Biphonic calls as signature whistles in a free-ranging bottlenose dolphin

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Abstract

Biphonic signals are non-linear phenomena occurring in the repertoire of several animal groups and widespread across mammalian species. Although the mechanism of production is unclear, an adaptive meaning was suggested by their communication function, such as group or individual recognition. We document the rare recording event of a biphonic whistle emitted within a free-ranging population of bottlenose dolphins in the waters of southwestern Sicily, Italy. The whistle was recorded in three different years, always when a photo-identified individual was present. A quantitative description of the signal is provided. The signal presents some unique characteristics in its frequency modulation pattern which is stable for a long period. Furthermore, the synchronized beginning of the two fundamental frequencies, the signal repetition within few seconds and in its emission in freely interacting contexts suggest that biphonation is neither temporary nor involuntary. Also, we propose that biphonation can be produced via multiple mechanisms in bottlenose dolphins and that Non Linear Phenomena could represent natural recognizing marks that play a role in communication between bottlenose dolphins.

Running Title

Biphonation in free-ranging bottlenose dolphins

Key words

Biphonation, non-linear phenomenon, signature whistle, bottlenose dolphin, *Tursiops truncatus*, Sicily Channel

Introduction

Biphonation is a non-linear phenomenon (NLP) consisting of the simultaneous occurrence of two independent fundamental frequencies (Wilden et al. 1998, Fitch et al. 2002, Tokuda et al. 2002, Volodina et al. 2006). Biphonation events in animal vocalizations are relatively common and have been described for fishes (Rice at al 2011), frogs (Feng et al. 2009), and birds (Fee et al. 1998, Fletcher 2000, Fletcher 2010, Digby et al. 2014). They are also widespread across mammalian taxa such as primates (Owren and Rendall 2001, Riede et al. 2004, 2007), dogs (Wilden et al. 1998, Volodin et al. 2002, Volodina et al. 2006), deer (Facchini et al. 2003), cetaceans (Tyson et al. 2007, Filatova et al. 2009, Nemiroff et al. 2009), and manatees (Mann et al. 2006).

Nevertheless, the mechanisms by which biphonation is produced are still poorly understood.

Across species, vocalizations may involve different sound production mechanisms, thus we can assume that biphonation events are the result of various non-linear self-oscillating systems (Mergell and Herzel 1997, Volodin et al. 2002, Wilden 1998). Desynchronization of vocal folds that are not harmonically related (Berry et al. 1994) is generally thought to produce biphonic vocal patterns. For birds, in particular, the definition of this phenomenon was split into two different events in relation to syrinx oscillation due to air flow (Zollinger 2007):

- *biphonation:* the air flows in only one side of the organ involved, but produces two independent fundamental frequencies;

- *two-voices phenomenon*: each side of the organ involved produces a distinct and unrelated fundamental frequency.

Anatomical structures could also influence the generation of a second frequency through independent vibrations of the vocal lips, as described for non human primate phonation (Brown

76 and Cannito 1995). In some cases, animals capable of producing biphonic signals use the larynx

77 and/or phonic lips (Tyson et al. 2007).

78 According to previous studies, biphonation events during vocal production are essentially

79 peripheral and therefore not under the control of the central nervous system (Fitch et al. 2002).

80 Since biphonation frequently appears in infants and in pathological individuals (including humans,

81 Herzel et al. 1995), it is still uncertain whether it could play a role in mammalian communication

82 (Wilden et al. 1998).

83 However, an adaptive meaning is apparent because of the communication function described for a

84 number of species. In social species, such as dholes and killer whales, which present complex vocal

85 communication systems, it has been proposed that biphonic signals enhance the potential for

86 recognition of individuals or pods, since their two fundamental frequencies may increase the

87 probability of identification (Volodina et al. 2006, Filatova et al. 2009, Filatova et al. 2012). Filatova

88 et al. (2009) also hypothesized their function as signals to discern group members, because

89 biphonic calls could be employed as markers of pod and a matriline affiliation.

