

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Geographic variability in the acoustic parameters of striped dolphin's (*Stenella coeruleoalba*) whistles.**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/129351> since

*Published version:*

DOI:10.1121/1.4774274

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

E. Papale; M. Azzolin; I. Cascão; A. Gannier; M.O. Lammers; V.M. Martin; J. Oswald; M. Perez-Gil; R. Prieto; M.A. Silva; C. Giacoma. Geographic variability in the acoustic parameters of striped dolphin's (*Stenella coeruleoalba*) whistles.. THE JOURNAL OF THE ACOUSTICAL SOCIETY OF AMERICA. 133 (2) pp: 1126-1134.  
DOI: 10.1121/1.4774274

The publisher's version is available at:

<http://scitation.aip.org/content/asa/journal/jasa/133/2/10.1121/1.4774274>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/129351>



25 **Julie Oswald**

26 Bio-Waves, Inc., 517 Cornish Drive, Encinitas, California 92024

27

28 **Monica Perez-Gil**

29 Society for the Study of Cetaceans in the Canary Archipelago (SECAC). Edif. Antiguo Varadero 1<sup>a</sup>

30 planta Local 8 B, Urb. Puerto Calero, 35571 Yaiza. Isla de Lanzarote.

31

32 **Rui Prieto**

33 Centro do Instituto do Mar (IMAR) da Universidade dos Açores, Departamento de Oceanografia e

34 Pescas, Rua Prof. Doutor Frederico Machado, 9901-862 Horta, Portugal.

35

36 **Monica A. Silva**

37 Centro do Instituto do Mar (IMAR) da Universidade dos Açores, Departamento de Oceanografia e

38 Pescas, Rua Prof. Doutor Frederico Machado, 9901-862 Horta, Portugal.

39 Biology Department, Woods Hole Oceanographic Institution, MS#33, Woods Hole, MA 02543, USA.

40

41 **Cristina Giacoma**

42 University of Torino, Life Sciences and Systems Biology Department, Via Accademia Albertina 13,

43 10123 Torino, Italy.

44

45

46

47

48

49

50

51 **ABSTRACT**

52

53 Geographic variation in the acoustic features of whistles emitted by the striped dolphin (*Stenella*  
54 *coeruleoalba*) from the Atlantic Ocean (Azores and Canary Islands) and the Mediterranean was  
55 investigated. Ten parameters (signal duration, beginning, end, minimum and maximum frequency, the  
56 number of inflection points, of steps, of minima and maxima in the contour and the frequency range)  
57 were extracted from each whistle. Discriminant Function Analysis correctly classified 73% of sounds  
58 between Atlantic Ocean and Mediterranean Sea. A cline in parameters was apparent from the Azores  
59 to the Mediterranean, with a major difference between the Canaries and the Mediterranean than  
60 between Azores and Canaries. Signal duration, maximum frequency and frequency range measured in  
61 the Mediterranean sample were significantly lower compared to those measured in the Atlantic.  
62 Modulation parameters played a considerable role in area discrimination and were the only parameters  
63 contributing to highlight the differences within the Atlantic Ocean. Results suggest that the acoustic  
64 features constrained by structural phenotype, such as whistle's frequency parameters, have a major  
65 effect on the Atlantic and Mediterranean separation while behavioural context, social and physical  
66 environment may be among the main factors contributing to local distinctiveness of Atlantic areas.  
67 These results have potential passive acoustic monitoring applications.

68

69

70 **KEY WORDS:** whistles, geographic variation, striped dolphin, Atlantic Ocean, Mediterranean Sea

71

72

73

74

75

76

77 **I. INTRODUCTION**

78

79 Geographic variation in the acoustic features of signals of a species have been investigated for several  
80 delphinids, including *Tursiops* sp. (May-Collado and Wartzok 2008, Morisaka et al. 2005, Hawkins et  
81 al. 2010, Baron et al. 2008, Jones and Sayigh 2002, Wang et al. 1995), *Stenella coeruleoalba* (Azzolin  
82 2008), *Stenella frontalis* (Baron et al. 2008), *Stenella longirostris* (Bazua-Duran et al. 2004),  
83 *Delphinus delphis* (Petrella 2009, Griffiths 2009), *Orcinus orca* (Ford et al. 1983), *Sotalia guianensis*  
84 (Rossi-Santos and Podos 2006), *Pseudorca crassidens*, *Grampus griseus*, *Globicephala melas* and  
85 *Globicephala macrorhynchus* (Rendell et al. 1999). Geographic variation in signal acoustic structure  
86 results from the combination of genetic and environmental characteristics, both physical and social.  
87 Moreover, acoustic parameters are under morpho-physiological constraints and different selective  
88 pressures (Gerhardt 1991). Isolation due to geographic or cultural factors can lead to acoustic  
89 diversification (Janik and Slater 2000). The role of geographic distance in acoustic differentiation has  
90 already been assessed in species distributed in continuous basins or in contiguous areas. Wang and  
91 colleagues (1995) reported a continuous variation for bottlenose dolphins with a change in acoustic  
92 parameters between the oceans, whereas Rossi-Santos and Podos (2006) found differences in an  
93 apparently continuous geographic distribution of *Sotalia fluviatilis* in Brazil. For the Mediterranean  
94 striped dolphins, Azzolin (2008) highlighted that advancing in the Mediterranean Sea from the  
95 Gibraltar Strait most of the whistle parameters gradually change.

96 The species analyzed in this study, the striped dolphin (*Stenella coeruleoalba*, Meyen, 1833), is a  
97 cosmopolitan species and occurs in tropical, sub-tropical and temperate pelagic waters (Folkens and  
98 Reeves 2002). The species is abundant in the Atlantic and considered the most common species in the  
99 Mediterranean (Forcada et al 1994 and Panigada et al 2011). Striped dolphins are sometimes observed,  
100 both in Mediterranean Sea and in the Atlantic Ocean, in mixed groups with common dolphin, Risso's  
101 dolphin and other species of the genus *Stenella*. The species is gregarious with pods varying in size

102 between a few and over 1000 individuals with average school sizes ranging between 100-500  
103 individuals.

104 Striped dolphins are classified as ‘lower risk’ but ‘conservation dependent’ at the oceanic level by  
105 IUCN experts (Cetacean Specialist Group 1996), and in the Mediterranean Sea they are considered  
106 “Vulnerable” (Reeves and Notarbartolo di Sciara 2006).

107 Significant genetic differentiation was detected between the Mediterranean and the Atlantic  
108 populations using five polymorphic microsatellite loci (Bourret et al 2007). No haplotype was shared  
109 between Mediterranean and Atlantic areas, indicating the existence of two different populations. A  
110 very limited amount of gene flow across the Strait of Gibraltar is hypothesised (Garcia-Martinez et al.  
111 1999). Moreover, skull dimensions of Mediterranean striped dolphins are smaller than their  
112 counterparts in the Eastern North Atlantic (Archer 2002). To date, geographic variation in striped  
113 dolphin acoustic behaviour has been studied only in the Ligurian Sea (Gitter 2009) and in the  
114 Mediterranean (Azzolin 2008).

115 In this study we evaluate for the first time if whistles produced by *Stenella coeruleoalba* across the  
116 Mediterranean Sea are different from the eastern North Atlantic Ocean. We then estimate patterns of  
117 geographic variability in the acoustic features, comparing the acoustic structure of whistles from two  
118 archipelagos belonging to Macaronesia (the Azores and the Canaries) in relation to the Mediterranean.

119

## 120 **II. MATERIALS AND METHODS**

121

### 122 *Study area*

123 Recordings were collected from two macro-geographic areas: the Mediterranean Sea and the Atlantic  
124 Ocean (Fig. 1). From the Atlantic, sound recordings from two of the five archipelagos of the  
125 Macaronesian area were analyzed (the Azores Islands and the Canary Islands). Both archipelagos are  
126 located in the North Atlantic Ocean and are geological hot spots of the Mid-Atlantic ridge. They are  
127 separated by a distance of about 1200 km. The three areas considered for the analysis are:

128 1) The Azores, located approximately 1500 km west of Portugal between 36° and 40° latitude North  
129 and 24° and 32° longitude West, composed of nine islands divided into 3 subgroups (western, central  
130 and eastern), extending about 600 km along a northwest-southeast axis. The seabed around the islands  
131 is deep (ca. 1500 m at 3.7 km) with numerous scattered seamounts (Santos et al. 1995, Morato et al.  
132 2008).

133 2) The Canary Archipelago, located between 27° and 30° North and 13° and 19° West, made up of  
134 seven main islands located 115 km away from the African coast. The archipelago extends north to  
135 south approximately 500 km and the bathymetry is characterized by steep island slopes with depths  
136 reaching 1000m only 1.8 km from the coast (Canales et al 1998).

137 3) The Mediterranean Sea which is a basin connected with the Atlantic Ocean by the Strait of  
138 Gibraltar. Two main sub-basins make up the Mediterranean: the Eastern and Western basins, separated  
139 by the Sicily Strait (Astraldi et al 1999). The bathymetry is deeper in the eastern, or Levantine, basin  
140 where the Hellenic trench reaches 5093 m depth, while in the western basin the deepest area is found  
141 in the Tyrrhenian Sea, around 3800 m.

