

Behavioral response of brown meagre (*Sciaena umbra*) to boat noise



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ABSTRACT

Underwater man-made noise is recognized as a major global pollutant in the 21st Century, and its reduction has been included in national and international regulations. Despite the fact that many studies have pointed out the ecological impact of noise on marine organisms, few studies have investigated - in a field context - the behavioral response to boat noise in fish. In the present study we measure how *Sciaena umbra* reacts to boat noise. We found that boat noise: i) increased duration of flight reactions and number of individuals performing them, ii) increased the frequency of hiding behaviors, and iii) did not elicit a change in fish activity level and sound emission. Flights and hiding behavior, usually related to predation risk, were not uniform between individuals and showed a quick recovery after noise exposure. On the basis of these results, potential metabolic, physiological and behavioral consequences are discussed and management recommendations are proposed.

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1. Introduction

In the marine environment, there are many human activities that are potentially able to affect the status of biodiversity. Man-made noise, produced by commercial shipping, recreational boating and other anthropogenic sources (pile driving, seismic exploration, energy production) has been occurring recently with increasing frequency and intensity in terms of sound levels (McDonald et al., 2006). Studies conducted over the last twenty years have pointed out that man-made noise has changed the acoustic landscape of many areas (Simpson et al., 2014) and that it is a cause of deterioration of the good status of species conservation, especially in proximity of populated coasts. Man-made noise is currently recognized as a major global pollutant in the 21st Century and is included in both national and international legislation (US National Environment Policy Act and the European Commission Strategy Framework Directive). According to European Directives, the introduction into the environment of energy, even in the form of noise/vibrations, should be maintained at levels which do not impair the marine environment (Directive 2008/56/EC of the European Parliament and of the Council - June 17, 2008). Most studies have highlighted the effect of underwater noise on aquatic mammals, although growing concern has been expressed for others zoological groups, such as fish (Normandeau Associates, Inc, 2012; Shannon et al., 2014). Increased noise might result in the masking of biologically relevant signals (e.g. communication calls), considerably reducing the range over which individuals are able to exchange information (Amoser et al., 2004; Vasconcelos et al., 2007). Furthermore, noise can cause

avoidance from favorable habitats (Hirst and Rodhouse, 2000; Mitson and Knudsen, 2003; Slotte et al., 2004), stress and physiological changes (Sverdrup et al., 1994; Smith et al., 2004; Wysocki et al., 2006), destruction of the auditory sensory cells (Hastings et al., 1996; McCauley et al., 2003) and temporary or permanent loss of hearing capability (McCauley et al., 2003; Popper et al., 2005; Popper and Hastings, 2009). Finally, exposure to high sound levels could affect animal behavior in various ways by: i) causing a reduction of activity and locomotion (Mendle, 1999); ii) acting as a distracting stimulus (Mendle, 1999); iii) masking crucial acoustic cues (Brumm and Slabbekoorn, 2005); iv) influencing settlement, foraging, social interactions and anti-predator behavior (e.g. Sarà et al., 2007; Purser and Radford, 2011; Bracciali et al., 2012; Brintjens and Radford, 2013; Holles et al., 2013).

One of the main sources of underwater noise in coastal area is recreational boat traffic, which has undergone a considerable increase over the past years (Graham and Cooke, 2008). Recreational boats generally produce noise in the frequencies below 1000 Hz (Codarin et al., 2009); this frequency range fall within the auditory capability of many fish species (Scholik and Yan, 2002). With the exception of the last few years, the impact of boat noise on fish behavior has been neglected, with very few exceptions, likely because of the difficulty of linking human activities to specific changes in animal behavior (Wysocki and Ladich, 2005). The majority of studies on this topic have been carried out under controlled laboratory conditions, which allow more detailed and accurate data collection than field-based studies. However, care must be taken when extrapolating findings to real-world situations, because captive conditions may represent a highly simplified and artificial environment. Here we present the outcome of a field-based experiment

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planned to investigate the effect of boat noise on the behavior of a commercially relevant species, the brown meagre (*Sciaena umbra*). To our knowledge, this represents one among the few studies for the determination of the behavioral response of fish to boat noise, conducted in a wild context where the animals were free of any constraints on movement (Sarà et al., 2007; Bracciali et al., 2012; Picciulin et al., 2012a).

The brown meagre is a common species in the Mediterranean Sea. However, due to fishing and habitat degradation, the species have suffered a reduction in stocks such as to be considered endangered and vulnerable (Mayol et al., 2000). Brown meagre is a gregarious and sedentary fish, who forms groups of 4–30 individuals and performs limited displacements (<1 km), with spatial extensions greater at night (Alos and Cabanellas-Reboredo, 2012). It is a vocally active species. During the spawning season (May–August; Grau et al., 2009) it produces short lasting broadband impulsive sounds with peaks at 250–300 Hz (Picciulin et al., 2012b) and main energy ranging from 118 to 336 Hz (Codarin et al., 2009). These calls match well with *S. umbra*'s hearing ability: the sound peak frequency (270 Hz) falls very close to its highest auditory sensitivity, identified at 300 Hz, measured in terms of both sound pressure level and particle acceleration level (Wysocki and Ladich, 2005). The energy of their call may span several kHz, in accordance with a hearing bandwidth that ranges up to 3 kHz (Picciulin et al., 2012b). The calls are produced mainly during night and in chorus, likely to facilitate aggregation of individuals and mating, as in other Sciaenidae. A previous study (Picciulin et al., 2012a) demonstrated that brown meagre exposed to multiple boat passages increased the mean pulse rate of their calls, a likely form of vocal compensation (Picciulin et al., 2012a). Despite this initial information on acoustic behavior, as far as we know, to this point no studies have investigated the behavioral response of brown meagre to boat noise in the wild and this aspect is still an unexplored field.

Present study was aimed at: i) measuring the behavioral response of brown meagre to boat noise in terms of fish activity level index;

ii) verifying the acute reactions of brown meagre to boat noise, in terms of flight and hiding reactions; iii) in case of a response, verifying if that response has a rapid recovery to the pre-exposure state, or if it persists over time; iv) quantifying boat traffic in the Marine Protected Area “Capo Caccia - Isola Piana”, as a function of a zone with different regimes of protection and type of boat, in the perspective to propose management actions.