90 Therefore, non-linear events may play a role in providing conspecifics with individual identity cues.

91 In dolphins, particularly bottlenose dolphins (*Tursiops* sp.), individual recognition can be

92 determined using signature whistles (Caldwell et al. 1990, Janik et al. 2006, Janik 2009). Signature

93 whistles have been studied extensively both in captivity (Tyack 1986, Miksis et al. 2002) during

94 temporary capture–release efforts (Sayigh et al. 1990, Sayigh et al. 1999, Fripp et al. 2005) and in

95 wild free-ranging populations (Cook et al. 2004, Watwood et al. 2005, Quick et al. 2012, Gridley et

96 al. 2013, Janik et al. 2013). In 1965, a signature whistle was defined as an individually distinctive

97 and stereotyped signal that is the predominant whistle type produced by an isolated bottlenose

98 dolphin (Caldwell and Caldwell 1965). Furthermore, dolphins were reported to use signature

99 whistles as a recognition system for maintaining contact between individuals (Smolker et al. 1993,

Janik and Slater 1998, Nakahara et al. 2011). The acoustic plasticity of these animals (May-Collado and Wartzok 2008) has been revealed in their ability to imitate vocalizations of conspecifics (Janik 2000), modify signals in relation to environmental and anthropogenic noise (Morisaka et al. 2005, May-Collado et al. 2008, La Manna et al. 2013) and emit different signals in relation to behaviour (Hawkins 2010), ecological and genetic factors (Papale et al. 2013a). Therefore, if biphonation signals could be produced by the anatomical structure of bottlenose dolphins, as well as by killer and pilot whales, and if they have identification function, we can suspect they could be found during signature whistles events.

The first evidence of the acquisition of a biphonic whistle was documented for a captive calf during the development of its signature whistle (Bojanowski 2000). Recently, Kriesell et al. (2014) reported evidence of a similar phenomenon in signature whistle production by bottlenose dolphins recorded in Namibia. In that case, the independent production of a high ascending and a lower descending fundamental frequencies was attributed to the two-voices phenomenon, as described for birds and was suggested that biphonic whistles are produced by the oscillation of both sets of phonic lips. The mechanism of sound production in dolphins remains unclear (Tyack and Miller 2002), but the most reliable hypothesis, supported by physiological experiments, identify the phonic lips as source of sound production with both the right and left lips capable of producing whistles by tissue vibration (Madsen et al. 2011). Madsen et al. (2011) suggest that the fundamental frequency is the result of the tension and mass of the source in addition to the passing air pressure. Also, the vibrating organ acts as the vocal folds of terrestrial mammals and the syrinx of birds, and some of the air sacs of the phonic lips could effect on timbre. Nevertheless, NLP could be potentially produced also by laryngeal folds. In this framework, we can suppose that different processes of biphonation are possible and may result in different biphonic signal structures.

124 In this work we document the features of a distinct biphonic event recorded in a whistle type of a

125 wild bottlenose dolphin in the Mediterranean Sea. We discuss its acoustic structure and phonation

126 mechanism, its occurrence and individual recognition function.

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128

129 **Material and methods**

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131 Data Collection

132 We acoustically recorded and collected behavioural contexts (by focal group follows, Altmann

133 1974) during 32 sightings of wild bottlenose dolphins (*Tursiops truncatus*) in 2011, 2012 and 2014.

134 Whistles were present only in 16 recordings (each corresponding to a separate sighting).

135 The study area was located in southwestern Sicily (Sicily Channel) in the vicinity of Capo Feto

136 (37°38'41.86"N- 12°31'18.90"E) and Capo San Marco (37°39'33.41"N - 12°30'53.81"E), up to 20 nm

137 from the coast. We recorded for about 13 h using a calibrated hydrophone (model 8104, Bruel and

138 Kjer, Nærum, Denmark) with a sensitivity of -205.6 dB re 1 V/l µPa ± 4.0 dB in the 0.1 Hz to 80 kHz

139 frequency band (+4 dB and -12 dB in the frequency range of 0.1 Hz to 120 kHz). The hydrophone

140 was connected to a digital acquisition card (USGH 416HB, Avisoft Bioacoustics, Berlin, Germany,

141 set at 40 dB gain) managed by dedicated Avisoft Recorder USGH software (Avisoft Bioacoustics,

142 Berlin, Germany). The signals were acquired at 300 kilosamples/s at 16 bits.

143 During the 16 sightings the mean group size was 6.93 (Sd = 3.37). Individuals were recognized

144 through photographic-identification methods (see Boldrocchi et al. 2013 for details) and 94

145 specimens were identified since 2004, while the population was estimated of 135 individuals.

