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Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.)

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ABSTRACT

The physiological responses of fish to underwater noise are poorly understood and further information is needed to evaluate any possible negative effects of sound exposure. We exposed European sea bass and gilthead sea bream to a 0.1–1 kHz linear sweep (150 dB_{rms} re 1 μPa). This band frequency is perceptible by many species of fish and is mainly produced by vessel traffic. We assessed the noise-induced motility reaction (analysing the movements) and the haematological responses (measuring blood glucose and lactate, and haematocrit levels). The noise exposure produced a significant increase in motility as well as an increase in lactate and haematocrit levels in sea bream and sea bass. A significant decrease of glucose was only observed in sea bream. A linear correlation between blood parameters and motility in fish exposed to the noise was observed. The acoustic stimulus produced intense muscle activity.

1. Introduction

The impact of human activity on marine habitats can produce adaptive alterations and other significant changes in animals (McIntyre, 1995; Myrberg, 1980; Popper et al., 2004). In recent years, many studies have been carried out with the aim of evaluating the effects of anthropogenic acoustic disturbance on marine organisms (Santulli et al., 1999; Sarà et al., 2007; Scholik and Hong Yan, 2001), thus increasing the awareness of the damage done to animals exposed to human related underwater sounds (National Research Council, 2003, 2005). These sounds are associated with shipping, seismic surveys, sonar, recreational boating and many other anthropogenic sources that are known to induce several types of responses in fishes (Bart et al., 2001; Engås et al., 1996; Myrberg, 1980; Popper et al., 2005; Sandström et al., 2005; Schwarz and Greer, 1984; Smith et al., 2004). Pacific herring (*Harengus pallasi*) exhibited alarm responses in reaction to motorboat noise (Schwarz and Greer, 1984). Engås et al. (1996) found that seismic shooting has effects on the local abundance and distribution of *Gadus morhua* and haddock (*Melanogrammus aeglefinus*). Smith et al. (2004) examined the short- and long-term effects of increased ambient noise on the behaviour and hearing of goldfish (*Carassius auratus*). They noted that goldfish exhibited an initial startle response with a rapid burst of erratic swimming followed by general increased swimming activity with the onset of an experimental noise (bandwidth ranging from 0.1 to 10 kHz at 160–170 dB re 1 μPa total sound pressure level). Kastelein et al. (2008) observed a behaviour response of sea bass exposed to pure tone signals ranging between 0.1 and 0.7 kHz at 0–30 dB above the hearing thresholds.

Since acoustic signals in the 100–500 Hz band are detected by many species of fish (Popper et al., 2003) and there is an increase in this low-frequency ambient noise as a result of increased international shipping (Ross, 2005), it is safe to assume that these noises are having an impact on the welfare of many fish species. While the effects of such anthropogenic sounds on marine mammals have been described (Myrberg, 1980; National Research Council, 2000, 2003, 2005; Richardson et al., 1995), the impact of underwater noise on marine fish is not understood sufficiently. Further information is needed to evaluate or predict any negative effects (Popper et al., 2004).

Previous studies have shown that acoustic stimulation can affect fish behaviour, but the physiological consequences have hardly been studied. Some studies have shown that acoustic stimulation can produce metabolic changes in fish. Smith et al. (2004)

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pointed out a significant modification of plasma cortisol and glucose levels in goldfish after exposure to white noise. Santulli et al. (1999) demonstrated variations in cortisol, glucose, lactate, AMP, ADP, ATP and cAMP levels in different tissues of sea bass, indicating a typical primary and secondary stress response to air gun detonations. Wysocki et al., 2006 indicated that ship noise constitutes a potential stressor for European freshwater fishes.

The present study aims to investigate the motility and haematological responses of European sea bass and gilthead sea bream exposed to an experimental acoustic stimulus in a low-frequency range using analyses of movement, blood glucose, blood lactate and haematocrit values. The correlation between movement and blood parameters was also investigated.

2. Materials and methods

2.1. Study animals

The experiment was carried out from July to September 2008 at the Istituto per l'Ambiente Marino Costiero of Consiglio Nazionale delle Ricerche (IAMC-CNR) – Laboratories of Capo Granitola (Trapani, Italy) using 14 sub-adult European sea bass (*Dicentrarchus labrax*) weighing 189.4 ± 80 g with body lengths of 26.2 ± 3.3 cm, and 14 sub-adult gilthead sea bream (*Sparus aurata*) weighing 172.6 ± 23.7 g with body lengths of 22.9 ± 0.9 cm.

