Combining whistle acoustic parameters to discriminate Mediterranean odontocetes during passive acoustic monitoring

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Acoustic observation can complement visual observation to more effectively monitor occurrence and distribution of marine mammals. For effective acoustic censuses, calibration methods must be determined by joint visual and acoustic studies. Research is still needed in the field of acoustic species identification, particularly for smaller odontocetes. From 1994 to 2012, whistles of four odontocete species were recorded in different areas of the Mediterranean Sea to determine how reliably these vocalizations can be classified to species. Recordings were attributed to species by simultaneous visual observation. The results of this study highlight that the frequency parameters, which are linked to physical features of animals, show lower variability than modulation parameters, which are likely to be more dependent on complex eco-ethological contexts. For all the studied species, minimum and maximum frequencies were linearly correlated with body size. DFA and Classification Tree Analysis (CART) show that these parameters were the most important for classifying species; however, both statistical methods highlighted the need for combining them with the number of contour minima and contour maxima for correct classification. Generally, DFA and CART results reflected both phylogenetic distance (especially for common and striped dolphins) and the size of the species. © 2014 Acoustical Society of America.

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I. INTRODUCTION

Most cetacean species are difficult to monitor, since they live at low density over large areas and spend much time underwater (Marques et al., 2009). Acoustic techniques can improve the effectiveness of visual monitoring by providing methods for detecting cetaceans when they are likely to be missed by visual observers (Oswald et al., 2003). Passive acoustic techniques have been employed to survey vocal marine mammals at longer ranges than is possible via visual monitoring (Carstensen et al., 2006; Mellinger et al., 2007; Oswald et al., 2007), thus increasing knowledge about cetacean occurrence (Lewis et al., 1998; Simon et al., 2010), density (Gordon et al., 2000) and habitat use (Mellinger and Barlow, 2003; Lammers et al., 2008).

A preliminary step for applying the Passive Acoustic Monitoring (PAM) techniques to a particular cetacean species is the ability to recognize that species acoustically. Odontocetes acoustic signals can be classified into three main categories: Tonal whistles, burst pulses, sounds and clicks (Janik, 2009). Generally studies involving odontocetes species identification employ spectrographic analysis of tonal whistle (Steiner, 1981; Schultz and Corkeron, 1994;...
Whistles are common signals of most odontocetes vocal repertoires and they may convey information about species identity, individual and population identity, and the behavioral state of the caller (Au et al., 2000). Whistles are thought to be important for regulating group organization (Norris et al., 1994) and maintaining social cohesion within groups (Lammers et al., 2006). The fundamental frequencies of whistles lie in the bandwidth between 800 Hz (Schultz and Corkeron, 1994) and 28.5 kHz (May-Collado and Wartzok, 2008) and durations range between 100 ms and just over 4 s (Buckstaff, 2004). In 1981, scientists began to examine inter-species variability in whistle frequency parameters, duration, and modulation (Steiner, 1981; Schultz and Corkeron, 1994; Wang et al., 1995a; Matthews et al., 1999; Rendell et al., 1999; Oswald et al., 2003, 2004, 2007; Gannier et al., 2010). More than a single factor may explain the observed variability in these whistle characteristics. As noted by Steiner (1981), taxonomic relations and morphology can be correlated to the differences in whistle characteristics among species. Gillooly and Ophir (2010) suggested that body mass and body size impose absolute constraints on the frequency and duration parameters of the acoustic signals of 500 diverse species of insects, fishes, reptiles, amphibians, birds, and mammals. Body mass correlated negatively with signal frequency and positively with signal duration. For the whistles of odontocetes, Wang (1993) hypothesized a limitation in sound production capability determined by body size, and Wang et al. (1995a) reported a negative correlation between frequency parameters and body size. Behavioral context (Norris et al., 1994; Driscoll, 1995; Herzing, 1996; Dudzinski, 1996; Janik, 2000; Acevedo-Gutierrez and Stienessen, 2004; Simon et al., 2007; Quick and Janik, 2008) and environmental and anthropogenic factors (Wang et al., 1995b; Morisaka et al., 2005) have also been shown to influence whistle characteristics within species, but the effect of these factors has not been investigated in relation to inter-specific variability.