146

147 Acoustic analyses

148   Recordings were analyzed using the spectrogram (time versus frequency graph) view in Raven Pro

149   1.4 (The Cornell Lab of Ornithology, Ithaca, N.Y., U.S.A.) with resolutions of 256–512 bands, 256

150   FFT size and a Hanning window. We analyzed all whistles with good signal-to-noise ratio (for

151   details see Papale et al. 2013b). We measured the same parameters described in Papale et al.

152   (2013b) to quantify the contour of each whistle.

153   We defined as biphonic whistles the signals made up of two simultaneous, but not obviously

154   interacting, fundamental frequencies (Digby et al. 2014). We considered as separate types of

155   signature whistles those that shared similar modulation contours and were repeated in bouts

156   within 1-10 s of each other (Janik et al. 2013).

157   For each biphonic signal, we evaluated if they were emitted in bouts and if the beginning of the

158   two frequency components was synchronized (table 1). We also evaluated the coefficients of

159   variation (standard deviation/mean value *100) of both components for each spectral and

160   temporal parameter, to assess variability among signals.

161

162   **Results and discussion**

163

164   Over 630 whistles were recorded in the free-ranging population, and approximately 60 whistle

165   types were identified. We found that 42 of these types were repeated in the specific 1-10 s

166   window of each other in at least the 75% of cases. One of these 42 types presented the

167   simultaneous occurrence of two independent fundamental frequencies with different contours of

168   frequency modulation in the spectrum, i.e. it was a biphonic signal. The biphonic signal was

169   identified 13 times in three recordings (three separate sightings), once in 2011 and twice in 2012.

170   None of the components of the signal was recorded alone and each one presented harmonics.

171   Details of the two fundamental frequencies are shown in Table 2 (the mean values and standard

172   deviations of each of ten parameters are considered). The signals we recorded had a repeated

173   structure and low variability among the frequency parameters (beginning, end, minimum and

174   maximum frequency). Although the coefficient of variation for the number of inflection points may

175   suggest high variability at the modulation level, this is due to the low mean value for these

176   parameters, which fluctuated around zero and one.

177   Since this particular tonal signal was recorded in different years with the same characteristics, its

178   stereotypy suggests that, in this case, biphonation is neither temporary nor randomly exhibited.

179   We found that biphonic whistles were emitted in different contexts. Twice the animals were found

180   following a trawler, while in another case, dolphins were involved in social activity and a calf was

181   present in the group. Even if the research boat could be a source of stress for an individual and

182   small changes in diving patterns can affect energy gain (Symons et al. 2014), the behavioural

183   activities of the animals were never disrupted by the presence of our vessel.

184   Exactly as Krisell et al. (2014), we found only one example of a biphonic whistle recorded over

185   multiple years. Nevertheless, we recorded that both fundamentals match temporally, which

186   means that this signal could differ in the production mechanism from the biphonic whistle

187   documented in Krisell et al. (2014). Since, as suggested for songbirds (Zollinger 2007), also

188   dolphins have two independent sound sources, these nonlinear events could be either unilaterally

189   or two-voice-produced. Even if for cetaceans these phenomena were not yet related with

190   fluctuations in air sac pressure, we can suppose that vocalizations are produced by unilateral or

191   bilateral airflow and are therefore resulting in signals characterized by spectrographically different

192   fundamental frequencies, modulated more or less independently.

193   Nevertheless, we cannot exclude the presence of internal deformities and, therefore, that this

194   whistle may be the result of some aberrance in the sound production system of a particular

195 animal. However, we never found biphonation in other whistles, either variable or showing the

196 signature pattern. The hypothesis that the signal could be an indication of poor physical condition

197 or of stress is weakened by the absence of any observation of external signs of illness in any

198 dolphin within the population, either in 2011, 2012, or in 2014.

199 A number of characteristics suggest that biphonation may play a role in communication among

200 bottlenose dolphins, as it does in several social species. First of all, non linearity is a trait of a

201 signature whistle emitted in freely interacting contexts and is characterized by a high degree of

202 stereotypy and stability of the frequency modulation pattern.

