, San Jose, CA, USA). Measurements of the foregut and mucosa areas (μm^2), mucosa lining (perimeter, μm), number of goblet cells and width of the lamina propria of mucosa folds were executed as illustrated in Fig. 2. The foregut area was determined by drawing a circumference outlining the serosa layer and then subtracting the lumen area. The mucosal area was determined by circumscribing the foregut in transverse sections so that the submucosa, muscle/serosa layers were excluded. The lumen and mucosa areas and the mucosa lining from each transverse section of the foregut were analysed using the Otsu method. The total number of goblet cells (irrespective of the staining reaction) per transverse section was counted and the width of the lamina propria (μm) of four mucosa folds (one per quadrant on transverse sections of the foregut) was measured at the base of each villous.

Mucosa area/lining and the goblet cell number were normalized by the foregut and the mucosa area, respectively, to compensate for changes in these parameters induced by variations in the foregut area. The results are presented using the parameters measured in the fish incubated as eggs at 13.5 °C (standard egg incubation temperature for European sea bass, (Morretti, 1999)) that were not exposed to food deprivation as the reference (normalized data was transformed into log2 fold change).

2.5. Molecular analysis

2.5.1. RNA extraction and cDNA synthesis

Total RNA was extracted from samples of the liver (Supp. Table S1) and foregut that had been fixed in RNAlater™ (Sigma-Aldrich) using an E.Z.N.A® Total RNA Kit I (R6834, Omega). Total RNA was extracted following the recommendations supplied with the kit. In brief, tissue samples (15–25 mg) were disrupted in lysis buffer containing β-mercaptoethanol using iron beads and a Tissue Lyser (Retsch, Germany) set at a frequency of 30 Hz. Liver samples were disrupted for 30 s with one iron bead and the foregut samples were disrupted with 2 iron beads for 3 × 30 seconds. RNA extracts were purified using the columns supplied with the E.Z.N.A® Total RNA Kit and treated with DNase to remove contaminating genomic DNA using an E.Z.N.A® RNase-Free DNase Set I (E1091, Omega) according to the manufacturer's instructions. Total

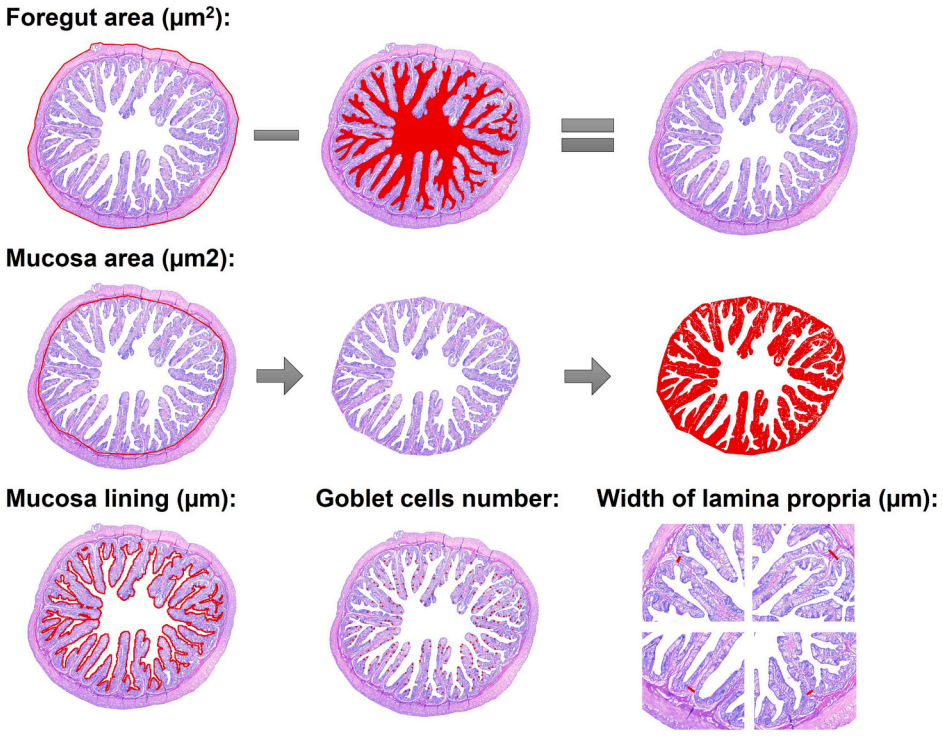

Fig. 2. Histomorphometry of foregut transverse sections. Measurements were performed with Fiji v1.52p software in the first section on 3 slides (sections analysed were separated by 25 μm) stained with ABPAS (100× magnification). Foregut area (μm^2) was measured by subtracting the lumen area from the total area confined within the circumference (red) outlining the serosa layer. Mucosa area (μm^2) was measured by cropping the area outside the circumference (red) to exclude submucosa, muscle and serosa layers and then the area obtained by the Otsu method. The mucosa lining or perimeter (μm) was determined with the Otsu method, which outlined the surface of the mucosa folds of the foregut. The lamina propria width was assessed at the base of 4 mucosa folds (one per quadrant of the section). Goblet cell number was obtained by manually counting the cells stained blue or purple. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

RNA quality and concentration were assessed with a Nanodrop spectrophotometer (Thermo Fisher Scientific), and the integrity of the extracted total RNA was verified by agarose gel (0.8%) electrophoresis. DNA-free total RNA (500 ng) was used for cDNA synthesis as previously described (Costa et al., 2017). The quality of the cDNA was verified by RT-PCR amplification of 18S ribosomal RNA (*18S*) with specific primers (Table 2) using the following thermocycle: 95 °C for 10 min, followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 20 s and a final extension step of 5 min at 72 °C. To confirm the absence of genomic DNA contamination a cDNA reaction in which reverse transcriptase was omitted (−RT control) was included in all PCR experiments and the PCR products were run on a 1% agarose gel to confirm amplicon size and the absence of genomic DNA contamination.

2.5.2. Analysis of gene expression by quantitative real-time PCR

Quantitative real-time PCR (RT-qPCR) was used, as previously described (Costa et al., 2017) to analyse the expression of target genes (Table 2) in the liver and foregut of fish from the three thermal regimes (11 °C, 13.5 °C and 16 °C) and different experimental groups, fed (control), food deprived (S) and food deprived/refed [R at 10 h (10hR) and 2 days (2dR) after refeeding]. Quantification was performed using a StepOnePLus thermocycler (Applied Biosystems) and was based on the standard-curve method using serial dilutions (1:10) of purified PCR product for each gene (obtained using the same species, tissues and primers for qPCR analysis, software StepOne™ Real-Time PCR Software v2.2). The thermocycle used was as follows: 30 s at 95 °C, 45 cycles of 5 s at 95 °C and 15 s at 60 °C, and a final melting curve performed between 60 °C and 95 °C which produced a single product dissociation curve for each primer pair. Reactions were performed in duplicate 10 μL reactions containing 10 ng of cDNA, 300 nM of specific primers (Table 2) and EvaGreen (Sso Fast EvaGreensupermix, Bio-Rad Laboratories, USA). Control reactions included a no-template control and a RT control. Relative gene expression was estimated after normalization of candidate gene expression with the geometric mean of *18S* and *ef1a*. The reference genes used were selected as they did not vary significantly ($p > 0.05$) between samples. Log2 fold change was then determined for each gene analysed in the liver and foregut in relation to fish from eggs of the 13.5 °C thermal regime, that had not been subjected to food deprivation (13.5-F, reference group). The fish from the 13.5 °C egg thermal regime was chosen as the reference group as this is the most common temperature for European sea bass egg incubation (Morretti, 1999).

2.6. Statistical analysis

All statistical analysis were performed using IBM® SPSS® Statistics 28.0 for Windows (IBM Corp., NY, USA) and after assessing if the data had a normal distribution. No significant differences were detected between tank replicates (*t*-test), so all data of fish from the same thermal background in each experimental group were pooled. Two-way ANOVA was performed to evaluate the impact of egg thermal regimes (11 °C, 13.5 °C and 16 °C), food deprivation and food deprivation/refeeding (F, S, 10hR and 2dR), and the interactions between these factors on the experimental parameters analysed. Simple main effects were performed for pairwise comparisons and a Bonferroni adjustment was used to minimise the effect of type I errors. The significance cut-off was set at $p < 0.05$ for all the analysis performed. Graphs were constructed using GraphPad Prism 6.01 for Windows (GraphPad Software, CA, USA).

Table 2
List of primers used for gene expression analysis by quantitative real-time PCR in the liver and foregut of thermally imprinted European sea bass (*Dicentrarchus labrax*). The gene symbol and name, accession number, primer sequence, annealing temperature (Ta, °C), qPCR efficiency (%) and R2 are indicated for each primer pair (F = forward and R = reverse primer).

Tissue	Gene symbol	Gene name	Accession number	Primer sequence (5` → 3`)	Amplicon (bp)	Ta (°C)	Eff (%)	R^2
Liver	*igf-1*	Insulin-like growth factor 1	AY996779	F: TGTCTAGCGCTCTTTCCTTTCA R: AGAGGGTGTGGCTACAGGAGATAC	84	60	83	1
	igf-ii	Insulin-like growth factor 2	AY839105	F: CCTGGCGCTCTACGTTGTGG R: CGGTTCTGGGTCCGTCTGTT	147	62	92	1
	igf-1r	Insulin-like growth factor receptor 1	DLAgn_00167690	F: TGGTGTCGTACTGTGGGAGA R: CTGCCAGCACATCCTCATCA	157	62	88	1
	ghr-1	Growth hormone receptor 1	DLAgn_00119640	F: AGCACCGACAGGCACGAA R: GCGTCACGGACCCGATTT	118	62	87	1
	ghr-2	Growth hormone receptor 2	DLAgn_00087370	F: TGAGGTCGTCCGGCAAGG R: GCTCTGAGGTGTAACCCCAATG	159	62	88	0.999
	ppara	Peroxisome proliferator-activated receptor alpha	AY590300	F: TGCTCAGACAAGGCTTCAGGC R: GTTGCGTTCACACTTATCATAATCC	114	60	76	0.998
	lipca	Lipase C, hepatic type a	DLAgn_00158270	F: TTTGTACGGCATCCGAGACC R: GACAAGCAGAGCATGGCCTA	171	60	95	1
Foregut	*pept1*	Solute carrier family 15 member 1 (SLC15A1)	FJ237043.2	F: ACCAAATTGTGAAAACAGCATCC R: GGGTGCCTGTGAGTAGGAGAAC	151	60	81	0.999
	hes-1	Hairy and enhancer of split-1	DLAgn_00148410	F: AACTCATCCCCGCAGGTCC R: CCGCATTGGGTATGAGGAAA	154	62	94	1
	cat	Catalase	DLAgn_00171080	F: TTTGCCTGATGGCTACCGC R: TGGCATAATCTGGGTTGGTG	173	62	87	0.999
	dio2	Deiodinase 2	DLAgn_00018780	F: CGCCTACAAGCAGGTAAAACTCG R: GGCGGCACTCGTCTCCAA	146	60	89	0.998
	pck1	Phosphoenolpyruvate carboxykinase 1	DLAgn_000978	F: GCTTTTAGCTGGCAACACGG R: TGTAGCCGAAGAAGGGACGC	127	60	95	1
Liver and Foregut	*sod1*	Superoxide dismutase 1	DLAgn_00043240	F: CTAAAGACGGGCAATGCTGG R: GGTCTTAAGTGCTGTGGGGAA	122	62	94	1
	18 s	18S ribosomal protein	(Pinto et al., 2010)	F: TGACGGAAGGGCACCACCAG R: AATCGCTCCACCAACTAAGAACGG	158	60	94	1
	ef1a	Elongation factor 1 alpha	AJ866727.1	F: GACACAGAGACTTCATCAAG R: GTCCGTTCTTAGAGATACCA	114	60	87	1

3. Results

3.1. Overall fish condition

Main effects analysis demonstrated that, irrespective of the egg thermal regime, there was an overall decrease in mass and size of the food deprived/refed fish irrespective of the time post-refeeding, 10hR and 2dR, when compared to fish that were fed (Table 1). Simple main effects analysis did not reveal significant differences between sampling times for any thermal regime.

Macroscopic observations of control and treated fish during sampling of the liver and foregut revealed all had a massive accumulation of fat in the abdominal cavity surrounding the intestine. After collecting the plasma of blood samples some were observed to be lipemic, which caused technical constraints during analysis of glucose and lactate in plasma.

Two-way ANOVA showed that fasting significantly ($p < 0.001$) influenced the yield of RNA extracted from liver samples (Supp. Fig. S1). One week of food deprivation caused a significant ($p < 0.05$) increase in μg of RNA extracted per mg of tissue in 11 °C-S and 13.5 °C-S fish, compared to the respective controls/fed fish. The quantity of RNA extracted per mg of liver was also significantly ($p = 0.002$) increased in 13.5 °C-S fish in relation to 16 °C-S fish. To enable comparison of gene expression between groups the same amount of RNA (500 ng) was used for cDNA synthesis, thus minimizing the effects of the difference in RNA yield.

3.2. Plasma parameters

Technical problems due to the high lipid content of the plasma in fed fish meant it was not possible to obtain valid results for all samples of the fed (control) group and anomalous samples had to be eliminated and led to a variable and generally low sample number from fed individuals of fish from the different egg thermal regimes. For this reason, the glucose and lactate levels of the fed fish were not included in the main statistical analysis, but the values obtained are presented in Supp. Fig. S2.

Two-way ANOVA revealed that all plasma parameters were significantly ($p < 0.01$) affected by food deprivation/refeeding. Lactate was also significantly ($p = 0.02$) affected by thermal regime and cortisol was significantly ($p < 0.01$) modified by the interaction between the two main factors (Fig. 3).

Cortisol levels were similar ($p > 0.05$) in fed fish irrespective of the thermal regime at egg incubation (11, 13.5 or 16 °C). After one week of food deprivation, cortisol levels were significantly ($p < 0.01$) increased in all groups, with the amplitude of the cortisol response higher in 11 °C-S and 13.5 °C-S. Ten hours after refeeding, cortisol levels returned to initial concentrations in all thermal groups, except for 16 °C-10hR, which exhibited elevated cortisol levels throughout the experiment compared to 16 °C-F fish. At the end of the trial (2dR), cortisol levels of 11 °C-2dR fish returned to basal levels and were significantly ($p < 0.01$) lower relative to other fish (13.5 °C-2dR and 16 °C-2dR).

Lactate levels of fish incubated as eggs at 11 °C were significantly ($p < 0.05$) modulated by food deprivation/refeeding, while fish incubated as eggs at 13.5 °C and 16 °C thermal regimes had similar lactate levels throughout the experiment. Lactate levels of 13.5 °C-S and 13.5 °C-10hR fish were significantly ($p < 0.05$) higher compared to 16 °C-S and 16 °C-10hR fish, respectively. Lactate levels were significantly increased ($p < 0.01$) in plasma from 11 °C-10hR compared to 11 °C-S and were significantly ($p < 0.01$) higher than lactate levels of 16 °C-10hR fish. Two days post-refeeding, lactate levels of 11 °C-2dR fish returned to initial levels. Glucose levels also significantly ($p < 0.01$) decreased at 2d post-refeeding when compared to food deprived fish and 10 h post-refed fish, irrespective of the egg thermal regime (Fig. 3).

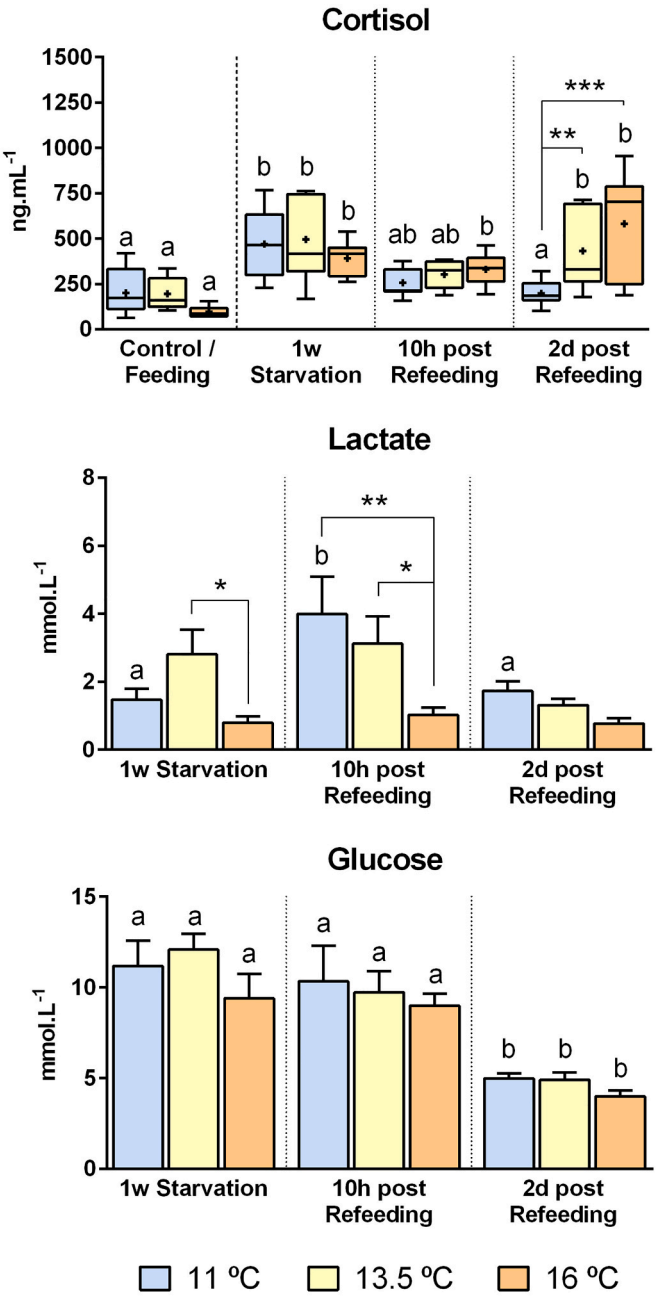

Fig. 3. Plasma levels of cortisol (ng.mL^{-1}), lactate (mmol.L^{-1}) and glucose (mmol.L^{-1}) of European sea bass from different thermal regimes. Cortisol levels are plotted in a Tukey box and whiskers plot and '+' represents the mean. Lactate and glucose results are represented as mean ± s.e.m. Three different thermal regime groups are plotted (11 °C, 13.5 °C and 16 °C) for each experimental group: before starvation (Control/Feeding), after one week of food deprivation (1w Starvation) and 10 h and 2 days after refeeding (10 h and 2d post refeeding). Due to technical constraints, lactate and glucose levels from the Feeding groups were not included in the analysis. Different letters denote significant differences between different time points, within the same thermal group. Asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) indicate significant differences between fish from different thermal regimes in each experimental group. Statistical significance (by two-way ANOVA) was set at $p < 0.05$, $n = 8$ per thermal regime/experimental group.