Three months before the beginning of the experiment, upon arrival at the laboratories from the marine fish farm of Trappeto (Palermo, Italy), fish were placed in circular tanks (diameter: 3 m, depth: 1 m, volume: 5000 L) at a low stock density (5 kg/m^3) with re-circulated and filtered seawater. Fish were exposed to the natural photoperiod and fed daily with commercial dry pellets. Feeding was stopped at least 48 h before the experiment.

2.2. Acoustic stimulus

Most fish are able to detect sound and the range of hearing is from 100 to 500 Hz (Popper et al., 2003). Keeping this in mind, as well as the fact that acoustic energy produced by vessel traffic is more intense at low frequencies (Ross, 2005), it was decided that an acoustic stimulus with a frequency band of 0.1–1 kHz would be used. A 1-second linear sweep was used to cover the frequency band. The linear sweep was repeated for 10 min without pause.

The signals, generated by a waveform generator (Model 33220A, Agilent Technologies, Santa Clara, CA, United States), were amplified (Model PA-4000 Inkel, Chonan City, Korea) and emitted using an underwater moving coil loudspeaker (Model UW30, Lubell, Columbus, Ohio, USA) with a 100 Hz–10 kHz rated frequency response.

The sound pressure level (American Acoustical Society, 1994) of the emitted signal was measured using an omni-directional calibrated hydrophone (TC4034, Reson, Slangerup, Denmark) positioned inside the cage (1.5 m deep) 5.5 m from the underwater speaker. Signals were pre-amplified (VP1000, Reson, Slangerup, Denmark) and were recorded using a DAQ card (Ni DAQ-Card-6062E, National Instruments, United States) using a sampling frequency of 100 kHz. Digital signals obtained were elaborated with a routine procedure developed by the Inter-disciplinary Group of Oceanography (GIO at CNR-IAMC, Capo Granitola, Italy) using LabView rel. 7.1 (National Instruments, United States). The maximum sound pressure level of a single sweep was 150 dB_{rms} re 1 μPa. The mean power spectrum and the spectrogram of 5 s of emitted signal are shown in Figs. 1 and 2.

2.3. Experimental procedure

During the experimental phase, sea bass and sea bream were randomly assigned to control and test groups. For each test, the fish was transported to an experimental sea cage (see Fig. 3) located in a circular natural harbour (with a diameter of about 200 m and a depth of 3 m) and left there to acclimate.

A research cabin was placed 8 m away from the sea cage. The cabin housed the sound generator, as well as video and sound recording equipment.

One hour later, the specimen was recorded (both audio and video) for 10 min with an acoustic acquisition system and two underwater video cameras (model RE-BCC6L, DSE, Italy) mounted outside the cage. One camera was mounted on the middle of the cage and the other camera was mounted on top of the cage (Fig. 3) so that the entire cage was visible. During video and sound recording, individuals in the test group were exposed to an acous-

