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Published version:

DOI:10.1121/1.4808329

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Marta Azzolin; Elena Papale; Marc O. Lammers; Alexandre Gannier; Cristina Giacoma. Geographic variation of whistles of the striped dolphin (Stenella coeruleoalba) within the Mediterranean Sea. THE JOURNAL OF THE ACOUSTICAL SOCIETY OF AMERICA. 134 (1) pp: 694-705.
DOI: 10.1121/1.4808329

Geographic variation of whistles of the striped dolphin (S*tenella coeruleoalba*) within the Mediterranean Sea

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ABSTRACT

The striped dolphin is a cosmopolitan species distributed worldwide. Morphological and genetic studies strongly suggest that the Mediterranean and eastern North Atlantic populations are isolated from each other. The Mediterranean population is considered a distinct conservation unit by IUCN experts, classified as “vulnerable”. This study describes the geographical variation of the striped dolphin whistles within the Mediterranean Sea. Recordings were collected from 1996 to 2003 throughout the whole basin, employing multiple platforms. Thirty seven independent sightings with acoustic data collection were made, and 599 whistles were extracted and considered for statistical analysis. Whistle analysis enabled the identification of sub-populations of striped dolphins within the Mediterranean Sea. Their acoustic diversity reflect the genetic differences recently found among striped dolphins inhabiting different Mediterranean regions. The results of this study support the hypothesis that gene flow reduction plays an important role in determining variation in whistle duration and frequency parameters, while ecological and social factors influence parameters of the modulation domains. The ability of acoustically identifying distinct geographic sub-populations could provide a useful tool for the management of this protected species.

Keywords: *Stenella coeruleoalba*, Mediterranean Sea, vocal behaviour, whistle, geographic variation, evolutionary units

1 INTRODUCTION

The striped dolphin *(Stenella coeruleoalba)* is a cosmopolitan species quite common worldwide in tropical and temperate pelagic waters. It is the most abundant cetacean of the Mediterranean Sea where it is typically found in productive, open waters beyond the continental shelf of the Alboran Sea, the Algerian-Provençal Sea and the Ligurian Sea (Forcada et al. 1994) and decreases in abundance in the Eastern Mediterranean. Striped dolphins face significant challenges worldwide, especially in the Mediterranean Sea, due to environmental changes and human activities.

The Mediterranean population of striped dolphins is considered a distinct conservation unit by International Union for the Conservation of Nature (IUCN) experts (Reeves and Notarbartolo di Sciara 2006) and its conservation status is classified as Vulnerable. Morphological and genetic studies strongly suggest that the Mediterranean and eastern North Atlantic populations are isolated from each other, with little or no gene flow across the Strait of Gibraltar. The maximum body length of eastern North Atlantic striped dolphins is 5-8 cm longer than the Mediterranean animals (Calzada and Aguilar 1995). Skull size is also smaller in Mediterranean specimens than in their neighbouring Atlantic counterparts (Archer 1997). Mitochondrial DNA analysis has yielded 27 haplotypes, none of which was shared between the two areas, thus supporting differentiation (García-Martínez et al. 1999). Within the Mediterranean there is some cline variation in body size suggestive of population structure and/or restriction in gene flow between areas (Calzada and Aguilar 1995). Gaspari et al. (2007) considered dispersal range sufficiently limited between sub-populations across the Mediterranean, and probably between inshore and offshore populations within the Ligurian Sea, to make genetic differentiation possible. The reduced dispersal range also appears to be confirmed by significant differences in tissue pollutant levels of Spanish and Italian striped dolphins (Monaci et al. 1998; A. Bellante, (2012):

Since the distribution, mobility and degree of separation of striped dolphins between different Mediterranean areas is still unknown – even though morphometric and genetic variations suggest restriction in gene flow between areas – more data about the biology of the species are needed to identify the Mediterranean Sea sub-populations and an acoustic analysis is likely to be insightful in this regard. Characteristics of acoustic behaviour have been used to distinguish populations of humpback whales (*Megaptera novaegliae*), fin whales (*Balaenoptera physalus*), blue whales (*Balaenoptera musculus*), sperm whales (*Physeter catodon*), and killer whales (*Orcinus orca*) among others (Ford

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and Fisher 1982; Stafford et al. 2001; Thompson et al. 1992; Weilgart and Whitehead 1997; Winn et al. 1981). Recently, there has been a growing interest in intraspecific variations in the signalling structure of *Delphinidae* (Bazúa-Durán and Au 2004), as illustrated in several studies on the bottlenose dolphin (*Tursiops truncatus*). Research on this species has identified acoustic differences between social groups (Janik 2000), localities (Wang et al. 1995a, Morisaka et al. 2005) and between males and females (Sayigh et al. 1995). However, there are no published studies on geographic variation in the signalling structure of the Mediterranean striped dolphin.

Striped dolphin acoustic signals can be classified into two main categories: tonal whistles and pulsed sounds. Whistles are frequency modulated tones with durations varying from less than a second to several seconds (Bazúa-Durán and Au 2002, Wang et al. 1995b). In other species of the same genus, such as spinner dolphins (*Stenella longirostris*), they are considered to be signals used to regulate group organization (Norris et al. 1994, Janik and Slater 1998) and they are believed to be particularly important for maintaining social cohesion within groups (Lammers et al. 2006). Pulsed sounds, on the other hand, are primarily characterized by trains of short (<100 µs) broadband clicks used in echolocation (Au 1993), but are also produced as “burst pulses”, which are believed to play a role in intra-group communication (Lammers et al. 2003).

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This paper describes the geographical variation of the acoustic structure of striped dolphin whistles within the Mediterranean Sea and analyses the factors that influence this variability. We first analysed the differences between striped dolphins belonging to the eastern and western basins. We then examined the variation between striped dolphins belonging to the following two regions of the western basin: a) Alboran Sea, Spanish and Balearic waters; and b) the Algerian-Provençal Sea, and Tyrrhenian Sea. After that we investigated the variation between inshore and offshore striped dolphins.

Subsequently, we considered the variation of whistle parameters in relation to distance from the geographical barrier represented by the Strait of Gibraltar. Finally, we tested the influence of environmental factors (depth and wind intensity) and social factors (group size) on the variability of whistles acoustic parameters.

2 MATERIALS AND METHODS

2.1 Study area

For the purpose of this study, the Mediterranean Sea was divided into two basins at the level of the Sicilian Strait, which we will refer to as the western Mediterranean and eastern Mediterranean (Fig. 1). The western basin includes the following sub-basins: Alboran Sea, Algerian-Provençal Sea and Tyrrhenian Sea. This basin is characterized by current coming from Gibraltar. The eastern basin includes the following sub-basins: Adriatic Sea, Ionian Sea, Aegean Sea and Levantine Sea, and is influenced by a water stream coming west from the deeper water layer of the Levantine Sea (Miller 1983).

2.2 Data collection

Acoustic data were collected from 1996 to 2003 in different areas of the Mediterranean Sea from multiple platforms, thanks to the cooperation of the two following research groups: the International Fund for Animal Welfare (IFAW, UK), and the Groupe de Recherche sur les Cétacés (GREC, France).

Visual sightings of the recorded animals enabled identification of the species. Thirty seven independent acoustic detections associated with visual sightings were made in an area delimited by 36.83N, -3.950W, and 36.66N, 20.688E (Fig. 1).

Sound recordings were made using a variety of recording equipment, all of which had a flat frequency response ($\pm$3dB) up to 22KHz (Table 1). The coordinates of each sighting, all referred to the WGS84 system, were collected using a variety of Garmin™ GPS units.

To calculate the progressive distance from the Atlantic Ocean, the distance of each sighting from the Strait of Gibraltar was measured using ARCGIS 9.0.

To attribute a depth (m) to each sighting, the navigation charts of 1) the Italian *Istituto Idrografico* and 2) the English Hydrographic Office were used.

