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Contexts of emission of non-signature whistles in bottlenose dolphins (*Tursiops truncatus*) under human care

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ABSTRACT

Bottlenose dolphins are social cetaceans that strongly rely on acoustic communication and signaling. The diversity of sounds emitted by the species has been structurally classified into whistles, clicks and burst-pulsed sounds. Although click sounds and individually-specific signature whistles have been largely studied, not much is known about non-signature whistles. Most studies that link behavior and whistle production conduct aerial behavioral observations and link the production of whistles to the general category of social interactions. The aim of this study was to determine if there was a correlation between the non-signature whistle production and the underwater behaviors of a group of bottlenose dolphins (*Tursiops truncatus*) under human care, during their free time in the absence of trainers. To do this we made audio-video recordings 15 min before and after 10 training sessions of eight dolphins in Boudewijn Seapark (Belgium). For the behavioral analysis we conducted focal follows on each individual based on six behavioral categories. For the acoustical analysis, carried out at the group level, we used the SIGID method to identify non-signature whistles ($N = 661$) and we classified them in six categories according to their frequency modulation. The occurrences of the six categories of whistles were highly collinear. Most importantly, non-signature whistle production was positively correlated with the time individuals spent slow swimming alone, and was negatively correlated with the time spent in affiliative body contact. This is the first analysis that links the production of non-signature whistles with particular underwater behaviors in this species.

1. Introduction

Bottlenose dolphins (*Tursiops truncatus*) are social cetaceans that exhibit a system of fission-fusion grouping patterns, in which individuals associate in small groups that often vary in composition with respect to age, sex, reproductive status and activity (Connor et al., 2000; Mann, 2000; Gibson and Mann, 2008; Tsai and Mann, 2013). In this mobile species, individuals of the same group can be separated by hundreds of meters within a habitat of limited visibility (Connor et al., 1998). Under these conditions, interactions among conspecifics based on the use of

acoustic signals seem to be the most effective strategy to assess their social and natural environments (Janik, 2009).

The diversity of the sounds emitted by this species has been classified into three structural categories and two functional classes. Structurally, the sounds emitted by bottlenose dolphins are categorized in whistles or tonal sounds (reviewed in Janik, 2009), clicks or pulsed sounds (Au, 2012), and burst-pulsed sounds (López and Shirai, 2009). Functionally, sound emissions may be used for echolocation (involved in orientation and navigation), or may play a role in communication and social interactions (Herzing, 2000).

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Whistles are continuous narrow-band frequency modulated signals that range from 800 Hz to 28.5 kHz and last between 100 ms up to over 4 s (Evans and Prescott, 1962). These sounds are associated with various social situations, with some whistles being individual-specific (Caldwell et al., 1990) and functioning to maintain group cohesion (Janik and Slater, 1998). These whistles, also called “signature whistles”, have been widely studied (Caldwell et al., 1990; Janik, 2000; Janik and Sayigh, 2013; King et al., 2014). Their production rate varies with context: signature whistles can represent more than 90 % of whistles produced by temporarily restrained dolphins (Caldwell and Caldwell, 1965; Sayigh et al., 1990), between 30–70 % of whistles emitted by free ranging dolphins (Cook et al., 2004) and less than 1% in dolphins under human care (Janik and Slater, 1998). The Whistles that are not signature whistles (called “non-signature whistles”) are commonly described qualitatively by the classification of their contour shape. Shape categories include upsweep, downsweep, convex, concave and sinusoid patterns (Bazúa-Duran and Au, 2002; Hickey et al., 2009; López, 2011). A study in bottlenose dolphins suggests that whistle types are shared between individuals within a group (McCowan and Reiss, 1995).

To date, not much is known about the behavioral contexts of emission of non-signature whistles in dolphins, and the few available studies are done from the surface, taking into account only general behavioral categories such as traveling, resting, socializing and foraging (Herzing, 2015). For instance, in free-ranging bottlenose dolphins in the Mediterranean, foraging behavior was associated with sinusoid whistles, while upsweep whistles were associated with social behaviors (López, 2011).

Some studies have shown that the schedule of human-controlled periods modulates the behavior of animals and is also associated with vocalization patterns (Clegg et al., 2017). The management of bottlenose dolphins in captivity is largely based on positive reinforcement training (Laule, 2003; Brando, 2010), and often several training or feeding sessions are held per day. These training sessions can be rewarding, as animals voluntarily take part and participate in order to obtain rewards (Laule and Desmond, 1998). In the daily life of dolphins under human care, training sessions could represent outstanding events that involve feeding and interaction with humans. For example, it has been observed that anticipatory behaviors are higher before training sessions (Jensen et al., 2013), while synchronized swimming peaks shortly after these sessions (Clegg et al., 2017). With respect to the general whistle vocalization rate (signature and non-signature whistles), it has been found that upsweep whistles are mainly produced during feeding sessions, while convex and sinusoid whistles are more frequently observed before the feeding session (Akiyama and Ohta, 2007). However, detailed studies linking the dolphins’ behavioral interactions to their non-signature whistle production are still missing and might provide valuable information to better understand the communication system of dolphins.

