TECHNICAL REPORT

Physiological responses of *Sparus aurata* and *Dicentrarchus labrax* exposed to an acoustic stimulus

Authors

Giuseppa Buscaino, Francesco Filiciotto, Vincenzo Di Stefano, Gaspare Buffa, Antonio Bellante, Salvatore Mazzola

IAMC-CNR Capo Granitola

November 2013

Many of the designations used by the manufacturers and sellers to promote their products are claimed as trademarks. Where those designations appear in the Report and CNR was aware of a trademark claim the designations have been printed in all caps. In addition, we have reported some of them in the Production.

The data presented hereafter is the property of CNR. Unauthorized use of the data would be considered unfair.

Many of the systems and programs used to generate data are ’free’ because either they are either public domain or the licences are roughly equivalent to the GNU Public License. Some programs are either commercial or have more restrictive licenses and may require payment. Where known, programs and systems that are not ‘free’ are acknowledged.

ACKNOWLEDGMENTS

Many people contributed to the success of the research. This investigation was financially supported by the detached branch of the Capo Granitola of the IAMC-CNR, through funds made available from the ‘‘Acoustic Dispositive for the Reduction of Fishery and Marine Mammals Interaction – DAIMAR” project.

Contents

ABSTRACT

Several field and laboratory studies evaluating the effects of sound on fish have shown that increased ambient sound levels could alter their habitat selection, behaviour, and ecology. Maritime traffic, even if it is characterized by low powers of emissions, is undoubtedly the most impactful environmental source because the most widespread, continuous and omni-directional. The impact of underwater noise on marine fishes is not enough understood and further information is needed to evaluate or predict any negative effects. The present study aims at investigating the motility and biochemical responses of 14 sub-adult European sea bass (*Dicentrarchus labrax*) and 14 sub adult gilthead sea bream (*Sparus aurata*) exposed to an experimental acoustic stimulus, mainly produced by vessel traffic and perceptible by majority of fishes, To evaluate the effect of the acoustic stimulus, the movement, blood glucose, blood lactate, haematocrit, albumin, cholesterol, total proteins, total bilirubin and tryglycerids values were assessed.

Fishes were singularly placed into a sea cage in a natural harbour and exposed to a linear sweep of 1 second with an initial and final frequency respectively of 0.1 and 1 kHz repeated for 10 minutes without pause. The maximum sound pressure level of the single sweep was 150 (dB_{rms} re 1µPa). After 10 minutes of stimulus projection, fish was captured for blood sampling procedure. All specimens were recorded for 10 minutes with two underwater video cameras for the evaluation og the fish mobility.

On whole blood the immediate assessment of glucose and lactate with a portable blood glucose analyser and blood lactate analyser were performed. On serum samples were measered Albumin, Cholesterol, Total Proteins, Total Bilirubin and Tryglycerids contents.

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. No statistical differences were observed in Albumin, Cholesterol, Total Proteins, Total Bilirubin, Tryglycerids values between Control and Test groups both of sea bream and sea bass. The analysis of sea bream and sea bass motility and some haematic parameters indicates a disturb impact on the subjects exposed to acoustic stimulus.

1. INTRODUCTION AND BACKGROUND

During the last 50 years, the increase of anthropogenic activities led to a considerable increase in the ambient noise (Hildebrand 2009, Ross 2005) that altered the "soundscape" on a global scale and the current estimate is that the noise in the oceans due to shipping is increasing at about 0.4 dB per year (Ross, 2005). The noise pollution produced by the maritime traffic is characterized by signals that cover a wide range of frequencies. The signals generated mainly by container ships, ferry boats, boats for recreational activity, fishing boats and research vessels are focused around the low frequencies. Other acoustic signals generated at low and high frequencies are produced by the equipment used by ships, fishermen, oil industry, oceanographers, geologists, meteorologists. For example, the sonar used by fishermen (sonar) became an essential tool for searching fish schools.

Recently, there has been an increased interest in the effects of anthropogenic noise on marine fish (Popper, 2003). Several field and laboratory studies evaluating the effects of sound on fish have shown that increased ambient sound levels could alter their habitat selection, behaviour, and ecology (Engås et al., 1996; Knudsen et al., 1994; Pearson et al., 1992; Popper, 2003; Sand et al., 2000; Tolimieri et al., 2002). Noise pollution can cause negative effects on fish physiology and welfare, such as reduced growth rates (Sun et al., 2001), hearing damage (Amoser and Ladich, 2003; Codarin et al., 2009; Enger, 1981; Hastings et al., 1996; McCauley et al., 2003; Scholik and Yan, 2001) and stress response (Bart et al., 2001; Engas et al., 1996; Myrberg, 1980; Popper et al., 2005; Smith et al., 2004; Wysocki et al., 2006).

For example, pacific herring (*Harengus pallasi*) exhibited alarm responses in reaction to motorboat noise (Schwarz and Greer, 1984). Pearson et al. (1992) found that sounds from seismic surveys can affect rockfish (*Sebastes* spp.) behaviour, and similar effect on Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus)* was documented (Engås et al., 1996). Smith (2004) examined the short- and long-term effects of increased ambient sound on the stress and hearing of goldfish (*Carassius auratus*). Kastelein et al. (2008) demonstrated that the 50 % of startle responses of sea bass to pure tone signals ranged between 0.1 and 0.7 kHz at 0-30 dB above the hearing thresholds. Moreover, Santulli et al. (1999) observed variations in typical primary and secondary stress parameters in different tissues of *Dicentrarchus labrax* exposed to air gun detonations.

Maritime traffic, even if it is characterized by low powers of emissions, is undoubtedly the most impactful environmental source because the most widespread, continuous and omni-directional (Figure 1).