To attribute yearly mean wind speed to the area of each sighting, wind statistics from the Offenbach Synoptic Centre, available at the "windfinder" web site were used.

2.3 Acoustic analysis procedures

Approximately 19 hours of recordings, associated with 37 acoustic detections, 21 for the western and 16 for the eastern Mediterranean were examined (Table 1). A total of 1045 whistles were extracted for manual measurement based on two criteria: 1) the signal to noise ratio needed to be sufficiently high so that timing and frequency parameters could be unambiguously discerned from background noise; and 2) whistles that had similar contours were discarded in order to increase the whistle variability of each acoustic detection and to minimize the possibility of oversampling the whistles belonging to an individual, to a group or to a specific behavioural context. Eight scalar parameters were obtained for each whistle using CoolEdit™, through manual measurements of the whistle contour, according to Oswald and colleagues (2003) (Fig. 2). These parameters included: 1) duration, 2) beginning frequency, 3) final frequency, 4) minimum frequency, 5) maximum frequency, 6) frequency range (calculated by subtracting the values of the maximum and minimum frequency), 7) number of inflection points in the frequency contour and 8) number of steps (a discontinuous change in frequency). Two more parameters were also added to further describe the whistle contour: 9) number of contour minima and 10) number of contour maxima.

Whistles that presented interruptions within the contour shorter than 200ms were considered “discontinuous”. In order to consider a whistle contour belonging to two different whistles the duration of a break along the contour should have been 200 ms (Bazua-Duran and Au 2002) or greater. Since discontinuous whistles could have been collected from animal not pointing directly towards the recording system they were not considered for statistical analysis, because the whistle contour could have been not always clearly identifiable.

Whistles that went off scale (above 22 KHz) were not included in statistical analysis.

To reduce over-representation of the most “vocal” dolphins, the maximum number of whistles to be analysed per group was set to four times the number of individuals present in the group (Azevedo and Van Sluys 2005).

Following Gaspari and colleagues (2007), we classified striped dolphins as inshore when found within a depth of 600 m and offshore when found beyond 2000 m depth. The whistles recorded between a depth of 600 and 2000 m were not considered for the comparison of inshore and offshore animals, because in this particular case the attribution to inshore or offshore sub-populations was not considered reliable.

2.4 Statistical analyses

The following statistical methods were used to document and test the variation of whistle parameters: 1) a descriptive statistic was generated to describe mean variation of whistle parameters of the striped dolphin within the Mediterranean Sea and CVs were calculated to evaluate the variation within parameters with strong or low morphophysiological constraint; 2) a univariate non-parametric statistic (Mann-Whitney test) was used to compare acoustic structure of whistles recorded in the eastern and western basins, in inshore and offshore waters, in the inshore and offshore waters of each basin, and in the following two regions of the western basin: a) Alboran Sea, Spanish and Balearic waters; and b) Algerian-Provençal Sea, and Tyrrhenian Sea); 3) a multivariate statistic (Discriminant Function Analysis – stepwise method), was used to highlight the possibility of correctly classifying whistles to the eastern and western animals, to the inshore and offshore animals, to the inshore and offshore animals of each basin, and to the two regions considered in the western basin. For cross-validating the DFA the leave-one-out procedure was applied; 4) a Spearman's Rho test was performed to investigate the correlation between the considered parameters and the progressive distance from the Strait of Gibraltar.

The variability of the environmental and social factors of the present Mediterranean dataset was investigated by applying the following statistical methods: 1) a univariate statistic (Mann-Whitney test) was used to compare the environmental (depth, mean yearly wind intensity) and social factors (group size) recorded in the eastern and in western basins of the Mediterranean Sea, in the inshore and offshore waters, and in the two regions considered in the western basin; and 2) a Spearman's Rho test was performed to investigate the correlation between the environmental and social factors and the progressive distance from the Strait of Gibraltar.

Statistical analysis was carried out using the software SPSS 16.0.

3 RESULTS

After sub-sampling data 599 whistles were considered for statistical analysis, 384 belonged to the western basin of the Mediterranean Sea and 215 to the eastern one, 127 fit in the inshore area and 269 in the offshore one.

The normality of the whistle parameters considered in this study was checked by applying the Kolmogorov-Smirnov Test: duration (N=599; Z=1.310; P=0.065), final frequency (N=599; Z=1.121; P=0.162), minimum frequency (N=599; Z=0.900;

P=0.392), and frequency range (N=599; Z=0.677; P=0.750) resulted normally distributed; beginning frequency (N=599; Z=1.879; P=0.002), maximum frequency (N=599; Z=1.492; P=0.023), No of Inflection points (N=599; Z=5.460; P=0.000), No of Steps (N=599; Z=5.892; P=0.000), No of Minima (N=599; Z=9.537; P=0.000); No of Maxima (N=599; Z=9.001; P=0.000) resulted not normally distributed.

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3.1 Variability among striped dolphins belonging to the eastern and western basins

In order to investigate whether the Italian Peninsula represents a geographical barrier for the movement and exchange of striped dolphins within the Mediterranean Sea, we analysed the whistle characteristics of the distinct eastern and western striped dolphin sub-populations (Table 2). The whistles of the eastern striped dolphins have lower CVs for most of the parameters except for beginning frequency, final frequency and no. of inflection points. The Mann-Whitney test indicates that duration (N=599; Z=-7.130; P<0.001), minimum frequency (N=599; Z=-2.391; P<0.05), maximum frequency (N=599; Z=-7.909; P<0.001), frequency range (N=599; Z=-7.071; P<0.001), no. of inflection points (N=599; Z=-2.252; P<0.05), no. steps (N=599; Z=-3.810; P<0.001), no. of contour minima (N=599; Z=-2.292; P<0.05), and no. of contour maxima (Mann-Whitney test N=599; Z=-4.878; P<0.001) are significantly greater for eastern animals. DFA (Wilks' Lamda = 0.88; F = 77; df = 597; P < 0.005) correctly assigns whistles to the area they were recorded in the 64% of cases, when running the cross-validated procedure, with a percentage of correct classification significantly greater than that expected by chance. The whistles of the western basin (N=384) are correctly classified in 65% of cases, while those of the eastern basin (N=215) are correctly classified in 63% of cases. Three parameters contribute to the discriminant model of the two sub-populations: maximum frequency (0.78), duration (0.46) and final frequency (-0.23).

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3.2 Variability among striped dolphins belonging to the following two regions of the western basin: a) Alboran Sea, Spanish and Balearic waters; and b) Algerian-Provençal Sea, and Tyrrhenian Sea

In order to investigate the existence of an acoustic differentiation between striped dolphins belonging to the two distinct regions of the western basin where data were collected (Fig.1) we applied both univariate and multivariate statistics. Table 2 shows the descriptive statistic for each region. The Mann-Whitney test indicates that duration is significantly greater in the Alboran Sea and the Spanish and Balearic waters ($N=384$; $Z=-3.398$; $P<0.005$), as well as final frequency ($N=384$; $Z=-4.261$; $P<0.001$), maximum frequency ($N=384$; $Z=-6.219$; $P<0.001$) and frequency range ($N=384$; $Z=-6.403$; $P<0.001$). DFA (Wilks' Lamda = 0.92; $F = 30.48$; $df = 382$; $P < 0.005$) correctly classifies the whistles to the two regions in 68% of cases, when running the cross-validated procedure, with a percentage of correct classification significantly greater than that expected by chance. Whistles of the Alboran Sea, Spanish and Balearic waters ($N=152$) are correctly classified in 66% of cases, while those of the Algerian-Provençal Sea, and Tyrrhenian Sea ($N=232$) are correctly classified in 70% of the cases. Three parameters contribute to the discriminant model: maximum frequency (1.19), beginning frequency (-0.60), and no. of inflection points (-0.55)