Several different statistical approaches have been employed to classify whistles to species based on frequency, duration, and modulation parameters. Using multivariate discriminant function analysis (DFA), Steiner (1981) correctly classified the whales of five western North Atlantic (NA) odontocete species for 70% of the records; Wang et al. (1995a) correctly classified 65% of the whales of seven odontocete species from diverse geographic locations; Rendell et al. (1999) correctly classified 55% of the whales of five odontocete species from several geographic locations; Oswald et al. (2003) correctly classified 41.1% of the whales of nine Eastern Tropical Pacific (ETP) odontocete species. In contrast, Matthews et al. (1999) examined the potential for acoustic species recognition using published spectrographic measurements for ten cetacean species (nine odontocetes and one mysticete) using DFA and achieved only 28% correct classification. Employing classification tree analysis (CART), Oswald et al. (2003) correctly classified 51.4% of the whales of nine ETP odontocete species and Gannier et al. (2010) correctly classified 62.9% of the whales of five West Mediterranean odontocete species. Finally, Schultz and Corkeron (1994) employed principal component analysis and canonical discriminant analysis to highlight the differences in whistles produced by two odontocete species of East Australia.

In this paper we describe the characteristics of whistles produced by the four delphinid species most common in the Mediterranean Sea: Bottlenose dolphin (Tursiops truncatus), long-finned pilot whale (Globicephala melas), short-beaked common dolphin (Delphinus delphis), and striped dolphin (Stenella coeruleoalba). We then use DFA and CART to examine how the acoustic parameters of the whistles of these Mediterranean odontocete species can be combined and employed for acoustical species identification. Using DFA and CART allows comparisons with previous studies.

Mediterranean odontocetes are morphologically (Calzada and Aguilar, 1995; Archer, 1997), genetically (Garcia-Martinez et al., 1995, 1999; Hoelzel et al., 1998; Natoli et al., 2004; Valsecchi et al., 2004), and acoustically (Azzolin, 2008; Papale et al., 2013) differentiated from Atlantic odontocetes. IUCN classifications for Mediterranean odontocetes range from “vulnerable” to “critically endangered” (Reeves and Notarbartolo di Scia, 2006). The ability to acoustically recognize these species would allow them to be monitored over time and space and would allow verification that undertaken management measures are effective. Recently, Gannier et al. (2010) contributed to this aim, focusing their attention on the identification of whistles produced by species in the western portion of the Mediterranean Sea. However, whistles have been shown to vary geographically (Wang et al., 1995b; Sayigh et al., 1995; Janik, 2000; Morisaka et al., 2005; Bazúa-Durán and Au, 2004; Azzolin, 2008; Azzolin et al., 2013; Papale et al., 2013), and therefore our analysis of whistles recorded in the whole basin will provide additional insights.

II. METHODS

A. Data collection

From 1994 to 2012 the most common odontocete species found in the Mediterranean Sea were recorded in different portions of the basin (Fig. 1): Bottlenose dolphin, long-finned pilot whale, short-beaked common dolphin, and striped dolphin. Each acoustic detection belonged to a distinct group of animals recorded while monitoring for cetaceans on a research vessel. Visual sightings of the recorded animals enabled the identification of the species. All acoustic detections involving more than one species present were discarded from the analysis. In order to avoid over-sampling groups or individuals odontocete whales were collected in multiple areas as suggested by Oswald et al. (2003), data collected by the following research groups were analyzed: International Fund for Animal Welfare (IFAW, United Kingdom), Alnitak (Spain), Groupe de Recherche sur les Cétacés (France), and Bioacoustics Lab of National Research Council (CNR, Italy) (Table I). Moreover, since whales produced by dolphins show great plasticity (May-
Collado, 2010), the recording sessions considered for the analysis belonged to different years of study. This allowed possible intra-species variability due to cultural factors or recording conditions to be taken into account.