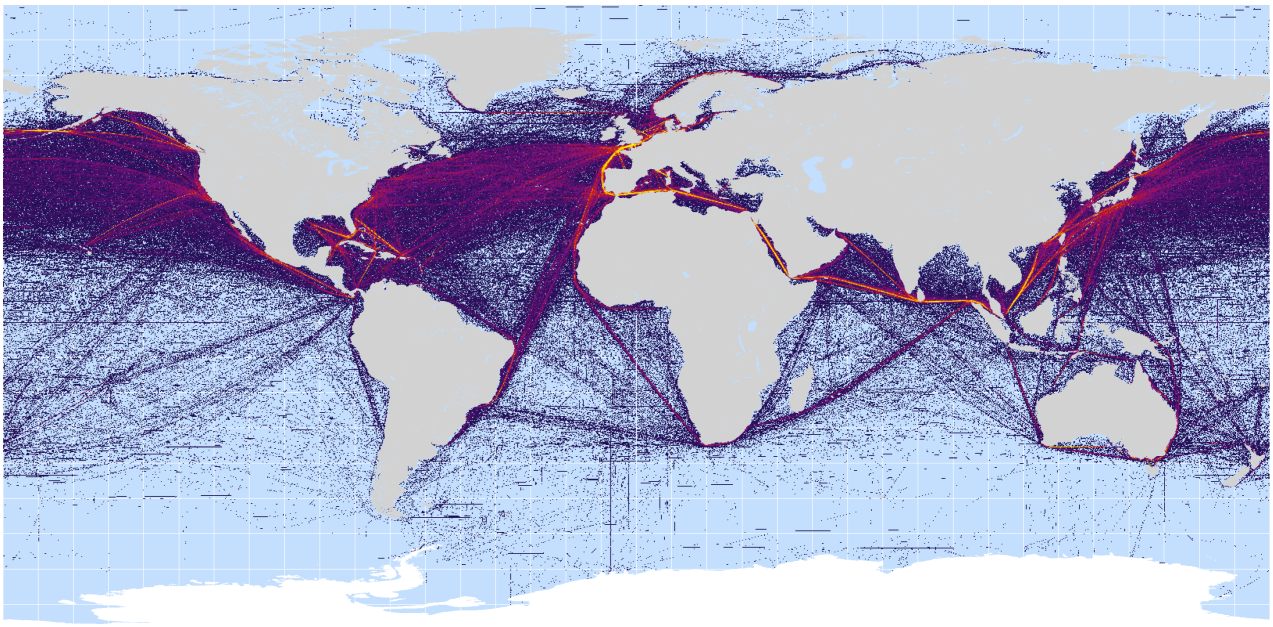

Figure 1. Global marine traffic representation (Halpern et al., 2008)

Since acoustic signals in the band 50-1000 Hz are perceived by the majority of fish species (Popper et al., 2003) and there is an increase in low-frequency ambient noise at an average of about 0.4 dB per year, as results of increase of world shipping (Ross, 2005), an increasing impact on fish welfare could be assumed. 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 fishes is not enough understood and further information is needed to evaluate or predict any negative effects (Popper et al., 2004).
In this contest, previous studies have shown that acoustic stimulation can affect fish behaviour but the physiological consequences were poorly studied.

1.1 Objectives

The present study aims at investigating the motility and biochemical responses of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) exposed to an experimental acoustic stimulus, mainly produced by vessel traffic (Ross, 2005) and perceptible by majority of fishes, To evaluate the effect of the acoustic stimulus, the movement, blood glucose, blood lactate, haematocrit, albumin, cholesterol, total proteins, total bilirubin and tryglycerids values were assessed.

2. MATERIALS AND METHODS

2.1. Collection and housing of animals

The study was carried out from July to September 2008 at the harbor of IAMC–CNR Detached Unit of Capo Granitola (Campobello di Mazara, Italy) (Fig. 1).

Figure 2. Harbor of IAMC-CNR, Detached Unit of Capo Granitola

14 sub-adult European sea bass (*Dicentrarchus labrax*) weighing 189.4 ± 80 g with a body length of 26.2 ± 3.3 cm, and 14 sub adult gilthead sea bream (*Sparus aurata*) weighing 172.6 ± 23.7 g with a body length of 22.9 ± 0.9 cm were used for the study.

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 acclimation circular tanks (diameter: 3 m, depth: 1 m, volume: 5000 l) (Fig. 3), with re-circulated and filtered seawater with a constant flow of water at a rate of 25 ± 3.7 l min^{-1} (mean ± SD). The animals were fed commercial pellets daily. The specimens were deprived of food for 2 days before the start of the experimental trials. All animals were kept under natural photoperiods.

Figure 3. Acclimation circular tank

2.2. Rationale and experimental procedures

During the experimental phase, sea bass and sea bream were randomly assigned to two different groups: control (group A) and experimental (group B). Fishes were singularly transported into plastic floating platform (Fig. 4) that supported a sea cage as shown in Figures 5 and 6. This structure was located in the circular natural harbour of Capo Granitola (with a diameter of about 200 m and a bathymetry of 3 m).