3.3. Liver histology and histomorphometry

The hepatic parenchyma of fed fish (before food deprivation) from all egg thermal regimes presented pronounced vacuolization due to lipid

accumulation, which caused hypertrophy of the hepatocytes, displacement of the nucleus to the periphery of the cell and reduced eosinophilic cytoplasm (Fig. 4A - F fish). Hepatocyte nuclei were round and basophilic with evident nucleolus inside them. Discreet sinusoids with red blood cells were observed between hepatocyte cords. Two-way ANOVA demonstrated that the number of nuclei and infiltration of the liver with lipid (percentage of area) were significantly affected by food deprivation/refeeding ($p < 0.001$) and by the interaction of food deprivation/refeeding with egg thermal regime ($p = 0.025$ and $p = 0.002$, respectively). The area occupied by lipid was also significantly ($p < 0.001$) affected by egg thermal regime. The area of the nuclei was not significantly modified during the experiment ($p > 0.05$, Fig. 4B). One week after food deprivation, there was a significant reduction ($p < 0.001$) in the area occupied by lipid in the liver and a significant increase ($p < 0.001$) in the number of nuclei, irrespective of the egg thermal regime. The reduction in the area occupied by lipid was significantly higher ($p < 0.001$) in 16 °C-S fish compared to 11 °C-S fish (Fig. 4A – S fish). In the parenchyma of 11 °C-S fish intermixed areas were observed, containing hepatocytes with a single, large lipid droplet or hepatocytes with many, small lipid droplets.

The percentage of the liver occupied by lipid progressively increased to before-food deprivation levels during refeeding, irrespective of the

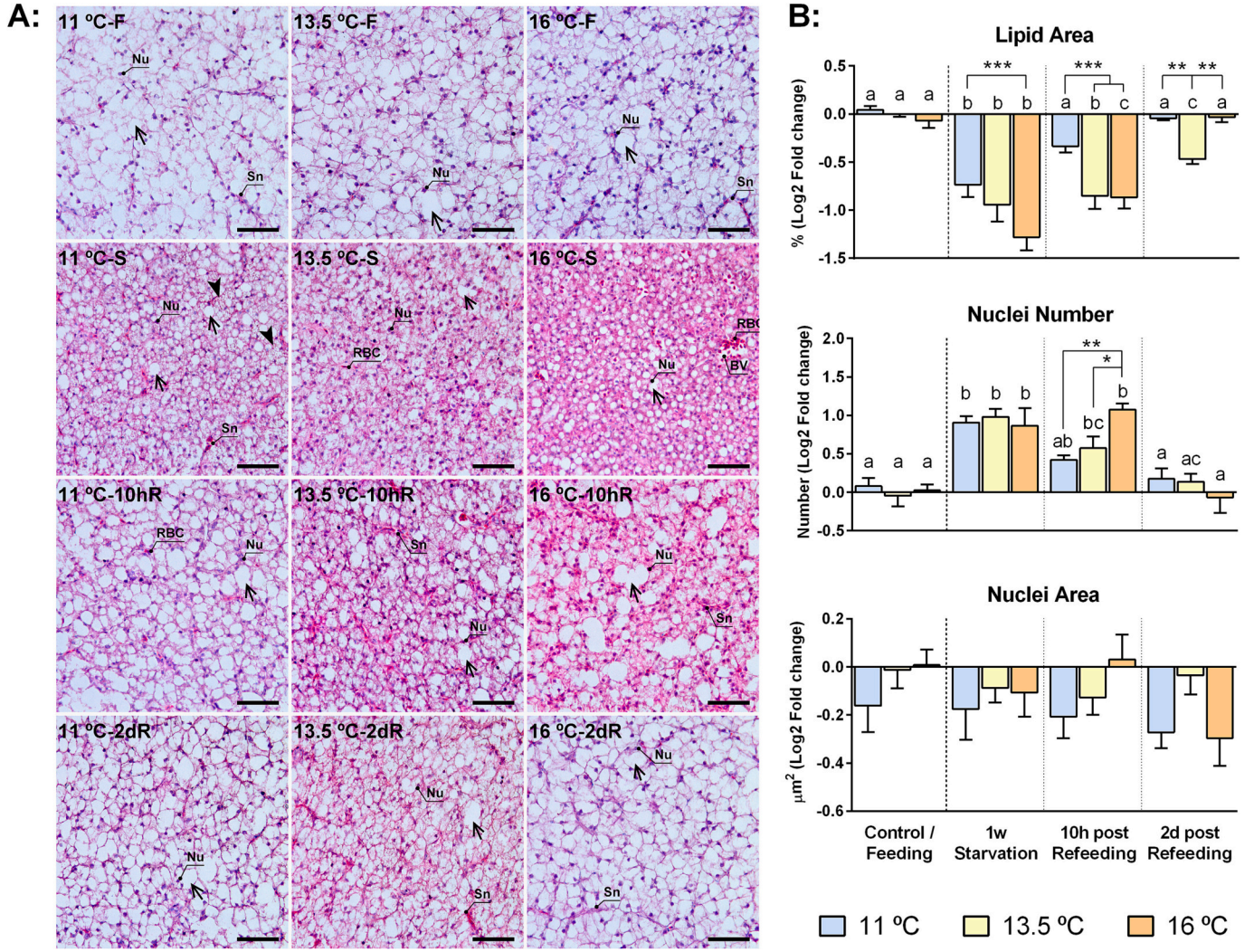

Fig. 4. Histomorphology of European sea bass liver in juveniles raised from eggs incubated at different temperatures (11 °C, 13.5 °C and 16 °C) and challenged with one week of food deprivation and then refeeding (F = fed/control fish; S = starved fish; 10hR = 10 h post refeeding; 2dR = 2 days post refeeding). A: Morphology of the liver, stained with H&E. The liver parenchyma was composed of enlarged hepatocytes due to the large lipid droplets (arrow) that pressed the eosinophilic cytoplasm and basophilic nucleus (Nu) to the periphery of the hepatocytes, in fed fish (F). Occasionally, blood sinusoids (Sn) with red blood cells (RBC) were observed along hepatocyte cords. One week of starvation decreased the lipid vacuolization of the parenchyma, increasing the eosinophilic cytoplasmic area within the hepatocytes (S fish). In the hepatic parenchyma of 11-S fish there were hepatocytes with large, single lipid vacuoles (arrow) and hepatocytes with multiple, small lipid vacuoles (arrowhead). After resumption of feeding, the parenchyma started to acquire the before fasting tissue morphology, with an increased area of lipids within the hepatocytes. The exception was the 13.5-2dR fish, which maintained a significantly reduced lipid area compared to initial levels and to fish from the groups 11–2dR and 16-2dR. BV = blood vessel. Scale bar = 50 µm. B: Histomorphometric parameters analysed in the liver. Lipid area (µm^2) and nuclei number and area (µm^2) were measured in a captured field of liver taken at 400×, with approximately 89 × 10^3 µm^2 area, and was performed in 3 sections/fish (n = 8/thermal regime/experimental group) spaced by 15–20 µm between the sections analysed, using the Otsu method (Papadopulos et al., 2007; Zhang and Hu, 2008). Results are presented as mean ± s.e.m. and were calculated relative to fish from eggs incubated at the most common temperature for European sea bass that were not exposed to food deprivation as adults (13.5 °C-F). Different letters denote significant differences between different experimental groups, within the same thermal regime group. Asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) indicate significant differences between fish from different thermal regimes in each experimental group. Statistical significance (by two-way ANOVA) was set at $p < 0.05$, n = 8 per thermal regime/experimental group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

egg thermal regime (Fig. 4A – 10hR and 2dR fish, Fig. 4B). The exception was the 13.5 °C-2dR fish, in which the lipid area in the liver remained significantly ($p < 0.01$) decreased compared to control fish (13.5 °C-F) and the 11 °C-2dR and 16 °C-2dR refed fish. The number of nuclei for a given area of the liver also progressively decreased during refeeding, to levels found in fed fish. The exception was the 16 °C-10hR fish that had significantly ($p < 0.05$) more nuclei/area than the fish of the 11 °C-10hR and 13.5 °C-10hR groups.

3.4. Foregut histology and histomorphometry

The morphology of the foregut was similar between fish from different egg thermal regimes at each time point. The foregut had a well conserved structure with elongated branching mucosa folds covered in a luminal facing mucosa layer, the submucosa, muscle layers and the encapsulating serosa layer (Fig. 5). The mucosa was highly folded and contained simple columnar enterocytes and scattered goblet cells that stained dark purple by AB-PAS. Analysis by two-way ANOVA showed that all the histomorphometric parameters analysed in the foregut were significantly ($p < 0.001$) modified by the food deprivation/refeeding challenge. The exception was the mucosa area and the width of the lamina propria, which were not significantly ($p > 0.05$) modified during the experiment.

In fed fish, the foregut and mucosa areas (μm^2), mucosa lining (perimeter, μm), the number of goblet cells and the width of the lamina propria were similar in fish from different egg thermal regimes (Fig. 6). Food deprivation caused a significant ($p < 0.01$) reduction in the foregut area and a significant ($p < 0.05$) increase in the number of goblet cells in 13.5 °C-S and 16 °C-S compared to the fed fish. In fish incubated as eggs at 11 °C, a significant ($p < 0.001$) decrease in the foregut area was observed 10 h after refeeding compared to 11 °C-F. Two days post-refeeding (2dR) the foregut area and the number of goblet cells, returned to the levels seen in the fed fish, irrespective of the egg thermal regime. The exception was the 16 °C-2dR fish, in which the foregut area was significantly ($p = 0.02$) reduced compared to 16 °C-F. At 2d post-refeeding, the perimeter of the mucosa was significantly ($p < 0.05$) increased in 13.5 °C-2dR fish compared to 11 °C-2dR and 16 °C-2dR.

3.5. Gene expression

3.5.1. Expression of growth axis-related genes in the liver

Analysis by two-way ANOVA revealed that the egg thermal regime did not significantly ($p > 0.05$) affect the expression of genes of the growth axis in the liver, but that food deprivation/refeeding had a significant ($p < 0.01$) impact on the expression of genes *ghr-2, igf-1* and *igf-2* (Fig. 7). The expression of *ghr-2* was significantly ($p < 0.05$) down-regulated in 13.5 °C-10hR relative to 13.5 °C-F and 13.5 °C-S fish. Although two-way ANOVA revealed a significant impact of food deprivation/refeeding on the expression of *ghr-1* ($p = 0.005$), simple main effects corrected with Bonferroni did not identify significant differences between fish with different egg thermal regimes within each experimental group. Analysis of main effects revealed that *ghr-1* in the fed fish was significantly different ($p < 0.05$) in food deprived and 10 h post-refeeding fish from all egg thermal regimes.

The expression of *igf-1* was significantly increased ($p < 0.05$) in 11 °C-S fish compared to 11 °C-F but returned to levels of fed fish 10 h after the start of refeeding (11 °C-10hR). Expression of *igf-1* in the 13.5 °C-2dR fish was significantly lower ($p < 0.05$) than in the fed fish. Food deprivation and refeeding caused a significant ($p < 0.05$) up-regulation of *igf-2* in 11 °C-10hR and 16 °C-10hR fish compared to levels in 11 °C-F and 16 °C-F, respectively. The expression of *ghr-1* and *igf-1r* was not significantly modified ($p > 0.05$) throughout the experiment in any of the egg thermal groups.

3.5.2. Expression of genes associated with metabolism

3.5.2.1. Expression of dio2 and pck1 in liver and foregut. Two-way ANOVA revealed that food deprivation/refeeding significantly ($p < 0.05$) modified the expression of *dio2* and *pck1* in the liver (Fig. 8). Egg thermal regime also significantly ($p = 0.001$) affected the expression of *pck1*. In the foregut, the expression of *dio2* and *pck1* was significantly ($p < 0.001$) modified by food deprivation/refeeding and by egg thermal regime ($p = 0.03$ and $p = 0.001$, respectively, two-way ANOVA, Fig. 8). Expression of *pck1* in the foregut was also significantly modified ($p < 0.05$) by the interaction between food deprivation/refeeding and the egg thermal regime.

Expression of *dio2* in the liver was significantly ($p < 0.05$) up-regulated in 16 °C-10hR and in all fish at 2dR, irrespective of egg thermal regime compared to fed fish. In the foregut, one week of food deprivation caused a significant up-regulation ($p < 0.05$) of *dio2* in 13.5 °C-S relative to 13.5 °C-F fish. *Dio2* mRNA levels returned to control levels in the 13.5 °C-10hR group and were significantly down-regulated ($p < 0.05$) compared to 11 °C-10hR and 16 °C-10hR.

In the liver of fish from all egg thermal regimes one week of food deprivation caused a significant up-regulation ($p < 0.001$) in the expression of *pck1* compared to levels in fed fish and levels stayed significantly up-regulated ($p < 0.001$) until 10 h post-refeeding (Fig. 8). At the end of the experiment, the expression of *pck1* was significantly down-regulated ($p < 0.05$) in 11 °C-2dR and 13.5 °C-2dR compared to the levels in fed fish. The fish 16 °C-2dR had significantly up-regulated ($p = 0.006$) expression of *pck1* compared to 11 °C-2dR fish. In the foregut of fish from eggs incubated at 16 °C, *pck1* was significantly up-regulated ($p < 0.05$) compared to fish from eggs incubated at 11 °C, before (fed) and after food deprivation. After 1 week of food deprivation there was a significant up-regulation ($p < 0.05$) of *pck1* in the foregut of 13.5 °C-S fish compared to the foregut of fed fish (13.5 °C-F). Ten hours post-refeeding, *pck1* was significantly up-regulated ($p < 0.05$) in the foregut of all fish compared to the fed fish and 16 °C-10hR fish had significantly ($p < 0.05$) increased levels of *pck1* compared to 13.5 °C-10hR. Two days post-refeeding, the expression of *pck1* in the foregut returned to the levels detected in fed fish but remained significantly up-regulated ($p < 0.05$) in the foregut of 16 °C-2dR compared to 11 °C-2dR fish.

3.5.2.2. Expression of pparα and lipcα in liver. Two-way ANOVA revealed that food deprivation/refeeding only caused a significant modification (up-regulation, $p < 0.05$) in the expression of *pparα* in the liver of fish from the 16 °C egg thermal regime (Fig. 8). A significant up-regulation in *pparα* ($p < 0.05$) transcription in the liver was observed between 16 °C-10hR and 16 °C-F and returned to that of fed fish in the 16 °C-2dR fish. Expression of *lipc* was not significantly affected by the main factors nor by the interaction between them.

3.5.2.3. Expression of pept1 and hes1 in foregut. Two-way ANOVA revealed that the expression of *pept1* and *hes1* in the foregut was significantly ($p < 0.001$ and $p = 0.01$, respectively) affected by food deprivation/refeeding and by the egg thermal regime ($p < 0.05$; Fig. 8). No significant differences were found in the expression of *pept1* and *hes1* between fish from eggs exposed to different thermal regimes in the fed group. One week of food deprivation caused a significant up-regulation ($p < 0.01$) of *pept1* in the foregut of 13.5 °C-S and 16 °C-S fish compared to the fed fish, and 10 h after refeeding the expression of *pept1* had returned to the same level as found in fed fish. In the food deprived/refed fish 10 h after refeeding the *pept1* was significantly up-regulated ($p < 0.05$) in the foregut of 16 °C-10hR fish compared to 13.5 °C-10hR. By the end of the experiment after 2 days of refeeding (2dR) the expression of *pept1* was significantly decreased ($p < 0.01$) in the foregut of 11 °C-2dR and 13.5 °C-2dR fish compared to the 11 °C-F and 13.5 °C-F, respectively.