The aim of this study was to fill this gap. To this end, we observed a group of eight bottlenose dolphins under human care to assess whether there was an association between the animals’ non-signature whistle production and their underwater behaviors. In particular, we assessed whether specific social contexts (e.g., alone vs. synchronous swimming) were associated with the production of specific non-signature whistle types.

2. Methodology

2.1. Study subjects

The study was conducted during January and February 2017 at the Boudewijn Seapark (Bruges, Belgium). The complex was inhabited by eight Atlantic bottlenose dolphins (*T. truncatus*), five adult females aged 51, 41, 32, 19 and 14 years, one adult male aged 12 years, and two calves, one male and one female, that were born at the park in the summer of 2015. Two of the adult females were born at the park, the

adult male was born at another facility and the three oldest females originated from the wild. All dolphins were subject to the same management schedule based on positive reinforcement training methods. Every day dolphins took part in six to eight training sessions approximately at the same time during which their trainers fed them after they performed several exercises aimed to facilitate the husbandry and medical care procedures and to prepare them for presentations to the public. The adult animals were familiar with 100–130 behaviors upon trainers’ commands plus the newly trained behaviors they learned. The sequence, frequency and duration of these commands changed every day in every training session. The repertoire of the animals involved underwater/aerial behaviors and solitary/group behaviors.

The data used in the present study was collected before and after the training sessions, during which time the trainers mainly stayed in the office and food preparation area and thus were not visible or audible to the dolphins. At the beginning of each training session, the trainers emerged from the food area carrying fish buckets and stood at the edge of the pool. During training sessions, the animals were divided into subgroups, with each subgroup in a different interconnected pool depending on whether they were performing exercises or not. Separations into subgroups were obtained by positive reinforcements and the animals remained within acoustical reach of one another at all times.

Overall this facility consists of five interconnected pools: a main pool, two holding pens, a medical pool and a quarantine pool (Fig. 1). The quarantine pool and main pool are connected by a channel. The depth of the pools is at least 3 m in the shallowest areas and 5.6 m at its deepest point in the main pool. The total volume is 2896 m³ or approximately 3 million liters. Training sessions with caregivers took place in all of the pools.

During the recordings, the audio-video device (see description below) was placed in the main pool, always at the same location, and the animals could move freely between the five pools. The facility was closed to the public at the time of the recordings. Between the recording sessions the animals were provided with enrichment items that were alternated on a daily basis.

2.2. Recording device

Simultaneous audio and video recordings were carried out using a waterproof 360° audio-video system known as BaBeL (Lopez-Marulanda

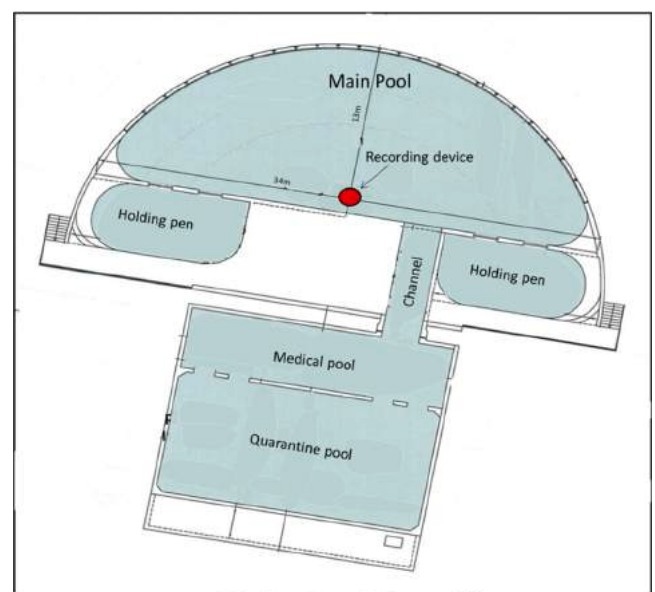


Fig. 1. Top view of the enclosure at the Boudewijn Seapark (Belgium). The location of the hydrophone array during the recordings is marked by a red dot. Animals have access to all the pools except the two holding pens.

et al., 2017). Video data were collected using one wide angle camera (GIROPTIC) consisting of three objectives that allowed a 360° range of view. This camera was positioned under the waterproof housing of a digital recorder ZOOM H6 plugged into four calibrated and automatically synchronized Aquarian H2a-XLR hydrophones. Audio recordings were conducted at a 96 kHz sampling frequency and coded on 24bits.

At the beginning of each recording session, the video and audio recordings were manually synchronized for a *posteriori* analysis with a specific video editing software (Final Cut Pro X 10.1.3 © Apple Inc.). A single video file was created from the GIROPTIC camera and was associated with one of the four audio tracks and the video of its corresponding spectrogram (FFT size: 1024, overlap 50 %, Hanning window) provided by the software Audacity 2.0.6 (GNU General Public License). The recording apparatus was placed close to the wall of the pool in order to not to block the dolphins' movements.