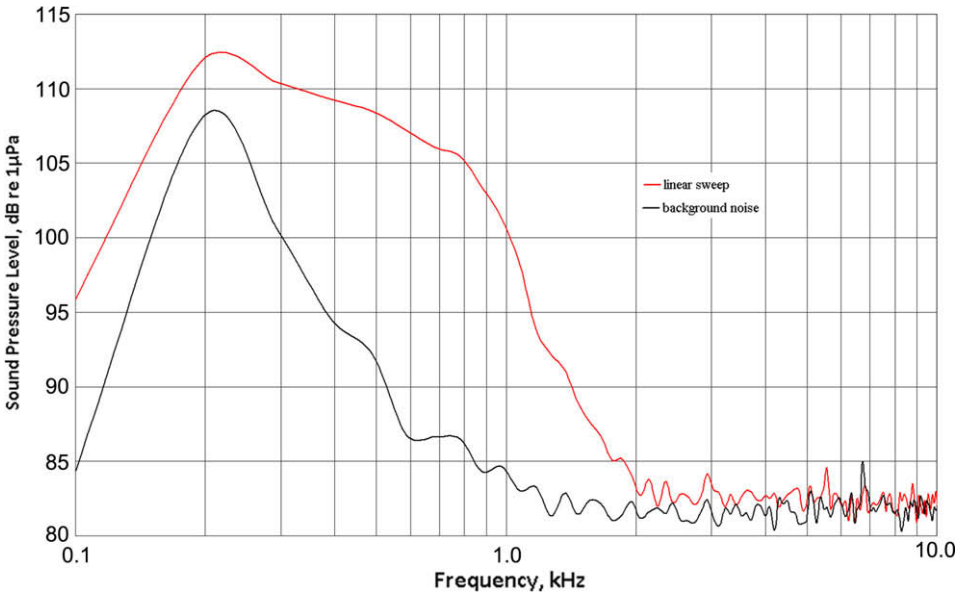

Fig. 1. Mean power spectrum of sweep signal and background noise. The sampling frequency of the signal was 100 kHz. The size of Fast Fourier Transform (FFT) was 1024 points and the window type was cosine tapered.

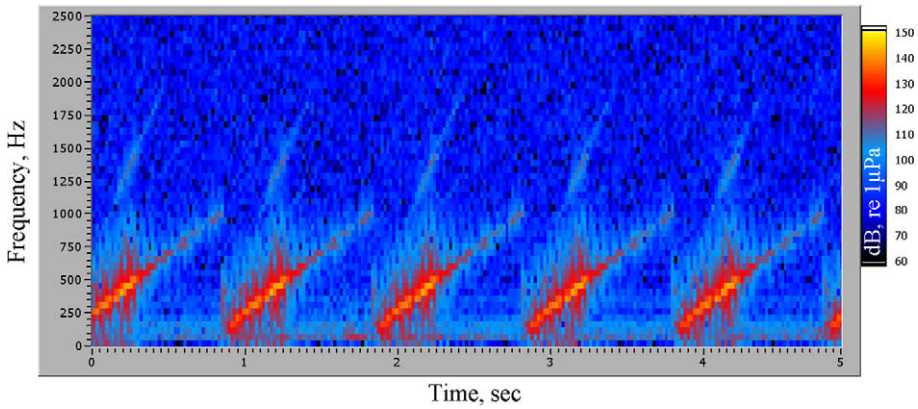

Fig. 2. Spectrogram of the sweep signal. Time in seconds and frequencies in hertz are shown along the *x*-axis and *y*-axis, respectively. Amplitude was measured in dB (re 1 µPa) using a colour scale. The sampling frequency of the signal was 100 kHz, the size of FFT was 2048 points and the window type was cosine tapered.

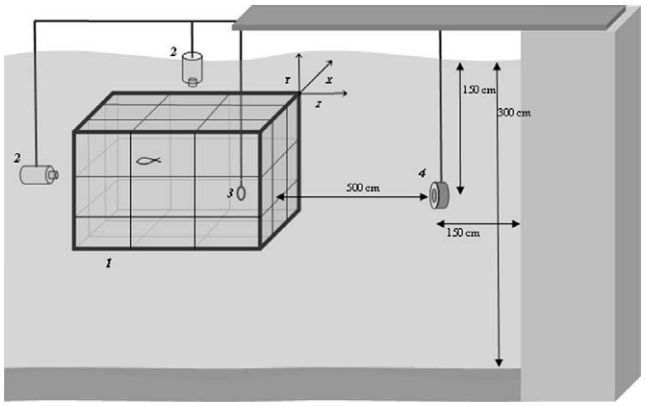

Fig. 3. A schematic view of the sea cage (1) with two underwater video cameras (2), a hydrophone inside the cage (3) and the underwater speaker (4).

tic stimulus while members of the control group had no stimulus applied. The fish were then captured with a net to take blood samples. After this, the fish were transferred into a small tank and then released after recovery. The glucose, lactate and haematocrit levels were measured in the fish blood samples, while fish motility was evaluated using the video recordings (see Section 2.5).

Blood parameters and motility data recorded in the control and test group of sea bass and sea bream were statistically analysed to assess possible differences between the groups. Moreover, a statistical correlation was applied to the total number of movements and blood parameters. The entire work presented here complied with current regulations regarding animal experimentation in Italy.