203 Furthermore, these signals were emitted in the typical signature whistle pattern (i.e. emitted in

204 bouts containing repetition within 1-10 s of each other).

205 During our observation, the pods, composed by 10.33 (SD= 1.15) adult dolphins on average (calves

206 were recorded only once) were always different except for one individual. Therefore, the emission

207 of this signal is not related to group membership and is more likely to represent an individual

208 identification signal rather than a group recognition signal, as was described for killer whales

209 (Filatova et al. 2009). In the three sightings involved, we photo-identified more than 93% of the

210 individuals. The only animal in common for all three sightings was seen only during those sightings

211 when a biphonic whistle was recorded and this signal was never collected in absence of this

212 animal. Nevertheless, since whistles can be transmitted underwater over several kilometres and it

213 is possible that the signal was produced by an animal not photo-identified, the allocation to a

214 given individual needs further studies. However, differently from previous studies, its possible

215 assignment shows that the individual could (potentially) be tracked and monitored.

216 This supports not only the individual recognition hypothesis, but also that biphonation could be a

217 component of signature whistles concurring to the distinctiveness of signals. Even if bottlenose

218 dolphin calves model their signature whistles on whistles of community members they rarely

219   interact with (Fripp et al. 2005) in order to ensure individual distinctiveness, biphonation could be

220   a characteristic strengthening individual identification, since it includes a larger amount of

221   information compared to a monophonic signal. The biphonic signal could improve correct

222   identification as hypothesized for a captive calf by Bojanowski (2000).

223   Similar phenomena have been described for penguins and dhole (Aubin et al. 2000, Lengagne et

224   al. 2001, Aubin and Jouventin 2002, Volodina et al. 2006). In these cases, biphonation was

225   suggested to provide additional cues for mate-mate and/or parent-chick recognition.

226   In a coastal bottlenose dolphin population with resident and transient (Boldrocchi et al. 2013)

227   individuals, a clear distinction from similar signature whistles could be useful and could make this

228   signal more suitable to individually recognize the emitter.

229

230

231 Conclusions

232   We provide the second description of the occurrence of a biphonic event in wild adult bottlenose

233   dolphins. Our results strengthen the hypothesis that biphonic whistles are not only produced

234   within the first year of life, as described by Bojanowski (2000), or by anomalous individuals, but

235   also during adulthood and by healthy individuals in a natural context.

236   These results support the possibility that NLPs play a communicative role, that they are produced

237   by more than one mechanism and suggest that their rarity may be partially a consequence of a

238   lack of attention. We suggest that NLPs are worth of more consideration because they could

239   potentially represent natural recognizing marks and convey relevant information to conspecifics.