3.3 Variability among inshore and offshore striped dolphins

To investigate the existence of an acoustic differentiation between inshore and offshore Mediterranean striped dolphins we applied both univariate and multivariate statistics. The Mann-Whitney test indicates that whistles of Mediterranean offshore striped dolphins show significantly lower beginning frequency ($N=396$; $Z=-4.524$; $P<0.005$), minimum frequency ($N=396$; $Z=-6.142$; $P<0.001$), maximum frequency ($N=396$; $Z=-4.833$; $P<0.001$), no. of inflection points ($N=396$; $Z=-2.306$; $P<0.05$), and no. of steps

(N=396; Z=-3.685; P<0.001), compared to those of inshore animals (Tables 3). The DFA (Wilks' Lamda = 0,92; F = 36,44; df = 394; P < 0,005) correctly assigns to the different areas in the 68% of the cases, when running the cross-validated procedure, with a percentage of correct classification significantly greater than that expected by chance. The whistles of inshore animals (N=127) are correctly classified in 65% of the cases, while those of offshore animals (N=269) are correctly classified in 70% of the cases. Five parameters contribute to the discriminant model of the two sub-populations: minimum frequency (0.64), maximum frequency (0.57), final frequency (-0.54), no of inflection points (-0,38), n° of steps (0,31). The differences between inshore and offshore animals are consistent within the two basins (Table 3). The Mann-Whitney test indicates that the whistles of western offshore striped dolphins show significantly lower beginning frequency (N=332; Z=-3.452; P<0.005), minimum frequency (N=332; Z=-5.977; P<0.001), maximum frequency (N=332; Z=-2.912; P<0.005), and no. of steps (N=332; Z=-2.218; P<0.05). For the eastern striped dolphins the Mann-Whitney test confirms that the whistles of offshore animals show significantly lower beginning frequency (N=92; Z=-3.587; P<0.001), maximum frequency (N=92; Z=-3.466; P<0.005), and no. of steps (N=92; Z=-4.169; P<0.001), and highlight that they present also lower final frequency (N=92; Z=-3.952; P<0.001), frequency range (N=92; Z=-3.135; P<0.005), no. of inflection points (N=92; Z=-4.477; P<0.001) and no. of contour minima (N=92; Z=-2.114; P<0.05). DFA confirms the existence of significant differences in the whistle structure of inshore and offshore animals of both basins. In the western basin the whistles are correctly assigned to different areas of depth in 65% of cases, when running the cross-validated procedure, with a percentage of correct classification significantly greater than that expected by chance (Wilks' Lamda = 0.91; F = 33.34; df = 330; P < 0.005). In detail, the whistles of inshore animals (N=91) are correctly classified in 67% of cases, while those of offshore animals (N=241) are

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correctly classified in 65% of cases. Three parameters contribute to the discriminant model of the two sub-populations: minimum frequency (0.84), no. of steps (0.46) and duration (-0.34). In the eastern basin the whistles are correctly assigned to different area of depth in 75% of cases, when running the cross-validated procedure, with a percentage of correct classification significantly greater than that expected by chance (Wilks' Lamda = 0.83; F = 24.08; df = 116; P < 0.005). More in detail, the whistles of inshore animals (N=72) are correctly classified in 72% of cases, while those of offshore animals (N=46) are correctly classified in 80% of cases. Three parameters contribute to the discriminant model of the two sub-populations: final frequency (0.77), no. of inflection points (0.74), and maximum frequency (-0.58).

3.4 Intra-species variability of the parameters in the entire basin

Average values and variance of all acoustic parameters measured are shown in Table 2. Duration and frequency parameters (beginning frequency, final frequency, minimum frequency and maximum frequency) are characterised by relatively low intra-specific coefficients of variation (CV from 23% to 47%). Parameters of frequency modulation, show higher intra-specific variability (CV from 103% to 164%) than the frequency and duration ones.

3.5 Traits variability in relation to the distance from the geographical barrier represented by the Strait of Gibraltar

We analysed the correlation between whistle parameters and distance from the Strait of Gibraltar, in order to investigate whether the Strait represents a geographical barrier for movement and exchange between populations of striped dolphins of the Atlantic Ocean and the Mediterranean Sea. Distance from Gibraltar is significantly correlated to the value of all whistle parameters, except beginning and minimum frequency (Spearman’s rho test, Table 4). The correlation is significantly positive for duration, maximum frequency, frequency range, n° of inflection points, n° of steps, n° of contour minima, n° of contour maxima and it is significantly negative for final frequency. Since the data of the distance from Gibraltar are normally distributed (Kolmogorov-Smirnov Test: N=37; Z=1.126; P=0.158) a linear regression was also ran among normally distributed whistles parameters (i.e.: duration, final frequency, minimum frequency, frequency range) and this geographical factor. Duration resulted to be the only parameter to be linearly correlated to distance from Gibraltar (P=0.040; y=0.728+7.17E-005x; $R2=0.142$).

3.6 Traits variability in relation to environmental factors such as depth and wind intensity

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To investigate whether environmental factors affect the acoustic structure of striped dolphins, we ran a series of Spearman's rho tests between the values of the whistle acoustic parameters and the values of site depth and mean yearly wind intensity of all acoustic detections. The results of the tests are shown in Table 4. In summary, site depth is significantly negatively correlated with beginning frequency, minimum and maximum frequency, no. of steps and no. of contour maxima; wind intensity shows a negative correlation with duration and a positive correlation with final frequency. Since the data of site depth and yearly mean wind intensity are normally distributed (Kolmogorov-Smirnov Test: N=31; Z=1.000; P=0.270; N=33; Z=0.912; P=0.376) a linear regression was ran among the whistles parameters that resulted normally distributed (i.e.: duration, final frequency, minimum frequency, frequency range) and these environmental factors. The only linear correlation found was the one among duration and yearly mean wind intensity (negative correlation: P=0.038; y=1.168-0.33x; R2=0.144).

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3.7 Traits variability in relation to social factors, such as group size

To investigate whether the group size (i.e. the number of animals present for each acoustic detection) affects whistle variability we compared the CVs obtained for five categories of groups with an increasing number of animals: 1) one individual, 2) 2-5 individuals, 3) 6-10 individuals, 4) 11-50 individuals, 5) >50 individuals. As shown in Table 5, the CVs do not increase with the number of animals.

To investigate whether striped dolphins modify whistle characteristics as a result of social factors, such as group size, we ran a Spearman's rho test between the values of the whistle acoustic parameters and the number of animals present in all sightings. The results of the tests are shown in Table 4. Group size is significantly negatively correlated with all parameters except final frequency, minimum frequency and no. of inflection points.

3.8 Variability of environmental and social factors for the Mediterranean dataset of this project

We analysed how the environmental and social factors varied within the basin for our Mediterranean dataset.

A Mann-Whitney test was performed to analyse the differences in environmental factors (depth, mean yearly wind intensity) and social factors (group size) between western and eastern acoustic detections. Site depth (N=599; Z=-6.406; P<0.001), mean yearly wind intensity (N=599; Z=-9.309; P<0.001) and group size (N=599; Z=-13.209; P<0.001) were significantly greater for the western detections (Table 6). The Spearman's rho test indicates that site depth (N=521; Correlation Coefficient =-0.280; P<0.001), mean yearly wind intensity (N=593; Correlation Coefficient =-0.529; P<0.001), and no. of animals (N=599; Correlation Coefficient =-0.279; P<0.001) show all a negative correlation with the distance from the Strait of Gibraltar.

A Mann-Whitney test was performed also to analyse the difference in environmental and social factors between the two regions of the western Mediterranean basin. Site depth (N=380; Z=-8.55; P<0.001), mean yearly wind intensity (N=380; Z=-9.56; P<0.001) and group size (N=380; Z=-10.89; P<0.001) were all significantly different between the two regions (Table 6). According to the Spearman's rho test all variables decrease from the western to the eastern groups, except for the number of animals, which shows its highest value in the Ligurian Provençal and Tyrrhenian Sea basin.

