In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area

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ABSTRACT

The short-term behavioural effects of two types of boat noise were tested on *Gobius cruentatus* and *Chromis chromis*, i.e. one permanently and one temporarily benthic vocal fish species living inside the WWF-Natural Miramare Marine Reserve (Northern Adriatic Sea, Italy). The underwater noises produced by a 26-m tourist ferry and a 5-m fiberglass boat were recorded inside the core zone of the reserve. Each type of boat noise was subsequently played back *in situ* to 10 animals *per* species (*C. chromis* males caring their nests or *G. cruentatus* in their shelters).

The 1/3 octave spectra of recorded sound pressure levels were compared to the underwater ambient noise level and to sound pressure level measured at the hearing threshold of the two species. The boat noise levels have been calculated in terms of particle acceleration for both field measurements and *in situ* playback projections and subsequently compared to the available measured values of particle acceleration at the hearing threshold.

The animals were free to move in all directions during the whole experimental session. The behaviour of each fish was videotaped by an underwater camera for a total of 10 min (5 min before and 5 min during the noise playback). No short-term behavioural reaction (aversion) was observed in any of the specimen of the two species during the playback of the recorded noises, therefore suggesting no impact. However a time-budget analysis revealed a significant change in the total time spent in caring their nests (*C. chromis*) or inside their shelters (*G. cruentatus*). This result highlighted how analyzing fish reaction on a short-term might underestimate the effects of noise disturbance and indicated that the overall fish behaviour should be considered to assess noise impact.

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1. Introduction

Worldwide concern about the impact of noise pollution on aquatic fauna is growing in these years. There is an increasing amount of scientific evidence that anthropogenic noise can harm marine species (Tyack, 2008). While noise pollution has been recognised to be steadily growing in the world's oceans (Andrew et al., 2002), this phenomenon is still largely unmonitored in coastal areas. A major source of low-frequency noise (under 1000 Hz) in marine species living in highly anthropized coastal areas comes from boats and vessels, since their number, distribution and mobility are very high (Greene and Moore, 1995; Richardson and Würsig, 1997).

Boat noise represents a chronic source of harassment (Haviland-Howell et al., 2007) for fish species (Popper, 2003), whose communication for inter- and intra-sexual selection is mainly based on low-frequency sound signals (Ladich and Myrberg, 2006; Myrberg and Lugli, 2006). It has been recently shown that boat noise may induce endocrine stress response (Wysocki et al., 2006), as well as diminish hearing ability and mask intra-specific relevant signals in exposed fish species (Scholik and Yan, 2002; Amoser et al., 2004; Vasconcelos et al., 2007; Codarin et al., 2009). In addition, boat and vessel noises have the capacity to provoke short-term changes in the spatial position and group structure of pelagic fish in the water column, as shown by many studies carried out since the 1960s (for example, Buerkle, 1974; Olsen et al., 1983; Schwarz and Greer, 1984; Engås et al., 1995; Soria et al., 1996; Vabø et al., 2002; Mitson and Knudsen, 2003; Ona et al., 2007; Sarà et al., 2007). The most common boat-induced behavioural changes in fish include the temporary cessation of activities, alarm response, flight reaction or the so-called 'startle' response, i.e. a powerful flexion of the body followed by a few seconds of faster

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swimming (Boussard, 1981). In many species, fish behaviour is affected by noise only when a certain threshold in pressure level is reached. Very often, the previously mentioned short-term changes in swimming speed have been used to fix the threshold of fish behavioural reaction to human noise (Kastelein et al., 2008a), but such noise-response studies on marine fish are rare (Akamatsu et al., 1996) and they show marked differences in the reaction of various species, depending on the threshold levels of the noise frequencies, the threshold levels at which a reaction occurs varying *per* frequency for each species (Kastelein et al., 2008b). The relationship between the strength of short-term responses and the underlying sensitivity of wildlife is unlikely to be straightforward (Gill et al., 2001) and fish reactions depend not only on the properties of noise but also on the individual context (e.g. location, temperature, physiological state, age, body size, etc.). As result, much more information is still needed to understand the behavioural consequences of anthropogenic noise exposure (Popper et al., 2004).

Interestingly, the application of a time-budget analysis has recently proved to be a useful tool for assessing human disturbance in several cetacean species (e.g., Williams et al, 2006; Hodgson and Marsh, 2007; Dans et al., 2008; Stockin et al., 2008). This technique has never been applied to fish species so far, therefore, the aim of this study was (1) to record, inside a core zone of a coastal reserve, the noise produced by a tourist ferry and a fiberglass boat moving along and inside the Marine Protected Area (MPA); (2) to field-test, through the time-budget method, short-term effects of both boat noise types on a permanently and a temporarily benthic soniferous fish species (*Gobius cruentatus* and *Chromis chromis*) living inside the MPA.

Investigating the impact of boat noise on target fish species is particularly relevant for coastal MPAs, which are biologically rich locations in highly populated regions and deserve protection from anthropogenic pollutants. Managers of MPAs have recently begun to study noise (Agardy et al., 2007; Haren, 2007) but far too little is known about animals hearing capacity, behaviour and ecology to set a standard or apply an exposure limit with confidence (Popper and Løkkeborg, 2008). This high level of uncertainty underlines the need for local assessment in noise pollution as well as a precautionary principle as management rule for sensitive areas (Horowitz and Jasny, 2007).