Sound recordings were made using a variety of recording equipment, all of which had a flat frequency response (±1 to 3 dB) up to 24 kHz or greater (Table I).

B. Acoustic analysis procedures

Approximately 40 h of recordings, associated with 47 acoustic detections, were examined (Table I). Spectrograms were produced using CoolEdit™. Only loud and clear whistles that did not overlap extensively with other whistles and were easily detected aurally and by visual inspection of the spectrogram were considered for parameter measurement. For the purpose of this project, whistles that contained interruptions within the contour were considered “discontinuous” and not taken into account for parameter measurement. When the break in a contour was greater than 200 ms, the contours were considered to be two separate whistles (Bazúa-Durán and Au 2002).

Following Oswald et al. (2003) eight scalar parameters were obtained for each whistle through manual measurements.
C. Statistical analyses

Of the whistle contour (Fig. 2). These parameters included: (1) Beginning frequency, (2) final frequency, (3) minimum frequency, (4) maximum frequency, (5) frequency range (calculated by subtracting the values of the maximum and minimum frequency), (6) duration, (7) number of inflection points, and (8) number of steps. The definition adopted for “step” is different from the one described by Oswald et al. (2003), since “steps” were considered as all the rapid changes in frequency with few or without any continuity in frequency. The aforementioned parameters were chosen because they can be easily and reliably measured from a spectrogram and to allow comparisons of the results of this research with previous studies (Steiner, 1981; Wang et al., 1995a; Rendell et al., 1999; Matthews et al., 1999; Gannier et al., 2010). Since the low correct classification scores of previous studies may be due to the wrong or uncompleted combination of the parameters, as suggested by Janik (2009), in this study two additional parameters were added: (9) Number of contour minima and (10) number of contour maxima. Considering the whistle contour as a mathematical function, a minimum or a maximum are a point on a curve where the tangent is horizontal (Fig. 2).

III. RESULTS

Recordings from at least 10 and up to 16 different acoustic detections were analyzed for each species. A total of 874 whistles met the “loud and clear” criteria for parameter manual measurement. After frequency truncation, and omitting the whistles with frequencies above 24000 Hz (1.5% of the whistles), the dataset was reduced to 861 whistles. After a random selection of a maximum of 35 whistles for each recording session, the final number of whistles analyzed whistle.

The normality of the whistle parameters considered in this study was evaluated by applying the Kolmogorov-Smirnov Test. The results of the Kolmogorov-Smirnov Test show that: (a) Final frequency (N = 683; Z = 1.270; P = 0.080), minimum frequency (N = 683; Z = 0.995; P = 0.276), and frequency range (N = 683; Z = 1.060; P = 0.212) were normally distributed; (b) duration (N = 683; Z = 2.524; P = 0.000), beginning frequency (N = 683; Z = 2.114; P = 0.000), maximum frequency (N = 683; Z = 2.906; P = 0.000), number of inflection points (N = 683; Z = 5.607; P = 0.000), number of steps (N = 683; Z = 6.496; P = 0.000), number of minima (N = 683; Z = 7.017; P = 0.000), and number of maxima (N = 683; Z = 7.190; P = 0.000) were not normally distributed.