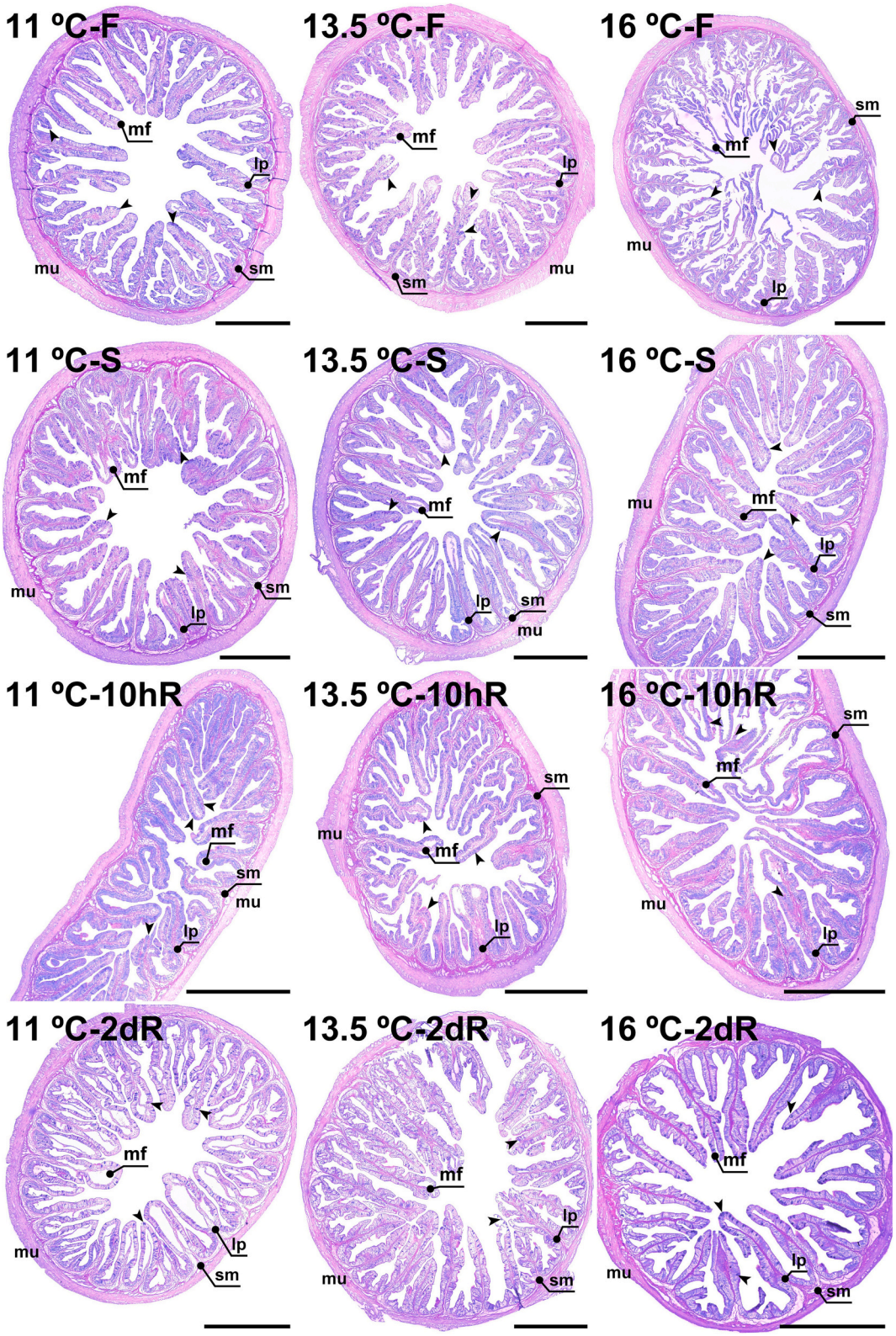

Fig. 5. Morphology of the European sea bass foregut stained with AB-PAS, from juvenile fish incubated as eggs at different temperatures (11 °C, 13.5 °C and 16 °C) and challenged with one week of food deprivation and then refeeding (F = fed/control fish; S = starved fish; 10hR = 10 h post refeeding; 2dR = 2 days post refeeding). The morphology of the foregut was similar between fish from different thermal regimes in each experimental group and consisted of an inner layer of mucosa, submucosa (sm), muscle (mu) and then an outer layer of serosa (not visible). The mucosa was comprised of several mucosal folds (mf) projecting into the lumen that were lined by simple columnar enterocytes and scattered goblet cells that stained dark purple (arrowheads). Scale bar = 500 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

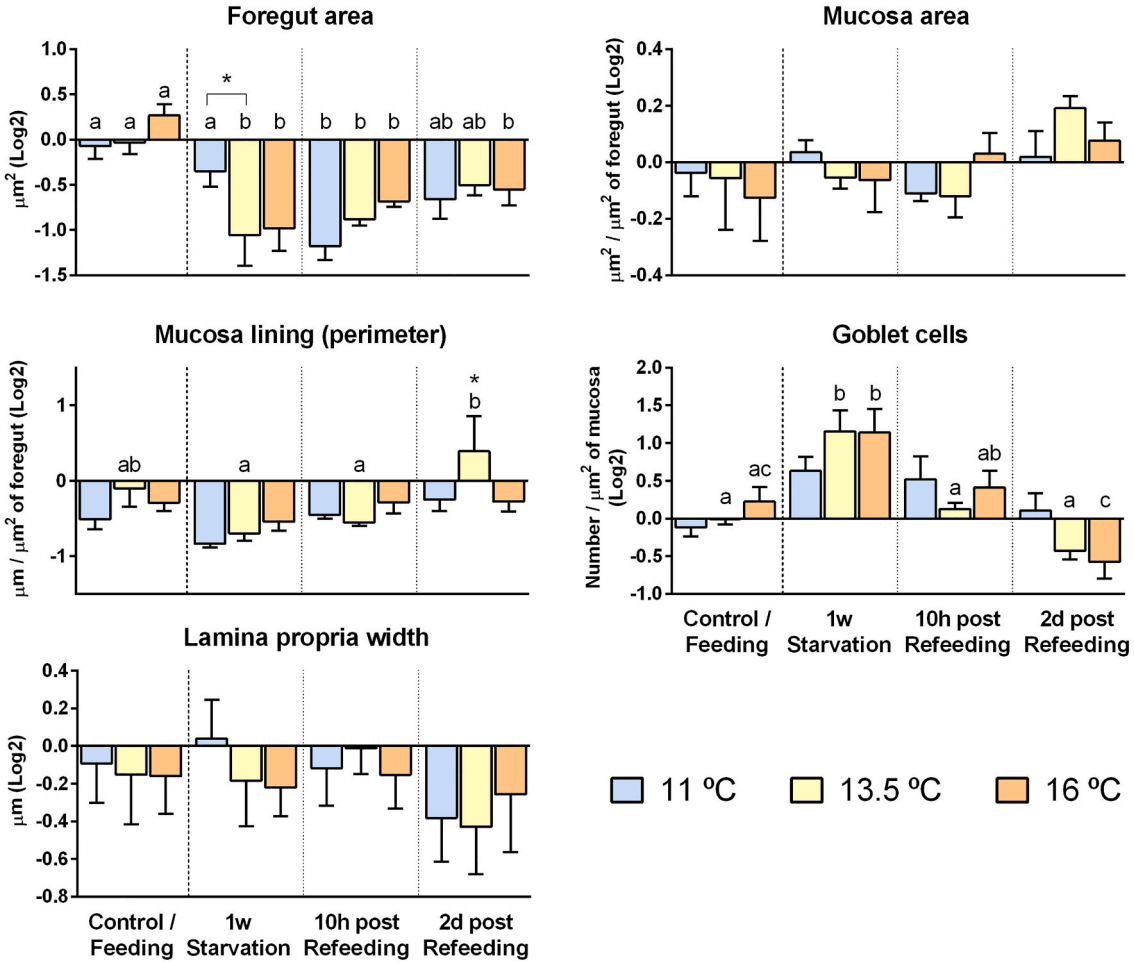

Fig. 6. Histomorphometric analysis of the foregut. The parameters evaluated included foregut transverse section and the area of the mucosa folds (μm^2), perimeter of the mucosa folds (mucosa lining, μm), lamina propria width (μm) and total number of goblets cells per section, using Fiji v1.52p software (Schindelin et al., 2012). The foregut area was used to normalize the mucosa area and lining (perimeter), and the goblet cell number was normalized by the mucosa area. Methodological details are provided in section 2.4.1. Results of all parameters were normalized against the designated reference group (13.5-F), transformed in Log2 fold change and presented as mean ± s.e.m. Three different thermal regime groups are plotted (11 °C, 13.5 °C and 16 °C) for each experimental group: before food deprivation (Control/Feeding), after one week of food deprivation (1w Starvation) and 10 h and 2 days after refeeding (10 h and 2d post refeeding). Different letters denote significant differences between different experimental groups, within the same thermal regime group. Statistical significance (by two-way ANOVA) was set at $p < 0.05$, $n = 8$ per thermal regime/experimental group.

Expression of *hes1* in the foregut was significantly down-regulated ($p < 0.05$) in 16 °C-10hR and 16 °C-2dR fish compared to the fed fish. Expression of *hes1* in fish from eggs incubated at 11 °C and 13.5 °C was not significantly altered during the food deprivation/refeeding experiment, but at 10 h post-refeeding, the expression of *hes1* in the foregut of 11 °C-10hR fish was significantly up-regulated ($p < 0.05$) compared to 13.5 °C-10hR and 16 °C-10hR fish.

3.5.3. Expression of antioxidant enzymes in liver and foregut

Expression of *sod1* and *cat* in both liver and foregut (Fig. 9) was significantly ($p < 0.001$) affected by food deprivation/refeeding as demonstrated by two-way ANOVA. In the liver, *sod1* was significantly down-regulated ($p < 0.05$) in 16 °C-S fish, compared to 16 °C-F fish. Ten hours post-refeeding, all groups of fish irrespective of egg thermal regime had significantly down-regulated ($p < 0.01$) *sod1* in the liver compared to fed fish. The expression of *sod1* returned to the levels typical of fed fish, except for 13.5 °C-2dR fish. In the foregut, the only fish that had significant down-regulation ($p < 0.05$) of *sod1* during the experiment compared to the fed fish was 13.5 °C-S and 13.5 °C-10hR fish. Two days after refeeding, the expression of *sod1* in the foregut was significantly up-regulated ($p < 0.05$) in 11 °C-2dR compared to 11 °C-S and 11 °C-10hR.

Expression of *cat* in the liver was significantly down-regulated ($p < 0.05$) in 13.5 °C-10hR and 16 °C-10hR fish compared to fed fish (Fig. 9). In the foregut, one week of food deprivation provoked a significant up-regulation ($p = 0.02$) of *cat* in 11 °C-S fish, compared to 11 °C-F. Ten hours post-refeeding, 13.5 °C-10hR fish had a significant down-regulation ($p < 0.05$) of *cat* in the foregut compared to fish from other thermal regimes (11 °C-10hR and 16 °C-10hR). Expression levels of *cat* were re-established to levels found in fed fish and in both tissues after 2 days refeeding in all groups.

4. Discussion

Brief manipulation of temperatures during incubation of European sea bass eggs was sufficient to cause phenotypic plasticity in some of the juvenile responses to food deprivation and refeeding. Significant changes occurred in the morphology of the liver during food deprivation and refeeding. However, it was the foregut that exhibited the most notable molecular response to food deprivation and refeeding between fish from different egg incubation temperature regimes, suggesting imprinting occurred. This is a very intriguing aspect of our study, since the liver is recognized as fundamental for adaptation to food deprivation, and the foregut as the segment of the intestine mainly responsible

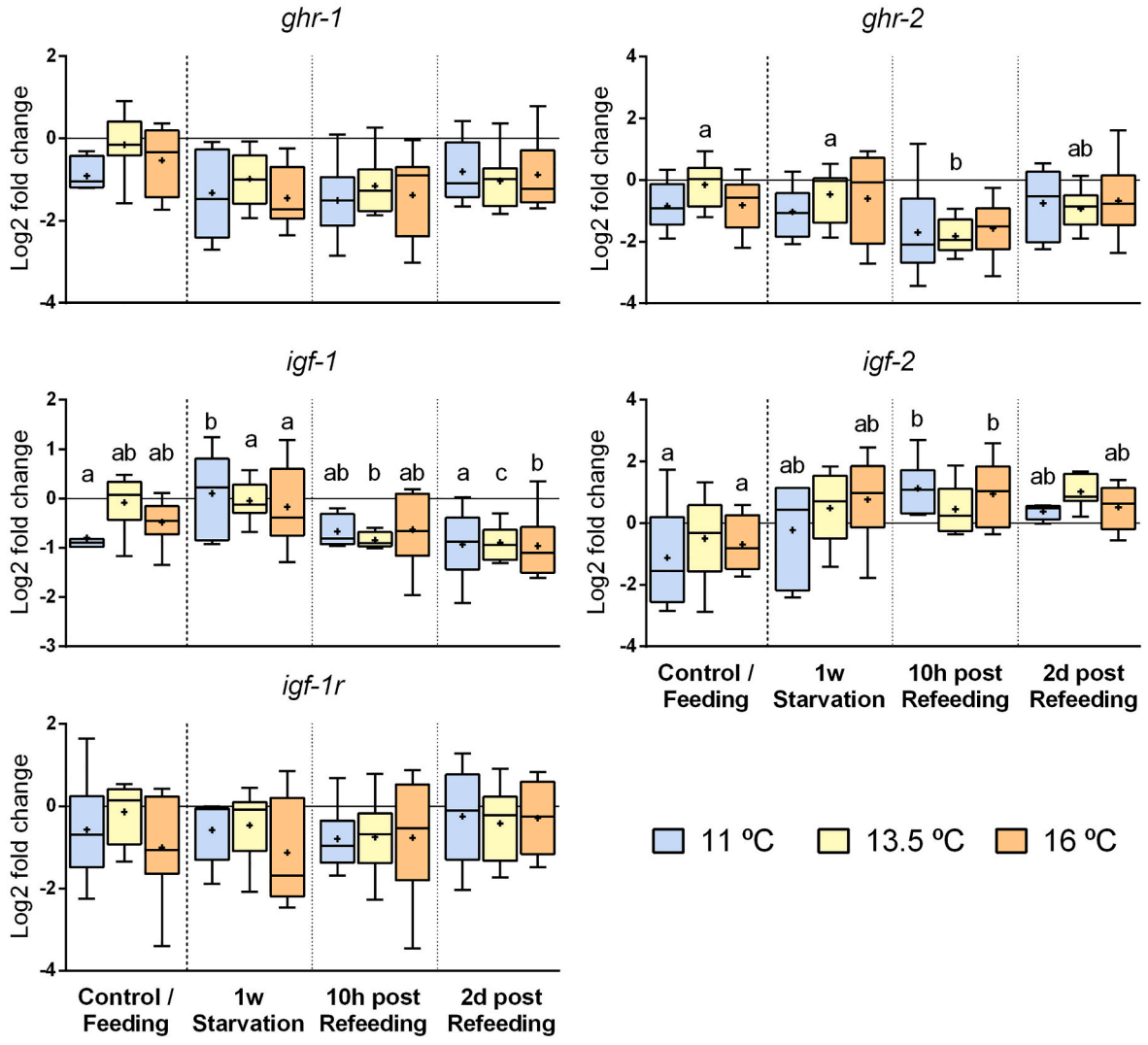

Fig. 7. Relative expression of transcripts of the growth axis analysed by qPCR in the liver of thermally imprinted European sea bass during the food deprivation and refeeding experiment. Results obtained for *ghr-1*, *ghr-2*, *igf-1*, *igf-2* and *igf-1r* were normalized by the geometric mean of *18 s* and *ef1a*, and then expressed as Log2 fold change, calculated relative to fish from eggs incubated at the most common temperature for European sea bass that were not exposed to food deprivation as juveniles (13.5 °C-F). Each thermal regime group (11 °C, 13.5 °C and 16 °C) for each experimental group (before food deprivation [Control/Feeding], after one week of food deprivation [1w Starvation] and 10 h and 2 days after refeeding [10 h and 2d post refeeding]) are plotted in Tukey box and whiskers graphs and '+' represents the mean. Different letters denote significant differences between different experimental groups, within the same thermal regime. Statistical significance (by two-way ANOVA) was set at $p < 0.05$, $n = 8$ per thermal regime/experimental group.

for nutrient digestion/absorption and is one of the first organs to sense nutritional modifications (Rašković et al., 2011). Different responses between the foregut and liver we speculate may be related to the timing of their organogenesis, and in the case of the liver primordium in European sea bass it emerges from the thickening of the hindgut wall several hours after hatching (Diaz and Connes, 1997) when in our experiments the thermal regime was common for all groups.

Fish from eggs exposed to the highest thermal regime (16 °C) were the fish that underwent the most morphological changes in the liver and foregut after food deprivation, while fish incubated as eggs at lower temperatures (11 °C) exhibited less changes after the experimental treatments. Specifically, in 11 °C-10hR fish the appearance of the hepatic parenchyma recovered earlier than fish from other egg thermal regimes. Fish from eggs exposed to the thermal regime normally used in European sea bass hatcheries (13.5 °C) did not recover after 2 days of refeeding and had a significantly reduced lipid area compared to the pre-food deprivation levels and to fish from other egg thermal regimes. Moreover, fish from eggs hatched under a standard temperature regime, 13.5 °C after refeeding (13.5 °C-10hR) had significantly lower mRNA levels of gene transcripts encoding metabolic and the antioxidant enzyme, *cat*, in the foregut compared to fish from other egg thermal regimes. Even though recovery of metabolic and endocrine responses after food deprivation may differ between the onset of refeeding and later during refeeding (Jia et al., 2019; Power et al., 2000), our results suggest that the egg thermal regime influenced the rate of recovery in the European sea bass and that temperatures below and above (11 and 16 °C, respectively) the current optimum used in hatcheries (13.5 °C) recovered sooner.