2. Methods

2.1. Study area

The field-work has been run at the WWF-Miramare Natural Marine Reserve, an UNESCO-MAB Biosphere Reserve located in the Gulf of Trieste (Northern Adriatic Sea, Italy) at 45°42′08″ N and 13°42′42″ E. The area is divided in a core (30 ha) and in a buffer zone where the maximum depth reaches 18 m. The level of human presence around Miramare MPA is extremely high compared to more remote Mediterranean MPAs. The site is less than 8 km away from the city of Trieste, an important seaport with more than 48 million tons of ship traffic per year. The site is also very close to a tourist port characterized by high recreational boat traffic. However, the coastline of the reserve (1700 m) and its offshore area (120 ha) are densely populated by several fish species (Guidetti et al., 2005), most of which spawn during summer. This makes the Miramare Reserve an important seasonal nursery area in the North Adriatic Sea. A recent study, which assessed the number of vessels moving in the Gulf of Trieste and their distance from the reserve (Codarin et al., 2008), showed that tourist ferry boats that connect different ports along the local coastline during the summer are a possible source of noise disturbance for local fish species, due to their frequency (4 times a day) and their route, which is very close to the limit of the core zone of the MPA. In addition, a fiberglass boat used by the MPA staff for moving inside the area during research and educational activities has been labelled as another source of annoyance.

2.2. Target species

The red mouthed goby, *G. cruentatus*, is a small benthic member of the Family Gobiidae and is common in Mediterranean Sea and in Western Atlantic Ocean. During the year, it lives in rock crevices (Wilkins and Myers, 1993), defending itself from intruders using visual and acoustic displays (Picciulin et al., 2006). Acoustic displays consist in four different types of sounds, ranging in peak frequency from 82 Hz to 185 Hz (Sebastianutto et al., 2008). Recently the species' audiogram, as well as the audiogram of *C. chromis*, has been described in terms of both sound pressure and particle acceleration (Wysocki et al., 2009). The hearing range of *G. cruentatus* reaches 700 Hz, with highest sensitivity to sound pressure at 300 Hz and highest sensitivity to sound particle acceleration at 200 Hz (Wysocki et al., 2009). Unlike other goby species, *G. cruentatus* has a swim bladder (Gil et al., 2002).

The Mediterranean damselfish, *C. chromis*, is a common small fish that lives in shoals in the Mediterranean Sea, between 3 and 30 m deep. From June to September males synchronously establish territories, prepare nests and court females through visual displays (Abel, 1961) and acoustic signals, i.e. broadband pulses, called "pops," peaking at about 400 Hz (Picciulin et al., 2002). Females lay demersal eggs that are guarded and fanned by males until hatching. When hatching of the eggs is concluded, males leave the nests and rejoin the feeding school; males remain on their territory for about 10 days. *C. chromis*' audiogram reaches an upper frequency limit of 600 Hz and shows highest sensitivity to both particle acceleration and sound pressure at 200 Hz (Wysocki et al., 2009).

In the study area, both species live and reproduce in waters between 3 and 7 m deep on a 150-m coastal rocky reef located inside the core zone of the Natural Marine Reserve of Miramare. Being as far as possible from the limits of the core area, this reef can be considered the most protected area. It can be used as a reference point for boat noise recordings, assuming that if a disturbance from noise recorded at this point is noticeable, a much larger impact of the stimulus can be expected along the whole MPA.

2.3. Boat noise recordings

The noise emissions of a 26-m tourist ferry (TF) with inboard diesel engine moving at 6 kn along the perimeter of the core zone of the Miramare MPA and a 5-m fiberglass boat (FB) with 40 HP outboard engine moving at 15 kn were recorded on the 6th June 2004 during daytime in the coastal rocky reef where fish density of target species was high (Fig. 1). A calibrated Reson TC4032 hydrophone (sensitivity −170 dB re 1 V/µPa; frequency range: 5 Hz–120 kHz) was placed underwater, 4 m deep from a boat (bottom depth: 8 m) and connected to a Pioneer DC-88 DAT recorder (sample rate 44.1 kHz, 16-bit) operating on batteries. During the recordings, water temperature — equal to 18 °C — was measured at the same depth using a multiparametric 316 CTD-Idronaut probe. The distances of the hydrophone between TF and FB were 82 m and 1 m, respectively. Distances were calculated by hand-held GPS. The recording conditions were: sea state 0–1 (Douglas scale), wind speed 7–15 km/h, and few clouds (5% clouds at maximum). Each recording lasted 60 s. As a comparison, 60-second-long samples of the sea ambient noise (SAN) were collected at the same site and depth when no boats were moving in a range of 10 nautical miles from the recording point.