Statistics analyses were carried out using the software PASW STATISTICS 18.0 (SPSS Institute Inc., Chicago, IL).
TABLE II. Dataset description: Number of acoustic detections for each species; number of measured whistles = number of whistles manually measured for each species; number of whistles in scale = number of whistles manually measured for each species that do not contain energy above 24 kHz; number of whistles after random selection = number of whistles remaining after randomly selecting a maximum of 35 whistles from each acoustic detection.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recording sessions</th>
<th>Number of measured whistles</th>
<th>Number of whistles in scale</th>
<th>Number of whistles after random selection</th>
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<tr>
<td>Bottlenose dolphin</td>
<td>11</td>
<td>258</td>
<td>258</td>
<td>164</td>
</tr>
<tr>
<td>Long-finned pilot whale</td>
<td>10</td>
<td>206</td>
<td>203</td>
<td>133</td>
</tr>
<tr>
<td>Short-beaked common dolphin</td>
<td>16</td>
<td>198</td>
<td>195</td>
<td>188</td>
</tr>
<tr>
<td>Striped dolphin</td>
<td>10</td>
<td>212</td>
<td>205</td>
<td>198</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>874</td>
<td>861</td>
<td>683</td>
</tr>
</tbody>
</table>

A. Descriptive and monovariate statistics

Descriptive statistics for the ten scalar whistle variables are presented in Table III.

The parameters that describe the shape of the whistle contour (number of steps, number of inflection points, number of contour minima, number of contour maxima) show the highest coefficients of variation for all the species (CVs from 82 to 179). Of the four studied species, whistles produced by a long-finned pilot whale showed the highest coefficients of variation and the lowest mean values for frequency parameters. Short-beaked common dolphin showed the highest mean values for most of the frequency parameters (beginning, final, minimum frequency), while a striped dolphin showed the highest mean values for maximum frequency and a bottlenose dolphin for frequency range. Concerning whistle duration, the bottlenose dolphin and long-finned pilot whale had the highest and the lowest mean values, respectively. The bottlenose and striped dolphin had the highest and the lowest coefficients of variation, respectively. The bottlenose dolphin generally had high mean values and low CVs for modulation parameters, while striped and short-beaked common dolphins generally had low mean values and high CVs for the same parameters.

B. Monovariate statistics

The results of the monovariate non-parametric analysis (Kruskall-Wallis Test) are presented in Table III. All parameters were significantly different among the four species.

TABLE III. Descriptive statistics for whistle parameters produced by bottlenose dolphins, long-finned pilot whales, short-beaked common dolphins, and striped dolphins. Key of abbreviations for parameters: Beg. Freq. = beginning frequency; Final Freq. = final frequency; Min. Freq. = minimum frequency; Max Freq. = maximum frequency; Freq. Range = frequency range; No. I.P. = number of inflection points.