2. Methods

2.1. Behavioral sampling and analysis

Data were collected inside the boundaries of the MPA “Capo Caccia - Isola Piana” (Fig. 1) between May–September 2014–2016. After a pilot survey, aimed at identifying suitable sites where the species were consistently present, the area (about 4000 m²) inside the Bay of Porto Conte, with the greatest number of brown meagre groups was selected. This area was located in the C zone (partial protection) of the MPA, in sites with a depth between 2 and 8 m. Within the C zone, navigation is allowed with a speed of <10 knots and mooring is permitted. Observational sites were localized close to reef, boulders and crevices covered by macro algae, such as *Padina pavonica*, *Laurencia* spp., *Dictyota* spp., *Sphacelariales*, algal turfs and encrusting invertebrates, interspersed with *Posidonia oceanica* habitats. Behavioral observations were carried out between 10 am and 3 pm, the hours with the best light, in almost calm conditions (wind speed <5 knots [$\sim 9 \text{ km h}^{-1}$] and wave height <0.25 m). We conducted one or two trials per day. In each trial, a different group of brown meagres (based on number and size of individuals and their position on the reef) was selected. However, given the ability of this species to move in a range of about 1 km (Alos and Cabanellas-Reboredo, 2012) and since the morphological distinction was not possible, we were not able to exclude the possibility that the same individual was sampled in more than one trial. As brown



Fig. 1. Study area. Capo Caccia - Isola Piana Marine Protected Area inside the solid line: i) A zone (integral protection); ii) B zone (partial protection); iii) C zone (partial protection); iv) Co (corridor, no protection). Inside the dotted line: area of video and audio recording of brown meagre groups. The filled points correspond to the sites of boat traffic recording (white = B zone; black = C zone; grey = corridor).

meagre can be shy in the presence of scuba divers and in order to avoid any interference in their natural behavior, behavior was recorded by two fixed underwater cameras (GO PRO Black 3+) placed on the sea floor. The cameras were deployed by divers so as to record all the area used by the brown meagre group subject of the trial (about 100 m²). At the beginning of each trial, behavior and movements of the animals were monitored in order to fix the cameras in the best position to perform continuous behavioral observation. In cases in which the animals continuously moved outside the field of view of both cameras, the experimental trial was interrupted.

In each trial, the behavioral observation started 10 min after the divers had fixed cameras in their final positions and lasted 60 min. We were interested in measuring the behavioral response of fish to boat noise and whether, eventually, the response persisted after noise exposure or recovered quickly to the pre-exposure state. Thus, each trial was divided into three phases: pre-exposure (boat navigating at a distance >500 m from the group or not visible at all); exposure (boat navigating at a distance between 200 m and 5 m from the group); post-exposure (boat navigating at >500 m from the group) with a similar design already adopted in companion studies (e.g. Sarà et al., 2007). We were not able to prevent the entry of boats into the bay or control the boat traffic in any way. The boats considered in the experiment were those spontaneously navigating in the proximity of the study area, particularly inflatable or motor boats of small size (<10 m) with engine between 40 and 270 hp, sailing at speed between 3 and 12 knots (Table 3). Special attention was reserved for sample the choice: i) for the sound condition, only trials with just the experimental boat and without any other boats sailing within the distance of 3000 m from the brown meagre group under observation were included in the analyses; ii) for the ambient condition, only trials with no boats sailing within the distance of 3000 m from brown meagre group under observation were included in the analyses. Data were collected following the group focal sampling method in sessions of 2 min for each phase (Martin and Bateson, 2007). The interval between the experimental phases was 2 min. Control conditions were standardised in order to carry out behavioral data collection when no boat had passed the group under observation for at least 10 min (Sarà et al., 2007). Thus as control, we used a trial of 10 min, without any boat passages, that was divided into the same 3 experimental phases 2 min in length (pre-exposure, exposure and post-exposure). We obtained 30 trials, 15 for the sound condition and 15 for the control, over 21 days of observations.

As the ethogram of brown meagre had not been previously described in scientific literature, we used pre-survey sessions, with and without boat noise, in order to define a list of behavioral states and events to be analyzed (Sarà et al., 2007). To measure the behavioral response of fish to boat noise the following variables were considered: the activity level of each fish, measured as an activity index where “0” represents a fish that is motionless, “1” a fish that is moving fins or body without moving forward, and “2” a fish that is swimming (Johnsson et al., 2001); flight reaction; hiding reaction; sound emission (Table 1). The software BORIS was used to measure behavioral variables (©Olivier Friard - Università degli Studi di Torino).

Table 1
Behavioral states and events considered in the analyses.

Behavior	Description	Unit
Activity Level	A0 = Fish are motionless A1 = Fish move fins or body without moving forward A2 = Fish swim actively	Total duration (sec)
Flight reaction	Time elapsed from the start of a flight reaction until the fish ceased active swimming	Total duration (sec) Percentage of individuals performing a flight reaction
Hiding reaction	Fish swim toward cave or crevice	Frequency (number of hiding events per min)
Sound	Brown meagre calls	Number of pulse per min

Table 2
Outcomes of one-way ANOVA run on the boats h⁻¹.

Source	MS	df	F	P	Source	MS	df	F	P
Small motor boat					Sail boat				
Zone	8.7	2.0	8.3	***	Zone	1.4	2.0	2.3	ns
Residuals	1.0	42.0			Residuals	0.6	42.0		
Source					Source				
Cabin cruiser					Tourist boat				
Zone	3.1	2.0	1.7	ns	Zone	0.4	2.0	0.5	ns
Residuals	37.3	42.0			Residuals	0.8	42.0		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns'

We tested the hypothesis that the behavior of brown meagre would not have changed in response to boat noise. Behavioral variables (activity index, flight reaction, hiding, emission of sound) were analyzed using two way ANOVA, where sound condition (ambient and boat) and phase (pre-exposure, exposure and post-exposure) were considered as fixed factors. To test for normality and homogeneity of variances, the Shapiro Wilk test and Levene's test were run respectively. No data were transformed. All descriptive statistics and analyses were done using R for OSX.

2.2. Acoustic sampling and analyses

Sea ambient and boat noise were measured by means of a PAM (Passive Acoustic Monitoring) device, named RASP, equipped with programmable underwater acoustic recorders (M-Audio MicroTrack II) and hydrophones with bandwidth between 10 Hz and 96 kHz (Sensor Technology SQ26-08; sensitivity 168.8 dB re 1 V/1 μPa; for details see La Manna et al., 2014). The hydrophone had a flat frequency response curve over the range of 10 Hz to 20 kHz, and its sensitivity was measured with spot calibration. Before each trial, the recording system was calibrated by applying a sinusoidal voltage of 0.1 V_{RMS} to the transducer input of the system by means of a signal generator. RASP was deployed on the sea floor, as near as possible to the mean position of the brown meagre group under observation. Data were stored as WAV files (16 bit sampled at 96 kHz).

Acoustic analyses were performed using PAMGuide (1024-point fast Fourier transform (FFT), 50% Overlap, Hanning window), a template code provided in R (Merchant et al., 2015). Boat and ambient noise

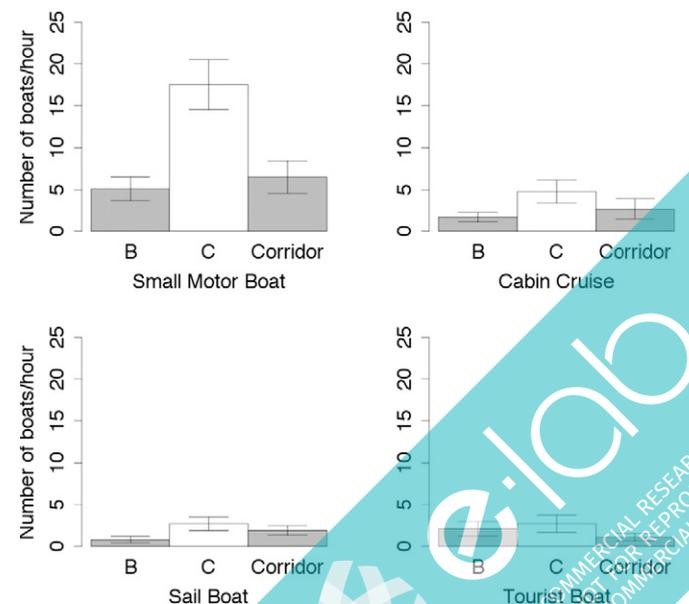


Fig. 2. Mean ± SE of boats h⁻¹ as a function of zone of the MPA Capo Caccia - Isola Piana (N = 90).