Figure 4. Plastic floating platform placed into the harbor of Capo Granitola

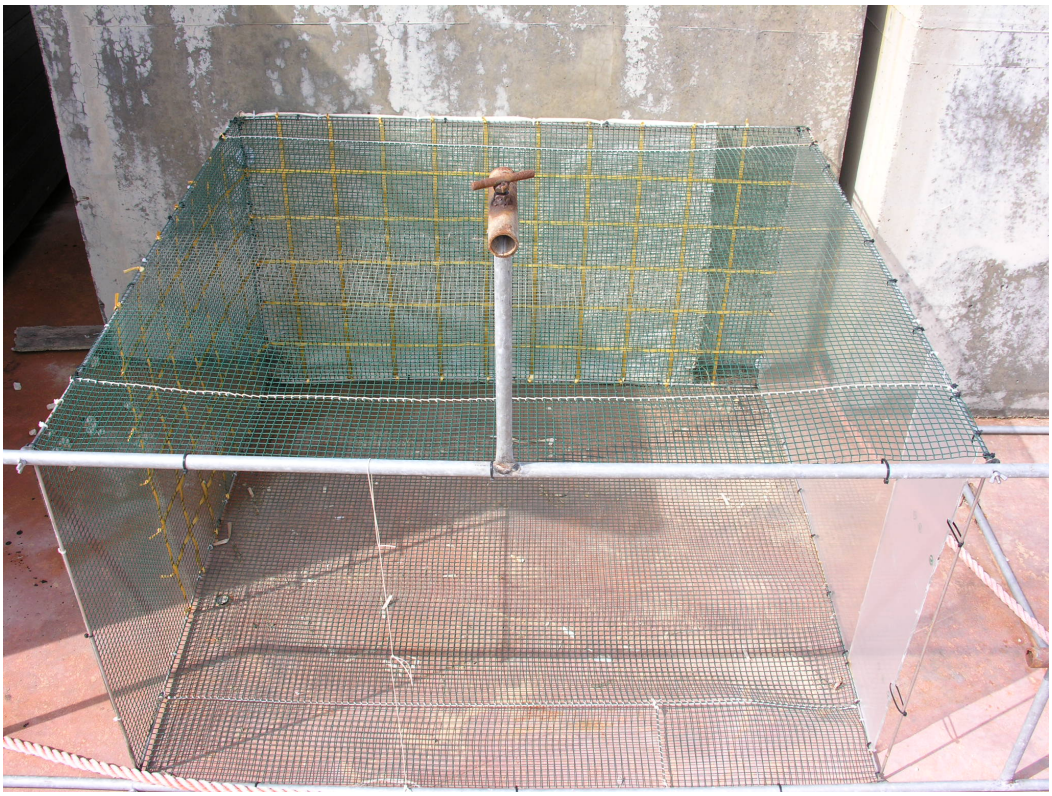

Figure 5. View from above of the sea cage

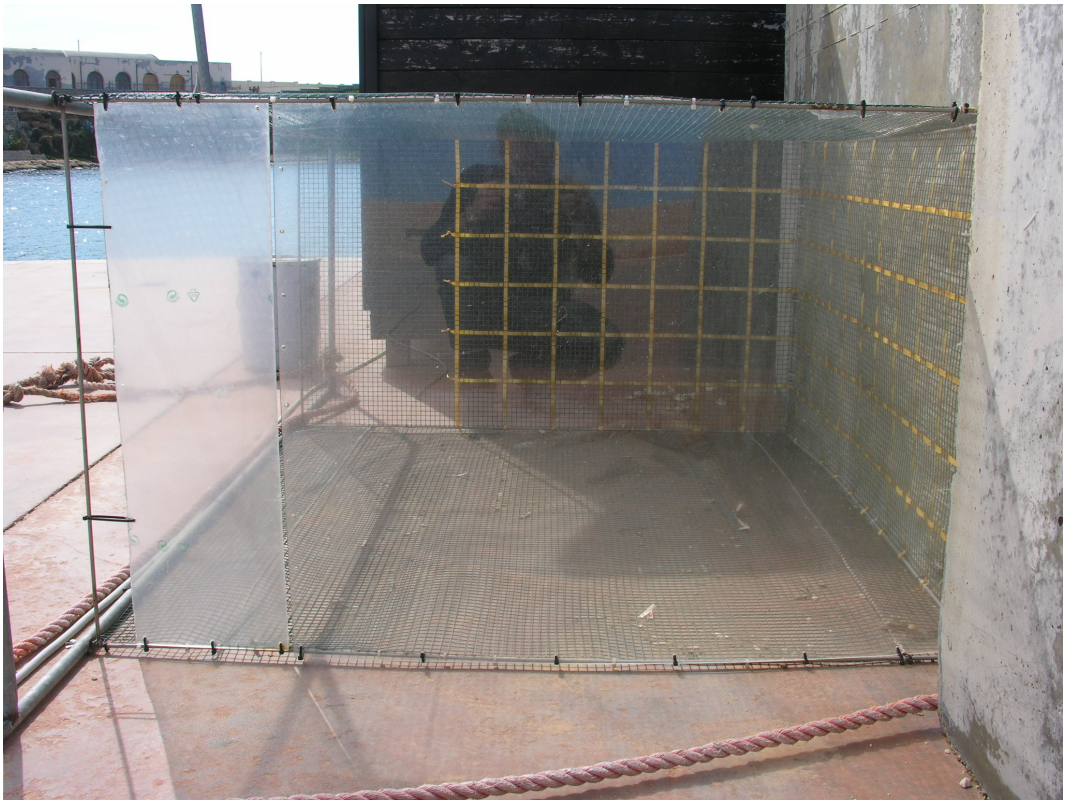

Figure 6. Lateral view of the sea cage

Fish were put into the cage and left there for one hour acclimation (Fig. 7)

Figure 7. The operator is putting the fish inside the cage

A research cabin was placed 8 m apart of the sea cage (Fig. 8). The cabin housed the sound generation, video and sound recording equipment.

Figure 8. The wood research cabin

One hour later, specimen was recorded for 10 minutes with two underwater video cameras (model RE-BCC6L, DSE, Italy), both mounted out of the cage, respectively at the middle of the depth, and at the top of the cage (Fig. 9), so that the total of water volume in the cage was visible. During video and sound recording, the specimen of group B was submitted to acoustic stimulus.

