



Stress in rearing: Biochemical and growth impacts of acoustic exposure in juvenile *Dicentrarchus labrax*

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ABSTRACT

Acoustic pollution is an emerging concern in aquaculture, where continuous exposure to noise can affect the welfare of farmed fish. Juvenile fish may be more susceptible to acoustic disturbances during their growth phases, which could potentially affect their physiological responses. The European seabass (*Dicentrarchus labrax*) is an important species in aquaculture due to its economic value and widespread farming, making it a relevant model for studying the effects of acoustic pollution on farmed species. This study aimed to evaluate the short-term effects (30 min, 3 h, 24 h, and 7 days) of traffic noise exposure on the cellular responses of juvenile *Dicentrarchus labrax*, using whole-body analyses. Acoustic emissions were recorded from four different fishing vessels at distances between 10–15 m, with recordings played back randomly separated by 10 to 30-second intervals of silence between consecutive emissions. The biomarkers tested included heat shock protein 70 (HSP70), superoxide dismutase (SOD), glutathione peroxidase (GPx), adrenocorticotrophic hormone (ACTH), cortisol, glucose, esterase, and alkaline phosphatase. The results revealed significant alterations in these parameters after just 30 min of exposure, with a tendency toward recovery after 7 days. Furthermore, a statistically significant reduction in growth rates was observed in exposed individuals. Although these findings may not apply to all species of farmed fish, they provide valuable insights into minimizing acoustic impacts and improving animal welfare in aquaculture environments.

1. Introduction

Growing global demand for seafood, combined with the progressive decline of natural stocks and the effects of climate change on aquatic ecosystems, is making aquaculture's contribution to ensuring food security and helping to reduce pressure on marine ecosystems increasingly important (Verdegem et al., 2023; FAO, 2024; Troell et al., 2014; Poore and Nemecek, 2018; Golden et al., 2021). In this context, it is important to ensure sustainable production by adopting husbandry practices that promote animal welfare (Ashley, 2006). In line with this objective, in recent years aquaculture facilities have implemented strategies to improve animal welfare and production sustainability (Holmer, 2010; Pelegri et al., 2006; Khan et al., 2021). However, despite growing attention to key stressors, the impact of noise in aquaculture environments remains poorly studied. This becomes even more relevant when considering onshore and offshore facilities, which inevitably also influence the marine environments in which they are

located. In intensive aquaculture systems, the continuous operation of pumps, aerators, and filtration systems represents a major source of low-frequency underwater noise (Wysocki et al., 2006; Davidson et al., 2009; Craven et al., 2009; Gutscher et al., 2011; Anderson, 2013; Barnes et al., 2015; Kathy et al., 2016; Voorhees and Barnes, 2017; Lara and Vasconcelos, 2018; Radford and Slater, 2019). In offshore systems, animals are exposed to both natural marine sounds and anthropogenic noise from marine traffic, including commercial vessels and fishing vessels operating in close proximity to breeding cages (Wysocki et al., 2007; Davidson et al., 2009; Radford and Slater, 2019). Comparing noise across aquaculture production systems, ambient noise in both recirculating aquaculture systems (RAS) and net pens lay in the 100 to 500 Hz range. The main pattern is that open cages and net pens are usually more variable and often louder in the 100 to 500 Hz, driven by marine traffic, while in recirculating aquaculture systems is

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typically more continuous, equipment-driven, and louder for the low-frequency at 20 to 100 Hz range (Radford and Slater, 2019; Slater et al., 2020). The unresolved part is not whether systems differ, but how those different soundscapes translate into chronic welfare and production effects across species and life stages. These aspects are particularly relevant considering the crucial role of sound in the survival of aquatic organisms: acoustic signals between 50 Hz and 10 kHz are essential for communication, reproduction, feeding, and defense (Chahouri et al., 2021). In fish, prolonged exposure to high levels of noise can induce chronic stress, with consequences on physiology, growth, and survival (Banner and Hyatt, 1973; Davis and Schreck, 1997; Lara and Vasconcelos, 2021). Anthropogenic noise has also been associated with alterations in hearing and behavior, as well as effects on reproduction and growth performance (Sverdrup et al., 1994; Mardi et al., 1996; Santulli et al., 1999; McCauley et al., 2003; Kunc et al., 2016). Focusing on the physiological level, the stress response in these organisms involves a complex cascade of cellular and humoral changes. Activation of the neuroendocrine axis leads to increased corticosteroids, particularly cortisol, and catecholamines, while secondary responses include the induction of heat shock proteins and antioxidant enzyme systems (Sapolsky et al., 2000; Schreck and Tort, 2016; Tudorache et al., 2015). Although several studies have investigated the effects of acoustic stress in adult fish, often considered more sensitive due to their swim bladder characteristics (Casper et al., 2013), results remain mixed. In several species, including common carp, gudgeon (*Gobio gobio*), and European perch (*Perca fluviatilis*), ship noise induces increased cortisol levels, suggesting activation of the stress response (Wysocki et al., 2007). However, physiological effects are variable: while some studies report alterations in blood parameters, enzymatic activity and antioxidant defenses, others do not highlight significant changes, as observed in *Salmo salar* exposed to typical aquaculture noise (Slater et al. 2020). Conversely, negative effects on antioxidant defenses and immune function have been described in *Micropterus salmoides*, highlighting species-specific responses that are still difficult to compare and generalize (Hang et al., 2021; Zhang et al., 2022). Further evidence in *Sparus aurata* exposed to noise typical of coastal aquaculture shows alterations in oxidative status and immune response, with variations in lysozyme activity, antiproteases, leukocyte count, and albumin/globulin ratio (Filiciotto et al., 2017). However, these results are part of a still fragmented context, in which the effects of noise on physiological parameters are often inconsistent across different studies and species. The response to noise stress also appears to be modulated by multiple factors — individual, environmental, and related to adaptive capacity — that have not yet been systematically standardized or integrated into different experimental models (Koolhaas et al., 1999; Øverli et al., 2007; Conrad et al., 2011). Similarly, biological variables such as body size and physiological status appear to influence noise sensitivity (Engås et al., 1996; Kastelein et al., 2017; Purser et al., 2016), but their role has not yet been quantified in a comparable way across different species. Furthermore, although it has been suggested that continuous noise may cause more severe effects than intermittent noise (de Jong et al., 2020), evidence remains limited and difficult to compare, especially in aquaculture environments characterized by prolonged and multifactorial exposures. The duration of exposure is also crucial: in *Dicentrarchus labrax*, Spiga et al. (2017) observed an acute response to stress, with an initial increase in the frequency of opercular beats followed by an attenuation over time, potentially interpretable as adaptation. However, it is still unclear whether this attenuation reflects a true physiological adaptation or rather a desensitization with possible biological costs that are not immediately evident. Overall, available evidence indicates that different sound sources and intensity levels can induce heterogeneous physiological responses (Hawkins et al., 2015), but the strong variability between species and experimental conditions limits the ability to draw generalizable conclusions. In this context, juvenile fish stages represent a particularly sensitive period. At this

stage, increased vulnerability is generally associated with high energy requirements, ongoing developmental processes, and an immature capacity to cope with environmental stress. Even the structure and function of the swim bladder reveal a mixed picture regarding acoustic sensitivity: while some authors suggest greater vulnerability in adult fish in relation to bladder size (Casper et al., 2013), others highlight that the coupling between the bladder and otoliths can reduce with growth and that the bladder itself can attenuate vibrations thanks to its viscoelastic properties, making juveniles the most sensitive (Fine et al., 2016; Parmentier and Fine, 2016; Salas et al., 2019). Furthermore, during early development, the auditory system develops within a few days of hatching and undergoes rapid morphological and functional changes, making the juvenile stages particularly relevant for assessing sound sensitivity (Leis et al., 2011). Overall, early life stages constitute a critical period in which physiological and sensory alterations can have long-term effects on fitness.