Table 3
Boats used in the experimental trials.

Trial	Type of boat, size and engine horse power	SPL (dB re 1 μ Pa RMS)	Max instantaneous SPL (dB re 1 μ Pa)
1	Motor boat, 4 m, 40 hp	140.80	146.63
2	Inflatable boat, 5 m, 80 hp	145.10	153.20
3	Inflatable boat 5 m, 50 hp	146.10	150.32
4	Inflatable boat 5 m, 50 hp	146.10	150.32
5	Motor boat 5 m, 50 hp	137.70	145.13
6	Motor boat, 9 m, 270 hp	137.20	145.91
7	Motor boat, 9 m, 270 hp	137.20	145.91
8	Motor boat 5 m, 50 hp	141.80	147.75
9	Motor boat, 5 m, 80 hp	142.20	148.30
10	Inflatable boat, 5 m, 80 hp	144.90	150.35
11	Inflatable boat, 5 m, 80 hp	133.70	145.00
12	Motor boat 5 m, 50 hp	144.00	151.03
13	Motor boat 5 m, 80 hp	143.00	154.56
14	Motor boat 5 m, 50 hp	141.30	146.44
15	Motor boat, 6 m, 80 hp	142.50	148.51

were measured as broadband sound pressure level (SPL). SPL, the most ubiquitous acoustic metric, expresses the root-mean-square (RMS) sound amplitude within a given time window (2 min in the present study) and frequency range as a single decibel (dB) level. Each acoustic sample was described both in terms of 1/3 octave band and in the frequency range from 10 to 2500 Hz, corresponding to the hearing bandwidth of brown meagre. To estimate the efficiency of the sampling design, noise levels (SPL for each 1/3 octave band and for the wideband 10–2500 Hz) were tested by means of a two way ANOVA, where sound (ambient and boat) and phase (pre-exposure, exposure, post-exposure) were considered as fixed factors. To test for normality and homogeneity of variances, the Shapiro Wilk test and Levene's test were run. No data were transformed. All descriptive statistics and analyses were done using R for OSX.

2.3. Boat traffic

Behavioral and acoustic data were measured together with boat traffic in order to quantify its intensity. The area of MPA visible from the study area (Bay of Porto Conte) included three zones, with different regimes of protection: 1) C zone; 2) B zone; 3) corridor zone (Fig. 1). Inside the B zone navigation by recreational motorboats (at a speed not exceeding 5 knots) and motorboats used as collective transport for guided tours (at a speed not exceeding 8 knots) is permitted. Inside the C zone navigation by motorboats (at a speed not exceeding 10 knots) is permitted. The corridor zone is not included within the boundaries of the MPA; from this corridor vessels can access (at speeds up to 20 knots) the port of Porto Conte and the mooring points defined by the Maritime Authority. All vessels in navigation or moored inside 3 sites per zone (Fig. 1) were logged by one observer with the aid of binoculars, over a period of 60 min overlapping acoustic and behavioral

Table 4
Outcomes of two-way ANOVA run on the SPL (dB re 1 μ Pa) for the band 50–2500 and the 1/3 octave bands. Only bands with significant results are shown in the table.

Source	MS	Df	F	P	Source	MS	Df	F	P	Source	MS	Df	F	P
50–2500 Hz					100 Hz					125 Hz				
Phase (Ph)	164.7	2	5.0	**	Phase (Ph)	88.4	2	4.2	*	Phase (Ph)	174.1	2	6.0	**
Sound (So)	101.1	1	6.1	*	Sound (So)	75.8	1	7.3	**	Sound (So)	98.9	1	6.8	*
Ph X So	136.7	2	4.1	*	Ph X So	217.5	2	10.4	***	Ph X So	261.8	2	9.1	***
Residuals	1393.4	84			Residuals	876.9	84			Residuals	1213.2	84		
Source	MS	Df	F	P	Source	MS	Df	F	P	SPL 63 Hz	MS	Df	F	P
160 Hz					200 Hz					250 Hz				
Phase (Ph)	127.8	2	5.9	**	Phase (Ph)	67.4	2	3.5	*	Phase (Ph)	21.0	2	1.5	ns
Sound (So)	79.6	1	7.3	**	Sound (So)	49.1	1	5.1	*	Sound (So)	17.3	1	2.5	ns
Ph X So	242.5	2	11.1	***	Ph X So	99.5	2	5.1	**	Ph X So	42.0	2	3.1	*
Residuals	915.8	84			Residuals	814.5	84			Residuals	573.8	84		

Significance codes: 0 **** 0.001 *** 0.01 ** 0.05 * 0.1 ns.

Table 5
Outcomes of two-way ANOVA run on the effect of boat noise on the activity index of brown meagre. A0 = fish are motionless; A1 = fish move fins or body without moving forward; A2 = fish swim actively.

Source	MS	df	F	P
A0				
Sound (So)	8914	2	2.5	.
Phase (Ph)	755	1	0.4	ns
Ph X So	8244	2	2.3	ns
Residuals	152,575	84		
A1				
Sound (So)	7003	2	1.7	ns
Phase (Ph)	412	1	0.2	ns
Ph X So	1270	2	0.3	ns
Residuals	176,308	84		
A2				
Sound (So)	2486	2	1.1	ns
Phase (Ph)	6217	1	5.4	*
Ph X So	2267	2	1.0	ns
Residuals	97,392	84		

Significance codes: 0 **** 0.001 *** 0.01 ** 0.05 * 0.1 ns.

recording. Boats were classified in the following categories: small motor boat (motorized boats with size <10 m, SMB); cabin cruiser (CC); sail boat (SB); tourist boat (motor boats bigger than 10 m performing excursions along the coast, TB). Boat traffic was expressed as number of boats per hour (boats h^{-1}). Data were analyzed in order to test the null hypothesis that there was no difference in the number of boats among zones with different regimes of protection, using a simple one-way ANOVA, where zone (three levels) was considered as a fixed factor. To test for normality and homogeneity of variances, the Shapiro Wilk test and Levene's test were run. All data were logarithmically transformed. All descriptive statistics and analyses were done using R for OSX.

3. Results

From May–September 2014–2016 we carried out 21 days of observations and we obtained 1260 min of acoustic and video recordings. From these recordings we extracted thirty 10 min trials (15 trials for each of the 2 sound conditions), respecting the sampling protocol. Each trial consisted of 6 min (360 s) of behavioral data collection, 120 s for each of the 3 experimental phases, and 4 min of interval between phases without data collection.

3.1. Boat traffic

Boat traffic was measured for 15 h contemporary to the behavioral data collection. Considering all types of boats in navigation or moored inside the Bay of Porto Conte (Fig. 1), we recorded a mean of 16.5 ± 15 boats h^{-1} and a maximum of 56 boats h^{-1} . SMB was the most abundant boat type in the study area, with a mean of 9.7 ± 10 and a

Table 6

Outcomes of two-way ANOVA run on the effect of boat noise on hiding, flight reaction (total duration in second) and proportion of individuals performing a flight reaction.

