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Geographic variation of whistles of the striped dolphin (Stenella coeruleoalba) within the Mediterranean Sea

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1	Geographic variation of whistles of the striped dolphin (Stenella coeruleoalba)
2	within the Mediterranean Sea
3	
4	Marta Azzolin ¹
5	University of Torino, Life Sciences and Systems Biology Department, Via Accademia
6	Albertina 13, 10123 Torino, Italy.
7	
8	Elena Papale
9	University of Torino, Life Sciences and Systems Biology Department, Via Accademia
10	Albertina 13, 10123 Torino, Italy.
11	
12	Marc O. Lammers
13	Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI 96744, USA.
14	
15	Alexandre Gannier
16	Groupe de Recherche sur les Cétacés (GREC), Antibes, France.
17	
18	Cristina Giacoma
19	University of Torino, Life Sciences and Systems Biology Department, Via Accademia
20	Albertina 13, 10123 Torino, Italy.
21	

¹ Author to whom correspondence should be addressed. Electronic mail: tursiope.ve@libero.it

22 ABSTRACT

23

24 The striped dolphin is a cosmopolitan species distributed worldwide. Morphological and 25 genetic studies strongly suggest that the Mediterranean and eastern North Atlantic 26 populations are isolated from each other. The Mediterranean population is considered a 27 distinct conservation unit by IUCN experts, classified as "vulnerable". This study 28 describes the geographical variation of the striped dolphin whistles within the 29 Mediterranean Sea. Recordings were collected from 1996 to 2003 throughout the whole 30 basin, employing multiple platforms. Thirty seven independent sightings with acoustic 31 data collection were made, and 599 whistles were extracted and considered for 32 statistical analysis. Whistle analysis enabled the identification of sub-populations of 33 striped dolphins within the Mediterranean Sea. Their acoustic diversity reflect the 34 genetic differences recently found among striped dolphins inhabiting different 35 Mediterranean regions. The results of this study support the hypothesis that gene flow 36 reduction plays an important role in determining variation in whistle duration and 37 frequency parameters, while ecological and social factors influence parameters of the 38 modulation domains. The ability of acoustically identifying distinct geographic sub-39 populations could provide a useful tool for the management of this protected species.

40

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

44 **1 INTRODUCTION**

45

45		
46	The striped dolphin (Stenella coeruleoalba) is a cosmopolitan species quite common	
47	worldwide in tropical and temperate pelagic waters. It is the most abundant cetacean of	
48	the Mediterranean Sea where it is typically found in productive, open waters beyond the	
49	continental shelf of the Alboran Sea, the Algerian-Provençal Sea and the Ligurian Sea	
50	(Forcada et al. 1994) and decreases in abundance in the Eastern Mediterranean. Striped	
51	dolphins face significant challenges worldwide, especially in the Mediterranean Sea,	
52	due to environmental changes and human activities.	
53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70	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; <u>A. Bellante</u> , (2012); <u>Since</u> the distribution, mobility and degree of separation of striped dolphins between	
72	different Mediterranean areas is still unknown - even though morphometric and genetic	
73	variations suggest restriction in gene flow between areas - more data about the biology	
74	of the species are needed to identify the Mediterranean Sea sub-populations and an	
75	acoustic analysis is likely to be insightful in this regard. Characteristics of acoustic	
76	behaviour have been used to distinguish populations of humpback whales (Megaptera	
77	novaegliae), fin whales (Balaenoptera physalus), blue whales (Balaenoptera musculus),	
78	sperm whales (Physeter catodon), and killer whales (Orcinus orca) among others (Ford	

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80 and Fisher 1982; Stafford et al. 2001; Thompson et al. 1992; Weilgart and Whitehead 81 1997; Winn et al. 1981). Recently, there has been a growing interest in intraspecific 82 variations in the signalling structure of Delphinidae (Bazúa-Durán and Au 2004), as 83 illustrated in several studies on the bottlenose dolphin (Tursiops truncatus). Research on 84 this species has identified acoustic differences between social groups (Janik 2000), 85 localities (Wang et al. 1995a, Morisaka et al. 2005) and between males and females 86 (Sayigh et al. 1995). However, there are no published studies on geographic variation in 87 the signalling structure of the Mediterranean striped dolphin.