Despite the observed atrophy of the foregut 10 h after refeeding in fish from eggs incubated at 16 °C, a significant up-regulation of *pept1* and *pck1* occurred compared to fish from eggs hatched under standard hatchery conditions (13.5 °C-10hR), suggesting that oligopeptide absorption (Daniel, 2004; Wang et al., 2017) and extrahepatic gluconeogenesis (Yang et al., 2009) were compensated, if the capacity of the intestine to produce glucose is assumed despite the current lack of consensus about this subject (Croset et al., 2001; Martin et al., 2010; Mithieux, 2005; Mutel et al., 2011; Penhoat et al., 2014; Potts et al., 2018; Rajas et al., 2000). In analogy with the up-regulation of *Pck1* in

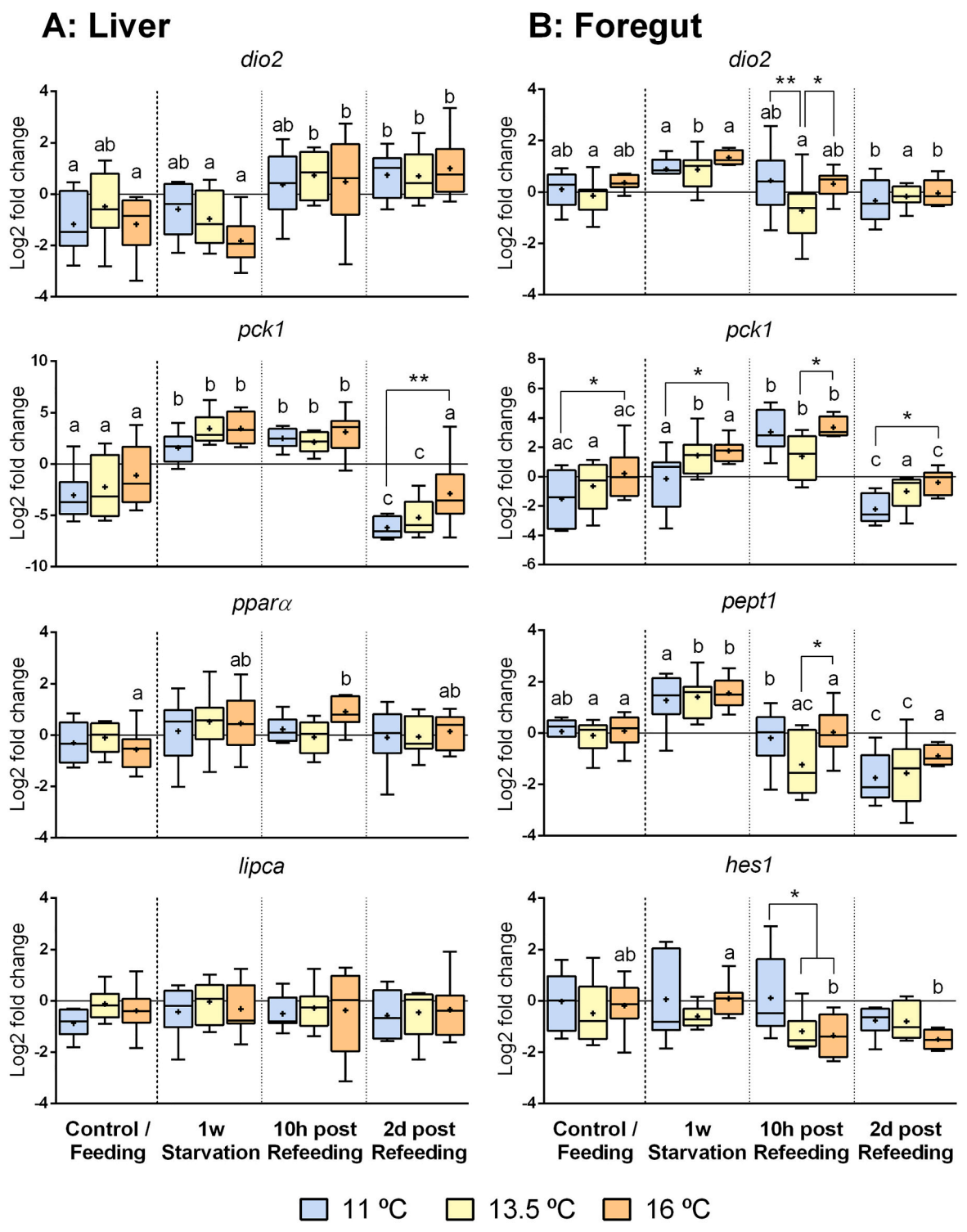

Fig. 8. Relative expression of transcripts related to metabolism analysed by qPCR in the liver and foregut of thermally imprinted European sea bass during the food deprivation and refeeding experiment. A: Transcripts associated with liver metabolism (*dio2*, *pck1*, *pparα* and *lipca*). B: Transcripts associated with foregut metabolism (*dio2*, *pck1*, *pept1* and *hes1*). Results were normalized by the geometric mean of *18 s* and *ef1a*, and then expressed as Log2 fold change, calculated relative to fish from eggs incubated at the most common temperature for European sea bass that were not exposed to food deprivation as juveniles (13.5 °C-F). Each thermal regime group (11 °C, 13.5 °C and 16 °C) for each experimental group (before food deprivation [Control/Feeding], after one week of food deprivation [1w Starvation] and 10 h and 2 days after refeeding [10 h and 2d post refeeding]) are plotted in Tukey box and whiskers graphs and '+' represents the mean. Different letters denote significant differences between different experimental groups, within the same thermal regime. Asterisks (*$p < 0.05$; **$p < 0.01$) indicate significant differences between fish from different thermal regimes in each experimental group. Statistical significance (by two-way ANOVA) was set at $p < 0.05$, $n = 8$ per thermal regime/experimental group.

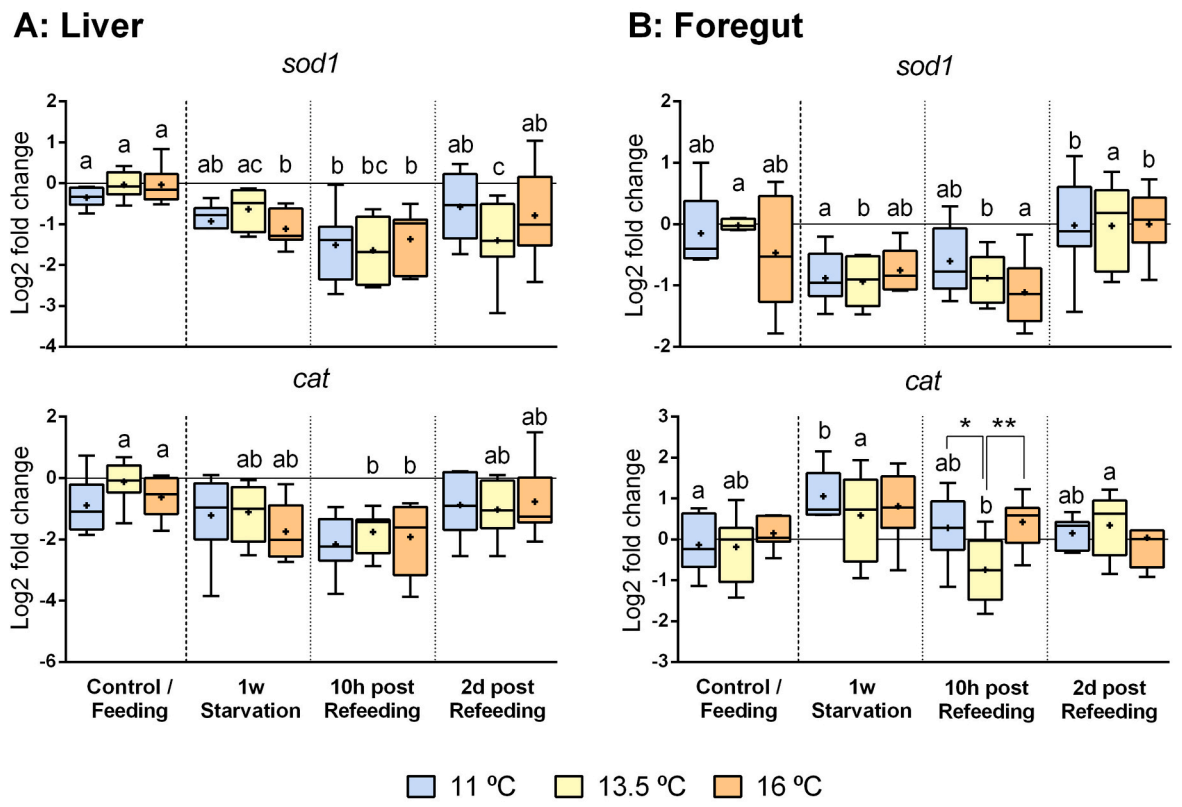

Fig. 9. Relative expression of antioxidant enzymes *sod1* and *cat* analysed by qPCR in the liver (A) and foregut (B) of thermally imprinted European sea bass during the food deprivation and refeeding experiment. Results were normalized by the geometric mean of *18 s* and *ef1a*, and then expressed as Log2 fold change, calculated relative to fish from eggs incubated at the most common temperature for European sea bass that were not exposed to food deprivation as juveniles (13.5 °C-F). Each thermal regime group (11 °C, 13.5 °C and 16 °C) for each experimental group (before food deprivation [Control/Feeding], after one week of food deprivation [1w Starvation] and 10 h and 2 days after refeeding [10 h and 2d post refeeding]) are plotted in Tukey box and whiskers graphs and '+' represents the mean. Different letters denote significant differences between different experimental groups, within the same thermal regime. Asterisks (*$p < 0.05$; **$p < 0.01$) indicate significant differences between fish from different thermal regimes in each experimental group. Statistical significance (by two-way ANOVA) was set at $p < 0.05$; n = 8 per thermal regime/experimental group.

mammals by thyroid hormones (TH) (Hanson and Reshef, 1997; Hasan Al-bayati and ML AL-Khateeb, 2021; Matosin-Matekalo et al., 1998), we speculate that the up-regulation of *pck1* might be related to the up-regulation of *dio2* in 16 °C-10hR fish compared to 13.5 °C-10hR. Up-regulation of *dio2* in the foregut suggests T3 increased in enterocytes and that TH signalling was enhanced (Bianco et al., 2019; Deal and Volkoff, 2020; Jarque and Piña, 2014; Orozco and Valverde, 2005). Interestingly, it has previously reported that fish (Carballo et al., 2018; Mateus et al., 2017a; Politis et al., 2018) and chicken (Loyau et al., 2014; Nassar et al., 2015) embryos incubated at higher temperatures had significantly up-regulated *dio2*. Taking into consideration the essential role of THs in fish development and metamorphosis (Power et al., 2001), it is not surprising that phenotypic plasticity induced by thermal imprinting during embryonic development of fish affects, and may be mediated in part, by the hypothalamic-pituitary-thyroid axis (HPT) (Carballo et al., 2018; Lema, 2020; Mateus et al., 2017a; Politis et al., 2018; Salis et al., 2021).

4.1. Temporary stimulation of igf-1 following food deprivation in fish from lower eggs thermal regime

Higher temperatures during fish egg incubation are known to accelerate hatching and increase the growth rate and size of fish during their development (Ayala et al., 2001; Jennings and Pawson, 1991; Koumoundouros et al., 2001; Saka et al., 2001), a response mediated by the GH-IGF axis (Gabillard et al., 2003a; Konstantinidis et al., 2021; Li and Leatherland, 2008; Li et al., 2007). In the present study, fish from eggs incubated at higher temperatures (16 °C) hatched two days earlier than fish from eggs hatched at 11 °C (data not shown). But nine months later, body mass and size of fish from different egg thermal regimes that were used in the experiments were not significantly different, nor was the expression of genes of the GH-IGF axis.

After one week of starvation, mRNA levels of *igf-1* were significantly up-regulated in fish incubated at 11 °C as eggs (11 °C-S) compared to 11 °C-F fish. The expression of *igf-2* was also up-regulated in fish from the 11 °C egg incubation regime but only when feeding was resumed (11 °C-10hR). A significant down-regulation of *igf-1* gene expression has previously been observed in fish deprived of food, and mRNA levels were reported to achieve basal mRNA levels very soon after refeeding or after several days delay (Hack et al., 2019; Terova et al., 2007; Tian et al., 2015). However, like in our study previous studies have also reported an increase in *igf-1* and *igf-2* expression after food deprivation (Ayson et al., 2007; Kaneko et al., 2023). Although there is no clear rationale to explain the up-regulation of *igf-1* after one week of food deprivation in our study, these results suggest that a temporary stimulation of the GH-IGF axis occurred in fish from 11 °C egg incubation regime. The receptors for GH (*ghr-1* and *ghr-2*) that regulate IGFs in the liver (Triantaphyllopoulos et al., 2020), were not significantly affected by food deprivation in our study, suggesting that these receptors were most likely not involved in the endocrine response to fasting (Delgadin et al., 2015).

4.2. Modulation of the intestinal response to food deprivation in fish from higher thermal regimes

Food deprivation caused a significant reduction in the area of the

foregut in fish from eggs maintained under control and higher temperatures (13.5 °C-S and 16 °C-S, respectively), as previously reported in fish deprived of food (Emadi Shaibani et al., 2013; Hall and Bellwood, 1995; Krogdahl and Bakke-McKellep, 2005; Zeng et al., 2012). No significant changes were seen in the mucosa area, suggesting a proportional shrinkage of all components of the mucosal mass (Sokolović et al., 2007; Zeng et al., 2012). AB-PAS of goblet cells revealed that food deprivation stimulated the number of these cells in 13.5 °C-S and 16 °C-S fish despite the reduction in the foregut area, as previously reported in other fish species deprived of food (Baeverfjord and Krogdahl, 1996; Emadi Shaibani et al., 2013; Li et al., 2017). The presumptive increase in goblet cell differentiation may have been stimulated by inhibition of the Notch-Hes1 pathway, as seen in mammals (Jensen et al., 2000; van Es et al., 2005; Zhou et al., 2015) and other fish (Crosnier et al., 2005; Wang et al., 2016), since in our experiment *hes1* was significantly down-regulated in 13.5 °C-10hR and 16 °C-10hR sea bass compared to 11 °C-10hR. Surprisingly, in the sea bass from eggs maintained at 11 °C, one week of food deprivation did not significantly modify the histological parameters analysed in the intestine.

Sea bass with a significant reduction in the foregut area (13.5 °C-S and 16 °C-S) compared to the fed fish also had a significant up-regulation of *pept1*, a previously characterised transporter localised on the brush border of the enterocytes that takes up oligopeptides from the intestinal lumen (Daniel, 2004; Wang et al., 2017). In mice, food deprivation increased the expression of *PepT1*, in parallel with an enhanced absorption rate (Ma, 2010; Ma et al., 2012; Naruhashi et al., 2002; Thamotharan et al., 1999) and loss of mucosal mass (Habold et al., 2007; Ihara et al., 2000). This suggests that in the sea bass from eggs incubated at 13.5 °C and 16 °C, up-regulation of *pept1* may reflect a compensatory mechanism for the presumptive reduction in small peptide absorptive capacity that is associated with intestinal atrophy. However, in other species of fish, variable results were obtained for *pept1* expression during long- or short-term food deprivation and in some it decreased (Bucking and Schulte, 2012; Orozco et al., 2017; Terova et al., 2009; Tian et al., 2015) while in others it increased (Bucking and Schulte, 2012; Hakim et al., 2009), suggesting the response of *pept1* may depend on the duration of the fasting. Further studies are required on the effect of food deprivation in fish so that the results of gene and protein expression are coupled to function. In mice (Okamura et al., 2014; Shimakura et al., 2006) and chicken (Madsen and Wong, 2011), it was reported that starvation markedly increased the amount of PEPT1 mRNA and protein in the intestine, via increased expression of *Pparα*. However, in the present study of the European sea bass if a similar connection exists between the expression of *pept1* and *pparα* in the foregut was not established since the latter gene was not measured.

4.3. Differential modification of liver parenchyma following food deprivation in fish from different egg thermal regimes

Food deprivation for one week severely affected the liver parenchyma of all fish irrespective of the egg thermal regime, with a significant reduction in the lipid area measured and a significant increase in the number of hepatocyte nuclei, two morphometric indicators of altered liver metabolism previously reported to be induced by starvation in fish (Hammock et al., 2020; Hur et al., 2006; Mohapatra et al., 2017; Power et al., 2000; Zeng et al., 2012). The increased number of nuclei was probably due to atrophy of hepatocytes given the loss of lipid and glycogen vacuoles, which typically occurs after food deprivation (Hammock et al., 2020; Hur et al., 2006; Power et al., 2000; Zeng et al., 2012). Although the hepatocyte size was reduced in the sea bass liver in the present study the size of the nucleus was unaffected, which has been taken to indicate continued nuclear activity during food deprivation (Power et al., 2000).

Studies of the impact on the juvenile/adult liver of egg and larval thermal regimes in fish are scarce. One study in Atlantic cod (*Gadus morhua*) indicated that higher egg incubation temperatures enhanced the expression of several micro RNA (miR) in the liver of juveniles (Bizuayehu et al., 2015). Modified miR included miR-7a, previously reported in zebrafish as a regulator of lipid metabolism in times of liver cellular stress (Lai et al., 2018), and miR-221, which in chicken is described to increase lipid metabolism in liver (Zhang et al., 2020) via PPAR activated lipid catabolism (Chen et al., 2015). Activation of PPARα has previously been reported in the liver of fish deprived of food (Mohapatra et al., 2015; Ning et al., 2016), but in our study of the European sea bass, modulation of *pparα* mRNA levels in the liver only occurred in one group of fish, 16 °C-10hR. At the time of thermal imprinting (egg stage), the biliary tract and exocrine pancreas (Beccaria et al., 1991; Diaz et al., 2002; Diaz and Connes, 1997), which synthesize the enzymes involved in lipid metabolism (e.g. *lipc*), only exist as a primordium, and may not have been significantly affected by the thermal regime and this may explain the absence of change in *lipc* expression in the liver in the present study. These observations raise interesting questions about how thermal imprinting during the egg stage affects the liver parenchyma and modifies the hepatic response in juvenile fish. However, a strong possibility that explains the lasting effects of egg thermal imprinting may be epigenetic regulation of PPARα by miRNAs or epigenetic marks (Aibara et al., 2022; Peyrou et al., 2012). Further studies will be required to explore this possibility.

On the resumption of feeding, the organisation of the liver parenchyma in fish from eggs of the 11 °C thermal regime (11–10hR) recovered before that of the fish from the other thermal backgrounds. There is evidence in mice that activation of the Notch signalling pathway in the intestine enhances lipid uptake by the liver (Fowler et al., 2011). Intriguingly, in the foregut of the 11 °C-10hR fish the expression of *hes1*, a well-known Notch target (Wilson and Radtke, 2006), was significantly up-regulated compared to the fish from other thermal regimes and this may be a putative mechanism explaining the difference observed in the liver. Nonetheless, since the expression of *lipc* and *pparα* in the liver of fish from eggs incubated at 11 °C did not change, further studies will be required to explore the mechanism underpinning the modified lipid metabolism in response to food deprivation in European sea bass from eggs exposed to different thermal regimes.