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration (s)</th>
<th>Minimum freq. (kHz)</th>
<th>Mean freq. (kHz)</th>
<th>Maximum freq. (kHz)</th>
<th>Frequency range (kHz)</th>
<th>No. I.P.</th>
<th>No. steps</th>
<th>No. minima</th>
<th>No. maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottlenose dolphin (N = 164)</td>
<td>Range</td>
<td>2.66</td>
<td>18.122</td>
<td>9816</td>
<td>17.844</td>
<td>18.115</td>
<td>15</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.09</td>
<td>18.46</td>
<td>3219</td>
<td>18.46</td>
<td>5021</td>
<td>1092</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.75</td>
<td>22.865</td>
<td>21.341</td>
<td>11.662</td>
<td>22.865</td>
<td>19.207</td>
<td>15</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Mean</td>
<td>1.04</td>
<td>8130.10</td>
<td>10.207.74</td>
<td>6165.93</td>
<td>15.250.20</td>
<td>9084.27</td>
<td>3.51</td>
<td>2.09</td>
<td>1.50</td>
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<td>Std. error</td>
<td>0.04</td>
<td>270.66</td>
<td>371.75</td>
<td>156.82</td>
<td>272.44</td>
<td>277.81</td>
<td>0.25</td>
<td>0.25</td>
<td>0.11</td>
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<tr>
<td>CV</td>
<td>53.13</td>
<td>42.63</td>
<td>46.64</td>
<td>32.57</td>
<td>22.88</td>
<td>39.16</td>
<td>89.72</td>
<td>151.74</td>
<td>92.38</td>
</tr>
<tr>
<td>Long-finned pilot whale (N = 133)</td>
<td>Range</td>
<td>2.09</td>
<td>43.686</td>
<td>21.859</td>
<td>11.267</td>
<td>20.704</td>
<td>17.972</td>
<td>18</td>
<td>18</td>
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<tr>
<td>Minimum</td>
<td>0.11</td>
<td>553</td>
<td>788</td>
<td>535</td>
<td>2704</td>
<td>274.44</td>
<td>0.25</td>
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<td>Maximum</td>
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<td>22.647</td>
<td>11.802</td>
<td>23.408</td>
<td>16.507</td>
<td>18</td>
<td>18</td>
<td>9</td>
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<td>Mean</td>
<td>0.90</td>
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<td>6983.55</td>
<td>4062.67</td>
<td>9309.56</td>
<td>5246.89</td>
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<td>1.61</td>
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<tr>
<td>Std. error</td>
<td>0.04</td>
<td>478.32</td>
<td>425.89</td>
<td>250.63</td>
<td>471.64</td>
<td>299.38</td>
<td>0.21</td>
<td>0.23</td>
<td>0.11</td>
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<tr>
<td>CV</td>
<td>46.54</td>
<td>96.99</td>
<td>70.33</td>
<td>71.15</td>
<td>58.43</td>
<td>65.80</td>
<td>104.12</td>
<td>162.25</td>
<td>117.12</td>
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<td>Range</td>
<td>2.91</td>
<td>21.134</td>
<td>18.173</td>
<td>14.197</td>
<td>15.250</td>
<td>176.94</td>
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<td>Minimum</td>
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<td>5126</td>
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<td>Maximum</td>
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<td>23.780</td>
<td>23.299</td>
<td>16.843</td>
<td>23.780</td>
<td>17.694</td>
<td>8</td>
<td>21</td>
<td>9</td>
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<tr>
<td>Mean</td>
<td>0.92</td>
<td>11902.27</td>
<td>12168.89</td>
<td>8312.84</td>
<td>16078.12</td>
<td>7765.28</td>
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<td>1.41</td>
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<td>Std. error</td>
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<td>319.64</td>
<td>274.53</td>
<td>161.97</td>
<td>220.78</td>
<td>239.63</td>
<td>0.12</td>
<td>0.19</td>
<td>0.07</td>
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<td>51.39</td>
<td>36.82</td>
<td>30.93</td>
<td>26.72</td>
<td>18.83</td>
<td>42.31</td>
<td>82.35</td>
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<td>5243</td>
<td>3226</td>
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<tr>
<td>Maximum</td>
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<td>22991</td>
<td>13512</td>
<td>22.991</td>
<td>15530</td>
<td>11</td>
<td>12</td>
<td>3</td>
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<tr>
<td>Mean</td>
<td>1.00</td>
<td>11022.64</td>
<td>11874.66</td>
<td>7989.80</td>
<td>16997.13</td>
<td>8987.46</td>
<td>1.69</td>
<td>2.20</td>
<td>0.48</td>
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<tr>
<td>Std. error</td>
<td>0.01</td>
<td>301.23</td>
<td>288.18</td>
<td>136.03</td>
<td>205.97</td>
<td>198.09</td>
<td>0.11</td>
<td>0.17</td>
<td>0.05</td>
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<tr>
<td>CV</td>
<td>31.21</td>
<td>38.45</td>
<td>34.15</td>
<td>23.96</td>
<td>17.05</td>
<td>31.01</td>
<td>91.89</td>
<td>111.84</td>
<td>152.33</td>
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<tr>
<td>Kruskall-Wallis Test</td>
<td>Chi-square</td>
<td>9.37</td>
<td>191.51</td>
<td>110.93</td>
<td>220.47</td>
<td>165.87</td>
<td>110.63</td>
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<td>P</td>
<td>0.025</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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</tr>
</tbody>
</table>
C. DFA

Using all the parameters, except frequency range, that is correlated to minimum and maximum frequency, the DFA shows that it is possible to correctly classify whistles to species in 58.5% of cases, with a percentage of correct classification greater than expected by chance alone ($X^2$ Test; $P = 0.0002$), (Wilks’ Lambda = 0.631; $F = 132.029$; $P < 0.001$).