D. labrax is a euryhaline and eurythermic species belonging to the Moronidae family, characterized by a remarkable capacity to adapt to diverse environmental conditions. It is widespread throughout the North Atlantic, the Mediterranean, and the Black Sea, inhabiting various demersal habitats up to 100 m deep (Varsamos et al., 2001). This study aimed to evaluate the physiological response of juvenile *D. labrax* exposed to acoustic stress due to well-characterized non-regular intermittent vessels noise over different time intervals (30 min, 3 h, 24 h, and 7 days) through an integrated analysis of endocrine, metabolic, protein, and enzymatic markers at the whole-organism level. In particular, it is hypothesized that noise exposure induces the activation of the primary stress response, with variations in cortisol and ACTH levels, indicative of activation of the hypothalamic–pituitary–interrenal (HPI) axis. At the same time, an increase in glucose levels is expected, as an indicator of energy reserve mobilization, and the induction of HSP70 as a protective cellular response to stress. It is also hypothesized that noise stress alters the redox balance, resulting in changes in reduced glutathione (GSH) levels and the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase, as well as changes in the activity of esterases and phosphatases, considered indicators of metabolic and cellular alterations. Overall, it is hypothesized that noise exposure induces an integrated systemic response, detectable through coordinated changes in endocrine, metabolic, antioxidant, and cellular biomarkers, and potential effects in growth indicators.

2. Materials and methods

2.1. Experimental plan

A total of 144 juvenile *Dicentrarchus labrax* (1.5 ± 0.5 g, 4.7 ± 1.0 cm) individuals sourced from a commercial aquaculture farm were used for the experimental plan carried out at the Polytechnic University of Valencia (UPV, Gandia Campus, Spain). The fish were maintained in a tank with internal dimensions $137 \times 108 \times 71$ cm (Length $\times$ Width $\times$ Height) with filtered and recirculated sea water (salinity 36.5 ppt) with a constant temperature of 19 ± 1 °C and a dissolved oxygen of 8 mg/L, in a natural photoperiod and fed with commercial pellets (0.5% of their body weight). The feeding protocol was identical across all groups (experimental, control, and reservoir): a fixed daily ration of pellets (pellet size=1 mm) was administered to all tanks at the same time, at a rate of 0.45 mg of pellet per 1 g of fish, once per day, which the fish ate voluntarily.

Before starting the experimental procedure, the animals were transferred to the corresponding experimental tank. Each group was located in a separate individual tank, which was identical to the maintenance tank. Animals were not fed for 24 h prior to the experiment. For the experimental setup, a cylindrical net with 40 cm diameter and a submerged height of 60 cm was used to maintain the fish with a density of 0.3 kg/m³. Then animals were subjected to acoustic stress for 30 min, 3 h, 24 h, and 7 days. For each time point, three replicates

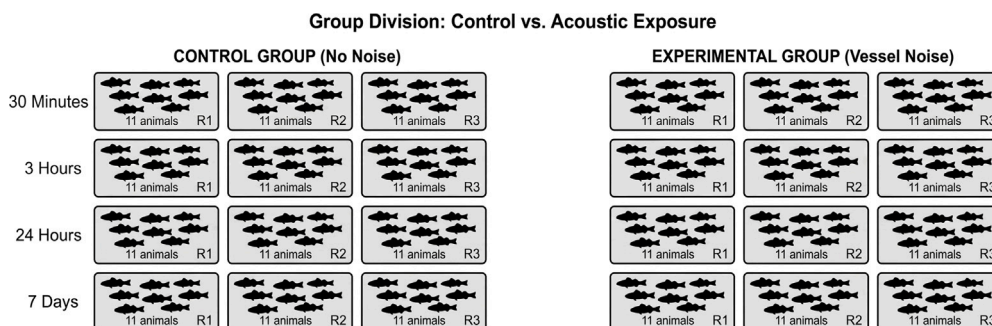


Fig. 1. Schematic representation of the experimental design and group distribution across tanks. Juvenile *D. labrax* were divided into Control and Experimental (noise-exposed) groups, with three replicates (R1, R2, R3) per time point (30 min, 3 h, 24 h, and 7 days). Each replicate tank contained 11 individuals.

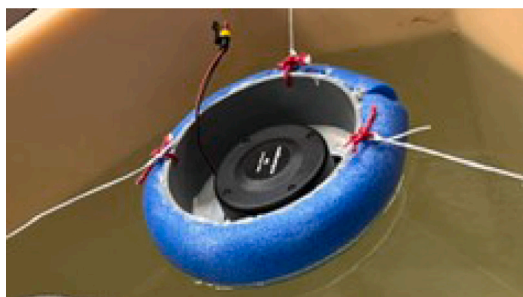


Fig. 2. The floating Distributed Mode Loudspeaker (DML) prototype used for acoustic playback positioned in a corner of the experimental tanks.

were performed and three control groups were included. Each replica corresponding to an experimental or control group, was located in a separate and individual tank with 11 individuals (see Fig. 1). All the replicas corresponding to the same time interval ran simultaneously, to avoid interference with any environmental factor. After the considered time interval, all the individuals were removed from the tank to be anesthetized and sampled. All the individuals were measured and weighted, while 6 animals per replicate (18 animals in total for each experimental duration and the corresponding 18 animal for the paired control replicas) were used in the biochemical analyses.

All procedures were conducted in accordance with institutional guidelines and were approved by the Ethics Committee of the Universitat Politècnica de València (approval reference: P08_27-05-2025).

2.2. Acoustic emission

Noise was generated inside the tanks using an electroacoustic system composed of a digital acquisition card (DAQ), an amplifier (FA-TE370 Sony Integrated Stereo Amplifier, Sony Corporation, Tokyo, Japan), and a cost-effective Distributed Mode Loudspeaker DML (prototype UA-UPV) placed floating in a corner of the experimental tank without contact with the walls (Fig. 2). The loudspeaker was previously calibrated with a reference hydrophone (Reson TC4034, Teledyne Reson, Slangerup, Denmark).

Acoustic recordings from four bottom-trawl fishing vessels were randomly broadcast. The recordings were previously obtained using a SoundTrap 300 compact self-contained underwater sound recorders (Ocean Instruments, Auckland, New Zealand) off the coast of Murcia, Spain. The characteristics of the vessels, which passed at a minimum distance of 10–15 m from the recording device, are summarized in Table 1. The recordings ranged from 90 to 150 s in duration and covered a frequency bandwidth between 50 and 2000 Hz. To simulate a high-density maritime traffic scenario comparable to that found in the vicinity of a harbor during the departure and arrival periods of the fishing fleet, while also preventing potential biases caused by fish

Table 1

Principal characteristics of the fishing vessels used in the experiments.