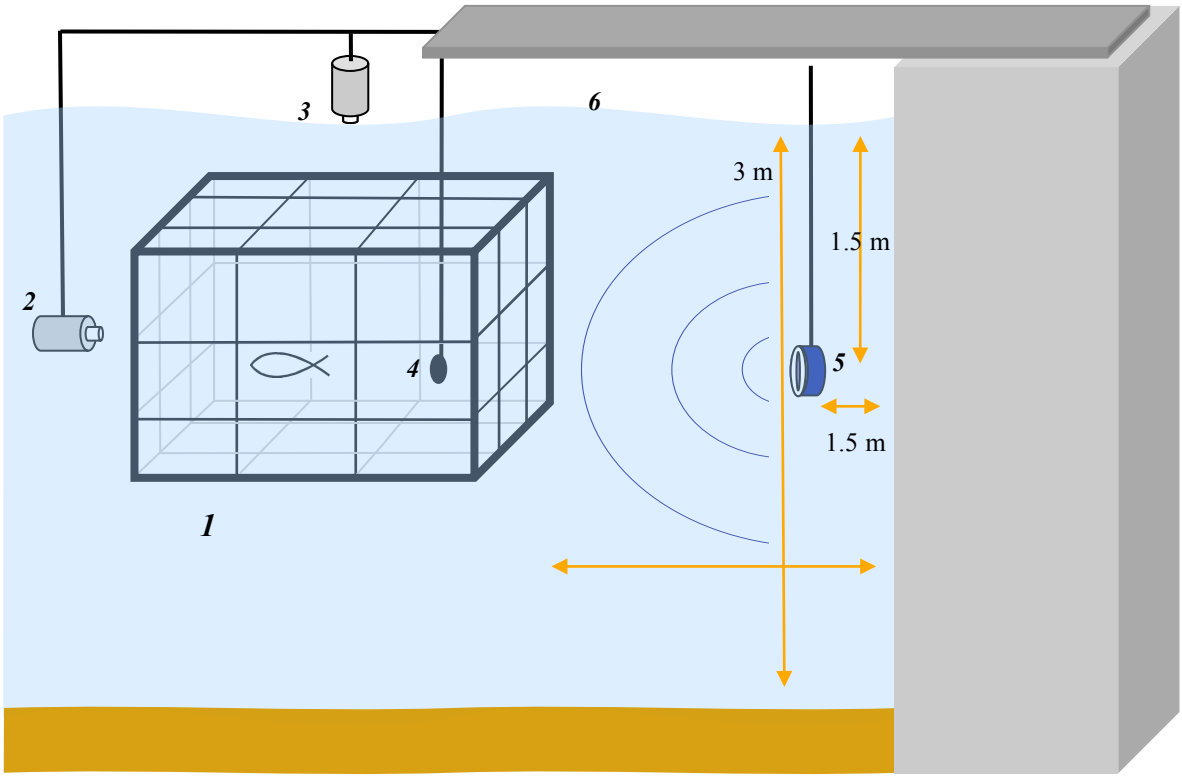

Figure 9. Schematic view of the experimental design: (1) sea cage, (2) two underwater video-cameras, (3) hydrophone inside the cage, (4) underwater loudspeaker.

2.3. Acoustic stimulus

A linear sweep of 1 second with an initial and final frequency respectively of 0.1 and 1 kHz was emitted. Sweep was repeated for 10 minutes without pause. The spectrogram of 5 seconds of emitted signal is shown in Figure 10.

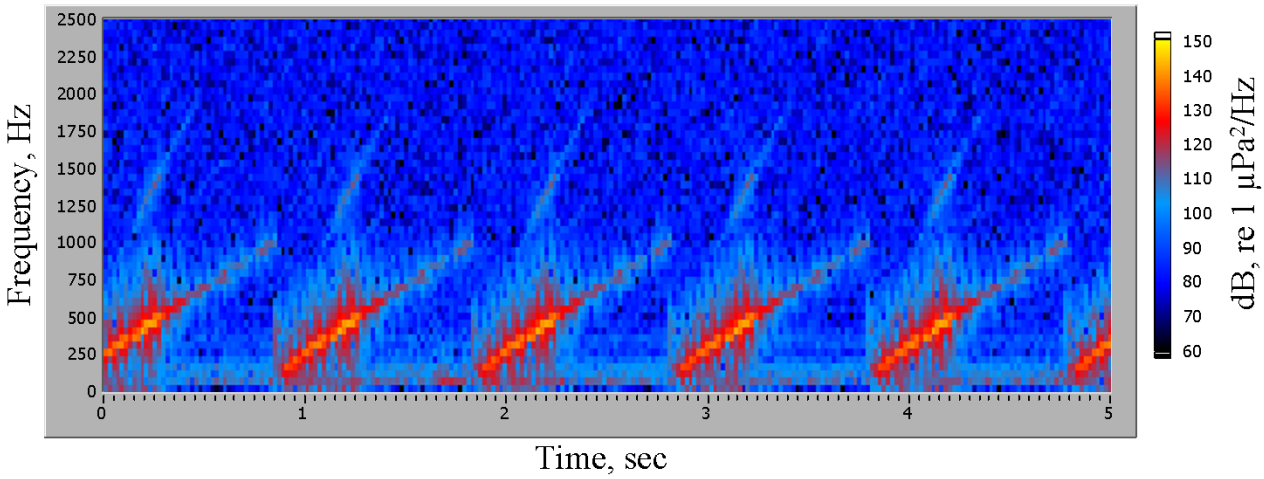

Figure 10. Spectrogram of the sweep signal. Time in seconds and frequencies in Hertz, are given respectively along x and y-axis. Amplitude is done in dB (re 1µPa^2/Hz) by color scale. The sampling frequency of signal is 100 kHz, the size of FFT is 2048 points and the window type is cosine tapered.

Signals, generated by a waveform generator (model 33220A, Agilent Technologies, Santa Clara, CA, United States) (Fig. 11), were amplified (model PA-4000 Inkel, Chonan City, Korea) and emitted using an underwater loudspeaker (model UW30, Lubell, Columbus, Ohio, USA) (Fig. 12) with a 100Hz - 10kHz rated frequency response.

Figura 11. Waveform generator

Figura 12. Underwater loudspeaker

The acoustic stimulus was recorded using a calibrated hydrophone (TC4034, Reson, Slangerup, Denmark) positioned inside the cage (1 m deep) at 5 m from the underwater speaker. The hydrophone was connected to a digital acquisition card (Ni DAQCard-6062E, National Instruments, United States). The signals were acquired at 100 kilosamples per second at 16 bits and analysed 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 the single sweep was 150 (dB_{rms} re 1µPa).

2.4. Blood collection procedure and biochemical analysis

After 10 minutes of stimulus projection, fish was captured for blood sampling procedure.

A standardized handling procedure for each sea bass and sea bream was applied in order to minimize the stress produced during the blood sampling. After of video recording, the fish was captured from the experimental cage and immediately anesthetized with 2-phenoxyethanol (0.4 ml L^{-1}) in a 10-litre tank (Fig. 13).