A Mann-Whitney test was performed to investigate whether the environmental (mean yearly wind intensity) and social factors (group size) considered for the analysis were significantly different between the inshore and the offshore acoustic detections. The results of the test showed that none of factors is significantly different between the two areas of depth.

4 DISCUSSION

In this paper we analyse how the geographic whistle variation of the Mediterranean striped dolphins (*Stenella coeruleoalba*) is the result of genetic pressure and ecological and social factors that act on different traits of the whistle structure.

With regard to geographic variations of animal sounds, which are usually divided microgeographically and macrogeographically (Mundinger 1982), these can result from various factors such as morphology, genetics, ecology, sociality and culture (Catchpole et al. 1995). For birds (Marler 1955) and other vertebrates (Klumpt and Shalter 1984, Gerhardt 1991) there is evidence that multiple types of selection pressures act on distinct traits of the acoustic signals. For cetaceans, a wide range of factors is considered to contribute to the diversification of whistles among odontocetes (Rossi-Santos and Podos 2006); nevertheless the selection pressures that act on distinct traits have not yet been investigated. Recent studies have revealed the existence of geographic variations in the whistles of different odontocete species (Rendell et al. 1999; Wang et al. 1995a,b; Bazúa-Durán 2004; Morisaka et al. 2005; Bazúa-Durán 2004) due to the reduced individual exchange, different behavioural context, different emotional state, adaptation to different habitats, adaptation to environmental noise, society structure, and group size.

This study highlights first, that whistle acoustic structure of striped dolphin varies within the Mediterranean Sea and second, that the progressive distance from the Strait of Gibraltar and the environmental parameters affect acoustic traits differently one from the other.

Concerning within basin variability, whistle acoustic structure enabled the identification of distinct sub-populations within the Mediterranean Sea, such as the eastern and western sub-populations, two sub-populations within the Mediterranean western basin and the inshore and offshore sub-populations. Concerning traits variability: duration and all frequency parameters show low variability (CVs lower than 57%), while modulation parameters present a high degree of intra-species variation (CVs higher than 88%); duration, maximum frequency, frequency range, and all the modulation parameters gradually increase, and final frequency gradually decreases, with the progressive advance into the Mediterranean Sea from the Strait of Gibraltar; 3 frequency parameters (beginning frequency, minimum frequency and maximum frequency) and 2 modulation parameters (no of steps and no of contour maxima) are negatively correlated with site depth; duration and final frequency are respectively negatively and positively correlated with wind intensity; duration, 3 modulation parameters (no of steps, no of contour minima, no of contour maxima), and 3 frequency parameters (beginning frequency, maximum frequency and frequency range) are all negatively correlated with the number of animals.

Concerning the genetic influence on whistle structure, Wang and colleagues (1995a) proposed that for bottlenose dolphins the genetic geographic isolation could lead to differences of whistles of sub-populations belonging to non-adjacent areas. If a low level of individual interchange is responsible for whistle geographical variation of striped dolphins within the Mediterranean Sea, we would expect that whistle acoustic structure is different between western and eastern basins as a consequence of the presence of the Italian Peninsula. The delimitation of the Sicily Strait, in fact, can cause the reduction of individuals rate of exchange and consequently of genetic flux between striped dolphins present in the two different basins, similarly to the effect of the Gibraltar strait for Atlantic and Mediterranean individuals. Differentiation between

Mediterranean and North Atlantic striped dolphins, based on microsatellite DNA analyses, is well documented (Garcia-Martinez et al. 1999). Recently, Gaspari and colleagues (2007) found that striped dolphins inhabiting the eastern side of the Mediterranean Sea are genetically differentiated from the western individuals, and that the two sub-populations of Spain and of the Tyrrhenian Sea are genetically distinct. The findings of this projet went in the same direction of this last genetic study, providing an important support for the hypothesis of a genetic basis of the acoustic structure of whistles. In fact significant difference are found between the whistles belonging to the western and eastern basins and to the two regions of the western basin (Alboran Sea, Spanish and Balearic waters, and Algerian-Provençal Sea, and Tyrrhenian Sea) for which genetic differences have been highlighted.

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Most of the considered whistle parameters are positively correlated with distance from Gibraltar. This gradual geographical gradient supports as well the genetic explanation, indicating that dolphins whistle differences gradually increase as striped dolphins advance into the Mediterranean Sea, diversifying themselves from the Atlantic individuals. A recent study (Papale et al. 2013) on the whistle characteristics of striped dolphins belonging to the Atlantic ocean and to the Mediterranean Sea showed significant differences among the whistles of the two populations and the possibility of correctly assign the whistle to both area in the 73% of the cases.

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Acoustic signals in which elements are correlated with measures of body size are prevalent among mammals (e.g. Clutton-Brock & Albon 1979; August & Anderson 1987; Gouzoules & Gouzoules 1990), amphibians (e.g. Davies & Halliday 1978; Robertson 1986) and birds (e.g. Barabraud et al. 2000). Body mass and body size have been suggested to impose absolute constraints on the frequency and duration parameters of the acoustic signals of 500 diverse species among insects, fishes, reptiles, amphibians, birds and mammals (Gillooly J.F. and Ophir A.G. 2010), with body mass correlating negatively with signal frequency and positively with signal duration. Mager and colleagues (2007) pointed out for birds a negative relation among the frequency parameters and the body mass and conditions. For the whistles of odontocetes Wang (1993) hypothesised a limitation of sound production capability determined by body size, and Wang and colleagues (1995b) highlighted a negative correlation among frequency parameters and body size.

If the genetic factor is at least partly responsible for whistle parameters variation, we would expect that parameters under strong morphophysiological constraint are characterised by lower intra-specific CV than other parameters (Mousseau et al 1987). The results of this study highlight that frequency parameters and duration, that are the most subject to morphophysiological constraint, are characterised by lower intra-specific and intra sub-population CVs (CV from 23% to 47%), than the parameters of frequency modulation, which may be affected by social and/or ecological factors (May-Collado 2007), and that show higher intra-specific variability (CV from 103% to 164%). These results support the hypothesis that genetic differences may contribute to the geographic variation of this whistle traits. Moreover maximum frequency and duration allow the correct assignment of whistles emitted by western and eastern striped dolphins in 64% of cases, and the whistles of eastern animals are significantly higher in frequency of the western ones. On this basis we can predict that they may belong to smaller animals and it will very interesting to be able to verify this hypothesis together with the hypothesis that they can be genetically differentiated from the western animals. In addition, the generally higher CVs shown by the western animals may be related to higher genetic variability of the animals closer to the Atlantic Ocean, with greater chance of contact with the Ocean individuals.

The differences found between the whistles of inshore and offshore animals may also be linked to a genetic effect on whistle variation. Gaspari and colleagues (2007) did not

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specify whether the genetic differences between inshore and offshore samples they found were linked to ecotypes with different morphologies. However, for bottlenose dolphins, Perrin (2002) pointed out that the western Atlantic coastal ecotype is smaller than the pelagic ecotype. In this study, the offshore animals produce whistles with significantly lower minimum and maximum frequencies than the inshore animals. These differences in whistle characteristics may be linked to the different size of the animals belonging to the two areas, with larger animals inhabiting deeper waters.

Comment [c6]: Elena mi diceva che è uscito un lavoro che dice che nella costa Per tursiope nella costa ovest la situazione è diversa. Forse dovresti aggiungere una frase che dice che però non sempre è così e altri fattori possono influire. Qualcosa del genere

Concerning the influence of environmental factors, Wang and colleagues (1995a) suggest also a relationship between higher-frequency whistling and higher level of human industrial noise. In contrast, Morisaka and colleagues (2005) found that bottlenose dolphin whistle acoustic structure diversifies in order to adapt to different acoustic environments, with a decrease in frequency and whistle modulation as a consequence of an increase in ambient noise.

