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Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy)

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ABSTRACT

The WWF-Natural Marine Reserve of Miramare (Trieste, Italy) is located in a major industrial and vacation area in the Adriatic Sea. Consequently, noise emanating from boating and shipping is an inevitable factor for local fishes. This study investigates the effects of ambient and ship noise on representatives of three vocal fish families with different hearing abilities. Ambient and ship noise were recorded, their sound pressure levels measured and played back in the lab. Auditory sensitivity was determined in *Chromis chromis*, *Sciaena umbra* and *Gobius cruentatus*, utilizing the auditory evoked potential recording technique. Compared to lab conditions, hearing thresholds determined during ambient noise playbacks were barely masked. Contrary, the noise emanating from a cabin-cruiser substantially reduced auditory sensitivity relative to thresholds in ambient noise. This masking effect was most pronounced in the frequency range where acoustic communication takes place. Boat noise potentially affects acoustic communication in fishes inhabiting the reserve.

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1. Introduction

Noise can be defined as an “unwanted sound” that affects animals by causing stress, increasing the risk of mortality by unbalancing predator/prey detection, and by interfering with orientation and sound communication, especially in the reproductive context. The amount of marine noise pollution generated by humans has been increasing significantly within the last decades not only in highly populated coastal areas, but also in the open ocean (Andrew et al., 2002; Ross, 2005; Tyack, 2008). This raises a broad concern on the extent of negative impacts on marine life.

Human-made noise in the sea can be categorized as high-intensity and acute such as the noise produced by military sonar, pile driving and seismic explorations, or lower-level and chronic such as ship noise. Most studies have focused on the high-intensity acute noise emanating from air guns or military sonars (Pearson et al., 1992; Wardle et al., 2001; Popper et al., 2005, 2007). They demonstrate that noise exposure can cause temporary hearing loss (Amoser and Ladich, 2003; Scholik and Yan, 2001; Smith et al., 2004), impaired temporal resolution ability (Wysocki and Ladich,

2005a), damage to the sensory epithelia of the inner ear (Hastings et al., 1996; McCauley et al., 2003) and endocrinological stress responses (Santulli et al., 1999; Sverdrup et al., 1994).

Only few studies, however, have addressed lower-level and chronic noise pollution, such as that due boat traffic (Haviland-Howell et al., 2007). Such noise can reduce the effective range of communication signals and therefore the signalling efficiency between individual fish (Amoser et al., 2004; Vasconcelos et al., 2007). Masking is not the only potential effect of boat noise: displacement of fish, impaired hearing ability as well as endocrinological stress response have been described in fish exposed to boat noise (Mitson and Knudsen, 2003; Sarà et al., 2007; Scholik and Yan, 2002; Wysocki et al., 2006).

Investigating the impact of boat noise on fish species is particularly relevant for sensitive areas located in highly populated coastal zones, such as the WWF-Miramare Natural Marine Reserve. The latter is an UNESCO-MAB Biosphere reserve located in the Gulf of Trieste (North Adriatic Sea, Italy). Compared to more remote Mediterranean Marine Protected Areas (MPAs), the level of human presence around Miramare MPA is extremely high. The site is close to a touristic port characterized by high recreational boat traffic. It is also less than 8 km away from the city of Trieste, an important seaport with more than 48 million tons of ship traffic per year. Nonetheless, the reserve's coastline (1700 m) and offshore area (120 ha) are densely populated by different fish species (Guidetti et al., 2005), most of which spawn during summer. This makes

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Miramare Reserve an important nursery area in the North Adriatic Sea. Known vocal fishes living in the Miramare MPA are the brown meagre (*Sciaena umbra*), included in the Barcelona Convention since 1995, the Mediterranean damselfish (*Chromis chromis*) and the red-mouthed goby (*Gobius cruentatus*). These species vocalize in agonistic and reproductive contexts and the frequency range of their vocalizations overlap with the low-frequency noise generated by boats and ships (Bonacito et al., 2002; Picciulin et al., 2002; Sebastianutto et al., 2008).

The aims of the present study were to (1) investigate whether three representative vocalizing fish species in Miramare, i.e. *S. umbra*, *C. chromis* and *G. cruentatus*, are adapted to the ambient noise and (2) determine the degree to which the noise of a cabin-cruiser – the typical boat type used both inside and around the reserve (Codarin et al., 2008; Picciulin and Codarin, 2008) – affects both their hearing sensitivity and their ability to detect conspecific sounds and thus their intraspecific acoustic communication.

2. Materials and methods

2.1. The tested species

S. umbra (Linnaeus 1758) is one of the five species of the family Sciaenidae (drums, croakers) living in shallow coastal waters of the Mediterranean Sea. During the reproductive period (May–August; Chauvet, 1991), *S. umbra* generates a chorus consisting of overlapping, short-lasting broadband pulses with main energies below 1 kHz (Bonacito et al., 2002). Preliminary observations showed that boat noise can mask the chorus, especially between 200 and 300 Hz (Picciulin et al., 2008).