Figura 12. Capturing and stunning procedures

Fish reached stage V of anaesthesia (Summerfelt and Smith, 1990) within 1-2 minutes. Immediately after the stunning procedure, sea bream and sea bass were weighed and measured, and finally underwent venipuncture for blood collection. The blood samples were collected the from the caudal vein using 1-ml syringes with 25 G X 1 $^{1}/_{2}$ needles in less than 2 min for each fish (Fig. 14). Time elapsing from capture to blood collection was less than 3 minutes.

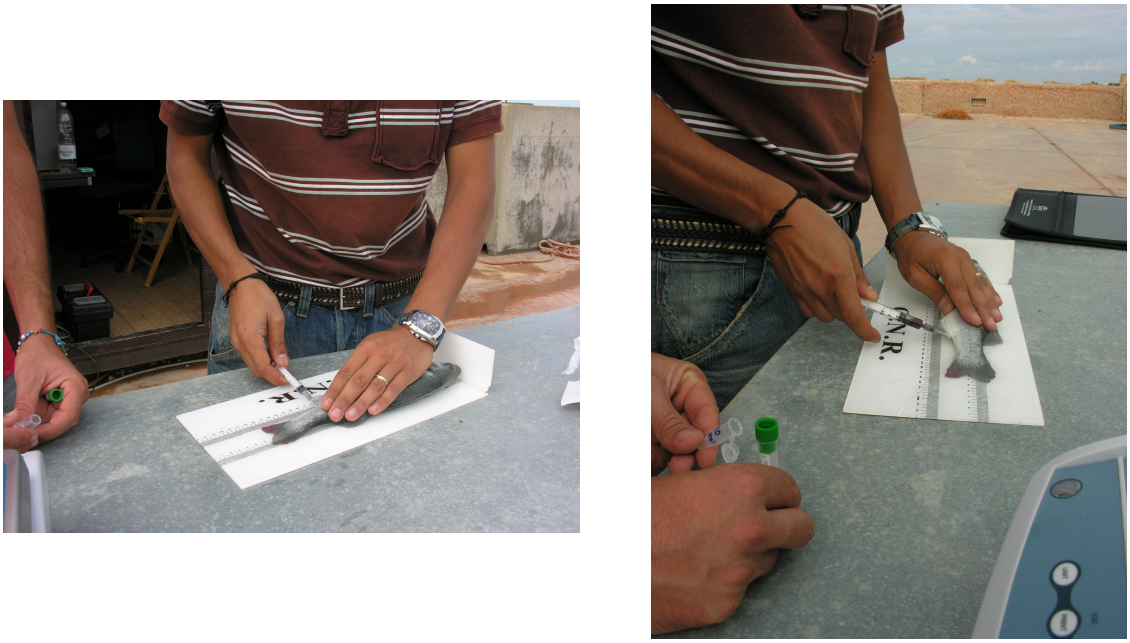

Figure 13. Blood sampling collection

On whole blood the immediate assessment of glucose and lactate with a portable blood glucose analyser (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and blood lactate analyser (Accusport, Boehringer Mannheim, Germany) were performed (Fig. 15).

Figura 14. Glucose- and Lactate-meter

A Select-A-Fuge Model 24 blood micro hematocrit centrifuge (Bio-Dynamics, Inc., Indianapolis, United States) at 3600 rpm for 5 minutes was used to assess the haematocrit value.

For each fish, a part of blood sample was stored in eppendorf tubes (1 ml Eppendorf, MBL International Corporation, Woburn, MA USA) with no additive after clotting and centrifugation at 3000 rpm for 10 min at 4 °C measurements. Serum samples were split into several aliquots and frozen at -20 °C for determination of Albumin, Cholesterol, Total Proteins, Total Bilirubin and Tryglycerids values.

After blood collection procedure, the fish was transferred into a tank where was released after complete recovery. The experimental procedure was replicated for each specimen of sea bream and sea bass.

2.5. Video monitoring system and analysis

Video recording was carried out to evaluate the fish motility.

Two grids were affixed on the sides of the sea cage monitored by the cameras, each comprising nine quadrants, to analyze the movements of fish along the three axes of the space: x, y and z (see Fig. 16).

Figure 16. Grids affixed on the sides of the sea cage

The images from the two cameras were synchronized and fish movements were analysed from the video recordings (Fig. 17).

Figure 17. Video-recording desk

The transit of the specimen from one quadrant to another of the grid was recorded and noted manually (Fig. 18). A focal animal sampling technique of Altmann (1974) modified was used in continuous during the 10 minutes of the recording period, analyzing the images in slow motion.
For each minute of sampling we recorded a value (as sum of the movements along x, y, and z axis), to obtain 10 values for specimen.

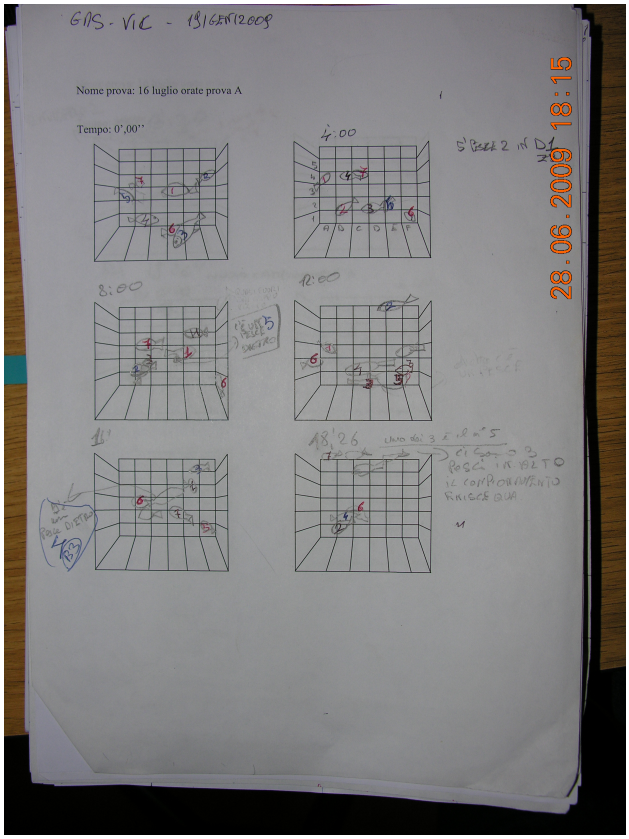

Figure 18. Movements noting process example

2.6. Statistical analysis

Unpaired T-test was used to determine significant differences in blood glucose, blood lactate, haematocrit Albumin, Cholesterol, Total Proteins, Total Bilirubin, Tryglycerids values and Movements (as the total movements during each minute of sampling for all specimens) between group A and group B of sea bass and sea bream.