88 Striped dolphin acoustic signals can be classified into two main categories: tonal 89 whistles and pulsed sounds. Whistles are frequency modulated tones with durations 90 varying from less than a second to several seconds (Bazúa-Durán and Au 2002, Wang et 91 al. 1995b). In other species of the same genus, such as spinner dolphins (Stenella 92 longirostris), they are considered to be signals used to regulate group organization 93 (Norris et al. 1994, Janik and Slater 1998) and they are believed to be particularly 94 important for maintaining social cohesion within groups (Lammers et al. 2006). Pulsed 95 sounds, on the other hand, are primarily characterized by trains of short (<100 µs) 96 broadband clicks used in echolocation (Au 1993), but are also produced as "burst 97 pulses", which are believed to play a role in intra-group communication (Lammers et al. 98 2003).

99 This paper describes the geographical variation of the acoustic structure of striped 100 dolphin whistles within the Mediterranean Sea and analyses the factors that influence 101 this variability. We first analysed the differences between striped dolphins belonging to 102 the eastern and western basins. We then examined the variation between striped 103 dolphins belonging to the following two regions of the western basin: a) Alboran Sea, 104 Spanish and Balearic waters; and b) the Algerian-Provençal Sea, and Tyrrhenian Sea. 105 After that we investigated the variation between inshore and offshore striped dolphins. Formatted: Font: Italic

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.

110

111 2 MATERIALS AND METHODS

112

113 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).

121

122 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).

- 130 Sound recordings were made using a variety of recording equipment, all of which had a
- 131 flat frequency response (+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 eachsighting from the Strait of Gibraltar was measured using ARCGIS 9.0.

To attribute a depth (m) to each sighting, the navigation charts of <u>1</u>) the Italian *Istituto Idrografico* and <u>2</u>) 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.

140

141 2.3 Acoustic analysis procedures

142 Approximately 19 hours of recordings, associated with 37 acoustic detections, 21 for 143 the western and 16 for the eastern Mediterranean were examined (Table 1). A total of 144 1045 whistles were extracted for manual measurement based on two criteria: 1) the 145 signal to noise ratio needed to be sufficiently high so that timing and frequency 146 parameters could be unambiguously discerned from background noise; and 2) whistles 147 that had similar contours were discarded in order to increase the whistle variability of 148 each acoustic detection and to minimize the possibility of oversampling the whistles 149 belonging to an individual, to a group or to a specific behavioural context. Eight scalar 150 parameters were obtained for each whistle using CoolEditTM, through manual 151 measurements of the whistle contour, according to Oswald and colleagues (2003) (Fig. 152 2). These parameters included: 1) duration, 2) beginning frequency, 3) final frequency, 153 4) minimum frequency, 5) maximum frequency, 6) frequency range (calculated by 154 subtracting the values of the maximum and minimum frequency), 7) number of 155 inflection points in the frequency contour and 8) number of steps (a discontinuous 156 change in frequency). Two more parameters were also added to further describe the 157 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.

165 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<u>depth</u>. 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.

174

175 2.4 Statistical analyses

176 The following statistical methods were used to document and test the variation of 177 whistle parameters: 1) a descriptive statistic was generated to describe mean variation of 178 whistle parameters of the striped dolphin within the Mediterranean Sea and CVs were 179 calculated to evaluate the variation within parameters with strong or low 180 morphophysiological constraint; 2) a univariate non-parametric statistic (Mann-Whitney 181 test) was used to compare acoustic structure of whistles recorded in the eastern and 182 western basins, in inshore and offshore waters, in the inshore and offshore waters of 183 each basin, and in the following two regions of the western basin: a) Alboran Sea,

184 Spanish and Balearic waters; and b) Algerian-Provençal Sea, and Tyrrhenian Sea); 3) a 185 multivariate statistic (Discriminant Function Analysis - stepwise method), was used to 186 highlight the possibility of correctly classifying whistles to the eastern and western 187 animals, to the inshore and offshore animals, to the inshore and offshore animals of 188 each basin, and to the two regions considered in the western basin. For cross-validating 189 the DFA the leave-one-out procedure was applied; 4) a Spearman's Rho test was 190 performed to investigate the correlation between the considered parameters and the 191 progressive distance from the Strait of Gibraltar.

192 The variability of the environmental and social factors of the present Mediterranean 193 dataset was investigated by applying the following statistical methods: 1) a univariate 194 statistic (Mann-Whitney test) was used to compare the environmental (depth, mean 195 yearly wind intensity) and social factors (group size) recorded in the eastern and in 196 western basins of the Mediterranean Sea, in the inshore and offshore waters, and in the 197 two regions considered in the western basin; and 2) a Spearman's Rho test was 198 performed to investigate the correlation between the environmental and social factors 199 and the progressive distance from the Strait of Gibraltar.

200 Statistical analysis was carried out using the software SPSS 16.0.

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202 3 RESULTS
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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.