4.4. Gluconeogenic response to food deprivation in thermally imprinted fish

The expression of *pck1*, a gluconeogenic rate-limiting enzyme (Yang et al., 2009), was significantly up-regulated following one week of food deprivation in the liver of all fish irrespective of the egg thermal regime. The impact of food deprivation on the expression of *pck1* has previously been reported in other fish (Li et al., 2018; Tian et al., 2015) and mammals (Sokolović et al., 2008). In the present study the expression of *pck1* in the foregut of food deprived European sea bass was also affected by the egg thermal regime; fish incubated as eggs at 13.5 °C had a significant up-regulation of *pck1* after one week of food deprivation, while the other fish had a significant up-regulation 10 h post-refeeding (11 °C-10hR and 16 °C-10hR), and the increase was greatest in 16 °C-10hR compared to 13.5 °C -10hR. Two days after the resumption of feeding, the expression of *pck1* returned to pre-fasting levels in both the liver and foregut, but fish incubated at 16 °C as eggs (16-2dR) had a significant up-regulation of *pck1* relative to 11 °C-2dR fish (a pattern repeated in fed fish, 16 °C-F versus 11 °C-F). The impact of thermal imprinting on gluconeogenesis is essentially unknown, but epigenetic changes in the promoter of PEPCK and decreased expression of miRNAs controlling post-transcriptional levels of PEPCK was reported in the offspring of rats with restricted food intake during pregnancy (Imam and Ismail, 2017; O'Sullivan et al., 2012; Saghazadeh et al., 2019) or exposed to psychological stress (Wu et al., 2016), and was associated with increased expression of *Pepck* and hyperglycaemia.

The liver is the main organ contributing to glucose homeostasis by production of endogenous glucose when dietary glucose is unavailable

(Klover and Mooney, 2004; Miyamoto and Amrein, 2017; Polakof et al., 2012). However, in rats controversy surrounds the question of glucose production by the intestine during prolonged fasting (Croset et al., 2001; Martin et al., 2007; Mithieux, 2005; Mutel et al., 2011; Penhoat et al., 2014; Potts et al., 2018; Rajas et al., 2000). In our study, despite the suggestion from molecular analysis that the gluconeogenic response was probably changed by the egg thermal regime, no significant differences in plasma glucose levels were identified between fish from eggs imprinted with different thermal regimes. There was no evidence in the present study that egg thermal regimes affected glucose homeostasis and modified plasma glucose levels. Unfortunately, due to technical problems with the plasma samples, linked to small sample volumes and hyperlipidaemia in control/fed sea bass meant we could not establish if significant differences in glucose existed between control groups. Nonetheless, taking into consideration the average glucose levels reported in the literature for sea bass (Echevarria et al., 1997; Gutiérrez et al., 1991; Peres et al., 2014; Pérez-Jiménez et al., 2007; Viegas et al., 2013), in our study the sea bass deprived of food had higher levels of glucose. Stress is the most common cause of elevated blood glucose levels in European sea bass (Acerete et al., 2009; Samaras et al., 2021; Samaras et al., 2018; Simontacchi et al., 2008), and the significantly elevated plasma cortisol following one week of food deprivation tends to support the idea that food deprived sea bass had elevated glucose.

The notion that the gluconeogenic response was probably changed by the egg thermal regime is further supported by the significant change in plasma lactate between the sea bass from different thermal regimes during the experiment. Fish from 13.5 °C-S and 13.5 °C-10hR groups had significantly increased lactate levels compared to 16 °C-S and 16 °C-10hR fish, respectively. Lactate is both a gluconeogenic substrate for mitochondrial PEPCK (Stark and Kibbey, 2014) and a product of enhanced expression of *Pepck1* in the murine intestine in short-term fasting (Mithieux et al., 2006; Mutel et al., 2011; Sokolović et al., 2007). Interestingly, although no significant differences were seen in the expression levels of *pck1* in the intestine between 13.5 °C-S and 16 °C-S fish, significant up-regulation of *pck1* occurred in 16 °C-10hR compared to 13.5 °C-10hR fish when feeding was resumed. Divergent responses in *pck1* expression and plasma lactate levels between food deprived and refed fish from different egg thermal regimes leads us to speculate that egg thermal regimes modified the metabolic response in juveniles.

Although not established in fish, there is evidence in mammals that the adipose tissue is capable of lactate production independent of glucose metabolism (Jansson et al., 1990; Krycer et al., 2020). One very intriguing aspect of our study was the substantial perivisceral fat observed during dissection, the subcutaneous adipose observed in histology (Mateus et al., 2023) and the evident lipemia in plasma samples from fed fish. Aside from the impact of egg thermal regimes on the expression of *pck1* in the intestine, fish from eggs incubated at higher temperatures (16 °C) also had the greatest depletion of the liver lipid reserves following food deprivation. These observations lead us to speculate that the modulation of lactate may come from modified adipocyte metabolism. Further studies will be required to determine if modifications in plasma lactate resulted from modulation of *pck1* in the intestine or from the HPT axis and why egg incubation temperatures modulated *pck1* expression in the intestine but not in the liver.

4.5. Different modulation of antioxidant enzymes in liver and foregut

In fish, food deprivation often leads to oxidative damage, and one of the responses of the liver to counteract increased ROS is to up-regulate the activity of antioxidant enzymes, such as Sod1 and Cat (Bayir et al., 2011; Choi et al., 2012; Dar et al., 2019; Hammock et al., 2020; Sakyi et al., 2020; Zheng et al., 2016). However, in our study, one week of food deprivation did not modify the expression of *sod1* and *cat* in the liver and instead a significant down-regulation of *sod1* and *cat* occurred 10 h post-refeeding compared to fed fish. Down-regulation of antioxidant gene transcripts and/or decreased activity of antioxidant enzymes have previously been reported in fish under a prolonged fast (Drew et al., 2008; Furné et al., 2009; Malandrakis et al., 2014; Pascual et al., 2003; Rossi et al., 2015; Waagbø et al., 2017; Yang et al., 2019). Further studies will be needed to establish if 7 days of food deprivation induced oxidative stress in the European sea bass and if down-regulation of *sod1* and *cat* was the consequence of substrate induced negative feedback or due to a possible antioxidant defence failure (Pippenger et al., 1998).

Food deprivation has also been associated with oxidative stress at the level of the intestinal mucosa and with increased levels of antioxidant enzymes in the intestine of fish (Antonopoulou et al., 2013; Bu et al., 2021; Shi et al., 2022). Yet, in our experiments the expression of *sod1* was significantly down-regulated in 13.5 °C-S and 13.5 °C-10hR groups of fish compared to 13.5 °C-F, which is similar to what was observed in the liver. In contrast, *cat* had a different modulation in the foregut compared to the liver. Food deprivation did not significantly modify the expression of *cat* in the liver of fish incubated as eggs at 11 °C, but in the foregut *cat* was significantly up-regulated in 11 °C-S compared to 11 °C-F. In previous studies of the European sea bass and the Yangtze Sturgeon (*Acipenser dabryanus*) *cat* had a higher expression in the intestine compared to the liver when fish were deprived of food (Antonopoulou et al., 2013; Shi et al., 2022), suggesting that a tissue-specific response to food deprivation occurs and in our study the intestine seemed to be more sensitive to food deprivation mediated oxidative stress than the liver, despite the latter tissue's vital role in antioxidant defence in fish (Furné et al., 2009; Morales et al., 2004).

5. Conclusion

This study evaluated for the first time if egg incubation temperature in European sea bass modified their response to food deprivation as juveniles (9 months old). The most divergent responses were found between fish incubated at higher and lower temperatures (16 °C and 11 °C) than are normally used in aquaculture for incubation of European sea bass eggs (13.5 °C). Fish incubated as eggs at the lowest temperature (11 °C) had a temporary response of *igf-1* after one week of food deprivation and this was not observed in fish from other egg thermal regimes. The fish incubated at 11 °C as eggs experienced less atrophy of the foregut and the liver parenchyma returned to that typical of fed fish when feeding was resumed, earlier than the juvenile fish from eggs incubated at 13.5 °C and 16 °C. The sea bass incubated as eggs at the most commonly used (13.5 °C) and highest temperatures (16 °C) underwent the most pronounced morphological changes after one week of food deprivation, including atrophy of the foregut and a significant depletion of liver lipid reserves. Associated with the pronounced histological changes in the foregut and liver the sea bass from eggs incubated at 16 °C experienced the most pronounced molecular modifications and significant up-regulation of transcripts associated with gluconeogenesis, peptide absorption and antioxidant defence compared to sea bass incubated as eggs at 13.5 °C. Despite the tissue specific morphological changes, fish from eggs incubated at 16 °C were able to recover following 2 days of refeeding, while the liver parenchyma of food deprived fish incubated as eggs at control temperatures (13.5 °C) did not fully recover during refeeding.

In the context of global climate change, shifting thermal regimes in the marine environment will affect early development in ectotherms and will affect food availability either by changed biogeography of nutrients or by induced fasting periods due to lower temperatures during temperature shifts. Therefore, it is of utmost importance to understand if the response of the European sea bass to fasting-refeeding cycles under different egg thermal regimes could lead to beneficial metabolic adaptations or to a detrimental stress response with consequences for general fish welfare and survival. This study raises three exciting hypotheses for further investigation: 1) that the foregut displays more pronounced adaptations to food deprivation than the liver, 2) that egg thermal regimes induce changes in fat and lactate metabolism in juvenile fish and 3) that there is a role for the HPT axis in the phenotypic plasticity that

thermally imprinted juvenile fish show in cycles of food deprivation and refeeding.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739806.

CRediT authorship contribution statement

Ana Patrícia Mateus: Methodology, Formal analysis, Validation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Rita A. Costa:** Methodology, Formal analysis, Validation, Investigation, Visualization, Writing – original draft. **Javier Jiménez Herrero:** Methodology. **Bastien Sadoul:** Methodology, Writing – review & editing. **Marie Laure Bégout:** Writing – review & editing, Project administration. **Xavier Cousin:** Conceptualization, Methodology, Writing – review & editing. **Adelino V.M. Canario:** Resources, Writing – review & editing, Supervision, Project administration. **Deborah M. Power:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors 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

This study received Portuguese National funds from FCT - Foundation for Science and Technology through the COFASP ERA-NET project "SUStainable production of High-quality aquaculture FISH using innovative tools and production strategies and integrating novel processing methods and cold chain management (Acronym: SUSHIFISH, COFASP/0002/2015) and the project UIDB/04326/2023 and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121. RAC was funded by a research assistant grant CCMAR/BI/0008/2016.

References

Abdel, I., Abellán, E., López-Albors, O., Valdés, P., Nortes, M.J., García-Alcázar, A., 2004. Abnormalities in the juvenile stage of sea bass (*Dicentrarchus labrax* L.) reared at different temperatures: types, prevalence and effect on growth. Aquac. Int. 12 (6), 523–538. https://doi.org/10.1007/s10499-004-0349-9.

Acerete, L., Reig, L., Alvarez, D., Flos, R., Tort, L., 2009. Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*). Aquaculture 287 (1–2), 139–144. https://doi.org/10.1016/j.aquaculture.2008.10.012.

Agius, C., Roberts, R.J., 1981. Effects of starvation on the melano-macrophage centres of fish. J. Fish Biol. 19 (2), 161–169. https://doi.org/10.1111/j.1095-8649.1981.tb05820.x.

Aibara, D., Takahashi, S., Yagai, T., Kim, D., Brocker, C.N., Levi, M., Matsusue, K., Gonzalez, F.J., 2022. Gene repression through epigenetic modulation by PPARA enhances hepatocellular proliferation. iScience 25 (5), 104196. https://doi.org/10.1016/j.isci.2022.104196.

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. https://doi.org/10.1111/jfb.14599.

Alix, M., Blondeau-Bidet, E., Grousset, E., Shiranghi, A., Vergnet, A., Guinand, B., Chatain, B., Boulo, V., Lignot, J.H., 2017. Effects of fasting and re-alimentation on gill and intestinal morphology and indicators of osmoregulatory capacity in genetically selected sea bass (*Dicentrarchus labrax*) populations with contrasting tolerance to fasting. Aquaculture 468, 314–325. https://doi.org/10.1016/j.aquaculture.2016.10.016.

Anastasiadi, D., Díaz, N., Piferrer, F., 2017. Small ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. Sci. Rep. 7 (1), 12401. https://doi.org/10.1038/s41598-017-10861-6.

Antonopoulou, E., Kentepozidou, E., Feidantsis, K., Roufidou, C., Despoti, S., Chatzifotis, S., 2013. Starvation and re-feeding affect Hsp expression, MAPK activation and antioxidant enzymes activity of European sea bass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165 (1), 79–88. https://doi.org/10.1016/j.cbpa.2013.02.019.

Ayala, M.A.D., López-Albors, O., Gil, F., Garcia-Alcázar, A., Abellán, E., Alarcón, J.A., Álvarez, M.A.C., Ramirez-Zarzosa, G., Moreno, F., 2001. Temperature effects on muscle growth in two populations (Atlantic and Mediterranean) of sea bass, *Dicentrarchus labrax* L. Aquaculture 202 (3), 359–370. https://doi.org/10.1016/S0044-8486(01)00785-2.

Ayson, F.G., de Jesus-Ayson, E.G.T., Takemura, A., 2007. mRNA expression patterns for GH, PRL, SL, IGF-I and IGF-II during altered feeding status in rabbitfish, *Siganus guttatus*. Gen. Comp. Endocrinol. 150 (2), 196–204. https://doi.org/10.1016/j.ygcen.2006.08.001.

Azzurro, E., Sbragaglia, V., Cerri, J., Bariche, M., Bolognini, L., Ben Souissi, J., Busoni, G., Coco, S., Chryssanthi, A., Fanelli, E., Ghanem, R., Garrabou, J., Gianni, F., Grati, F., Kolitari, J., Letterio, G., Lipej, L., Mazzoldi, C., Milone, N., Pannacciulli, F., Pešić, A., Samuel-Rhoads, Y., Saponari, L., Tomanic, J., Eda Topçu, N., Vargiu, G., Moschella, P., 2019. Climate change, biological invasions, and the shifting distribution of Mediterranean fishes: A large-scale survey based on local ecological knowledge. Glob. Chang. Biol. 25 (8), 2779–2792. https://doi.org/10.1111/gcb.14670.

Baeverfjord, G., Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19 (5), 375–387. https://doi.org/10.1046/j.1365-2761.1996.d01-92.x.

Bagni, M., 2021. Cultured Aquatic Species Information Programme. *Dicentrarchus labrax*. https://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en.

Bar, N., Volkoff, H., 2012. Adaptation of the physiological, endocrine, and digestive system functions to prolonged food deprivation in fish. In: Comparative Physiology of Fasting, Starvation, and Food Limitation. Springer, pp. 69–89.

Bayir, A., Sirkecioglu, A.N., Bayir, M., Haliloglu, H.I., Kocaman, E.M., Aras, N.M., 2011. Metabolic responses to prolonged starvation, food restriction, and refeeding in the brown trout, *Salmo trutta*: oxidative stress and antioxidant defenses. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 159 (4), 191–196. https://doi.org/10.1016/j.cbpb.2011.04.008.

Beccaria, C., Diaz, J.P., Connes, R., Chatain, B., 1991. Organogenesis of the exocrine pancreas in the sea bass, *Dicentrarchus labrax* L., reared extensively and intensively. Aquaculture 99 (3), 339–354. https://doi.org/10.1016/0044-8486(91)90254-5.

Bermejo-Poza, R., De la Fuente, J., Pérez, C., Lauzurica, S., González de Chávarri, E., Diaz, M., Villarroel, M., 2016. Reducing the effect of pre-slaughter fasting on the stress response of rainbow trout (*Oncorhynchus mykiss*). Anim. Welf. 25 (3), 339–346. https://doi.org/10.7120/09627286.25.3.339.

Bianco, A.C., Dumitrescu, A., Gereben, B., Ribeiro, M.O., Fonseca, T.L., Fernandes, G.W., Bocco, B., 2019. Paradigms of dynamic control of thyroid hormone Signaling. Endocr. Rev. 40 (4), 1000–1047. https://doi.org/10.1210/er.2018-00275.

Bizuayehu, T.T., Johansen, S.D., Puvanendran, V., Toften, H., Babiak, I., 2015. Temperature during early development has long-term effects on microRNA expression in Atlantic cod. BMC Genomics 16 (1), 305. https://doi.org/10.1186/s12864-015-1503-7.

Blaxter, J.H.S., Ehrlich, K.F., 1974. Changes in behaviour during starvation of herring and plaice larvae. In: Blaxter, J.H.S. (Ed.), The Early Life History of Fish. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 575–588.

Boglione, C., Costa, C., 2011. Skeletal deformities and juvenile quality. Sparidae. 233–294.

Bu, T., Xu, L., Zhu, X., Cheng, J., Li, Y., Liu, L., Bao, L., Chu, W., 2021. Influence of short-term fasting on oxidative stress, antioxidant-related signaling molecules and autophagy in the intestine of adult *Siniperca chuatsi*. Aquacult. Reports 21, 100933. https://doi.org/10.1016/j.aqrep.2021.100933.

Bucking, C., Schulte, P.M., 2012. Environmental and nutritional regulation of expression and function of two peptide transporter (PepT1) isoforms in a euryhaline teleost. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 161 (4), 379–387. https://doi.org/10.1016/j.cbpa.2011.12.008.

Butt, R.L., Volkoff, H., 2019. Gut microbiota and energy homeostasis in fish. Front. Endocrinol. (Lausanne) 10. https://doi.org/10.3389/fendo.2019.00009.

Carballo, C., Firmino, J., Anjos, L., Santos, S., Power, D.M., Manchado, M., 2018. Short- and long-term effects on growth and expression patterns in response to incubation temperatures in Senegalese sole. Aquaculture 495, 222–231. https://doi.org/10.1016/j.aquaculture.2018.05.043.

Cardoso, J.C.R., Félix, R.C., Costa, C., Palma, P.F.S., Canário, A.V.M., Power, D.M., 2018. Evolution of the glucagon-like system across fish. Gen. Comp. Endocrinol. 264, 113–130. https://doi.org/10.1016/j.ygcen.2017.10.003.

Caruso, G., Denaro, M.G., Caruso, R., Mancari, F., Genovese, L., Maricchiolo, G., 2011. Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*). Mar. Environ. Res. 72 (1–2), 46–52. https://doi.org/10.1016/j.marenvres.2011.04.005.

