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EMISSION OF SIGNATURE WHISTLES BY 8 BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) UNDER HUMAN CARE

by

Nikolaas Colpaert

Promoters: Dr. Piet De Laender
Prof. Christel Moons

Research Report as part
of the Master's Dissertation

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PREFACE

This master's thesis would not have come about without the sustained support and guidance of a great many people. Firstly, I would like to thank my supervisor, Dr. Piet De Laender, both for his invaluable feedback throughout the writing process, and for including me in a number of fascinating veterinary interventions involving marine mammals. These experiences were as enriching as I could have ever hoped for. Secondly, I would like to thank my co-supervisor, Prof. Christel Moons, for allowing me to pursue this research topic and for her insightful comments on the text that follows. Thirdly, I would like to thank the marvellous team of animal trainers at the Boudewijn Seapark for their hospitality and for putting so much effort into accommodating this research. Fourthly, I want to thank my parents Karel Colpaert and Katrien Veramme, my brother Pieterjan, my sister-in-law Laurence De Sloovere, my dear friend Floortje Vernooij and especially my partner Jack McMartin for their tireless support over the years I spent in vet school. Finally, and most importantly, I want to thank the wonderful Juliana Lopez-Marulanda. Working with her was nothing but delightful, and without her friendship and guidance this dissertation would not have been possible.

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SUMMARY IN DUTCH

Tuimelaars (*Tursiops truncatus*) zijn sociale en fundamenteel vocale dieren die geluid gebruiken in zeer uiteenlopende gedragscontexten zoals navigatie, forageergedrag en communicatie. Hun vocalisaties omvatten enerzijds klikgeluiden die voornamelijk gebruikt worden voor echolocatie en anderzijds fluitgeluiden die vooral een rol spelen in communicatie. Onder de fluitgeluiden zijn met name de zogeheten 'signature whistles' uitvoerig beschreven in de wetenschappelijke literatuur. Deze individu-gebonden fluitjes ontwikkelen tuimelaardolfijnen in hun eerste levensmaanden en ze blijven grotendeels stabiel doorheen hun leven. Tuimelaars gebruiken deze fluitjes voor zover geweten om hun identiteit en locatie mee te delen aan soortgenoten en om de cohesie van de groep te bevorderen. Omdat ze als dermate belangrijk worden beschouwd voor deze soort, worden tuimelaar-fluitgeluiden over het algemeen ingedeeld in 'signature whistles' enerzijds en 'non-signature whistles' anderzijds in de wetenschappelijke literatuur over dolfijnen.

Nauwkeurigere studies die nagaan onder welke concrete gedragscontexten deze geluiden voorkomen ontbreken echter. De reden hiertoe is dat dolfijnen geen uitwendige tekens van geluidsproductie vertonen wanneer zij vocaliseren. Hierdoor was het tot nog toe onmogelijk voor wetenschappers om vocaliserende dieren met zekerheid te identificeren wanneer meer dan één dolfijn tegelijk geobserveerd werd. Om aan dit probleem tegemoet te komen werd in 2016 door *L'Institut des Neurosciences Paris-Saclay* een apparaat ontwikkeld dat toelaat de exacte geluidsbronnen te identificeren in video-opnames onder water. Dit stelt waarnemers in staat om geregistreerde geluiden te correleren met individuele dieren en bij uitbreiding met het gedrag dat deze dieren vertoonden wanneer zij vocaliseerden. Op die manier kan de betekenis en rol van geluiden nauwkeuriger worden bestudeerd. In het voorjaar van 2017 werden met dit apparaat audio- en videodata verzameld voor een etho-akoestisch onderzoek bij de acht tuimelaardolfijnen in het Boudewijn Seapark in Brugge, België. Voor dit onderzoek werd ongeveer 30 uur aan audio- en video-opnames gemaakt vlak vóór, tijdens en vlak na trainingssessies, welke de voornaamste menselijke interacties zijn voor tuimelaars in gevangenschap. De geluids- en video-opnames werden afzonderlijk geanalyseerd en de audio-analyse is het onderwerp van deze masterproef. Naast het uitvoeren en presenteren van de audio-analyse werd voor deze thesis ook nagegaan of de gedragscontexten (pre-training, training en post-training) op zich een invloed hadden op de hoeveelheid en het type fluitgeluiden dat door de dieren werd geproduceerd. Hiermee werd gezocht naar een antwoord op de vraag of menselijke interacties het akoestisch gedrag van tuimelaardolfijnen in gevangenschap significant beïnvloeden.