If striped dolphins could adapt the characteristics of their whistles to the habitat they live in, we would expect to find a significant correlation between whistle characteristics and depth or wind intensity, which is the most significant cause of natural sea environmental noise. Our results indicate that most of the frequency parameters show a negative correlation with depth. This result is consistent with the difference between inshore and offshore animals, indicating that adaptation to this particular ecological condition (deeper waters) may have been directionally selected, leading to genetic difference between inshore and offshore individuals.

With regard to the modulation parameters, the no. of steps and the no. of contour maxima show a negative correlation with depth, indicating that whistle pattern is simplified in deeper waters. A specific behavioural study would be useful to investigate the behaviour and ecology of striped dolphins in deeper waters.

The negative correlation between duration and mean wind intensity and the positive correlation between final frequency and mean wind intensity may represent a form of adaptation to the physical environment, with animals emitting shorter whistles in a noisy environment, in order to better transmit their signals. A higher final frequency has already been hypothesised by Wang and colleagues (1995a) for animals inhabiting areas with a high level of human industrial noise.

The analysis of variability of environmental factors for the Mediterranean dataset of this project indicate that the previously identified differences between the western and eastern animals may be the result of genetic isolation acting on animals directionally selected by particular ecological conditions, such as coastal waters with lower wind intensity. Finally, we cannot exclude that the positive correlation between signal duration and distance from the Strait of Gibraltar may be a secondary effect of the linear decrease in wind intensity from west to east.

Concerning the influence of social factors, Bazua-Duran (2004) suggested that geographic differences may not occur solely due to geographic isolation, and that the difference in whistle variability of bottlenose and spinner dolphin may be linked to other factors, such as differences in the population structure of species.

If striped dolphins could adapt the characteristics of their whistles as a response to different numbers of animals present in a group, for example to avoid masking effects, we would expect to find a correlation between whistle characteristics and group size, due to different communication functions and/or context.

The statistical analyses show a negative correlation between most whistle parameters and group size, except for final frequency, minimum frequency and no. of inflection points. The whistles of animals belonging to larger groups show shorter duration, lower frequency parameters and lower whistle modulation, indicating that dolphins may produce shorter and simpler signals with lower frequency in the presence of many animals and social environmental noise.

In the present study we didn't take into account the influence of dolphin behaviour on whistle characteristics, even if the behavioural context is for sure a variable that can affect whistle variability. A focused study on this topic would be object of future researches.

Comment [c7]: Questo lo metterei nella rebuttal letter, ricordando che hai cercato d rappresentare la massima variabilità

5 CONCLUSION

More than just a single cause may explain the whistle geographical variability of the Mediterranean striped dolphin.

Genetic isolation is probably the major cause of geographic variation in the frequency parameters, which may reflect an evolutionary adaptation to particular ecological conditions of the environment or may be the by-product of an evolutionary morphological adaptation, such as a constraint in sound production related to body length. For the parameters describing sound modulation (duration, number of steps, number of minima and number of maxima) the variability may be attributable to ecological, cultural and social factors such as depth, wind intensity and number of animals.

This study shows that the analysis of the whistles of striped dolphins may be useful in understanding the population structure and dynamics of the species within the Mediterranean Sea, by highlighting differences between western and eastern animals and between inshore and offshore animals.

The ability to acoustically identifying distinct geographic sub-populations could enable their monitoring over time and provide a useful tool for managing protected species. The study of complex, stable and sympatric vocal behaviours should therefore be integrated into studies of conservation biology of acoustically active animals such as delphinids.

ACKNOWLEDGEMENTS

This project was carried out thanks to the support of IFAW, ALNITAK and GREC, which supplied the recordings and data collected during their scientific cruises.

We would like to thank Prof. Marie A. Roch for her constructive comments and the final manuscript revision and Dr Sergio Castellano and Andrea Giovannini for their valuable help. We would also like to thank Dr. Julie Oswald for her assistance in setting up the acoustic work.

REFERENCES

Archer F.I. 1997 Osteological variation in striped dolphins (*Stenella coeruleoalba*). Paper presented to the International Whaling Commission Scientific Committee SC/49/SM28 30 pp.

Au W.W.L. 1993 The sonar of dolphins. Springer-Verlag, New York, USA 277 pp.

Azevedo A.F., Van Sluys M. 2005 Whistles of tucuxi dolphins (*Sotalia fluviatilis*) in Brazil: Comparisons among populations. J Acoust Soc Am 117(3), 1456-1464

August P.V. and Anderson J.G.T. 1987 Mammal sounds and motivation-structural rules: a test of the hypothesis. Journal of Mammalogy, 68, 1-9.

Bahri-Sfar L., Lemaire C., Ben Hassine O.K., Bonhomme F. 2000 Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proc R Soc Lond B Biol Sci 267, 929–935

Barabraud C. Mariani A. Jouventin J. 2000 Variation in call properties of the snow petrel, *Pagodroma nivea*, in relation to sex and body size. Australian Journal of Zoology, 48, 421-430.

Bazúa-Durán M.C. 2004 Differences in the whistles characteristics and repertoire of the bottlenose and spinner dolphins. An Braz Acad Sci 76, 386-392

Bazúa-Durán M.C., Au W.W.L. 2002 The whistles of Hawaiian Spinner Dolphins. J Acoust Soc Am 112, 3064-3072

Bazúa-Durán M.C., Au W.W.L. 2004 Geographic variations in the whistles of spinner dolphins (*Stenella longirostris*) of the Main Hawaiian Islands. J Acoust Soc Am 116(6), 3757-3769

Bellante

Calzada N., Aguilar A. 1995 Geographical variation of body size in Western Mediterranean striped dolphins (*Stenella coeruleoalba*). Z Saugetierkd 60, 257-64

Catchpole C.K., Slater P.J.B. 1995 Bird Song, Biological Themes and Variations. Cambridge University Press, Cambridge 348 pp.

Clutton-Brock T.H. and Albon S.D. 1979 The roaring of red deer and the evolution of honest advertisement. Behaviour, 69, 145-170

Cognetti G., Sarà M., Magazzù G. 1999 Biologia marina / Marine Biology. Calderini, Bologna, Italy 596 pp.

Davies N.B. Halliday T.R. 1978 Deep croaks and fighting assessment in toads *Bufo bufo*. Nature, 274, 683-685.

Forcada J., Aguilar A., Hammond P., Pastor X., Aguilar R. 1994 Distribution and numbers of striped dolphins in the western Mediterranean after the 1990 epizootic outbreak. Mar Mamm Sci 10, 137-150

Ford J.K.B., Fisher H.D. 1982 Killer whale (*Orcinus orca*) dialects as an indicator of stocks in British Columbia. Rep Int Whal Comm 32, 671-679

García-Martinez J., Moya A., Raga J.A., Latorre A. 1999 Genetic differentiation in the striped dolphins (*Stenella coeruleoalba*) from European waters according to mitochondrial DNA (mtDNA) restriction analysis. Mol Ecol 8, 1069-1073

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Gaspari F., Azzellino A., Airoldi S., Hoelzel A.R. 2007 Social kin associations and genetic structuring of striped dolphin populations (*Stenella coeruleoalba*) in the Mediterranean Sea. Mol Ecol 16, 2922-2933

Gerhardt H.C. 1991 Female choice in treefrogs: static and dynamic acoustic criteria. Anim Behav 42, 615-635.

Gillooly J.F. and Ophir A.G. 2010 The energetic basis of acoustic communication Proc. R. Soc. B 277, 1325-1331

Gouzoules H. and Gouzoules S. 1990 Body size effects on the acoustic structure of pigtail macaque (*Macaca nemestrina*) screams. Ethology, 85, 324-334.