Correct classification scores for individual species ranged from 50.5% for the short-beaked common dolphin to 75.4% for the long-finned pilot whale. Because four species were included in the DFA, there were three canonical discriminant functions (Table IV). The first function explained 77.8% of the variance, the second 15.7%, and the third 6.4%. Seven parameters contributed to the three discriminant functions (Table IV). The most important parameters in the first function were maximum frequency (0.598) and minimum frequency (0.554), two parameters with a low CV for all species. Duration (0.407), number of minima ($-0.293$), and number of maxima ($-0.278$) were also important in this function. Maximum frequency (0.792) and number of maxima (0.644) were the most important parameters in the second function. Number of minima (0.964) was the most relevant parameter in the third function.

An examination of misclassification scores in Table V suggests similarities in whistles produced by the short-beaked common dolphin and the striped dolphin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Functions</th>
</tr>
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<tr>
<td>Duration</td>
<td>0.407</td>
</tr>
<tr>
<td>Beginning frequency</td>
<td>-0.039</td>
</tr>
<tr>
<td>Minimum frequency</td>
<td>0.554</td>
</tr>
<tr>
<td>Maximum frequency</td>
<td>0.598</td>
</tr>
<tr>
<td>Number of steps</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of minima</td>
<td>-0.293</td>
</tr>
<tr>
<td>Number of maxima</td>
<td>-0.278</td>
</tr>
</tbody>
</table>

D. Classification trees

Using all the parameters, except frequency range, the optimal classification tree consisted of seven terminal nodes and produced an overall correct classification score of 58.1%, that is greater than the 25% expected by chance alone ($X^2$ Test; $P = 0.0001$). Correct classification scores for individual species range from 43.1% for short-beaked common dolphins to 69.7% for a striped dolphin (Table VI). Maximum and minimum frequencies were the most important discriminating parameters, as judged by their performance as primary and secondary splitters (Fig. 3). The number of maxima and number of minima were also important parameters for species classification.

E. Correlation among mean body size and frequency parameters

Mean body sizes were attributed to species according to Notarbartolo di Sciaara and Demma (1994) who reported the following mean lengths for Mediterranean odontocetes: Bottlenose dolphin, 3 m; long-finned pilot whale, 5.5 m; short-beaked common dolphin, 2 m; striped dolphin, 2.2 m. Statistical analysis highlights that minimum (Linear correlation; $P = 0.000$; $y = -1206.25 - 0.568x$; $R^2 = 0.321$) and maximum frequencies (Spearman’s Rho Test; $P = 0.000$; Correlation Coefficient = $-0.368$) were both negatively correlated with the mean predicted body size for the examined species.

IV. DISCUSSION AND CONCLUSIONS

This paper investigates characteristics of the whistles of the most common species of Mediterranean odontocetes: Bottlenose dolphin, long-finned pilot whale, short-beaked common dolphin, and striped dolphin. For the first time acoustic data have been collected in the Mediterranean Sea on a basin scale, and with a sampling frequency up to 48 kHz or greater. A comparison of whistle parameters...
measured during this study with whistle parameters measured by other researchers in other locations suggests the existence of geographic variation in whistle structure for these species. For example, for the bottlenose dolphin, Mediterranean whistles were shorter in duration and lower in minimum, maximum, and beginning frequency than those reported for bottlenose dolphins in the ETP (Oswald et al., 2003) and NA (Steiner, 1981). For the long-finned pilot whale, characteristics of the whistles recorded for this study were similar to those presented by Rendell et al. (1999) for their single Mediterranean acoustic detection. Mediterranean whistles had similar duration, higher frequency (minimum, maximum, beginning, and final), and a greater number of inflection points compared to those recorded in the NA (Steiner, 1981; Rendell et al., 1999). For the short-beaked common dolphin, Mediterranean whistles were similar to those recorded in the ETP (Oswald et al., 2004). For the striped dolphin, whistles recorded in the Mediterranean were slightly longer, with higher beginning frequency, maximum frequency, and frequency range and lower final and minimum frequency compared to those recorded in the ETP (Oswald et al., 2004).