Samples of 25 s from SAN, FB and TB were considered for the analysis, the boat samples included the highest amplitude value of the noise. The noises were analysed looking at instantaneous sound pressure levels (SPL, L-weighted, 20 Hz to 20 kHz, RMS fast) with Spectra RTA (Sound Technology) spectral analyser, operating in 1/3 octave bands. Each recording was previously calibrated with a signal of 100 mV RMS at 1 kHz recorded at the start of each tape. The equivalent continuous SPL (L_{Leq}) was calculated averaging the instantaneous SPLs values over 25 s.
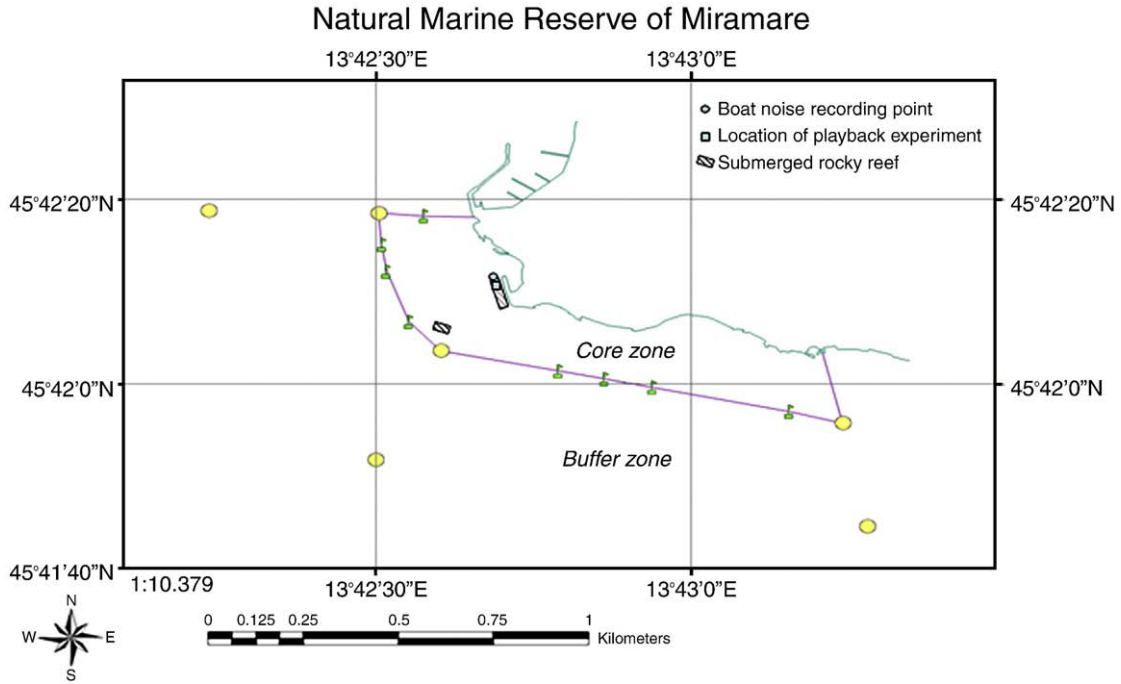
Fig. 1. Map of the Natural Marine Reserve of Miramare. The circle shows the recording point of ambient and boat noises; the square indicates the location of the playback experiment. The two submerged rocky reefs inside the core area of the reserve are also indicated.

2.4. Relationship between sound pressure, particle velocity and particle acceleration

In underwater acoustics, till now most of the work considered just the sound pressure field. Transducers (hydrophones) are sensitive directly to sound pressure, and propagation models are usually dealing only with calculation of the sound pressure as a function of the source–receiver distance. On the other hand, we have increasing scientific evidence (Fay and Edds-Walton, 1997; Popper and Fay, 1999; Bass and McKibben, 2003) that particle motion plays an important role in fish hearing and, within the context, that particle acceleration may be the most appropriate component for describing underwater sound.

Water particle motion can indifferently be expressed in terms of displacement x (measured in m), velocity v (m/s) or acceleration a (m/s^2). Let's focus just on velocity and acceleration. We usually compute levels in decibel scale according with the following formulas:

$$SPL = 20 \cdot \log_{10}\left[\frac{p_{RMS}}{p_o}\right] \; ; \; p_o = 1 \cdot 10^{-6}\,\text{Pa}$$
$$L_v = 20 \cdot \log_{10}\left[\frac{v_{RMS}}{v_o}\right] \; ; \; v_o = 1 \cdot 10^{-9}\,\text{m/s} \qquad (1)$$
$$L_a = 20 \cdot \log_{10}\left[\frac{a_{RMS}}{a_o}\right] \; ; \; a_o = 1 \cdot 10^{-6}\,\text{m/s}^2.$$

It must be noticed that these reference quantities define dB scales which provide a value of 120 dB when, respectively, pressure equates 1 Pa and acceleration equates 1 m/s^2. The velocity level is equal to the acceleration level just at a very specific frequency, i.e. 159.15 Hz (so that $\omega = 2\pi f = 1000$ rad/s). At other frequencies, the particle acceleration level increases by 6 dB/octave with respect to particle velocity level:

$$L_a = L_v + 20 \cdot \log_{10}\left[\frac{f}{159.15}\right]. \qquad (2)$$

2.4.1. Plane-wave (far field) relationship

Usually, the relationship between the sound pressure level, the particle velocity level and particle acceleration level is found considering a plane wave field. For a plane, progressive wave, the ratio between sound pressure and particle velocity is the "characteristic acoustic impedance" of water:

$$\frac{p}{v} = z = \rho \cdot c = \left(1000\frac{\text{kg}}{\text{m}^3}\right) \cdot \left(1500\frac{\text{m}}{\text{s}}\right) = 1,500,000\,\text{rayls}. \qquad (3)$$

Therefore the relationship between SPL and L_v is:

$$L_v = SPL - 63.5\,\text{dB}. \qquad (4)$$

Once L_v is known, we can easily compute also the acceleration level L_a, thanks to Eq. (2):

$$L_a = SPL - 63.5 + 20 \cdot \log_{10}\left[\frac{f}{159.15}\right]. \qquad (5)$$

The above formulas hold also for spherical waves, if the distance between source and receiver is much larger than the wavelength (at least 5 times, that is, "far field"). Dealing with the recordings of passing-by boats in Miramare MPA, since the average distance between source and receiver was several meters, it can be assumed that far-field conditions occurred. Hence, for the boat noise measurements (and SAN, too), the values of L_a are obtained by Eq. (5). Table 1 (column 2) reports the values of SPL $- L_a$ computed according to Eq. (5).

