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Temperature and salinity are abiotic factors that affect physiological responses in aquaculture species. The European sea bass (*Dicentrarchus labrax*) is a temperate species that is generally farmed at 18 °C in seawater (SW). In the wild, its incursions in shallow habitats such as lagoons may result in hyperthermal damage despite its high thermal tolerance. Meanwhile, the milkfish (*Chanos chanos*), a tropical species, is generally reared at 28 °C, and in winter, high mortality usually occurs under hypothermal stress such as cold snaps. This study compared changes in hepatic antioxidant enzymes (superoxide dismutase, SOD; and catalase, CAT) in these two important marine euryhaline aquaculture species in Europe and Southeast Asia, respectively, under temperature challenge combined with hypo-osmotic (fresh water, FW) stress. After a four-week hyper- or hypo-thermal treatment, hepatic SOD activity was upregulated in both species reared in SW and FW, indicating enhanced oxidative stress in European sea bass and milkfish. The expression profiles of *sod* isoforms suggested that in milkfish, the increase in reactive oxygen species (ROS) was mainly at the cytosol level, leading to increased sod1 expression. In European sea bass, however, no obvious difference was found between the expression of *sod* isoforms at different temperatures. A lower expression of *sod2* was observed in FW compared to SW in the latter species. Moreover, no significant change was observed in the mRNA expression and activity of CAT in the livers of these two species under the different temperature treatments, with the exception of the lower CAT activity in milkfish challenged with SW at 18 °C. Taken together, our results indicated that the antioxidant responses were not changed under long-term hypoosmotic challenge but were enhanced during the four-week temperature treatments in livers of both the temperate and tropical euryhaline species.

1. Introduction

Estuarine and lagoon environments are highly dynamic and characterized by fluctuations in temperature and oxygen levels. In these habitats, salinity also drastically changes within hours (Freire et al., 2012), imposing physiological, metabolic, and molecular adjustments to organisms inhabiting such unstable habitats. In temperate areas such as the Mediterranean Basin, coastal lagoons are often warmer in spring and summer, sometimes reaching up to 28 °C (Ifremer, 2013), compared to the much more stable and generally lower temperatures recorded in the open sea during the summer months. The European sea bass (*D. labrax*) is a temperate marine species that migrates to such lagoon habitats in spring to use them as feeding grounds or nurseries, and sometimes migrate to river mouths (Barnabé, 1989; Dufour et al., 2009). The salinity of lagoon environments can strongly vary and sometimes reach levels close to those of fresh water (FW), notably following extreme precipitation events in spring (Mariotti et al., 2008). In autumn, when temperatures reach levels below 10 °C, *D. labrax* migrates back to deeper

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warmer seawater (SW) (Pickett et al., 2004). Previous studies have shown that the standard metabolic rate increased in *D. labrax* between 10 and 25 °C (Claireaux and Lagardère, 1999), and maximal growth rate was reported at 26 °C (Person-Le Ruyet et al., 2004). In addition, compared to SW at 18 °C, exposure to 24 °C causes a higher nitrogen excretion and increased branchial mRNA expression in genes involved in the maintenance of hydromineral balance and nitrogen excretion (Person-Le Ruyet et al., 2004; Masroor et al., 2019). *D. labrax* has been reported to tolerate full FW (Nebel et al., 2005); however, due to osmoregulatory requirements, sea basses must adjust their physiological machinery to hyperosmoregulate, which might be energetically costly. The above-mentioned studies refer to increased metabolic and physiological activity at temperatures up to 24 °C, particularly in FW.

Milkfish (*C. chanos*), in contrast, is a tropical marine teleost of Southeast Asia and Taiwan, where it is an important economic species. It reproduces in shallow marine areas of coastal waters (lagoons and atolls) and uses FW habitats as nursery grounds (Bagarinao, 1994). According to the Aquaculture Condition Reports (Fisheries Agency, Council of Agriculture, Taiwan), the percentage of FW milkfish ponds has increased in this decade, probably linked to FW-acclimated milkfish having a better feed conversion rate than SW-acclimated milkfish (Martinez et al., 2006). The optimal temperature range for milkfish aquaculture is 24–33 °C with limits at 15 °C during cold snaps (Hanke et al., 2019). In winter, high mortalities have been recorded during cold snaps, which have caused enormous economic losses in Taiwan (Liao, 2016). Previous studies have shown that SW-acclimated milkfish exhibit a better cold tolerance than FW-acclimated fish (Kang et al., 2015), notably through differential energy utilization pathways (Chang et al., 2019, 2020).