Ship	Material	Length (m)	Power (CV)	Speed	Distance (m)
1	Wood	18.25	139.9	8.4	10
2	Polyester	17.00	128.5	7.2	14
3	Wood	20.50	165.0	10	12
4	Polyester	22.70	399.9	8.5	15

habituation to a predictable temporal noise pattern (Neo et al., 2014; Wong et al., 2022), the vessel sounds were separated by randomly assigned silent intervals ranging from 10 to 30 s.

The acoustic field was characterized in terms of the power spectral density (PSD), the sound pressure level (SPL), and the Sound Exposure Level (SEL). Fig. 3 shows the power spectral density spectrum and the sound pressure level.

The Sound Exposure Level (SEL), Fig. 4 was calculated following the ISO 18405:2017 standard, representing the integral of the squared sound pressure over a stated time interval T , normalized to a reference duration of 1 s ($\text{dB re } 1 \mu\text{Pa}^2\text{s}$). Time interval was considered 1 h.

To prevent acoustic transmission between tanks, tank supports were isolated from the floor with an acoustic absorber to reduce vibration transfer. A preliminary test was conducted by broadcasting recordings in one experimental tank (ET-test) while monitoring sound in the others. Comparisons between recordings with and without emission confirmed no detectable transmission. To ensure a sufficiently homogeneous sound field within the cylindrical net cage, the acoustic field was first mapped by measuring the sound pressure level (SPL) at eight different locations within the water volume using a hydrophone (Aquarian AS-1). These measurements were used to identify the natural modes of the enclosure and to validate a numerical fluid–structure interaction (FSI) model. A detailed analysis of the sound pressure field within the fish enclosure was then performed using the validated model, showing that the standard deviation of the SPL remained below 1 dB across the range of measurement depth for a fixed frequency. During the experiments, each tank was continuously monitored using an independent Aquarian AS-1 hydrophone.

2.3. Animal sampling and analysis

At the end of each experimental time point, fish were euthanized using tricaine methanesulfonate (MS-222) and stored at -20°C . Tissue extracts were subsequently prepared by homogenization in 1X phosphate-buffered saline (PBS, pH 7.4). The levels of cortisol, heat shock protein 70 (HSP70), superoxide dismutase (SOD), glutathione peroxidase (GPx), and adrenocorticotrophic hormone (ACTH) were assessed using ELISA Kits (Cusabio). Additionally, parameters including glucose, esterase, and alkaline phosphatase (ALP) were evaluated. Glucose levels were determined using Gluco Test (Pic). ALP and Esterase activities were quantified following Ross et al. (2000). The ALP reaction

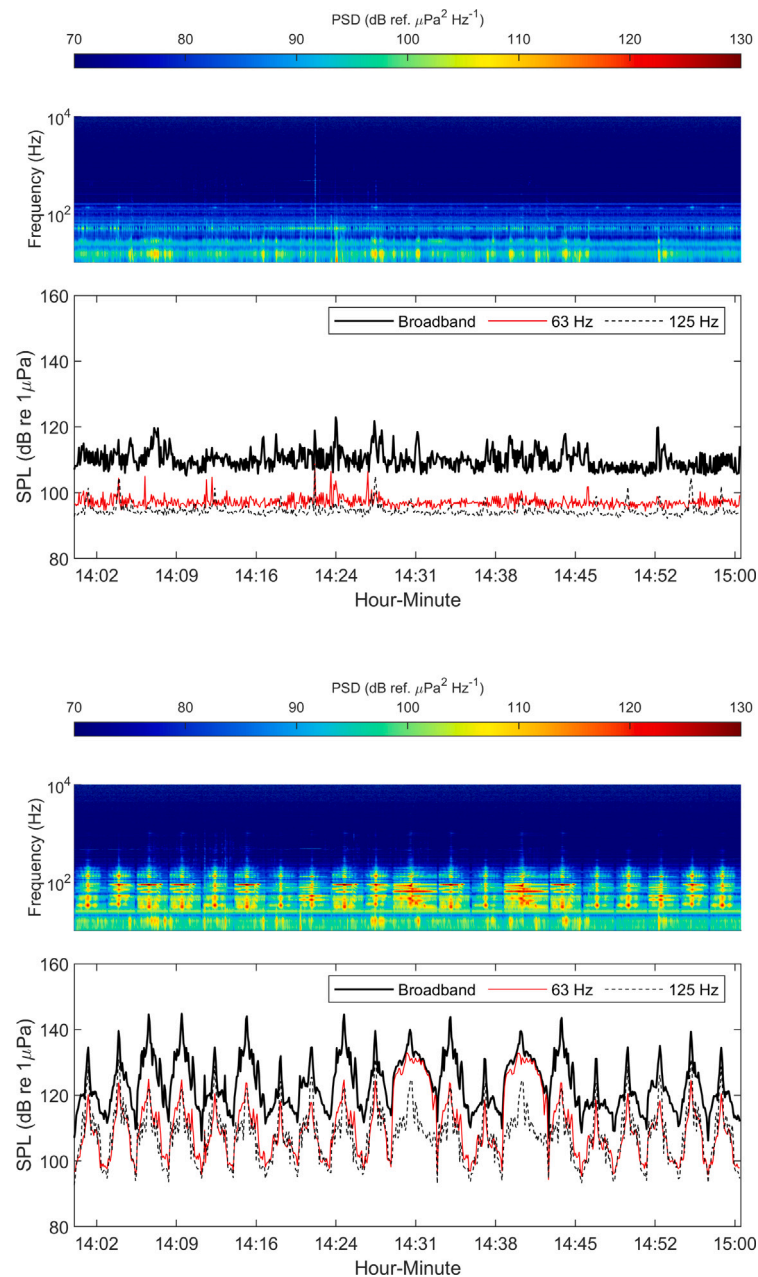


Fig. 3. Power spectral density (PSD) spectrograms and sound pressure levels (SPL) recorded over a 1-hour interval in control (top) and experimental (bottom) tanks. The plots display broadband SPL (20 Hz–20 kHz, black line) alongside 1/3-octave band levels at 63 Hz (red line) and 125 Hz (dashed black line), illustrating the intermittent nature of the broadcasted fishing vessel noise. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mixture contained 4 mM p-nitrophenyl phosphate (Sigma, Italy) in a 100 mM ammonium bicarbonate buffer 1 mM MgCl₂, pH 7.8, 30 °C. Esterase activity was assessed using 0.4 mM p-nitrophenyl myristate in a 100 mM ammonium bicarbonate buffer (0.5% Triton X-100, pH 7.8, 30 °C). Optical density (O.D.) was monitored at 405 nm using a GloMax™ Multi Detection System (Promega). Enzyme activity was expressed as U/μg of protein.