Source	MS	df	F	P
Hiding				
Sound (So)	1.7	2	8.5	***
Phase (Ph)	0.7	1	7.1	***
Ph X So	0.2	2	0.9	ns
Residuals	8.5	84		
Flight reaction (total duration)				
Sound (So)	90.1	2	0.9	ns
Phase (Ph)	45.7	1	3.4	.
Ph X So	285.2	2	5.3	**
Residuals		84		
Flight reaction (% individuals)				
Sound (So)	0.1	2	0.2	ns
Phase (Ph)	0.0	1	1.4	ns
Ph X So	0.7	2	5.4	**
Residuals		84		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns'.

maximum of 34 boats h^{-1} , followed by CC, with a mean of 3.1 ± 4.4 and a maximum peak of 18 boats h^{-1} . Tourist and sail boats were more sporadically present with, respectively, a mean of 1.9 ± 3.1 and 1.8 ± 2.4 boats h^{-1} and maximum of 14 and 10 boats h^{-1} respectively. No

significant difference (ANOVA, $P = n.s.$) was found in the boats h^{-1} as a function of MPA zones with the only exception being SMB. SMB were more abundant inside the C zone respect to the other zones (ANOVA - Table 2, Fig. 2).

3.2. Boat noise

The noise level of the boat expressed as SPL (mean and instantaneous maximum) are showed in Table 2. The distance between the boats and the hydrophone ranged between 5 m and 200 m. The mean SPL ranged from 134 to 146 dB re 1 μPa with maximum SPL between 145 and 154 dB re 1 μPa . To show how sound level varies with frequency, the power spectral density (PSD - 1 Hz bandwidth) of the 15 trials of the exposure phase for both sound condition (boat and ambient) were plotted in Fig. 3. RMS level, together with the 95% and 99% percentiles, were higher in boat condition compared to ambient in the frequency below 250 Hz.

To verify the efficiency of the experimental design we measured SPL for each phase (pre-exposure, exposure and post-exposure) and in each sound condition (boat and ambient) in the frequency range 10–2500 Hz and in the 1/3 octave band between 50 and 2500 Hz.

No statistical differences were found among phase and sound condition with the exception of the frequency bands centered in 100, 125,

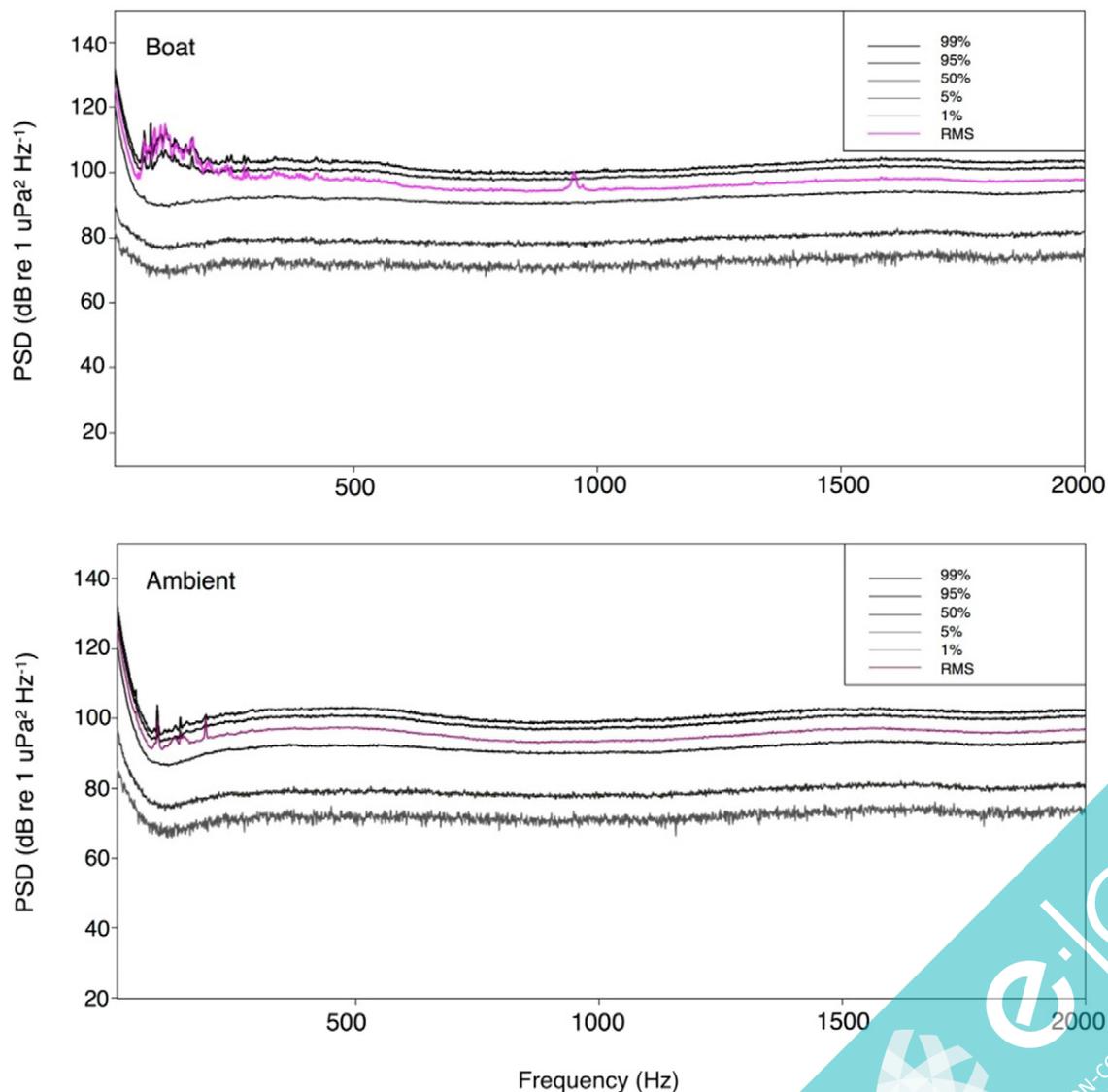


Fig. 3. PSD (Power Spectral Density) of the 15 2 min trials of the exposure phase for both treatments, boat and ambient.

160, 200 and 250 Hz, and in the wideband 50–2500 Hz (Table 4, Fig. 4). Since these frequencies correspond to the auditory sensitivity of brown meagre (Wysocki et al., 2009), the experimental design was properly elaborated to measure the response of the species to boat noise.