Exposure to different environmental factors such as temperature, salinity, and dissolved oxygen can influence the oxidative status and cellular production of reactive oxygen species (ROS). These can trigger the activation of antioxidant defense mechanisms in different organs to prevent and intercept such oxidative species (Martínez-Álvarez et al., 2005). Among antioxidant enzymes that intercept ROS, superoxide dismutase (SOD) and catalase (CAT) form an important part of the antioxidant responses in fish (Lesser, 2006). Temperature increase or decrease has been shown to induce oxidative stress and trigger antioxidant defenses in fish, including the two analyzed species (Malek et al., 2004; Lushchak, 2011; Vinagre et al., 2012; Chang et al., 2020). The liver is an essential organ involved in antioxidative mechanisms (Martínez-Álvarez et al., 2005). It is not yet clear how temperature alone and synergically combined with salinity affects antioxidant mechanisms in these two species, which are both extremely euryhaline but originate from habitats characterized by different temperature regimes and different temperature tolerances. Increased gene expression does not always lead to enhanced protein activity because of the translational and posttranslational mechanisms of enzyme activity regulation (O'Brien, 2010; Song et al., 2019). It is thus essential to simultaneously analyze the responses at the gene expression and protein activity levels.

In this study, the hepatic redox status was analyzed by investigating two key antioxidants at the molecular and protein levels in order to compare the responses in the livers of a temperate and a tropical euryhaline aquaculture fish to warm and cold temperatures combined with salinity changes.

2. Material and methods

2.1. Experimental design

Juvenile sea bass (*D. labrax*) from a West Mediterranean population was brought to the Montpellier University and maintained for one week in 3500-L tanks containing natural seawater (SW) from the Mediterranean Sea at 38‰ and 18 °C according to the natural habitat in a temperate climate. Fish were transferred to 200-L tanks (14 fish/tank, density of 6–7 kg/m^3, two replicates per condition) to be acclimated either to 24 °C or kept at 18 °C as a control condition, as in Masroor et al. (2019). Milkfish (*C. chanos*) were maintained in 100-L tanks holding SW at 35‰ prepared from dechlorinated tap water (fresh water; FW) with proper amounts of Blue Treasure Sea Salts (New South Wales, Australia) at 28 °C according to natural habitat in subtropical climate. Fish (N = 6 fish/tank) were then acclimated progressively to either 18 °C or kept at 28 °C as a control. In both cases, temperature acclimation was progressive (2 °C/h) either by cooling (*C. chanos*) (PF-225M, PRINCE, Tainan, Taiwan) or warming (*D. labrax*) (Eheim Thermocontrol) the water. After two weeks of temperature acclimation, fish were transferred directly either to FW or kept in SW (sea bass: 7 fish/tank, two replicates for each condition; milkfish: 6 fish/tank, two replicates for each condition) at the respective temperature and maintained in these temperature-salinity conditions for two weeks before sampling (Fig. 1). For both species, a 12 h light/12 h dark photoperiod was used, and water was aerated and mechanically/biologically filtered (Eheim System, Lens, Pas-de-Calais, France). Temperature (28 ± 0.5 °C and 18 ± 0.3 °C), salinity (0‰ and 35 ± 0.5‰), oxygen, and nitrogen levels were checked daily. A quarter of the water volume was changed every two days. Fish were fed with fish granules (for sea bass, Aphymar feed, Meze, Herault, France; for milkfish, FwuSow, Taichung, Taiwan) twice a week (for sea bass) or every day (for milkfish) until 2 days before sampling. At the end of the experiments, fish were anesthetized in a solution of phenoxy-2-ethanol (240 ppm) prior to tissue collection. European sea bass and milkfish used in the experiments had a length of 19.2 ± 1.3 cm (mean±SD) and 10.2 ± 0.2 cm and an average weight of 86.87 ± 20.23 g and 13.7 ± 1.3 g, respectively. Four groups were compared: SW at 18 °C, SW at 24 °C or 28 °C for sea bass and milkfish, respectively, FW at 18 °C