2.4. Growth performance

After being euthanized, individuals were weighed using a high-precision balance (Ohaus Navigator, accuracy: 0.1 g), and the length measured using a caliper (accuracy: 0.1 cm). The growth performance was evaluated by considering the evolution of fish weight and length, in

terms of three growth rates: absolute growth rate, relative growth rate and specific growth rate. The absolute growth increase $\Delta M(t_f)$ is defined as the difference between the final individual (M) measurement at final time t_f and the mean baseline measurement $\overline{M}(t_i)$ measured at the initial time $t_i = 30$ min. The variable M refers to the weight or length, depending on the growth variable under consideration (Lugert et al., 2016). The absolute growth increase is defined as:

$$\Delta M(t_f) = M(t_f) - \overline{M}(t_i) \quad (1)$$

The absolute growth rate (AGR) includes the temporal information

$$AGR_M = \frac{\Delta M(t_f)}{(t_f - t_i)} \quad (2)$$

where M refers to the weight/length, being AGR_W measured in g/day and AGR_L measured in cm/day. The Relative Growth Rate (RGR),

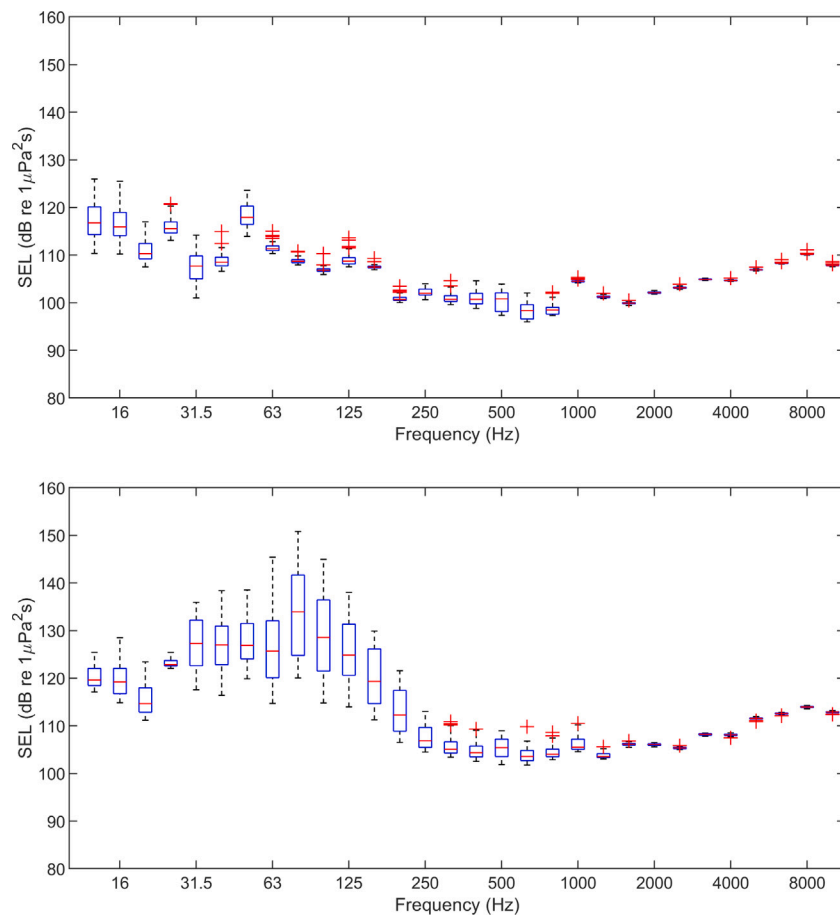


Fig. 4. Box plots of 1/3-octave Sound Exposure Levels (SEL) for a 1-hour temporal interval ($T = 1$ h) in control (top) and experimental (bottom) tanks. The boxes represent the interquartile range and the red lines indicate the median SEL across the frequency spectrum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expressed as a percentage increase, provides the absolute increase in relation to the initial weight/length at a fixed duration time and was calculated as (Lugert et al., 2016):

$$RGR_M = \frac{M(t_f) - M(t_i)}{M(t_i)} \times 100. \quad (3)$$

Finally, although the specific growth rate (SGR) relies on the absolute growth rate, it uses the natural logarithmic functional relation. Expressed in percentage increase per day, the mathematical expression for SGR is:

$$SGR_M = \frac{\log(M(t_f)) - \log(\bar{M}(t_i))}{(t_f - t_i)} \quad (4)$$

being the units % per day. The growth study utilized a destructive, cross-sectional, time-course design because the biochemical analyses required terminal sampling. We evaluated whether exposed groups exhibited a lower group-level relative growth rate than controls, assuming the groups were comparable at baseline. Growth metrics were calculated from initial and final group means rather than from the same terminally sampled individuals. Therefore, growth differences reflect comparisons between independent groups, rather than repeated measures on the same fish (Opinion et al., 2023).

2.5. Statistical analysis

Three replicates were performed for each duration. Normality was assessed with the Shapiro–Wilk test. For normal distributions, a one-way ANOVA was conducted to compare groups (control, 30', 3 h, 24 h, and 7d). When non-normality was detected, a Kruskal–Wallis test was

used. Comparisons between specific experimental times and the control group were performed using the Mann–Whitney U test (for medians) or post-hoc tests (for means). Significant differences were considered at $p < 0.05$. All statistical analyses were executed using (R Core Team, 2022) and Wickham (2016) for plotting the results. For the biochemical analyses, the control groups were compared to each other, with no statistically significant differences over different times. Therefore, for clarity, a unique control value was considered for further comparison with the experimental groups. For the growth studies, the experimental and control groups were compared at each sampling time.

3. Results

3.1. Biochemical analysis

Normality was verified for the majority of the datasets using the Shapiro–Wilk normality test. The results are presented in Table 2. In some cases, non-normality appears to be driven by outlier data points. Statistical significance was set at a 95.0% ($p < 0.05$) when the control and the experimental groups were compared. Results are shown in Fig. 5.

Whole-body analysis of HSP70 levels (Fig. 5(a)), expressed in pg/mL, revealed significant increases after 30 min of acoustic exposure (232.70 pg/mL; $p < 0.001$) compared to the control group (28.01 pg/mL). Significantly higher levels were also maintained three hours after exposure to acoustic stress. However, both after 24 h and 7 days, heat shock protein levels returned to baseline values similar to those observed in the control group.