C. chromis (Linnaeus 1758) is the only representative of the family Pomacentridae (damselfishes) in the Mediterranean Sea (Allen, 1991). It lives in shoals at depths ranging from 3 to 30 m. During the spawning season (June–September), males prepare a benthic nest and court females using visual displays, known as signal jumps (Abel, 1961). These are accompanied by acoustic signals, i.e. broadband single pulses called “pops”, peaking at about 400 Hz (Picciulin et al., 2002).

G. cruentatus (Gmelin 1789) is a small benthic member of the family Gobiidae (gobies) common in the Mediterranean Sea and in the Western Atlantic Ocean. Throughout the year, it uses crevices in the rocks as shelters (Wilkins and Myers, 1993), defending them vigorously from intruders with both visual and acoustic displays (Picciulin et al., 2006). The acoustic repertoire of *G. cruentatus* consists of four low-frequency sound types, emitted during territorial encounters (Sebastianutto et al., 2008).

None of the three species is known to possess accessory hearing structures (air-filled cavities connected to the inner ear, sensu Ladich and Popper, 2004) to enhance their auditory sensitivities. This was expected for *C. chromis* and *G. cruentatus* because no connection between the bladder and the auditory endorgans or any other accessory auditory structure has been previously described in other pomacentrids or gobiids (Lugli and Fine, 2003; Myrberg and Spires, 1980; Myrberg et al., 1986). Although some sciaenids have anterior swim bladder appendages expanding rostrally to various degrees towards the inner ear (Ramcharitar et al., 2001), *S. umbra* has a simple, well-developed, carrot-shaped swim bladder without appendages; it also has a rather thick, large and ovoid saccus in the sacculus of the inner ear (Chao, 1986; pers. obs.).

2.2. Field recordings of noise

The noise emission of a cabin-cruiser, 8.5 m long with a 163 HP inboard diesel engine (Fig. 1) operating at maximum speed (6 knots), was recorded on 29 December 2006, with good weather

and sea conditions (5% clouds, 0–1 Douglass sea state, wind speed 3–4 km/h). The recordings were conducted inside the Marine Reserve of Miramare at a submerged rocky reef where fish density is high (at 45°42'08" N latitude and 13°42'42" E longitude, Fig. 2). A calibrated Reson TC4032 hydrophone (sensitivity – 170 dB re 1 V/μPa) connected to a Pioneer DC-88 DAT (sample rate 44.1 kHz, 16-bit) operating on batteries was used. The hydrophone was placed at 10 m depth (bottom depth: 18 m). The minimum distance of the boat to the hydrophone was 10 m during its passage. The noise was recorded for 60 s.

Sea Ambient Noise (SAN) was also recorded for 5 min using the same procedure at the same site and depth. This was done in the absence of boats moving within a range of 10 nautical miles from the recording point.

Both ambient and boat noises were analysed in terms of instantaneous SPL (L-weighted, 40 Hz to 20 kHz, RMS fast) using a Spectra RTA (Sound Technology) spectral analyser calibrated with a signal of 100 mV RMS @1 kHz and hydrophone sensitivity. The equivalent continuous SPL (L_{Leq}), a measure commonly used to assess environmental noise (ISO 1996), was calculated by averaging the instantaneous SPL values over 60 s.

2.3. Field recordings of fish sounds

Sounds of *S. umbra* were recorded at night (between 21:00 and 23:00) on 26 April and 23 July 2007 from a boat in water depths of 4–12 m at four different locations (Fig. 2). The experiments were conducted within a range of 30 m to artificial rocky reefs located in the core zone of the reserve in which fish are abundant, but at unknown distances to the individual fish. The recording conditions were: sea state 1 (Douglas scale), wind speed 10–15 km/h, 15% clouds in April, and sea state 1, wind speed 7–10 km/h, 0% clouds in July.

Sounds of *C. chromis* were recorded on 25 July during day time at three different locations within one nesting area (Fig. 2) in a water depth of 4 m. Additional recordings were done at distances of 30–50 cm from nesting males displaying courtship behaviour (signal jumps). The recording conditions were: sea state 0, wind speed 1–2 km/h, 0% clouds.

Sounds were recorded either on a Marantz PMD 660 digital recorder (in April) or on a DAT recorder Sony TCD 100 (in July) using a hydrophone Brüel & Kjaer 8101 powered by a power supply Brüel & Kjaer 2804. Absolute sound pressure levels (L_{LFP} , L-weighted, 5 Hz–20 kHz, RMS fast) of the sounds were simultaneously measured with a sound level meter (Brüel & Kjaer 2238) connected to the second output of the power supply.

2.4. Experimental animals

Six adults of each species, i.e. the brown meagre (*S. umbra*, standard length (SL) 14.2–17.3 cm), the Mediterranean damselfish (*C. chromis*, SL 7.2–8.9 cm) and the red-mouthed goby (*G. cruentatus*, SL 9.7–12.1 cm), were captured with trap nets at rocky reefs facing the Trieste Gulf (North Adriatic Sea, Italy) and then transported to the University of Vienna.