Geographic variation in the acoustic features of whistles has been investigated for several odontocetes species, including bottlenose dolphin (May-Collado and Wartzok, 2008; Morisaka et al., 2005; Hawkins and Gartside, 2010;
Baron et al., 2008; Wang et al., 1995b), striped dolphin (Azzolin, 2008; Azzolin et al., 2013; Papale et al., 2013), Atlantic spotted dolphin (Stenella frontalis, Baron et al., 2008), spinner dolphin (Stenella longirostris, Bazúa-Durán and Au, 2004), short-beaked common dolphin (Azzolin, 2008; Griffiths, 2009), killer whale (Orcinus Orca, Ford and Fisher, 1983), Guiana dolphin (Sotalia guianensis, Ross-Santos and Podos, 2006), false killer whale (Pseudorca crassidens), Risso’s dolphin (Grampus griseus), long-finned pilot whale, and short-finned pilot whale (Globicephala macrocephalus) (Rendell et al., 1999). Geographic variation in signal acoustic structure likely results from the combination of genetic and environmental characteristics, both physical and social. Moreover, acoustic parameters are under morphophysiological constraints and different selective pressures (Gerhardt, 1991). Isolation due to geographic or cultural factors can lead to acoustic diversification (Janik and Slater, 2000). Significant genetic differentiation has been detected between the Mediterranean and the Atlantic populations of short-beaked common dolphin (Natoli et al., 2008), striped dolphin (Bouret et al., 2007; Garcia-Martinez et al., 1999), and bottlenose dolphin (Natoli et al., 2005). Differences among the whistles of Mediterranean and Atlantic populations have been already highlighted for bottlenose dolphin (Azzolin, 2008) striped dolphin (Papale et al., 2013), short-beaked common dolphin (Azzolin, 2008), and pilot whale (Rendell et al., 1999). It would be valuable to include ETP datasets in future comparisons.

An examination of the characteristics of whistles of the four examined species highlights the fact that the whistles produced by the long-finned pilot whale had the lowest mean frequency values, while the short-beaked common dolphin and striped dolphin had the highest mean values for most of the frequency parameters. This pattern is consistent through all previous studies including these species (Steiner, 1981; Oswald et al., 2004; Gannier et al., 2010).

In this study, a negative correlation was found between mean body size and minimum and maximum frequency for all four species. As a result, a strong morphophysiological constraint on frequency parameters can be hypothesized. Acoustic signals in which elements are correlated with measures of body size are prevalent among mammals (Clutton-Brock and Albon, 1979; August and Anderson, 1987; Gouzoules and Gouzoules, 1990), amphibians (Davies and Halliday, 1978; Robertson, 1986), and birds (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 of insects, fishes, reptiles, amphibians, birds, and mammals (Gillooly and Ophir, 2010), with body mass correlating negatively with signal frequency and positively with signal duration. Mager et al. (2007) report that for birds a negative correlation exists among frequency parameters and body mass and conditions. Frequency parameters have been shown to be related to body length for several marine mammal species (Wang, 1993; Wang et al., 1995a; Matthews et al., 1999). Furthermore, the coefficient of variation of frequency parameters is low while it is high for the modulation parameters that are likely to be more dependent on the complex eco-ethological characteristics of the species (Rendell et al., 1999).

