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Research Article

Monitoring the habitat use of common Bottlenose Dolphins (Tursiops truncatus) using passive acoustics in a Mediterranean marine protected area

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Abstract

The Mediterranean *Tursiops truncatus* subpopulation has been classified as Vulnerable on the IUCN Red List because of its decline. This species in coastal areas is exposed to a wide variety of threats: directed kills, bycatch, reduced prey availability caused by environmental degradation and overfishing, habitat degradation including disturbances from boat traffic and noise. Despite the increase in boat traffic in the Mediterranean Sea, the effect on *T. truncatus'* habitat use has not been studied in detail and published data are limited. This study represents the first attempt to characterise spatial and temporal habitat use by *T. truncatus* and its relation to boat traffic in the Isole Pelagie Marine Protected Area (Italy) on the basis of an originally developed passive acoustic monitoring system (PAM). The devices were deployed in 2 areas in the southern waters of Lampedusa, during 2 separate years (2006 and 2009), each time for 3 months (from July to September) and in 6 time slots (3 diurnal and 3 nocturnal). Acoustic analysis showed that *T. truncatus* used the Southern coastal area of Lampedusa independently of the year, primarily during the early summer, a period coinciding with the peak of the calving season. Dolphin occurrences appeared independent of boat traffic, with the exception of the smallest temporal scale (time slots); dolphin occurrences were more prevalent during the night when the level of boat traffic was lower. This study provides evidence on *T. truncatus* habitat use in the Mediterranean Sea and reveals that boat traffic could be one of the factors influencing it, thus stressing the need for further detailed investigation regarding this topic.

Keywords: *Tursiops truncatus*, habitat use, displacement, passive acoustic monitoring, Mediterranean Sea, marine protected area.

Introduction

Tursiops truncatus is among the most common cetaceans in the Mediterranean Sea. The Mediterranean subpopulation appears to be fractioned into isolated units (Natoli *et al.,* 2005) and, due to its general decline, the *International Union for the Conservation of Nature (IUCN*) has been listed it as a Vulnerable species under the Red List of Threatened Species category (Bearzi & Fortuna, 2006). While directed kills by fishermen represented the most important source of population decline until the 1960s, currently by-catch in fishing gear (Buscaino *et al.,* 2009) and habitat degradation (chemical pollution, boat traffic and underwater noise) (Bearzi & Fortuna, 2006; Bearzi *et al.*, 2008) are recognised as important causes of disturbance and loss of life. Most marine animals are very sensitive to sound, particularly marine mammals, because they use acoustic signals to communicate with one another, locate food and navigate underwater. As a consequence, anthropogenic sounds may elicit many detrimental effects on marine mammals: physical injury, physiological effects (temporary or permanent loss of hearing sensitivity), behavioural modification (for example, changes in foraging or habitat-use patterns, separation of groups or mother-calf pairs, changes in the acoustic features of vocalizations), and masking (Richardson *et al.*, 1995; Weilgart, 2007). In the last decades, boat traffic has increased exponentially in the Mediterranean Sea (Abdulla & Linden, 2008) leading to a parallel rise in noise and related effects on local fauna (e.g. Bracciali *et al.*, 2012). Nevertheless, the effects on Mediterranean habitat use and behaviour of *T. truncatus* have never been studied in detail and very little data has been published in peer-reviewed journals. Permanent or temporary displacement of bottlenose dolphins from specific areas of the Mediterranean, as a consequence of boat traffic, has only been reported in the coastal waters of Croatia (Rako *et al.,* 2013) and the Lampedusa Islands (Italy) (La Manna *et al.*, 2013), though similar negative effects have been reported for other places around the world. Allen & Read (2000), for instance, provided evidence that Florida bottlenose

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dolphins reduced the use of some feeding sites in relation to the density of boats, while Lusseau (2005) observed that the seasonal residence index of dolphins and the time spent by animals in the area were negatively correlated with the number of boat trips. Furthermore, decline in the relative abundance of bottlenose dolphins with the increasing number of whale-watching boats was also observed by Bejder *et al.* (2006).

In 2006, a LIFE NAT/IT/000163 European project provided a great opportunity to study the impact of boat traffic on the *Tursiops truncatus* community of the Southern Mediterranean (Lampedusa Island, Strait of Sicily, Italy). Temporal and spatial trends of dolphin habitat use were monitored using moored autonomous recorders for Passive Acoustic Monitoring (PAM). PAM is a good technique for studying cetaceans because acoustics is often the only tool allowing the study of submerged animals that are not visible to human observers and it does not interfere with animal behaviour (Zimmer, 2011). In particular, autonomous recorders were chosen because they are more flexible as regards configuration, timing, and location of deployment, and they are less obtrusive to both animals and boat traffic when compared to other types of PAM systems (Sousa-Lima *et al.*, 2013). Thus, the main aims of this study were: i) to detect the spatial and temporal trends in bottlenose dolphin habitat use and boat traffic and ii) to assess if boat traffic elicited displacement responses in bottlenose dolphins, influencing habitat use.