2.4.2. Spherical-wave (near-fields) relationship

At distances shorter than 5 times the wavelength, the ratio between sound pressure and particle velocity is not simply equal to the characteristic acoustic impedance of water. Instead, the following equation holds at a distance r from a point source, radiating a spherical sound wave:

$$\frac{p}{v} = \frac{\rho \cdot c}{1 + \frac{1}{j \cdot k \cdot r}} \qquad (6)$$

in which k is the wave number: $k = \frac{\omega}{c} = \frac{2 \cdot \pi \cdot f}{c}$.

Table 1
Sound pressure level (SPL, dB re 1 µPa) *versus*, particle acceleration (L_a, dB re 1 µm/s^2) conversion factors; * from Wysocki et al. (2009).

Column	1	2	3
Frequency (Hz)	$SPL - L_a$ (dB) In pool*	$SPL - L_a$ (dB) Far field	$SPL - L_a$ (dB) Near field (0.5 m)
100	40	68	54
200	38	62	53
300	34	58	53
500	36	54	51
600	36	52	50
700	38	51	49
1000	35	48	47

As we are only interested in the magnitude of the velocity signal compared to the magnitude of the pressure signal, we can discard the phase, and we obtain:

$$|v| = \frac{|p|}{\rho \cdot c} \cdot \left| 1 + \frac{1}{j \cdot k \cdot r} \right| = \frac{|p|}{\rho \cdot c} \cdot \sqrt{1 + \frac{1}{(k \cdot r)^2}}. \tag{7}$$

Transforming to the dB scale, and converting, as done previously, velocity to acceleration, we get:

$$L_a = L_p - 63.5 + 10 \cdot \log_{10}\left[1 + \left(\frac{c}{2 \cdot \pi \cdot f \cdot r} \right)^2 \right] + 20 \cdot \log_{10}\left[\frac{f}{159.15} \right]. \tag{8}$$

The spherical wave model works reasonably well for the *in situ* playback experiments performed at the Miramare Marine Reserve: the loudspeaker-receiver distance was 0.5 m and hence the expected difference between SPL and L_a can be computed with Eq. (8), as reported in column 3 (Table 1).

2.4.3. Test pool for hearing threshold measurements

Inside the small basin where the hearing threshold experiments have been performed, none of the above relationships holds. The true values of sound pressure and particle acceleration audiograms have been measured by Wysocki et al. (2009), and reported in their paper, along with the SPL—L_a difference: hence these values are also employed here, as reported in column 1 (Table 1).

2.5. Experimental set-up

An electroacoustics playback system was used, with the aim of ensuring that each fish specimen was exposed to a fairly constant noise level during the whole test.

25-seconds of boat noise was played back in loop for 5 min with a 20 cm diameter underwater speaker (AQ339 Clark Synthesis; frequency range 35 Hz–17 kHz), connected to a Sony TCD-D 100 DAT recorder and a power amplifier. The underwater speaker was the greatest constraint on the frequency response of the system; for example, its sensitivity cut-off of 35 Hz removed the noise produced by the ferry propeller blade rate. However, the maximum hearing sensitivity to sound pressure of the target species is located at 200 Hz (*C. chromis*) and 300 Hz (*G. cruentatus*) with an upper frequency limit of 600–700 Hz (Codarin et al., 2009; Wysocki et al., 2009), where the speaker does not produce distortion in the reproduced signal.

A series of preliminary tests were run in order to set the volume of the projected stimuli. Several combinations were tested using the same experimental settings in terms of distance and position of the speaker to the recording hydrophone (Reson TC4032), the latter 'mimicking' the position of the tested fish. The played-back noise was re-recorded, the power spectra calculated with Spectra RTA (Sound Technology) spectral analyser and the spectra superimposed on the

spectra of the boat noises, in order to compare SPLs and frequency distributions; the stimuli with the most similar spectrum to the recorded one were chosen. Finally, the particle acceleration level of the projected noises at each frequency was calculated applying spherical-field Eq. (8).

Playback experiments were run from July to beginning of August along the 150-m coastal rocky reef located inside the core zone of the Natural Marine Reserve of Miramare, which has a maximum water depth of 7 m. The noises were projected to 10 different animals *per* noise type and *per* considered species (for a total of 20 tested animals *per* species), distributed along a section of 35 m of length of reef, at a depth ranging from 3 to 6 m. The tested fish were spaced out by at least 2 m of distance. This allowed the observer to track the animal individually using focal sampling method.

One fish *per* species was tested *per* day (one session was run during the morning, another during the afternoon, with a randomization between the two species, completing all experiments between 10:00 and 16:30 local time) in order to prevent any possible effect due to noise exposure from testing of neighbouring fish. The order of the two boat stimuli was randomized across subjects. Due to enforced restrictions within the naturally protected areas (Ciriaco et al., 1998), we avoided physically handling animals and/or marking the nest for multiple testing of the same specimen. Therefore the age/length and sex of each tested fish was not sampled and just one replicate *per* animal was possible.