The eighteen fish were kept in three different 250 l aquaria fitted with external filters and protein skimmers. The bottoms of the aquaria were covered with sand and equipped with several plastic shelters. The tanks were illuminated by automatically regulated lights with a 12 h:12 h L:D cycle. Animals were maintained at 20 ± 1 °C and fed with a combination of mussels (*Mytilus galloprovincialis*), crustaceans (*Penaeus* spp. and *Daphnia* spp.) and commercial food for aquaria (TetraMin®, TetraWerke, Germany).

All animal experiments were performed with the permission of the Austrian Federal Ministry for Education, Science and Culture (GZ 66.006/2-BrGT/2006).



Fig. 1. The cabin-cruiser used in this experiment for the noise emission recording (8.5 m in length, 163 HP inboard diesel engine; for more details see text).

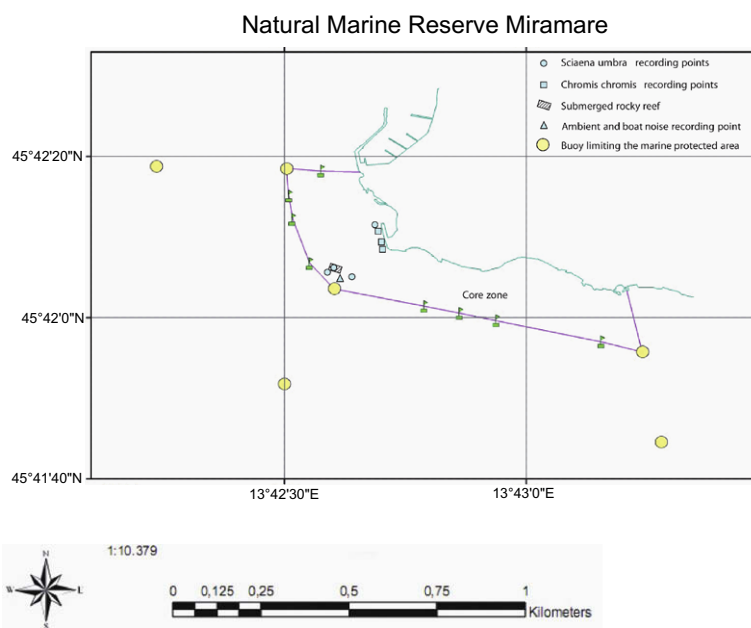


Fig. 2. The Natural Marine Reserve of Miramare. Squares (*C. chromis*) and circles (*S. umbra*) indicate the locations of fish sound recordings, the triangle indicates the recording point of ambient and boat noise.

2.5. Auditory sensitivity measurements

Hearing thresholds to tone bursts of varying frequencies (100–3000 Hz; $n = 6$ for all three species) and to conspecific sounds (only in *S. umbra* and *C. chromis*; $n = 5$ per species) were determined by using the Auditory Evoked Potential (AEP) recording technique under laboratory conditions and in the presence of both ambient and boat noise. The protocol closely followed that developed by Kenyon et al. (1998) and modified by Wysocki and Ladich (2003), Wysocki and Ladich (2005b) and Vasconcelos et al. (2007).

Test subjects were mildly immobilized with Flaxedil (gallamine triethiodide; Sigma–Aldrich, Vienna, Austria) diluted in a Ringer solution for sea water fish (Walsh, 1987). The dosage applied ($7 \pm 2 \mu\text{g g}^{-1}$ body mass for *S. umbra*; $4.8 \pm 1.5 \mu\text{g g}^{-1}$ for *C. chromis*; $12.37 \pm 0.7 \mu\text{g g}^{-1}$ for *G. cruentatus*) allowed fish to still perform slight opercular movements but not to initiate significant myogenic noise that could interfere with the AEP-recordings.

The subjects were placed just below the water surface in the center of an oval plastic tub ($45 \times 30 \text{ cm}$; water depth 12 cm; 1.5 cm layer of sand) lined on the inside with acoustically absor-

bent material (air-filled packing wrap) to reduce resonances and reflections (Wysocki and Ladich, 2002). The contacting points of the electrodes were maximally 1–2 mm above the water surface. Tissue paper (Kimwipes®) was placed on the fish head to keep it moist and ensure proper contact of electrodes. Respiration was achieved through a temperature-controlled (20.7 ± 0.1 °C) gravity-fed seawater circulation system using a pipette inserted into the subject's mouth.

The AEPs were recorded by using silver wire electrodes (0.38 mm diameter) pressed firmly against the skin: the recording electrode was placed over the region of the medulla and the reference electrode cranially between the nares. Shielded electrode leads were attached to the differential input of an AC preamplifier (Grass P-55, gain 100x, high-pass at 30 Hz, low-pass at 1 kHz), with a ground electrode placed in the water near the fish body. A hydrophone (Brüel & Kjaer 8101) was placed close to the inner ear of the animals (~1 cm away) in order to determine absolute stimulus SPLs.