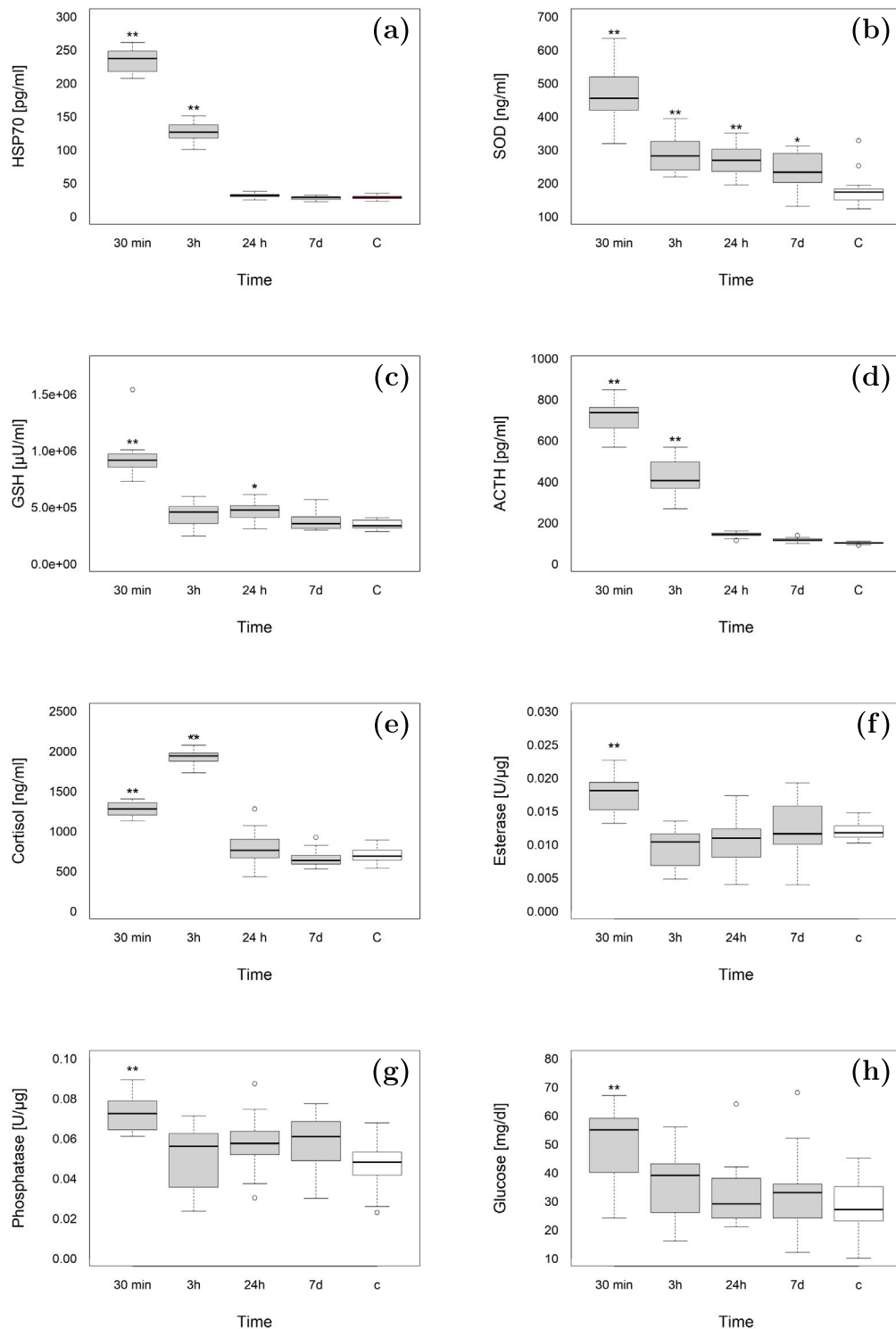


Fig. 5. Box-and-whisker plots showing the whole-body levels of (a) HSP70, (b) SOD, (c) GSH-PX, (d) ACTH, (e) cortisol, (f) esterase, (g) alkaline phosphatase, and (h) glucose in juvenile fish. Samples were collected at 30 min, 3 h, 24 h, and 7 days of exposure, alongside a unified control group (C). Asterisks indicate statistically significant differences relative to the control (* $p < 0.05$, ** $p < 0.01$).

Superoxide dismutase (SOD) levels (Fig. 5(b)), expressed in ng/mL, showed a significant increase after 30 min of acoustic exposure (456.36 ng/mL; $p < 0.001$). Although the values began to decrease after 3 h and 24 h, they remained significantly higher compared to the controls (178.18 ng/mL). After 7 days, the levels returned to those similar to the control group.

GSH-PX levels (Fig. 5(c)), expressed in μU/mL, showed a significant increase after 30 min of acoustic exposure (934,840 μU/mL; $p < 0.001$). The values began to decrease after 3 h and after 7 days, the levels returned to those similar to the control group.

ACTH levels (Fig. 5(d)), measured in pg/mL, exhibited a significant increase following 30 min of acoustic exposure (722.49 pg/mL; $p < 0.001$).

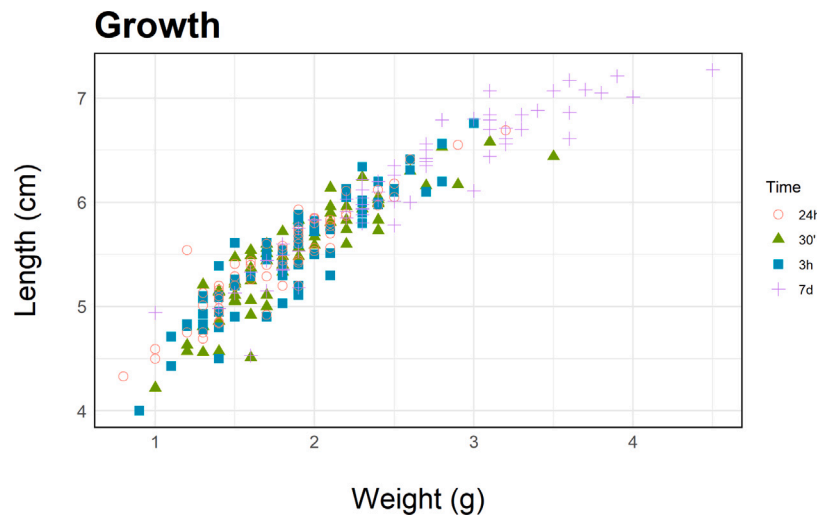


Fig. 6. Relationship between body weight (g) and length (cm) of fish across different sampling intervals. Data are categorized by sampling time: 24 h (red circles), 30 min (green triangles), 3 h (blue squares), and 7 days (purple crosses). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.001). Even though the levels started to decrease after 3 and 24 h, the values remained significantly elevated compared to the control group (99.83 pg/mL). By 7 days, ACTH levels had returned to baseline values, similar to those observed in the controls.

Cortisol levels (Fig. 5(e)), expressed in ng/mL, exhibited a significant increase after 30 min of acoustic exposure (1269.45 ng/mL; $p < 0.001$). Unlike the other parameters, cortisol levels peaked after 3 h of exposure (1931.86 ng/mL; $p < 0.001$). Following this, the values began to decrease after 24 h, and by the 7seventh day, the levels returned to those like the control group (701.51 ng/mL).

Esterase levels (Fig. 5(f)) measured in U/ μ g protein, exhibited a significant increase following 30 min of acoustic exposure (0.018 U/ μ g; $p < 0.001$) suggesting an early induction of esterase activity. In contrast, the 3 h, 24 h and 7 days groups showed a significant decrease, returning to levels similar to the control group's (0.012 U/ μ g); no significant differences were observed compared to the control group.

Alkaline phosphatase levels (Fig. 5(g)) measured in U/ μ g protein, exhibited the highest increase after 30 min of acoustic exposure (0.073 U/ μ g; $p < 0.001$), clearly surpassing the other groups. The 3 h and 24 h groups showed intermediate levels, with a decrease compared to the 30 min group. Despite the progressive decrease, they did not return to levels like those of the control group (0.047 U/ μ g).

Glucose levels (Fig. 5(h)) measured in mg/dL, exhibited the highest increase after 30 min of acoustic exposure (49.47 mg/dL; $p < 0.001$). Compared to the control group, the 3 h and 7 days groups also showed higher median values. However, the 24 h group showed levels similar to the control group. Results reveal a progressive decline from the peak at 30 min, with values remaining above the control group (27.47 mg/dL) in almost all treated groups.

3.2. Growth analysis

The fish were measured considering the variables weight (g) and length (cm). The scatter plot (Fig. 6) shows the positive relationship between weight and length, with increasing variability over time, as expected with the natural growth trend.