2.3. Desensitization process

During four weeks prior to the recording sessions, animal caregivers desensitized the animals to the presence of the recording device in the water. The desensitization process consisted of 6 steps, which were gradually built up over the weeks prior to our recordings. The first step involved placing the device on the side of the main pool, out of the water, but within sight of the animals. For the second step, an animal caregiver put the device into the water, standing on the underwater platform in the channel that connected the main show pool to the quarantine pool, manually holding the device in the main show pool. During this phase, the animals could see the device in the water, but were kept under control by other trainers. During step three, the animals were allowed to swim freely for limited times in the presence of the device, which was held by a trainer as described in step 2. These times were gradually prolonged and animals were rewarded when ignoring the device. During step four, the device was placed in the water without a trainer while the animals were kept under control. In step five, the device was left in the water while the animals swam freely and were given enrichment items to distract them from showing interest in the recording device. Finally, during the last phase, the device was frequently placed in the water by the animal trainers at different times of the day, with or without the presence of enrichment items.

2.4. Audio-video recordings

Recording sessions were carried out approximately 15 min before and 15 min after ten training sessions that took place on six days. During the recordings, the device was suspended from a buoy and kept in place at the side of the main pool (Fig. 1) by two ropes and a pole manipulated by one observer who remained at the edge of the pool avoiding visual contact with the animals.

2.5. Whistle categorization

Recorded whistles were analyzed by visual inspection of spectrograms (FFT size: 1024, overlap 50 %, Hamming window) using the software Audacity 2.06 (GNU General Public License). A graph of the spectrogram of each whistle was registered giving special attention to standardize the x- and y-axes (1 s long, with a frequency range of 0 Hz to 48 kHz) to prevent distortion of whistles caused by axes of differing length influencing the categorizing process. Whistles with a negative signal-to-noise ratio or overlapping with other whistles were not included in the categorization.

To categorize the whistles we first identified signature whistles within our recordings based on two criteria (SIGID method; Janik et al., 2013): firstly, signature whistles were whistles repeated at least four times in a recording session, and secondly, at least on one occasion the whistles were produced in a sequence in which 75 % or more repetitions occurred within 1–10 s of one other. Such whistles were not considered

in any further analyses in this paper. The whistles not categorized as signature whistles using this above described method were categorized as non-signature whistles. These were visually categorized into one out of six fundamental shapes: upsweep, downsweep, flat, convex, concave and sinusoid (with more than one inflection point).

To verify the reliability of our classification method, five experts, all affiliated to the acoustic communication team of NeuroPSI laboratory (Orsay, France) and working on bioacoustics in classification of birds or cetacean sounds, performed one visual classification tasks using the identified non-signature whistles of our dataset [see Kriesell et al., 2014]. For each non-signature whistle type, six whistle repetitions were randomly selected: one to act as a template and five to be classified by the experts. Each signature whistle repetition was surrounded by the signature whistle templates and was presented to each expert on a Microsoft Power Point slide. The experts were asked to assign to each whistle repetition the most similar template category. The ratings were compared between experts using Fleiss' Kappa statistics (Siegel and Castellan, 1988) to determine inter-observer agreement in whistle classification and consistency in categorization. When experts are in complete agreement Fleiss' Kappa statistics (*k*) is equal to 1; if agreement between experts is the same as expected by chance, then *k* is equal to 0 (Landis and Joch, 1977).

2.6. Behavioral analysis

For each video, we recorded the occurrence and duration of the most frequent social and anticipatory behaviors displayed by the animals by focal animal sampling (Altmann, 1974; Mann, 1999). We took into account the swim style (alone or synchronous), the different speeds of swim (slow: around 2 m/s or less, minimal tail beats; fast: more than 2 m/s and stronger tail beats), play, positive social body contact, agonistic, sexual and anticipatory behaviors (Table 1). The definitions of behaviors were taken from a published ethogram built to analyze the effect of training sessions on the behavior of dolphins under human care (Clegg et al., 2017). For each behavior we calculated the percentage time spent by the focal individual within the range of view of the camera (observation time).

No individual marking was applied. Each dolphin could be recognized by the use of patterns such as the general coloration of the body, patches of permanent skin discoloration, body size, body shape and

Table 1
Catalogue and definitions of behavioral variables used for this study, based on an ethogram proposed by Clegg et al. (2017).