Uit de opnames werden 6202 fluitgeluiden geïsoleerd, waarvan 5160 (83,2%) van voldoende kwaliteit waren om te analyseren en categoriseren. Met behulp van de 'Signature Identification Method' (Janik, 2013) werden van deze fluitjes 4917 (95,3%) als non-signature whistles ingedeeld en 243 (4,7%) als signature whistles. De signature whistles konden in zes verschillende types worden ingedeeld die worden verondersteld overeen te komen met zes verschillende dieren. Uit de statistische analyses bleek dat zowel non-signature whistles als signature whistles significant meer voorkwamen tijdens trainingssessies dan vóór en na trainingssessies. Verder werd aangetoond dat non-signature whistles significant meer geproduceerd werden vóór trainingssessies dan na training-sessies. Hieruit blijkt dat menselijke interacties het akoestisch gedrag van tuimelaars in gevangenschap significant beïnvloeden.

We stellen dat de verklaring voor de verhoogde non-signature whistles vóór trainingen te wijten kan zijn aan anticiperend gedrag en opwinding in het vooruitzicht van het krijgen van voedsel en interageren met

dierverzorgers. Anticipatie kan ook de verklaring zijn van de verhoogde non-signature whistles *tijdens* trainingen omdat de dieren niet allemaal tegelijkertijd deelnamen aan de trainingssessies, maar in kleinere groepjes na elkaar. Hierin werd ook de meest waarschijnlijke verklaring gezien voor de verhoogde signature whistles tijdens training sessies: aangezien de dieren tijdelijk opgesplitst werden in kleinere groepen is het waarschijnlijk dat zij signature whistles tijdens trainingen gebruikten om contact met elkaar te houden wanneer een aantal dieren uit elkaars gezichtsveld waren, wat als normaal gedrag voor deze soort kan worden beschouwd. De aanstaande uitgebreide etho-akoestische analyse van de verzamelde data zal deze veronderstellingen mogelijk staven.

INTRODUCTION

In the last few decades, much scientific work has been devoted to understanding the complex social behavior and communication strategies of whales and dolphins. However, studying these marine mammals in the wild poses many challenges. Firstly, finding reliable study groups can prove difficult for mobile species with dispersed distributions. Secondly, even when dependable study groups can be found for systematic observations, their submarine environment is often inaccessible, making it practically impossible to make direct observations. To overcome these difficulties, scientists have often turned to zoological facilities that exhibit marine mammals. Such settings provide reliable study groups where systematic and repeated observations can be made and fundamental research can be carried out. Since bottlenose dolphins (*Tursiops truncatus*) are the most common cetaceans kept in captivity,[1] most cetacean studies, including the present one, address this species.

The use of audio and video recordings rather than direct observations has made it possible to more accurately study dolphin behavior and dolphin vocalizations because data can be analyzed systematically by multiple observers. Despite these practical advancements, scientific studies on dolphin vocalizations are still limited by one particular problem: bottlenose dolphins generally do not show any external cues when they vocalize. Scientists have thus struggled to attribute particular recorded dolphin sounds to individual animals in video recordings, leaving much of the acoustic repertoire of the species yet to be understood. However, as data-gathering techniques improve, so too do the prospects for a more complete comprehension of dolphin behavior and communication. Eventually, these techniques may enable researchers to more effectively study bottlenose dolphins and other cetacean species in the wild.

One particularly promising technological innovation came in 2016 from researchers at the *Institut des Neurosciences Paris-Saclay*, who developed an audio-video device that accurately identifies the source of recorded sounds in simultaneous video recordings. The device is the first of its kind and opens the way for new insights into the use and meaning of cetacean vocalizations. A preliminary study to asses the functionality of this device was carried out by a team of researchers from the *Université Paris-Sud*, published by Lopez-Marulanda et al. in 2017. They concluded that the device was both functional and practical for studying bottlenose dolphin vocalizations. In a first application in an experiment setting, Lopez-Marulanda used the audio-video device to carry out data-collecting sessions at the Boudewijn Seapark in Bruges, Belgium in early spring of 2017. This implied recording the park's dolphins both during training sessions and at times when the animals were undisturbed. Analyzing the collected data involves processing the audio and video data separately and then correlating one with the other: first, the audio recordings are parsed for relevant vocalizations; second, the video recordings are transcribed into a comprehensive behavioral description of each dolphin's actions during the recordings; third, the sound and video recordings are correlated to associate individual vocalizations to individual dolphins' behaviors.