Guarniero I., Franzellitti S., Ungaro N., Tommasini S., Piccinetti C., Tinti F. 2002 Control region haplotype variation in the central Mediterranean common sole indicates geographical isolation and population structuring in Italian stocks. J Fish Biol 60, 1459-1474

Janik V.M. 2000 Whistle matching in wild bottlenose dolphins (*Tursiops truncatus*). Science 289, 1355-1357

Janik V.M., Slater P.J.B. 1998 Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. Anim Behav 56, 829-838

Klumpt G.M., Shalter M.D. 1984 Acoustic behaviour of birds and mammals in the predator context. I Factors affecting the structure of alarm calls. II The functional significance and evolution of alarm signals. Z Tierpsychol 66, 189–226

Lammers M.O., Au W.W.L., Herzing D.L. 2003 The broadband social acoustic signalling behaviour of spinner and spotted dolphins. J Acoust Soc Am 114, 1629-1639

Lammers M.O., Schotten M., Au W.W.L. 2006 The spatial context of whistle and click production in pods of Hawaiian spinner dolphins (*Stenella longirostris*). J Acoust Soc Am 119, 1244-1250

Loy A., Tamburelli A., Carlini R., Slice D.E. 2011. Craniometric variation of some Mediterranean and Atlantic populations of *Stenella coeruleoalba* (Mammalia, Delphinidae): a three-dimensional geometric morphometric analysis. Mar Mammal Sci 27(2), 65-78

Mager J.N., Walcott C., Piper W.H. 2007 Male common loons, *Gavia immer*, communicate body mass and condition through dominant frequencies of territorial yodels. Animal Behav 73(4), 683-690

May-Collado L.J., Agnarsson I., Wartzok D. 2007 Reexamining the relationship between body size and tonal signals frequency in whales: a comparative approach using a novel phylogeny. Mar Mammal Sci 23, 524–552

Marler P. 1955 Characteristics of some animal calls. Nature 176, 6-8

Miller A.R. 1983 The Mediterranean Sea, a physical aspects. In: Estuaries and Enclosed Seas. Ketchum B.H. Editor. Elsevier Scientific Publishing Company, Amsterdam, pp 219-238

Monaci F., Borrel A., Leonzio C., Marsili L., Calzada N. 1998 Trace elements in striped dolphins *(Stenella coeruleoalba)* from the western Mediterranean. Environ Pollut 99, 61-68

Morisaka T., Shinohara M., Nakahara F., Akamatsu T. 2005 Geographic variation in the whistles among three Indo-Pacific bottlenose dolphin *Tursiops truncatus* population in Japan. Fishery Sci 71, 568-576

Mousseau T.A., Roff D.A. 1987 Natural selection and the heritability of fitness components. Heredity 59, 181-197

Mundinger P.C. 1982 Microgeographic and macrogeographic variation in the acquired vocalizations of birds. In: Acoustic Communication in Birds. Kroodsma D.E., Miller E.H. Editors. Academic Press, New York, pp 147-208

Natoli A., Birkun A., Aguilar A., Lopez A., Hoelzel A.R. 2005 Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). Proc R Soc Lond B 272, 1217-1226

Norris K.S., Würsig B., Wells R.S., Würsig M. 1994 The Hawaiian Spinner Dolphin. University of California Press, Berkeley and Los Angeles, CA 436 pp.

Oswald J.N., Barlow J., Norris T.F. 2003 Acoustic identification of nine delphinid species in the eastern tropical pacific ocean. Mar Mammal Sci 19, 20-37

Papale E., Azzolin M., Cascao I., Gannier A., Lammers M.O., Martin V.M., Oswald J., Perez-Gil M., Prieto R., Silva M.A. Giacoma C. 2013 Geographic variability in the acoustic parameters of striped dolphin's (*Stenella coeruleoalba*) whistles J. Acoust. Soc. Am. 133 (2), 1126-1134

Perrin W.F., Würsig B., Thewissen J.G.M. 2002. Encyclopedia of Marine Mammals. Academic Press. London, UK. 1414pp.

Reeves R., Notarbartolo Di Sciara G. 2006 The status and distribution of cetaceans in the Black Sea and Mediterranean Sea. IUCN Centre for Mediterranean Cooperation, Malaga, Spain 142 pp.

Rendell L.E., Matthews J.N., Gill A., Gordon J.C.D., Macdonald D.W. 1999 Quantitative analysis of tonal calls from five odontocete species, examining interspecific and intraspecific variation. J Zool, Lond 249, 403-410

Robertson J.G.M. 1986 Male territoriality, fighting and assessment of fighting ability in the Australian frog *Uperoleia rugosa*. Animal Behaviour, 34, 763-772

Rossi-Santos M.R., Podos J. 2006 Latitudinal variation in whistle structure of the estuarine dolphin *Sotalia guianensis.* Behaviour 143, 347-364

Sayigh L.S., Tyack P.L., Wells R.S., Scott M.D., Irvine B.A. 1995 Sex difference in signature whistle production of free-ranging bottlenose dolphins, *Tursiops truncatus.* Behav Ecol Sociobiol 36, 171-177

Stafford K.M., Nieukirk S.L., Fox C.G. 2001 Geographic and seasonal variation of blue whale calls in the North pacific. J Cetacean res Manage 3, 65-76

Thompson P.O., Findley L.T., Vidal O. 1992 20 Hz pulses and other vocalizations of fin whales, *Balaenoptera physalus*, in the Gulf of California, Mexico. J Acoust Soc Am 92, 3051-3057

Wang 1993 Dolphin whistles: comparisons between populations and species. PhD dissertation. Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, P.R. China

Wang D., Würsig B., Evans W. 1995a Whistle of bottlenose dolphins: comparisons among populations. Aquatic Mammals 21(1), 65-77

Wang D., Würsig B., Evans W. 1995b Comparisons of whistles among seven odontocete species. In: Sensory Systems of Aquatic Mammals. Kastelein R.A., Thomas J.A., Nachtigall P.E. Editors. De Spil, Woerden, The Netherlands, pp 299-323

Weilgart L., Whitehead H. 1997 Group-specific dialects and geographical variation in coda repertoire in South Pacific sperm whales. Behav Ecol Sociobiol 40, 277-285

Windfinder (Offenbach Synoptic Centre), (date last viewed 18/12/2009), www.windfinder.com

Winn H.E., Thompson T.J., Cummings W.C., Hain J., Hudnall J., Hays H., Steiner W.W. 1981 Song of the humpback whale population comparisons. Behav Ecol Sociobiol 8, 41-46

TABLES

Table 1 Details of the data collection

Mediterranean Sea	**Region**	**Location**	**No. of acoustic detections**	**Period**	**Research group**	**Instrument**	**Equipment**
Western basin	Alboran Sea, Spanish and Balearic waters	Strait of Gibraltar	2	2004	IFAW	Omnidirectional hydrophone and towed array with two hydrophones	Flat response: 61 dB between 1Hz and 15 kHz, and of 63 dB between 15 and 30 kHz. Sampling rate: 48 kHz
		Alboran Sea	1				
			1	1999	GREC	Dual channel hydrophone (Magrec Ltd) towed on a 100m cable	Flat response: 62 dB from 200 Hz to 30 kHz. Sampling rate: 44,1 kHz
		Spanish Waters	5				
		Balearic Sea	1				
			1	2004	IFAW	Omnidirectional hydrophone and towed array with two hydrophones	Flat response: 61 dB between 1Hz and 15 kHz, and of 63 dB between 15 and 30 kHz. Sampling rate: 48 kHz
	Algerian-Provençal Sea, and Tyrrhenian Sea	Provençal Sea	1	1996	GREC	Dual channel hydrophone (Magrec Ltd) towed on a 100m cable	Flat response: 62 dB from 200 Hz to 30 kHz. Sampling rate: 44,1 kHz
		Ligurian Sea	5				
		Sardinian Channel	2	2004	IFAW	Omnidirectional hydrophone and towed array with two hydrophones	Flat response: 61 dB between 1Hz and 15 kHz, and of 63 dB between 15 and 30 kHz. Sampling rate: 48 kHz
		Tyrrhenian Sea	2	2003			
Eastern basin	Ionian Sea	Ionian Sea	14				
		Ionian Sea	2	1998	GREC	Dual channel hydrophone (Magrec Ltd) towed on a 100m cable	Flat response: 62 dB from 200 Hz to 30 kHz. Sampling rate: 44,1 kHz

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Table 2 Results of descriptive statistic for whistle parameters of striped dolphins belonging to the whole Mediterranean Sea, to the western and eastern basins, to the two regions of the western basin (Alboran Sea, Spanish and Balearic waters; and the Algerian-Provençal and Tyrrhenian Sea). (Key of abbreviations for parameters: Beg. Freq.=beginning frequency; Final Freq.=final frequency; Min. Freq.=minimum frequency; Max Freq.=maximum frequency; Freq. Range=frequency range; No. I. P.=number of inflection points). ***=P<0,001; **=P<0,005; *=P<0,05.