Materials and Methods

Study Area

This study was carried out in the waters surrounding the island of Lampedusa (Pelagie Archipelago, Italy), located on the north African continental shelf, about 130 km from the Tunisian coast and 250 km from the Sicilian coast. A portion of this area was declared a Marine Protected Area by the Italian Ministry of the Environment in 2002 and a Site of Community Importance (SIC - ITA04013) for the Sicily Region in 2005. The area is inhabited by a rather abundant population of common bottlenose dolphins (*Tursiops truncatus*). Through the analysis of photo-identification and monitoring data, an image of *T. truncatus* habitat use has begun to emerge. A variable presence of the animals has been observed over the years, with a portion of them more resident than others and a growing presence of animals in areas beyond 3 miles from the coast (AAVV, 2007). Dolphin group size is highly variable in this population, ranging between 1 and 30 animals (on average 3). The estimated population size - from 1998 data - was 103 individuals (CIs: 79–134) in an area of 500 km^2 surrounding Lampedusa and including the MPA. More recent estimates (Pulcini *et al.,* 2010, 2013) are 176 individuals (CIs: 131–236) suggesting that the dolphins frequenting the archipelago are likely to be part of a larger population (Pulcini *et al.,* 2010, 2013).

From May to October, Lampedusa is visited by a large number of tourists, and its inhabitants rely on boat-related tourism as one of their main economic resources. Boat traffic in the waters of Lampedusa is very dense throughout the summer as shown by La Manna *et al.* (2010) who counting more than 2,229 boats in the summer of 2006.

Autonomous recorders and sampling

In this study, we employed new autonomous fixed recorders, called RASP. Eight RASPs were used and equipped with programmable underwater acoustic recorders (M-Audio MicroTrack II; data format 16-24 bit WAV) and hydrophones with bandwidth between 10 Hz and 96 kHz (Sensor Technology SQ2; sensitivity -169 dB re 1V/1uPa). The recorders had a custom timer control board offering full programmability for recording times and intervals, and were equipped with hard disks offering from 4 to 32 Gb of memory *per* deployment. The battery pack had a 12,000 mA nominal capacity and was made of NiMH fast rechargeable cells with an ultra-low-dropout voltage regulator.

Thus, the RASP components were: an aluminium cylindrical watertight case contained inside the hydrophone, the recorder and the battery package (Fig. 1); an anchor weight, which allows the RASP to sink and remain fixed on the sea floor; an electrolytic acoustic release, which could receive remote commands from the control station to drop the anchor; and a flotation device which kept the assembly upright from the sea floor. RASPs were hand-deployed from a 5.70 m Zodiac equipped with a 50 hp engine.

After a pilot study in which RASPs were deployed randomly all around Lampedusa at different sites located from 0.2 to 7.0 miles off the coast, we decided to reduce the sampling area in order to minimise the risk of trawlers fishing in the area picking up the instruments, and to allow deployment of RASPs almost independently of weather conditions. Thus, RASPs were deployed in two macro-areas of about 17 km^2 each, off the Southern part of the island (Fig. 2). Both areas were characterised by a sandy bottom, 35–40 m deep. Distance from the coast ranged between 0.1 and 0.8 nautical miles. The SW area included zones A (full protection), and zones B and C (partial protection) of the Marine Protected Area Isole Pelagie. Navigation was either forbidden (zone A) or allowed at speeds no greater than 6 knots (zones B and C) and professional fishing was not permitted (zones A, B and C). In contrast, the SE area was outside the perimeter of the MPA, including the harbour and fishing was allowed below a depth of 50 m. The deployment sites were chosen randomly inside

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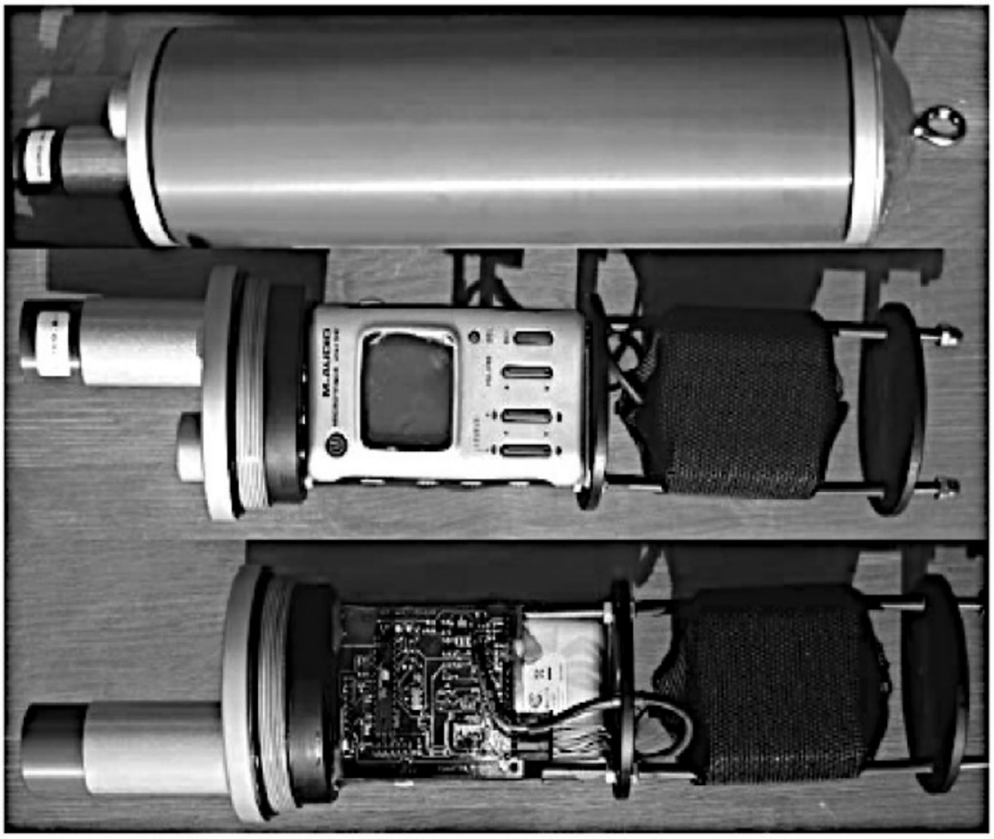