The experimental tub was positioned on an air table (TMC Micro-g 63Y540, Technical Manufacturing Corporation, Peabody, MA, USA) which rested on a vibration-isolated concrete plate; the entire setup was enclosed in a walk-in soundproof room, which was constructed as a Faraday cage (interior dimensions: $3.2 \times 3.2 \times 2.4$ m). For technical reasons, hearing thresholds are given in terms of sound pressure level in dB re 1 μ Pa (RMS), although it is unknown whether the species investigated are pressure sensitive or only respond to particle motion. However, because our main interest was to investigate relative threshold changes in the presence of masking noise, use of a pressure measure is a valid approach for comparative purposes. Moreover, we previously calibrated the sound field in terms of sound pressure and particle acceleration to describe the audiograms in these three species using the same experimental setup and the same tone burst stimuli. This showed that sound pressure and particle acceleration levels de- and increase proportionally to each other. Accordingly, relative hearing sensitivity and audiogram shape are independent of the physical unit measured (Wysocki et al., in press).

2.6. Sound stimuli and masking noise presentation

Acoustic stimuli were presented at a repetition rate of 21 s^{-1} . The duration of the tone burst stimuli increased from two cycles at 100 Hz up to eight cycles at 3 kHz. All bursts were gated using a Blackman window. The duration of the conspecific sounds was 49 ms for *S. umbra* and 34 ms for *C. chromis*. Sound pressure levels were attenuated in 4-dB steps.

For each test condition, one thousand stimuli were presented at opposite polarities, i.e. 90° and 270° , and were averaged together by the BioSig RP Software, yielding a 2000-stimulus trace to eliminate any stimulus artifact. Close to hearing threshold, this procedure was performed twice and the AEP traces were overlaid to visually check if they were repeatable. The lowest SPL at which a repeatable AEP trace could be obtained, as determined by overlaying replicate traces, was defined as the threshold.

For determining hearing thresholds under noise conditions, a sample of 17 s from the recorded ambient noise or a 10 s section of the boat noise (including the maximum amplitude) were played back in a continuous loop during the AEP-recordings. Noise, as well as tone bursts and conspecific sounds, were presented through two speakers (Fostex PM-0.5 Sub and PM-0.5 MKII, Fostex Corporation, Tokyo, Japan). These were positioned 0.5 m above the water surface to achieve low-frequency noise spectral amplitudes similar to those of the field recordings.

Ambient and boat noise stimuli waveforms were generated using TDT Sig-Gen software sent to a 30-band equalizer (Alesis MEQ 230, Alesis Corp., Los Angeles, CA, USA) and adjusted to en-

sure that the spectra in the experimental tub were similar in frequency content and level to those recorded in the field. The average equivalent continuous SPLs ($L_{\text{Leq, 1 min}}$) measured over 1 min was 84.5 dB for the lab noise, 99.5 for the playback of ambient noise, and 136.5 dB for the playback of boat noise inside the tub at the position of the tested fish. Absolute sound spectra were calculated as described in Wysocki and Ladich (2005b).

Both fish sound and noise stimuli presentations, as well as AEP waveform recordings, were accomplished using a Tucker-Davis Technologies (Gainesville, FL, USA) modular rack-mount system (TDT System 3) controlled by a Pentium 4 PC containing a TDT digital processing board and running TDT BioSig RP and Sig-Gen RP Software.

2.7. Statistical analysis

Hearing thresholds recorded under different conditions (i.e. baseline, ambient and ship noise audiograms) were compared using a repeated measures ANOVA based on two within-subjects factors (frequency and noise condition) with Bonferroni post hoc tests.

Hearing threshold to conspecific sounds recorded under different conditions (i.e. baseline, ambient and ship noise audiograms) were compared using a repeated measures ANOVA based on one within-subjects factor (noise condition) with a Bonferroni post hoc test.

Data were normally distributed (Kolmogorov–Smirnov test) and variances were homogeneous. Statistical tests were run using Statistica 6.0 for Windows (StatSoft, Inc.).

3. Results

3.1. Ambient and boat noise

The equivalent continuous SPL ($L_{\text{Leq, 1 min}}$) of the recorded ambient noise was 97 dB re 1 μ Pa. Its sound power spectrum showed main energies below 1000 Hz and was rather flat up to higher frequencies. The $L_{\text{Leq, 1 min}}$ of the boat noise recorded in the field was 132 dB, with a maximum instantaneous SPL ($L_{\text{LFP, L-weighted}}$, 5 Hz–20 kHz, RMS fast) of 138 dB. The cabin-cruiser noise spectrum ranged from 0.3 to 10 kHz, with a peak at 100 Hz, and presented the main energy below 1500 Hz. Spectral energies of the boat noise were 35–40 dB above those of ambient noise between 100 Hz and 5 kHz (Fig. 3).