		Duration (s)	Beg. Freq. (KHz)	Final Freq. (KHz)	Min. Freq. (KHz)	Max Freq. (KHz)	Freq. Range (KHz)	No. I. P.	No. steps	No. Minima	No. Maxima
Mediterranean Sea (N=599)	Minimum	0.04	1.47	2.78	1.47	3.52	0.14	0.00	0.00	0.00	0.00
	Maximum	3.03	23.09	21.45	13.62	23.09	17.04	11.00	13.00	4.00	5.00
	Range	2.99	21.62	18.67	12.15	19.57	16.91	11.00	13.00	4.00	5.00
	Mean	**0.84**	**10.70**	**11.31**	**7.91**	**15.03**	**7.11**	**1.39**	**1.61**	**0.46**	**0.48**
	Standard Error of Mean	0.02	165.95	141.40	80.84	143.84	134.65	0.06	0.09	0.03	0.03
	CV	**47.32**	**37.96**	**30.59**	**25.00**	**23.42**	**46.34**	**103.41**	**133.84**	**164.33**	**155.39**
Western basin (N=384)	Minimum	0.036	1.468	2.779	1.468	3.52	0.135	0.00	0.00	0.00	0.00
	Maximum	2.162	22.084	21.45	13.619	22.285	17.044	7.00	9.00	4.00	3.00
	Range	2.126	20.616	18.671	12.151	18.765	16.909	7.00	9.00	4.00	3.00
	Mean	**0.75*** **	**10.33**	**11.25**	**7.74***	**14.14*** **	**6.41*** **	**1.23***	**1.33*** **	**0.39***	**0.37*** **
	Standard Error of Mean	0.02	194.00	172.40	100.96	180.26	168.93	0.06	0.10	0.03	0.03
	CV	**49.75**	**36.80**	**30.04**	**25.57**	**24.98**	**51.67**	**92.94**	**142.23**	**169.25**	**169.99**
Eastern basin (N=215)	Minimum	0.26	3.23	5.24	3.23	10.18	1.21	0.00	0.00	0.00	0.00
	Maximum	3.03	23.09	20.07	13.51	23.09	16.24	11.00	13.00	4.00	5.00
	Range	2.76	19.87	14.82	10.29	12.91	15.02	11.00	13.00	4.00	5.00
	Mean	**1.00*** **	**11.36**	**11.43**	**8.23***	**16.62*** **	**8.37*** **	**1.68***	**2.11*** **	**0.59***	**0.68*** **
	Standard Error of Mean	0.03	301.58	246.05	132.55	197.01	195.84	0.12	0.17	0.06	0.06
	CV	**39.01**	**38.94**	**31.56**	**23.62**	**17.38**	**34.29**	**108.39**	**117.76**	**151.76**	**130.81**
Alboran Sea, Spanish and Balearic waters (N=152)	Minimum	0.05	1.47	2.87	1.47	3.52	0.23	0	0	0	0
	Maximum	1.77	22.08	18.71	13.62	22.29	14.42	4	9	2	3
	Range	1.73	20.62	15.84	12.15	18.77	14.19	4	9	2	3
	Mean	**0.82*** *	**10.27**	**12.17*** **	**7.68**	**15.33*** **	**7.65*** **	**1.10**	**1.47**	**0.30**	**0.38**
	Standard Error of Mean	0.03	0.35	0.28	0.17	0.25	0.25	0.08	0.16	0.04	0.05
	CV	**40.76**	**42.32**	**27.90**	**26.86**	**19.73**	**39.97**	**88.11**	**133.66**	**173.15**	**162.24**
Algerian-Provençal and Tyrrhenian Sea (N=232)	Minimum	0.04	3.04	2.78	2.78	3.52	0.14	0	0	0	0
	Maximum	2.16	21.78	21.45	13.42	21.78	17.04	7	8	4	3
	Range	2.13	18.74	18.67	10.64	18.26	16.91	7	8	4	3
	Mean	**0.71*** *	**10.37**	**10.64*** **	**7.78**	**13.37*** **	**5.59*** **	**1.31**	**1.23**	**0.45**	**0.36**
	Standard Error of Mean	0.03	0.22	0.21	0.13	0.24	0.21	0.08	0.12	0.05	0.04
	CV	**55.47**	**32.85**	**30.40**	**24.75**	**27.16**	**57.60**	**94.17**	**148.62**	**163.03**	**175.73**

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Table 3 Results of descriptive statistic for whistle parameters of striped dolphins belonging to the inshore and offshore area, to the western inshore and offshore area, and to the eastern inshore and offshore area. (Key of abbreviations for parameters: Beg. Freq.=beginning frequency; Final Freq.=final frequency; Min. Freq.=minimum frequency; Max Freq.=maximum frequency; Freq. Range=frequency range; No. I. P.=number of inflection points). ***=P<0,001; **=P<0,005; *=P<0,05.