The present master's thesis discusses the acoustical portion of the study, which was carried out by the

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[1] No reliable data exist concerning the exact number of cetaceans in captivity worldwide. As of June 21, 2017, approximately 481 bottlenose dolphins, 80 beluga whales, 25 killer whales, 17 Pacific white-sided dolphins, 6 rough-toothed dolphins, 6 short-finned pilot whales, 4 Commerson's dolphins, 2 false killer whales, 1 Atlantic spotted dolphin and 1 melon-headed whales were kept under human care in the United States and Canada (Captive Cetacean Database, 2017).

author in cooperation with Lopez-Marulanda. While the acoustical analysis has been completed, the behavioral analysis is ongoing and it is not yet possible to report on correlations between particular vocalizations and behaviors. However, the same acoustical data can be used in advance of the final results to evaluate if the broader contexts in which recordings were made significantly influenced the vocal behavior of our study subjects. To this end, this master's thesis addresses the research question: 'Does human interaction during training affect the vocal behavior of the dolphins under study?' Since our data was collected directly before, during and directly after training moments, they lend themselves particularly well to this question. In what follows, we first present a literature review of the relevant work carried out on bottlenose dolphin vocalization, discuss our methodology and describe our results before moving on to a statistical analysis and discussion.

LITERATURE REVIEW

Bottlenose dolphin vocalizations

Physiology

Unlike terrestrial mammals, who produce sound waves by propelling air flows over vocal folds in their larynx, bottlenose dolphins vocalize through 'nasal complexes' located below their blowhole (Madsen et al., 2012). These complexes consist of an intricate network of nasal passages, air sacs and specialized anatomical structures called 'monkey lips' or 'phonic lips' (Fig. 1). Dolphin vocalizations are generated when air is pushed through the phonic lips in a process that is analogous to the way terrestrial mammals push air past their vocal cords (Madsen et al. 2012). This passage of air causes the phonic lips and surrounding tissues to vibrate, which results in click or whistle sounds (Cranford et al., 1996). The acoustical quality of click sounds is controlled by changing the volume and resulting resonance of the air sacs, whereas whistles are frequency-modified by changing the configuration and tension of the phonic lips (Madsen et al., 2012). After air has passed through the phonic lips, it can either be expelled through the blowhole or recycled back to the air sacs for the production of new sounds (Dormer et al., 1979). Since dolphins possess two sets of independent phonic lips, whistles and click sounds can be produced simultaneously (Fitch et al., 2002).

The melon is a lipid-rich structure that dolphins use like an acoustic lens: it allows them to focus sound beams in a specific direction (Harper et al. 2008). This ability is particularly important in echolocation, when the animals emit click sounds and register the echoes of these sounds in order to visualize their surroundings (Au, 1993). Echoes are detected through specialized structures around the lower jaw, guiding them to the animals' inner ear (Fig 1.)
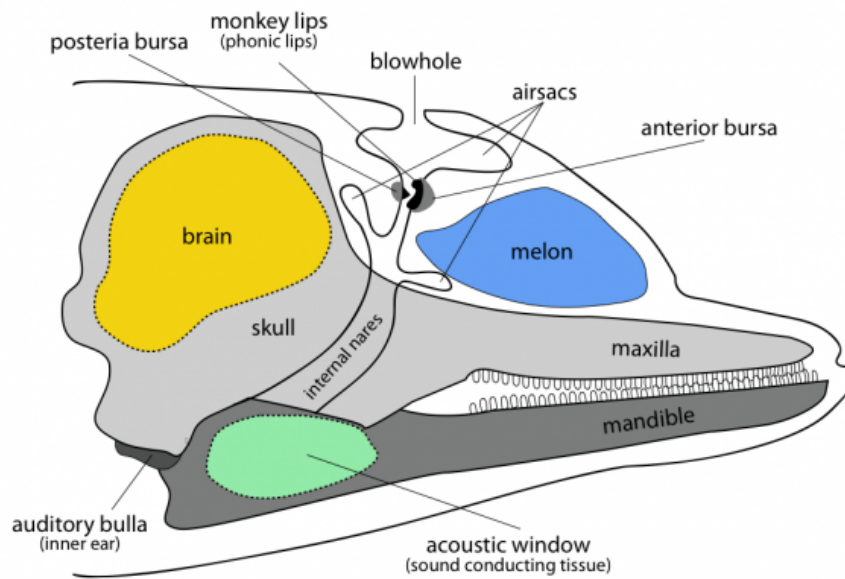
Fig. 1: *Anatomy of sound production in bottlenose dolphins. (Source: Whale and Dolphin Conservation, 2017)*

Behavioral context

Bottlenose dolphins are highly vocal animals and use sound in a wide range of behavioral contexts, including navigation, communication, foraging and identification (Herzing, 2009). Their vocalizations range from well-defined whistles and clicks to more ambiguously described sounds such as 'quacks', 'barks' and 'thunks' (Boisseau, 2005). The dependency of the species on sound for various behavioral purposes can be attributed to the submarine habitat they inhabit. In these surroundings, visibility is often limited, rendering visual perception insufficient to survive (Janik, 1999). In addition, bottlenose dolphins live in fluctuant social groups, described as a fission-fusion society (Mann et al., 2000). Such a social structure involves animals frequently leaving associated members of their species before reuniting with them and requires communication methods that function over longer distances in order to track down conspecifics (Janik, 1999). Under these circumstances, acoustic communication appears to be the most reliable strategy for maintaining contact with other individuals (Janik, 1999).