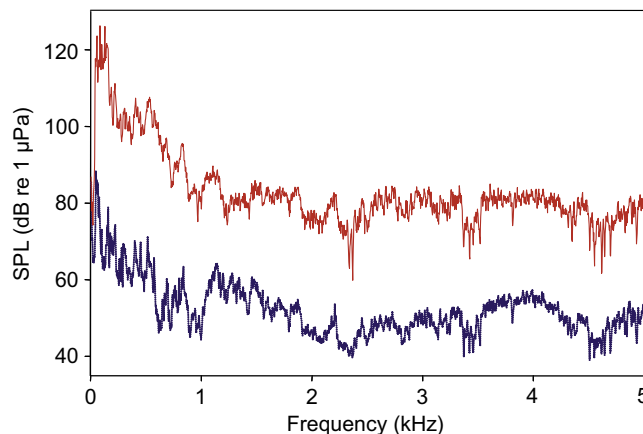


Fig. 3. Sound spectra of ambient (lower line) and cabin-cruiser (upper line) noise recorded in the core zone of the Natural Marine Reserve of Miramare.

3.2. Fish vocalizations in the field

Sounds of *S. umbra* consisted of a variable number (three to more than 35) of knocks. Their main energy was significantly (unpaired *t*-test: $T = -9.57$; $df = 15$, $P \leq 0.001$) lower in April (average: 166 ± 11 Hz; range: 118–194 Hz; $n = 6$) than in July (average: 262 ± 10 Hz; range: 213–336 Hz; $n = 11$). The average L_{LFP} was 124.4 ± 0.22 dB re 1 μ Pa RMS fast ($n = 121$ measurements, range: 119–129 dB).

Sounds of *C. chromis* were short pulses emitted either alone or in a series (up to four pulses). Their main energy ranged from 121 to 530 Hz (average: 327 ± 25 Hz; $n = 25$). The average L_{LFP} was 131.4 ± 0.42 dB ($n = 65$ measurements, range: 124–141 dB).

3.3. Hearing under ambient and boat noise conditions

S. umbra showed lowest hearing thresholds at 300 Hz, whereas both *G. cruentatus* and *C. chromis* had a maximum auditory sensitivity at 200 Hz (Fig. 4). The drum showed a broader hearing bandwidth and lower thresholds than the damselfish and the goby, detecting tone bursts up to 3000 Hz. On the other hand, no consistent AEPs could be obtained for frequencies higher than 600 Hz in *C. chromis* and higher than 700 Hz in *G. cruentatus* at the highest possible test level (136 dB re 1 μ Pa).

Baseline hearing thresholds recorded under quiet lab conditions were barely masked by ambient noise in all three species (Fig. 4; Tables 1–3). In contrast, auditory thresholds increased considerably during boat noise exposure at all frequencies by up to 35 dB in *S. umbra*, 20 dB in *C. chromis*, and 10 dB in *G. cruentatus* as compared to the sensitivity under ambient noise conditions. The largest threshold shifts between ambient and boat noise conditions occurred in the frequency range in which all three species were most sensitive to sound. This threshold shift decreased with increasing frequency (35 dB at lower frequencies vs. 10 dB at 1 kHz) in *S. umbra*. Similarly, in *G. cruentatus*, the smallest difference between the BN and the AN thresholds was at the highest frequency tested (700 Hz).

A repeated measures ANOVA revealed a significant effect of the three noise conditions (baseline, AN, BN) on auditory sensitivity in *S. umbra* (tested frequencies: 100, 200, 300, 500, 1000 Hz; $F_{2,10} = 140.14$, $P < 0.001$), *C. chromis* ($N = 6$, tested frequencies 100, 200, 300, 500, 600 Hz; $F_{2,10} = 113.53$, $P < 0.001$), and *G. cruentatus* ($N = 6$, tested frequencies, 100, 200, 300, 500, 700 Hz; $F_{2,10} = 49.84$, $P < 0.001$). A Bonferroni post hoc test revealed that baseline and AN audiograms differed significantly from BN ($P < 0.001$ for both cases for all the three species), whereas baseline and AN did not differ ($P = \text{n.s.}$ for *G. cruentatus*, *C. chromis* and *S. umbra*). A significant statistical interaction between the factors “noise” and “frequency” was observed for two species, i.e. *S. umbra* ($F_{8,40} = 10.93$, $P < 0.001$) and *G. cruentatus* ($F_{8,40} = 3.22$, $P = 0.006$), indicating that boat noise affects hearing thresholds differently at different frequencies. This was not the case in *C. chromis*, where no significant statistical interaction between the two factors was found ($F_{8,40} = 1.56$, $P = 0.165$), suggesting that the increase of hearing thresholds due to the noise exposure is similar within the frequency range (100–600 Hz).

3.4. Hearing threshold to conspecific sounds in the presence of noise

The mean hearing threshold to their conspecific sounds was 98 dB for *S. umbra* and 101 dB for *C. chromis* under both quiet lab noise and AN conditions, but increased in the presence of boat noise by approximately 20 dB (Fig. 5).