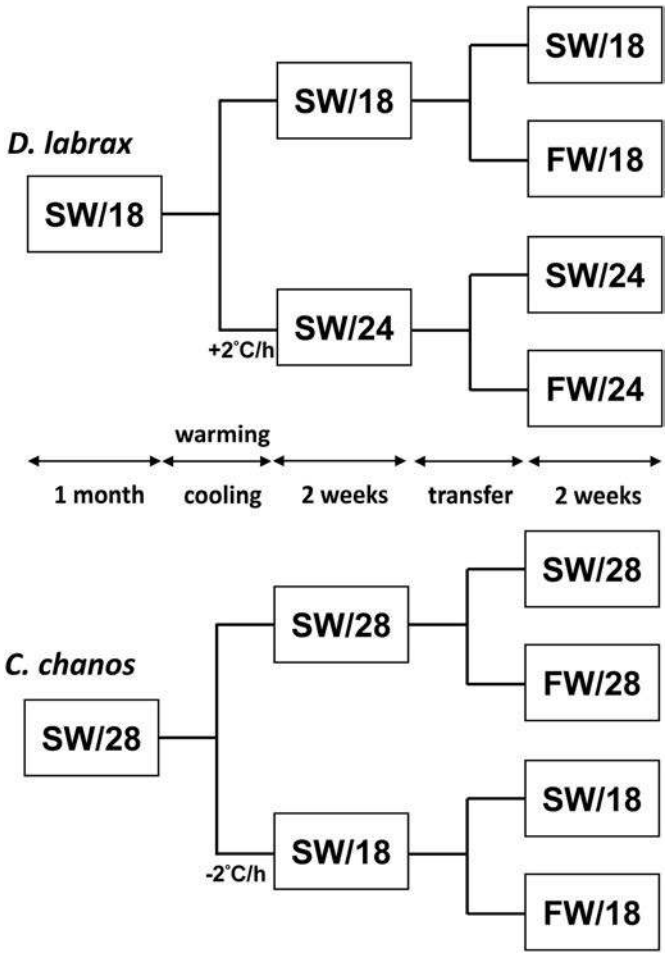

Fig. 1. Experimental design for temperature and salinity treatments of European sea bass (*D. labrax*) and milkfish (*C. chanos*).

and FW at 24 °C or 28 °C for sea bass and milkfish, respectively. Following anesthesia, liver tissues were immediately collected from SW- and FW-exposed sea bass and milkfish acclimated at 18 °C and 24 °C or 28 °C, respectively. Liver samples were immersed in Trizol® reagent (for gene expression analyses) or flash frozen in liquid nitrogen (for quantification of antioxidant activities). All tissues were stored at −80 °C until analysis.

These experiments respected the guidelines of the European Union (directive 86/609), the French law (decree 87/848) regulating animal experimentation, and Institutional Animal Care and Use Committee (IACUC) of the National Chung Hsing University (IACUC Approval No. 105-024 to T.H.L.).

2.2. RNA extraction and reverse transcription

Total RNA was extracted using Trizol® reagent according to the manufacturer's instructions. RNA quantity and purity were assessed by measuring the A260/A280 ratio using a NanoDrop® ND-1000 V3300 spectrometer (Nanodrop Technology Inc., Wilmington, Delaware, USA). RNA quality was checked in some samples using an Agilent bioanalyzer (Agilent) using the electrophoretic trace method. One microgram of total RNA was treated with DNase I amplification grade (Invitrogen™, Life Technologies). Reverse transcription was performed using 200 U M-MLV reverse transcriptase (Invitrogen™), and the first strand of complementary DNA (cDNA) was generated using 250 ng of random primers (Invitrogen™), dNTPs (10 mM), and 40 U of RNase OUT (Invitrogen™), following the manufacturer's instructions.

2.3. Gene expression analyses

All primer pairs used for qPCR are listed in Table 1. The amplification efficiencies of the primer pairs used in the qPCR that utilized serial cDNA dilution were predicted to be 92–104%. The PCR products were double-checked by melting curve, 1.5% agarose gel electrophoresis, and sequencing. The qPCR mixture contained 8 μL of cDNA, 2 μL of 1 μM primer pairs, and 10 μL of 2 × KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems, Wilminton, MA, USA). The qPCR was performed using the MiniOpicon Real-Time PCR system (Bio-Rad, Hercules, CA, USA). The cDNA samples in all experimental groups were mixed as an internal control to reduce artificial effects in every reaction. The formula $2^{-[(Ct\ target,\ n\ -\ Ct\ ef1\alpha,\ n)\ -\ (Ct\ target,\ ic\ -\ Ct\ ef1\alpha,\ ic)]}$ was used for calculating relative mRNA expression, where "Ct" is the threshold cycle number, "n" indicates each sample, and "ic" indicates the internal control.

2.4. Antioxidant enzyme activities

Frozen liver samples were maintained at −80 °C until analysis of SOD and CAT activities using a TECAN spectrophotometer (Infinite®200 PRO, Männedorf, Switzerland). Tissues were homogenized in KPi buffer (50 mM KH_2PO_4, 50 mM K_2HPO_4, pH = 7.3) containing Triton X-100, 120 mM KCL, and protease inhibitors (1 μg/mL) using a Mixer Mill 400 (Retsch GmbH, Haan, Germany, 30 s at 30 beats s^{-1}) with steel balls (Retsch). Following centrifugation at 14,000 rpm for 3 min (4 °C), the supernatant was divided into two parts to assess SOD and CAT activities.