3.3. Behavioral response

The behavior of 65 brown meagres was sampled. The mean group size was 4 (range 1–14). In general, brown meagre group spent the majority of their time in activity index A1 (fish moves fins or body without moving forward), or A0 (fish is motionless) and very little time in A2 (active swimming). Boat noise did not elicit any significant statistical variation in the activity index of brown meagres, with the only exception of A2 which is lower in condition where a boat is present (Table 5). Although there was no statistical difference, A0 decreased during noise exposure while A1 increased (Fig. 5). In the post-exposure phase, A0 and A1 returned to the levels of the pre-exposure phase. In

total only 76 sounds were recorded. The majority of sounds were produced when fish were in state A0 and A1, respectively 30% and 46% of the total, and only 18% when fish were in state A2. The sound emission frequency was in general really low, with a mean n° of pulse/min of 0.65 ± 1.11 , 0.23 ± 0.56 and 0.70 ± 0.92 in the pre-exposure, exposure and post-exposure phases of the sound condition and a mean n° of pulse/min of 0.50 ± 1.1 , 0.13 ± 0.52 and 0.57 ± 0.90 in the pre-exposure, exposure and post-exposure phases of the ambient condition. No significant difference (ANOVA, $P = n.s.$) was found in sound emission frequency as a function of sound condition and phase. Nevertheless, the analysis showed that both flight and hiding reactions were significantly affected by boat noise (Table 5). The total duration of flight reactions was longer during exposure to boat noise (Table 6, Fig. 6), as well as the number of individuals that performed a flight reaction (Table 6, Fig. 7). The flight reaction was not uniform among individuals of the same group: on average 37% of individuals in a group reacted to boat noise. At the end, frequency of hiding (n° of events/min) was

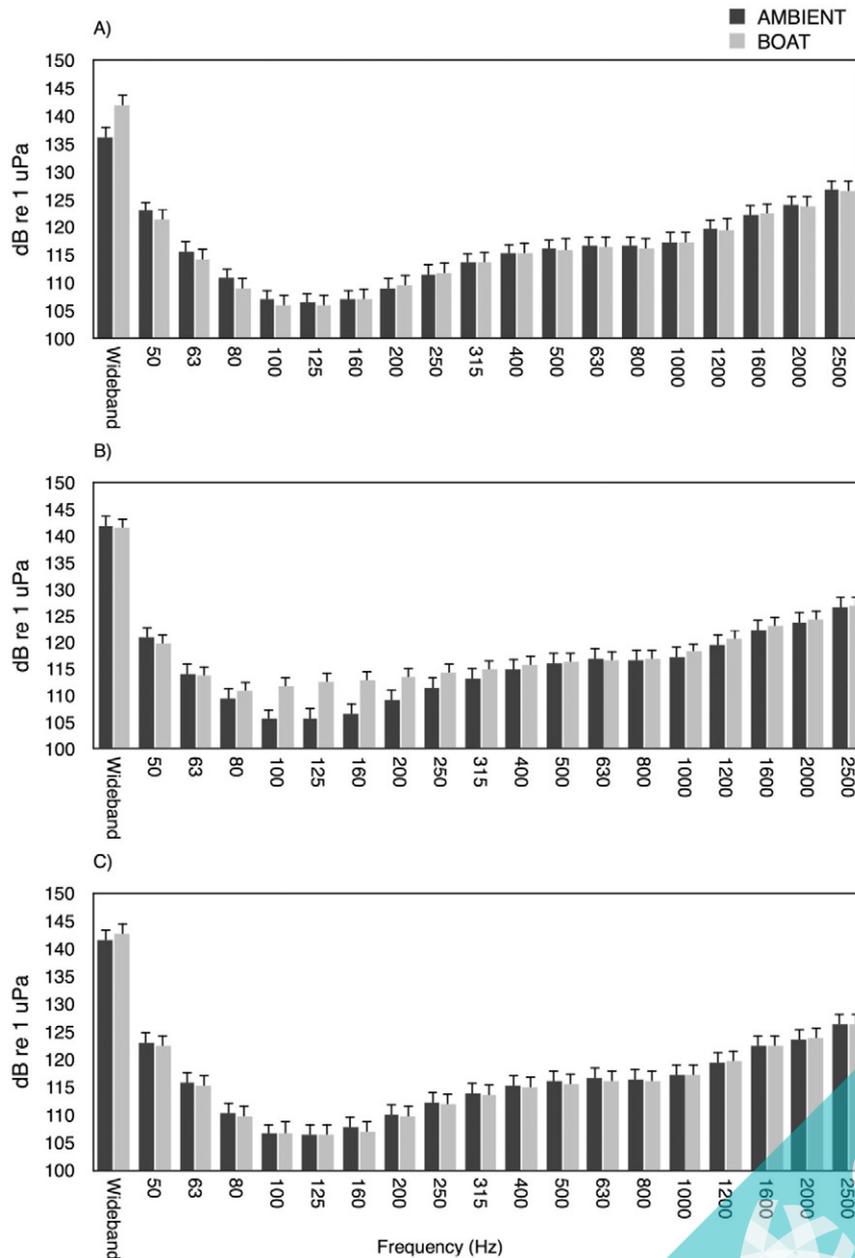
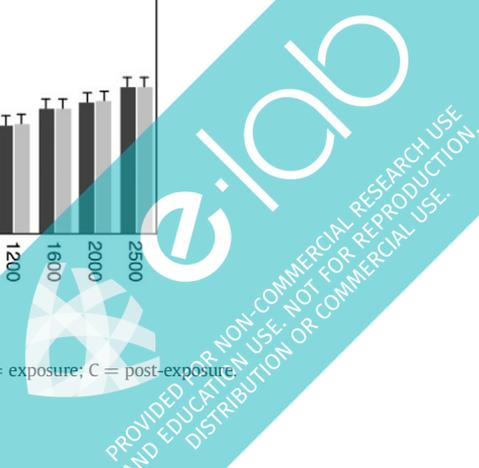


Fig. 4. Mean ± SE of SPL in the wideband 50–2500 Hz and for 1/3 octave bands. A = pre-exposure; B = exposure; C = post-exposure.