142

#### 143 *Data collection*

144 Four research groups participated in the data collection for this study: IMAR-DOP/UAç (Centre of  
145 Institute of Marine Research, Department of Oceanography and Fisheries, University of the Azores,  
146 Portugal), SECAC (Society for the study of Cetaceans in the Canary Archipelago, Spain), IFAW  
147 (International Fund for Animal Welfare, United Kingdom) and GREC (Research Group about  
148 Cetaceans, France) (Table I). GREC used a mono towed hydrophone with *Benthos* AQ4 in 1999 and a  
149 stereo towed with the same hydrophone elements the other years with a flat response of  $\pm 2$  dB from  
150 200Hz to 30 kHz, a 29 dB pre-amplifier and 200Hz high-pass filter. An external high-pass filter unit  
151 (Magrec Ltd) set to 1kHz was used on the hydrophone output to improve the quality of recording.

152 IMAR-DOP/UAç and IFAW recorded with either an omnidirectional hydrophone (*HTI-94-SSQ*) or a  
153 towed array with 2 hydrophones (*Benthos* AQ4 with a linear flat response of  $\pm 1$  dB between 1 Hz and



154 15 kHz, and of  $\pm 3$  dB between 15 kHz and 30 kHz. Recordings were made with a digital tape recorder  
155 *Tascam* ®*DA-PI*, with a sampling frequency of 48 kHz, 16 bits resolution and frequency responses  
156 from 20 Hz to 20 kHz  $\pm 0.5$  dB) and SECAC utilized a towed array with 4 elements: 2 hydrophones  
157 *Benthos* AQ4 and 2 spherical ceramic hydrophone elements with a frequency response of ~2-150 kHz  
158 (Seiche UK Ltd). Sounds were digitalized at a sampling rate of 48 kHz by IMAR-DOP/UAç, IFAW  
159 and GREC, and 192 kHz by SECAC. No recording off scale was considered in the analysis.

160 Data from the Mediterranean (Alboran Sea, Balearic and Spain, Corso-ligure-provençal basin,  
161 Tyrrhenian, Ionian Sea) were collected in 1996, 1998 and 1999 by GREC and in 2003-2004 by IFAW.  
162 Data from the Azores were collected by IMAR-DOP/UAç in the summers 2000 and 2002, and year-  
163 round in 2003. In the Canary Islands data were recorded by SECAC from 2008 to 2011 (Table I).

164 All the recordings were collected when only one group of animals formed by only one species was  
165 within visual range in order to identify the species and avoid the risk of considering emissions by  
166 mixed groups of sympatric species.

167

#### 168 *Data analysis*

169 Each whistle was also classified by assigning a quality index between 0 and 3 (Fig. 2). The assigned  
170 score was: 0) when detection of the complete time-frequency contour of the whistle was impossible  
171 because of overlapping with other sounds and low intensity; 1) when low intensity and/or low signal to  
172 noise ratio prevented to recognise the complete contour, 2) when the complete contour can be  
173 recognised but intensity was low and 3) when intensity was high and time-frequency contour well  
174 defined. Only whistles classified as 2 or 3 were considered of high quality and used in the analysis.  
175 Moreover, sounds with similar time-frequency contours were considered only once to avoid potential  
176 autocorrelation effects.

177 Recordings were analyzed by extracting whistle parameters using the spectrogram view in the program  
178 CoolEdit 2000 (Syntrillium Software, U.S.A.). Ten signal parameters (duration, 5 for frequency and 4  
179 for modulation of the sound) were manually measured from each whistle after the method of Oswald

180 et al (2003, 2007) and Azzolin (2008). These included: signal duration, beginning frequency, end  
181 frequency, minimum frequency, maximum frequency, the number of inflection points (change from  
182 positive to negative or negative to positive slope), the number of steps (a discontinuous change in  
183 frequency) and the number of minima and maxima in the contour (Fig. 3). We calculated also the  
184 frequency range as maximum frequency minus minimum frequency.

185 The statistical software package PASW Statistics 18.0 (SPSS Institute Inc., Chicago, Illinois, USA)  
186 was used for the descriptive (mean, standard deviation) and comparative statistical analysis of whistle  
187 parameters. Once it was verified that the data distribution was not normal, the nonparametric Mann-  
188 Whitney and Kruskal-Wallis tests were used to determine whether parameters varied between  
189 populations. The analysis of the Coefficient of Variation (CV) provided a fine scale estimation of the  
190 variability of the parameters between and within the three areas. We also performed a Discriminant  
191 Function Analysis (DFA) in order to evaluate whether recorded whistles could be correctly assigned  
192 first between the Mediterranean Sea and the Atlantic Ocean, and then between the Mediterranean Sea,  
193 the Azores Archipelago and the Canary Archipelago. The leave-one-out procedure (Lachenbruch and  
194 Mickey, 1968) was then used for cross-validation. For all the multivariate statistics we did not use  
195 frequency range as a predictor variable due to its dependence from maximum and minimum frequency  
196 parameters.

197

198

### 199 **III. RESULTS**

200

#### 201 *Study effort*

202 We used 3.70 hours of recordings from 13 sightings for the Atlantic area. We extracted 1141 whistles  
203 but used only 553 high quality whistles (scored 2 and 3) for the analyses (111 from the Azores and 442  
204 from the Canary Islands). From the Mediterranean area, we used 18.71 hours of recordings from 38

205 sightings. We extracted 1802 whistles and analyzed 1062 (346 in the Eastern basin and 716 in the  
206 Western basin).

207

#### 208 *Whistle diversity between Atlantic Ocean and Mediterranean Sea (Table II)*

209 An analysis of parameters variability within the basins showed higher values in the parameters  
210 associated with signal modulation (92-166) and lower CVs associated with variables related to signal  
211 frequency (20-50). Whistles produced by striped dolphins in the Atlantic showed significantly higher  
212 values compared to the Mediterranean ones for duration (Mann-Whitney test  $N=1615$ ;  $Z=-4.726$ ;  
213  $P<0.001$ ), maximum frequency (Mann-Whitney test  $N=1615$ ;  $Z=-7.889$ ;  $P<0.001$ ) and frequency  
214 range (Mann-Whitney test  $N=1615$ ;  $Z=-8.671$ ;  $P<0.001$ ). Modulation parameters, like number of steps  
215 (Mann-Whitney test  $N=1615$ ;  $Z=-13.502$ ;  $P<0.001$ ), number of maxima (Mann-Whitney test  $N=1615$ ;  
216  $Z=-7.407$ ;  $P<0.001$ ) and number of minima (Mann-Whitney test  $N=1615$ ;  $Z=-7.557$ ;  $P<0.001$ ), were  
217 significantly higher in the Atlantic Ocean whistles while the number of inflection points were  
218 significantly lower (Mann-Whitney test  $N=1615$ ;  $Z=7.962$ ;  $P<0.001$ ). The results of the cross  
219 validated Discriminant Function Analysis performed between whistles recorded in the Atlantic and in  
220 the Mediterranean, showed that 73.4 % of signals could be correctly classified to the study areas on the  
221 basis of the number of inflection points, number of maxima, number of minima and minimum  
222 frequency (coefficients:  $n^\circ$  inflection points= -1.05,  $n^\circ$  of maxima= 0.75,  $n^\circ$  of minima=0.48,  
223 minimum frequency =-0.42).

224

#### 225 *Whistle variation among the Azores, Canaries and Mediterranean Sea*

226 The same pattern of differences found between the sounds of the Atlantic and the Mediterranean was  
227 evident when we analyzed separately whistles recorded at the Azores and Canaries in comparison with  
228 the Mediterranean Sea recordings. The analysis of the degree of variability within the three areas  
229 showed higher CVs in parameters associated with signal modulation and lower variability for  
230 frequency-related parameters (Table III). All the frequency parameters had inter areas CVs ranging

231 from 3.80 to 12.33. Frequency parameters did not differ significantly between sounds from the  
232 Atlantic Archipelagos but again, both Atlantic Archipelagos recordings differed significantly from the  
233 Mediterranean (Fig. 4). Between the Azores Islands and Mediterranean Sea sounds significant  
234 differences were found in the number of steps (Mann-Whitney test  $N=1173$ ;  $Z=-10.791$ ;  $P<0.001$ ),  
235 maxima ( $Z=-2.167$ ;  $P=0.03$ ), the frequency range ( $Z=-6.316$ ;  $P<0.001$ ), signal duration ( $Z=-3.271$ ;  
236  $P=0.01$ ) and the maximum frequency ( $Z=-5.153$ ;  $P<0.001$ ) that presented values significantly lower in  
237 the Mediterranean sea, while the number of inflection points ( $Z=9.447$ ;  $P<0.001$ ) were higher in the  
238 Mediterranean sea whistles. Between the Canary Islands and the Mediterranean Sea sounds significant  
239 differences occurred in the number of steps (Mann-Whitney test  $N=1504$ ;  $Z=-10.965$ ;  $P<0.001$ ),  
240 maxima ( $Z=-7.662$ ;  $P<0.001$ ), minima ( $Z=-8.972$ ;  $P<0.001$ ), the frequency range ( $Z=-7.255$ ;  $P<0.001$ )  
241 signal duration ( $Z=-4.030$ ;  $P<0.001$ ), and maximum frequency ( $Z=-6.863$ ;  $P<0.001$ ), all significantly  
242 lower in the Mediterranean whistles but the number of inflection points was significantly higher  
243 ( $Z=5.021$ ;  $P<0.001$ ) (Fig. 4).