SOD activity was measured using the protocol of McCord and Fridovich (1969), modified by Livingstone et al. (1992). CAT activity was determined by measuring the decrease in absorbance at 240 nm resulting from H_2O_2 consumption, as described by Aebi (1984). All measurements were carried out in triplicate. Throughout the procedure, samples were kept on ice and the results were expressed per unit (U) of activity per mg of protein, following protein quantification according to the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as standard. One U SOD corresponds to the amount of enzyme causing a 50% inhibition of nitroblue tetrazolium reduction under assay conditions. One U CAT is defined as the amount of enzyme required to reduce 1 μmol of H_2O_2 per min.

2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism (version 6, GraphPad Software Incorporated, La Jolla, CA, USA). Normality and homogeneity of variance were checked using the D'Agostino-Pearson test and Bartlett test. A two-way factorial analysis of variance with temperature and salinity as the main factors was performed. Critical differences between groups were evaluated using the Fisher's least-square difference test. A non-parametric Mann-Whitney test was performed for SOD and CAT protein activity data. Data are presented as box and whisker plots showing median, minimum, and maximum values. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. The mRNA levels and protein activity of SOD in livers

In livers of *D. labrax*, a salinity effect was observed for *Dlsod1* (superoxide dismutase 1 of *D. labrax*) and *Dlsod2* (superoxide dismutase 2 of *D. labrax*) expression (Table 2, two-way ANOVA, $p < 0.05$). *D. labrax* challenged to FW at 18 °C displayed 37% and 46% significantly lower *Dlsod1* and *Dlsod2* mRNA expression levels compared to the SW group at 18 °C (Fig. 2A and B). At 24 °C, a significant 32% lower *Dlsod2* mRNA expression was measured following FW transfer; no change in *Dlsod1* mRNA expression was observed at this temperature. In livers of *C. chanos*, a highly significant salinity and temperature effect was detected for *Ccsod1* (superoxide dismutase 1 of *C. Chanos*; Table 2, two-way ANOVA, $p < 0.0001$). The interaction between temperature and salinity had a significant effect on *Ccsod1* expression ($p < 0.01$). *C. chanos* challenged from SW 28 °C to SW 18 °C showed a 208% higher *Ccsod1* mRNA expression compared to the control but no change in *Ccsod2* (superoxide dismutase 2 of *C. chanos*) mRNA levels. Following FW transfer, no difference in mRNA expression was observed at 28 °C (Fig. 2C). At 18 °C, however, *Ccsod1* mRNA levels were 46% lower in FW

Table 1
Primer sequences used in the gene expression analysis.

Target gene	Primer name	Sequence ID	Sequence (from 5' to 3')	Efficiency
sod 1	*D.l. sod 1*-F	DLA_LG14_005480	AACCATGGTGATCCACGAGA	98.8%
	D.l. sod 1-R		ATGCCGATGACTCCACAGG	
sod 2	*D.l. sod 2*-F	DLAgn_00071530	TGCCCTCCAGCCTGCTCT	92.0%
	D.l. sod 2-R		CTTCTGGAAGGAGCCAAAGTC	
cat	*D.l. cat*-F	DLAgn_00171080	GGCTGGGAGCCAACTATCTG	94.8%
	D.l. cat-R		GGAGCTCCACCTTGGTTGTC	
sod 1	*C.c. sod 1*-F	XP_030648381	GCATGTTGGGGACCTTGG	98.3%
	C.c. sod 1-R		TCAGCCTTCTCATGGATCACC	
sod 2	*C.c. sod 2*-F	XP_030627172	GGCTTTGAAAAGGAGAGTGG	102.3%
	C.c. sod 2-R		CTGCAGATAATAAGCGTGCTC	
cat	*C.c. cat*-F	XP_030641298	ATGCCCCCTGGCATTGAG	104.1%
	C.c. cat-R		GGCCCTGAAGGGACAGTT	

Table 2
Two-way ANOVA results of different gene expressions analyzed with salinity and temperature as the main factors. ns: not significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. N = 8–13 per condition.