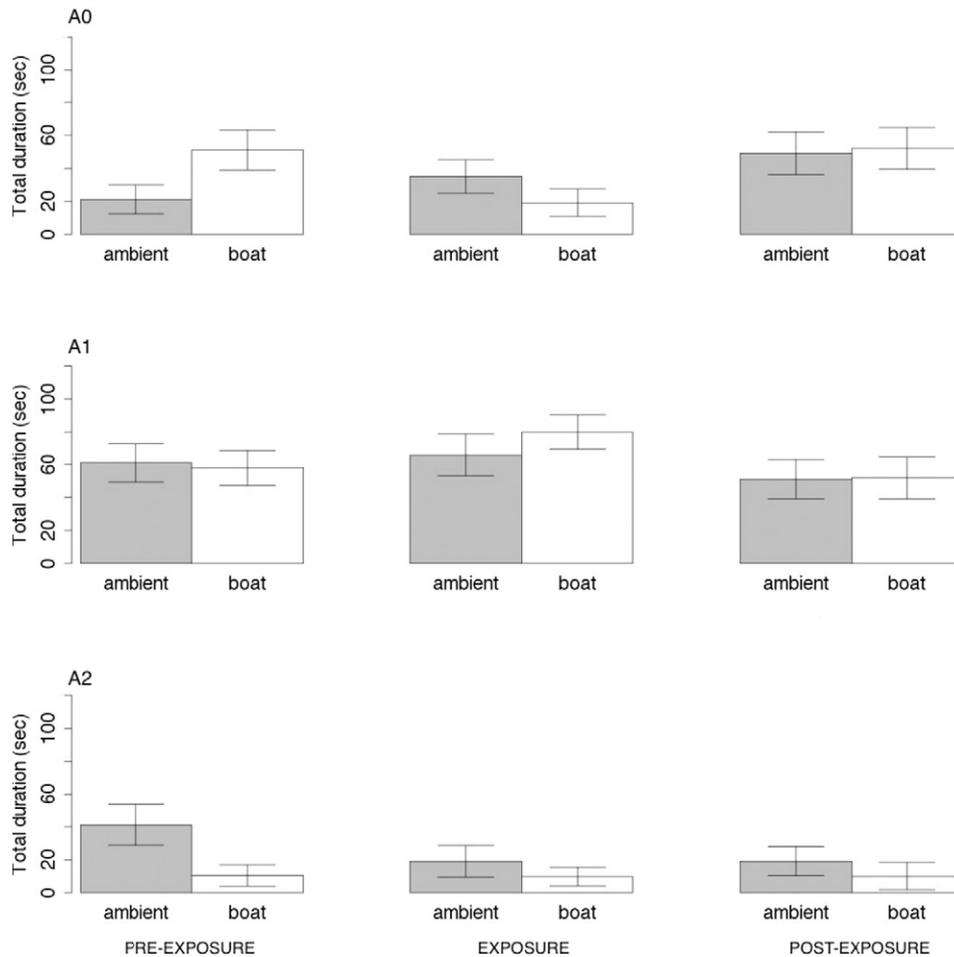


Fig. 5. Mean \pm SE of activity index as a function of sound condition (boat, ambient) and phase (pre-exposure, exposure, post-exposure) of the experimental design (N = 90). A0 = fish are motionless; A1 = fish move fins or body without moving forward; A2 = fish swim actively.

statistically different between phase and sound condition. Particularly, hiding increased during exposure to boat noise. This increase disappeared in the post exposure phase. (See Fig. 8.)

4. Discussions

4.1. Behavioral response of brown meagre to boat noise

In the present study brown meagre showed no significant changes in activity levels as a consequence of boat noise. Groups were moderately more active during noise exposure though they did not display any increase in active swimming or displacement from the area monitored by the cameras. In this case, displacement from the area is not a valuable strategy for coping with noise exposure since the entire area is affected by high frequency of boating. Inside the MPA and during the tourist season, brown meagre might experience on average 18.7 boats h^{-1} inside the MPA (B and C zones averaged). In addition, inside the study area brown meagre groups were more abundant compared to other sites investigated during the pilot surveys, and used the same reefs and crevices year by year. It is likely that, this area hosts some crucial resources (prey and/or refuge availability and presence of conspecifics grouped for the spawning season) of extreme relevance for the species. The benefits of this preferred habitat may enhance tolerance to the short term cost of the noise disturbance. We also found no change in sound emission frequency as a consequence of noise exposure. This result is consistent with previous studies (e.g. Picciulin et al., 2012a) finding no immediate effects on *S. umbra* mean pulse rate during a single boat

passage. Nevertheless, *S. umbra* mean pulse rate increased over multiple boat passages (Picciulin et al., 2012a). In the present study, we cannot exclude the possibility that the result achieved was the consequence of the experimental design, which provided a single noise exposure, rather than a total lack of vocal response to noise. Furthermore our study was conducted during the day, while the study of Picciulin et al. was conducted at night. Thus, the absence of an acoustic response to the passage of boats could also be linked to a different behavioral state of brown meagres during daytime. Conversely, brown meagre groups showed increased flight reactions and hiding behavior as a response to boat noise. These reactions, flights and hiding, are usually related to predation risk (Höjesjö et al., 1999): brown meagre seems to react to boat noise as if it were a predator attack. In other words, as in other fish studies, this brought us to infer that brown meagre used analogous decision processes to evaluate responses to the risks presented by natural predators and those presented by anthropogenic agents of disturbance (Bejder et al., 2009). This kind of reaction to short-term exposure to boat noise was consistent with other studies. European eels (*Anguilla anguilla*) and sea bass (*Dicentrarchus labrax*) were affected by additional noise (playback of recordings of ship noise) in their anti-predator startle behavior and ventilation rate (Bruinjes et al., 2016). Defense against predator of *Neolamprologus pulcher* was negatively affected by playback of the noise of a passing boat in a tank (Bruinjes and Radford, 2013). High speed boats elicited a flight response in two cyprinid species (Boussard, 1981). Vessel noise can elicit a response similar to the hiding behavior induced by predators in *Thunnus thynnus* (Sarà et al., 2007) and *Chromis chromis* (Bracciali et al.,

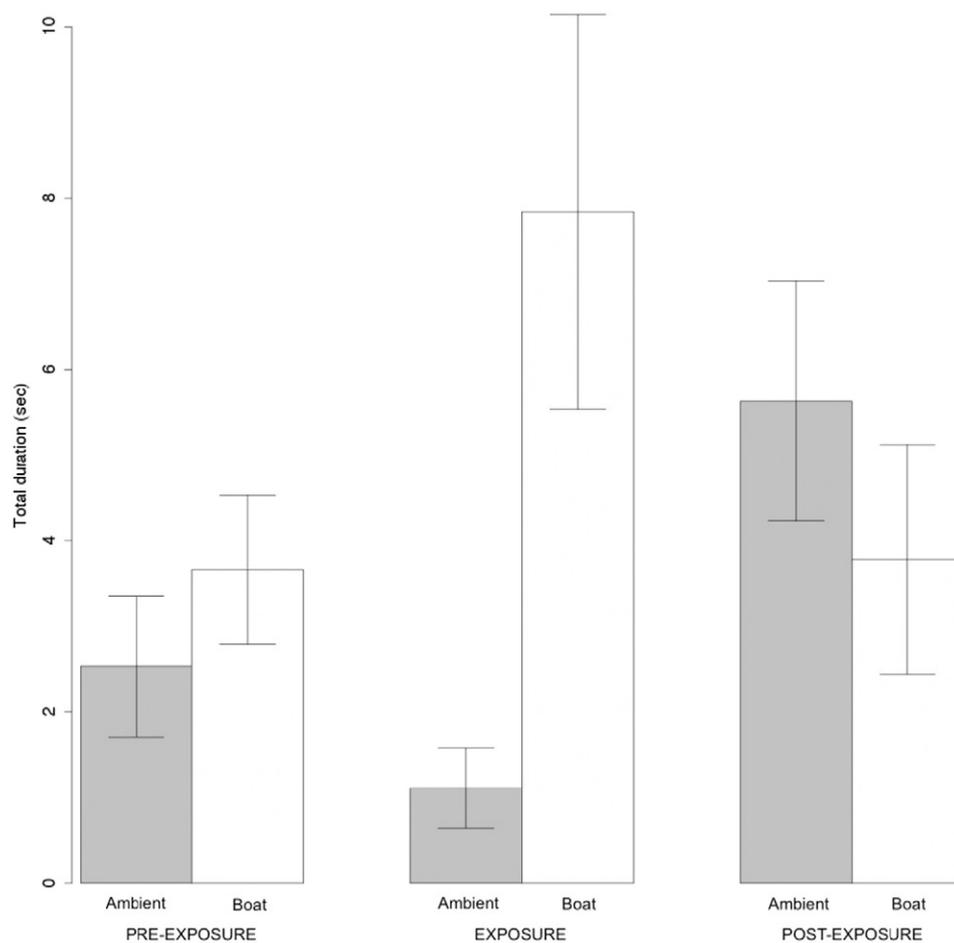


Fig. 6. Mean \pm SE of flight duration (in sec) as a function of sound condition (boat, ambient) and phase (pre-exposure, exposure, post-exposure) of the experimental design (N = 90).

