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

Papale E.¹

Bioacoustics Lab, IAMC Capo Granitola, National Research Council, Via del Mare 3, 91021 Torretta Granitola (TP), Italy and Department of Life Sciences and Systems Biology, University of Torino, Via Accademia Albertina 13, 10123 Torino, Italy

Buffa G., Filiciotto F., Maccarrone V., Mazzola S.,

Bioacoustics Lab, IAMC Capo Granitola, National Research Council, Via del Mare 3, 91021 Torretta Granitola (TP), Italy

Ceraulo M.,

Bioacoustics Lab, IAMC Capo Granitola, National Research Council, Via del Mare 3, 91021 Torretta Granitola (TP), Italy and Department of Basic Sciences and Foundations, University of Urbino, Campus Scientifico Enrico Mattei, 61029 Urbino, Italy.

Giacoma C.

Department of Life Sciences and Systems Biology, University of Torino, Via Accademia Albertina 13, 10123 Torino, Italy

Buscaino G.,

Bioacoustics Lab, IAMC Capo Granitola, National Research Council, Via del Mare 3, 91021 Torretta Granitola (TP), Italy

¹ Corresponding author: Elena Papale, *Bioacoustics Lab, IAMC Capo Granitola, National Research Council, Via del Mare 3, 91021 Torretta Granitola (TP), Italy* E-mail: elena.papale@iamc.cnr.it

29 **Abstract**

30

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

44

45 **Running Title**

46 Biphonation in free-ranging bottlenose dolphins

47

48 **Key words**

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

51

52

53 Introduction

54

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

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

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

- 70 • *biphonation*: the air flows in only one side of the organ involved, but produces two
71 independent fundamental frequencies;
- 72 • *two-voices phenomenon*: each side of the organ involved produces a distinct and unrelated
73 fundamental frequency.

74 Anatomical structures could also influence the generation of a second frequency through
75 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,

100 Janik and Slater 1998, Nakahara et al. 2011). The acoustic plasticity of these animals (May-Collado
101 and Wartzok 2008) has been revealed in their ability to imitate vocalizations of conspecifics (Janik
102 2000), modify signals in relation to environmental and anthropogenic noise (Morisaka et al. 2005,
103 May-Collado et al. 2008, La Manna et al. 2013) and emit different signals in relation to behaviour
104 (Hawkins 2010), ecological and genetic factors (Papale et al. 2013a). Therefore, if biphonation
105 signals could be produced by the anatomical structure of bottlenose dolphins, as well as by killer
106 and pilot whales, and if they have identification function, we can suspect they could be found
107 during signature whistles events.

108 The first evidence of the acquisition of a biphonic whistle was documented for a captive calf
109 during the development of its signature whistle (Bojanowski 2000). Recently, Kriesell et al. (2014)
110 reported evidence of a similar phenomenon in signature whistle production by bottlenose
111 dolphins recorded in Namibia. In that case, the independent production of a high ascending and a
112 lower descending fundamental frequencies was attributed to the two-voices phenomenon, as
113 described for birds and was suggested that biphonic whistles are produced by the oscillation of
114 both sets of phonic lips. The mechanism of sound production in dolphins remains unclear (Tyack
115 and Miller 2002), but the most reliable hypothesis, supported by physiological experiments,
116 identify the phonic lips as source of sound production with both the right and left lips capable of
117 producing whistles by tissue vibration (Madsen et al. 2011). Madsen et al. (2011) suggest that the
118 fundamental frequency is the result of the tension and mass of the source in addition to the
119 passing air pressure. Also, the vibrating organ acts as the vocal folds of terrestrial mammals and
120 the syrinx of birds, and some of the air sacs of the phonic lips could effect on timbre. Nevertheless,
121 NLP could be potentially produced also by laryngeal folds. In this framework, we can suppose that
122 different processes of biphonation are possible and may result in different biphonic signal
123 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.

127

128

129 **Material and methods**

130

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 μ Pa \pm 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

Recordings were analyzed using the spectrogram (time versus frequency graph) view in Raven Pro 1.4 (The Cornell Lab of Ornithology, Ithaca, N.Y., U.S.A.) with resolutions of 256–512 bands, 256 FFT size and a Hanning window. We analyzed all whistles with good signal-to-noise ratio (for details see Papale et al. 2013b). We measured the same parameters described in Papale et al. (2013b) to quantify the contour of each whistle.

We defined as biphonic whistles the signals made up of two simultaneous, but not obviously interacting, fundamental frequencies (Digby et al. 2014). We considered as separate types of signature whistles those that shared similar modulation contours and were repeated in bouts within 1-10 s of each other (Janik et al. 2013).

For each biphonic signal, we evaluated if they were emitted in bouts and if the beginning of the two frequency components was synchronized (table 1). We also evaluated the coefficients of variation (standard deviation/mean value *100) of both components for each spectral and temporal parameter, to assess variability among signals.