Fig. 1: RASP: cylinder shaped watertight case (on the top), recorder and hydrophone (in the middle), timer (on the bottom).

these two macro-areas. After the pilot study (May and June 2006), we decided to collect data at equal intervals, 10:10, i.e. 10 min of recordings followed by 10 min of pause (sampling rate of 48 kHz, 24 bit rate) collecting a total of 3.5 hours in 1 day. This schedule seemed to us as the best compromise between the likelihood of capturing signals of interest, battery power consumption and hard disk storage capacity.

Acoustic and statistical analysis

The acoustic environment of the study area had never been studied before; therefore, we manually analysed all the recordings collected, despite the large volume of data, with the aim of retaining as much information as possible. We divided all the recordings into slots of 5 min. We calculated the occurrence frequencies of biological and anthropogenic signals, particularly bottlenose dolphin vocalisations and motor boat noise, as the number of seconds of dolphin acoustic sound (click train or tonal sound) or boat noise per slot. Thus, two frequencies of occurrence were obtained: the DFREQ (dolphin frequency) and the BFREQ (boat frequency), ranging from 0 (no dolphin sounds or boat noise) to 1 (dolphin sounds or boat noise during the entire slot). All frequencies were tested for the normality and homogeneity of the variance, with the Shapiro-Wilk test and Cochran's C test, respectively. Neither frequency was normally distributed; therefore, a Multivariate Analysis of Variance with Permutation (PERMANOVA) was applied to test them as functions of spatial and temporal factors. The independent variables were: i) Year (fixed factor, two levels: 2006 and 2009), to test the permanence of dolphins in the area year by year and the annual trend in boat traffic; ii) Area (fixed factor, two levels: SW and SE), to verify an eventual habitat preference of dolphins and the spatial trend in boat traffic related to the MPA; iii) Period (factor nested in Year, three levels: A from 20 to 31 of July; B from 10 to 21 August; C from 5 to 16 September) to test habitat use and boat traffic level as a function of different periods; iv) Time slot (fixed factor, six levels: 5–9 am; 9 am–1 pm; 1–5 pm; 5–9 pm; 9 pm – 1 am; 1–5 am) to determine the dolphins' diurnal and nocturnal habitat use and boat traffic level. The factor Period had three levels of twelve days each. The factor time slot was divided into three diurnal levels, the first of which included sunrise (from 5:30 to 6:20 am), and three nocturnal levels, the first of which included sunset (from 8:15 to 9:00 pm). This additional factor was introduced in the 2009 sampling design, and was thus tested in the corresponding dataset. PERMANOVA variables were *a priori* transformed to their square roots and data were transformed with Euclidean distance. Analyses were carried out with 9999 permutations of