2.4. Blood sampling procedure and chemical analyses

A standardized handling procedure for each sea bass and sea bream was applied in order to standardize the potential stress produced during the blood sampling. After 10 min of audio and video recording, the fish was captured with a net from the experimental cage and immediately anesthetized with 2-phenoxyethanol (0.4 ml L^{-1}) in a 10-litre tank. Fish reached stage V of anaesthesia (Summerfelt and Smith, 1990) within 1–2 min after which they were weighed and measured. Later, blood samples were collected from the caudal vein (using a 2.5 ml syringe with a 22 G × 1 ½″ needle) for the immediate assessment of glucose and lactate on whole blood with a portable blood glucose analyser (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and blood lactate analyser (Accusport, Boehringer Mannheim, Germany). The time between capture and blood collection was less than 5 min. A Select-A-Fuge Model 24 blood micro haematocrit centrifuge (Bio-Dynamics, Inc., Indianapolis, United States) running at 3600 rpm for 5 min was used to assess the haematocrit value.

2.5. Motility sampling procedure

Two grids were affixed on the sides of the sea cage monitored by the cameras, each comprising nine regions, to analyse the movements of fish along the three axes of space (see Fig. 3). The images from the two cameras were synchronized and fish movements were analysed from the video recordings. The transit of the specimen from one region to another region of the grid was recorded. A focal animal sampling technique modified from Altmann (1974) was used to analyse the images in slow motion. For each minute of sampling, we recorded a value (as sum of the movement across the *x*, *y* and *z* axes) for a total of 10 values for each specimen.

2.6. Statistical analysis

An unpaired *t*-test was used to determine significant differences in blood glucose, blood lactate and haematocrit levels as well as differences in movement (as the total movement during each minute of sampling for all specimens) between the control groups and the test groups. A linear regression model ($y = a + bx$) was applied to the total movement during the 10 min of sampling and the values for the blood parameters (glucose, lactate and haematocrit) of each fish in order to determine the degree of correlation in the control and test groups. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Motility

The results obtained during the motility sampling procedure showed that the amount of movement observed in the test groups of sea bream and sea bass were significantly higher ($P < 0.0001$ and $P < 0.001$, respectively) than those of the control groups. The total movement for the control and test groups of sea bream and sea bass are shown in Table 1 while the mean values of total movement per minute and the statistical significance are shown in Fig. 4.

3.2. Blood parameters

In sea bream, glucose levels were significantly lower ($P < 0.005$) in the test group than in the control group, with a difference of 34.41 mg dL^{-1}. Blood lactate exhibited significantly increased val-

Table 1
Total movement and means ± standard error of the mean (SEM) for blood glucose, blood lactate and haematocrit levels in the control and test groups of gilthead sea bream and European sea bass.

	Gilthead sea bream		European sea bass	
	Control group	Test group	Control group	Test group
Motility				
Total movements	2451	3182	648	889
Blood parameters	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Glucose (mg dL^{-1})	116.02 ± 7.42	81.61 ± 6.58	131.86 ± 5.47	132.61 ± 7.04
Lactate (mmol L^{-1})	1.92 ± 0.27	3.05 ± 0.38	13.54 ± 1.59	16.56 ± 1.80
Haematocrit (%)	18.57 ± 2.30	33.88 ± 3.61	21.71 ± 2.2	30.57 ± 1.98

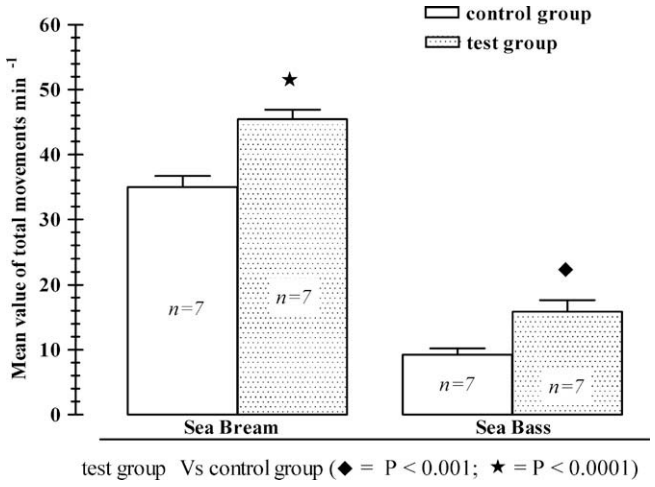

Fig. 4. Mean values (±standard error of the mean, SEM) of total movement per minute for sea bream and sea bass with the statistical significance values.

ues ($P < 0.05$) in the control group compared to the test group (difference of 1.13 mmol L^{-1}).