The speaker was positioned *in situ*, i.e. on the rocks of the artificial reef located inside the core zone of the Reserve, 3–6 m deep, at a distance of 50 cm from the tested animal (*C. chromis* male caring its nest or *G. cruentatus* in its shelter) and suspended from a PVC frame so that the centre of its membrane was at the same level and facing the fish. The fish were free to move in any direction during the whole experimental session.

The behaviour of each fish was videotaped by an underwater camera SONY VIDEO 8 — TR805 (10×) for a total of 10 min (5 min of silence and 5 min of playback). Before starting the field video recordings we set a distance, near enough to get sufficient information, overcoming the problem of poor water visibility, but not too close to disturb the fish. As we worked in a protected area where fish are quite acquainted with SCUBA-divers, this shortened their flight-distance. Eventually a distance of 1.3–1.5 m from the nest was the best range. Ten minutes of acclimatization with camera and loudspeaker turned off occurred before each playback trial; this allowed the fish to habituate to the apparatus. In addition, previous observations indicated that the whole recording and projecting system did not have an over-riding effect on the fish behavioural response (Picciulin, 2004).

2.6. Behaviour data analysis

In order to obtain the time budget of fish behaviour, all audio-video recordings were analysed frame-by-frame and behaviour was classified and logged on previously prepared *ad hoc* check-lists using a software widely used for ethological observations, EthoLog 2.2 (Ottoni, 2000). More in detail, two behavioural units, i.e. 'stationary position' (the fish stays close to the shelter, above the substrate in a horizontal position with the pectoral fins spread) and 'in the shelter' (the fish is inside the shelter, therefore it is not visually inspecting its territory) were considered for *G. cruentatus* behavioural analysis according to the ethogram of the species (Picciulin et al., 2006). These two are critical behaviours for Gobiids, since they rely mainly on visual perception for social and other activities (Kinzer, 1960). In fact, when a goby does not patrol its territory by visual inspection, peripheral overlap of territories between neighbours is observed (Wilkins and Myers, 1995).

The 'nest caring' unit was considered in the analysis of *C. chromis* behaviour. This unit includes all the single patterns and postures

associated both with cleaning of the spawning-place and with egg care and is easily recognizable due to the contact or to the very close proximity of the *C. chromis* male to its nest (Verginella et al., 1999).

A Wilcoxon signed-ranks test was applied during the observation of behaviour in the two conditions (the 5-min period of silence *versus* the 5-min period of noise playback). Statistical tests were run using Statistica 6.0 for Windows (StatSoft, Inc.).

3. Results

3.1. Boat noise characterization

The equivalent continuous SPL ($L_{Leq, 25 s}$) of the tourist ferry (TF, recorded at 82 m of distance) and the fiberglass boat (FB, recorded at 1 m of distance) were 140.3 and 158.8 dB re 1 μPa, with a maximum instantaneous SPL of 147.7 and 162.2 dB re 1 μPa, respectively. Assuming a cylindrical spreading (10 log R, meaning a loss of 3 dB per doubling of distance) as the best transmission loss model in shallow water (Richardson et al., 1995), the source level of TF noise can be estimated 160 dB re 1 μPa at 1 m. It is however possible that this SPL value is much higher if recorded closer to the ferry boat, due to several variables affecting the sound propagation in coastal area (i.e. bottom morphology, absorption, shadow zones due to refraction, salinity, thermal clines, etc). Both noise spectra had the main energy content below 1.5 kHz, with a peak at 1033 Hz and 602 Hz for TF and FB, respectively. Below 1.5 kHz, the spectral energies of TF and FB noises were 13 and 33 dB re 1 μPa higher than Sea Ambient Noise (SAN). The $L_{Leq, 25 s}$ of the recorded SAN was 132.3 dB re 1 μPa. The 1/3 octave band noise pressure spectra of the two boats and of SAN are shown in Fig. 2.

Fig. 3 compares the 1/3 octave band noise spectra of the boats and of SAN in the frequency range below 1 kHz with the published *G. cruentatus* and *C. chromis* audiograms measured with the AEP methods in terms of sound pressure level (Codarin et al., 2009). The FB noise exceeded the SPL threshold of *C. chromis* and *G. cruentatus* up to a maximum of 45 dB (at the 160 Hz-1/3 octave band) and 43.3 dB (at the 160 Hz-1/3 octave band) and the TF noise exceeded up to a maximum of 22.3 (at the 160 Hz-1/3 octave band) and 21.6 dB (at the 125 Hz-1/3 octave band), respectively.

Fig. 4 represents the boat noise and SAN spectra in terms of particle acceleration (L_a) compared to the *C. chromis* and *G. cruentatus* audiograms measured with the AEP methods (Wysocki et al., 2009).
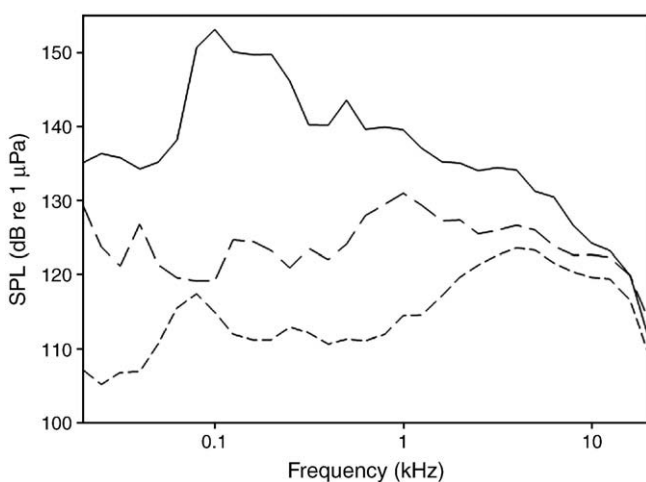

Fig. 3. 1/3 octave band Sound Pressure Level spectra (logarithmic scale) of ambient (short dash line), tourist ferry (dash–dotted line) and fiberglass (continuous line) boat noises compared to hearing thresholds of *Chromis chromis* (circles) and *Gobius cruentatus* (triangles) (sampling frequency 44.1 kHz).