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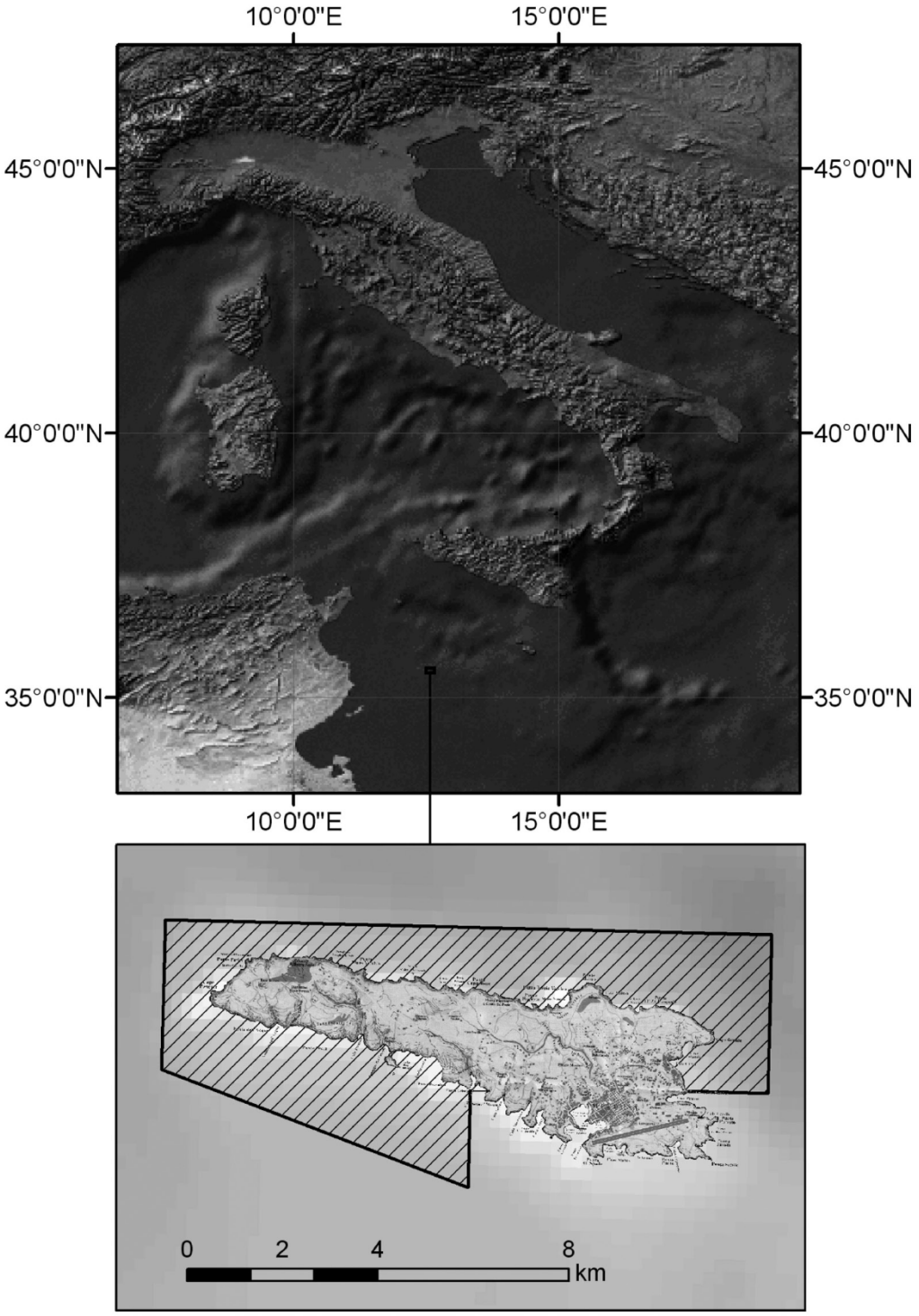


Fig. 2: Study area. The striped zone is the marine protected area. SW area: from the most western point of the island to the southern limit of the MPA; SE area: south-eastern no protected zone of the island.

the residuals under a reduced model (Anderson, 2001). Differences between levels of a factor (area, year, period and time slot) were tested in detail using Pair-wise tests. All descriptive statistics and analyses were carried out using R for Mac, with the exception of PERMANOVA, which was carried out in Primer 6+ (licensed to Chiara Romano).

Results

From July to September 2006 and from July to September 2009, we collected 119 hours of recordings, during 34 days of sampling. Due to logistical limits and weather conditions, the number of deployment days between different levels of the factors was not equal, but

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we obtained forty-two replicates (5 min slot) for each sampling day.

The recordings collected contained anthropogenic signals, mostly belonging to boats, but also to sonars and fish-finders in a small percentage of instances. In addition, we detected biological signals, mainly bottlenose dolphin vocalisations, but also snapping shrimp (*Alpheidae spp*) and choruses of the fish *Dactylopterus volitans* (La Manna, *pers. obs.*). We analysed the biological signal of interest to the study, bottlenose dolphin vocalizations, and compared its frequency (DFREQ) with that of boat traffic (BFREQ), the most commonly recurring anthropogenic signal.

DFREQ had a mean value of 0.12 (± 0.26) and the results were statistically different with respect to all the factors with the exception of Year (Table 1). DFREQ was slightly larger in the SE (0.13 ± 0.30) compared to the SW (0.11 ± 0.23), but considering the interaction between year and area, DFREQ was larger in the SW (protected area) compared to the SE in 2006, while in 2009 the opposite was true. Finally, considering the period as a factor, in 2006 in the SW, periods A and B were statistically larger than C, while in 2009 A was larger than B and C. In 2006, in the SE, the periods did not differ significantly, while in 2009, A and B were larger than C (Fig. 3).

BFREQ had a high value (0.82 ± 0.36) and was statistically different as a function of all factors, except Area (Table 2). BFREQ was larger in 2006 (0.98 ± 0.11) compared to 2009 (0.73 ± 0.41). In 2006 there was no difference between SE and SW, but in 2009 SE was significantly larger than SW. Considering the period factor, the results did not show a regular trend in either area or year. In 2006, in the SW, A and B were significantly

Table 1. Outcomes of PERMANOVA carried out on the DFREQ Euclidean matrix, root-square transformed using 9999 permutations. MS = Mean Square.