207 The normality of the whistle parameters considered in this study was checked by

208 applying the Kolmogorov-Smirnov Test: duration (N=599; Z=1.310; P=0.065), final

209 frequency (N=599; Z=1.121; P=0.162), minimum frequency (N=599; Z=0.900;

- 214 Maxima (N=599; Z=9.001; P=0.000) resulted not normally distributed.
- 215

216 3.1 Variability among striped dolphins belonging to the eastern and western basins 217 In order to investigate whether the Italian Peninsula represents a geographical barrier for 218 the movement and exchange of striped dolphins within the Mediterranean Sea, we 219 analysed the whistle characteristics of the distinct eastern and western striped dolphin 220 sub-populations (Table 2). The whistles of the eastern striped dolphins have lower CVs 221 for most of the parameters except for beginning frequency, final frequency and no. of 222 inflection points. The Mann-Whitney test indicates that duration (N=599; Z=-7.130; 223 P<0.001), minimum frequency (N=599; Z=-2.391; P<0.05), maximum frequency 224 (N=599; Z=-7.909; P<0.001), frequency range (N=599; Z=-7.071; P<0.001), no. of 225 inflection points (N=599; Z=-2.252; P<0.05), no. steps (N=599; Z=-3.810; P<0.001), 226 no. of contour minima (N=599; Z=-2.292; P<0.05), and no. of contour maxima (Mann-227 Whitney test N=599; Z=-4.878; P<0.001) are significantly greater for eastern animals. 228 DFA (Wilks' Lamda = 0.88; F = 77; df = 597; P < 0.005) correctly assigns whistles to 229 the area they were recorded in the 64% of cases, when running the cross-validated 230 procedure, with a percentage of correct classification significantly greater than that 231 expected by chance. The whistles of the western basin (N=384) are correctly classified 232 in 65% of cases, while those of the eastern basin (N=215) are correctly classified in 233 63% of cases. Three parameters contribute to the discriminant model of the two sub-234 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) AlgerianProvençal Sea, and Tyrrhenian Sea

239 In order to investigate the existence of an acoustic differentiation between striped 240 dolphins belonging to the two distinct regions of the western basin where data were 241 collected (Fig.1) we applied both univariate and multivariate statistics. Table 2 shows 242 the descriptive statistic for each region. The Mann-Whitney test indicates that duration 243 is significantly greater in the Alboran Sea and the Spanish and Balearic waters (N=384; 244 Z=-3.398; P<0.005), as well as final frequency (N=384; Z=-4.261; P<0.001), maximum 245 frequency (N=384; Z=-6.219; P<0.001) and frequency range (N=384; Z=-6.403; 246 P<0.001). DFA (Wilks' Lamda = 0.92; F = 30.48; df = 382; P < 0.005) correctly 247 classifies the whistles to the two regions in 68% of cases, when running the cross-248 validated procedure, with a percentage of correct classification significantly greater than 249 that expected by chance. Whistles of the Alboran Sea, Spanish and Balearic waters 250 (N=152) are correctly classified in 66% of cases, while those of the Algerian-Provencal 251 Sea, and Tyrrhenian Sea (N=232) are correctly classified in 70% of the cases. Three 252 parameters contribute to the discriminant model: maximum frequency (1.19), beginning 253 frequency (-0.60), and no. of inflection points (-0.55)

254

255 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

262 (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 263 264 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 265 266 chance. The whistles of inshore animals (N=127) are correctly classified in 65% of the 267 cases, while those of offshore animals (N=269) are correctly classified in 70% of the 268 cases. Five parameters contribute to the discriminant model of the two sub-populations: 269 minimum frequency (0.64), maximum frequency (0.57), final frequency (-0.54), no of 270 inflection points (-0.38), n° of steps (0.31). The differences between inshore and 271 offshore animals are consistent within the two basins (Table 3). The Mann-Whitney test 272 indicates that the whistles of western offshore striped dolphins show significantly lower 273 beginning frequency (N=332; Z=-3.452; P<0.005), minimum frequency (N=332; Z=-274 5.977; P<0.001), maximum frequency (N=332; Z=-2.912; P<0.005), and no. of steps 275 (N=332; Z=-2.218; P<0.05). For the eastern striped dolphins the Mann-Whitney test 276 confirms that the whistles of offshore animals show significantly lower beginning 277 frequency (N=92; Z=-3.587; P<0.001), maximum frequency (N=92; Z=-3.466; 278 P<0.005), and no. of steps (N=92; Z=-4.169; P<0.001), and highlight that they present 279 also lower final frequency (N=92; Z=-3.952; P<0.001), frequency range (N=92; Z=-280 3.135; P<0.005), no. of inflection points (N=92; Z=-4.477; P<0.001) and no. of contour 281 minima (N=92; Z=-2.114; P<0.05). DFA confirms the existence of significant 282 differences in the whistle structure of inshore and offshore animals of both basins. In the 283 western basin the whistles are correctly assigned to different areas of depth in 65% of 284 cases, when running the cross-validated procedure, with a percentage of correct 285 classification significantly greater than that expected by chance (Wilks' Lamda = 0.91; F 286 = 33.34; df = 330; P < 0.005). In detail, the whistles of inshore animals (N=91) are 287 correctly classified in 67% of cases, while those of offshore animals (N=241) are