161

162 **Results and discussion**

163

Over 630 whistles were recorded in the free-ranging population, and approximately 60 whistle types were identified. We found that 42 of these types were repeated in the specific 1-10 s window of each other in at least the 75% of cases. One of these 42 types presented the simultaneous occurrence of two independent fundamental frequencies with different contours of frequency modulation in the spectrum, i.e. it was a biphonic signal. The biphonic signal was identified 13 times in three recordings (three separate sightings), once in 2011 and twice in 2012. 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.

240

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245

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447 **Figures**

448

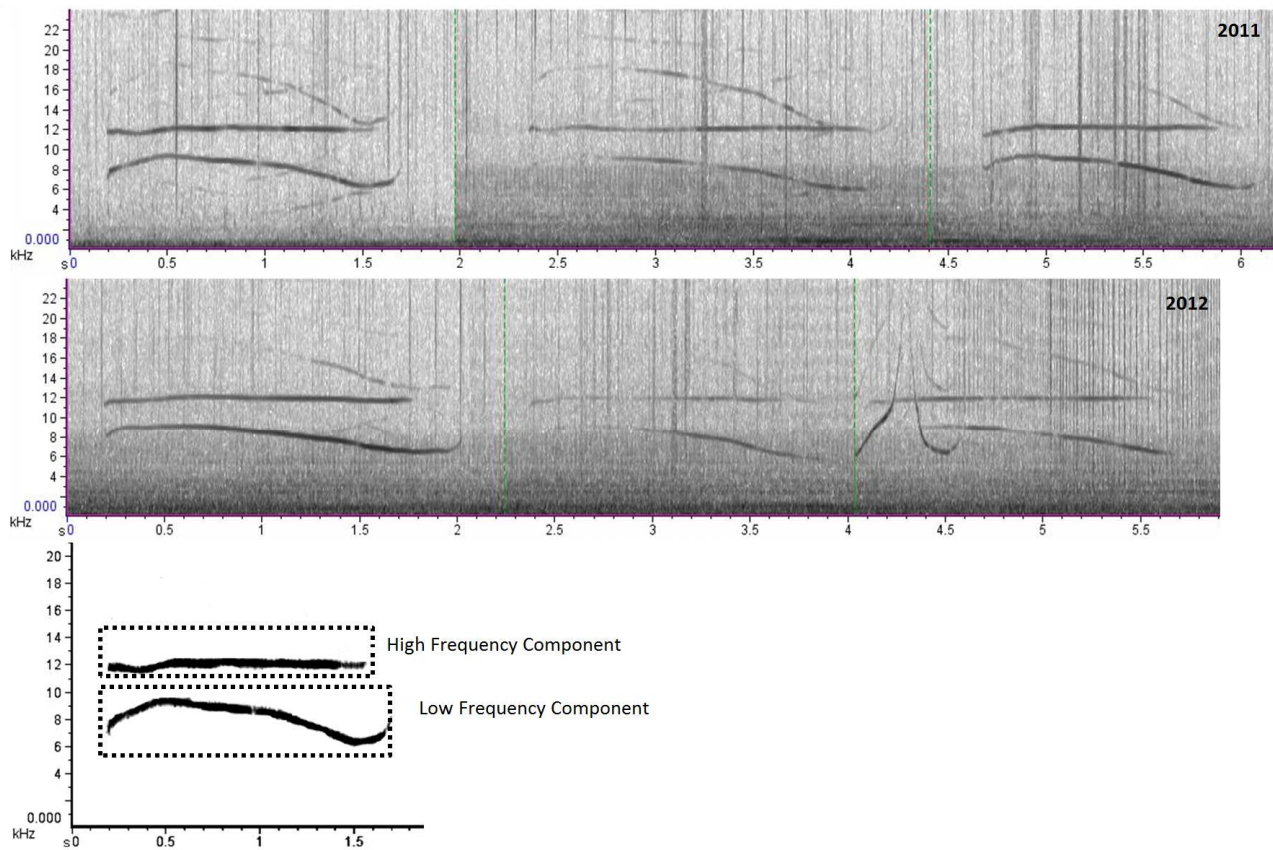


Figure 1 Three biphonic signature whistle of a bottlenose dolphin, identified in the same recordings collected in the Sicily Channel, Italy in 2011 and 2012. The three whistles were repeated within 10 s from each other. In the last spectrogram the High Frequency Component and Low Frequency Component are squared.

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

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Table 1 Details of the recordings in which the biphonic whistles occurred; synchronized beginning of the two components and the presence of emission in bouts (occurring within the 1-10 s of each other as specified in Janik et 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

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Table 2. Mean values and standard deviations of the acoustic measurements considered from the two components. The coefficients of variation of both components for each parameter are also shown in the table.