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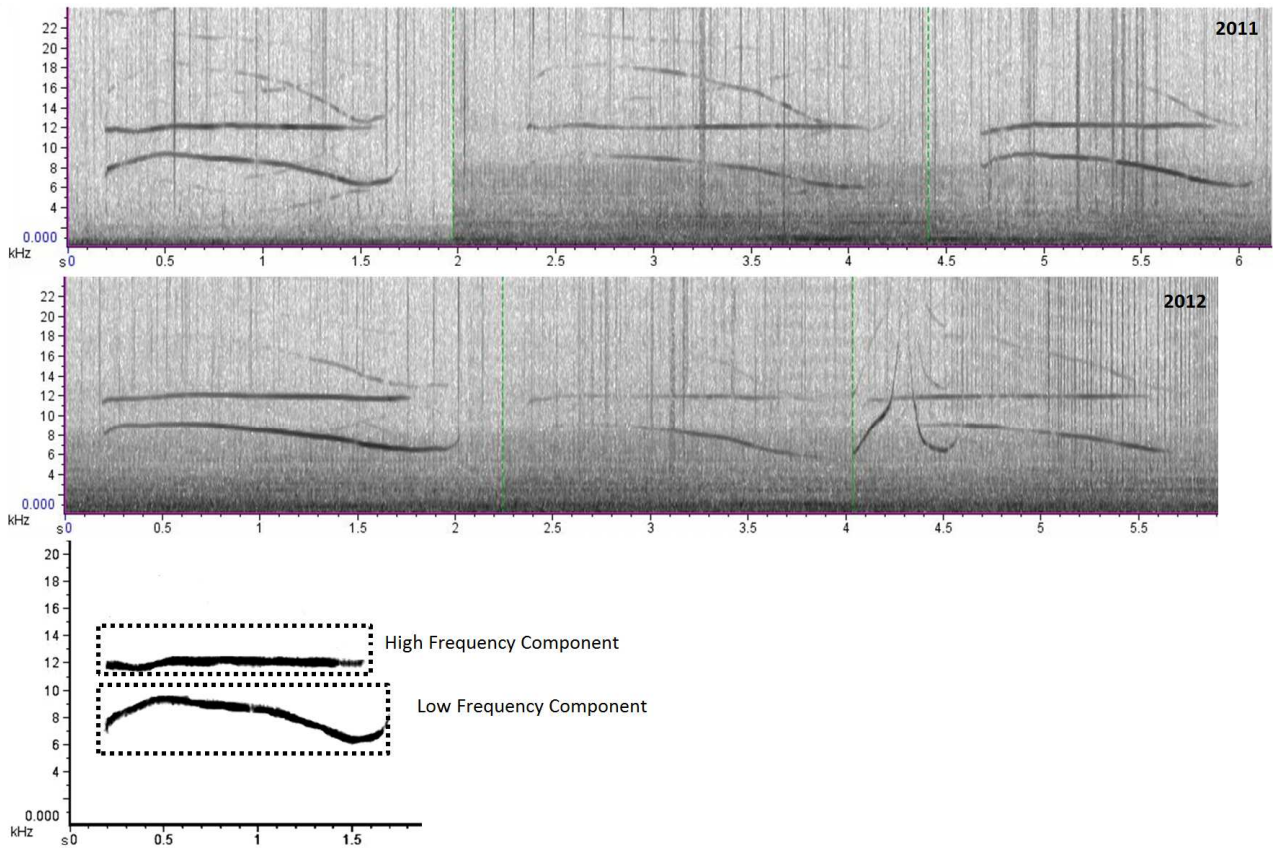
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446

447 **Figures**

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450

451   Figure 1 Three biphonic signature whistle of a bottlenose dolphin, identified in the same recordings collected in the

452   Sicily Channel, Italy in 2011 and 2012. The three whistles were repeated within 10 s from each other. In the last

453   spectrogram the High Frequency Component and Low Frequency Component are squared.

454

Number of signal	Number of Recording (sighting)	Year	Synchronized beginning	In bout
1	1	2011	Y	N
2	1	2011	Y	Y
3	1	2011	Y	Y
4	1	2011	N	Y
5	2	2012	Y	N
6	3	2012	Y	Y
7	3	2012	Y	Y
8	3	2012	Y	Y
9	3	2012	Y	Y
10	3	2012	Y	Y
11	3	2012	N	Y
12	3	2012	Y	Y
13	3	2012	N	N

455

456   Table 1 Details of the recordings in which the biphonic whistles occurred; synchronized beginning of the two

457   components and the presence of emission in bouts (occurring within the 1-10 s of each other as specified in Janik et

458   al. 2013)

Signal parameters	Mean value High-frequency component (SD)	CV	Mean value Low-frequency component (SD)	CV
Duration (s)	1.21 (0.33)	27.29	1.29 (0.36)	28.07
Beginning Frequency (Hz)	11619 (336.08)	2.89	8050 (410.01)	5.09
End Frequency (Hz)	11948 (269.14)	2.25	6994 (899.37)	12.86
Maximum Frequency (Hz)	12327 (277.61)	2.25	9189 (191.55)	2.08
Minimum Frequency (Hz)	11255 (254.45)	2.26	6347 (615.97)	9.70
Frequency Range (Hz)	1071 (227.48)	21.22	2841 (756.91)	26.64
Number of Inflection points	0.31 (0.48)	156.12	0.38 (0.51)	131.66
Number of steps	0.00 (0.00)	0.00	0.00 (0.00)	0.00

459

460   Table 2. Mean values and standard deviations of the acoustic measurements considered from the two components.

461   The coefficients of variation of both components for each parameter are also shown in the table.