A linear regression model ($y = a + bx$) was applied on the total movements (during 10 minutes of sampling) and the values of biochemical of each fish, in order to determine the correlation degree in group A and group B.

$P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The results obtained during motility sampling procedure showed that the movements observed in group B of sea bream and sea bass were significantly higher (respectively $P < 0.0001$ and $P < 0.001$) than those regarding the group A subjects (Fig. 19).

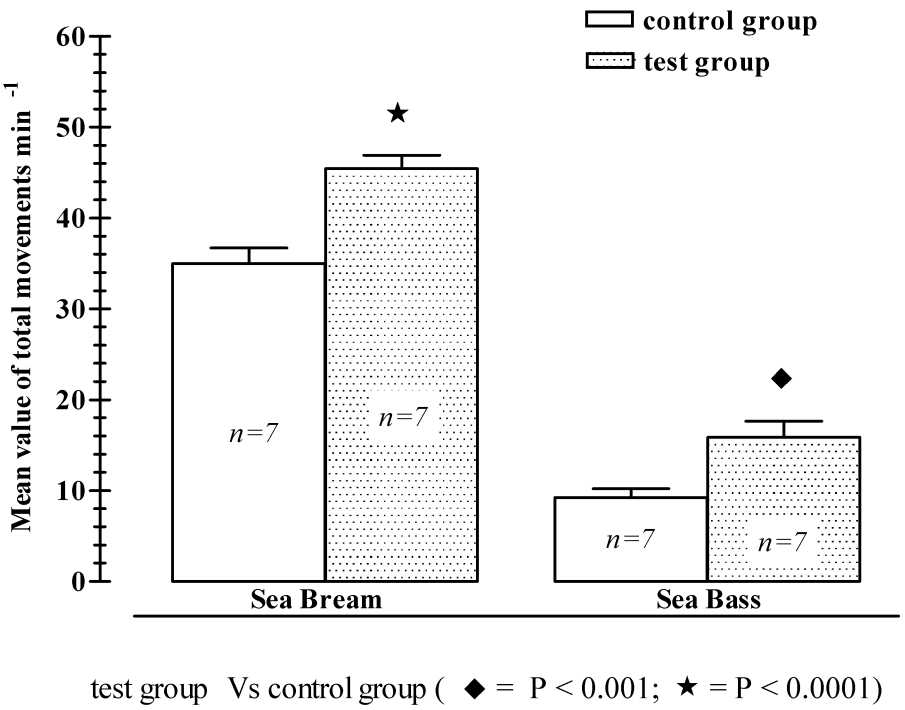

Figure 19. Mean value of total movements per minute and statistical significance

In sea bream, statistical differences with significantly ($P<0.05$) lower glucose levels were observed in group B in comparison to group A levels, with a difference of 34.41 mg dl^{-1}. Blood lactate exhibited significantly increased values ($P < 0.05$) in group B compared to group A.

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

In both species, significantly higher levels of haematocrit value were recorded in group B in comparison to group A levels (sea bass: $P < 0.05$; sea bream: $P < 0.01$), with an increase of 15.3 % and 8.9% for sea bream and sea bass respectively.

Mean ± SEM of blood lactate, haematocrit value and blood glucose recorded in group A and group B of sea bream and sea bass are showed in Figure 20.