2012). Vabø et al. (2002) found that the most common behavioral reactions of herring (*Clupea harengus*) to a passing vessel was body tilting and vertical or horizontal swimming which is a reflection of predator avoidance reaction.

The defense against predation represents a high but necessary cost for the prey. If brown meagre perceives boat noise as stimuli of a predator attack, hiding and flight reactions result in unexpected and unnecessary energy expenditure that can have consequences on the total energy budget of the individuals with ultimate effects on fitness.

4.2. Individual response and behavioral recovery

The present study highlighted a different individual response to high noise level. The percentage of individuals that performed a flight reaction increased under boat noise, although the flight reaction was not uniform among all the individuals of the group, with the exception of only few insignificant cases. In the remaining cases, on average, 37% of individuals in a group reacted. Animals in one group showed a different level of tolerance to this kind of disturbance. It is clear, from other research fields, that factors such as sex, dominance status, age, size, and health may all influence how members of the same population are affected by a given stimulus (Radford et al., 2012). In a recent study, behavioral reactions to boat noise showed to be dependent on the context and physiological conditions of individuals. For example, under lower relative body conditions, the ventilation rate of eels increased when exposed to additional noise (Purser et al., 2016). Also, Bruinjtjes and Radford (2013) showed that dominant males and females of a cooperatively breeding cichlid fish species (*Neolamprologus pulcher*) exhibited different behavioral responses to the same playback of boat noise. These observations corroborate the outcome of the present

study and suggest that a correct assessment of intra-population variations plays crucial role in understanding how anthropogenic noise impacts on marine biota (Purser et al., 2016). The individual reaction of brown meagre to boat noise deserves further examination.

Our study also demonstrated the potential of brown meagre to resume behavior quickly after noise exposure. This ability may play an important role in the resilience of the species to impacts of noise and may be the result of the rapidly changing acoustic conditions of brown meagre's habitats (sensu Bruinjtjes and Radford, 2013). Although the observed behavioral rapid recovery could be positively interpreted, we cannot exclude that the measured acute reaction of brown meagre to boat noise can lead to physiological and metabolic consequences, or to stronger effects at a population rather than individual level.

From this point of view, the results of this study represent the first step in understanding the effect of noise on this protected and commercially relevant species. Future work will be necessary to assess the long term biological significance of this short term response (Bejder et al., 2009).

Finally, with the present experimental design we could not test the response of repeated boat noise exposure, due to logistic limits related to field conditions and the difficulty to control experimental conditions over a longer period of time. Future work should investigate how repeated noise exposure influences the responses of fish over periods longer than those investigated.

4.3. Boat traffic, noise and implications for management

Present results can easily be translated in terms of implications for marine resource management. The study area, the Capo Caccia - Isola Piana MPA, is heavily congested by boat traffic during the tourist

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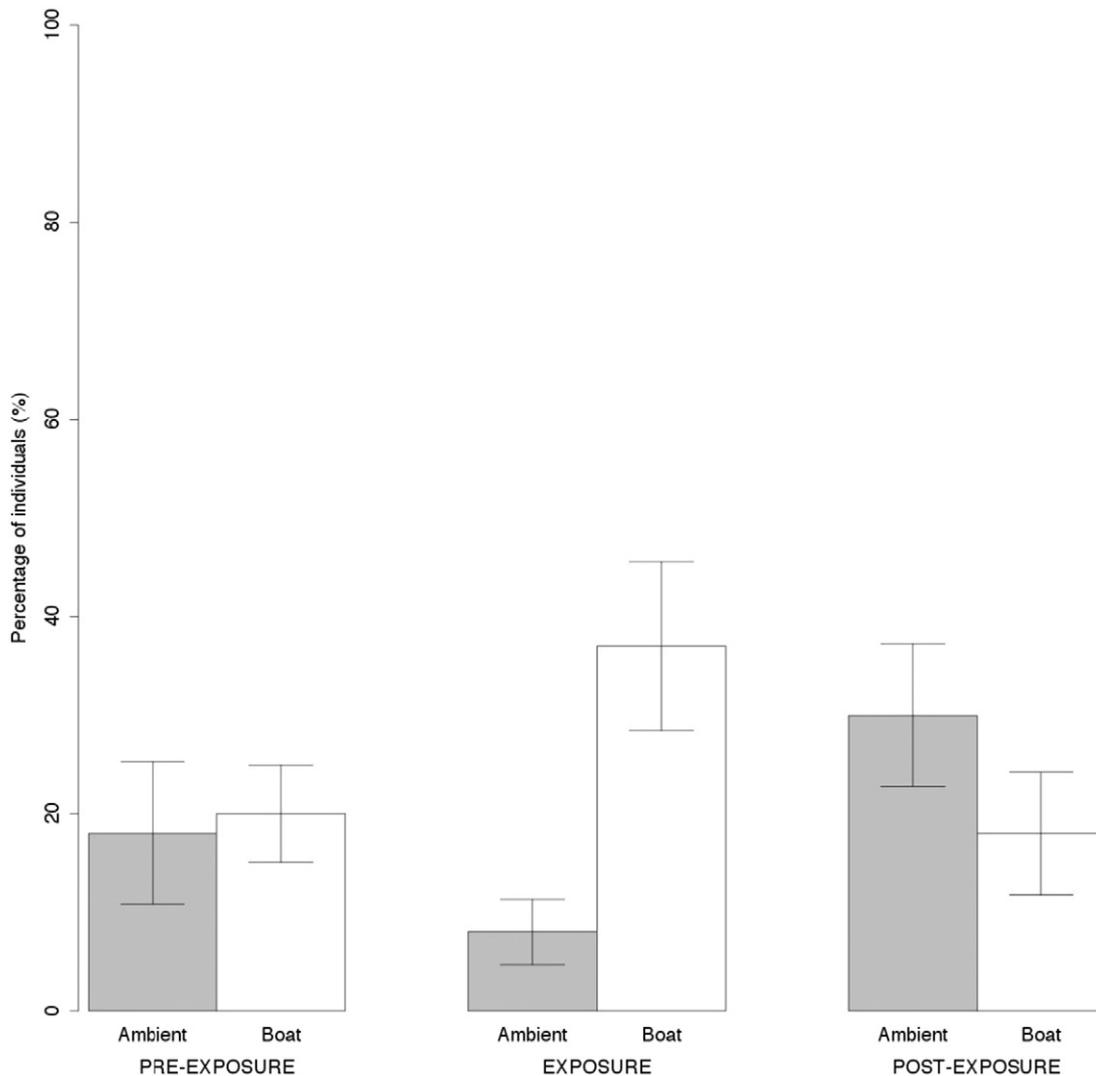


Fig. 7. Mean \pm SE of percentage of individuals performing a flight reaction as a function of sound condition (boat, ambient) and phase (pre-exposure, exposure, post-exposure) of the experimental design (N = 90).