In particular, the calculated values for the boat noise measurements as well as the playback noise projections are shown.

3.2. Performances of the playback system

The equivalent wideband continuous SPL ($L_{Leq, 1 min}$) of the projected noise was equal to 162.4 (FB) and 142.2 (TF) dB re 1 μPa, whereas the equivalent continuous particle acceleration level of the projected noise ($L_{a,eq, 1 min}$) was equal to 110 (FB) and 88 (TF) dB re 1 μm/s^2.

The playback SPL values are just slightly larger than the measured values during the boat pass-by, the playback error in terms of wideband SPL was just +3.5 dB for FB and +2.1 dB for TF. On the other hand, the calculated values of particle acceleration generated by the loudspeaker are much larger than those which were present during the recording of real boats. In this case the error, in terms of wideband particle acceleration level, was calculated to be respectively 10.2 dB for FB and 3.8 dB for TF. The spectra (Fig. 4) clearly shows that the acceleration level in the near field boosts significantly at very low frequency and the calculated values of L_a (particle acceleration level)

Fig. 2. 1/3 octave band sound pressure level spectra (logarithmic scale) of ambient (SAN, short dash line), tourist ferry (TF, dash–dotted line) and fiberglass (FB, continuous line) boat noises recorded in the core zone of Natural Marine Reserve of Miramare (sampling frequency 44.1 kHz).
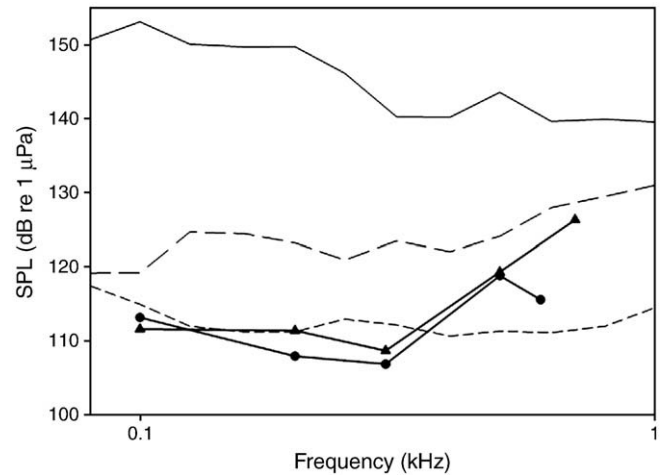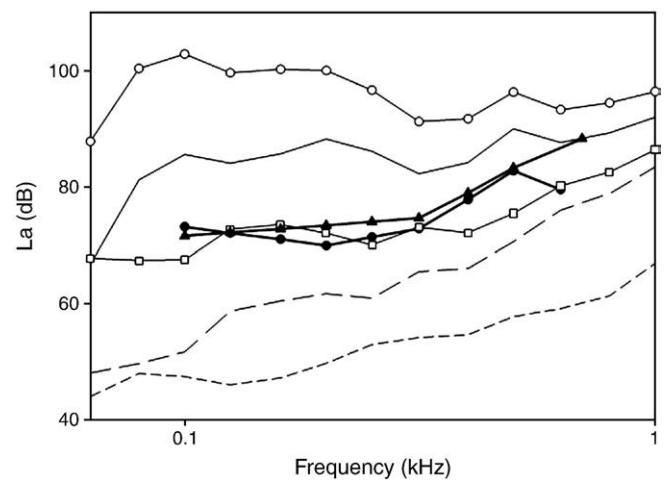

Fig. 4. 1/3 octave band Particle Acceleration Level spectra (logarithmic scale) of ambient (short dash line), tourist ferry (dash–dotted line; playback: open square) and fiberglass (continuous line; playback open square) boat noises calculated in terms of particle acceleration (L_a) compared to the *Chromis chromis* (filled circles) and *Gobius cruentatus* (filled triangles) audiograms measured with the AEP methods as particle acceleration.
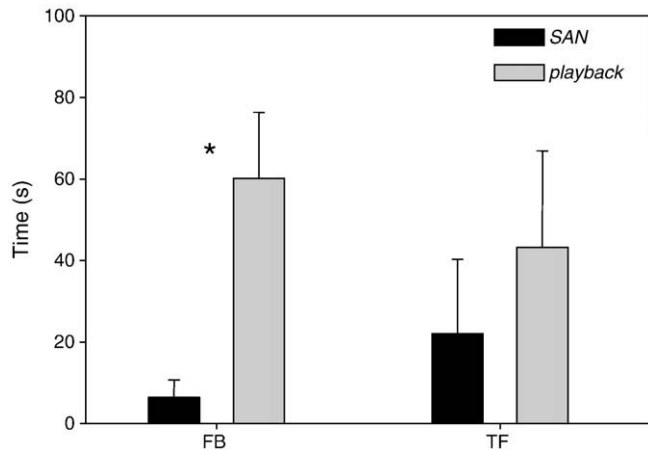
Fig. 5. Average time + standard error spent by *G. cruentatus* inside its shelter when exposed to natural sea ambient noise (SAN) and during playback of either tourist ferry (TF) or fiberglass boat (FB) noises. * indicates significant statistical differences highlighted by Wilcoxon test.

generated by the loudspeaker are much larger than the values estimated by the recording of the boat noises.