In sea bass, noise exposure did not significantly affect glucose levels between the control and test groups ($P < 0.94$). Blood lactate showed significantly higher levels ($P < 0.01$) in the test group.

In both species, significantly higher levels of haematocrit were recorded for the test group compared with the control group (sea bream: $P < 0.01$; sea bass: $P < 0.05$), with an increase of 15.3% and 8.9% for sea bream and sea bass, respectively. Mean blood glucose, blood lactate and haematocrit levels of sea bream and sea bass are shown in Table 1, while the mean values and the statistical significance are shown in Fig. 5.

3.3. Correlation between movements and blood parameters

In the control groups of both species, the application of a linear regression showed no significant correlation between the values for total movement and the values for the blood parameters. The total value of the movement for the sea bream test group had a significant correlation with the values of blood glucose and lactate (Fig. 6a and c). A significant correlation between the total movement and blood lactate levels was observed in sea bass (Fig. 6d) while no significant correlation between movement and blood glucose was recorded in sea bream (Fig. 6b). Haematocrit levels and movement were highly correlated for both test groups (Fig. 6e and f).

4. Discussion

The present study was conducted in a confined space, so that the obtained fish responses may be different to those observed in the wild. The analysis of sea bream and sea bass motility showed increased swimming activity, indicating a disturbance due to the

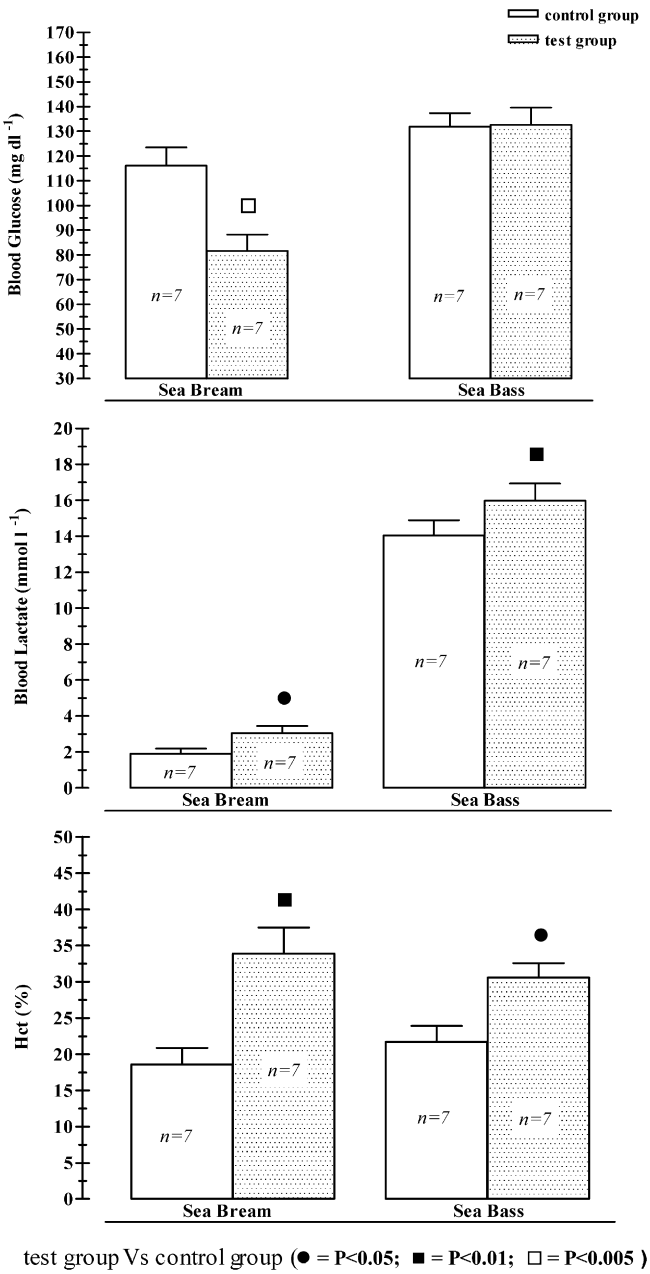

Fig. 5. Mean values (±standard error of the mean, SEM) of blood glucose, lactate and haematocrit levels for sea bream and sea bass together with the statistical significance.