Categorizing bottlenose dolphin vocalizations

Overall, researchers have divided bottlenose dolphin sounds into three categories based on their acoustic properties: whistles (1), clicks (2) and burst-pulsed sounds (3). This division can be found in early studies like Lilly and Miller (1961) and is maintained in relatively more recent publications (e.g. Jones and Sayigh, 2002; Acevedo-Guttierez, 2004; Louis, 2016). Studies published in the decades after Lilly and Miller 1961 categorized clicks and burst-pulsed sounds under the common denominator of 'broad-band pulsed sounds' (Caldwell and Caldwell, 1977; Herman and Tavolga, 1980; Richards et al., 1984; Schultz, 1995; McCowan, 1995; Connor, 1996), but the threefold division was eventually maintained due to the fact that clicks and burst-pulsed sounds serve very different behavioral purposes (cf. infra).

From a functional point of view, the categorization of dolphin vocalizations is more ambiguous, as the behavioral purpose of a large proportion of the bottlenose dolphin vocal repertoire remains debatable. First, the category of 'burst-pulsed sounds' is still particularly open to question, as it comprises a variety of vocalizations that are used in diverse behavioral contexts. Authors have linked some of these sounds to aggression (Overstrom, 1983) or parental disciplinary behavior (McCowan, 1995), whereas other burst-pulsed sounds might be used as part of a hunting technique or in associative behavior, for example to summon pod members to a feeding site (Janik, 2000).

In contrast, the role of 'click' sounds in dolphin behavior is relatively well understood: these compact broad-band pulsed sounds are essentially used by the animals in echolocation, allowing them to navigate under water by registering the echoes of emitted clicks (Au, 1993). The animals' ability to navigate using sonar has drawn considerable scientific interest in the past and will not be further discussed in this introduction.

Finally, the last sound category of 'whistles' or 'tonal sounds' has been the subject of both considerable research and substantial debate. In particular, abundant fieldwork has been done to examine a certain type of individual-specific whistles called *signature whistles* (Caldwell and Caldwell, 1965)*. Bottlenose dolphins develop these individualized whistles as juveniles by the age of four to six months (Caldwell and Caldwell, 1970) and they can remain stable for at least 12 years (Sayigh et al., 1990). Scientists have attached so much importance to these particular whistles that the class of tonal sounds is now commonly divided into *signature whistles* on the one hand and *non-signature whistles* or *variants* on the other (Sayigh et al., 1990)*. The former will be the focus of the remainder of this introduction, which will provide a largely chronological overview of the relevant research that has been carried out with regard to these individualized sounds.

Signature whistles

Signature whistles were first described by Caldwell and Caldwell in 1965. These researchers recorded vocalizations of 5 recently captured bottlenose dolphins in a variety of situations. In each of these animals, they described "a tendency to emit the same whistle regardless of the situation" (Caldwell and Caldwell, 1965). When visualizing these recorded whistles in spectrogram graphs, they observed a distinct shape for each animal's individualized whistle. In addition, the whistles varied only slightly in number per minute, intensity, duration and the point at which started whistles were interrupted. They recorded these whistles under a variety of circumstances. These included feeding sessions, confrontation with familiar stimuli such as the introduction known pod members and unfamiliar stimuli such as unfamiliar tanks and human divers in the water. They also subjected the dolphins to 'disturbing stimuli' like dead calves from the same wild school (Caldwell and Caldwell, 1965). According to these findings, the individual-specific sound emissions comprised over 90% of the dolphins' vocalizations (Caldwell and Caldwell, 1965). Functionally speaking, Caldwell and Caldwell suggested that these whistles are used to broadcast the identity of the animal emitting them, thus inaugurating the signature whistle hypothesis.

In 1986, Tyack pointed out that Caldwell and Caldwell (1965) had merely recorded dolphins that had been forced into isolation and proposed to observe dolphin vocalizations in a relatively more natural setting. For this purpose, he made recordings of two captive animals swimming together undisturbed (Tyack, 1986). To identify

the animal that was vocalizing, he used a device called a 'vocalight' (Tyack, 1986). The device was attached to the dolphins' heads with a suction cup and used LED lights to signal when an animal was vocalizing. Like the Caldwells in 1965, he observed an individual preference for certain whistle types, but noticed that both animals also produced their tankmate's preferred whistle in addition to their own. He therefore did not only confirm the signature whistle hypothesis, but also suggested that the animals might copy each other's signature whistles (Tyack, 1986). Nevertheless, hypothesizing that the animals might share signature whistles, Tyack did not do away with the assumption that these whistles are used to convey an individual's identity to conspecifics. His research suggests that the association between a signature whistle and a particular animal might still be realized, first, by the higher frequency at which an animal emits its preferred whistle and, second, by individual acoustic features which can allow a distinction between animals even when they emit a whistle of the same contour (Tyack, 1986). He further suggests that this mimicry might actually serve one animal to address a particular conspecific (Tyack, 1986). In any case, his hypothesis of vocal mimicry or vocal modification of signature whistles based on social relationships was later corroborated in several more research studies (e.g. Janik, 2000; Watwood et al., 2004; Fripp et al., 2005).