244 In addition, all whistle modulation parameters had higher inter areas CVs than frequency parameters  
245 and they were all significantly different not only between Atlantic archipelagos and the Mediterranean  
246 whistles, but also between the recordings from the two Atlantic archipelagos (Fig. 4). Significant  
247 differences existed between the Azores and the Canary Islands sounds for number of steps (Mann-  
248 Whitney test  $N=553$ ;  $Z=4.576$ ;  $P<0.001$ ), which was significantly lower in the Canary Archipelago.  
249 Inflection points ( $Z=-6.131$ ;  $P<0.001$ ), minima ( $Z=-5.258$ ;  $P<0.001$ ) and maxima ( $Z=-2.236$ ;  $P=0.02$ )  
250 were significantly higher in the Azores archipelago whistles. Number of steps and of minima were  
251 also the only parameters contributing to the Discriminant Function Analysis performed between the  
252 Canaries and the Azores (coefficients:  $n^\circ$  steps= 0.72,  $n^\circ$  of minima= -0.69).

253 The cross-validated Discriminant Function Analysis correctly assigned 63% of whistles recorded in  
254 the different areas. Mediterranean Sea whistles had a correct assignment rate of 73%, the Azores of  
255 55% and Canary Islands 40%. For the Canary Islands, 30.1% of whistles were misclassified to the  
256 Azores and 29.9% to the Mediterranean Sea, while for Azores 27% were misclassified to the Canary

257 Islands and only 18% to the Mediterranean (Table IV). The parameters that contributed most to the  
258 analysis were the number of inflection points, maxima, steps and minimum frequency (coefficients: n°  
259 of inflection points=-1.02, n° of maxima=0.77, steps=0.45 and minimum frequency=-0.41).

260 The canonical DFA scatter plot (Fig. 5) displayed a clear pattern of difference among areas. The  
261 whistles recorded in the Azores and in the Mediterranean Sea were definitely separated while the  
262 Canaries signals showed an intermediate group centroid between them.

263

#### 264 **IV. DISCUSSION**

265

266 The communication signals of delphinids play an important role in both species and group recognition,  
267 although the degree of modulation of signals can vary widely among populations (Janik 2009).  
268 Furthermore each parameter can vary independently under the pressure of different selective forces  
269 (Wang et al 1995).

270 Atlantic and Mediterranean whistles exhibited characteristics that were significantly different, both in  
271 parameters related to morphological constraint (such as frequencies), duration and in modulation  
272 parameters, which are more influenced by the physical and social environments (May-Collado and  
273 Wartzok 2008). DFA correctly classified 73% of whistles emitted in the two basins. Our findings are  
274 in agreement with differences described by genetic studies (Garcia-Martinez et al 1999, Bourret et al  
275 2007) which identified two populations inhabiting the two sides of the Gibraltar Strait. These two  
276 populations are partially reproductively isolated.

277 The analyses carried out among the recordings of the three study areas showed significant differences  
278 within the Atlantic Ocean samples only in modulation parameters, suggesting the presence of a  
279 gradual variation in the vocal characteristics and a possible pattern linked to geographic distance. A  
280 cline in parameters is apparent from the Azores to the Mediterranean, with a major difference between  
281 the Canaries and the Mediterranean than between Azores and Canaries whistles. As shown in the DFA  
282 classification, whistles can be discriminated between the areas but the whistles collected at the Canary

283 Islands have a correct assignment of just 40%. The misclassification score is subdivided into about  
284 30% of wrong assignments to either the Azores or to the Mediterranean Sea. Parameters recorded in  
285 whistles of the Canary Islands striped dolphins have intermediate values between the Azores and the  
286 Mediterranean, with the exception of the beginning and end frequency (which do not contribute to the  
287 discrimination) and the number of maxima and minima. A likely explanation for this result is the  
288 geographic position of the Canary Islands. This Archipelago is located at about midway between the  
289 other two sites, at a distance around 1000 km from the Gibraltar Strait and of 1200 km from the  
290 Azores Archipelago. Striped dolphins are sighted throughout the year both in the Azores (Hartman et  
291 al 2008) and the Canary Islands. The year-round occurrence of striped dolphins in the Gibraltar Strait  
292 has yet to be evaluated. The Canary Islands may represent an overlap area between striped dolphins  
293 inhabiting waters around the Strait of Gibraltar, that are likely to have Mediterranean acoustic features,  
294 and oceanic animals carrying Azorean characteristics.

295 Nevertheless, within the Atlantic Ocean differences are limited to modulation parameters while the  
296 signal duration, maximum frequency and range of frequency measured in whistles from the  
297 Mediterranean are significantly different than in the Canaries and Azores whistles. Since modulation  
298 parameters show a higher degree of variability between the areas as compared to frequency  
299 parameters, as well as the highest values of intra area variability, they may be linked to factors  
300 unrelated to those deriving from genetic relatedness. Furthermore no studies evidenced the occurrence  
301 of genetic differences between the two archipelagos. Considering that modulation parameters  
302 contribute the most to the differentiation within the Atlantic Ocean it is possible that behavioural  
303 context, social and physical environment may be among the main factors influencing the separation  
304 between Azores and Canaries sounds.

305 The separation between the Atlantic Ocean and the Mediterranean Sea can instead be related also to  
306 other factors including genetic variability. Minimum frequency, considered as the parameter most  
307 constrained by the structural phenotype (May-Collado et al 2007), displays the lowest inter area and  
308 low intra area CVs and is the only parameter of frequency contributing to the Discriminant Function.

309 The maximum frequency, another morphological constraint parameter with a negative relationship  
310 with body size (Wang et al 1995), differs significantly between the basins. Also the range of frequency  
311 (a parameter dependent from the maximum and minimum frequency values), shows significant inter-  
312 basins differences, but not within Atlantic. Furthermore the signal duration displays inter-basins  
313 differences between sounds recorded in the waters of Mediterranean and Canaries or Azores whistles  
314 but not between the Azores and the Canary Archipelagos.

315 Within-basin geographic variation among acoustic structure of whistles emitted in different areas was  
316 obtained for the Mediterranean by Gitter (2009). He found acoustic differences between inshore and  
317 offshore striped dolphins of the Ligurian Sea a difference that parallel the genetic differences reported  
318 by Gaspari (2004). Azzolin (2008) confirmed the acoustic differences for the whistles emitted by in-  
319 off-shore animals of the whole basin, and found differences in vocalizations between the Eastern and  
320 the Western basins according to the genetic differences hypothesized by Valsecchi et al (2004). In the  
321 present study the geographic barrier of Gibraltar can be an obstacle similarly to what was proposed for  
322 the Italian peninsula in the Mediterranean Sea by Azzolin (2008).

323 We can conclude that the acoustic features such as the whistle's frequency parameters, which have a  
324 lower variation, are more strongly constrained by the structural phenotype and are likely to be  
325 genetically related, are significantly different between the Atlantic and the Mediterranean. On the  
326 other hand, modulation parameters, which are less dependent on structural phenotype are probably  
327 more tied to context, social, environmental and random individual variation (May-Collado and  
328 Wartzok 2008) and consequently show higher variability. According to Bazua-Duran and Au (2004)  
329 the acoustic characteristics of whistles may be important in defining the limits and arrangement of a  
330 school, especially if acoustic signals are learned socially (Janik and Slater 2000, Sayigh et al 1995,  
331 Watwood et al 2004, Riesch 2006), and may reveal affiliative relationships (Watwood et al 2004).  
332 Also Rossi-Santos and colleagues (2006) suggest that the social interactions and the sharing of sounds  
333 among dolphins which spend time in the same group could be the cause of the geographic gradient.

334

335 **V. ACKNOWLEDGEMENTS**

336

337 The authors are grateful to IFAW research group for its contribution. Without its support this project  
338 could not have been realized. Data collection and processing in the Azores was funded by FEDER  
339 funds, through the Competitiveness Factors Operational Programme – COMPETE, and by national  
340 funds, through FCT – Foundation for Science and Technology, under projects CETAMARH  
341 (POCTI/BSE/38991/01) and TRACE (PTDC/MAR/74071/2006). M.A.S. was supported by an FCT  
342 postdoctoral grant (SFRH/BPD/29841/2006). I.C. and R.P. were supported by FCT doctoral grants  
343 (respectively SFRH/BD/41192/2007 and SFRH/BD/32520/2006). IMAR- DOP/UAç is the R&D Unit  
344 #531 and part of the Associated Laboratory ISR funded through the Pluri-annual and Programmatic  
345 funding schemes of FCT-MCTES and DRCT-Azores. Data collection by SECAC was funded by the  
346 U.E. LIFE programme – project LIFE INDEMARES (LIFE 07/NAT/E/000732)- and the Fundación  
347 Biodiversidad, under the Spanish Ministry of Environment, Rural and Marine Affairs (project ZEC-  
348 TURSIOPS). Many thanks also to Alice Galli for the GIS representation, Peter Tyack for his  
349 constructive suggestions and two anonymous reviewers.

350

351 **VI. REFERENCES**

352

353 Archer, F. I. II (2002). “Striped dolphin,” in *Encyclopedia of marine mammals*, edited by W. R. Perrin,  
354 B. Wursig, J. G. M. Thewissen, (Academic Press), pp 1201-1203.

355

356 Astraldi, A., Balopoulos, S., Candela, J., Font, J., Gacic, M., Gasparini, G. P., Manca, B., Theocharis,  
357 A., Tintore, J. (1999). “The role of straits and channels in understanding the characteristics of  
358 Mediterranean circulation,” *Progress in Oceanography* **44**, 65–108.



359 Azzolin, M. (2008). "Acoustic identification of Mediterranean odontocetes as a prerequisite for their  
360 passive acoustic monitoring," PhD Thesis, School of science and high technology, University of  
361 Torino, Italy pp. 203.