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290 correctly classified in 65% of cases. Three parameters contribute to the discriminant 291 model of the two sub-populations: minimum frequency (0.84), no. of steps (0.46) and 292 duration (-0.34). In the eastern basin the whistles are correctly assigned to different area 293 of depth in 75% of cases, when running the cross-validated procedure, with a 294 percentage of correct classification significantly greater than that expected by chance 295 (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 296 297 animals (N=46) are correctly classified in 80% of cases. Three parameters contribute to 298 the discriminant model of the two sub-populations: final frequency (0.77), no. of 299 inflection points (0.74), and maximum frequency (-0.58).

300

301 *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.

308

309 3.5 Traits variability in relation to the distance from the geographical barrier
310 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

316	rho test, Table 4). The correlation is significantly positive for duration, maximum
317	frequency, frequency range, n° of inflection points, n° of steps, n° of contour minima,
318	n° of contour maxima and it is significantly negative for final frequency. Since the data
319	of the distance from Gibraltar are normally distributed (Kolmogorov-Smirnov Test:
320	N=37; Z=1.126; P=0.158) a linear regression was also ran among normally distributed
321	whistles parameters (i.e.: duration, final frequency, minimum frequency, frequency
322	range) and this geographical factor. Duration resulted to be the only parameter to be
323	linearly correlated to distance from Gibraltar (P=0.040; y=0.728+7.17E-005x;
324	R2=0.142).
325	

326 3.6 Traits variability in relation to environmental factors such as depth and wind327 intensity

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Comment [MA4]: Il referee mi aveva chiesto di aggiungere, ma forse lo lascerei solo nella rebuttal letter? Va bene anche qua, io lo lascerei 331 To investigate whether environmental factors affect the acoustic structure of striped 332 dolphins, we ran a series of Spearman's rho tests between the values of the whistle 333 acoustic parameters and the values of site depth and mean yearly wind intensity of all 334 acoustic detections. The results of the tests are shown in Table 4. In summary, site 335 depth is significantly negatively correlated with beginning frequency, minimum and 336 maximum frequency, no. of steps and no. of contour maxima; wind intensity shows a 337 negative correlation with duration and a positive correlation with final frequency. Since 338 the data of site depth and yearly mean wind intensity are normally distributed 339 (Kolmogorov-Smirnov Test: N=31; Z=1.000; P=0.270; N=33; Z=0.912; P=0.376) a 340 linear regression was ran among the whistles parameters that resulted normally 341 distributed (i.e.: duration, final frequency, minimum frequency, frequency range) and 342 these environmental factors. The only linear correlation found was the one among 343 duration and yearly mean wind intensity (negative correlation: P=0.038; y=1.168-0.33x;

344

R2=0.144).

345

346 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 **Comment [MA5]:** Il referee mi aveva chiesto di aggiungere, ma forse lo lascerei solo nella rebuttal letter? Va bene qui ma forse toglierei dai materiali e metodi a questo punto 356 correlated with all parameters except final frequency, minimum frequency and no. of357 inflection points.

358

359 3.8 Variability of environmental and social factors for the Mediterranean dataset of this
360 project

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

363 A Mann-Whitney test was performed to analyse the differences in environmental factors 364 (depth, mean yearly wind intensity) and social factors (group size) between western and 365 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) 366 367 were significantly greater for the western detections (Table 6). The Spearman's rho test 368 indicates that site depth (N=521; Correlation Coefficient =-0.280; P<0.001), mean 369 yearly wind intensity (N=593; Correlation Coefficient =-0.529; P<0.001), and no. of 370 animals (N=599; Correlation Coefficient =-0.279; P<0.001) show all a negative 371 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 twoareas of depth.