Characteristics of signature whistles

With the existence of signature whistles firmly established, researchers in the nineties discovered that the properties of these sounds can vary according to three factors. Firstly, some of the intraspecific variation appears to be individual. Sayigh et al. (1990) described that the frequency of bottlenose dolphin signature whistles can range from 5 to 20 kHz and that the preferred number of signature whistle repetitions per emission varies with individual animals. Secondly, the age of the animals can influence their signature whistles: Caldwell et al. (1990) discovered that older dolphins produce more loops in each signature whistle compared to younger ones. Thirdly, gender was shown to be a significant factor as well. Sayigh et al. (1990) showed that male calves develop signature whistles that resemble those of their mothers, whereas female calves produce whistles that are notably different from their mothers'. A follow-up study by the same researchers about male-female differences in vocalizations confirmed these findings (Sayigh et al., 1995). This distinction between the sexes was attributed to the fact that matrilineally related females tend to reside in the same social groups after becoming independent. They therefore require to be distinctive within that group in order to facilitate contact with their own calves when they themselves start reproducing (Sayigh et al., 1995). After all, it is crucial for a species with high maternal investment that calves can clearly distinguish their mothers' calls from those of other related females. Males, on the other hand, do not face this problem and can develop signature whistles that can either be similar or different from those produced by their mothers.

Signature whistles and associated behaviors

In the wake of studies about the differences in use of signature whistles between bottlenose dolphins of certain ages and sexes, researchers started specifically investigating the *behavioral contexts* in which these sounds were emitted. Caldwell and Caldwell (1965) had already unwittingly discovered that signature whistles are often emitted in contexts of isolation, since they made recordings of animals that had only recently been captured and were therefore separated from their pod members (Caldwell and Caldwell, 1965). In addition, they individually isolated the dolphins from their study group to be able to make recordings of specific animals.

The association between signature whistle emission and the behavioral context of isolation has been confirmed in many subsequent studies. Some studies explicitly set out to evaluate this correlation by monitoring the animals' signature whistle emission rate before and after separating the animals from one another (e.g. Janik et. al. 1994). Their findings confirmed that the study subjects produced more signature whistles when separated compared to when they were swimming with their tankmates. Other studies indirectly corroborated this context by making recordings under conditions that necessarily implied forced isolation such as during brief captures of wild dolphins (Sayigh et al., 1990). Because such forced separations imply a certain degree of stress, it has been suggested that the emission of signature whistles could be an indicator of stress in the species (Esch et al., 2009). Even though this may very well be the case, researchers also noticed an increased production of signature whistles during spontaneous separations in the wild (Smolker at al., 1993) or in captivity (Janik and Slater, 1998), proving that the expression of stress does not suffice to explain the use of these sounds.

The purpose of producing signature whistles in isolation would be to convey the animals' identify (Caldwell and Caldwell, 1965) and location (Smolker, 1993) to group members. Such emissions could prompt individuals to reunite with other members in their pod: Smolker (1993) observed that the distance between mothers and calves decreased after signature whistles were emitted. Likewise, Herzing (1996) described that older dolphins retrieved younger dolphins by emitting their signature whistles. Finally, Quick and Janik (2012) observed that bottlenose dolphins also exchange signature whistles when members of different pods meet at sea, implying these sounds are not exclusively used to stay in contact with direct group members.

Other than stress, isolation and regrouping, the behavioral contexts that scientist have correlated with signature whistle emission appear to be limited. Janik and Slater (1998) describe an incident where the bottlenose dolphins in their study group produced a large number of signature whistles while not being separated from any group members. This production of signature whistles took place while the animals were facing their caretakers on the other side of underwater windows when a feeding session was delayed. According to the researchers, this incident suggests that signature whistles could be used contexts other than isolation, but they did not specifically investigate a correlation between signature whistles and such contexts as, for example, anticipation or frustration (Janik and Slater, 1998). In other dolphin species, signature whistles have been observed in notably different contexts than isolation. For example, in spotted dolphins (*Stenella frontalis*), signature whistles have been described as part of courtship (Herzing, 1996). Such findings indicate that more specific research into the behavioral contexts associated with signature whistle production could yield new insights into the purpose and meaning of these sounds.