362

363 Baron, S. C., Martinez, A., Garrison, L. P., Keith, E. O. (2008). "Differences in acoustic signals from  
364 Delphinids in the western North Atlantic and northern Gulf of Mexico," *Marine Mammal Science* **24**,  
365 42–56.

366

367 Bazua-Duran, C., Au W. W. L. (2004). "Geographic variations in the whistles of spinner dolphins  
368 (*Stenella longirostris*) of the Main Hawaiian Islands," *Journal of the Acoustic Society of America* **116**,  
369 3757–3769.

370

371 Bouret, V. J. R., Macé, M. R. J. M., Crouau-Roy, B. (2007). "Genetic variation and population  
372 structure of western Mediterranean and northern Atlantic *Stenella coeruleoalba* populations inferred  
373 from microsatellite data," *Journal of the Marine Biological Association of the United Kingdom* **87**,  
374 265–269.

375

376 Canales, J. P., Dañobeitia, J. J. (1998). "The Canary Islands swell: a coherence analysis of bathymetry  
377 and gravity," *Geophysical Journal International* **132**, 479–488.

378

379 Folkens, P., Reeves, R. (2002). *Guide to Marine Mammals of the World* (New York, Alfred A. Knopf,  
380 Inc.), pp527.

381

382 Forcada, J., Aguilar, A., Hammond, P., Pastor, X., Aguilar, R. (1994). "Distribution and numbers of  
383 striped dolphins in the Western Mediterranean Sea after the 1990 epizootic outbreak," *Marine*  
384 *Mammal Science* **10**, 137–150.

385

386 Ford, J. K. B., Fisher, H. D. (1983). “Group-specific dialects of killer whales (*Orcinus orca*) in British  
387 Columbia,” in *Communication and behaviour of whales*, edited by R.S. Payne, (Boulder, CO:  
388 Westview press), pp. 129-161.

389

390 García Martínez, J., Moya, A., Raga, J. A., Latorre, A. (1999). “Genetic differentiation in the striped  
391 dolphin *Stenella coeruleoalba* from European waters according to mitochondrial DNA (mtDNA)  
392 restriction analysis,” *Molecular Ecology* **8**, 1069–1073.

393

394 Gaspari, S. (2004). “Social and population structure of striped and Risso’s dolphins in the  
395 Mediterranean Sea,” Ph.D Thesis, School of Biological and Biomedical Sciences, University of  
396 Durham, United Kingdom, pp 184.

397

398 Gerhardt, C.H. (1991). “Female mate choice in treefrogs: static and dynamic acoustic criteria”, *Animal*  
399 *Behaviour* **42**, 615-635.

400

401 Gitter, S. J. (2009). “Whistle repertoire and geographic variation of striped dolphins (*Stenella*  
402 *coeruleoalba*) in the Ligurian Sea of the Mediterranean,” Thesis presented to the faculty of the school  
403 of graduate studies of western Illinois University, pp50.

404

405 Griffiths, E. T. (2009). “Whistle repertoire analysis of the short-beaked common dolphin, *Delphinus*  
406 *delphis*, from the Celtic Deep and the Eastern Tropical Pacific Ocean,” thesis of the Master of Science,  
407 Marine Biology School of Ocean Sciences Bangor University, United Kingdom pp 97.

408

409 Hartman, K. L., Visser, F., Hendriks, A. J. E. (2008). “Social structure of Risso’s dolphins (*Grampus*  
410 *griseus*) at the Azores: a stratified community based on highly associated social units,” Canadian  
411 Journal of Zoology **86**, 294-306.

412

413 Hawkins, E. R., Gartside, D. F. (2010). “Whistle emissions of Indo-Pacific bottlenose dolphins  
414 (*Tursiops aduncus*) differ with group composition and surface behaviours,” Journal of the Acoustic  
415 Society of America **127**, 2652–2663.

416

417 Janik, V. M. (2009). “Acoustic communication in delphinids,” In *Advances in the study of behaviour*.  
418 *Vocal communication in birds and mammals*, edited by K. Zuberbuhler, N. S. Clayton, V. M. Janik,  
419 M. Naguib, (Academic press), Elsevier, Amsterdam Vol. 40, 123-157.

420

421 Janik, V. M., Slater, P. J. B. (2000). “The different roles of social learning in vocal communication,”  
422 *Animal Behaviour* **60**, 1–11.

423

424 Jones, G. J., Sayigh, L. S., (2002). “Geographic variation in rates of vocal production of free ranging  
425 bottlenose dolphins,” *Marine Mammal Science* **18**, 374–393.

426

427 Lachenbruch, P. A., Mickey, M. R. (1968) “Estimation of error rates in discriminant analysis”.  
428 *Technometrics* **10**, 1-11.

429

430 May-collado, M. J., Wartzok, D. (2008). “A comparison of bottlenose dolphin whistles in the Atlantic  
431 Ocean: factors promoting whistle variation,” *Journal of Mammology* **89**, 1229–1240.

432

433 Morato, T., Machete, M., Kitchingman, A., Tempera, F., Lai, S., Menezes, G., Pitcher, T. J., Santos,  
434 R. S. (2008). "Abundance and distribution of seamounts in the Azores," Marine Ecology Progress  
435 Series **357**, 17–21.

436

437 Morisaka, T., Shinohara, M., Nakahara, F., Akamatsu, T. (2005). "Geographic variations in the  
438 whistles among three Indo-Pacific bottlenose dolphin *Tursiops aduncus* populations in Japan," Fishery  
439 Science **71**, 568–576.

440

441 Oswald, J. N., Barlow, J., Norris, T. F. (2003). "Acoustic identification of nine delphinid species in the  
442 eastern tropical Pacific Ocean," Marine Mammal Science **19**, 20-37.

443

444 Oswald, J. N., Rankin, S., Barlow, J., Lammers, M. O. (2007). "A tool for real-time acoustic species  
445 identification of delphinid whistles", Journal of the Acoustic Society of America **122**, 587-595.

446

447 Panigada, S., Lauriano, G., Pierantonio, N., Donovan, G. (2011). "Monitoring cetaceans populations  
448 through aerial surveys in the central Mediterranean Sea," 25th Conference of the European Cetacean  
449 Society, 21st-23rd March 2011 Cadiz, Spain.

450

451 Petrella, V. (2009). "Whistle characteristics of common dolphins (*Delphinus sp.*) in the Hauraki Gulf,  
452 New Zealand," PhD thesis, University of Naples. pp.133.

453

454 Reeves, R., Notarbartolo Di Sciara, G. (2006). "The status and distribution of cetaceans in the Black  
455 Sea and Mediterranean Sea," International Union for Conservation of Nature and Natural Resources  
456 (IUCN), Centre for Mediterranean Cooperation, Malaga, Spain. pp. 137.

457

458 Rendell, L. E., Matthews, J. N., Gill, A., Gordon, J. C. D., Macdonald, D. W. (1999). "Quantitative  
459 analysis of tonal calls from five odontocete species, examining interspecific and intraspecific  
460 variation," *Journal of Zoology* **249**, 403-410.

461

462 Riesch, R., Ford, J. K. B., Thomsen, F. (2006). "Stability and group specificity of stereotyped whistles  
463 in resident killer whales, *Orcinus orca*, off British Columbia," *Animal Behaviour* **71**, 79-91.

464

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

467

468 Santos, R. S., Hawkins, S., Monteiro, L. R., Alves, M., Isidro, E. J. (1995). "Case studies and reviews:  
469 marine research, resources and conservation in the Azores," *Aquatic Conservation* **5**, 311-354.

470

471 Sayigh, L. S., Tyack, P. L., Wells, R. S., Scott, M. D., and Irvine, A. B. (1995). "Sex difference in  
472 whistle production in free-ranging bottlenose dolphins, *Tursiops truncatus*," *Behavioral Ecology and*  
473 *Sociobiology* **36**, 171-177.

474

475 Valsecchi, E., Amos, W., Raga, J. A., Podestà, M., Sherwin, W. (2004). "The effects of inbreeding on  
476 mortality during a morbillivirus outbreak in the Mediterranean striped dolphins (*Stenella*  
477 *coeruleoalba*)," *Animal Conservation* **7**, 139-146.

478

479 Wang, D., Würsig, B., Evans, W. (1995). "Comparisons of whistles among seven odontocete species,"  
480 in *Sensory Systems of Aquatic Mammals*, edited by R. A. Kastelien, J. A. Thomas, and P. E.  
481 Nachtigall, (De Spil, Woerden, The Netherlands), pp. 299-323.