The sound energies of *S. umbra* knock sounds were up to 25 dB above the hearing thresholds in the frequency range between 180 and 300 Hz, whereas in *C. chromis*, pop sound energies were up to

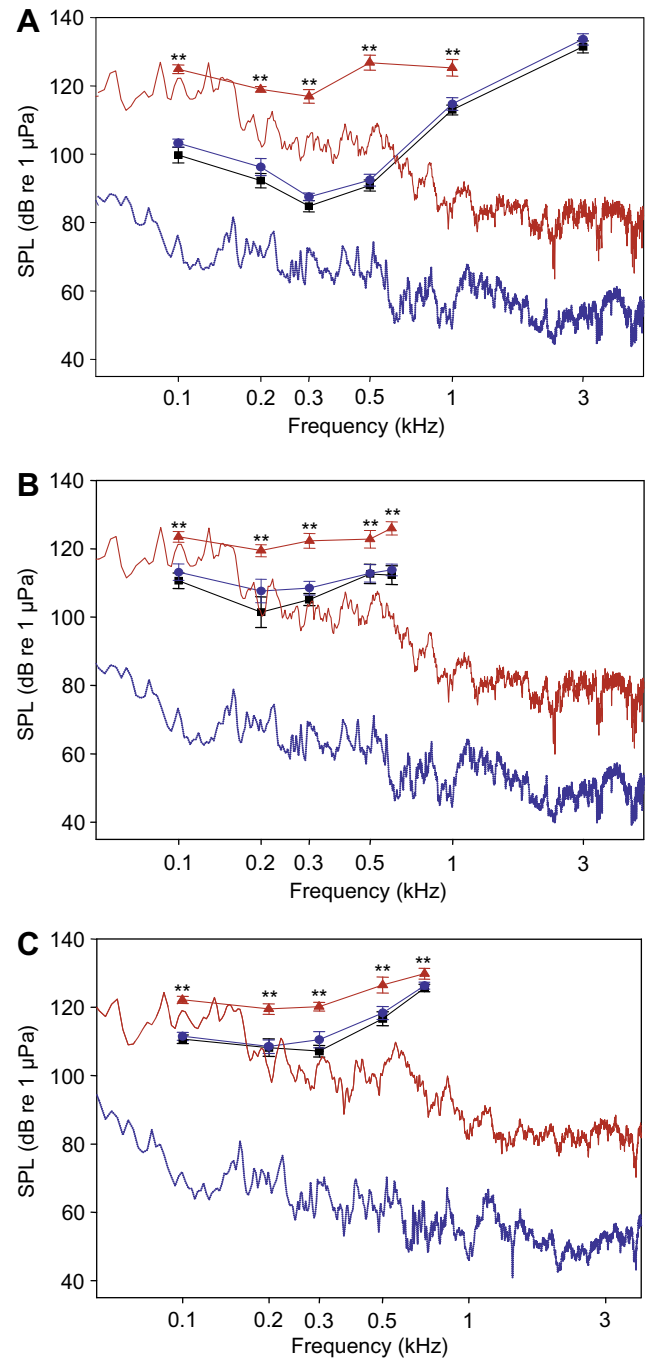


Fig. 4. Mean (\pm S.E.) hearing thresholds of (A) the brown meagre, (B) the Mediterranean damselfish and (C) the red-mouthed goby under laboratory conditions (“baseline”; squares) and during playback of the ambient (circles) and cabin-cruiser (triangles) noise compared to sound spectra of ambient (dotted line) and cabin-cruiser (continuous line) noises. ** = $p < 0.001$ (repeated measure ANOVA).

8 dB above the hearing thresholds in the frequency range from 300 to 400 Hz, where the main energies of sounds were concentrated. However, conspecific sounds were no longer detectable in the presence of the BN (Fig. 6).

A repeated measures ANOVA revealed a significant effect of the three noise conditions (baseline, AN, BN) on hearing thresholds to conspecific sounds in *S. umbra* ($N = 5$; $F_{2,8} = 98.72$, $P < 0.001$) and *C. chromis* ($N = 5$; $F_{2,8} = 50.75$, $P < 0.001$). A Bonferroni post hoc test indicated that thresholds to conspecific sounds under BN conditions were significantly different from baseline and AN ($P < 0.001$ for both cases for all the two species), whereas baseline and AN

Table 1
Mean hearing thresholds (\pm standard error) under different noise conditions (baseline, ambient and ship noise) and mean threshold shifts between the AN and BN audiograms of the brown meagre *S. umbra* ($N = 6$). N.R. = not responding at highest level tested.

Frequency (Hz)	Baseline		Ambient noise		Boat noise		
	Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Threshold shift (dB)
100	97.8	±2.4	101.5	±1.2	124.2	±1.4	22.7
200	90	±2.2	94.2	±2.6	118	±0.9	23.8
300	82.2	±1.8	84.8	±1.2	115.8	±2.1	31
500	88.5	±1.7	90.2	±1.8	116.2	±2.3	26
1000	111.6	±1.5	113.5	±1.9	124.6	±2.6	11.1
3000	131	±1.9	133.3	±1.7	N.R.	N.R.	/

Table 2
Mean hearing thresholds (\pm standard error) under different noise conditions (baseline, ambient and ship noise) and mean threshold shifts between the AN and BN audiograms of the Mediterranean damselfish *C. chromis* ($N = 6$).