Studying signature whistles

A common methodological difficulty in etho-acoustical studies is the classification of recorded sounds. In the case of studies about signature whistles, this proves to be particularly challenging since bottlenose dolphins produce a wide range of whistles of different acoustic contours. Signature whistles are commonly described as whistles with strikingly similar contours that are repeated in bouts (Janik, 2013). Similarity can be evaluated acoustically, but is usually assessed graphically by rendering the sound recordings in spectrograms (Fig. 2) and comparing individual whistles visually.
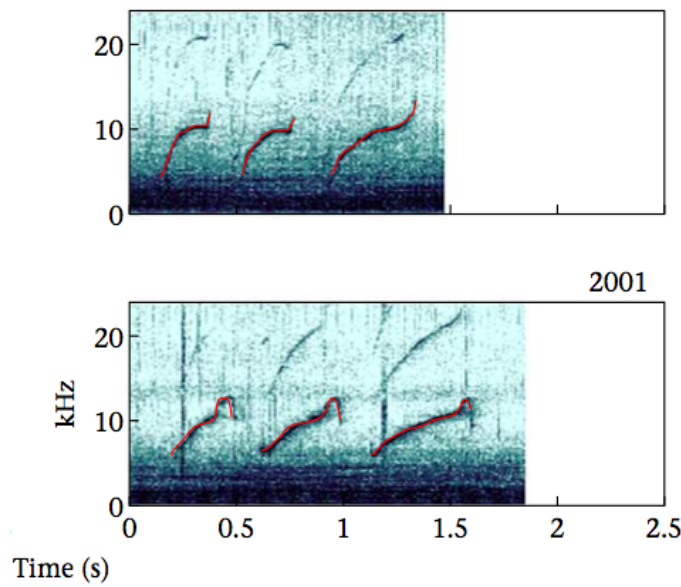

Fig. 2: Example of 2 types of signature whistles in spectrograms as recorded by Watwood et al. (2005). Sound frequency is shown in the Y-axis and time is presented in the X-axis.

Judging similarity of dolphin signature whistles can be done either by human or automated evaluation. Initially, researchers limited themselves to a classification based on a personal inspection of the spectrograms (e.g. Smolker at al., 1993; Janik et al., 1994). Particularly similar calls that were frequently repeated by the same animal were then classified as signature whistles. A more objective human evaluation was proposed by Sayigh et al. (1995). These scientists asked 74 independent human judges to score the similarity between random whistle pairs to asses their similarity. It should be noted that their goal was not to identify signature whistles from a recording that contained signature whistles and non-signature whistles. Instead, the researchers themselves first identified signature whistles from recordings and asked the independent judges to compare the similarity between those produced by dolphin mothers and those emitted by their offspring. In any case, later studies frequently employed human judges in blind classification studies to identify signature whistles by eye (Janik, 2013).

A second way to classify dolphin vocalizations is to use computer software (e.g. McCowan, 1995). Even though using a non-human categorizer might seem an ideal way to cancel out subjectivity, such programs often appear to fail in practice when compared to human observers (Janik, 2013). After all, dolphins can compress or stretch the overall pattern of their signature whistles, resulting in longer or shorter whistles but still containing the same contours. Computer software needs to be quite advanced to account for such modifications, whereas the similarity of the overall pattern will still remain noticeable to human observers (Janik, 2013).

An additional pitfall in studying signature whistles and dolphin vocalizations in general is identifying the individual animal that produces a particular sound. Since bottlenose dolphins do not show any reliable external cues when producing whistles, it is particularly challenging for observers to attribute a particular signature whistle to a specific animal. The most straightforward strategy to overcome such a problem is to record animals in isolation so that recorded sounds inherently belong to the isolated individual. Such a tactic, however, does not allow observers to record vocalizations in a natural, social setting. A rather invasive strategy to deal with this shortcoming can be found in Tyack's use of 'vocalights' (cf. supra; Tyack, 1986). Nevertheless, placing a device on a dolphin's head can be problematic and might influence the animal's behavior, leading to biased observations.

Keeping to a less intrusive method, several studies have used the release of bubble streams from the animals' blowholes as an external cue to identify whistling animals (e.g. McCowan and Reiss, 1995), basing themselves on the observation that dolphins consistently release small streams of bubbles when whistling. However, Janik (2013) pointed out that such a method leads to inaccurate results because bubble streams are primarily released when dolphins produce upsweep whistles. Since signature whistles can have a variety of contours, only relying on bubble streams to identify vocalizers creates a biased sample of upsweep whistles.