482

483 Watwood, S. L., Tyack, P. L., Wells, R. S. (2004). “Whistle sharing in paired male bottlenose  
484 dolphins, *Tursiops truncatus*”, Behavioral Ecology and Sociobiology **55**, 531–543.  
485

Table I Details of the data collection

Area	Period	Instruments	Research Group	Equipment	
Atlantic Ocean (3.70 hours)	Azores Archipelago	2000-2002-2003	Omnidirectional hydrophone and towed array with 2 hydrophones	DOP/Uaç	Flat response: $\pm 1$ dB between 1Hz and 15kHz, and of $\pm 3$ dB between 15kHz and 30kHz. Sampling frequency: 48kHz
	Canary Archipelago	2008-2011	Towed array with 4 hydrophones	SECAC	Flat response: $\pm 1$ dB between 1Hz and 15kHz, and of $\pm 3$ dB between 15kHz and 30kHz and $\sim 2$ -150 kHz Sampling frequency: 192kHz
Mediterranean Sea (18.71 hours)	Alboran Sea	1999	Mono towed hydrophone	GREC	Flat response of $\pm 2$ dB from 200Hz to 30 kHz Sampling frequency: 48kHz
	Balearic and Spain	1999	Mono towed hydrophone	GREC	
	Corso-ligure-provençal basin	1996-1998-1999	Stereo towed hydrophone	GREC	
	Tyrrhenian	2003	Omnidirectional hydrophone and towed array with 2 hydrophones	IFAW	Flat response: $\pm 1$ dB between 1Hz and 15kHz, and of $\pm 3$ dB between 15kHz and 30kHz. Sampling frequency: 48kHz
	Ionian Sea	2003-2004	Omnidirectional hydrophone and towed array with 2 hydrophones	IFAW	
		1998	Stereo towed hydrophone	GREC	

488 Table II Descriptive statistic for each parameter in the two areas (\*\*\*)significant differences  $P < 0.001$ )

489

(frequency parameters in Hz)

	Atlantic Ocean				Mediterranean Sea				Z
	Min	Max	Mean (SD)	CV	Min	Max	Mean (SD)	CV	
Maximum freq. ***	7636	30743	17171 (3500)	20.39	3520	24000	15597 (3501)	22.45	-7.89
Minimum freq.	1108	13487	7882 (1723)	21.86	1468	13619	7977 (1935)	24.26	0.88
Frequency Range ***	598	23603	9288 (3506)	37.75	135	19764	7616 (3388)	44.50	-8.67
Duration ***	0.15	2.23	0.95 (0.33)	34.54	0.04	4.61	0.90 (0.45)	50.46	-4.73
Beginning freq.	4264	24000	11753 (4772)	40.61	1468	24000	11012 (4054)	36.82	-1.76
End freq.	3371	30743	11897 (4420)	37.15	2420	23092	11288 (3543)	31.39	-1.76
Number of Inflection points ***	0	11	1.02 (1.42)	139.40	0	13	1.50 (1.54)	102.92	7.96
Number of Steps ***	0	21	3.84 (3.54)	91.93	0	16	1.73 (2.27)	131.59	-13.50
Number of maxima ***	0	5	0.77 (0.85)	109.65	0	8	0.49 (0.79)	159.00	-7.41
Number of minima ***	0	6	0.84 (0.95)	112.41	0	9	0.56 (0.93)	165.80	-7.56



490 Table III Descriptive statistics for each parameter in the three areas of the analysis and results of the Kruskal-Wallis test ( $X^2$ , significant P (P<  
 491 0.001) value is represented by stars in the table) (frequency parameters in Hz)

492

	Azores Archipelago				Canary Archipelago				Mediterranean Sea				CV inter areas	$X^2$
	Min	Max	Mean (SD)	CV	Min	Max	Mean (SD)	CV	Min	Max	Mean (SD)	CV		
Maximum freq. ***	10016	23107	17439 (3403.29)	19.52	7636	30743	17104 (3525.10)	20.61	3520	24000	15597 (3501.80)	22.45	5.87	63.66
Minimum freq.	1108	11950	7727 (1877.82)	24.30	3371	13487	7921 (1682.02)	21.23	1468	13619	7977 (1935.22)	24.26	1.67	0.9
Frequency Range ***	3074	21602	9712 (2924.44)	30.11	598	23603	9182 (3633.85)	39.57	135	19764	7616 (3388.86)	44.50	12.33	79.28
Duration ***	0.34	2.23	0.97 (0.26)	27.06	0.15	2.21	0.95 (0.34)	36.26	0.04	4.61	0.90 (0.45)	50.46	3.89	22.98
Beginning freq.	4264	22710	11433 (5108.49)	44.68	4661	24000	11833 (4686.90)	39.61	1468	24000	11012 (4054.92)	36.82	3.59	4.57
End freq.	4710	23107	11627 (4836.09)	41.59	3371	30743	11965 (4312.93)	36.04	2420	23092	11288 (3543.32)	31.39	2.91	4.75
Number of Inflection points ***	0	5	0.39 (0.82)	212.22	0	11	1.18 (1.50)	126.84	0	13	1.50 (1.54)	102.92	55.99	100.76
Number of Steps ***	0	18	5.28 (4.04)	76.67	0	21	3.49 (3.30)	94.77	0	16	1.73 (2.27)	131.59	50.80	201.31
Number of maxima ***	0	2	0.59 (0.65)	111.55	0	5	0.82 (0.88)	107.85	0	8	0.49 (0.79)	159.00	26.28	59.61
Number of minima ***	0	3	0.47 (0.72)	152.52	0	6	0.94 (0.97)	104.00	0	9	0.56 (0.93)	165.80	37.78	85.87

493

494 Table IV Results of cross-validate DFA analysis among the Canary Archipelago, Azores

495 Archipelago and Mediterranean Sea

496

		Predicted Group Membership		
Area		Canary	Azores	Mediterranean
		Archipelago	Archipelago	Sea
Cross-validated	Canary Archipelago	40.0 %	30.1 %	29.9 %
	Azores Archipelago	27.0 %	55.0 %	18.0 %
	Mediterranean Sea	15.8 %	11.3 %	72.9 %

497 **VIII. FIGURES**

498

499 Figure 1 – Map of the recording locations analyzed for Atlantic Ocean and Mediterranean  
500 Sea. Black dots represent the approximate position of sightings. Inserts show major details of  
501 areas where sightings were closer. Depth contour of 200 meters is shown.

502

503 Figure 2 –Figure including spectrograms of all different levels of quality of the whistles.  
504 Only whistles with quality 2 and 3 have been analyzed in the study.

505

506 Figure 3 - A sample spectrogram representing a striped dolphin whistle. Parameters manually  
507 measured for each whistle are shown: signal duration, beginning frequency, end frequency,  
508 minimum frequency, maximum frequency, the number of inflection points, the number of  
509 steps and the number of minima and maxima in the contour. Frequency range was calculated  
510 as maximum frequency minus minimum frequency.

