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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-

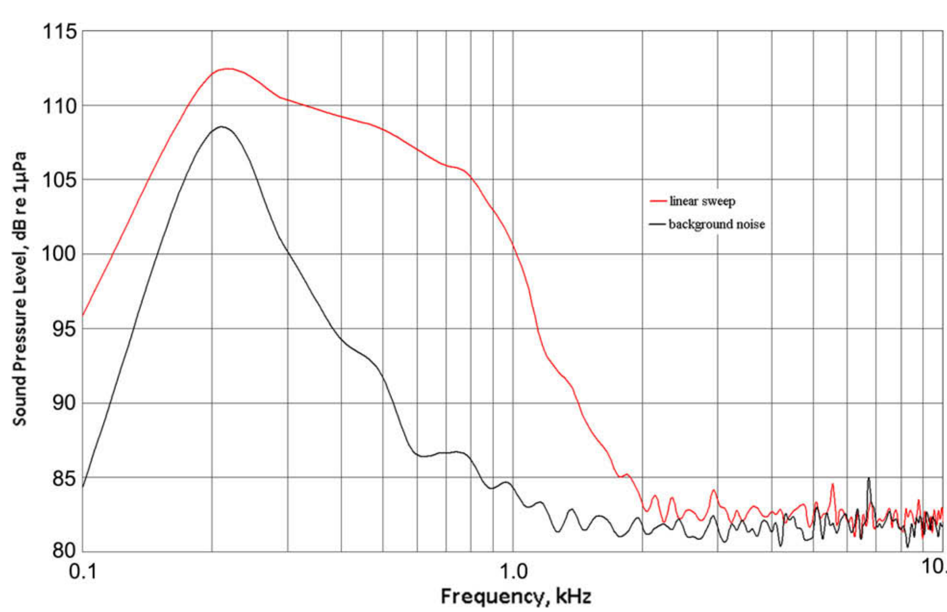

Fig. 1. Mean power spectrum of sweep signal and background noise. The sampling frequency of the signal was 100 kHz. The size of Fast Fourier Transform (FFT) was 1024 points and the window type was cosine tapered.