Frequency (Hz)	Baseline		Ambient noise		Boat noise		
	Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Threshold shift (dB)
100	110.7	±2.3	113.2	±2.4	123.5	±1.5	10.34
200	101.5	±3.4	107.7	±3.4	119.5	±1.8	11.84
300	105.2	±1.8	108.5	±2.0	122.3	±2.1	13.83
500	112.7	±2.8	112.8	±2.6	122.8	±2.5	10
600	112.3	±2.7	113.9	±1.7	126	±1.9	12.14

Table 3
Mean hearing thresholds (\pm standard error) under different noise conditions (baseline, ambient and ship noise) and mean threshold shifts between the AN and BN audiograms of the red-mouthed goby *G. cruentatus* ($N = 6$).

Frequency (Hz)	Baseline		Ambient noise		Boat noise		
	Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Hearing threshold (dB re 1 μPa)		Threshold shift (dB)
100	110.7	±1.3	111.5	±1.2	122.2	±1.0	10.76
200	108.2	±2.6	108.5	±2.3	119.5	±1.5	11
300	107.2	±1.7	110.5	±2.2	120.2	±1.3	10.1
500	116.7	±2.0	118.3	±1.9	126.5	±2.3	8.2
700	125.7	±1.1	126.3	±1.0	129.8	±1.6	3.5

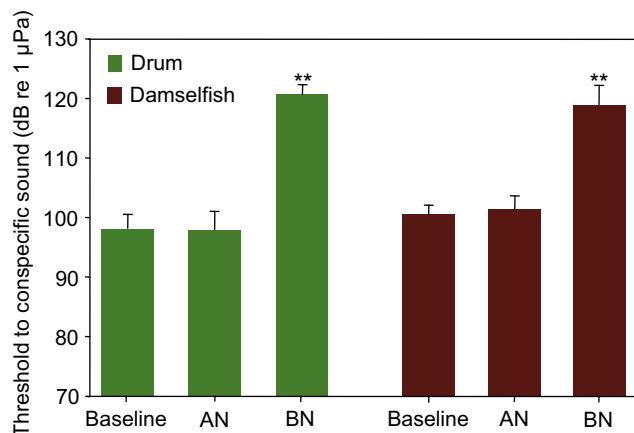


Fig. 5. Mean thresholds (\pm S.E.) to conspecific sounds under different noise conditions (baseline, AN = ambient noise, BN = boat noise) in two of the tested species. $^{**} = p < 0.001$ (repeated measure ANOVA).

did not differ from each other ($P = 0.997$ for *S. umbra* and $P = 0.998$ for *C. chromis*).

4. Discussion

4.1. Marine protected areas and background noise

Noise pollution is a serious threat to marine animals. Its effects are less perceptible than more visible pollutants such as oil spills

and marine debris and, even if noticed, cannot be easily stopped or confined to the outside of sensitive or protected areas. Marine Protected Areas (MPAs) are designated portions of the seas established as a policy tool for protecting and preserving living species and ecosystems. There are many types of MPAs all over the world. Although the potential to use them as a policy tool to regulate underwater noise is significant, only few protected areas are managed for noise impacts (Haren, 2007). Despite intrinsic difficulties in accurately defining a continuous and extremely variable phenomenon such as marine background noise (Cato, 1976; Urlick, 1983; Greene, 1995), a preliminary study showed that the Miramare MPA is exposed year-round to considerable underwater noise (Codarin et al., 2008). This is not surprising considering the high level of shipping and recreational boat activities in the Gulf of Trieste.

Living in constantly noisy conditions theoretically represents an environmental constraint for the local fish fauna, for example by reducing the range of sound detectability (Sprague and Luczkovich, 2004) or by triggering an endocrinological stress response (Wysocki et al., 2006). Our data demonstrate that the hearing abilities of the drum *S. umbra*, the damselfish *C. chromis* and the goby *G. cruentatus* are well adapted to the local background noise recorded at calm sea conditions (auditory sensitivities may change in relation to other sea conditions, as demonstrated by Chapman and Hawkins (1973)). We also showed that AN does not cause significant masking and thus does not impact detection thresholds of conspecific sounds in all three tested species. This agrees well with data on several other teleost families such as cods, cyprinids, perc-