A more reliable method to identify vocalizing animals can be found in a study done by Lopez-Marulanda et al. (2017). These researchers employed an audio-video device that combined a five-hydrophone array with two HD underwater cameras. Customized software analyzes the information from all five hydrophones to precisely locate the source of particular sounds in the video recordings, allowing the observers to accurately identify vocalizing animals. Such technological advancements allow researchers to precisely associate individual sounds with particular behaviors for the first time and therefore mark a breakthrough in etho-acoustical research. Such precise observations allow for more in-depth analyses than ever before.

As we mentioned in our introduction, the present study is part of a larger project that set out to employ this new technology to identify specific behaviors associated with particular tonal sounds in a group of 8 bottlenose dolphins under human care (Lopez-Marulanda; forthcoming). This research project involves an acoustical analysis in which whistle sounds will be identified and categorized on the one hand, and a behavioral analysis on the other hand. The two data sets will eventually be correlated to each other using the information from the five-hydrophone array. The present master's thesis discusses the acoustical portion of the study, which was carried out by the author in cooperation with Lopez-Marulanda. While the acoustical analysis has been completed, the behavioral analysis is ongoing and it is not yet possible to report on correlations between particular vocalizations and behaviors. However, the same acoustical data can be used in advance of the final results to evaluate if the broader contexts in which recordings were made significantly influenced the vocal behavior of our study subjects. To this end, this master's thesis addresses the research question: 'Does human interaction during training affect the vocal behavior of the dolphins under study?' Since our data was collected directly before, during and directly after training moments, they can be used to address this question. After all, training sessions are the principal reoccurring human interactions for captive bottlenose dolphins and as such can be considered significant events in the daily lives of these animals (Lopez-Marulanda et al., 2016). Previous studies (e.g. Sekiguchi and Kohshima, 2003; Akiyama and Ohta, 2007; Therien et al. 2012; Lopez-Marulanda et al., 2016) demonstrated that human interactions significantly influence the vocalizations of captive bottlenose dolphins. Interestingly, these studies came up with different results: Sekiguchi and

Kohshima (2003), Akiyama and Ohta (2007) and Therien et al. (2012) all found increased whistle rates during human interactions whereas Lopez-Marulanda et al. (2016) observed the highest rates for signature whistles directly after rather than during training sessions. We will therefore evaluate whether the vocal behavior observed in our study subjects corroborates or contradicts past findings.

MATERIAL AND METHODS

Study subjects

The study took place between 14 February and 3 March 2017 at the Boudewijn Seapark in Bruges, Belgium. The study group consisted of six adult Atlantic bottlenose dolphins and two calves. Among the adult animals were five females, aged 51, 41, 32, 19 and 14 years and one male, aged 12 years. The calves were 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.

The dolphins at the facility are trained using positive reinforcement training methods, in which individual behaviors are gradually trained using reinforcers such as food, water squirts from spray bottles and physical and auditory contact. The adult animals are familiar with approximately 100 to 130 commands from trainers, whereas the two calves know approximately 20 to 30 taught behaviors. Behaviors include jumps, gatings,[2] waterwork with trainers,[3] body positioning for medical care, coordinated behaviors and other exercises. All animals are continuously learning new behaviors according to objectives set by medical and husbandry requirements. The dolphins each partook in six to eight training sessions every day during which various behaviors were practiced. The type of exercises, their durations and their sequence varied per training and individual. Training sessions lasted between 5 and 45 minutes, depending on the time schedule of the trainers and the type of behaviors that was practiced. Each session was led by two to three trainers. Commands were provided by hand signals from caretakers and whistles were used as secondary reinforcers to mark the instant at which behaviors were considered successfully executed. During the trainings, the animals were usually temporarily divided over the different pools, depending on whether they were performing exercises or not. The animals were guided into the separate pools using trained cues and positive reinforcements, and the animals remained within acoustical reach of one another at all times.

Boudewijn Seapark facility

The Boudewijn Seapark dolphin enclosure consists of 5 connected indoor pools: a main show pool (SP), two holding pens (HP), a medical pool (MP) and a quarantine pool (QP) with a total water volume of 2896m³ or approximately 3 million liters (Fig. 3). The quarantine pool and main show pool are connected by a channel. The depth of the pools is at least 3m in the shallowest areas and 5.6m at its deepest point in the main show pool. Although training sessions with caregivers can take place in any one of the pools, the training sessions that were recorded for analysis all took place in the main show pool. The range of the video cameras covered the show pool, whereas the audio recordings covered all of the pools since these were not acoustically isolated.

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[2] Gating refers to the animals' learned behavior of swimming through the gateways that connect the different pools of the facility.
[3] Waterwork refers to any husbandry activities where trainers enter the water with the animals.