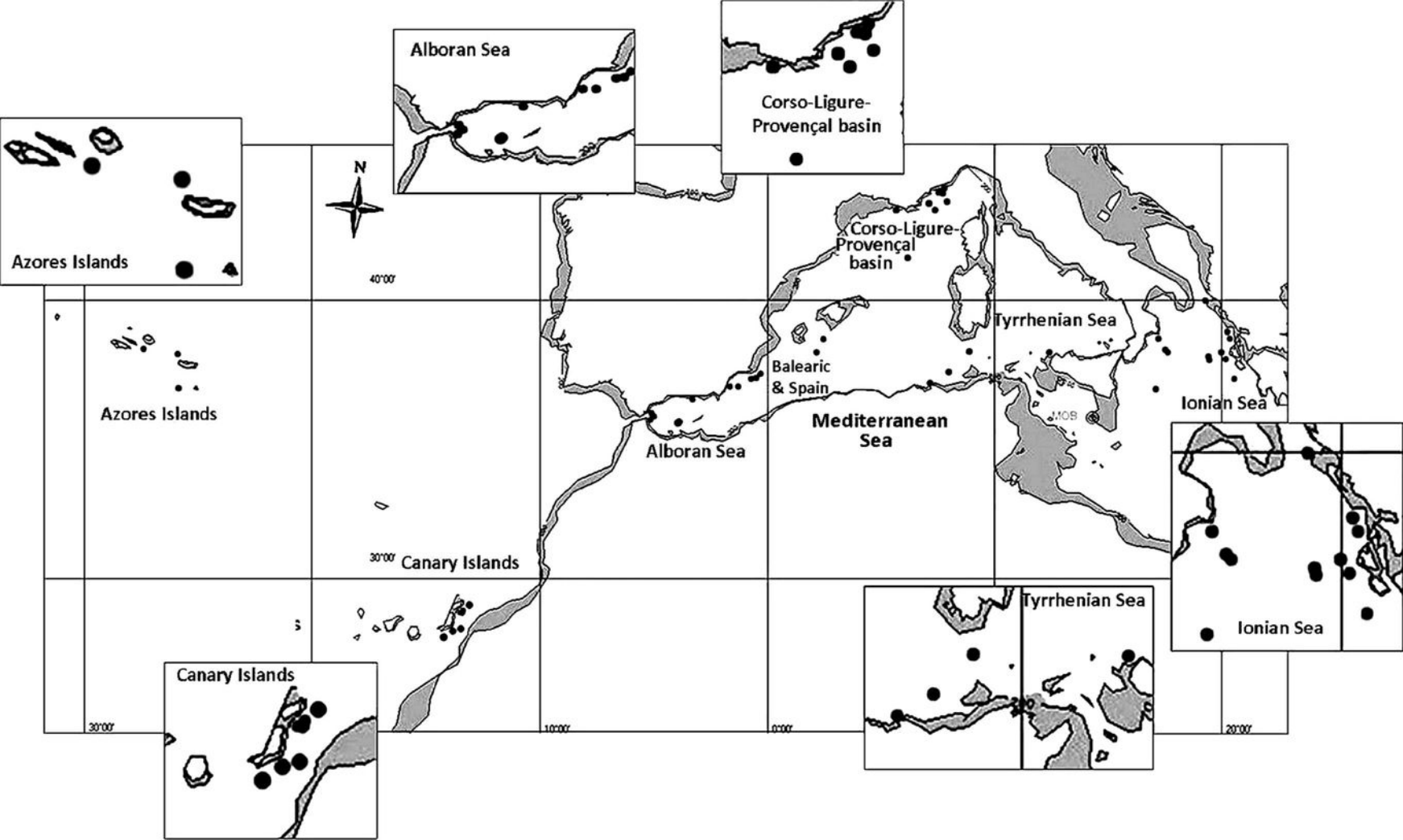
511

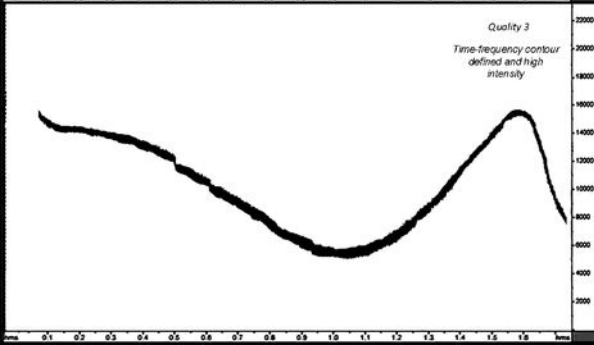
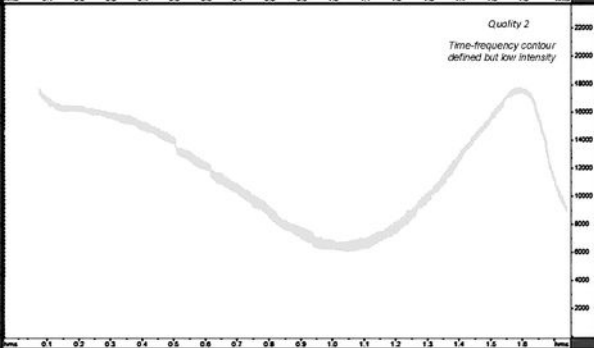
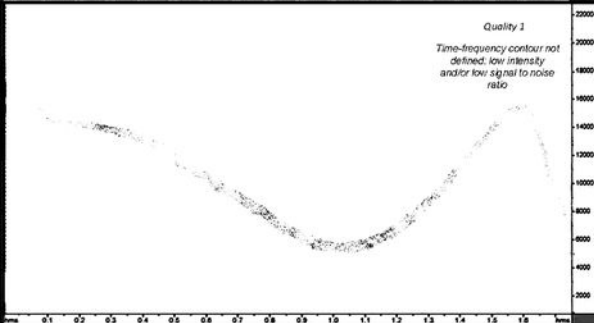
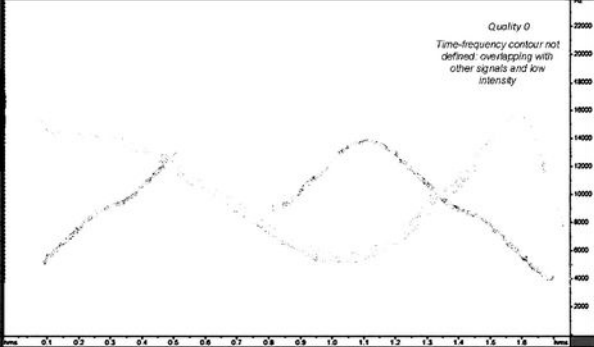
512 Figure 4 – Box plot graphs showing minimum, first quartile, median, third quartile and  
513 maximum values of maximum frequency (in Hz), frequency range (in Hz), signal duration (in  
514 seconds), the number for inflection points, the number of steps and the number of maxima in  
515 the contour. Significant differences among sounds of Azores islands, Canary islands and  
516 Mediterranean Sea are represented by stars (one star  $P < 0.05$ , two stars  $P < 0.001$ ).