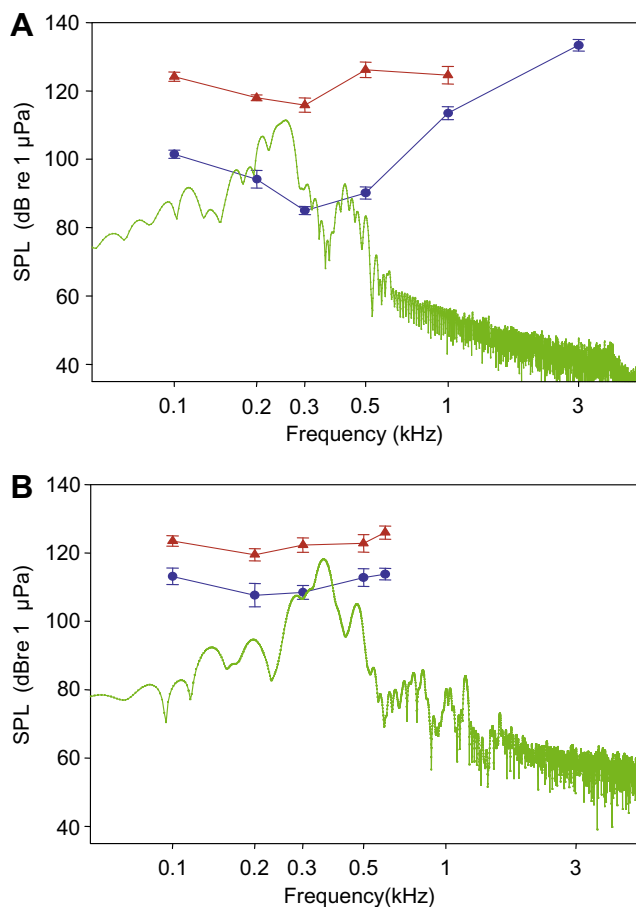


Fig. 6. Mean (\pm S.E.) hearing thresholds of (A) the brown meagre (B) and the Mediterranean damselfish measured during playback of ambient (circles) and cabin-cruiser (triangles) noise compared to sound spectra of the two respective conspecific sounds (continuous lines).

ids, batrachoidids and freshwater gobiids (Amoser and Ladich, 2005; Chapman and Hawkins, 1973; Lugli and Fine, 2003; Scholz and Ladich, 2006; Vasconcelos et al., 2007).

4.2. Effect of boat noise on audition and communication in the tested vocal fishes

A cabin-cruiser passing at 6 knots 10 m away from the recording point increased the local AN of 97 dB re 1 μ Pa by about 40 dB. The noise source level, calculated from 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), equals 142.3 dB at 1 m. The playback of the noise at the recorded levels (138 dB re 1 μ Pa, L_{Leq} in 10 s) was already accompanied by an upward shift in auditory threshold by 10–35 dB depending on the species. Hearing thresholds to conspecific sounds measured under ambient noise conditions and under boat noise conditions were used to calculate the potential detection distance. Since the actual detection distance in the field depends on many factors influencing sound propagation and spreading loss, such as bottom morphology, absorption, shadow zones due to refraction, salinity, temperature clines, etc., this will be only a crude estimate. The main goal of this calculation is to show by how much the presence of boat noise can decrease the potential detection distance of conspecific signals compared to quiet ambient noise conditions. Again, a cylindrical spreading loss was assumed as most appropriate for these shallow waters where fish sounds were recorded (Richardson et al., 1995).

In *S. umbra*, the played back cabin-cruiser noise increased the detection threshold to conspecific sounds from 98 dB under ambient noise to 122 dB re 1 μ Pa. Assuming an underestimated value of 124 dB re 1 μ Pa as the source level for sounds emitted by *S. umbra*, this suggests that the distance at which sound pressure levels decrease from the source to the fish's hearing threshold levels will decrease from 500 m under ambient noise conditions to only about 1 m under this particular boat noise measured. This estimation reasonably matches the results of Sprague and Luczkovich (2004), who demonstrated that an individual sciaenid fish calling at 135 dB could be detected above the background noise over a maximum of 316 m in quiet sea conditions (110 dB re 1 μ Pa) but less than 1 m in a heavy noise sea background condition (147 dB re 1 μ Pa).

In *C. chromis*, the played back cabin-cruiser noise increased the detection threshold to conspecific sounds from 101 dB under ambient noise to 120 dB re 1 μ Pa. The calculated detection distance of the sound under ambient noise condition is at least 10 m, while boat noise would completely mask the signal even at a distance to the vocalizing fish of less than one meter. Similar calculations cannot be made for *G. cruentatus* due to the lack of SPL measurements of their sounds in the field.

Nevertheless, we can conclude that a cabin-cruiser passing along the perimeter of Miramare Reserve impacts the acoustic communication of resident fishes, interfering with the detection of conspecific sounds. The detection distance of conspecific sounds can be reduced by 10- to more than 100-fold depending on the species. This represents a considerable restriction for intraspecific acoustic communication. In addition, the main energy of vessel noise is in the low-frequency range (up to 300 Hz; Picciulin et al., 2008), i.e. the same range in which the three investigated species vocalize (Picciulin et al., 2002, 2008; Sebastianutto et al., 2008).

This calls for a set of mitigation measures. The Gulf of Trieste is characterized by a high amount of human activities but at the same time an important spawning area for many Mediterranean fish species. Our data show the importance of establishing core and buffer zones around protected areas and controlling them rigorously. Particularly during the summer, when the species of concern are present and/or are reproducing it may also be considered to reduce boat traffic as well as boat speed around the reserve. Future studies monitoring the long-term boat noise levels in the reserve are necessary to get insights on potential short- and long-term behavioural impacts of this disturbance. Depending on the resulting requirements more general management measures could also include a code of conduct for recreational and commercial ships in the Gulf of Trieste. Similar considerations should be taken into account for other important marine spawning areas.

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