Genes	Interaction	Salinity	Temperature
Dlsod 1	ns	*	ns
Dlsod 2	ns	*	ns
Dl SOD activity	ns	ns	ns
Ccsod 1	**	****	****
Ccsod 2	ns	ns	ns
Cc SOD activity	ns	ns	*
Dlcat	ns	ns	*
Dl CAT activity	ns	ns	ns
Cccat	ns	ns	ns
Cc CAT activity	ns	ns	*

fish than in SW individuals (Fig. 2C). We did not observe any significant differences in *Ccsod2* mRNA levels between the different salinity and temperature conditions in *C. chanos* (Fig. 2D).

In *D. labrax*, hepatic SOD activity was increased following acclimation to 24 °C under SW conditions (Fig. 3A). FW transfer did not change SOD activity levels at either temperature, but a higher variability of the data was detected in FW conditions at both temperatures. In *C. chanos*, SOD activity in the livers was significantly increased following temperature transfer from 28 °C to 18 °C in SW (Fig. 3B). FW acclimation did not affect SOD activity levels, regardless of the temperature.

3.2. Catalase mRNA levels and protein activity

In *D. labrax*, the effect of temperature on *Dlcat* (catalase of *D. labrax*) mRNA expression was measured (Table 2, $p < 0.05$), but there was no effect of salinity or the interaction between both parameters. The mRNA levels of *Dlcat* in livers were significantly different only between FW conditions, with a 52% higher expression at 24 °C than at 18 °C (Fig. 4A). In *C. chanos*, *Cccat* (catalase of *C. chanos*) mRNA expression in the livers did not show an effect of temperature, salinity, or the interaction between both parameters (Table 2). No significant differences were observed between temperature and salinity conditions (Fig. 4B).

CAT activity did not change in livers of *D. labrax* following temperature transfer in SW. Following FW transfer, however, a 30% decrease in CAT activity was observed at 18 °C, whereas no change in mRNA levels was observed at 24 °C (Fig. 5A). In *C. chanos*, a temperature effect was observed regarding hepatic CAT activity (Table 2, $p < 0.05$). CAT activity decreased by 47% following a temperature challenge from 28 °C to 18 °C in SW. Following FW transfer, however, no change in hepatic CAT activity was observed regardless of the temperature (Fig. 5B).

4. Discussion

This study compared antioxidant genes and enzyme activities in two important aquaculture species from different climate zones under different salinity environments and nonlethal temperature stress.

4.1. Antioxidant responses to hypoosmotic transfer

Oxidative stress can be categorized according to endogenous or exogenous sources. Both the endogenous source mainly from mitochondria and the exogenous source via cytosolic pathways lead to cytosolic oxidative stress (Chowdhury and Saikia, 2020). Therefore, mRNA expression of cytosolic superoxide dismutase (*sod1*) and mitochondrial superoxide dismutase (*sod2*) are indicators of oxidative stress from the cytosol (exogenous source) and mitochondria (endogenous source), respectively (Wang et al., 2018). Acute responses of the marine euryhaline European sea bass and milkfish when transferred to FW have been reported in previous studies focusing on osmoregulatory ability in