This paper describes also how the acoustic parameters of whistles can be combined and employed for acoustically classifying Mediterranean odontocetes species. According to Janik (2009), the recognition of species is one of the most fundamental functions of animal communication signals. It is also an essential prerequisite for the PAM of cetaceans. The element still lacking for the application of this technique in the field is the identification of functional parameters of a chosen vocalization for the recognition of species. A main goal of this study was to manually analyze the whistle features of four Mediterranean odontocete species to find a combination of parameters that would be useful for their identification. In order to capture more of the geographic and temporal variability in whistle structure that may exist within species, data were recorded in more than one sub-basin of the Mediterranean Sea and during several years of monitoring. The monovariate statistical analysis performed in this study highlights that all the considered whistle parameters were significantly different among the four studied odontocete species. The results of DFA and CART are comparable. Both statistical methods correctly classified whistles to species in 58% of cases. The DFA and the CART show that minimum and maximum frequencies were the most important parameters for species classification. Duration and the two new modulation parameters considered in this study (number of minima and number of maxima) were also relevant for species classification. Correct classification scores were slightly lower for the short-beaked common dolphin and the bottlenose dolphin, medium for the striped dolphin, and higher for the long-finned pilot whale with both statistical methods.

From a global point of view, the long-finned pilot whale and short-beaked common dolphin are, respectively, the most and less distinct species, as already highlighted by previous studies (Steiner, 1981; Oswald et al., 2004; Gannier et al., 2010). DFA and CART results reflect both the influence of the morphology of the species, as well as phylogenetic distance among species, especially for common and striped dolphin (May-Collado et al., 2007a,b). The misclassification of the short-beaked common dolphin and the striped dolphin as each other could be a consequence of their similar size, since the frequency parameters, which are the more relevant for species classification, are linked to this morphological feature, or to their closeness in phylogeny. A cause of misclassification of the short-beaked common dolphin with the striped and bottlenose dolphin could be also the adaptation of the whistle features to the similar environment that these three species live in. In the Mediterranean Sea the short-beaked common dolphin can be found in both inshore and offshore waters (Notartbartolo di Sciara and Demma, 1994), the striped dolphin is typically found in productive, open waters beyond the continental shelf (Forcada et al., 1994), and the bottlenose dolphin is the most common species of the continental platform (Notartbartolo di Sciara, 2002). In Mediterranean inshore waters the short-beaked common dolphin may be observed together with the bottlenose dolphin, while in offshore waters it may be associated
with the striped dolphin (Bearzi et al., 2003; Frantzis and Herzing, 2002).

As suggested by Oswald et al. (2004) and Gannier et al. (2010), future work should include exploring the use of new variables that have lower within-species and higher among-species variability, and ought to focus on all possible combinations of parameters describing whistling, to assess how these features have an effect on the process of species recognition.

The use of artificial neural networks, such as artificial neural networks, may be another way to increase the accuracy of whistle classification. Artificial neural networks operate in a non-linear, self-organizing way and therefore may be able to detect differences among species that would be missed by other statistical methods (Deecke et al., 1999). Artificial neural networks have been successfully utilized to recognize the calls of bowhead whales (Potter et al., 1994) and to measure the similarity of discrete calls of killer whales (Deecke et al., 1999).

An alternative approach for acoustically classifying species is to consider a type of vocalization other than whistles, such as clicks. In fact, Roch et al. (2011) recently showed that clicks can be useful for the identification of some odontocete species in some locations. However, the frequency bandwidth necessary for analyzing clicks is much higher than the optimal bandwidth for whistles, leading to storage and other problems for future PAM settings.

In order to improve the correct classification score for each species, other variables not necessarily related to a single vocalization (whistle or click) could be taken into consideration. A potential method for increasing the probability of correctly identifying whistles in the field is the use of classification models that take species distribution into account, as well as variables related to the depth of the acoustic detection, the whistle rate, etc. In the current DFA and CART models, each whistle is assigned to species without considering whether that species is common, rare, or even absent in the specific area where the whistle was recorded. Taking species distribution into account, and including additional variables describing acoustic encounters could improve classification capabilities.

In the future the results of the manual analysis presented here could be implemented into real-time software databases, improving their capability and robustness in classifying species for their PAM.

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