Behavior	Description
Alone swimming	Dolphin swims at more than one body length of any other dolphin in the pool and shows no synchronous movements with its conspecifics. (slow: around 2 m/s or less, minimal tail beats; fast: more than 2 m/s and stronger tail beats)
Synchronized swimming	Dolphin swims in synchronous manner within one body length of another dolphin, showing parallel movements and body axes. Breathing can be separated maximum by 2 s. (slow: around 2 m/s or less, minimal tail beats; fast: more than 2 m/s and stronger tail beats)
Play ¹	Dolphin engages with another dolphin in a sequence of chase, bite and/or hit behaviors that end with one of the dolphins swimming erratically in the vicinity of its conspecific (Serres and Delfour, 2017)
Positive social contact	Dolphin touches or rubs another dolphin with its rostrum, its pectoral fin or any other part of its body.
Agonistic behavior ¹	Dolphin engages with another dolphin in a sequence of chase, bite and/or hit behaviors that end with the abrupt flee of one of the individuals (Serres and Delfour, 2017)
Sexual behavior ¹	Dolphin touches other dolphin genitals with any part of its body or with its own genitals.
Anticipatory behavior	Dolphin directs its look out of the water towards the arrival point of the trainers by a simple surface look, spy hopping, jumping or body slapping close to the edge of the pool.

¹ These behaviors did not occur during the observations of the present study.

notches on the dorsal fin and tail. Before the data collection began, we verified that the observer (JLM) could identify dolphins with 100 % accuracy.

2.7. Data analysis and sample sizes

Individual-based behavioral data were collected during 10 sessions prior to and 9 sessions after the training sessions. As some dolphins were not present during the observations, the total number of observations (individual observations during different sessions) was $n = 75$ prior to the training and $n = 66$ after the training. At least six individuals were present in the main pool at all times during the recordings; in four sessions, two individuals were absent from the main pool. However, when excluding these data and re-running all analyses, by and large the same results were obtained. All statistical analyses were done with R, version 3.4.1. (R Core Team, 2017). Except for the principal component analysis PCA, we always used permutation tests for the calculation of P -values. Permutation tests for linear models are well adjusted for moderate sample sizes and do not require normal distribution of model residuals (Good, 2005). However, we verified homogeneity of variances for all models (linear models LM or linear mixed-effects models LMM) by plotting residuals versus fitted values (Faraway, 2005).

We ran a PCA (R package *prcomp*; Venables and Ripley, 2002), based on the different non-signature whistles, which were found to be highly collinear (correlation matrix in Table 2). The resulting first axis was used as a non-signature “whistle score” in further statistical analyses. See more details in results.

Correlations at the group level, i.e. between the number of occurrences of the different non-signature whistle types and comparisons of the “whistle score” (dependent variable) recorded before and after the training sessions (factor with 2 levels) were tested by linear models (LM).

Comparisons at the individual level, i.e. between the duration (% time) of the different behaviors before and after the training session were tested by linear mixed-effects models (LMM) based on restricted maximum likelihood estimates, using the *lme* function of the R package *nlme* (Pinheiro et al., 2015). Furthermore, we used LMM to test for associations between the group-level pattern of non-signature whistles production (using the whistle score as obtained by PCA) and the individual-level % time the individuals spent showing the different

Table 2

: Correlations between the different non-signature whistle types, calculated by LMs. All models included the interaction with timing (factor with 2 level), i.e. whether the whistles were recorded before or after the training sessions. These interactions were never significant (all $P > 0.10$) and were removed from the models before these were re-calculated. P values were calculated by 1000 Monte Carlo permutations. The R^2 and the regression slope (based on scaled data) are provided. Upsweep whistle: A, downsweep whistles: B, flat whistles: C, convex whistles: D, concave whistles: E, sinusoid whistles: F. All the analysis was made for 8 individuals, 10 sessions prior to the training and 9 sessions after.

Dependent variable	Independent variable	Slope ($\beta \pm SE$)	R^2	P
A	B	0.799 ± 0.146	0.638	< 0.001
A	C	0.830 ± 0.136	0.688	< 0.001
A	D	0.777 ± 0.153	0.603	< 0.001
A	E	0.728 ± 0.166	0.530	< 0.001
A	F	0.770 ± 0.155	0.592	0.001
B	C	0.726 ± 0.167	0.527	0.001
B	D	0.534 ± 0.205	0.286	0.020
B	E	0.436 ± 0.218	0.190	0.064
B	F	0.479 ± 0.213	0.229	0.032
C	D	0.817 ± 0.140	0.667	< 0.001
C	E	0.516 ± 0.208	0.266	0.024
C	F	0.563 ± 0.200	0.317	0.021
D	E	0.599 ± 0.194	0.359	0.008
D	F	0.691 ± 0.175	0.478	0.003
E	F	0.933 ± 0.872	0.870	< 0.001

behaviors recorded. Analysis were done separately during the period prior to and after the training sessions. Training-session identity (thus pairing together observation sessions before and after a particular training session) and individual dolphin identity (thus allowing for repeated measurements at the individual level across different observation sessions) were used as random factors (random intercepts) in all LMMs. We used a nested random effects structure, i.e. individual identity was nested within training-session identity. P -values for LMM as well as for LM were calculated by Monte Carlo sampling with 1000 permutations, using the *PermTest* function of the R package *pgirmess* (Giraudoux, 2016).