Source	df	MS	Pseudo-F	P (perm)
AREA	1	2.2624	27.686	0.0001
YEAR	1	0.016818	0.20581	0.6438
PERIOD(YEAR)	4	1.9898	24.351	0.0001
AREA x YEAR	1	8.143	99.651	0.0001
AREA x PERIOD(YEAR)	4	0.44315	5.4231	0.0002
Residuals	1416	0.081715		

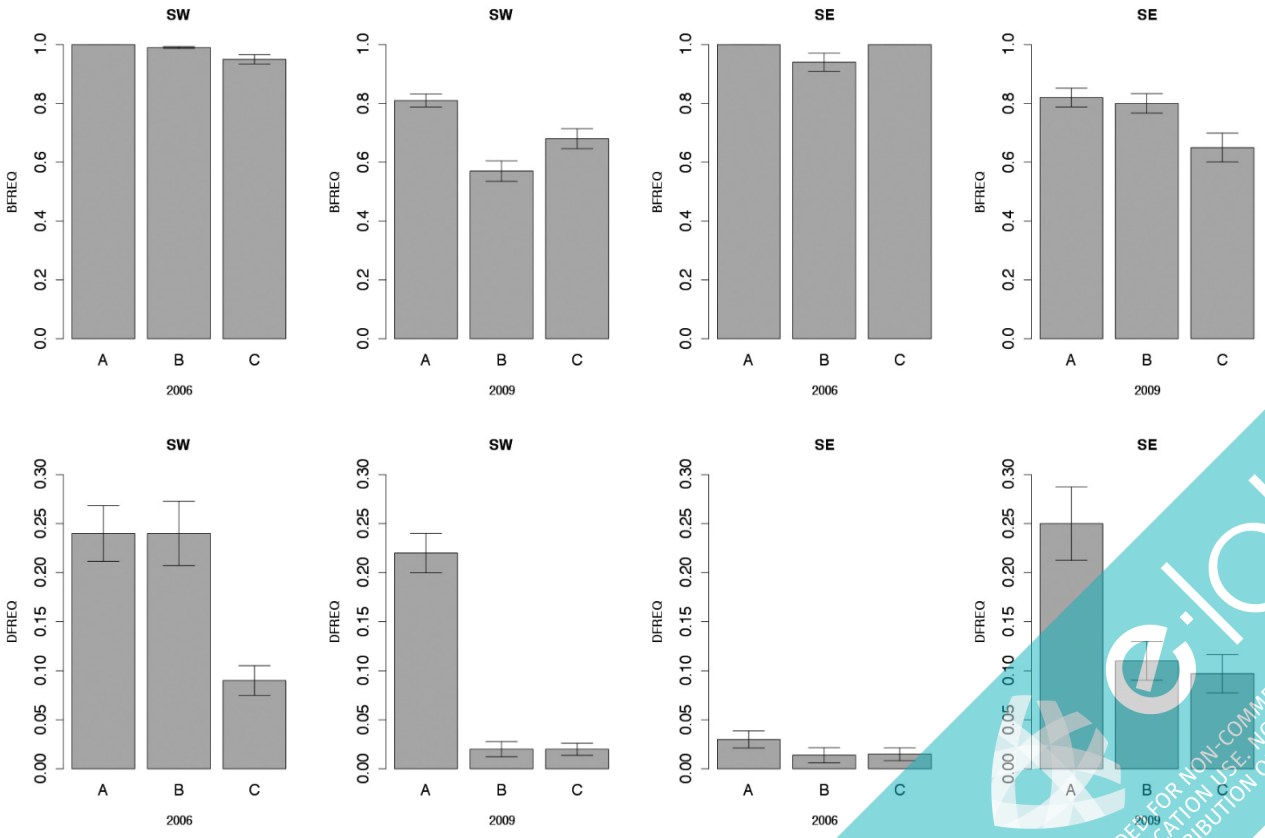


Fig. 3: DFREQ and BFREQ as a function of area (SW and SE), year (2006 and 2009) and period (A, B and C).

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Table 2. Outcomes of PERMANOVA carried out on the BFREQ Euclidean matrix, root-square transformed using 9999 permutations. MS = Mean Square.

Source	df	MS	Pseudo-F	P (perm)
AREA	1	0.28218	2.7753	0.0955
YEAR	1	16.848	165.7	0.0001
PERIOD(YEAR)	4	1.0171	10.004	0.0001
AREA x YEAR	1	0.33386	3.2835	0.0712
AREA x PERIOD (YEAR)	4	0.53361	5.2481	0.0005
Residuals	1416	0.10168		
Total	1427			

Table 3. Outcomes of PERMANOVA carried out on the DFREQ Euclidean matrix, root-square transformed using 9999 permutations. MS = Mean Square.

Source	df	MS	Pseudo-F	P (perm)
TIME	5	2.1404	33.561	0.0001
PERIOD	2	5.8676	92	0.0001
AREA	1	1.0489	16.446	0.0003
TIMExPERIOD	10	1.1658	18.279	0.0001
TIMExAREA	5	0.51744	8.113	0.0001
PERIODxAREA	2	0.77106	12.09	0.0001
TIMExPERIODxAREA	10	0.21791	3.4167	0.0002
Residuals	1514	0.06377		
Total	1549			

Table 4. Outcomes of PERMANOVA carried out on the BFREQ Euclidean matrix, root-square transformed using 9999 permutations. MS = Mean Square.