384

385 4 DISCUSSION

386

387 In this paper we analyse how the geographic whistle variation of the Mediterranean 388 striped dolphins (*Stenella coeruleoalba*) is the result of genetic pressure and ecological 389 and social factors that act on different traits of the whistle structure.

390 With regard to geographic variations of animal sounds, which are usually divided 391 microgeographically and macrogeographically (Mundinger 1982), these can result from 392 various factors such as morphology, genetics, ecology, sociality and culture (Catchpole 393 et al. 1995). For birds (Marler 1955) and other vertebrates (Klumpt and Shalter 1984, 394 Gerhardt 1991) there is evidence that multiple types of selection pressures act on 395 distinct traits of the acoustic signals. For cetaceans, a wide range of factors is considered 396 to contribute to the diversification of whistles among odontocetes (Rossi-Santos and 397 Podos 2006); nevertheless the selection pressures that act on distinct traits have not yet 398 been investigated. Recent studies have revealed the existence of geographic variations 399 in the whistles of different odontocete species (Rendell et al. 1999; Wang et al. 1995a,b; 400 Bazúa-Durán 2004; Morisaka et al. 2005; Bazúa-Durán 2004) due to the reduced 401 individual exchange, different behavioural context, different emotional state, adaptation 402 to different habitats, adaptation to environmental noise, society structure, and group 403 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. 408 Concerning within basin variability, whistle acoustic structure enabled the identification 409 of distinct sub-populations within the Mediterranean Sea, such as the eastern and 410 western sub-populations, two sub-populations within the Mediterranean western basin 411 and the inshore and offshore sub-populations. Concerning traits variability: duration and 412 all frequency parameters show low variability (CVs lower than 57%), while modulation 413 parameters present a high degree of intra-species variation (CVs higher than 88%); 414 duration, maximum frequency, frequency range, and all the modulation parameters 415 gradually increase, and final frequency gradually decreases, with the progressive 416 advance into the Mediterranean Sea from the Strait of Gibraltar; 3 frequency parameters 417 (beginning frequency, minimum frequency and maximum frequency) and 2 modulation 418 parameters (no of steps and no of contour maxima) are negatively correlated with site 419 depth; duration and final frequency are respectively negatively and positively correlated 420 with wind intensity; duration, 3 modulation parameters (no of steps, no of contour 421 minima, no of contour maxima), and 3 frequency parameters (beginning frequency, 422 maximum frequency and frequency range) are all negatively correlated with the number 423 of animals.

424 Concerning the genetic influence on whistle structure, Wang and colleagues (1995a) 425 proposed that for bottlenose dolphins the genetic geographic isolation could lead to 426 differences of whistles of sub-populations belonging to non-adjacent areas. If a low 427 level of individual interchange is responsible for whistle geographical variation of 428 striped dolphins within the Mediterranean Sea, we would expect that whistle acoustic 429 structure is different between western and eastern basins as a consequence of the 430 presence of the Italian Peninsula. The delimitation of the Sicily Strait, in fact, can cause 431 the reduction of individuals rate of exchange and consequently of genetic flux between 432 striped dolphins present in the two different basins, similarly to the effect of the 433 Gibraltar strait for Atlantic and Mediterranean individuals. Differentiation between 434 Mediterranean and North Atlantic striped dolphins, based on microsatellite DNA 435 analyses, is well documented (Garcia-Martinez et al. 1999). Recently, Gaspari and 436 colleagues (2007) found that striped dolphins inhabiting the eastern side of the 437 Mediterranean Sea are genetically differentiated from the western individuals, and that 438 the two sub-populations of Spain and of the Tyrrhenian Sea are genetically distinct. The 439 findings of this projet went in the same direction of this last genetic study, providing an 440 important support for the hypothesis of a genetic basis of the acoustic structure of 441 whistles. In fact significant difference are found between the whistles belonging to the 442 western and eastern basins and to the two regions of the western basin (Alboran Sea, 443 Spanish and Balearic waters, and Algerian-Provençal Sea, and Tyrrhenian Sea) for 444 which genetic differences have been highlighted.

445 Most of the considered whistle parameters are positively correlated with distance from 446 Gibraltar. This gradual geographical gradient supports as well the genetic explanation, 447 indicating that dolphins whistle differences gradually increase as striped dolphins 448 advance into the Mediterranean Sea, diversifying themselves from the Atlantic 449 individuals. A recent study (Papale et al. 2013) on the whistle characteristics of striped 450 dolphins belonging to the Atlantic ocean and to the Mediterranean Sea showed 451 significant differences among the whistles of the two populations and the possibility of 452 correctly assign the whistle to both area in the 73% of the cases.