For all significant covariate effects of LMM and GLMM, we provide the slopes (β ; based on scaled values) including their standard errors as a measure of (standardized) effects size.

3. Results

3.1. Patterns of whistles of the group

A total of 4 h 26 min (Table A, supplementary material) were recorded during the ten training sessions (2 h 30 min before and 1 h 56 min after) during which 776 whistles were identified: 95 (12.24 %) were classified as having a too low signal-to-noise ratio to be considered in this study, 9 (1.16 %) were classified as overlapping whistles, 11 (1.42 %) whistles were classified as signature whistles belonging to two different whistles types according to the SIGID method (Janik et al., 2013) (Fig. 2) and 661 (85.18 %) were classified as non-signature whistles. Only these non-signature whistles, which were visually assigned to one of the six categories (as given in Fig. 3) were considered for further analyses.

We found a very high inter-observer agreement of $k = 0.956$ between five different experts (Fleiss’ kappa statistics; $z = 28.7$, $P < 0.001$), indicating that by our visual classification the different whistle types could be reliably assigned.

3.1.1. Associations between different whistles

The most frequent category of non-signature whistle recorded in our study was the sinusoid whistle F with an occurrence of 1.12 min^{-1} , followed by the upsweep whistle A (0.52 min^{-1}), the concave whistle E (0.31 min^{-1}), the flat whistle C (0.19 min^{-1}), the convex whistle D (0.17 min^{-1}), and the downsweep whistle B (0.15 min^{-1}).

The occurrences of these different types of non-signature whistles recorded at the group level were statistically not independent, since there were various significant and positive correlations between them (Table 2). The non-significant interactions with the factor timing (factor with 2 levels; either before or after the training) indicate that these significant correlations given in Table 2 were not modulated by the timing of recording, i.e. whether the whistles were recorded before or after the training sessions.

Due to this high level of collinearity between the different non-signature whistle types, we decided to express the variation in whistle categories by a single score (from here on referred to as ‘whistle score’), calculated by the first axis of a PCA. This first axis explained 75.1 % of the variation of the data, and the eigenvalue of this axis was 4.5. All other axes had eigenvalues of ≤ 1 and thus were not considered for further analyses. The loadings of all whistle types included in the analysis were all positive (A: +0.450; B: +0.375; C: +0.409; D: +0.405; E: +0.394; F: +0.412).

3.1.2. Comparison of non-signature whistles production before and after training sessions

The whistle score, reflecting the totality of different non-signature whistles emitted by the group, was significantly higher prior to the training sessions than after the session (LM with 1000 permutations: $P < 0.001$; Fig. 4). That is, the dolphin group produced more non-signature whistles before than after the training sessions.

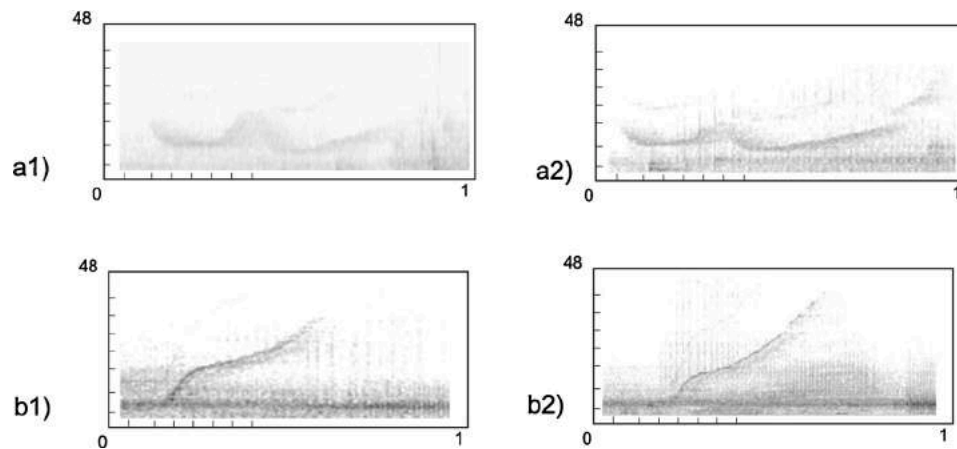


Fig. 2. Two randomly chosen spectrogram of each of the identified signature whistle types emitted by Boudewijn Seapark's bottlenose dolphins (Brugge, Belgium): (a1 and a2) signature whistle type 1 (b1 and b2) signature whistle type 2. Spectrograms are all presented in the same scaling. Frequency (kHz) is on the y-axis and ranges from 0 to 48 kHz. Time (s) is on the x-axis. FFT 1,024, Hanning window, overlap 50 %.