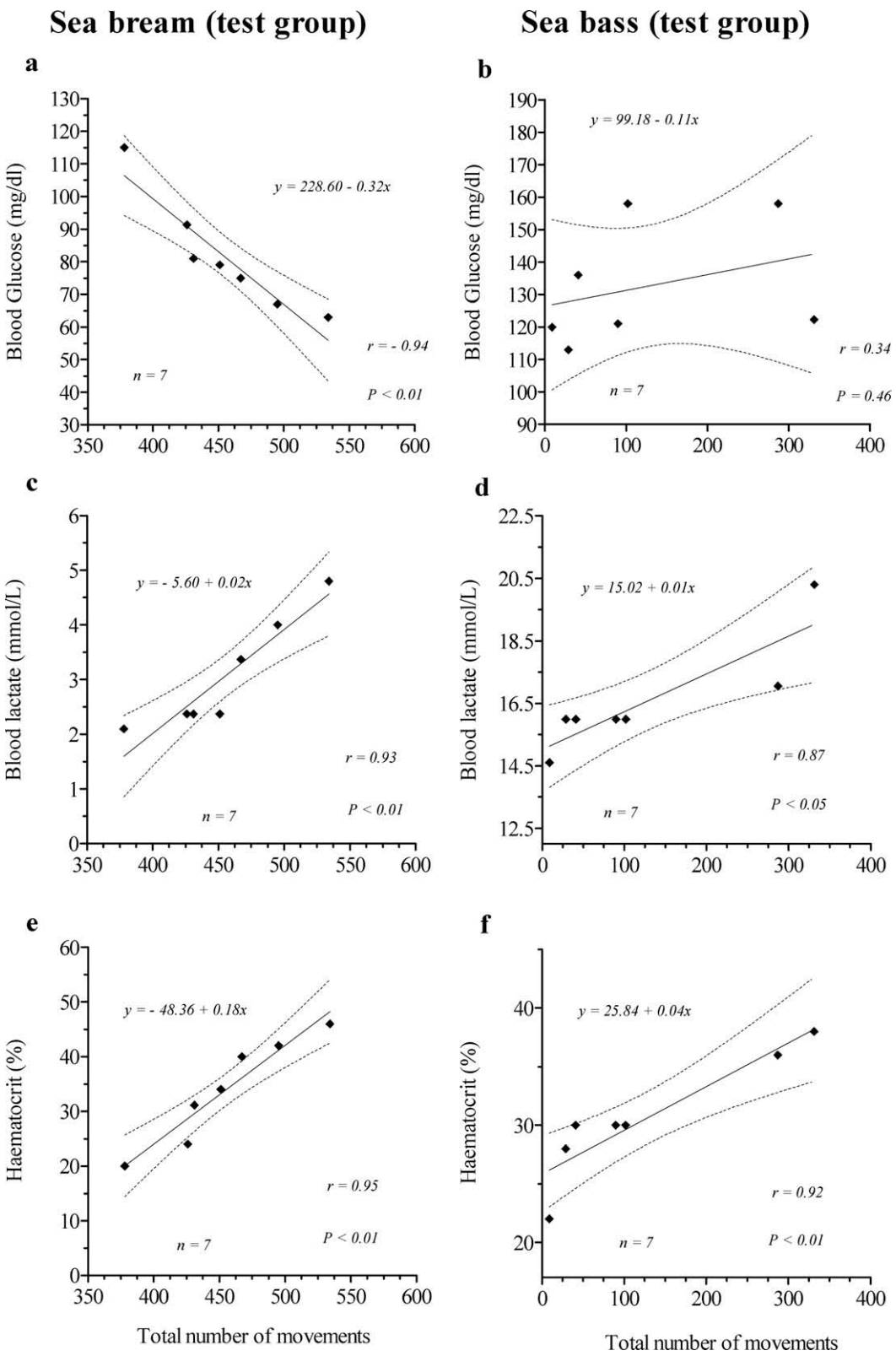

Fig. 6. Linear regression between the total number of movements and blood glucose (mg dL^{-1}) (a), blood lactate (mmol L^{-1}) (c) and haematocrit levels (%) (e) in the gilthead sea bream test group; (b), (d) and (f) are the same measurements for the European sea bass test group. The dashed lines delimit the 95% confidence interval.