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 Deleted: go

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465 correlating negatively with signal frequency and positively with signal duration. Mager 466 and colleagues (2007) pointed out for birds a negative relation among the frequency 467 parameters and the body mass and conditions. For the whistles of odontocetes Wang 468 (1993) hypothesised a limitation of sound production capability determined by body 469 size, and Wang and colleagues (1995b) highlighted a negative correlation among 470 frequency parameters and body size.

471 If the genetic factor is at least partly responsible for whistle parameters variation, we 472 would expect that parameters under strong morphophysiological constraint are 473 characterised by lower intra-specific CV than other parameters (Mousseau et al 1987). 474 The results of this study highlight that frequency parameters and duration, that are the 475 most subject to morphophysiological constraint, are characterised by lower intra-476 specific and intra sub-population CVs (CV from 23% to 47%), than the parameters of 477 frequency modulation, which may be affected by social and/or ecological factors (May-478 Collado 2007), and that show higher intra-specific variability (CV from 103% to 479 164%). These results support the hypothesis that genetic differences may contribute to 480 the geographic variation of this whistle traits. Moreover maximum frequency and 481 duration allow the correct assignment of whistles emitted by western and eastern striped 482 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 483 484 smaller animals and it will very interesting to be able to verify this hypothesis together 485 with the hypothesis that they can be genetically differentiated from the western animals. 486 In addition, the generally higher CVs shown by the western animals may be related to 487 higher genetic variability of the animals closer to the Atlantic Ocean, with greater 488 chance of contact with the Ocean individuals.

489 The differences found between the whistles of inshore and offshore animals may also be 490 linked to a genetic effect on whistle variation. Gaspari and colleagues (2007) did not Deleted: may be

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493 specify whether the genetic differences between inshore and offshore samples they 494 found were linked to ecotypes with different morphologies. However, for bottlenose 495 dolphins, Perrin (2002) pointed out that the western Atlantic coastal ecotype is smaller 496 than the pelagic ecotype. In this study, the offshore animals produce whistles with 497 significantly lower minimum and maximum frequencies than the inshore animals. These 498 differences in whistle characteristics may be linked to the different size of the animals 499 belonging to the two areas, with larger animals inhabiting deeper waters.

500 Concerning the influence of environmental factors, Wang and colleagues (1995a) 501 suggest also a relationship between higher-frequency whistling and higher level of 502 human industrial noise. In contrast, Morisaka and colleagues (2005) found that 503 bottlenose dolphin whistle acoustic structure diversifies in order to adapt to different 504 acoustic environments, with a decrease in frequency and whistle modulation as a 505 consequence of an increase in ambient noise.

506 If striped dolphins could adapt the characteristics of their whistles to the habitat they 507 live in, we would expect to find a significant correlation between whistle characteristics 508 and depth or wind intensity, which is the most significant cause of natural sea 509 environmental noise. Our results indicate that most of the frequency parameters show a 510 negative correlation with depth. This result is consistent with the difference between 511 inshore and offshore animals, indicating that adaptation to this particular ecological 512 condition (deeper waters) may have been directionally selected, leading to genetic 513 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. **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 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.

531 Concerning the influence of social factors, Bazua-Duran (2004) suggested that 532 geographic differences may not occur solely due to geographic isolation, and that the 533 difference in whistle variability of bottlenose and spinner dolphin may be linked to 534 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 manyanimals 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.

549

550 5 CONCLUSION

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

553 Genetic isolation is probably the major cause of geographic variation in the frequency 554 parameters, which may reflect an evolutionary adaptation to particular ecological 555 conditions of the environment or may be the by-product of an evolutionary 556 morphological adaptation, such as a constraint in sound production related to body 557 length. For the parameters describing sound modulation (duration, number of steps, 558 number of minima and number of maxima) the variability may be attributable to 559 ecological, cultural and social factors such as depth, wind intensity and number of 560 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 **Comment [c7]:** Questo lo metterei nella rebuttal letter, ricordando che hai cercato d rappresentare la massima variabilità

integrated into studies of conservation biology of acoustically active animals such asdelphinids.