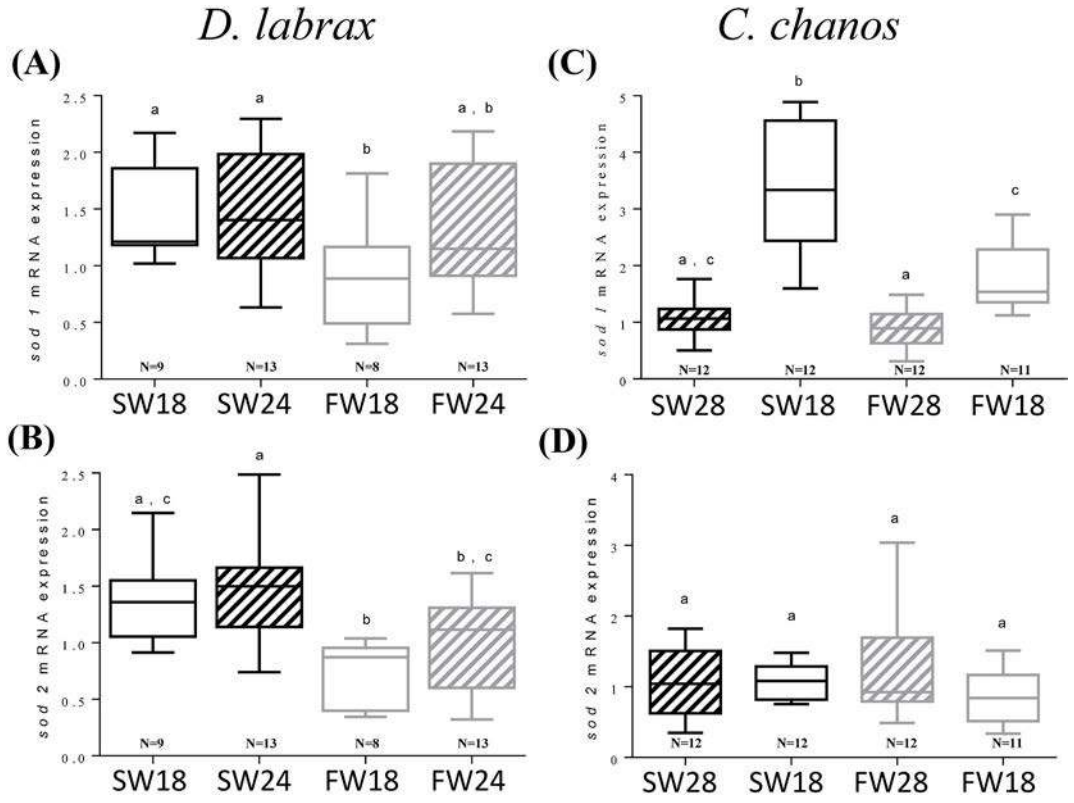

Fig. 2. Box and whiskers plot showing the median, minimum, and maximum mRNA expression levels of *sod 1* (A, C) and *sod 2* (B, D) in livers of *D. labrax* (A, B) and *C. chanos* (C, D) exposed to SW and FW at 18 °C and 24 °C (A, B) or 28 °C (C, D). mRNA levels were normalized to *ef1α*. Different letters indicate significant differences between conditions (two-way ANOVA followed by a Fisher Least Significant Difference (LSD) post hoc test, $p < 0.05$). FW, fresh water; SW, seawater.

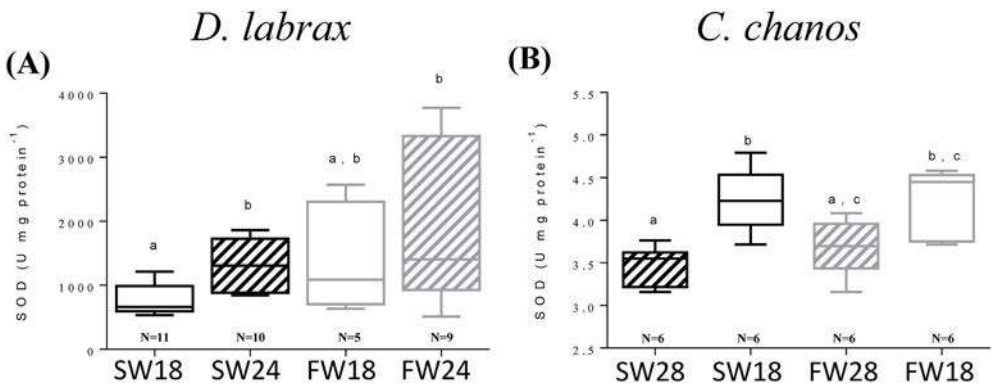

Fig. 3. Box and whiskers plot showing the median, minimum, and maximum SOD activity levels (U/mg proteins) in *D. labrax* (A) and *C. chanos* (B) exposed to SW and FW under hyperthermal acclimation (A) or hypothermal acclimation (B). Different letters indicate significant differences between conditions (Mann-Whitney test, $p < 0.05$). FW, fresh water; SW, seawater.

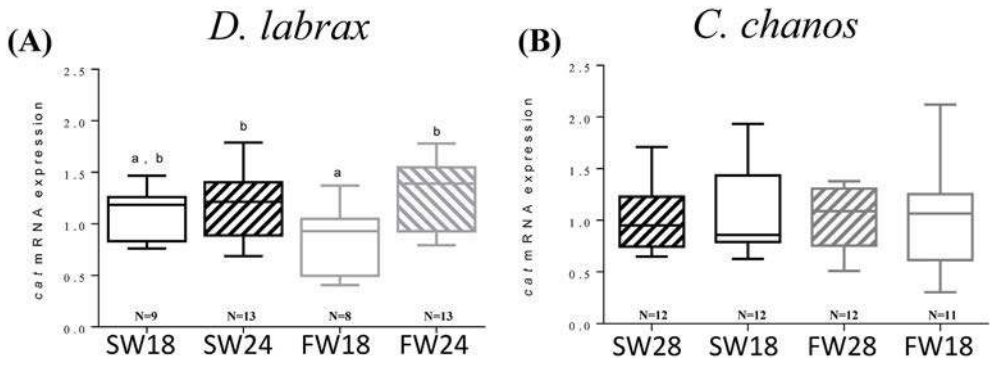

Fig. 4. Box and whiskers plot showing the median, minimum, and maximum catalase mRNA expression in *D. labrax* (A) and *C. chanos* (B) exposed to SW and FW at 18 °C and 24 °C (A) or 28 °C (B). mRNA levels were normalized to *ef1α*. Different letters indicate significant differences between conditions. FW, fresh water; SW, seawater. Two-way ANOVA followed by a Fisher Least Significant Difference (LSD) post hoc test, $p < 0.05$).