season although boat traffic was comparable to the levels recorded in other Mediterranean MPAs (La Manna et al., 2010; Bracciali et al., 2012). Close boat passages resulted in a mean SPL between 134 and 146 dB re 1 μ Pa and a maximum SPL between 145 and 154 dB re 1 μ Pa, corresponding to an enhancement of SPL from 2 up to 15 dB in the frequency bands from 80 to 250 Hz. These values roughly corresponded to those recorded in similar studies. Sarà et al. (2007) recorded a sound pressure between 137 and 125 dB re 1 μ Pa in the same frequency range during the passage of small boat, at a distance of 80 m from the hydrophone. The sound produced by a fast inflatable boat in the WWF-Natural Marine Reserve of Miramare produced a SPL between 120 and 150 dB re 1 μ Pa in the frequencies below 500 Hz (Picciulin et al., 2008). The equivalent continuous SPL value (80–2500 Hz) of the inflatable boat noise recorded by sonobuoys was equal to 135 dB re 1 μ Pa with a maximum instantaneous SPL of 150 dB re 1 μ Pa at a frequency of 160 Hz (Picciulin et al., 2012b). Local noise levels resulted similar to or higher than those measured in other Mediterranean sites, characterized by a similar boat traffic (Samuel et al., 2005; Picciulin et al., 2012a; Rako et al., 2013a, 2013b; Codarin and Picciulin, 2016). Such a high level of noise from 50 up to 800 Hz bands, recorded in absence of close boat passages, was due to small waves slamming on the reef (because of the coastal and shallow position of the recording system) and to low frequency noise from distant boating. The highest frequency band and the

wideband were dominated by snapping shrimps which were observed to be constantly present in every recording. Most noise recorded within the MPA occurred at frequencies below 2500 Hz, falling well within the range of sensitivity of brown meagre hearing.

MPAs are arguably the most powerful tools available to date for reducing the over-exposure to anthropogenic disturbance of marine resources, the degradation of marine habitats and for the maintenance and restoration of key species populations. Although the potential to use them as test sites where boat traffic and underwater noise are significantly regulated (Codarin et al., 2009), few protected areas have management measures in place for the reduction of noise impacts (Haren, 2007). MPAs habitat and species exert a strong attractive for tourists. Thus, they can play an important role in the development of seaside tourist destinations and consequently represent an important aspect of coastal economies. From this point of view, if the MPAs want to keep their main purpose of protecting and preserving the marine environment, the management of boat traffic and underwater noise should be taken into much greater consideration in the MPAs regulation and action plans. A monitoring scheme of boat traffic and noise levels inside the European MPAs would fulfill the request of the European MSFD to develop strategies in order to achieve and maintain Good Environmental Status (GES) in European Seas, with regard to underwater noise. In fact, between the two indicators that should be used to meet

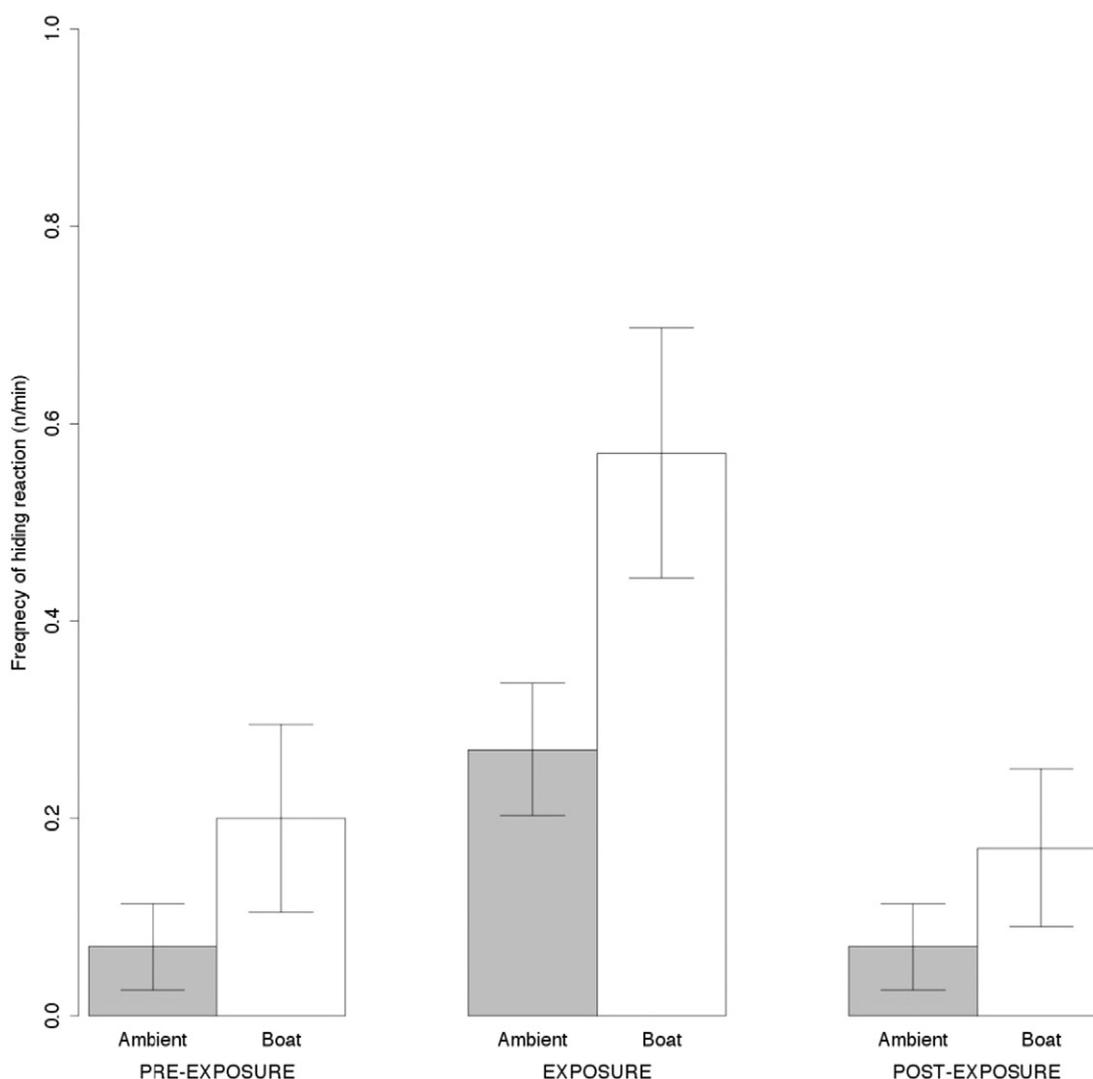


Fig. 8. Mean \pm SE of the frequency of hiding (number of events/min) as a function of sound condition (boat, ambient) and phase (pre-exposure, exposure, post-exposure) of the experimental design (N = 90).

the GES Descriptor 11 (Commission Decision 2010/477/EU on criteria and methodological standards on good environmental status, GES), the second is focused on low frequency ambient noise, with the main contributor being commercial shipping noise (van der Graaf et al., 2012). Preserving the acoustic landscape of marine protected areas should require: i) MPA management authorities to strongly uphold measures for reducing or regulating the most frequent noise-generating activities (usually recreational boat traffic); ii) the implementation of a proper and periodical educational program, containing awareness actions in order to change boaters' attitudes and behaviors regarding boat traffic in coastal areas, especially when protected; iii) the design of a periodic noise level monitoring plan should be established and considered as a priority.

Especially when they came from field-based experiments, results such as those obtained in the present study may provide new avenues for predicting responses at scales that are relevant to management and conservation in the context of Marine Spatial Planning.

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