570

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719 720 721 TABLES

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Table 1 Details of the data collection

Mediterrane an Sea	Region	Location	No. of acou stic detec tions	Period	Rese arch grou P	Instrument	Equipment
		Strait of Gibraltar	2			Omnidirectional	Flat response: 61 dB between 1Hz and
		Alboran Sea	1	2004	IFA W	hydrophone and towed array with two hydrophones	15 kHz, and of 63 dB between 15 and 30 kHz. Sampling rate: 48 kHz
	Alboran Sea,		1				
	Spanish and Balearic	Spanish Waters	5	1999	GRE C	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
	waters		1				
Western basin		Balearic Sea	1	2004	IFA W	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	1	1996	GRE C	Dual channel hydrophone (Magrec Ltd) towed on a	Flat response: 62 dB from 200 Hz to 30 kHz. Sampling rate: 44,1 kHz
	Provençal Sea, and	Ligurian Sea	urian Sea 5			100m cable	
	Tyrrhenian Sea	Sardinian Channel	2	2 2004			
		Tyrrhenian Sea	2		IFA W	Omnidirectional hydrophone and towed array with two	Flat response: 61 dB between 1Hz and 15 kHz, and of 63 dB between 15 and 30 kHz.
		Ionian Sea	14	2003		hydrophones	Sampling rate: 48 kHz
Eastern basin	Ionian Sea	Ionian Sea	2	1998	GRE C	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
723	3						

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

731 732

***=P<0,001; **=P<0,005; *=P<0,05.

132			Beg.	Final	Min.	Max	Ener	1			
.		Duration (s)	Бед. Freq. (KHz)	Final Freq. (KHz)	Freq. (KHz)	Freq. (KHz)	Freq. Range (KHz)	No. I. P.	No. steps	No. Minima	No. Maxima
	Minimum	0.04	1.47	2.78	1.47	3.52	0.14	0.00	0.00	0.00	0.00
1	Maximum	3.03	23.09	21.45	13.62	23.09	17.04	11.00	13.00	4.00	5.00
Ι	Range	2.99	21.62	18.67	12.15	19.57	16.91	11.00	13.00	4.00	5.00
Mediterranean Sea (N=599)	Mean	0.84	10.70	11.31	7.91	15.03	7.11	1.39	1.61	0.46	0.48
Sea (N=599)	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
I	Minimum	0.036	1.468	2.779	1.468	3.52	0.135	0.00	0.00	0.00	0.00
I	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
Western basin (N=384)	Mean	0.75***	10.33	11.25	7.74*	14.14***	6.41***	1.23*	1.33***	0.39*	0.37***
(11-30-1)	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
	Minimum	0.26	3.23	5.24	3.23	10.18	1.21	0.00	0.00	0.00	0.00
I	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
Eastern basin (N=215)	Mean	1.00***	11.36	11.43	8.23*	16.62***	8.37***	1.68*	2.11***	0.59*	0.68***
(11-215)	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
	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
Alboran Sea,	Range	1.73	20.62	15.84	12.15	18.77	14.19	4	9	2	3
Spanish and Balearic waters	Mean	0.82**	10.27	12.17***	7.68	15.33***	7.65***	1.10	1.47	0.30	0.38
(N=152)	Standard Error of Mean	0.03	0.35	0.28	0.17	0.25	0.25	0.08	0.16	0.04	0.05
I	CV	40.76	42.32	27.90	26.86	19.73	39.97	88.11	133.66	173.15	162.24
	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
Algerian-	Range	2.13	18.74	18.67	10.64	18.26	16.91	7	8	4	3
Provençal and	Mean	0.71 **	10.37	10.64***	7.78	13.37***	5.59***	1.31	° 1.23	0.45	0.36
Tyrrhenian Sea (N=232)	Standard	0./1**	10.37	10.04	1.10	13.37 ***	5.59	1.31	1.43	0.45	0.30
(11-434)	Error of Mean	0.03	0.22	0.21	0.13	0.24	0.21	0.08	0.12	0.05	0.04
733	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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735	Table 3 Results of descriptive statistic for whistle parameters of striped dolphins
736	belonging to the inshore and offshore area, to the western inshore and offshore
737	area, and to the eastern inshore and offshore area. (Key of abbreviations for
738	parameters: Beg. Freq.=beginning frequency; Final Freq.=final frequency; Min.
739	Freq.=minimum frequency; Max Freq.=maximum frequency; Freq.
740	Range=frequency range; No. I. P.=number of inflection points).
741	***=P<0,001; **=P<0,005; *=P<0,05.
742	
743	Beg. Final Min. Max Freq