Chatain, B., 1994. Estimation et amélioration des performances zootechniques de l'élevage larvaire de Dicentrarchus labrax et de Sparus auratus. PhD Thesis. Aix-Marseille 2. https://archimer.ifremer.fr/doc/00000/1750/.

Chen, C.F., Huang, J., Li, H., Zhang, C., Huang, X., Tong, G., Xu, Y.Z., 2015. MicroRNA-221 regulates endothelial nitric oxide production and inflammatory response by targeting adiponectin receptor 1. Gene 565 (2), 246–251. https://doi.org/10.1016/j.gene.2015.04.014.

Choi, C.Y., Shin, H.S., Choi, Y.J., Kim, N.N., Lee, J., Kil, G.S., 2012. Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion*

melanopus. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 163 (3–4), 357–363. https://doi.org/10.1016/j.cbpa.2012.07.005.
Costa, R.A., Cardoso, J.C.R., Power, D.M., 2017. Evolution of the angiopoietin-like gene family in teleosts and their role in skin regeneration. BMC Evol. Biol. 17 (1), 14. https://doi.org/10.1186/s12862-016-0859-x.
Croset, M., Rajas, F., Zitoun, C., Hurot, J.M., Montano, S., Mithieux, G., 2001. Rat small intestine is an insulin-sensitive gluconeogenic organ. Diabetes 50 (4), 740–746. https://doi.org/10.2337/diabetes.50.4.740.
Crosnier, C., Vargesson, N., Gschmeissner, S., Ariza-McNaughton, L., Morrison, A., Lewis, J., 2005. Delta-notch signalling controls commitment to a secretory fate in the zebrafish intestine. Development 132 (5), 1093–1104. https://doi.org/10.1242/dev.01644.
Cucchi, P., Sucré, E., Santos, R., Leclère, J., Charmantier, G., Castille, R., 2012. Embryonic development of the sea bass *Dicentrarchus labrax*. Helgol. Mar. Res. 66 (2), 199–209. https://doi.org/10.1007/s10152-011-0262-3.
Daniel, H., 2004. Molecular and integrative physiology of intestinal peptide transport. Annu. Rev. Physiol. 66, 361–384. https://doi.org/10.1146/annurev.physiol.66.032102.144149.
Dar, S.A., Srivastava, P.P., Varghese, T., Nazir, M.I., Gupta, S., Krishna, G., 2019. Temporal changes in superoxide dismutase, catalase, and heat shock protein 70 gene expression, cortisol and antioxidant enzymes activity of *Labeo rohita* fingerlings subjected to starvation and refeeding. Gene 692, 94–101. https://doi.org/10.1016/j.gene.2018.12.058.
Davis, K.B., Gaylord, T.G., 2011. Effect of fasting on body composition and responses to stress in sunshine bass. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 158 (1), 30–36. https://doi.org/10.1016/j.cbpa.2010.08.019.
Deal, C.K., Volkoff, H., 2020. The role of the thyroid Axis in fish. Front. Endocrinol. (Lausanne) 11. https://doi.org/10.3389/fendo.2020.596585.
Delgadin, T.H., Pérez Sirkin, D.I., Di Yorio, M.P., Arranz, S.E., Vissio, P.G., 2015. GH, IGF-I and GH receptors mRNA expression in response to growth impairment following a food deprivation period in individually housed cichlid fish *Cichlasoma dimerus*. Fish Physiol. Biochem. 41 (1), 51–60. https://doi.org/10.1007/s10695-014-0005-x.
Diaz, J.P., Connes, R., 1997. Ontogenesis of the biliary tract in a teleost, the sea bass *Dicentratchus labrax* L. Can. J. Zool. 75 (5), 740–745. https://doi.org/10.1139/z97-095.
Diaz, J.-P., Mani-Ponset, L., Blasco, C., Connes, R., 2002. Cytological detection of the main phases of lipid metabolism during early post-embryonic development in three teleost species: *Dicentrarchus labrax*, *Sparus aurata* and *Stizostedion lucioperca*. Aquat. Living Resour. 15 (3), 169–178. https://doi.org/10.1016/S0990-7440(02)01169-5.
Donelson, J.M., McCormick, M.I., Booth, D.J., Munday, P.L., 2014. Reproductive acclimation to increased water temperature in a tropical reef fish. PLoS One 9 (5), e97223. https://doi.org/10.1371/journal.pone.0097223.
Drew, R.E., Rodnick, K.J., Settles, M., Wacyk, J., Churchill, E., Powell, M.S., Hardy, R.W., Murdoch, G.K., Hill, R.A., Robison, B.D., 2008. Effect of starvation on transcriptomes of brain and liver in adult female zebrafish (*Danio rerio*). Physiol. Genomics 35 (3), 283–295. https://doi.org/10.1152/physiolgenomics.90213.2008.
Echevarria, G., Martinez-Bebiá, M., Zamora, S., 1997. Evolution of biometric indices and plasma metabolites during prolonged starvation in European sea bass (*Dicentrarchus labrax*, L.). Comp. Biochem. Physiol. A Physiol. 118 (1), 111–123. https://doi.org/10.1016/S0300-9629(96)00416-1.
EEA, 2021. Open ocean — Mean Ocean Temperature. https://www.eea.europa.eu/publications/europes-changing-climate-hazards-1/open-ocean/open-ocean-mean-ocean-temperature.
Emadi Shaibani, M., Mojazi Amiri, B., Khodabandeh, S., 2013. Starvation and refeeding effects on pyloric caeca structure of Caspian salmon (*Salmo trutta caspius*, Kessler 1877) juvenile. Tissue Cell 45 (3), 204–210. https://doi.org/10.1016/j.tice.2013.01.001.
Ensminger, D.C., Salvador-Pascual, A., Arango, B.G., Allen, K.N., Vázquez-Medina, J.P., 2021. Fasting ameliorates oxidative stress: A review of physiological strategies across life history events in wild vertebrates. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 256, 110929 https://doi.org/10.1016/j.cbpa.2021.110929.
van Es, J.H., van Gijn, M.E., Riccio, O., van den Born, M., Vooijs, M., Begthel, H., Cozijnsen, M., Robine, S., Winton, D.J., Radtke, F., Clevers, H., 2005. Notch/γ-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature 435 (7044), 959–963. https://doi.org/10.1038/nature03659.
FAO (Ed.), 2008. Climate Change and Food Security: a Framework Document. FAO, Rome.
Farming, C.I.W., 2018. Improving the Welfare of European Sea bass and Gilthead Sea Bream at Slaughter. https://www.compassioninfoodbusiness.com/media/7436994/improving-the-welfare-of-sea-bream-and-european-sea-bass-at-slaughter.pdf.
Fowler, J.C., Zecchini, V.R., Jones, P.H., 2011. Intestinal activation of notch Signaling induces rapid onset hepatic steatosis and insulin resistance. PLoS One 6 (6), e20767. https://doi.org/10.1371/journal.pone.0020767.
Fox, B.K., Riley, L.G., Hirano, T., Grau, E.G., 2006. Effects of fasting on growth hormone, growth hormone receptor, and insulin-like growth factor-I axis in seawater-acclimated tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol. 148 (3), 340–347. https://doi.org/10.1016/j.ygcen.2006.04.007.
Fraser, T.W., Hansen, T., Fleming, M.S., Fjelldal, P.G., 2015. The prevalence of vertebral deformities is increased with higher egg incubation temperatures and triploidy in Atlantic salmon *Salmo salar* L. J. Fish Dis. 38 (1), 75–89. https://doi.org/10.1111/jfd.12206.
Furne, M., Sanz, A., 2018. Starvation in fish – Sturgeon and rainbow trout as examples. In: Preedy, V., Patel, V.B. (Eds.), Handbook of Famine, Starvation, and Nutrient Deprivation: From Biology to Policy. Springer International Publishing, Cham, pp. 1–16.
Furné, M., García-Gallego, M., Hidalgo, M.C., Morales, A.E., Domezain, A., Domezain, J., Sanz, A.B., 2009. Oxidative stress parameters during starvation and refeeding periods in Adriatic sturgeon (*Acipenser naccarii*) and rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 15, 587–595. https://doi.org/10.1111/j.1365-2095.2008.00626.x.
Gabillard, J.C., Rescan, P.Y., Fauconneau, B., Weil, C., Le Bail, P.Y., 2003a. Effect of temperature on gene expression of the Gh/Igf system during embryonic development in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Zool. A Comp. Exp. Biol. 298 (2), 134–142. https://doi.org/10.1002/jez.a.10280.
Garcia de la Serrana, D., Vieira, V.L.A., Andree, K.B., Darias, M., Estévez, A., Gisbert, E., Johnston, I.A., 2012. Development temperature has persistent effects on muscle growth responses in Gilthead Sea bream. PLoS One 7 (12), e51884. https://doi.org/10.1371/journal.pone.0051884.
Gavery, M.R., Roberts, S.B., 2017. Epigenetic considerations in aquaculture. PeerJ 5, e4147. https://doi.org/10.7717/peerj.4147.
Georgakopoulou, E., Sfakianakis, D., Kouttouki, S., Divanach, P., Koumoundouros, G., 2007. The influence of temperature during early life on phenotypic expression at later ontogenetic stages in sea bass. J. Fish Biol. 70, 278–291. https://doi.org/10.1111/j.1095-8649.2007.01305.x.
Gutiérrez, J., Pérez, J., Navarro, I., Zanuy, S., Carrillo, M., 1991. Changes in plasma glucagon and insulin associated with fasting in sea bass (*Dicentrarchus labrax*). Fish Physiol. Biochem. 9 (2), 107–112. https://doi.org/10.1007/bf02265126.
Habold, C., Reichardt, F., Foltzer-Jourdainne, C., Lignot, J.H., 2007. Morphological changes of the rat intestinal lining in relation to body stores depletion during fasting and after refeeding. Pflugers Arch. 455 (2), 323–332. https://doi.org/10.1007/s00424-007-0289-0.
Hack, N.L., Cordova, K.L., Glaser, F.L., Journey, M.L., Resner, E.J., Hardy, K.M., Beckman, B.R., Lema, S.C., 2019. Interactions of long-term food ration variation and short-term fasting on insulin-like growth factor-1 (IGF-1) pathways in copper rockfish (*Sebastes caurinus*). Gen. Comp. Endocrinol. 280, 168–184. https://doi.org/10.1016/j.ygcen.2019.04.025.
Hakim, Y., Harpaz, S., Uni, Z., 2009. Expression of brush border enzymes and transporters in the intestine of European sea bass (*Dicentrarchus labrax*) following food deprivation. Aquaculture 290 (1), 110–115. https://doi.org/10.1016/j.aquaculture.2009.02.008.
Hall, K.C., Bellwood, D.R., 1995. Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. J. Fish Biol. 47 (3) https://doi.org/10.1111/j.1095-8649.1995.tb01913.x.
Hammock, B.G., Ramírez-Duarte, W.F., Triana Garcia, P.A., Schultz, A.A., Avendano, L.I., Hung, T.-C., White, J.R., Bong, Y.-T., Teh, S.J., 2020. The health and condition responses of Delta smelt to fasting: A time series experiment. PLoS One 15 (9), e0239358. https://doi.org/10.1371/journal.pone.0239358.
Hanson, R.W., Reshef, L., 1997. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. Annu. Rev. Biochem. 66, 581–611. https://doi.org/10.1146/annurev.biochem.66.1.581.
Hasan Al-bayati, D.A.A., ML AL-Khateeb, D.S., 2021. The effects of thyroid hormones and their abnormalities on intestinal and hepatic glucose metabolism. Schol. Intern. J. Biochem. https://doi.org/10.36348/sijb.2021.v04i03.002.
Hervant, F., 2012. Starvation in subterranean species versus surface-dwelling species: Crustaceans, fish, and salamanders. In: McCue, M.D. (Ed.), Comparative Physiology of Fasting, Starvation, and Food Limitation. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 91–102.
Hur, J.W., Jo, J.H., Park, I.-S., 2006. Effects of long-term starvation on hepatocyte ultrastructure of olive flounder *Paralichthys olivaceus*. Ichthyol. Res. 53 (3), 306–310. https://doi.org/10.1007/s10228-006-0348-0.
Hvas, M., Stien, L.H., Oppedal, F., 2021. The effect of fasting period on swimming performance, blood parameters and stress recovery in Atlantic salmon post smolts. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 255, 110913 https://doi.org/10.1016/j.cbpa.2021.110913.
Ibarz, A., Padrós, F., Gallardo, M.Á., Fernández-Borràs, J., Blasco, J., Tort, L., 2010. Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'winter syndrome'. Rev. Fish Biol. Fish. 20 (4), 539–556. https://doi.org/10.1007/s11160-010-9159-5.
Ihara, T., Tsujikawa, T., Fujiyama, Y., Bamba, T., 2000. Regulation of PepT1 peptide transporter expression in the rat small intestine under malnourished conditions. Digestion 61 (1), 59–67. https://doi.org/10.1159/000007736.
Imam, M.U., Ismail, M., 2017. The impact of traditional food and lifestyle behavior on epigenetic burden of chronic disease. Global Chall. 1 (8), 1700043. https://doi.org/10.1002/gch2.201700043.
IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC.
Jansson, P.A., Smith, U., Lönnroth, P., 1990. Evidence for lactate production by human adipose tissue in vivo. Diabetologia 33 (4), 253–256. https://doi.org/10.1007/BF00404805.
Jarque, S., Piña, B., 2014. Deiodinases and thyroid metabolism disruption in teleost fish. Environ. Res. 135, 361–375. https://doi.org/10.1016/j.envres.2014.09.022.
Jennings, S., Pawson, M.G., 1991. The development of bass, *Dicentrarchus labrax*, eggs in relation to temperature. J. Mar. Biol. Assoc. UK 71, 107–116. https://doi.org/10.1017/S0025315400037425.
Jensen, J., Pedersen, E.E., Galante, P., Hald, J., Heller, R.S., Ishibashi, M., Kageyama, R., Guillemot, F., Serup, P., Madsen, O.D., 2000. Control of endodermal endocrine development by Hes-1. Nat. Genet. 24 (1), 36–44. https://doi.org/10.1038/71657.
Jia, J., Qin, J., Yuan, X., Liao, Z., Huang, J., Wang, B., Sun, C., Li, W., 2019. Microarray and metabolome analysis of hepatic response to fasting and subsequent refeeding in