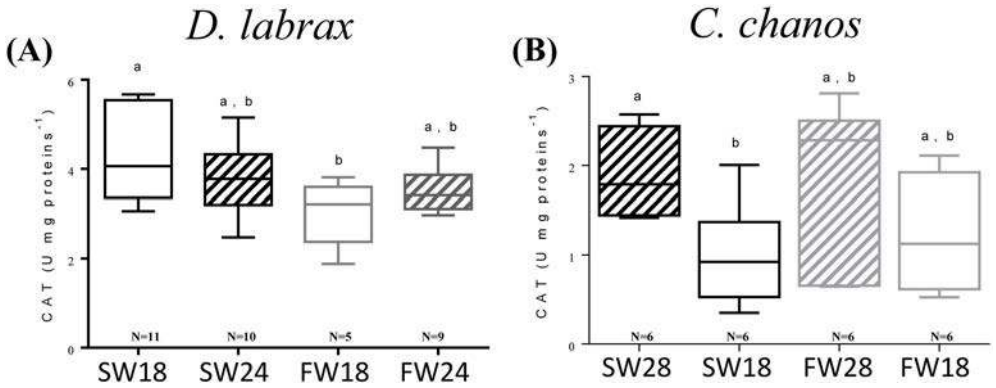

Fig. 5. Box and whiskers plot showing the median, minimum, and maximum CAT activity levels (U/mg proteins) in *D. labrax* (A) and *C. chanos* (B) exposed to SW and FW at 18 °C and 24 °C (A) or 28 °C (B). Different letters indicate significant differences between conditions (Mann-Whitney test, $p < 0.05$). FW, fresh water; SW, seawater.

gills as well as responses to oxidative stress in the liver (Kang et al., 2015; Sinha et al., 2015; Masroor et al., 2019; Chang et al., 2020; Islam et al., 2020). Sinha et al. (2015) reported that SOD and catalase (CAT) activities in the livers of European sea bass were not changed and downregulated, respectively, under short-term hypoosmotic challenge from 32 to 2.5‰. Synergic effects were observed when the hypoosmotic challenge was combined with ammonia treatment. In this study, hepatic SOD and CAT activity of the European sea bass acclimated to hypoosmotic environments revealed the same patterns. The gene expression of *sod* isoforms, however, was downregulated, which might have resulted from the negative feedback mechanisms that regulate the expression of *sod* isoforms (Wang et al., 2018). In milkfish, the acute salinity challenge did not affect oxidative stress or antioxidant mechanisms (Chang et al., 2017; 2020). Increased antioxidant responses, however, were usually found in the other euryhaline species upon hypoosmotic challenge at normal temperature. In the black porgy (*Acanthopagrus schlegeli*), genes and enzyme activities of SOD and CAT were upregulated after 1 day of hypoosmotic challenge (An et al., 2010). In the cinnamon clownfish (*Amphiprion melanopus*), enzyme activities of SOD, CAT, and glutathione peroxidase (GPx) were upregulated during

two days of hypoosmotic challenge (Park et al., 2011). In the rainbow trout (*Oncorhynchus mykiss*), hepatic CAT activity elevated but SOD activity did not change under two-days hypoosmotic challenge (Blewett et al., 2017). Moreover, all antioxidant responses in livers of the marine olive flounder (*Paralichthys olivaceus*) was increased under one- and two-week hypoosmotic stress (Kim et al., 2021). The long-term salinity changes in European seabass and milkfish did not affect their oxidative status, according to the enzyme activities of SOD and CAT. Compared to the other euryhaline species, the antioxidant capacity in the liver of European sea bass and milkfish was thus suggested to have the ability to maintain homeostasis when transferred to hypoosmotic environments.