To evaluate the potential effect of vessel noise exposure on growth, two groups, one corresponding to the control and the other to the experimental condition ($N = 33$ individuals each), were compared at the different sampling times. Fig. 7 shows the results of the absolute weight and length at 30 min (initial) and 7 days (end of the experiment).

Statistically significant differences between the experimental and control groups were assessed using a one-way ANOVA test, and the

normality of the data was evaluated through a Shapiro–Wilk test. The statistical results regarding the absolute growth between groups show a trend toward reduced growth in the experimental group compared with the control group, Table 3, although without reaching statistically significant levels of association equal to or greater than 95%. However, when growth rates were explored, a statistically significant decrease was observed in the experimental group compared with the control group after seven days of the experiment (final time). While at the beginning no statistical significant difference was reported, after 7 days SGR , AGR (both for length and weight) and RGR_W indicated a significant decrease in absolute and temporal rates for the exposed groups ($p < 0.05$), see Table 4.

4. Discussion

Anthropogenic underwater noise is a form of marine pollution affecting the physiology and behavior of aquatic organisms. Early fish developmental stages are particularly vulnerable to environmental stressors, making it essential to understand the impacts of acoustic pollution during these critical phases (Popper and Hawkins, 2019; de Jong et al., 2020; Blom et al., 2024). Working in relatively small tanks (1 m³) can create complex and spatially variable sound fields due to resonance and reverberation, which may strongly bias animal exposure (Akamatsu et al., 2002; Rogers et al., 2016; Duncan et al., 2016; Carbajo et al., 2025). The analyses of the vibrational modes of the tanks and the mapping of the sound field can confirmed that the region where the fish were confined was acoustically homogeneous over the working frequency range (Carbajo et al., 2025; Duncan et al., 2016; Rogers et al., 2016). This explicit acoustical characterization of the tank addresses common criticisms of small-tank studies, where spatial variation in sound pressure and particle motion is often unknown or ignored (Akamatsu et al., 2002; Campbell et al., 2019; Olivier et al., 2023; Rogers et al., 2016; Popper and Hawkins, 2019). A prototype floating distributed-mode loudspeaker was used as a cost-effective underwater source. Its output was calibrated in situ, rather than assuming appropriate playback fidelity, in line with recommendations that the reproduced signal should always be measured and compared to the original (de Jong et al., 2017; Mosharo and Lobel, 2023). The use of a low-cost prototype compared to conventional underwater speakers, specifically designed for ex-situ tank experiments, allows for an increase in the number of replicates while maintaining a balance between the statistical robustness required in these studies and economic expenditure (de Jong et al., 2017), facilitating the expansion

Table 2

Data groups involved in the statistical analysis and results for the statistical analysis performed on each control-experimental pair. Normality was assessed using the Shapiro–Wilk test. Comparison tests (t-test or Mann–Whitney) were applied based on distribution.

Variable	Group	Shapiro–Wilk test (p)	Test	p -value (vs Ctrl)
Cortisol	Control	$p = 0.5692$	–	–
	30'	$p = 0.3535$	t-test	< 0.001 ***
	3 h	$p = 0.9278$	t-test	< 0.001 ***
	24 h	$p = 0.9619$	t-test	0.1242
	7d	$p = 0.1921$	t-test	0.1981
ACTH	Control	$p = 0.4208$	–	–
	30'	$p = 0.7382$	t-test	< 0.001 ***
	3 h	$p = 0.5089$	t-test	< 0.001 ***
	24 h	$p = 0.9619$	t-test	0.1242
	7d	$p = 0.9818$	t-test	< 0.001 ***
GSH	Control	$p = 0.1070$	–	–
	30'	$p = 0.0001$ (Non-normal)	Mann–Whitney	< 0.001 ***
	3 h	$p = 0.3510$	t-test	0.0024 **
	24 h	$p = 0.9223$	t-test	< 0.001 ***
	7d	$p = 0.0426$ (Non-normal)	Mann–Whitney	0.1682
HSP70	Control	$p = 0.9435$	–	–
	30'	$p = 0.3300$	t-test	< 0.001 ***
	3 h	$p = 0.7698$	t-test	< 0.001 ***
	24 h	$p = 0.9947$	t-test	0.0043 **
	7d	$p = 0.1705$	t-test	0.6561
SOD	Control	$p = 0.0014$ (Non-normal)	–	–
	30'	$p = 0.9573$	Mann–Whitney	< 0.001 ***
	3 h	$p = 0.3420$	Mann–Whitney	< 0.001 ***
	24 h	$p = 0.5827$	Mann–Whitney	< 0.001 ***
	7d	$p = 0.1403$	Mann–Whitney	0.0013 **
Esterase	Control	$p = 0.7420$	–	–
	30'	$p = 0.2356$	t-test	< 0.001 ***
	3 h	$p = 0.2174$	t-test	0.0024 **
	24 h	$p = 0.9660$	t-test	0.0963
	7d	$p = 0.8130$	t-test	0.7378
Glucose	Control	$p = 0.7420$	–	–
	30'	$p = 0.1244$	t-test	< 0.001 ***
	3 h	$p = 0.3286$	t-test	0.0260 *
	24 h	$p = 0.0116$ (Non-normal)	Mann–Whitney	0.4691
	7d	$p = 0.2033$	t-test	0.2987
Phosphatase	Control	$p = 0.7024$	–	–
	30'	$p = 0.1271$	t-test	< 0.001 ***
	3 h	$p = 0.0509$	t-test	0.3282
	24 h	$p = 0.9315$	t-test	0.0240 *
	7d	$p = 0.2684$	t-test	0.0171 *

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

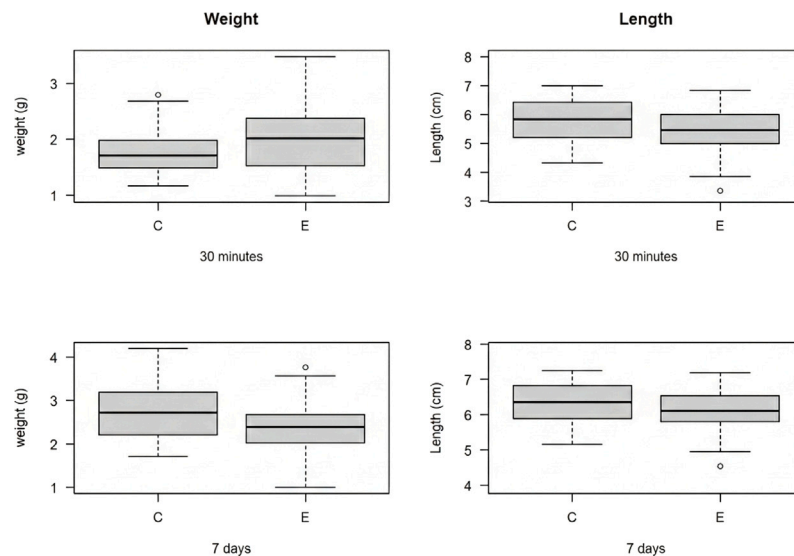


Fig. 7. Comparison of body weight (left) and total length (right) between control (C) and experimental (E) groups at the start (30 min) and conclusion (7 days) of the acoustic exposure period. No statistically significant differences in absolute measurements were observed between groups at either time point ($p > 0.05$).

Table 3

Length and Weight vs control. Normality assessed with Shapiro–Wilk test. Statistical test: ANOVA (both groups normal). No statistically significant differences ($p > 0.05$) were found.