3.3. Fish behavioural responses

The playback of the two noises did not elicit any instantaneous response, i.e. flight or rapid cessation of activities in any of the two species: the animals remained still and did not show any overt variation in activity. Nevertheless, only during the playback of FB noise the time spent by *G. cruentatus* inside the shelter increased (Wilcoxon signed-ranks test, $N = 10$, $P = 0.017$; Fig. 5), while the time spent in stationary position outside the shelter decreased (Wilcoxon signed-ranks test, $N = 10$, $P = 0.031$; Fig. 6). A significant decrease of the time spent caring their nests was observed in *C. chromis* during the playback of both boat noise types (signed-ranks test, $N = 10$, $P = 0.027$ for TF, $P = 0.041$ for FB; Fig. 7).

4. Discussion

Vessel traffic noise dominates the sea ambient noise of coastal areas mainly below 1 kHz (Richardson and Würsig, 1997). Although being extremely variable in relation to speed, load, pitch angle of propeller, vessel design and age (Mitson, 1993; Richardson et al.,
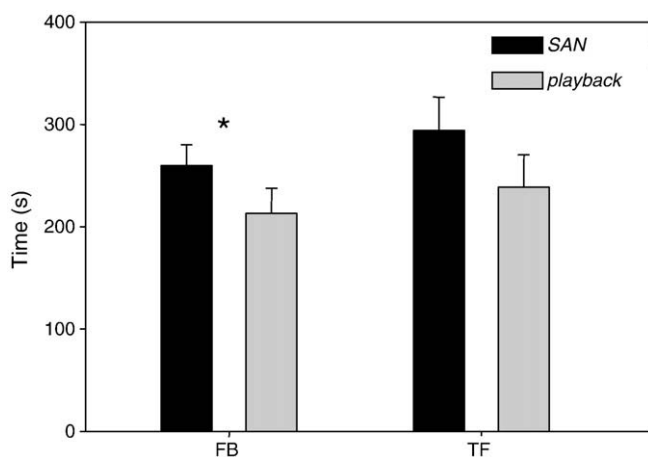

Fig. 6. Average time + standard error spent by *G. cruentatus* in stationary position when exposed to natural sea ambient noise (SAN) and during playback of either tourist ferry (TF) or fiberglass boat (FB) noises. * indicates significant statistical differences highlighted by Wilcoxon test.

Fig. 7. Average time with standard error spent by *C. chromis* males caring their nests when exposed to the natural sea ambient noise (SAN) and during playback of either tourist ferry (TF) or fiberglass boat (FB) noises. * indicates significant statistical differences highlighted by Wilcoxon test.
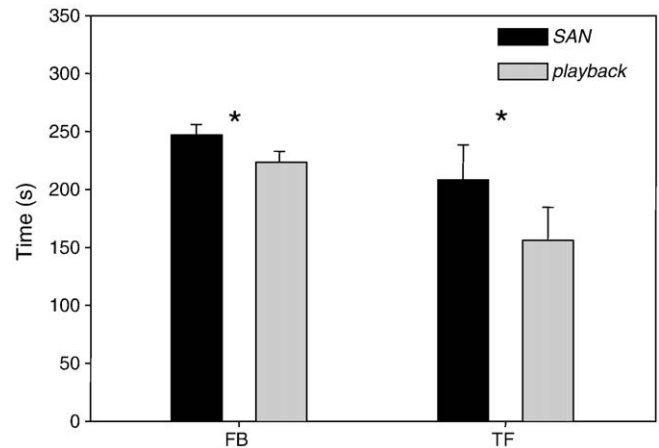
1995), boat source level (i.e. the amount of radiated sound measured at 1 m from the source, SL) generally ranges from 145 to 170 dB re 1 μPa at 1 m, with an average of 162 dB re 1 μPa at 1 m for speeds of around 50 km/h (Boussard, 1981; Greene and Moore, 1995; Erbe, 2002; Vasconcelos et al., 2007). These values are in accord with our recorded and estimated SL measurements of the noises produced by the fiberglass boat and the ferry boat.

In this study we were interested in the background noise and boat equivalent continuous sound pressure levels (i.e., the root mean square of the local instantaneous sound pressure calculated over a given interval of time) recorded in the reef under quiet condition inside the core zone of the Miramare MPA. Here the background noise was relatively high (about 132.3 dB re 1 μPa), also in comparison to other measurement done inside the core area, at 150 m of distance from the shoreline (97 dB re 1 μPa, 10 m depth, Codarin A., *personal communication*). Despite this, the boat noises resulted to be louder, especially below 1.5 kHz. In addition, the recorded boat noises, expressed in terms of pressure levels, largely exceed the best pressure-hearing thresholds of *C. chromis* and *G. cruentatus*, especially on the same frequency range of species-specific vocalizations (Picciulin et al., 2002; Sebastianutto et al., 2008), and have therefore the potential to be detected by these fish species inhabiting the Miramare marine protected area and to mask the intra-specific communication, as elsewhere reported (Vasconcelos et al., 2007; Luczkovich and Sprague, 2008; Codarin et al., 2009).