zebrafish (*Danio rerio*). BMC Genomics 20 (1), 919. https://doi.org/10.1186/s12864-019-6309-6.
Johnston, I.A., 2006. Environment and plasticity of myogenesis in teleost fish. J. Exp. Biol. 209 (12), 2249–2264. https://doi.org/10.1242/jeb.02153.
Jonsson, B., Jonsson, N., 2019. Phenotypic plasticity and epigenetics of fish: embryo temperature affects later-developing life-history traits. Aquat. Biol. 28, 21–32. https://doi.org/10.3354/ab00707.
Kaitetzidou, E., Xiang, J., Antonopoulou, E., Tsigenopoulos, C.S., Sarropoulou, E., 2015. Dynamics of gene expression patterns during early development of the European seabass (*Dicentrarchus labrax*). Physiol. Genomics 47 (5), 158–169. https://doi.org/10.1152/physiolgenomics.00001.2015.
Kaneko, N., Ishikawa, T., Nomura, K., 2023. Effects of the short-term fasting and refeeding on growth-related genes in Japanese eel (*Anguilla japonica*) larvae. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 265, 110826 https://doi.org/10.1016/j.cbpb.2023.110826.
Killen, S.S., Marras, S., McKenzie, D.J., 2011. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. J. Anim. Ecol. 80 (5), 1024–1033. https://doi.org/10.1111/j.1365-2656.2011.01844.x.
Klover, P.J., Mooney, R.A., 2004. Hepatocytes: critical for glucose homeostasis. Int. J. Biochem. Cell Biol. 36 (5), 753–758. https://doi.org/10.1016/j.biocel.2003.10.002.
Konstantinidis, I., Anastasiadi, D., Sætrom, P., Nedoluzhko, A.V., Mjelle, R., Podgorniak, T., Piferrer, F., Fernandes, J.M.O., 2021. Epigenetic mapping of the somatotropic axis in Nile tilapia reveals differential DNA hydroxymethylation marks associated with growth. Genomics 113 (5), 2953–2964. https://doi.org/10.1016/j.ygeno.2021.06.037.
Koumoundouros, G., Divanach, P., Anezaki, L., Kentouri, M., 2001. Temperature-induced ontogenetic plasticity in sea bass (*Dicentrarchus labrax*). Mar. Biol. 139 (5), 817–830. https://doi.org/10.1007/s002270100635.
Kourkouta, C., Printzi, A., Geladakis, G., Mitrizakis, N., Papandroulakis, N., Koumoundouros, G., 2021. Long lasting effects of early temperature exposure on the swimming performance and skeleton development of metamorphosing gilthead seabream (*Sparus aurata* L.) larvae. Sci. Rep. 11 (1), 8787. https://doi.org/10.1038/s41598-021-88306-4.
Krogdahl, A., Bakke-McKellep, A.M., 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 141 (4), 450–460. https://doi.org/10.1016/j.cbpb.2005.06.002.
Krycer, J.R., Quek, L.E., Francis, D., Fazakerley, D.J., Elkington, S.D., Diaz-Vegas, A., Cooke, K.C., Weiss, F.C., Duan, X., Kurdyukov, S., Zhou, P.X., Tambar, U.K., Hirayama, A., Ikeda, S., Kamei, Y., Soga, T., Cooney, G.J., James, D.E., 2020. Lactate production is a prioritized feature of adipocyte metabolism. J. Biol. Chem. 295 (1), 83–98. https://doi.org/10.1074/jbc.RA119.011178.
Lai, C.Y., Lin, C.Y., Hsu, C.C., Yeh, K.Y., Her, G.M., 2018. Liver-directed microRNA-7a depletion induces nonalcoholic fatty liver disease by stabilizing YY1-mediated lipogenic pathways in zebrafish. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1863 (8), 844–856. https://doi.org/10.1016/j.bbalip.2018.04.009.
Lema, S.C., 2020. Hormones, developmental plasticity, and adaptive evolution: endocrine flexibility as a catalyst for 'plasticity-first' phenotypic divergence. Mol. Cell. Endocrinol. 502, 110678 https://doi.org/10.1016/j.mce.2019.110678.
Li, H., Xu, W., Jin, J., Yang, Y., Zhu, X., Han, D., Liu, H., Xie, S., 2018. Effects of starvation on glucose and lipid metabolism in gibel carp (*Carassius auratus gibelio* var. CAS III). Aquaculture 496, 166–175. https://doi.org/10.1016/j.aquaculture.2018.07.015.
Li, M., Leatherland, J., 2008. Temperature and ration effects on components of the IGF system and growth performance of rainbow trout (*Oncorhynchus mykiss*) during the transition from late stage embryos to early stage juveniles. Gen. Comp. Endocrinol. 155 (3), 668–679. https://doi.org/10.1016/j.ygcen.2007.08.017.
Li, M., Raine, J.C., Leatherland, J.F., 2007. Expression profiles of growth-related genes during the very early development of rainbow trout embryos reared at two incubation temperatures. Gen. Comp. Endocrinol. 153 (1), 302–310. https://doi.org/10.1016/j.ygcen.2007.02.012.
Li, S., Li, J., Zhao, Y., Zhang, Q., Wang, Q., 2017. Nutrient sensing signaling integrates nutrient metabolism and intestinal immunity in grass carp, *Ctenopharyngodon idellus* after prolonged starvation. Fish Shellfish Immunol. 71, 50–57. https://doi.org/10.1016/j.fsi.2017.09.050.
Li, T., Qi, M., Gatesoupe, F.-J., Tian, D., Jin, W., Li, J., Lin, Q., Wu, S., Li, H., 2019. Adaptation to fasting in Crucian carp (*Carassius auratus*): gut microbiota and its correlative relationship with immune function. Microb. Ecol. 78 (1), 6–19. https://doi.org/10.1007/s00248-018-1275-0.
Liao, Z., Lin, D., Jia, J., Cai, R., Yu, Y., Li, W., 2021. Innate immune response to fasting and refeeding in the zebrafish kidney. Biomolecules 11 (6), 825. https://doi.org/10.3390/biom11060825.
Loyau, T., Métayer-Coustard, S., Berri, C., Crochet, S., Cailleau-Audouin, E., Sannier, M., Chartrin, P., Praud, C., Hennequet-Antier, C., Rideau, N., Couroussé, N., Mignon-Grasteau, S., Everaert, N., Duclos, M.J., Yahav, S., Tesseraud, S., Collin, A., 2014. Thermal manipulation during embryogenesis has long-term effects on muscle and liver metabolism in fast-growing chickens. PLoS One 9 (9), e105339. https://doi.org/10.1371/journal.pone.0105339.
Ma, K., 2010. Role, Relevance and Regulation of PEPT1 in Peptide Intestinal Absorption. The University of Michigan.
Ma, K., Hu, Y., Smith, D.E., 2012. Influence of fed-fasted state on intestinal PEPT1 expression and in vivo pharmacokinetics of glycylsarcosine in wild-type and Pept1 knockout mice. Pharm. Res. 29 (2), 535–545. https://doi.org/10.1007/s11095-011-0580-9.
Madsen, S.L., Wong, E.A., 2011. Expression of the chicken peptide transporter 1 and the peroxisome proliferator-activated receptor α following feed restriction and subsequent refeeding. Poult. Sci. 90 (10), 2295–2300. https://doi.org/10.3382/ps.2010-01173.
Malandrakis, E.E., Exadactylos, A., Dadali, O., Golomazou, E., Klaoudatos, S., Panagiotaki, P., 2014. Molecular cloning of four glutathione peroxidase (GPx) homologs and expression analysis during stress exposure of the marine teleost *Sparus aurata*. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 168, 53–61. https://doi.org/10.1016/j.cbpb.2013.11.005.
Martin, G., Ferrier, B., Conjard, A., Martin, M., Nazaret, R., Boghossian, M., Saadé, F., Mancuso, C., Durozard, D., Baverel, G., 2007. Glutamine gluconeogenesis in the small intestine of 72 h-fasted adult rats is undetectable. Biochem. J. 401 (2), 465–473. https://doi.org/10.1042/bj20061148.
Martin, S.A.M., Douglas, A., Houlihan, D.F., Secombes, C.J., 2010. Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (*Salmo salar*). BMC Genomics 11 (1), 418. https://doi.org/10.1186/1471-2164-11-418.
Mateus, A.P., Costa, R., Gisbert, E., Pinto, P.I.S., Andree, K.B., Estévez, A., Power, D.M., 2017a. Thermal imprinting modifies bone homeostasis in cold-challenged sea bream (*Sparus aurata*). J. Exp. Biol. 220 (19), 3442–3454. https://doi.org/10.1242/jeb.156174.
Mateus, A.P., Costa, R.A., Cardoso, J.C., Andree, K.B., Estevez, A., Gisbert, E., Power, D. M., 2017b. Thermal imprinting modifies adult stress and innate immune responsiveness in the teleost sea bream. J. Endocrinol. 233 (3), 381–394. https://doi.org/10.1530/JOE-16-0610.
Mateus, A.P., Costa, R.A., Sadoul, B., Bégout, M.-L., Cousin, X., Canario, A.V.M., Power, D.M., 2023. Thermal imprinting during embryogenesis modifies skin repair in juvenile European sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol. 134, 108647 https://doi.org/10.1016/j.fsi.2023.108647.
Matosin-Matekalo, M., Mesonero, J.E., Delezay, O., Poiree, J.C., Ilundain, A.A., Brot-Laroche, E., 1998. Thyroid hormone regulation of the Na+/glucose cotransporter SGLT1 in Caco-2 cells. Biochem. J. 334 (Pt 3), 633–640. https://doi.org/10.1042/bj3340633.
McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 156 (1), 1–18. https://doi.org/10.1016/j.cbpa.2010.01.002.
Metzger, D.C., Schulte, P.M., 2016. Epigenomics in marine fishes. Mar. Genomics 30, 43–54. https://doi.org/10.1016/j.margen.2016.01.004.
Mithieux, G., 2005. The new functions of the gut in the control of glucose homeostasis. Curr. Opin. Clin. Nutr. Metab. Care 8 (4), 445–449. https://doi.org/10.1097/01.mco.0000172587.17385.aa.
Mithieux, G., Gautier-Stein, A., Rajas, F., Zitoun, C., 2006. Contribution of intestine and kidney to glucose fluxes in different nutritional states in rat. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 143 (2), 195–200. https://doi.org/10.1016/j.cbpb.2005.11.007.
Miyamoto, T., Amrein, H., 2017. Gluconeogenesis: an ancient biochemical pathway with a new twist. Fly 11 (3), 218–223. https://doi.org/10.1080/19336934.2017.1283081.
Moghadam, H., Mørkøre, T., Robinson, N., 2015. Epigenetics—potential for programming fish for aquaculture? J. Marine Sci. Eng. 3 (2), 175–192. https://doi.org/10.3390/jmse3020175.
Moghadam, H.K., Johnsen, H., Robinson, N., Andersen, Ø., Jørgensen, H.E., Johnsen, H. K., Bæhr, V.J., Tveiten, H., 2017. Impacts of early life stress on the Methylome and transcriptome of Atlantic Salmon. Sci. Rep. 7 (1), 5023. https://doi.org/10.1038/s41598-017-05222-2.
Mohapatra, S., Chakraborty, T., Shimizu, S., Urasaki, S., Matsubara, T., Nagahama, Y., Ohta, K., 2015. Starvation beneficially influences the liver physiology and nutrient metabolism in *Edwardsiella tarda* infected red sea bream (*Pagrus major*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 189, 1–10. https://doi.org/10.1016/j.cbpa.2015.07.003.
Mohapatra, S., Chakraborty, T., Reza, M.A., Shimizu, S., Matsubara, T., Ohta, K., 2017. Short-term starvation and realimentation helps stave off Edwardsiella tarda infection in red sea bream (*Pagrus major*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 206, 42–53. https://doi.org/10.1016/j.cbpb.2017.01.009.
Morales, A.E., Pérez-Jiménez, A., Carmen Hidalgo, M., Abellán, E., Cardenete, G., 2004. Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. Compar. Biochem. Physiol. Part C: Toxicol. Pharmacol. 139 (1), 153–161. https://doi.org/10.1016/j.cca.2004.10.008.
Morretti, A., 1999. Manual on Hatchery Production of Seabass and Gilthead Seabream. Food & Agriculture Org.
Mutel, E., Gautier-Stein, A., Abdul-Wahed, A., Amigó-Correig, M., Zitoun, C., Stefanutti, A., Houberdon, I., Tourette, J.-A., Mithieux, G., Rajas, F., 2011. Control of blood glucose in the absence of hepatic glucose production during prolonged fasting in mice: induction of renal and intestinal gluconeogenesis by glucagon. Diabetes 60 (12), 3121–3131. https://doi.org/10.2337/db11-0571.
Myers, R.B., Fredenburgh, J.L., Grizzle, W.E., 2008. Carbohydrates. In: Bancroft, J.D., Gamble, M. (Eds.), Theory and Practice of Histological Techniques. Churchill Livingstone Elsevier, Philadelphia, USA.
Nadav, B., 2014. Physiological and hormonal changes during prolonged starvation in fish. Can. J. Fish. Aquat. Sci. 71 (10), 1447–1458. https://doi.org/10.1139/cjfas-2013-0175.
Naruhashi, K., Sai, Y., Tamai, I., Suzuki, N., Tsuji, A., 2002. PepT1 mRNA expression is induced by starvation and its level correlates with absorptive transport of cefadroxil longitudinally in the rat intestine. Pharm. Res. 19 (10), 1417–1423. https://doi.org/10.1023/a:1020436028194.
Nassar, M., Halle, I., Plagemann, A., Tzschentke, B., 2015. Detection of long-term influence of prenatal temperature stimulation on hypothalamic type-II

iodothyronine deiodinase in juvenile female broiler chickens using a novel immunohistochemical amplification protocol. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 179, 120–124. https://doi.org/10.1016/j.cbpa.2014.09.038.

Navarro, I., Gutiérrez, J., 1995. Chapter 17 fasting and starvation. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier, pp. 393–434.

Ning, L.J., He, A.Y., Li, J.M., Lu, D.L., Jiao, J.G., Li, L.Y., Li, D.L., Zhang, M.L., Chen, L.Q., Du, Z.Y., 2016. Mechanisms and metabolic regulation of PPARα activation in Nile tilapia (*Oreochromis niloticus*). Biochim. Biophys. Acta 1861 (9 Pt A), 1036–1048. https://doi.org/10.1016/j.bbalip.2016.06.005.

Okamura, A., Koyanagi, S., Dilxiat, A., Kusunose, N., Chen, J.J., Matsunaga, N., Shibata, S., Ohdo, S., 2014. Bile acid-regulated peroxisome proliferator-activated receptor-α (PPARα) activity underlies circadian expression of intestinal peptide absorption transporter PepT1/Slc15a1. J. Biol. Chem. 289 (36), 25296–25305. https://doi.org/10.1074/jbc.M114.577023.

Orozco, A., Valverde, R.C., 2005. Thyroid hormone deiodination in fish. Thyroid 15 (8), 799–813. https://doi.org/10.1089/thy.2005.15.799.

Orozco, Z.G.A., Soma, S., Kaneko, T., Watanabe, S., 2017. Effects of fasting and refeeding on gene expression of slc15a1a, a gene encoding an oligopeptide transporter (PepT1), in the intestine of Mozambique tilapia. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 203, 76–83. https://doi.org/10.1016/j.cbpb.2016.09.006.

O'Sullivan, L., Little, M.H., Combes, A.N., Moritz, K.M., 2012. Epigenetics and developmental programming of adult onset diseases. Pediatr. Nephrol. 27 (12), 2175–2182. https://doi.org/10.1007/s00467-012-2108-x.

Papadopulos, F., Spinelli, M., Valente, S., Foroni, L., Orrico, C., Alviano, F., Pasquinelli, G., 2007. Common tasks in microscopic and ultrastructural image analysis using ImageJ. Ultrastruct. Pathol. 31 (6), 401–407. https://doi.org/10.1080/01913120701719189.

Pascual, P., Pedrajas, J.R., Toribio, F., López-Barea, J., Peinado, J., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). Chem. Biol. Interact. 145 (2), 191–199. https://doi.org/10.1016/s0009-2797(03)00002-4.

Penhoat, A., Fayard, L., Stefanutti, A., Mithieux, G., Rajas, F., 2014. Intestinal gluconeogenesis is crucial to maintain a physiological fasting glycemia in the absence of hepatic glucose production in mice. Metabolism 63 (1), 104–111. https://doi.org/10.1016/j.metabol.2013.09.005.

Peres, H., Santos, S., Oliva-Teles, A., 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). Fish Physiol. Biochem. 40 (5), 1339–1347. https://doi.org/10.1007/s10695-014-9928-5.

Pérez-Jiménez, A., Guedes, M.J., Morales, A.E., Oliva-Teles, A., 2007. Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. Aquaculture 265 (1), 325–335. https://doi.org/10.1016/j.aquaculture.2007.01.021.

Peyrou, M., Ramadori, P., Bourgoin, L., Foti, M., 2012. PPARs in liver diseases and Cancer: epigenetic regulation by MicroRNAs. PPAR Res. 2012, 757803 https://doi.org/10.1155/2012/757803.

Piccinetti, C.C., Donati, M., Radaelli, G., Caporale, G., Mosconi, G., Palermo, F., Cossignani, L., Salvatori, R., Lopez, R.P., Olivotto, I., 2015. The effects of starving and feeding on Dover sole (*Solea solea*, Soleidae, Linnaeus, 1758) stress response and early larval development. Aquac. Res. 46 (10), 2512–2526. https://doi.org/10.1111/are.12410.

Pinto, M., Monteiro, J.N., Crespo, D., Costa, F., Rosa, J., Primo, A.L., Pardal, M.A., Martinho, F., 2021. Influence of oceanic and climate conditions on the early life history of European seabass *Dicentrarchus labrax*. Mar. Environ. Res. 169, 105362 https://doi.org/10.1016/j.marenvres.2021.105362.

Pinto, P.I., Matsumura, H., Thorne, M.A., Power, D.M., Terauchi, R., Reinhardt, R., Canário, A.V., 2010. Gill transcriptome response to changes in environmental calcium in the green spotted puffer fish. BMC Genomics 11, 476. https://doi.org/10.1186/1471-2164-11-476.

Pippenger, C.E., Browne, R.W., Armstrong, D., 1998. Regulatory antioxidant enzymes. Methods Mol. Biol. 108, 299–313. https://doi.org/10.1385/0-89603-472-0:299.

Pittman, K., Yúfera, M., Pavlidis, M., Geffen, A.J., Koven, W., Ribeiro, L., Zambonino-Infante, J.L., Tandler, A., 2013. Fantastically plastic: fish larvae equipped for a new world. Rev. Aquac. 5 (s1), S224–S267. https://doi.org/10.1111/raq.12034.

Polakof, S., Arjona, F.J., Sangiao-Alvarellos, S., Martín del Río, M.P., Mancera, J.M., Soengas, J.L., 2006. Food deprivation alters osmoregulatory and metabolic responses to salinity acclimation in gilthead sea bream *Sparus auratus*. J. Comp. Physiol. B. 176 (5), 441–452. https://doi.org/10.1007/s00360-006-0065-z.

Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: a review. J. Comp. Physiol. B. 182 (8), 1015–1045. https://doi.org/10.1007/s00360-012-0658-7.

Politis, S.N., Servili, A., Mazurais, D., Zambonino-Infante, J.L., Miest, J.J., Tomkiewicz, J., Butts, I.A.E., 2018. Temperature induced variation in gene expression of thyroid hormone receptors and deiodinases of European eel (*Anguilla anguilla*) larvae. Gen. Comp. Endocrinol. 259, 54–65. https://doi.org/10.1016/j.ygcen.2017.11.003.

Pottinger, T.G., Rand-Weaver, M., Sumpter, J.P., 2003. Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 136 (3), 403–417. https://doi.org/10.1016/s1096-4959(03)00212-4.

Potts, A., Uchida, A., Deja, S., Berglund, E.D., Kucejova, B., Duarte, J.A., Fu, X., Browning, J.D., Magnuson, M.A., Burgess, S.C., 2018. Cytosolic phosphoenolpyruvate carboxykinase as a cataplerotic pathway in the small intestine. American journal of physiology-gastrointestinal and liver. Physiology 315 (2), G249–G258. https://doi.org/10.1152/ajpgi.00039.2018.

Power, D., Melo, J., Santos, C., 2000. The effect of food deprivation and refeeding on the liver, thyroid hormones and transthyretin in sea bream. J. Fish Biol. 56 (2), 374–387. https://doi.org/10.1111/j.1095-8649.2000.tb02112.x.

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Björnsson, B.T., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Compar. Biochem. Physiol. Part C: Toxicol. Pharmacol. 130 (4), 447–459. https://doi.org/10.1016/S1532-0456(01)00271-X.

Rajas, F., Croset, M., Zitoun, C., Montano, S., Mithieux, G., 2000. Induction of PEPCK gene expression in insulinopenia in rat small intestine. Diabetes 49 (7), 1165–1168. https://doi.org/10.2337/diabetes.49.7.1165.

Rašković, B., Stanković, M., Marković, Z., Poleksić, V., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agricult. Sci. (Belgrade) 56 (1), 87–100. https://doi.org/10.2298/JAS1101087R.

Rescan, P.-Y., Montfort, J., Rallière, C., Le Cam, A., Esquerré, D., Hugot, K., 2007. Dynamic gene expression in fish muscle during recovery growth induced by a fasting-refeeding schedule. BMC Genomics 8 (1), 438. https://doi.org/10.1186/1471-2164-8-438.

Riera-Heredia, N., Martins, R., Mateus, A.P., Costa, R.A., Gisbert, E., Navarro, I., Gutiérrez, J., Power, D.M., Capilla, E., 2018. Temperature responsiveness of gilthead sea bream bone; an in vitro and in vivo approach. Sci. Rep. 8 (1), 11211. https://doi.org/10.1038/s41598-018-29570-9.