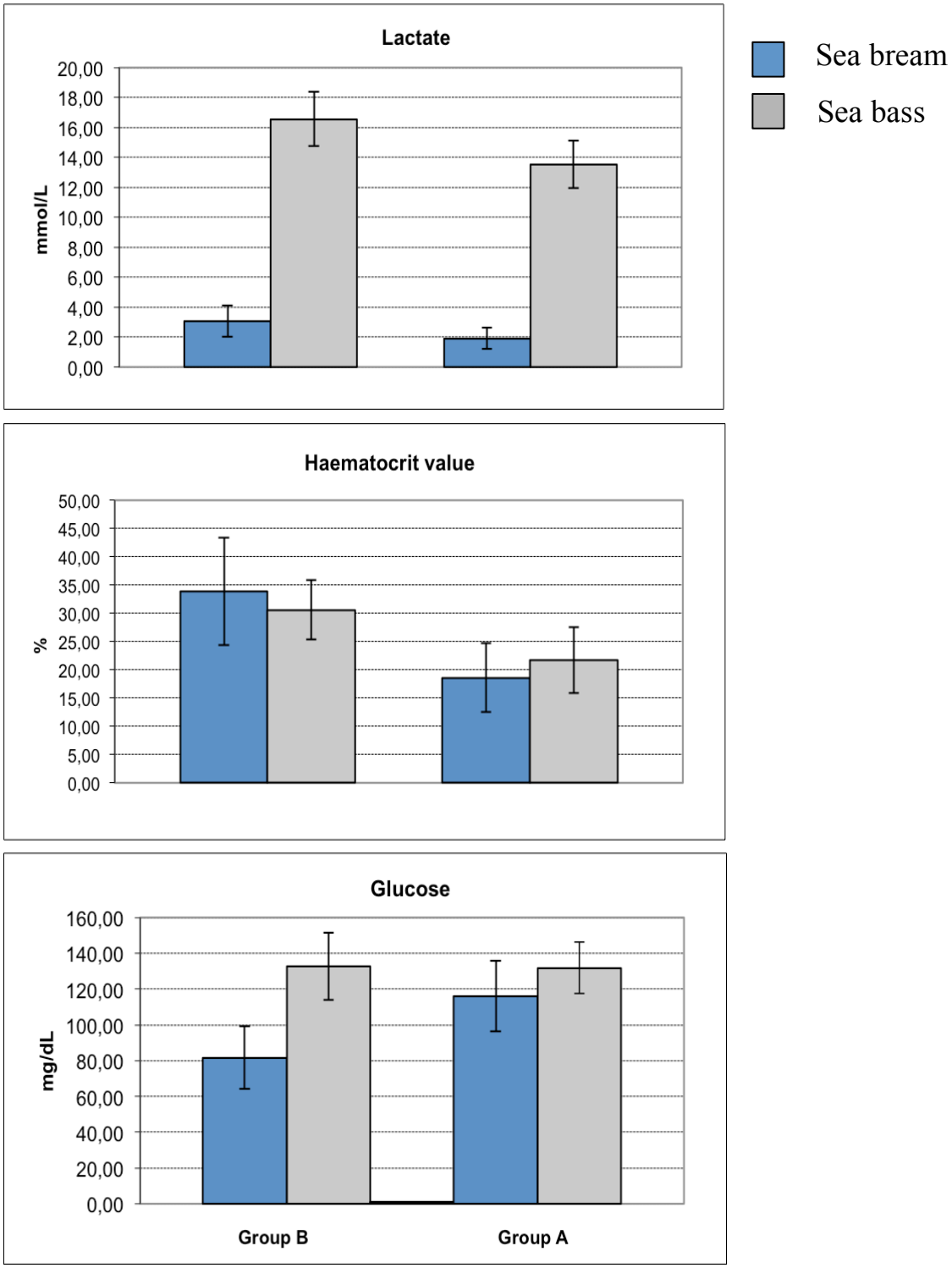

Figure 20. Mean ± SEM of blood lactate, haematocrit value and blood glucose of sea bream and sea bass

As reported in Figure 21, no statistical differences were observed in Albumin, Cholesterol, Total Proteins, Total Bilirubin, Tryglycerids values between Group A and Group B both of sea bream and sea bass.

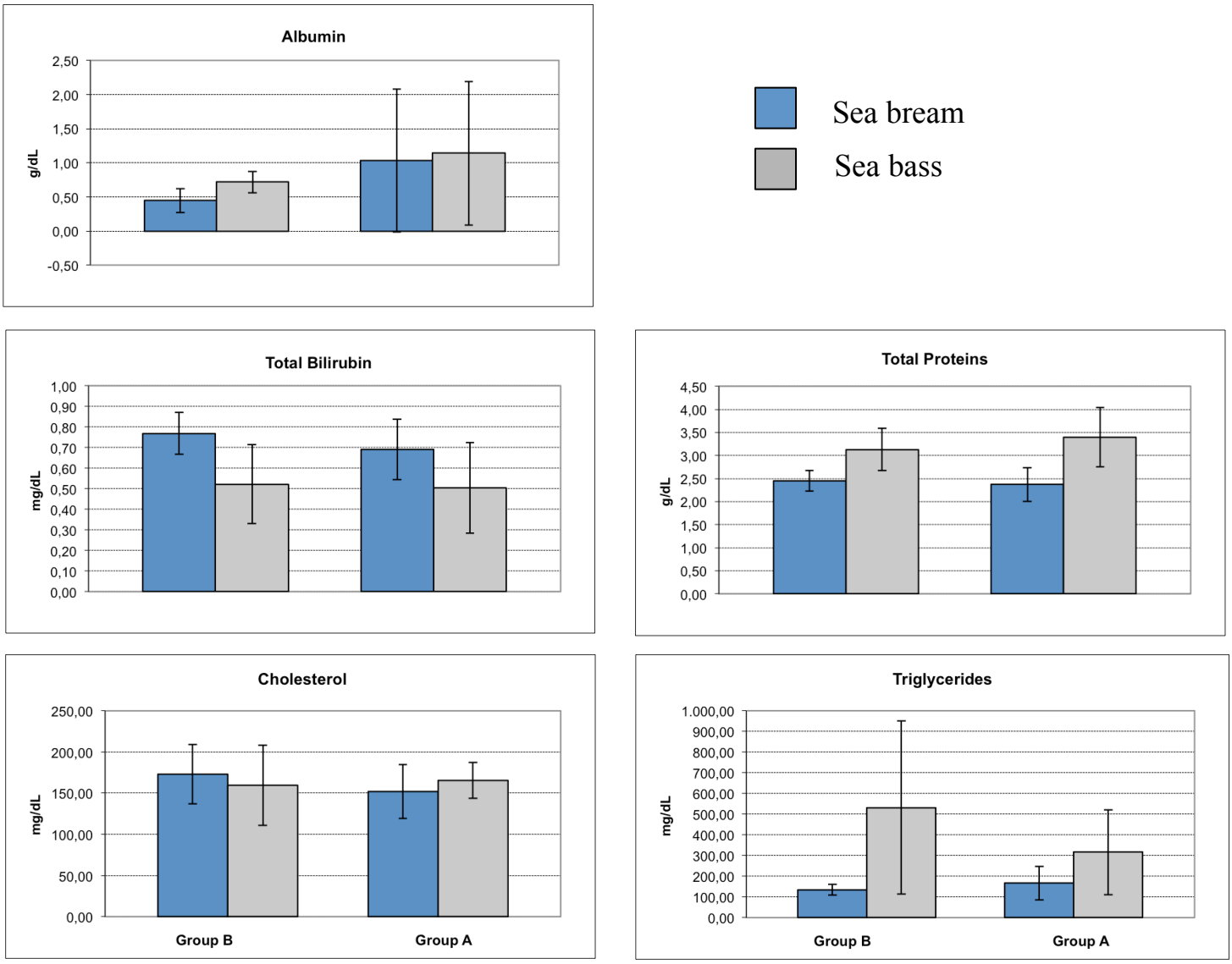

Figure 21. Mean ± SEM of Albumin, Cholesterol, Total Proteins, Total Bilirubin, Tryglycerids values of sea bream and sea bass

The analysis of sea bream and sea bass motility showed an increased swimming activity, indicating a disturb impact on the subjects exposed to acoustic stimulus.