Source	df	MS	Pseudo-F	P (perm)
TIME	5	2.9862	24.161	0.0001
PERIOD	2	3.0551	24.719	0.0001
AREA	1	3.0636	24.788	0.0001
TIMExPERIOD	10	1.8412	14.897	0.0001
TIMExAREA	5	0.46509	3.763	0.0021
PERIODxAREA	2	1.9132	15.479	0.0001
TIMExPERIODxAREA	10	0.66796	5.4044	0.0001
Residuals	1514	0.1236		
Total	1549			

larger than C, while in 2009, A and C were larger than B. In 2006, in the SE, A and C were larger than B, while in 2009, A and B were larger than C (Fig. 3).

In 2009, we introduced the "time slot" factor in the sampling design. Both BFREQ and DFREQ were tested in the 2009 dataset to verify their trends as a function of time slots, and their interaction with area and sampling period. Both BFREQ and DFREQ were statistically different in relation to all the factors (Table 3 and 4). Considering the time slots, DFREQ and BFREQ showed opposite trends; DFREQ increased from sunset to sunrise, while BFREQ increased from sunrise to sunset (Fig. 4). The results also showed the same opposing trends when considering the interaction with the factor Area; for both SW and SE, DFREQ increased from sunset to sunrise, and BFREQ increased from sunrise to sunset (Fig. 5). DFREQ and BFREQ were significantly different between all the time slots, with few exceptions.

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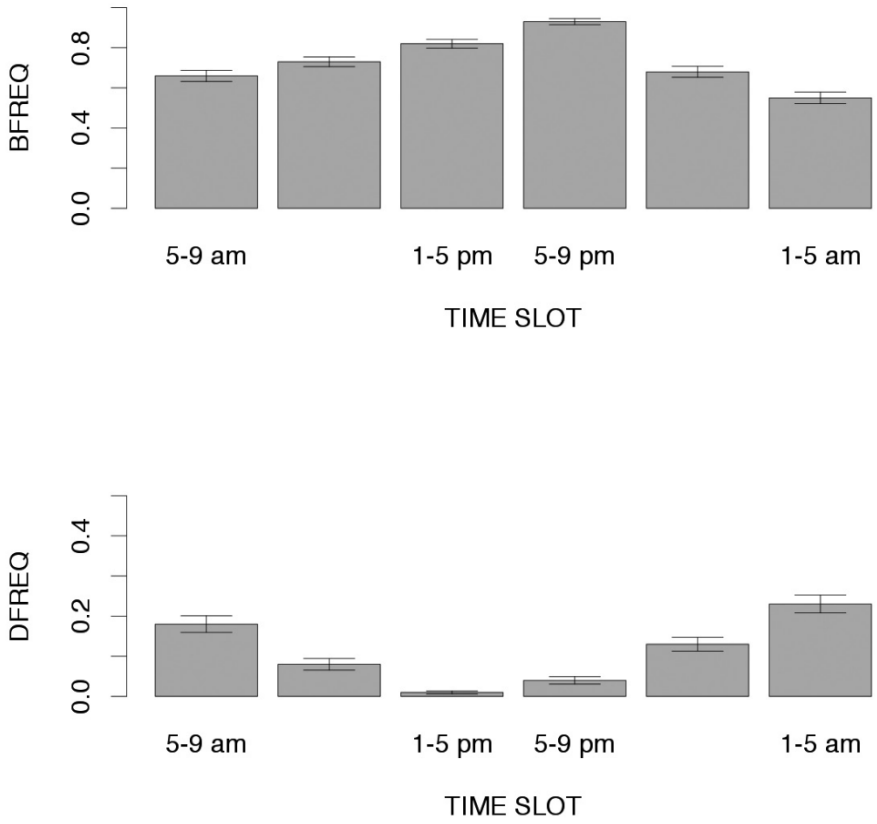


Fig. 4: BFREQ (on the top) and DFREQ (on the bottom) as a function of time slot.