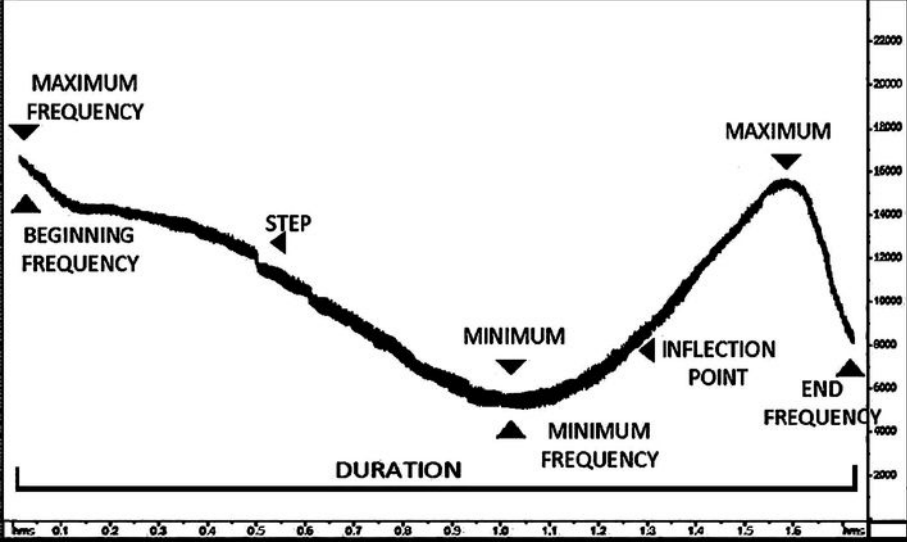
517

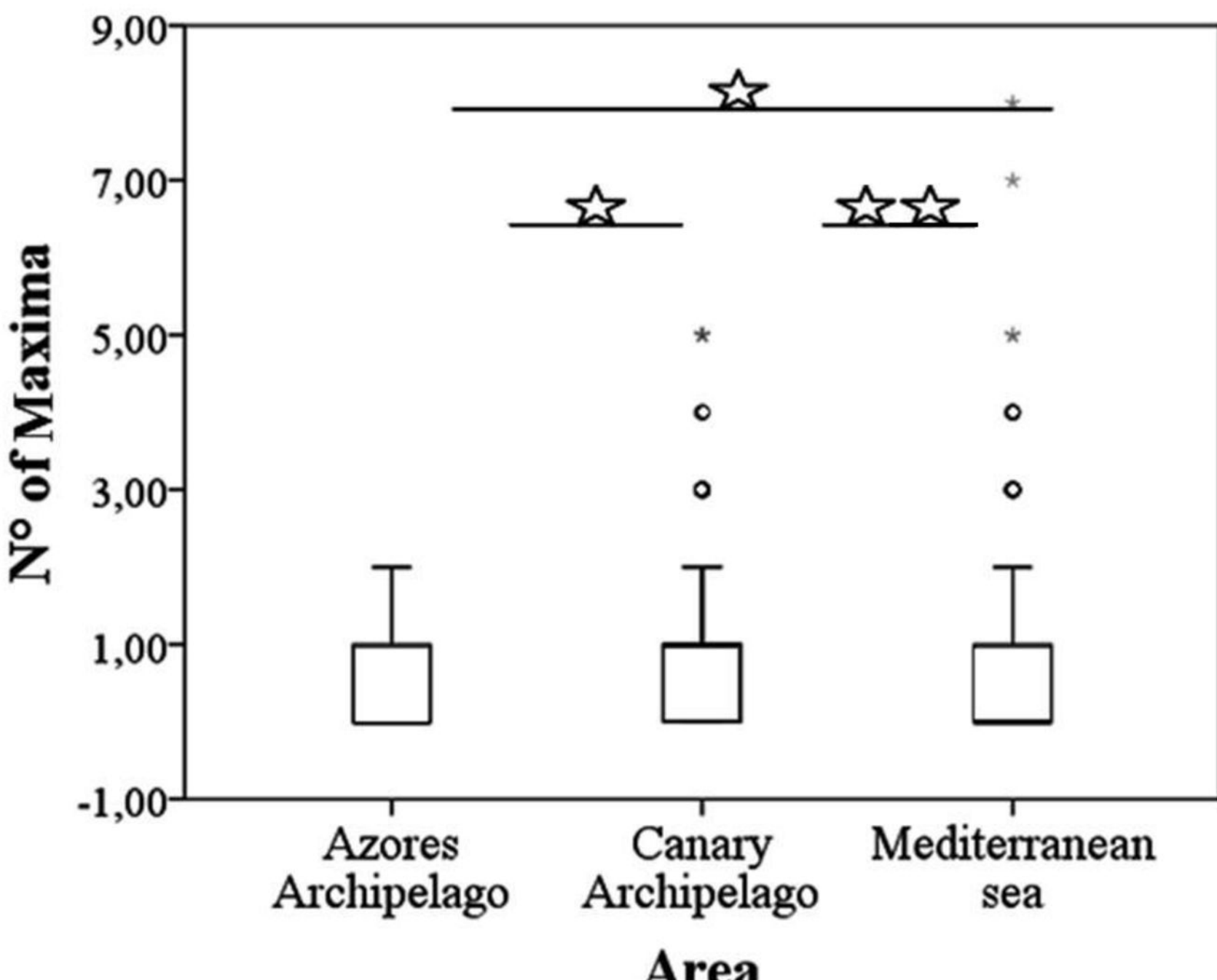
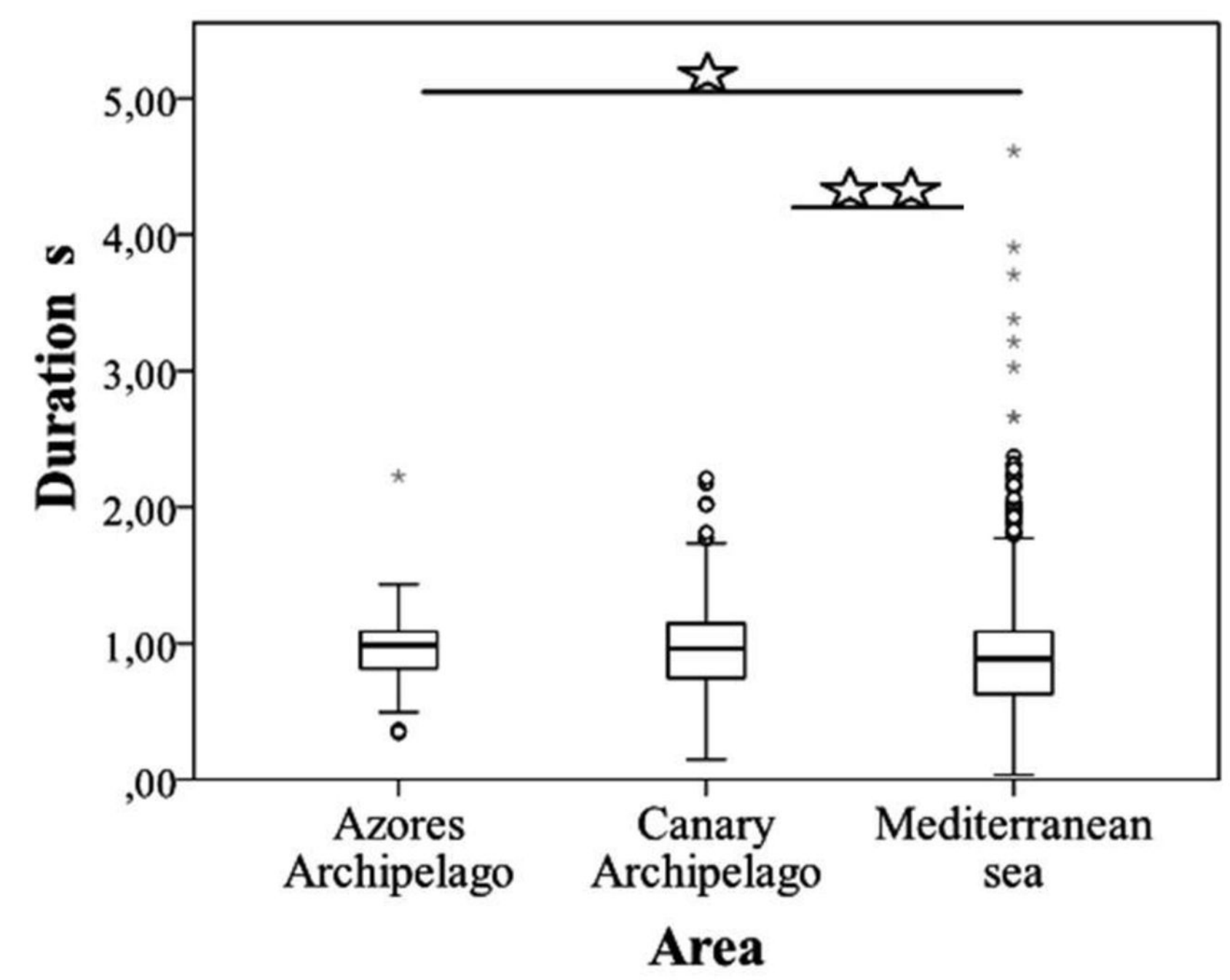
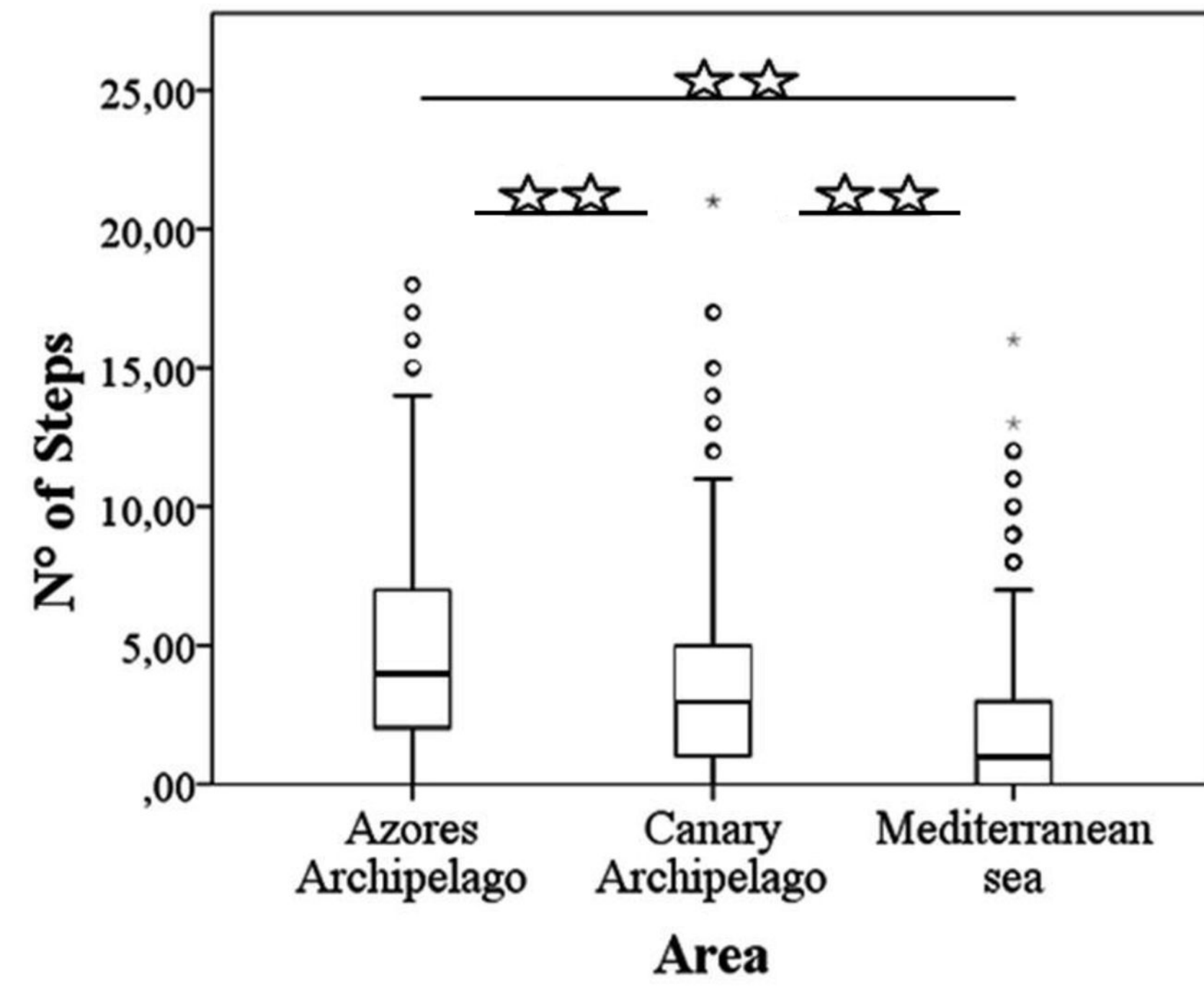
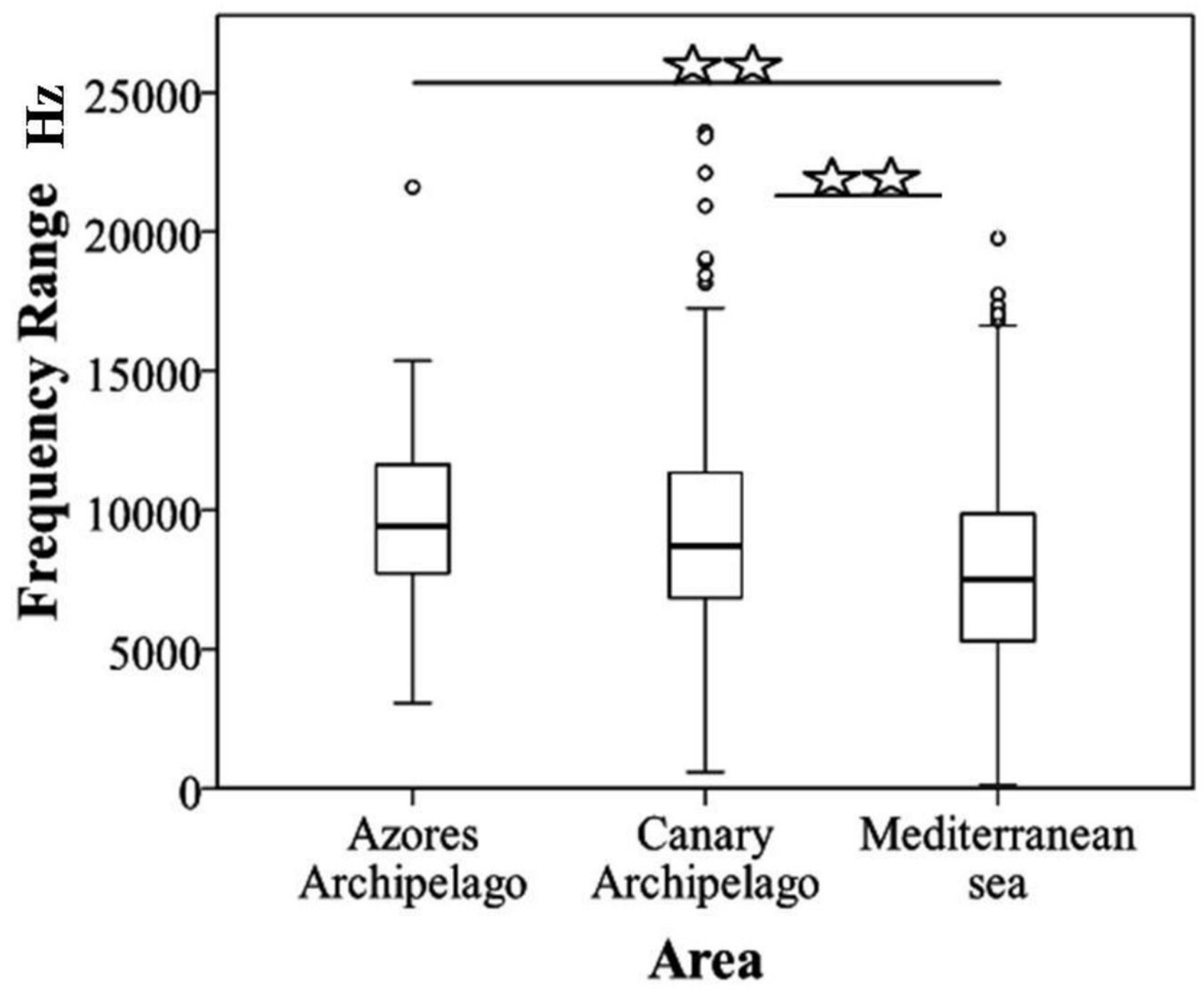
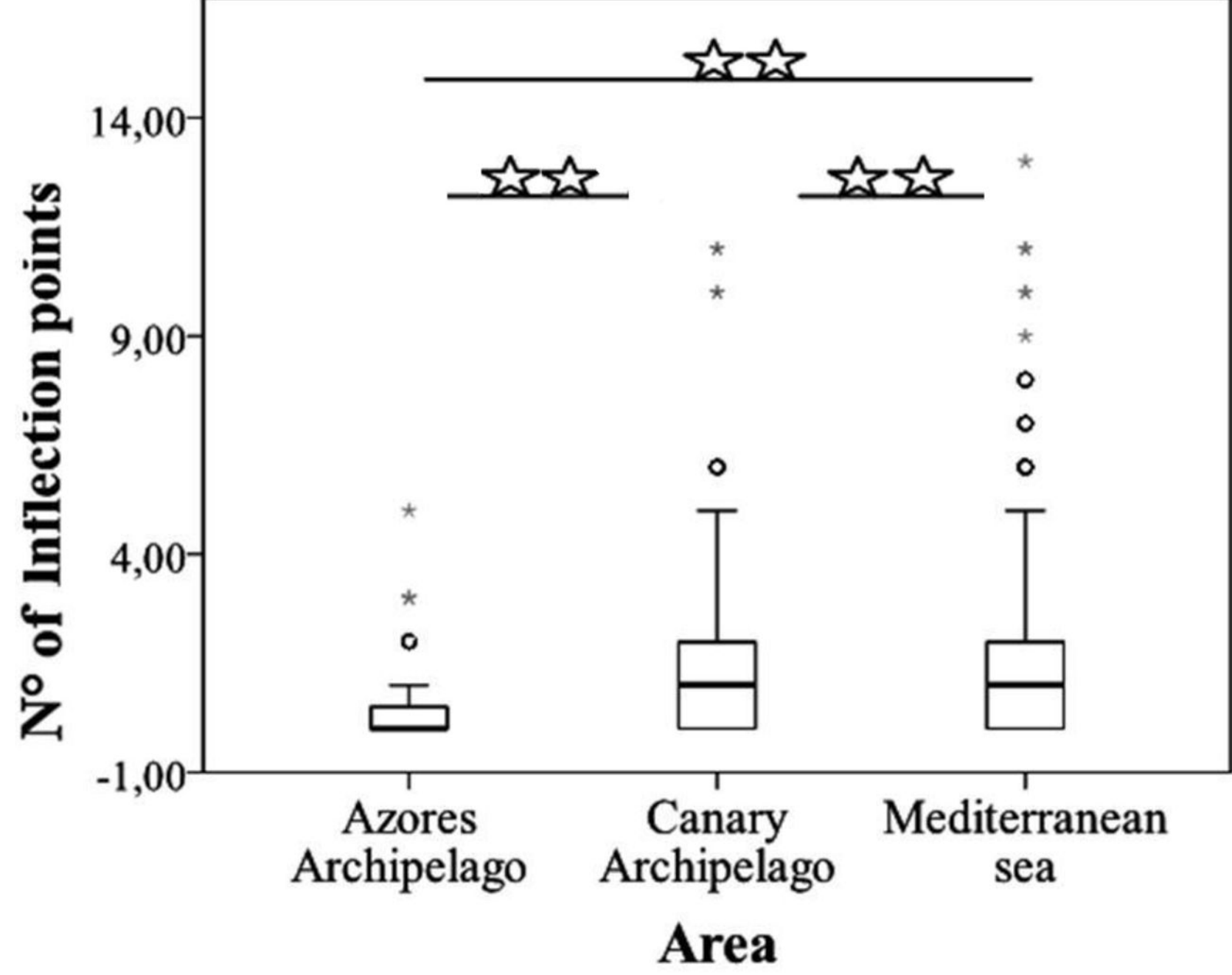
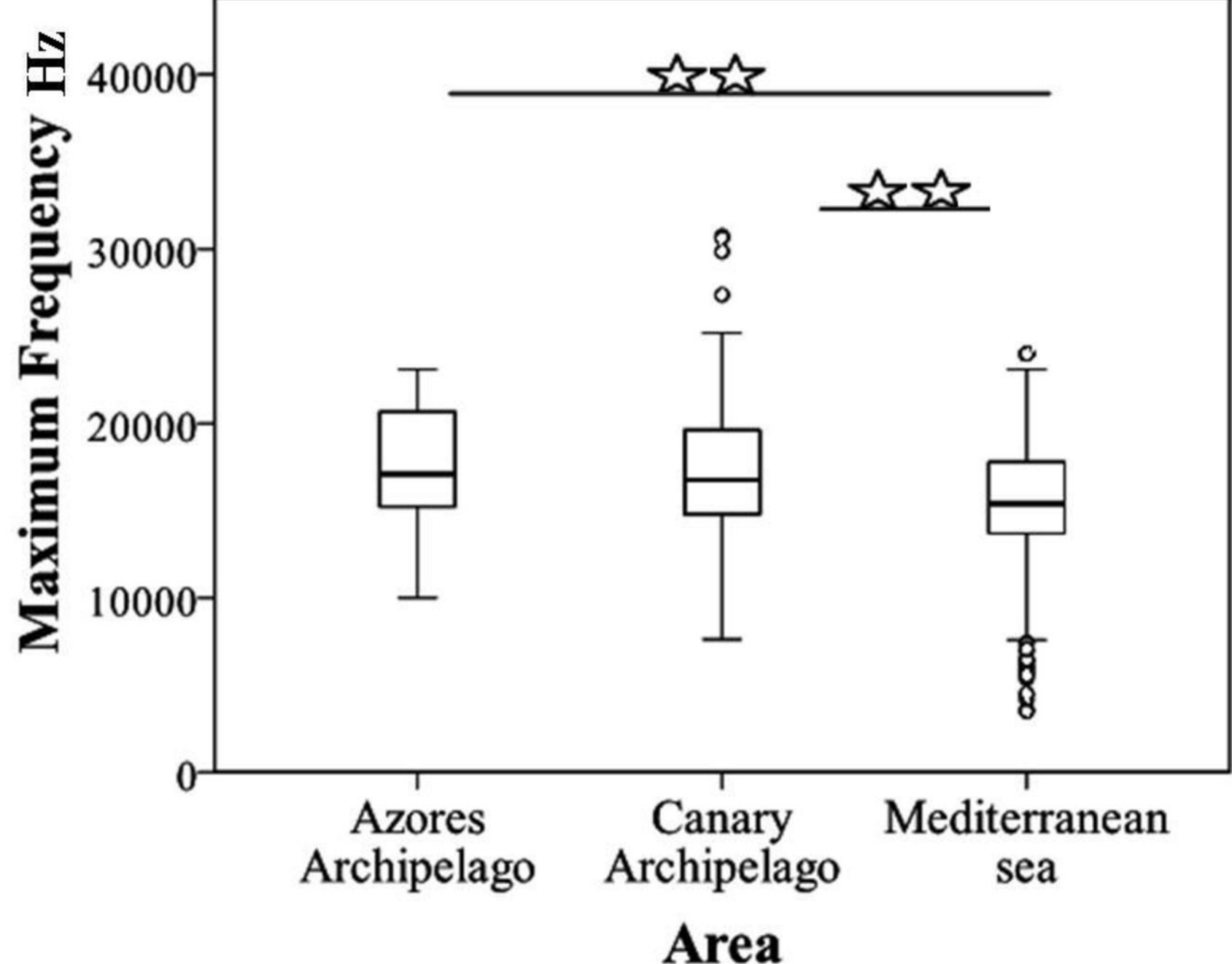
518 Figure 5 - Canonical Discriminant Function scatter plot of the striped dolphins populations of  
519 Canary islands, Azores islands and Mediterranean Sea, from the two functions that accounted  
520 for 100% of the observed variance (Coefficients Function 1: number of inflection points = -  
521 1.02, number of maxima = 0.77, number of steps = 0.45, minimum frequency = -0.41,  
522 number of minima = 0.40; Coefficients Function 2: number of minima = 0.70, number of

523 steps = -0.58, end frequency = 0.27, number of inflection points = -0.24, minimum frequency  
524 = -0.24).











# Canonical Discriminant Function