Although no changes were observed in Albumin, Cholesterol, Total Proteins, Total Bilirubin, Tryglycerids values of both species and blood glucose levels of sea bass, haematic parameters results with a decrease of blood glucose in the sea bream and an increase of blood lactate and haematocrit value in both species exposed to the acoustic stimulus, support and confirm the motility observations.

In conclusion, the results from this study showed that the anthropogenic noise at low frequencies could represent a disturb factor influencing swimming activity and energy budget of fishes.

4. REFERENCES

Altmann, J., 1974. Observational study of behaviour: sampling methods. Behav. 49, 227-265.

Amoser, S., Ladich, F., 2003. Diversity in noise-induced temporary hearing loss in otophysine fishes. J. Acoust. Soc. Am. 113, 2170–2179.

Bart A.N., Clark J., Young J., Zohar Y. (2001) Underwater ambient noise measurements in aquaculture systems: a survey. Aquacultural Engineering 25, 99–110.

Codarin A., Wysocki L.E., Ladich F., Picciulin M. 2010. Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Marine Pllution Bulletin, 52(12): 1880-1887.

Engås, A., Loîkkeborg, S., Ona, E., Soldal, A.V., 1996. Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Canadian Journal of Fisheries and Aquatic Science 53, 2238-2249.

Enger, P.S., 1981. Frequency discrimination in teleosts—central or peripheral? In: Tavolga, W.N., Popper, A.N., Fay, R.R. (Eds.), Hearing and Sound Communication in Fishes. Springer, New York, pp. 243–255.

Halpern BS., Walbridge S, Selkoe KA et al. A Global Map of Human Impact on Marine Ecosystems (2008) Science vo. 319 no. 5865 pp. 948-952.

Hastings, M.C., Popper, A.N., Finneran, J.J., Lanford, P.J., 1996. Effects of low-frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish Astronotus ocellatus. J. Acoust. Soc. Am. 99 (3), 1759–1766.

Hildebrand JA Anthropogenic and natural sources of ambient noise in the ocean. Mar Ecol Prog Ser Vol. 395: 5–20, 2009.

Kastelein, R.A., van der Heul, S., Verboom, W.C., Jennings, N., van der Veen, J., de Haan, D.,

2008. Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369-377.

Knudsen, F.R., Enger, P.S., Sand, O., 1994. Avoidance responses to low frequency sound in downstream migrating Atlantic salmon smolt, *Salmo salar*. Journal of Fish Biology 45, 227–233.

McCauley, R.D., Fewtrell, J., Popper, A.N., 2003. High intensity anthropogenic sound damages fish ears. J. Acoust. Soc. Am. 113, 638–642.

Myrberg, A.A.Jr., 1980. The effects of man-made noise on the behaviour of marine animals. Environ. Int. 16, 575-586.

National Research Council, 2000. Marine Mammals and Low Frequency Sound: Progress Since 1944, National Academy Press, Washington, DC.

National Research Council, 2003. Ocean Noise and Marine Mammals, National Academy Press, Washington, DC.

National Research Council, 2005. Marine Mammal Populations and Ocean Noise, Determining when Noise causes Biologically Significant Effects, National Academy Press, Washington, DC.

Pearson, W.H., Skalski, J.R., Malme, C.I., 1992. Effects of sounds from a geophysical survey device on behaviour of captive rockfish (*Sebastes* spp.). Can. J. Fish. Aquat. Sci. 49, 1343-1356.

Popper, A.N., 2003. Effects of anthropogenic sound on fishes. Fisheries 28, 24–31.

Popper, A.N., Fewtrell, J., Smith, M.E., McCauley, R.D., 2004. Anthropogenic sound: Effects on the behaviour and physiology of fishes. Mar. Tech. Soc. J. 37, 35-40.

Popper, A.N., Smith, M.E., Cott, P.A., Hanna, B.W., MacGillivray, A.O., Austin, M.E., Mann, D.A., 2005. Effects of exposure to seismic airgun use on hearing of 3 fish species. Journal of the Acoustical Society of America 117, 3958–3971.

Richardson, W.J., Greene, C.R., Malme, C.I., Thomson, D.H., 1995. Marine Mammals and Noise. Academic Press, London.

Ross, D., 2005. Ship Sources of Ambient Noise. J. Ocean. Eng. 30, 257-261.

Sand, O., Enger, P.S., Karlsen, H.E., Knudsen, F., Kvernstuen, T., 2000. Avoidance responses to infrasound in downstream migrating silver eels, *Anguilla anguilla*.

Santulli, A., Modica, A., Messina, C., Ceffa, L., Curatolo, A., Rivas, G., Fabi, G., D'Amelio, V., 1999. Biochemical Responses of European Sea Bass (*Dicentrarchus labrax L.*) to the Stress Induced by Offshore Experimental Seismic Prospecting in the Mediterranean sea. Mar. Pollut.

Bull. 38, 105-1114.

Scholik, A.R., Hong Yan, Y., 2001. Effects of underwater noise on auditory sensitivity of a cyprinid fish. Hear. Res. 152, 17-24.

Schwarz, A.L., Greer, G.L., 1984. Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds. Can. J. Fish. Aquat. Sci. 41, 1183-1192.

Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). The Journal of Experimental Biology 207, 427-435.

Summerfelt, R.C., Smith, L.S., 1990. Anaesthesia, surgery and related techniques, in: Schreck, C.B., Moyle, P.B. (Eds.), Methods in Fish Biology. American Fisheries Society, Bethesda, pp. 213-272.

Sun, Y., Song, Y., Zhao, J., Chen, J., Yuan, Y., Jiang, S., Zhang, D., 2001. Effect of drilling noise and vibration on growth of carp (*Cyprinus carpio*) by cut-fin marking. Marine Fisheries Research/Haiyang Shiuchan Yanjiu 22, 62–68.

Tolimieri, N., Haine, O., Montgomery, J.C., Jeffs, A., 2002. Ambient sound as a navigational cue for larval reef fish. Bioacoustics 12, 214–217.

Wysocki, L.E., Dittami, J.P., Ladich, F., 2006. Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.