Variable	Time	Mean (Min;Max)		Shapiro–Wilk (p)		p -value (Ctrl vs Exp)
		Ctrl	Exp	p(Ctrl)	p(Exp)	
Length (cm)	30'	5.45 (4.63;6.53)	5.60 (4.51;6.58)	0.867	0.150	0.392
	3 h	5.52 (4.00;6.76)	5.49 (4.43;6.56)	0.946	0.463	0.781
	24 h	5.43 (4.33;6.55)	5.51 (4.50;6.69)	0.834	0.993	0.586
	7d	6.32 (5.15;7.27)	6.06 (4.94;7.21)	0.118	0.737	0.101
Weight (g)	30'	1.80 (1.2;2.8)	2.01 (1.2;3.5)	0.051	0.453	0.095
	3 h	1.86 (0.9;3.0)	1.88 (1.1;2.8)	0.754	0.286	0.858
	24 h	1.77 (0.8;2.9)	1.82 (1.0;3.2)	0.262	0.103	0.677
	7d	2.79 (1.7;4.5)	2.44 (1.0;3.9)	0.249	0.804	0.060

Table 4

Growth rates RGR , AGR and SGR evaluated for weight (W) and length (L). Comparison between experimental and Control groups at each time point. Normality assessed with Shapiro–Wilk test. Statistical test: ANOVA (both groups normal).

Variable	Time	Mean		Shapiro–Wilk (p)		p -value (Ctrl vs Exp)
		Ctrl	Exp	p(Ctrl)	p(Exp)	
RGR_W (%)	3 h	3.45	−6.47	0.754	0.286	0.104
	24 h	−1.44	−9.46	0.262	0.103	0.191
	7d	55.09	21.55	0.249	0.804	0.001 *
RGR_L (%)	3 h	1.34	−1.47	0.946	0.463	0.251
	24 h	−0.30	−1.18	0.834	0.993	0.699
	7d	16.01	10.98	0.118	0.088	0.109
AGR_W (g/day)	3 h	0.496	−1.300	0.754	0.286	0.054
	24 h	−0.026	−0.223	0.262	0.103	0.094
	7d	0.141	0.062	0.249	0.892	0.003 *
AGR_L (cm/day)	3 h	0.586	−1.00	0.946	0.463	0.142
	24 h	−0.016	−0.109	0.834	0.993	0.460
	7d	0.125	0.073	0.118	0.624	0.024 *
SGR_W (% day ^{−1})	3 h	0.032	−0.937	0.636	0.232	0.060
	24 h	−0.046	−0.147	0.053	0.671	0.130
	7d	0.060	0.022	0.213	0.162	0.0005 *
SGR_L (% day ^{−1})	3 h	0.065	−0.219	0.361	0.469	0.156
	24 h	−0.007	−0.023	0.544	−0.999	0.476
	7d	0.020	0.012	0.066	0.349	0.002 *

of research on noise effects within controlled laboratory settings or ex-situ facilities (Olivier et al., 2023; Mosharo and Lobel, 2023). Although tank experiments remain subject to inherent limitations compared with open-water exposures (Rogers et al., 2016; Popper and Hawkins, 2019; Radford et al., 2016; Duncan et al., 2016), these methodological steps reduce major acoustic uncertainties identified in previous studies and strengthen the interpretation of the observed noise effects on juvenile fish.

In this study, we applied a whole-organism approach in juvenile *D. labrax*. While this method does not allow tissue-specific resolution, it is particularly appropriate during early developmental stages, when the small size of individuals limits organ-level sampling. Importantly, it provides an integrated overview of the organism's physiological status, capturing changes in key cellular markers and offering insight into the ability of young fish to cope with stress. The observed responses may reflect the activation of compensatory cellular mechanisms, in line with findings reported in other teleosts exposed to environmental stressors (Samara et al., 2024; Hayes et al., 2024).

Heat shock protein 70 (HSP70) is a molecular chaperone involved in cellular defense. It is essential for proper protein folding and repair and for preventing protein aggregation (Hassan et al., 2019; Hu et al., 2022). In fish, its expression has been widely documented in response to various stressors, including temperature changes, chemical contaminants, environmental disturbances, and anthropogenic noise (Tine et al., 2010; Jeyachandran et al., 2023). In the present study, the observed pattern indicates a rapid activation of the HSP70-mediated response following noise exposure, followed by a progressive return to baseline conditions within 24 h. This expression pattern suggests that, although noise exposure may have triggered an acute stress response,

juvenile *D. labrax* have the potential to activate compensatory mechanisms that could reduce cellular damage and support the restoration of homeostasis. Several studies have reported a significant increase in HSP production in fish exposed to various environmental stressors, such as rising temperatures and heavy metal contamination (Jeyachandran et al., 2023). In *Lateolabrax maculatus*, a strong induction of HSP70 expression was observed in the first hours of thermal exposure in several tissues, including gills, liver, and muscle (Sun et al., 2021), while in *Epinephelus coioides*, a time-dependent modulation of liver levels in response to acoustic stress has been reported (Lin et al., 2019). Similarly, exposure to ship traffic noise resulted in a significant increase in circulating HSP70 in *Sparus aurata* (Celi et al., 2016). Although differences between studies may reflect interspecies variability, developmental stage, exposure conditions, and the matrix analyzed, the overall picture is consistent with the early activation of the HSP70-mediated response following acoustic stress. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are key components of the endogenous antioxidant system and sensitive indicators of early cellular responses to environmental perturbations. In this study, whole-organism enzymatic activity showed a significant increase as early as 30 min after noise exposure, suggesting a rapid activation of cellular defenses potentially related to a transient redox imbalance rather than direct oxidative damage. Overall, the results are consistent with the literature describing a possible modulation of antioxidant defenses in different species exposed to noise, albeit with highly variable patterns. Increases in ROS levels have been reported in adult *Acanthopagrus schlegelii* (Chang et al., 2018), while similar responses have been observed in juvenile *Rutilus rutilus* and other marine species (Guh et al., 2021; Réalis-Doyelle et al., 2025). The decrease in enzymatic activity at later time points may