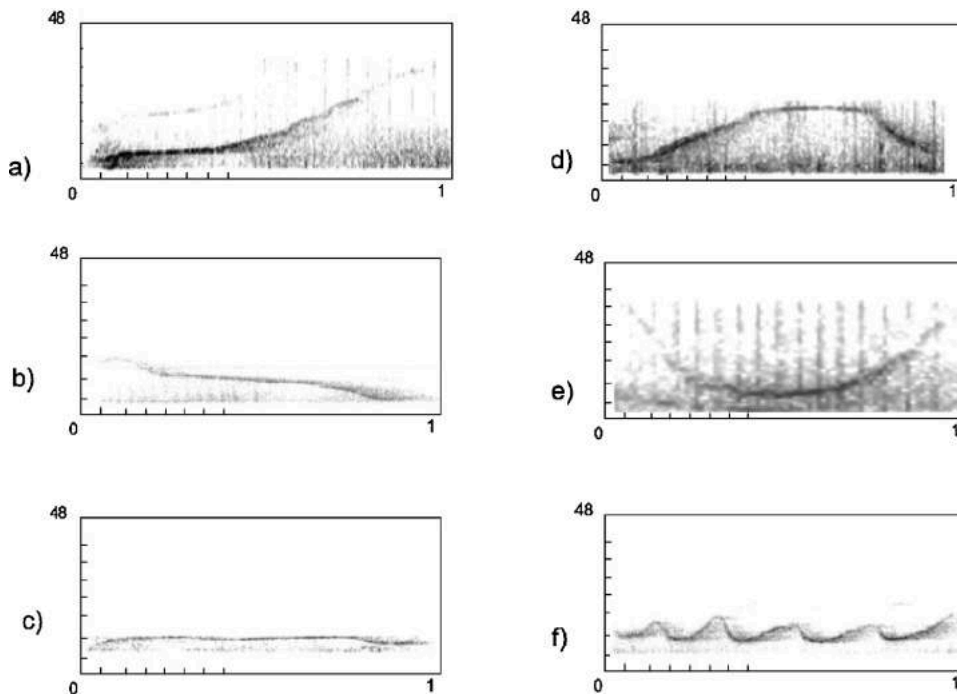


Fig. 3. One randomly chosen spectrogram of each of the identified non-signature whistles types emitted by Boudewijn Seapark's bottlenose dolphins (Brugge, Belgium): (a) Non signature whistle type A (upsweep), (b) non-signature whistle type B (downsweep), (c) Non-signature whistle type C (flat), (d) Non signature whistle type D (convex), (e) Non-signature whistle type E (concave), and (f) Non-signature whistle type F (sinusoid). Spectrograms are all presented in the same scaling. Frequency (kHz) is on the y-axis and ranges from 0 to 48 kHz. Time (s) is on the x-axis. FFT 1,024, Hanning window, overlap 50 %.

3.2. Individual-level behaviors

3.2.1. Comparison of individual-level behaviors before and after training sessions

A comparison of all observed individual-level behaviors revealed that only the % time of positive social body contacts differed significantly during the observations prior to and after the training sessions (LMM with 1000 permutations: $P = 0.027$). That is, the dolphins showed significantly more positive social body contacts after than before the training sessions.

There were some statistical tendencies indicating that the % time the animals spent swimming alone differed between the observations before and after the training session; however, this difference was modulated by the speed of swimming (Fig. 4). Fast swimming tended to be more frequent prior to the training sessions ($P = 0.051$), whereas slow swimming tended to be more frequent after the training sessions ($P = 0.055$). There were no significant differences with respect to the % time

the dolphins showed synchronous swimming, either slow ($P = 0.283$) or fast ($P = 0.544$), and the time they spent showing anticipatory behavior ($P = 0.663$; see Fig. 4). There were no significant effects of sex or of age class with respect to any of the behaviors tested (all $P > 0.10$; see details on statistics in Table B in Supplementary Material).

3.3. Associations between vocalization patterns and individual behaviors

There were significant associations between the group-level pattern of non-signature whistles (as assessed by the PCA-based whistle score) and certain of the different individual-level behaviors recorded, but only during the observations prior to the training sessions. The % time the animals spent swimming alone in a slow mode was significantly increased when more non-signature whistles were emitted by the group, as indicated by the significant and positive correlation between the % time spent swimming alone and the whistle score (LMM with 1000 permutations: $\beta = +0.387 \pm 0.179$ SE, $P = 0.017$; Fig. 5a).