4.2. Long-term temperature effects on antioxidant responses

Temperature is an important abiotic factor that leads to oxidative stress in aquatic organisms (Chowdhury and Saikia, 2020). Pörtner et al. (2007) reported that aquatic organisms under hyperthermal or hypothermal challenges increase their oxidative stress levels and induce antioxidant mechanisms due to the conceptual model of thermal limitation (Pörtner et al., 2007). In studies on teleosteans, the gene expression or enzyme activity of antioxidant mechanisms or oxidative damage markers are common indicators used to determine the redox status under short-term or long-term temperature treatments. The cold-water fish *Schizothorax prenanti* acclimated at 11 °C was determined to have an optimal raising temperature of 21 °C based on SOD and CAT activity as well as on the contents of MDA (Malondialdehyde; a marker for lipid peroxidation) (Yang et al., 2018). The mRNA expression profiles of the antioxidant mechanisms, including *sod* and *cat* in the liver of the pufferfish (*Takifugu obscurus*) under 1 h hot- or cold-temperature stress were upregulated, leading to apoptotic responses (Cheng et al., 2018a, 2018b). In the livers of the yellow drum (*Nibea albiflora*), the mRNA expression profiles of total *sod* isoforms (cytosolic superoxide dismutase, sod1; mitochondrial superoxide dismutase, sod2, extracellular superoxide dismutase, sod3) revealed that the elevated mRNA expression of *sod1* was highly related to upregulated SOD activity under hypothermal stress (Song et al., 2019). The thermal sensitivity of mitochondria in teleosts has been suggested to increase oxidative stress under hypothermal and hyperthermal stress (O'Brien, 2010; Banh et al., 2016). Most studies on temperature-induced oxidative stress in teleosts only determined one *sod* isoform as an indicator, either cytosolic or mitochondrial (Yang et al., 2018; Chang et al., 2017; Yu et al., 2017; Cheng et al., 2018b). However, in the present study, we highlight the interest in simultaneously carrying the analyses of both *sod* isoforms to provide a better understanding of the source of oxidative stress (Song et al., 2019; Maslanka et al., 2020).

During the four-week temperature treatments, the cytosolic and mitochondrial isoforms of *sod* had different responses in European sea bass and milkfish. In European sea bass, the expression of *sod* isoforms did not change under four-week hyperthermal stress, but SOD activity was upregulated under long-term heat stress in SW. Although the differences in the FW group were not significant, this might be due to the different ability of individuals to tolerate long-term hyperthermal challenge. Upregulation of CAT and glutathione peroxidase (GPx) activity as well as enhanced lipid peroxidation were reported in the liver of European sea bass under heat stress (25 °C) for 30 days (Almeida et al., 2015). In the present study, however, CAT activity did not change during four weeks (28-days) of hyperthermal stress (24 °C). The results suggested that 24 °C might be a critical temperature for European sea bass or dependent on fish size, leading to different abilities of temperature tolerance (Dahlke et al., 2020). In addition, short-term experiments might provide some information about the source of ROS for future studies. In milkfish, the mRNA expression of cytosolic *sod* showed synergistic interaction with temperature and salinity factors, while the mitochondrial *sod* did not show any change even upon acute hypothermal challenge (Chang et al., 2021). This finding suggested that the source of ROS was from the cytosol, because ROS induced extrinsic pathway of apoptosis in milkfish livers (Chang et al., 2021). The cytosol contains NADPH oxidase, the major ROS-producing enzyme correlated with immune responses under hypothermal stress (Singh et al., 2017; Kaushal et al., 2019). Therefore, immune responses of both FW and SW milkfish were enhanced by the upregulation of cytosolic SOD and the extrinsic apoptosis pathway from one week to one month under hypothermal stress (Chang et al., 2017, 2020). The *cat* expression profiles and CAT activity did not change after weeks of hypothermal stress. However, similar to the pattern of the European sea bass, some individual differences in enzyme activity under low-temperature treatment were found in the FW group.

Taken together, the results of this study revealed that changes in ambient temperatures enhanced responses of antioxidant enzymes in the livers of two marine euryhaline aquaculture species in different climate zones. After hypoosmotic challenge, variability in the responses was found in the FW group of both milkfish and European sea bass. Although synergistic responses of temperature and salinity were not found, ambient temperatures appeared to be the major factor regulating the activities of SOD in these two species. Meanwhile, milkfish may be more sensitive to the regulation of gene expression of antioxidative enzymes than European sea bass under long-term temperature stress, and the ROS may be derived from the cytosol. These two species have similar patterns of antioxidative mechanisms under four-week hyper- or hypo-thermal stress. The European sea bass, however, has more oxidative damage upon hyperthermal stress when acclimated to FW. This could be due to differential acclimation capacities in European sea bass from the same genetic lineage (Nebel et al., 2005). This study compared temperature-induced oxidative stress in the livers of two euryhaline species by elevated antioxidant mechanisms, providing a better understanding of future applications in their preferred aquaculture environments.

Declaration of competing interest

No conflicts of interest are declared by the authors.

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