suggest a temporal modulation of the antioxidant response, as described in *Cyprinus carpio* (Techer et al., 2017), although such dynamics are difficult to generalize. Discrepancies with other studies, such as (Pan et al., 2025) on *Acipenser sinensis*, may be related to methodological differences, particularly the use of whole-organism samples compared with tissue-specific or blood analyses. Moreover, studies on liver tissue (Amorim et al., 2022; Wang et al., 2025) indicate that antioxidant responses to noise may vary depending on species and experimental conditions. Taken together, these findings suggest that the antioxidant system may represent one of the early responses to acoustic stress in fish, although this interpretation should be considered with caution given biological variability and the lack of direct measures of oxidative damage. In fish, the physiological response to stress is primarily regulated by the hypothalamic–pituitary–interrenal (HPI) axis, through a neuroendocrine cascade culminating in the release of cortisol, the main glucocorticoid in teleosts and a key mediator of homeostasis restoration (Mommensen et al., 1999; Herman et al., 2016; Tsalafouta et al., 2014). In the present study, the significant increase in ACTH and cortisol observed, with different temporal dynamics, is consistent with the coordinated but non-synchronous activation of the HPI axis. In particular, ACTH appears to reflect an early phase of the stress response, while cortisol represents the systemic and integrated component of the endocrine response. Overall, these results suggest that underwater noise can rapidly activate the neuroendocrine response in juvenile fish, in agreement with what has been reported in *D. labrax* exposed to piling noise (Debusschere et al., 2016) and in other fish species, although with variations related to species, age and characteristics of the acoustic stimulus (Wysocki et al., 2006; Celi et al., 2016; Johansson et al., 2016; Samaras and Pavlidis, 2022). The differences compared to other studies, such as that of Pan et al. (2025) in *Acipenser sinensis*, in which the increase in cortisol was observed only after 24 h, suggest that the kinetics of the endocrine response may vary significantly as a function of the experimental and biological context. In our case, the rapid return to basal levels within 24 h is consistent with a transient response, underlining the importance of considering multiple markers and different time windows for a correct interpretation of the stress response. The activity of hydrolytic enzymes, particularly alkaline phosphatase and esterase, involved in dephosphorylation and hydrolysis processes and in key functions such as metabolism, detoxification, homeostasis, and stress response, was also assessed (Vazzana et al., 2020a,b; Parisi et al., 2017). In the present study, alkaline phosphatase activity increased in the initial phases of acoustic exposure, suggesting a rapid physiological response to the stimulus, followed by a progressive trend toward control values at later times. Overall, these changes could indicate a transient modulation of enzymatic activity in the early stages of acoustic stress. In literature, increases in alkaline phosphatase activity have been reported in *Oncorhynchus mykiss* under thermal stress conditions (Li et al., 2016), while more complex and non-linear patterns have been described in response to acoustic stress, as observed by Pan et al. (2025) in *Acipenser sinensis*. Furthermore, available evidence suggests a strong species-specific variability: Wang et al. (2025) reported different responses between *Acanthopagrus schlegelii* and *Larimichthys crocea* exposed to underwater wind turbine noise, indicating that physiological resilience may influence the extent of enzymatic variations. Similarly, to what observed for alkaline phosphatase activity, esterase activity also showed a significant increase already after the onset of acoustic exposure, returning to control values at subsequent experimental times. This could indicate an early and rapid induction of this enzymatic system. Similar results have been reported under chemical stress conditions in the same species (Sanahuja et al., 2020). Consistent with our data on hydrolase activity, Vazzana et al. (2020b) observed a significant increase in the levels of these enzymes in the coelomic fluid of *A. lixula* exposed to acoustic stress. In juvenile fish exposed to acoustic stress, such changes may reflect the activation of physiological processes involved in the handling of reactive metabolites, the modulation of cellular signaling

pathways related to detoxification, and acute metabolic adjustments associated with early stress responses, potentially contributing to the maintenance of cellular homeostasis (Rothman et al., 2023). Glucose is one of the most widely used biomarkers for assessing the physiological response to stress in fish, as its mobilization is closely associated with activation of the hypothalamic–pituitary–interrenal axis and cortisol release (Mommensen et al., 1999). In the present study, the increase in glucose levels observed early in the exposure could reflect an early metabolic response to the acoustic stimulus. However, this interpretation remains indirect and does not allow us to definitively attribute the hyperglycemia to an increase in energy demand, as the underlying metabolic processes were not directly assessed. The literature highlights considerable variability in the glycemic response to noise. Buscaino et al. (2010), for example, reported non-significant changes in adult *D. labrax* and a reduction in glucose levels in *S. aurata*, while Celi et al. (2016) and Filiciotto et al. (2013) observed a significant increase in blood glucose levels in *S. aurata* exposed to different noise sources. These differences suggest that the metabolic response may depend on multiple factors, including species, developmental stage, characteristics of the acoustic stimulus, and duration of exposure. Overall, our results indicate that glucose may be a sensitive indicator of early physiological changes associated with noise exposure in juvenile *D. labrax*.

Finally, the growth analysis followed the expected pattern. Although no statistically significant differences were found at the 95% confidence level ($p > 0.05$) for absolute length or weight, the shift in p -values from 30 min to 7 days ($p = 0.059$ for weight) suggests a trend that might become significant with longer exposure or larger sample sizes. This trend becomes evident when the relative growth rate was inspected. After 7 days, exposed group experienced minor growth rates compared to the control group with statistical significant difference. The experimental group exhibited reduced growth rates both for weight and length when the temporal information is considered. While some species like *Micropterus salmoides* show clear growth reduction under noise (Hang et al., 2021). Studies on *D. labrax* have often shown stress responses to noise without immediate growth effects (Radford et al., 2016; Santulli et al., 1999). Our data suggest that noise may transiently alter growth rates, a possibility that warrants further long-term investigation (Nedelec et al., 2015) including the potential relationship between growth rates and fish behavior. The role of swimming patterns and fish behavior should be considered as a potential driving factor for the lower growth rates reported, additionally and related to the biochemical parameters.

5. Conclusion

This study provides new evidence that anthropogenic underwater noise can rapidly modulate multiple physiological processes in juvenile of *D. labrax*. The temporal patterns observed across the measured biomarkers suggest that acoustic exposure elicits an early physiological response involving antioxidant defenses, energy metabolism, and endocrine regulation, followed by a progressive attenuation of these responses over time. Although several parameters tended to return toward baseline values, this pattern should be interpreted cautiously and may reflect temporal modulation of the physiological response rather than complete recovery or acclimation. Overall, our findings indicate that juvenile fish are capable of mounting a rapid and coordinated physiological response to acoustic disturbance, highlighting the sensitivity of early life stages to anthropogenic noise. These observations contribute to a better understanding of the biological effects of underwater noise pollution and support the inclusion of physiological biomarkers in ecological risk assessments and aquaculture monitoring programs. A major limitation of this study is the use of whole-body homogenates, a methodological choice dictated by the small size of the experimental animals, which precluded the assessment of tissue-specific responses. Consequently, the observed changes cannot be attributed to

specific organs or physiological systems. Although a significant reduction in growth rates was detected within seven days, further long-term investigations integrating a detailed characterization of fish behavior are essential to determine the persistence of these effects and their cumulative impact on the fitness and developmental trajectory of juvenile *D. labrax*. Future research should combine multi-tissue approaches with molecular, physiological, and behavioral endpoints, supported by multivariate analyses, to better resolve the mechanisms underlying acoustic stress responses and to clarify their ecological significance for marine fish.

CRedit authorship contribution statement

M. Mauro: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **P. Giner-Tarazona:** Writing – review & editing, Visualization, Methodology, Investigation. **V. Espinosa:** Writing – review & editing, Validation, Funding acquisition. **V. Puig-Pons:** Writing – review & editing, Software. **S. Morell-Monzó:** Writing – review & editing, Software. **M. Vazzana:** Writing – review & editing, Validation, Software, Methodology. **F. Longo:** Writing – review & editing, Validation, Software. **R. De Luca:** Writing – review & editing, Validation, Software. **J. Ramis:** Writing – review & editing, Supervision, Software, Methodology, Funding acquisition. **P. Poveda:** Writing – review & editing, Software, Methodology. **J. Carbajo:** Writing – review & editing, Software, Methodology. **I. Pérez-Arjona:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Gemini AI in order to improve and format the LaTeX document for the submission and to design some images in the Graphical Abstract. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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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.

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Data availability

Data will be made available on request.

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