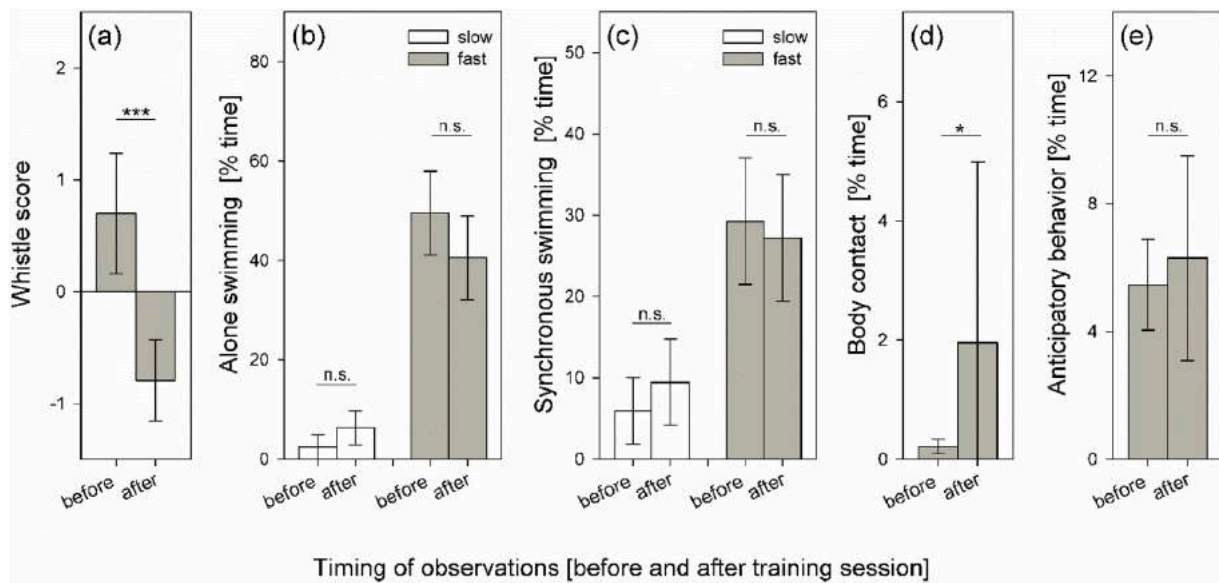


Fig. 4. Comparison between (a) the pattern of non-signature whistles at the group level (whistle score obtained by PCA, see text) and (b-e) individual-based behaviors prior to and after training sessions of bottlenose dolphins ($n = 8$ individuals, although not all individuals were observed in all sessions). Means with 95 % confidence intervals are given. Observation sessions ($n_{\text{before}} = 10$; $n_{\text{after}} = 9$) were around 15 min. Statistical comparisons by LMM; see text for details. Significant differences are indicated by asterisks (*** $P < 0.001$, * $P < 0.050$).

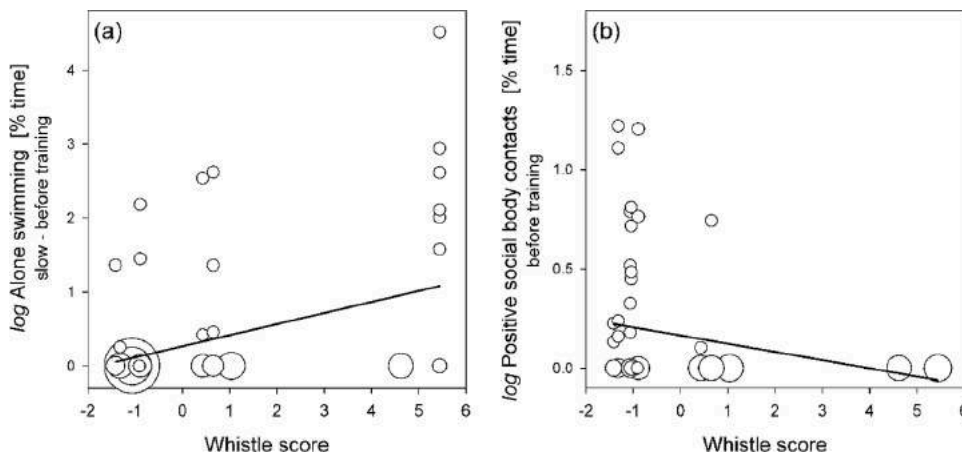


Fig. 5. Comparison between the pattern of non-signature whistles at the group level (whistle score obtained by PCA, see text) and the % time individuals (a) showed positive social contacts with conspecifics. Data are from 6 bottlenose dolphins observed during 10 sessions prior to training sessions ($n_{\text{total}} = 75$ observations). The size of dots indicate the number of overlapping cases. Parameters of regression lines obtained by LMM using log-transformation of dependent variables; see text for details.

Furthermore, the % time the animals spent showing positive social body contacts significantly decreased when more non-signature whistles were emitted by the group ($\beta = -0.286 \pm 0.120$ SE, $P = 0.014$; Fig. 5b).

Associations between the whistle score and any other behavior recorded prior to or after the training sessions were not statistically significant (all $P > 0.10$; see details in Table C in Supplementary Material). However, females showed significant more alone swim fast behavior than males ($\beta = +0.842 \pm 0.270$ SE, $P = 0.003$) and young dolphins showed significant more anticipatory behavior than adults ($\beta = +1.093 \pm 0.232$ SE, $P = 0.001$).