acoustic stimulus. Although no changes were observed in the blood glucose levels of sea bass, the others blood parameters corroborated the motility observations for both species. The blood parameter changes and motility responses reflected an intense metabolic activity involving white muscle anaerobic fibres during the acoustic exposure.

The muscle metabolism of fish has been thoroughly investigated in the past (Beamish, 1978; Brett and Groves, 1979; Fry,

1971). Fish, like other vertebrates, have several types of muscle fibre with different properties. Two fibre types are most important in locomotion: red or slow oxidative fibres (type IIb; low power system) and white or fast glycolytic fibres (type Ia; capacity-limited, high-power system).

Aerobic red muscle is used for routine swimming while anaerobic white muscle is used for bursts of activity (sprints and fast-start; Bennett and Licht, 1972; Goolish, 1991; Weiser et al., 1985). Anaerobic pathways can also fuel intermediate or prolonged swims that can be sustained for few minutes, but these pathways eventually tire the fish (Bone, 1975; Brett and Groves, 1979). In fact, white muscle glycogen reserves can be depleted by 50% in only 2 min of forced activity (Goolish, 1991). The capacity for anaerobic energy production has been estimated from decreases in substrate reserves and end products accumulation with a conversion of glucose to lactate followed by stimulation of pyruvate metabolism and oxygen debt (Goolish, 1991; Kauffman, 1990; Puckett and Dill, 1984, 1985; Weiser et al., 1985). Lactate produced in the myotome is released into the circulatory system following bursts of anaerobic activity.

In the present study, the statistically significant responses in motility and blood parameters (glucose and lactate) observed in both sea bass and sea bream exposed to the acoustic stimulus indicate increased muscle activity using anaerobic Ia fibres.

Furthermore, the increase of metabolic muscle activity implies a higher demand for oxygen, which is increased by increasing the respiratory rate. During this adaptive response, fish experience the haemopoietic activity of the spleen that encourages the production of red blood cells for oxygen transport (Franklin et al., 1993). This condition leads to an increase in the levels of blood corpuscular components such haematocrit, one of the most reliable indexes. This increase was also found in the present study (Fig. 5).

The significant correlation between the movement values and the selected blood parameters (Fig. 6) supports what has been described above. However, the different blood glucose trends for the two species should be highlighted. Sea bream experienced a significant decrease in blood glucose levels and these levels were highly correlated with motility, while sea bass experienced no significant differences in glucose levels and these levels were not correlated with motility. These responses may be explained by intrinsic species differences in recovery time and in the use of glucose for energy. These differences could also be due to a difference in sensitivity to sound exposure.

5. Conclusions

In conclusion, the results from this study showed that anthropogenic noise at low frequencies can influence the swimming activity of fish. Moreover, many previous studies have shown that muscle activity rates can be a very large part of the fish energy budget (Boisclair and Sirois, 1993; Koch and Wieser, 1983). Consequently, increased swimming activity and the associated metabolic costs could compromise other biological activities, such as food acquisition, regulation due to environmental perturbation, migration and reproduction. Banner and Hyatt (1973) and Lagardère (1982) observed a drastic reduction of egg survival and reproductive and growth rates in farmed fish species exposed to high sound levels.

The present study shows the relationship between behaviour and haematological parameters in relation to noise exposure. It also shows that the use of different study approaches (physiological or behavioural) can lead to similar results. Moreover, our results indicate that our real-time field assays were appropriate for measuring the physiological impact of the acoustic stimulus in sea bass and sea bream. Although this short-term noise experiment showed an increase in fish motility, lactate and haematocrit values, it would be useful to carry out future tests with a similar experimental procedure on other fish species using longer-term noise exposure to better understand the impact of anthropogenic noise on fish behaviour and physiology.

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