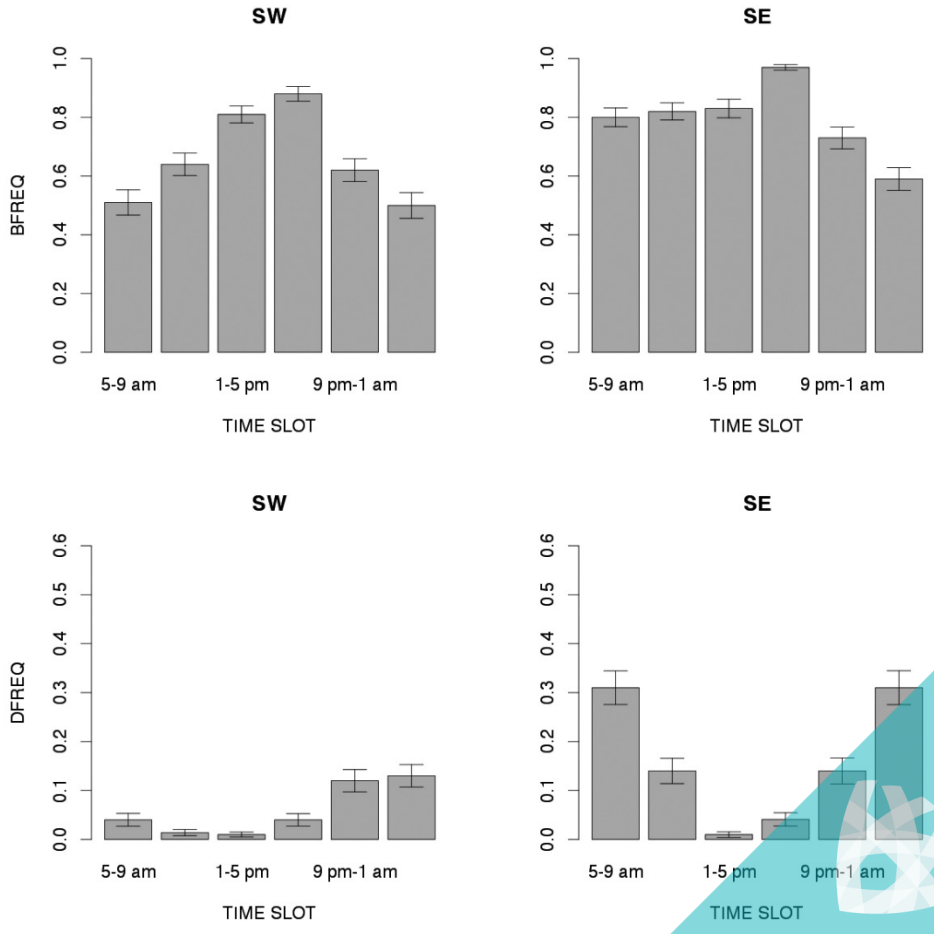


Fig. 5: BFREQ (on the left) and DFREQ (on the right) as a function of time slot and area.

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Discussion

The habitat use of Tursiops truncatus and the animal's interaction with boat traffic

This study represents the first attempt to characterise the habitat use of *Tursiops truncatus* and its relation to boat traffic in a Mediterranean Marine Protected Area, based on PAM technology. The species was observed using the southern zone of the study area, with a wider distribution within 1 mile from the coast than previously detected. Habitat use was independent of year. Considering the three different periods, we observed a progressive decline in the dolphins' occurrence from period A (July) to C (September). The dolphins' major use of coastal waters coincided with their calving season, which should peak in July and August (Evans *et al*., 2003). This could be related to the mothers' preference for shallower waters, which offer more protection for juveniles from strong currents and predators (Mann *et al.,* 2000). Finally, *T. truncatus* in Lampedusa did not have any consistent preference for either of the two investigated areas (SW and SE) as shown by the fact that the occurrence of dolphins was greater in the SW in 2006 and in the SE in 2009. These areas look very similar in terms of morphological and oceanographic features. Therefore, our experimental *a-priori* distinction would not be perceived as such by the species. However, the two sites do differ with regard to the protection regime. The first one is within the boundaries of the MPA and the second outside. Unfortunately, such a difference in protection does not seem to determine any stable preference in the species' habitat use, probably due to the small size of the protected area or the low efficiency of the protection regime. For example, the boat traffic level did not differ between SW and SE, proving that MPA management is not effective in reducing this kind of human disturbance. In our case, the yearly and seasonal dolphin habitat use appeared independent of the level of boat traffic, but the result appeared different when considered on a smaller temporal scale. In fact, the dolphins' daily habitat use was the opposite of the daily trend in boat traffic; boat traffic increased from 9 am to 5 pm, while the occurrence of dolphins increased from 5 pm to 5 am. These trends were also consistent considering the interaction with the other two independent variables: area and sampling period. Moreover, it appeared that dolphins tended to leave the coastal area just prior to the maximum peak in traffic. This behaviour could be explained in different ways. For instance, if animals perceive a threatening situation, they are more likely to adopt evasive tactics similar to those observed when escaping a predator (Lima & Dill, 1990; Bracciali *et al.,* 2012). A significant effect on habitat use as a result of disturbance from increasing boat traffic has been observed in other sites (Corkeron, 1995; Allen & Read*.,* 2000; Lusseau, 2003, 2005; Lamb & Ugarte*,* 2005; Sousa-Lima & Clark, 2008) such as in Lampedusa Island (Papale *et al*., 2011; La Manna *et al*., 2013). Analysing the effect of motorboats and trawlers on dolphins' acoustic permanence in the area and whistle parameters, a companion study found that the dolphins' behavioural strategies depend on the kind of boats present. In the case of motorboats, dolphins preferred to leave the area, probably because the disturbance became too heavy to be tolerated. While under trawlers, dolphins changed their acoustic behaviour to compensate for the masking noise (La Manna *et al.,* 2013). Another study using different methodology (land-based surveys) showed that the interaction between dolphins and silent sailboats was always neutral (i.e. no behavioural change), whereas fast motor boats caused the interruption of all activities and avoidance behaviour (Papale *et al.,* 2011). Despite these results, a variety of factors apart from level of boat traffic may also be included among factors influencing the daily short-term movements of dolphins. Firstly, the tidal cycle as tide-related movements of *Tursiops sp.* have been described by McBride & Hebb (1948), Caldwell & Caldwell (1972), Irvine & Wells (1972), and Shane (1977), though these studies involved the movement of dolphins into and out of macro-tidal channels, estuaries or canals, which are situations not directly comparable with the present study. In the Straits of Sicily, tides are semidiurnal and tidal amplitude is very narrow (not more than 0.25 m). However, if tides influence the behaviour of dolphins to some extent, we should have observed a bimodal daily trend in animal occurrence, instead of the modal one detected. Secondly, the cycle of prey could be another factor as the nocturnal dolphin movements in coastal waters may represent a searching pattern for fish moving from inshore to offshore and vice versa. For example, some species have diurnal or nocturnal peaks in activity, spending the day close to the seabed and dispersing at night to feed at the surface (Cardinale *et al.,* 2003). In some places, variations in the abundance and species composition between day and night have been demonstrated (Azzurro *et al.,* 2004). In Lampedusa, a study carried out with the aim of quantifying the interaction between dolphins and fishery revealed a different catch composition of the trawler fishing between night and day (Celoni *et al.,* 2009). This could explain the different level of dolphins' interaction with trawlers, which was observed to be higher during the night than the day. However, more research is needed to investigate the effect of trawlers on dolphin behaviour. Finally, to extrapolate data on dolphin habitat use from acoustic detections we need to assume that the vocalization rate is relatively constant throughout the day. In fact, differences in daily occurrence may depend on changes in vocal production rate. Increased echolocation has been documented at night in a single captive bottlenose dolphin (Akamtsu *et al*., 1996), and has been proposed to compensate for loss of visual cues (Carlström, 2005). However, some studies conducted on bottlenose dolphins and other species