4. Discussion

Bottlenose dolphins produced more non-signature whistles before than after the training sessions. These whistles were mostly sinusoid whistles and upsweep whistles. Upsweep whistles have been reported as the predominant whistle type shared between individuals within the same group (McCowan and Reiss, 2001). However, in our analysis we found strong correlations between all identified non-signature whistles types. This suggests that when non-signature whistles of any type of

contour occur, other non-signature whistles are also likely to occur.

The SIGID method allowed us to identify only two signature whistles (from eight individuals) during our recordings. This could be interpreted in different ways: on the one hand, it might be possible that only two dolphins used their signature whistle during our recordings. On the other hand, different individuals might have used the same type of signature whistle. We believe that the first option (i.e. that only signature whistles of 2 individuals were recorded) is more likely, as signature whistles in captivity are sparsely produced (less than 1% of time), in particular when animals are not isolated (Janik and Slater, 1998). Most probably, more recordings would have been necessary to identify a larger variety of signature whistles from a larger number of individuals.

Sinusoid and upsweep whistles were the most frequent non-signature whistle types produced during the recordings. These two kinds of whistles have been reported as the most common ones produced by bottlenose dolphins both in captivity (Akiyama and Ohta, 2007; McCowan and Reiss, 1995; 2001) and in the wild (Hickey et al., 2009; López, 2011). Our results suggest that these kinds of whistles play an important role in the natural communication system in this species (López, 2011). However, our findings indicate that there are differences

with respect to the behavioral context of whistle emission. In captivity, sinusoid whistles have been reported in as the most common ones, predominantly produced prior to the feeding sessions (Akiyama and Ohta, 2007), while in the wild, sinusoid whistles are more frequently produced during foraging (López, 2011). In captivity, upsweep whistles are reported to be most common during feeding sessions (Akiyama and Ohta, 2007), and in the wild, they are frequently associated to the display of social behaviors (López, 2011). These findings, together with the results obtained in the present study, suggest that the use of different types of non-signature whistles strongly depends on the context. We also found that the emission of the different non-signature whistle types were highly correlated, suggesting that the production of certain non-signature whistles might elicit the emission of others.

With respect to the behaviors observed, we found that positive social body contacts were more frequent after than before the training sessions. Positive social body contacts are known to play a role in restoring friendly relationships and have been shown to reduce conflicts between bottlenose dolphins in captivity (Tamaki et al., 2006) and spotted dolphins (*Stenella frontalis*) in the wild (Dudzinski, 1998). According to this, we suggest that the time after the training session plays an important role in maintaining positive social relationships between the individuals in our study, who were separated in subgroups during training. Moreover, during our recordings, positive body contacts were only observed between mothers and calves, an interaction that has been also reported as frequent in free ranging Indo-Pacific bottlenose dolphins (*Tursiops aduncus*; Sakai et al., 2006).

Fast swimming behavior was also more frequent during the period prior to the training sessions than after. This result matches with what has been described for bottlenose dolphins in captivity in a previous study, in which high-speed swimming was concurrent with periods of high production of vocalizations (Sekiguchi and Kohshima, 2003). However, this contrasts with what has been found in a study in other facilities, in which the speed of swimming did not notably differ between the time prior and after the training sessions (Clegg et al., 2017). We suggest that different groups of dolphins might differ in their expression of behaviors according to group composition, personalities and management. We suggest that in our study, the separation of animals during the training sessions may cause a certain level of excitation of the animals, reflected in the increase of fast swimming and of non-signature whistle emission.

5. Conclusions

Our results show associations between non-signature whistle production and certain behaviors. During the time prior to training sessions, significantly more non-signature whistles were produced while individuals were swimming alone in a slow manner. Also, a lower production of non-signature whistles coincided with the occurrence of more positive social body contacts. Even if this negative correlation cannot be interpreted as a causality, we suggest that when animals are already in contact with each other, they consequently will not need to produce such vocalizations. The fact that the animals do not use signature whistles as cohesion calls under these circumstances (Janik and Slater, 1998), could be explained by the fact that the animals are situated in the same pool and are in visual contact to each other. Thus, in such case, they possibly do not need to transfer information about their identity to regroup. However, in order to investigate the function of non-signature whistles in more detail, it will be necessary to localize and identify the individual producing a certain whistle and the behavioral responses to it by its conspecifics. Moreover, it will be necessary to observe these behaviors and vocalizations in a sufficient number of other groups of dolphins under human care as well as in the wild to determine the role of different non-signature whistles.

Ethical approval

This study adhered to the ASAB/ABS Guidelines for the Use of Animals in Research, and was reviewed and accepted by the scientific and animal welfare committees of Boudewijn Seapark. Our pre-established protocol dictated that if the dolphin showed signs of frustration or annoyance, the trial was stopped.

CRedit authorship contribution statement

Juliana Lopez-Marulanda: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Heiko G. Rödel:** Formal analysis, Writing - review & editing. **Nikolaas Colpaert:** Investigation, Data curation, Writing - review & editing. **Sander Vanderheul:** Investigation, Resources. **Olivier Adam:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition. **Fabienne Delfour:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.beproc.2020.104255>.

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