		Duration (s)	Beg. Freq. (KHz)	Final Freq. (KHz)	Min. Freq. (KHz)	Max Freq. (KHz)	Freq. Range (KHz)	No. I. P.	No. steps	No. Minima	No. Maxima
Inshore area (N=127)	Minimum	0.04	3.52	2.78	2.78	3.52	0.19	0.00	0.00	0.00	0.00
	Maximum	1.61	23.09	21.45	13.42	23.09	17.04	6.00	12.00	3.00	3.00
	Range	1.58	19.57	18.67	10.64	19.57	16.86	6.00	12.00	3.00	3.00
	Mean	**0.76**	**11.73****	**11.13**	**8.54*****	**15.48*****	**6.90**	**1.06***	**1.98*****	**0.33**	**0.43**
	Standard Error of Mean	0.03	0.38	0.30	0.17	0.35	0.32	0.10	0.21	0.05	0.06
	CV	**43.41**	**36.54**	**30.47**	**22.04**	**25.62**	**52.73**	**101.78**	**121.20**	**182.92**	**146.72**
Offshore area (N=269)	Minimum	0.05	1.47	2.87	1.47	3.52	0.14	0.00	0.00	0.00	0.00
	Maximum	2.16	19.92	21.38	13.62	22.18	13.71	7.00	8.00	4.00	3.00
	Range	2.12	18.45	18.51	12.15	18.66	13.58	7.00	8.00	4.00	3.00
	Mean	**0.78**	**9.56****	**11.31**	**7.33*****	**13.70*****	**6.37**	**1.36***	**1.01*****	**0.44**	**0.35**
	Standard Error of Mean	0.02	0.21	0.21	0.11	0.20	0.19	0.08	0.09	0.04	0.04
	CV	**50.27**	**36.49**	**29.94**	**25.53**	**23.46**	**49.65**	**91.13**	**150.83**	**158.92**	**179.18**
Western inshore area (N=91)	Minimum	0.04	3.52	2.78	2.78	3.52	0.19	0.00	0.00	0.00	0.00
	Maximum	1.61	21.78	21.45	13.42	21.78	17.04	6.00	8.00	3.00	3.00
	Range	1.58	18.26	18.67	10.64	18.26	16.86	6.00	8.00	3.00	3.00
	Mean	**0.68**	**11.31****	**11.48**	**8.61*****	**14.71****	**6.10**	**1.15**	**1.63***	**0.31**	**0.41**
	Standard Error of Mean	0.04	0.41	0.36	0.20	0.43	0.38	0.11	0.21	0.06	0.07
	CV	**49.06**	**34.32**	**29.85**	**22.15**	**28.05**	**59.36**	**93.99**	**125.56**	**191.92**	**163.87**
Western offshore area (N=241)	Minimum	0.05	1.47	2.87	1.47	3.52	0.14	0.00	0.00	0.00	0.00
	Maximum	2.16	19.92	21.38	13.62	21.38	13.71	7.00	8.00	4.00	3.00
	Range	2.12	18.45	18.51	12.15	17.86	13.58	7.00	8.00	4.00	3.00
	Mean	**0.76**	**9.56****	**11.19**	**7.26*****	**13.48****	**6.22**	**1.30**	**1.05***	**0.41**	**0.32**
	Standard Error of Mean	0.03	0.23	0.22	0.12	0.21	0.21	0.08	0.10	0.04	0.04
	CV	**52.09**	**36.87**	**30.48**	**26.14**	**23.87**	**51.95**	**92.52**	**151.78**	**168.02**	**183.61**
Eastern inshore area (N=36)	Minimum	0.62	4.84	5.55	4.84	12.61	2.42	0.00	0.00	0.00	0.00
	Maximum	1.54	23.09	19.97	11.50	23.09	13.61	5.00	12.00	2.00	1.00
	Range	0.92	18.25	14.42	6.66	10.49	11.19	5.00	12.00	2.00	1.00
	Mean	**0.95**	**12.79*****	**10.27*****	**8.37**	**17.42****	**8.94****	**0.83*****	**2.86*****	**0.39***	**0.47**
	Standard Error of Mean	0.04	0.85	0.53	0.31	0.45	0.47	0.18	0.49	0.11	0.08
	CV	**23.43**	**39.69**	**31.07**	**21.91**	**15.65**	**31.71**	**126.67**	**103.73**	**165.83**	**107.22**
Eastern offshore area (N=56)	Minimum	0.39	5.03	5.67	5.03	11.35	3.97	0.00	0.00	0.00	0.00
	Maximum	2.04	18.15	17.45	11.09	22.18	11.30	6.00	3.00	2.00	3.00
	Range	1.65	13.12	11.77	6.06	10.84	7.34	6.00	3.00	2.00	3.00
	Mean	**0.97**	**9.58*****	**12.38*****	**7.86**	**15.53****	**7.67****	**1.93*****	**0.75*****	**0.68***	**0.54**
	Standard Error of Mean	0.04	0.43	0.40	0.20	0.34	0.28	0.19	0.12	0.10	0.10
	CV	**32.32**	**33.39**	**24.25**	**19.25**	**16.26**	**27.59**	**75.21**	**117.21**	**105.55**	**146.61**

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Table 4 Correlation between whistle parameters and geographic, environmental and social parameters (Spearman's rho test). (Key of abbreviations for parameters: Beg. Freq.=beginning frequency; Final Freq.=final frequency; Freq. Min.=minimum frequency; Freq. Max=maximum frequency; Freq. range=frequency range; No. I. P.=number of inflection points)

		Duration	Beg. Freq.	Final Freq.	Freq. Min	Freq. Max	Freq. range	No. of I. P.	No. steps	No. contour minima	No. contour maxima
Distance from Gibraltar (Km)	Correlation Coefficient	0.184	0.038	-0.081	0.024	0.149	0.135	0.094	0.089	0.096	0.136
	Sig. (2-tailed)	0.000	0.350	0.047	0.555	0.000	0.001	0.021	0.030	0.018	0.001
	N	599	599	599	599	599	599	599	599	599	599
Depth	Correlation Coefficient	-0.028	-0.194	0.040	-0.274	-0.204	-0.052	0.061	-0.143	0.025	-0.091
	Sig. (2-tailed)	0.524	0.000	0.367	0.000	0.000	0.236	0.166	0.001	0.565	0.037
	N	521	521	521	521	521	521	521	521	521	521
Yearly mean wind intensity	Correlation Coefficient	-0.150	-0.026	0.088	0.025	-0.039	-0.060	-0.041	-0.079	-0.065	-0.061
	Sig. (2-tailed)	0.000	0.535	0.033	0.542	0.348	0.144	0.323	0.054	0.114	0.137
	N	593	593	593	593	593	593	593	593	593	593
Group size	Correlation Coefficient	-0.253	-0.128	-0.028	-0.076	-0.349	-0.314	-0.073	-0.117	-0.099	-0.174
	Sig. (2-tailed)	0.000	0.002	0.493	0.063	0.000	0.000	0.073	0.004	0.015	0.000
	N	599	599	599	599	599	599	599	599	599	599

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Table 5 CV of whistle parameters for groups of different size

	Mean CV per category of group size				
	1 individual (N=12)	2-5 individuals (N=129)	6-10 individuals (N=146)	11-50 individuals (N=279)	>50 individuals (N=33)
Duration	38.97	34.04	45.46	53.17	41.63
Beginning Freq.	40.46	36.83	36.19	37.77	37.40
Final Freq.	29.47	34.06	28.21	30.08	26.35
Minimum Freq.	17.40	25.45	21.28	26.74	21.85
Maximum Freq.	17.86	16.14	18.34	27.01	14.83
Freq. range	37.82	31.67	39.45	55.81	35.24
No. Inflection Points	80.35	116.21	97.93	97.22	79.24
No. Steps	120.00	125.78	118.46	144.76	117.52
No. Minimal Points	134.84	153.76	147.39	183.69	124.10
No. Maximal Points	134.84	137.74	125.56	188.72	137.60

Table 6 Results of descriptive statistic for environmental and social factors of western and eastern acoustic detections, and for the acoustic detections belonging to the two regions of the western basin. ***=P<0,001

		Depth (m)	Mean yearly wind intensity (Kts)	Group size
Eastern basin	N	139	215	215
	Minimum	480	7	1
	Maximum	3100	10	95
	Range	2620	3	94
	Mean	**1349.06*** **	**8.60*** **	**11.31*** **
	Standard Error of Mean	53.55	0.07	1.30
	CV	**46.80**	**11.93**	**168.21**
Western basin	N	382	378	384
	Minimum	180	7.6	1
	Maximum	2670	12	150
	Range	2490	4.4	149
	Mean	**1798.14*** **	**9.84*** **	**32.57*** **
	Standard Error of Mean	44.21	0.07	1.45
	CV	**48.05**	**13.95**	**87.21**
Alboran Sea, Spanish and Balearic waters	N	148	148	148
	Minimum	711	7.6	1
	Maximum	2670	12	85
	Range	1959	4.4	84
	Mean	**2085.77*** **	**10.59*** **	**18.24*** **
	Standard Error of Mean	50.75	0.13	1.72
	CV	29.80	14.91	116.05
Algerian-Provençal and Tyrrhenian Sea	N	232	232	232
	Minimum	180	8.8	1
	Maximum	2570	11	150
	Range	2390	2.2	149
	Mean	**1612.17*** **	**9.35*** **	**41.96*** **
	Standard Error of Mean	62.06	0.06	1.88
	CV	58.63	10.14	68.31

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FIGURES

Figure 1 Basins and sub-basins of the Mediterranean Sea. Line indicates division between western and eastern basins. Dotted lines indicate approximate divisions of the sub-basins: Alboran Sea, Algerian-Provençal Sea, Tyrrhenian Sea, Ionian Sea, Adriatic Sea

Figure 2 Depiction of the parameters manually measured for each analysed whistle