Rodgers, B.D., Weber, G.M., Kelley, K.M., Levine, M.A., 2003. Prolonged fasting and cortisol reduce myostatin mRNA levels in tilapia larvae; short-term fasting elevates. Am. J. Phys. Regul. Integr. Comp. Phys. 284 (5), R1277–R1286. https://doi.org/10.1152/ajpregu.00644.2002.

Rossi, A., Cazenave, J., Bacchetta, C., Campana, M., Parma, M.J., 2015. Physiological and metabolic adjustments of *Hoplosternum littorale* (Teleostei, Callichthyidae) during starvation. Ecol. Indic. 56, 161–170. https://doi.org/10.1016/j.ecolind.2015.04.001.

Rotllant, J., Guerreiro, P., Anjos, L., Redruello, B., Canario, A.V., Power, D., 2005a. Stimulation of cortisol release by the N terminus of teleost parathyroid hormone-related protein in interrenal cells in vitro. Endocrinology 146 (1), 71–76. https://doi.org/10.1210/en.2004-0644.

Rotllant, J., Redruello, B., Guerreiro, P., Fernandes, H., Canario, A.V., Power, D., 2005b. Calcium mobilization from fish scales is mediated by parathyroid hormone related protein via the parathyroid hormone type 1 receptor. Regul. Pept. 132 (1–3), 33–40. https://doi.org/10.1016/j.regpep.2005.08.004.

Rueda, F.M., Martinez, F.J., Zamora, S., Kentouri, M., Divanach, P., 1998. Effect of fasting and refeeding on growth and body composition of red porgy, *Pagrus pagrus* L. Aquac. Res. 29 (6), 447–452. https://doi.org/10.1046/j.1365-2109.1998.00228.x.

Sadoul, B., Alfonso, S., Cousin, X., Prunet, P., Bégout, M.-L., Leguen, I., 2021. Global assessment of the response to chronic stress in European sea bass. Aquaculture 544, 737072. https://doi.org/10.1016/j.aquaculture.2021.737072.

Sadoul, B., Alfonso, S., Goold, C., Pratlong, M., Rialle, S., Geffroy, B., Bégout, M.-L., 2022. Transcriptomic profiles of consistent risk-taking behaviour across time and contexts in European Sea bass. Proc. R. Soc. B Biol. Sci. 289 (1975), 20220399. https://doi.org/10.1098/rspb.2022.0399.

Saghazadeh, A., Mahmoudi, M., Rezaei, N., 2019. Nutriepigenomic immunity. In: Mahmoudi, M., Rezaei, N. (Eds.), Nutrition and Immunity. Springer International Publishing, Cham, pp. 483–501.

Saka, Ahin, Glrat, K.A., Kamad, H.O., 2001. The development of European Sea bass (*Dicentrarchus labrax* L., 1758) eggs in relation to temperature. Turk. J. Vet. Anim. Sci. 25, 139–147.

Sakyi, M.E., Cai, J., Tang, J., Abarike, E.D., Xia, L., Li, P., Kuebutornye, F.K.A., Zou, Z., Liang, Z., Jian, J., 2020. Effects of starvation and subsequent re-feeding on intestinal microbiota, and metabolic responses in Nile tilapia, *Oreochromis niloticus*. Aquacult. Reports 17, 100370. https://doi.org/10.1016/j.aqrep.2020.100370.

Salis, P., Roux, N., Huang, D., Marcionetti, A., Mouginot, P., Reynaud, M., Salles, O., Salamin, N., Pujol, B., Parichy, D.M., Planes, S., Laudet, V., 2021. Thyroid hormones regulate the formation and environmental plasticity of white bars in clownfishes. Proc. Natl. Acad. Sci. 118 (23), e2101634118 https://doi.org/10.1073/pnas.2101634118.

Samaras, A., Espírito Santo, C., Papandroulakis, N., Mitrizakis, N., Pavlidis, M., Höglund, E., Pelgrim, T.N.M., Zethof, J., Spanings, F.A.T., Vindas, M.A., Ebbesson, L. O.E., Flik, G., Gorissen, M., 2018. Allostatic load and stress physiology in European seabass (*Dicentrarchus labrax* L.) and gilthead seabream (*Sparus aurata* L.). Front. Endocrinol. (Lausanne) 9. https://doi.org/10.3389/fendo.2018.00451.

Samaras, A., Dimitroglou, A., Kollias, S., Skouradakis, G., Papadakis, I.E., Pavlidis, M., 2021. Cortisol concentration in scales is a valid indicator for the assessment of chronic stress in European sea bass, *Dicentrarchus labrax* L. Aquaculture 545, 737257. https://doi.org/10.1016/j.aquaculture.2021.737257.

Sarropoulou, E., Kaitetzidou, E., Papandroulakis, N., Tsalafouta, A., Pavlidis, M., 2019. Inventory of European Sea bass (*Dicentrarchus labrax*) snc RNAs vital during early teleost development. Front. Genet. 10, 657. https://doi.org/10.3389/fgene.2019.00657.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Scott, G.R., Johnston, I.A., 2012. Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. Proc. Natl. Acad. Sci. 109 (35), 14247–14252. https://doi.org/10.1073/pnas.1205012109.

Secor, S.M., Lignot, J.H., 2010. Morphological plasticity of vertebrate aestivation. Prog. Mol. Subcell. Biol. 49, 183–208. https://doi.org/10.1007/978-3-642-02421-4_9.

Shi, Q., Xiong, X., Wen, Z., Qin, C., Li, R., Zhang, Z., Gong, Q., Wu, X., 2022. Cu/Zn superoxide dismutase and catalase of Yangtze sturgeon, *Acipenser dabryanus*: molecular cloning, tissue distribution and response to fasting and refeeding. Fishes 7 (1), 35. https://doi.org/10.3390/fishes7010035.

Shimakura, J., Terada, T., Saito, H., Katsura, T., Inui, K.-I., 2006. Induction of intestinal peptide transporter 1 expression during fasting is mediated via peroxisome proliferator-activated receptor α. American journal of physiology-gastrointestinal and liver. Physiology 291 (5), G851–G856. https://doi.org/10.1152/ajpgi.00171.2006.

Siharath, K., Kelley, K.M., Bern, H.A., 1996. A low-molecular-weight (25-kDa) IGF-binding protein is increased with growth inhibition in the fasting striped bass, *Morone saxatilis*. Gen. Comp. Endocrinol. 102 (3), 307–316. https://doi.org/10.1006/gcen.1996.0074.

Simontacchi, C., Poltronieri, C., Carraro, C., Bertotto, D., Xiccato, G., Trocino, A., Radaelli, G., 2008. Alternative stress indicators in sea bass *Dicentrarchus labrax*. L. J. Fish Biol. 72 (3), 747–752. https://doi.org/10.1111/j.1095-8649.2007.01717.x.

Simpkins, D.G., Hubert, W.A., Del Rio, C.M., Rule, D.C., 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Trans. Am. Fish. Soc. 132 (3), 576–589. https://doi.org/10.1577/1548-8659(2003)132<0576:PROJRT>2.0.CO;2.

Sokolović, M., Wehkamp, D., Sokolović, A., Vermeulen, J., Gilhuijs-Pederson, L.A., van Haaften, R.I.M., Nikolsky, Y., Evelo, C.T.A., van Kampen, A.H.C., Hakvoort, T.B.M., Lamers, W.H., 2007. Fasting induces a biphasic adaptive metabolic response in murine small intestine. BMC Genomics 8, 361. https://doi.org/10.1186/1471-2164-8-361.

Sokolović, M., Sokolović, A., Wehkamp, D., van Themaat, E.V.L., de Waart, D.R., Gilhuijs-Pederson, L.A., Nikolsky, Y., van Kampen, A.H.C., Hakvoort, T.B.M., Lamers, W.H., 2008. The transcriptomic signature of fasting murine liver. BMC Genomics 9 (1), 528. https://doi.org/10.1186/1471-2164-9-528.

Stark, R., Kibbey, R.G., 2014. The mitochondrial isoform of phosphoenolpyruvate carboxykinase (PEPCK-M) and glucose homeostasis: has it been overlooked? Biochim. Biophys. Acta 1840 (4), 1313–1330. https://doi.org/10.1016/j.bbagen.2013.10.033.

Terova, G., Rimoldi, S., Chini, V., Gornati, R., Bernardini, G., Saroglia, M., 2007. Cloning and expression analysis of insulin-like growth factor I and II in liver and muscle of sea bass (*Dicentrarchus labrax*, L.) during long-term fasting and refeeding. J. Fish Biol. 70 (sb), 219–233. https://doi.org/10.1111/j.1095-8649.2007.01402.x.

Terova, G., Corà, S., Verri, T., Rimoldi, S., Bernardini, G., Saroglia, M., 2009. Impact of feed availability on PepT1 mRNA expression levels in sea bass (*Dicentrarchus labrax*). Aquaculture 294 (3–4), 288–299. https://doi.org/10.1016/j.aquaculture.2009.06.014.

Thamotharan, M., Bawani, S.Z., Zhou, X., Adibi, S.A., 1999. Functional and molecular expression of intestinal oligopeptide transporter (Pept-1) after a brief fast. Metabolism 48 (6), 681–684. https://doi.org/10.1016/s0026-0495(99)90164-6.

Tian, J., He, G., Mai, K., Liu, C., 2015. Effects of postprandial starvation on mRNA expression of endocrine-, amino acid and peptide transporter-, and metabolic enzyme-related genes in zebrafish (*Danio rerio*). Fish Physiol. Biochem. 41 (3), 773–787. https://doi.org/10.1007/s10695-015-0045-x.

Tran, N.T., Xiong, F., Hao, Y.-T., Zhang, J., Wu, S.-G., Wang, G.-T., 2018. Starvation influences the microbiota assembly and expression of immunity-related genes in the intestine of grass carp (*Ctenopharyngodon idellus*). Aquaculture 489, 121–129. https://doi.org/10.1016/j.aquaculture.2018.02.016.

Triantaphyllopoulos, K.A., Cartas, D., Miliou, H., 2020. Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? Rev. Aquac. 12 (3), 1637–1662. https://doi.org/10.1111/raq.12402.

Vagner, M., Zambonino-Infante, J.L., Mazurais, D., 2019. Fish facing global change: are early stages the lifeline? Mar. Environ. Res. 147, 159–178. https://doi.org/10.1016/j.marenvres.2019.04.005.

Vandeputte, M., Piferrer, F., 2018. Genetic and environmental components of sex determination in the European Sea bass. In: Wang, Han-Ping, Piferrer, Francesc, Chen, Song-Lin, Shen, Z.-G. (Eds.), Sex Control in Aquaculture, pp. 305–325.

Vandeputte, M., Clota, F., Sadoul, B., Blanc, M.-O., Blondeau-Bidet, E., Bégout, M.-L., Cousin, X., Geffroy, B., 2020. Low temperature has opposite effects on sex determination in a marine fish at the larval/postlarval and juvenile stages. Ecol. Evol. 10 (24), 13825–13835. https://doi.org/10.1002/ece3.6972.

Varsamos, S., Flik, G., Pepin, J., Bonga, S.W., Breuil, G., 2006. Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax*. Fish Shellfish Immunol. 20 (1), 83–96. https://doi.org/10.1016/j.fsi.2005.04.005.

Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. R. Soc. B Biol. Sci. 281 (1779), 20132612. https://doi.org/10.1098/rspb.2013.2612.

Viegas, I., Rito, J., González, J.D., Jarak, I., Carvalho, R.A., Metón, I., Pardal, M.A., Baanante, I.V., Jones, J.G., 2013. Effects of food-deprivation and refeeding on the regulation and sources of blood glucose appearance in European seabass (*Dicentrarchus labrax* L.). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166 (3), 399–405. https://doi.org/10.1016/j.cbpa.2013.07.013.

Vieira, F.A., Gregório, S.F., Ferraresso, S., Thorne, M.A.S., Costa, R., Milan, M., Bargelloni, L., Clark, M.S., Canario, A.V.M., Power, D.M., 2011. Skin healing and scale regeneration in fed and unfed sea bream, *Sparus auratus*. BMC Genomics 12 (1), 490. https://doi.org/10.1186/1471-2164-12-490.

Villamizar, N., Ribas, L., Piferrer, F., Vera, L.M., Sánchez-Vázquez, F.J., 2012. Impact of daily thermocycles on hatching rhythms, larval performance and sex differentiation of zebrafish. PLoS One 7 (12), e52153. https://doi.org/10.1371/journal.pone.0052153.

Waagbø, R., Jørgensen, S.M., Timmerhaus, G., Breck, O., Olsvik, P.A., 2017. Short-term starvation at low temperature prior to harvest does not impact the health and acute stress response of adult Atlantic salmon. PeerJ 5, e3273. https://doi.org/10.7717/peerj.3273.

Wang, J., Yan, X., Lu, R., Meng, X., Nie, G., 2017. Peptide transporter 1 (PepT1) in fish: a review. Aquacult. Fish. 2 (5), 193–206. https://doi.org/10.1016/j.aaf.2017.06.007.

Wang, J., Du, J.J., Jiang, B., He, R.Z., Li, A.X., 2019. Effects of short-term fasting on the resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus agalactiae* infection. Fish Shellfish Immunol. 94, 889–895. https://doi.org/10.1016/j.fsi.2019.09.055.

Wang, Q., He, G., Mai, K., Xu, W., Zhou, H., Wang, X., Mei, L., 2016. Chronic rapamycin treatment on the nutrient utilization and metabolism of juvenile turbot (*Psetta maxima*). Sci. Rep. 6 (1), 28068. https://doi.org/10.1038/srep28068.

Wang, T., Hung, C.C., Randall, D.J., 2006. The comparative physiology of food deprivation: from feast to famine. Annu. Rev. Physiol. 68, 223–251. https://doi.org/10.1146/annurev.physiol.68.040104.105739.

Wilson, A., Radtke, F., 2006. Multiple functions of notch signaling in self-renewing organs and cancer. FEBS Lett. 580 (12), 2860–2868. https://doi.org/10.1016/j.febslet.2006.03.024.

Wood, C.M., 2019. 7 - internal spatial and temporal CO2 dynamics: Fasting. In: Grosell, M., Munday, P.L., Farrell, A.P., Brauner, C.J. (Eds.), Feeding, Drinking, and the Alkaline Tide, in: Fish Physiology. Academic Press, pp. 245–286.

Wu, L., Lu, Y., Jiao, Y., Liu, B., Li, S., Li, Y., Xing, F., Chen, D., Liu, X., Zhao, J., Xiong, X., Gu, Y., Lu, J., Chen, X., Li, X., 2016. Paternal psychological stress reprograms hepatic gluconeogenesis in offspring. Cell Metab. 23 (4), 735–743. https://doi.org/10.1016/j.cmet.2016.01.014.

Xia, J.H., Lin, G., Fu, G.H., Wan, Z.Y., Lee, M., Wang, L., Liu, X.J., Yue, G.H., 2014. The intestinal microbiome of fish under starvation. BMC Genomics 15, 266. https://doi.org/10.1186/1471-2164-15-266.

Yang, J., Kalhan, S.C., Hanson, R.W., 2009. What is the metabolic role of phosphoenolpyruvate carboxykinase? J. Biol. Chem. 284 (40), 27025–27029. https://doi.org/10.1074/jbc.R109.040543.

Yang, S., He, K., Yan, T., Wu, H., Zhou, J., Zhao, L., Wang, Y., Gong, Q., 2019. Effect of starvation and refeeding on oxidative stress and antioxidant defenses in Yangtze sturgeon (*Acipenser dabryanus*). Fish Physiol. Biochem. 45 (3), 987–995. https://doi.org/10.1007/s10695-019-0609-2.

Zaldúa, N., Naya, D.E., 2014. Digestive flexibility during fasting in fish: A review. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 169, 7–14. https://doi.org/10.1016/j.cbpa.2013.12.006.

Zambonino Infante, J.L., Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. Compar. Biochem. Physiol. Part C: Toxicol. Pharmacol. 130 (4), 477–487. https://doi.org/10.1016/S1532-0456(01)00274-5.

Zeng, L.Q., Li, F.J., Li, X.M., Cao, Z.D., Fu, S.J., Zhang, Y.G., 2012. The effects of starvation on digestive tract function and structure in juvenile southern catfish (*Silurus meridionalis* Chen). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 162 (3), 200–211. https://doi.org/10.1016/j.cbpa.2012.02.022.

Zhang, D.D., Wang, D.D., Wang, Z., Wang, Y.B., Li, G.X., Sun, G.R., Tian, Y.D., Han, R.L., Li, Z.J., Jiang, R.R., Liu, X.J., Kang, X.T., Li, H.H., 2020. Estrogen abolishes the repression role of gga-miR-221-5p targeting ELOVL6 and SQLE to promote lipid synthesis in chicken liver. Int. J. Mol. Sci. 21 (5) https://doi.org/10.3390/ijms21051624.

Zhang, J., Hu, J., 2008. Image segmentation based on 2D Otsu method with histogram analysis, 2008 international conference on computer science and software engineering. IEEE 105–108.

Zheng, J.-L., Zhu, Q.-L., Shen, B., Zeng, L., Zhu, A.-Y., Wu, C.-W., 2016. Effects of starvation on lipid accumulation and antioxidant response in the right and left lobes of liver in large yellow croaker *Pseudosciaena crocea*. Ecol. Indic. 66, 269–274. https://doi.org/10.1016/j.ecolind.2016.01.037.

Zheng, Y.-H., Fu, S.-J., 2021. Effects of fasting on collective movement and fission–fusion dynamics in both homogeneous and heterogeneous shoals of a group-living cyprinid fish species. J. Fish Biol. 99 (5), 1640–1649. https://doi.org/10.1111/jfb.14872.

Zhou, Y., Rychahou, P., Wang, Q., Weiss, H.L., Evers, B.M., 2015. TSC2/mTORC1 signaling controls Paneth and goblet cell differentiation in the intestinal epithelium. Cell Death Dis. 6 (2), e1631. https://doi.org/10.1038/cddis.2014.588.