•		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
	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
Inshore	Range	1.58	19.57	18.67	10.64	19.57	16.86	6.00	12.00	3.00	3.00
area	Mean	0.76	11.73**	11.13	8.54***	15.48***	6.90	1.06*	1.98***	0.33	0.43
(N=127)	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
	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
Offshore	Range	2.12	18.45	18.51	12.15	18.66	13.58	7.00	8.00	4.00	3.00
area (N=269)	Mean	0.78	9.56**	11.31	7.33***	13.70***	6.37	1.36*	1.01***	0.44	0.35
(h=209)	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
	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
Western inshore	Range	1.58	18.26	18.67	10.64	18.26	16.86	6.00	8.00	3.00	3.00
area	Mean	0.68	11.31**	11.48	8.61***	14.71**	6.10	1.15	1.63*	0.31	0.41
(N=91)	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
	Minimum	0.05	1.47	2.87	1.47	3.52	0.14	0.00	0.00	0.00	0.00
Western	Maximum	2.16	19.92	21.38	13.62	21.38	13.71	7.00	8.00	4.00	3.00
offshore	Range	2.12	18.45	18.51	12.15	17.86	13.58	7.00	8.00	4.00	3.00
area (N=241)	Mean	0.76	9.56**	11.19	7.26***	13.48**	6.22	1.30	1.05*	0.41	0.32
(1)-241)	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
	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
Eastern inshore	Range	0.92	18.25	14.42	6.66	10.49	11.19	5.00	12.00	2.00	1.00
area	Mean	0.95	12.79***	10.27***	8.37	17.42**	8.94**	0.83***	2.86***	0.39*	0.47
(N=36)	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
	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
Eastern offshore	Range	1.65	13.12	11.77	6.06	10.84	7.34	6.00	3.00	2.00	3.00
area	Mean	0.97	9.58***	12.38***	7.86	15.53**	7.67**	1.93***	0.75***	0.68*	0.54
(N=56)	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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746 Table 4 Correlation between whistle parameters and geographic, environmental

747 and social parameters (Spearman's rho test). (Key of abbreviations for

parameters: Beg. Freq.=beginning frequency; Final Freq.=final frequency; Freq. 748 749

Min.=minimum frequency; Freq. Max=maximum frequency; Freq.

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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	Correlation Coefficient	0.184	0.038	-0.081	0.024	0.149	0.135	0.094	0.089	0.096	0.136
Gibraltar	Sig. (2-tailed)	0.000	0.350	0.047	0.555	0.000	0.001	0.021	0.030	0.018	0.001
(Km)	N	599	599	599	599	599	599	599	599	599	599
	Correlation Coefficient	-0.028	-0.194	0.040	-0.274	-0.204	-0.052	0.061	-0.143	0.025	-0.091
Depth	Sig. (2-tailed)	0.524	0.000	0.367	0.000	0.000	0.236	0.166	0.001	0.565	0.037
	Ν	521	521	521	521	521	521	521	521	521	521
Yearly mean	Correlation Coefficient	-0.150	-0.026	0.088	0.025	-0.039	-0.060	-0.041	-0.079	-0.065	-0.061
wind	Sig. (2-tailed)	0.000	0.535	0.033	0.542	0.348	0.144	0.323	0.054	0.114	0.137
intensity	Ν	593	593	593	593	593	593	593	593	593	593
Group	Correlation Coefficient	-0.253	-0.128	-0.028	-0.076	-0.349	-0.314	-0.073	-0.117	-0.099	-0.174
size	Sig. (2-tailed)	0.000	0.002	0.493	0.063	0.000	0.000	0.073	0.004	0.015	0.000
Ì	N	599	599	599	599	599	599	599	599	599	599

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	Mean CV per category of group size							
	1 2-5 6-10 11-50 >50							
	individual	individuals	individuals	individuals	individuals			
	(N=12)	(N=129)	(N=146)	(N=279)	(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 5 CV of whistle parameters for groups of different size

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	Formatted Table
	Ν	139	215	215	
İ	Minimum	480	7	1	
ĺ	Maximum	3100	10	95	
Eastern basin	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	
	Ν	382	378	384	
	Minimum	180	7.6	1	
	Maximum	2670	12	150	
Western basin	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	
	Ν	148	148	148	
	Minimum	711	7.6	1	
Alboran Sea, Spanish	Maximum	2670	12	85	
and Balearic waters	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	
	N	232	232	232	
	Minimum	180	8.8	1	
Algerian-Provençal and	Maximum	2570	11	150	
Tyrrhenian Sea	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	

765 766 FIGURES

767 Figure 1 Basins and sub-basins of the Mediterranean Sea. Line indicates division

- between western and eastern basins. Dotted lines indicate approximate divisions of 768
- 769 the sub-basins: Alboran Sea, Algerian-Provençal Sea, Tyrrhenian Sea, Ionian Sea,
- 770 **Adriatic Sea**
- 771
- Figure 2 Depiction of the parameters manually measured for each analysed whistle 772