Looking just at sound pressure data can be inappropriate, because both the tested species are probably primarily sensitive to the particle motion of sound, namely to particle acceleration. In fact, gobies are commonly considered to be hearing generalists (Lugli et al., 2003), i.e. they detect sound solely via their inner ears, and no other peripheral morphological structures are involved in the hearing process. Similarly, no swim bladder diverticula or other peripheral adaptations have been described so far in Pomacentrids. Nevertheless, it is often unknown which sound component (particle motion or sound pressure) is more relevant to most of the species for detecting sound at the hearing threshold (Horodysky et al., 2008). For example, hearing in *Stegastes* (syn. *Eupomacentrus*) *dorsopunicans* (Pomacentridae) resulted to be governed by particle motion around 100 Hz, but it was dominated by pressure detection at frequencies of 300 Hz (Myrberg and Spires, 1980).

The measures of particle motion signals from boats were not possible due to the lack of commercially available sensors that could be used in the field, but the particle acceleration (L_a) levels of boat noises have been here calculated for both field measurements and playback projections. The L_a spectra of the projected noises resulted to

be partially distorted in the very low-frequency range — in respect to the field recordings — due to the closeness of the sound source, resulting in a high exposure of the tested fish species to particle acceleration. This can be considered as an artifact of the system employed. Unfortunately, using one single loudspeaker and given the water characteristics, it is very difficult to generate the correct SPL and L_a spectra, simultaneously. Therefore the present paper highlighted the need to adopt in the future a system employing opposite couples of loudspeakers, such as using the Ambisonic technology (Gerzon, 1985), in order to successfully playback pressure and acceleration signals at sea. In addition, the influence of speaker location and fish position remains to be investigated in details when discussing the effect of the played-back sound on behaviour, whereas the same parameters have been recently considered in relation to the sound pressure and particle motion AEP hearing thresholds determination (Ladich and Wysocki, 2009).

The distortion in the relative proportion between the particle acceleration and sound pressure spectra also shows that it is very important to measure both sound pressure and particle acceleration, and to compare both of these quantities with the corresponding fish hearing thresholds. It has to be noticed that both the here considered boat noises, when measured as sound pressure levels, exceed the hearing thresholds of the two tested species, as above mentioned. On the contrary, when measured as acceleration levels, only the projected noise of the fiberglass boat (FB) significantly exceeded the *C. chromis* and *G. cruentatus* particle acceleration audiograms, whereas the recorded FB noise and the projected TF noise resulted only slightly above their hearing threshold.

Dealing with the behavioural modification, this field experiment did not evidence any short-term mild reactions, such as avoidance or escape, by any of the two tested species during playback. The lack of such reactions would be generally interpreted as the absence of behavioural impact of the considered noise types. However, the reason why *C. chromis* didn't escape from the noise source may be related to the high risk associated to such behaviour, which would imply sneaking, egg predations and aggressions by conspecifics (Picciulin et al., 2004). Similarly, *G. cruentatus* will reduce their ability in territorial defence in case of fleeing. In the benthic fish species, like the red mouthed goby or the Mediterranean damselfish during reproduction the behavioural response to disturbance is not a straightforward "cause–effect" relationship; conversely, animals may remain in a disturbed site if there are important resources, adopting a conditional behavioural strategy to cope with environmental modifications (Gill and Sutherland, 2000).

The time-budget analysis applied in this study indicated a non-negligible shift of relevant activities in both species, at least during the exposure to the fiberglass boat noise. Despite the low sample size, the effect on fish behaviour induced by boat noise can be deduced from the data. This highlighted how fish behaviour, in a broader sense, needs to be considered when defining the effects of man-made noise to fish fauna, as suggested by other authors (Shumway, 1999; Gill and Sutherland, 2000). It also stressed how behavioural responses to disturbance divert time and energy from other fitness-enhancing activities such as feeding, mating, defending territory, and this needs to be taken into account when evaluating disturbance on fish behaviour. In this regard, an interesting approach has been described by Frid and Dill (2002), who discussed the nonlethal human-caused disturbance in the theoretical framework of the economic models of anti-predator behaviour.

From a conservation point of view, it is seldom known how immediate behavioural responses are transformed into long-term-changes influencing reproduction, survival or population size (Gill et al., 2001; Bejder et al., 2006). Cumulative and long-term impacts to fish population of noise-induced behavioural shifts are very difficult to predict, the biological significance of a particular noise varying according to fish age (Egner and Mann, 2005), sex, ripeness and state of activity as well as location, season and time of day (McKibben and Bass, 1998; Sisneros et al., 2004a,b). This level of uncertainty nowadays requires the precautionary principle as management rule for sensitive areas (Horowitz and Jasny,

2007). As a consequence, even the small Miramare Marine Reserve has to reduce the unwanted noise input inside the area. Passive long-term monitoring programs, definition of noise-free areas or seasonal restriction of noise-producing activities during sensitive periods is a tool that can be exploited by the local management.

Besides the validity of these general rules, the managers are often asking for a metric that may help them to preventively define limits of noise exposure. Actually, fixed levels above the basic hearing threshold of animals have often been used as criteria for acceptable sound levels (Southall et al., 2007). However, these regulatory limits are expressed in terms that may have very little relevance for marine animals (Madsen et al., 2006), i.e. unweighted peak pressure, root mean square pressure or unweighted sound exposure level.

In conclusion, our results highlight that a larger number of behavioural studies on fish species should include simple disturbance measures in order to make mitigation measures more effective.

Acknowledgments

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