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of toothed whales showed no differences in the production rate of echolocation clicks (the most abundant type of vocalizations in the present study) between day and night (Bond, 2006; Philpott *et al.,* 2007; Bailey *et al*., 2009). In contrast, other studies demonstrated higher dolphin acoustic encounters during morning (Gregory & Rowden, 2001) or during night periods in the same area, but in different years (Alford, 2006), probably due to the availability and diurnal prey activity. However, such a fact deserves further investigation to discern those factors influencing the daily habitat use of bottlenose dolphins in Lampedusa Island, and which among prey availability and boat traffic plays a major role.

Management implications

Knowing the distribution and ranging patterns of cetaceans is important for implementing effective boundaries for marine protected areas and correct management. The present and other research on the Lampedusa *T. truncatus* population showed a possible negative effect of displacement from the coastal area, likely due to the high level of boat traffic, suggesting that the current boundaries and their regulation by the MPA are probably ineffective in protecting the species from this kind of human disturbance. Thus, we suggest: 1) the integration of mitigating actions for maritime noise and the physical impact of motor boats into the objectives and regulation of the existing MPA; 2) the extension of the boundaries of the protected area and the establishment of a SAC (Special Area of Conservation) for the conservation of bottlenose dolphins in this area, as also suggested by Pulcini *et al.* (2013); 3) the creation of boat traffic corridors and effective speed restrictions to minimise exposure to *T. truncatus*; 4) an increase in local surveillance of the MPA, also employing PAM technologies and video monitoring; 5) an increase in local public awareness regarding the effects of boat traffic on *T. truncatus* and other marine mammals.

Benefits, limits and potential development of the RASP

The RASP was effective in monitoring the presence and habitat use of dolphins as well as boat traffic trends. Thus, it responds well to the recent request by the scientific community to develop new technologies to be used for assessing presence and distribution of marine mammals in sensitive areas, and keeping track of changes in boat traffic and related underwater noise levels. Comparing these results with those obtained through forty-five hours of land observations conducted in 2006 in the same area and performed by a team of at least two or three observers per site (La Manna *et al*., 2010), we can highlight that: 1) The RASP detected the same level of traffic in the two studied areas established in 2006 (SE = SW); 2) The RASP detected the same evening decline in boat traffic, providing better temporal definition and adding the nocturnal trend; 3) The RASP detected the same major frequency occurrence of dolphins in SW. Thus, the RASP demonstrated that habitat use by the dolphins and boat traffic trends are consistent with those determined by visual observations, but it supplied information about the habitat use by dolphins as a function of period and time of day, which was not assessed in the visual land study. Despite these successes, the RASP has some limitations which suggest specific directions for its further development and use in marine biology research. Firstly, the large amount of acoustic data obtained requires automated analysis procedures in order to achieve the maximum benefit from such devices. Secondly, units with higher recording capacity than those used in this study will be very useful in providing monitoring for months without any research effort beyond deployment and recovery. Finally, the use of calibrated recorders (not available in our 2006 experiment) could provide estimates of ambient noise level to match against boat traffic trends.

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