The facility was closed to the public at the time of the recordings, with the exception of three animal presentations per week and a varying number of contact sessions such as animal therapy sessions for disabled children. However, our recordings never coincided with public presentations or therapy sessions so that no external persons were present during the recordings. Between recording sessions, the animals had free access to all five pools and were provided with enrichment items that were alternated on a daily basis.
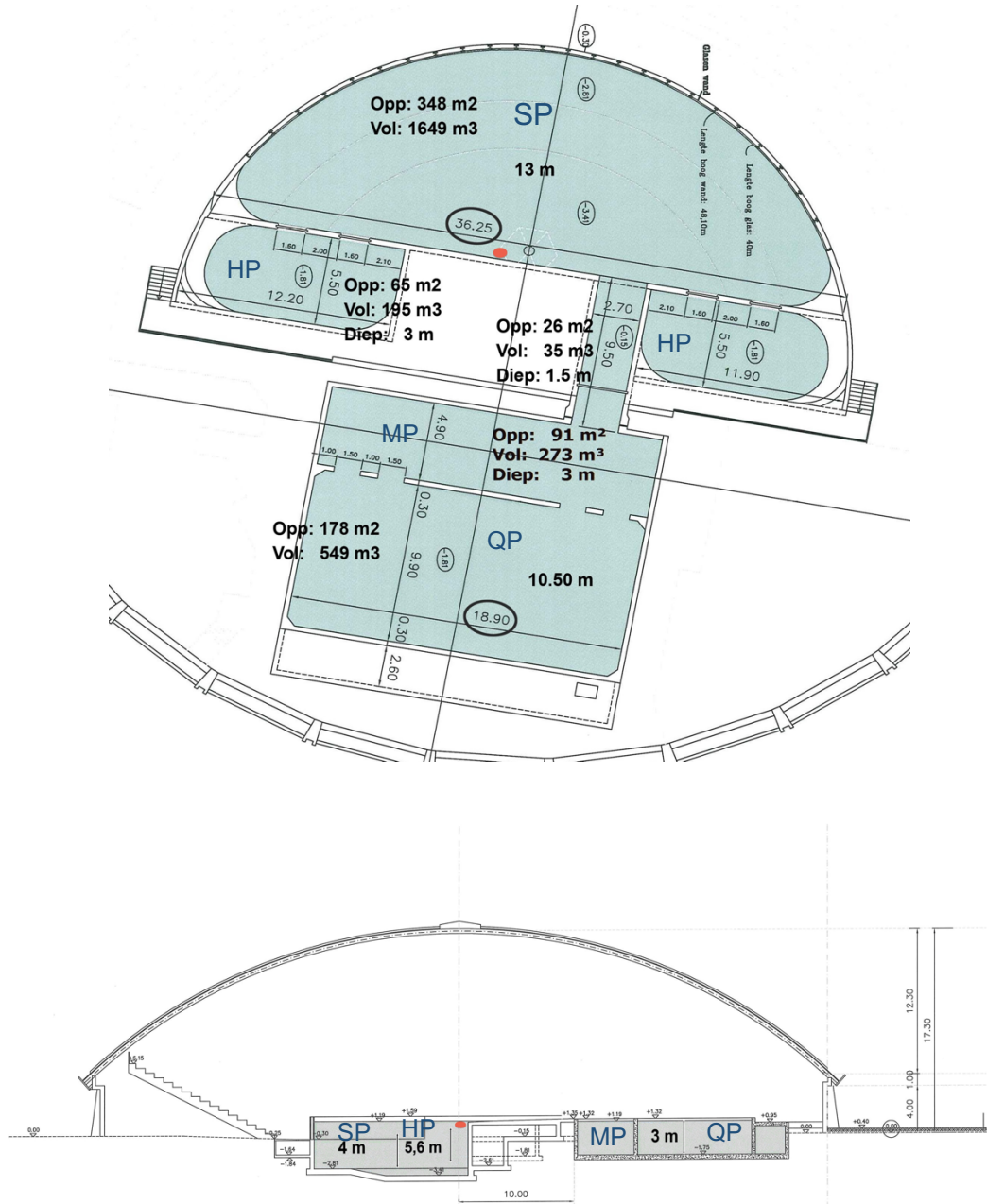
Fig. 3: Top- and side view of the enclosure at the Boudewijn Seapark. The location of the device during the recordings is marked by the red dot.

BaBeL recording device

For the comprehensive etho-acoustical analysis, audio and video recordings were carried out simultaneously using a purpose-built audio-video apparatus that combines 4 hydrophones with a 360° High Definition underwater camera (Fig. 4). This device, labeled BaBeL (BioAcoustique, Bien Être et Langage), was developed by researchers from *L'Institut des Neurosciences Paris-Saclay, Université Paris 13, Centre national de la recherche scientifique* and the *Laboratoire d'Ethologie Expérimentale et Comparée* and is a modified

version of the device employed by Lopez-Marulanda et al. (2017), our version having four instead of five hydrophones. Because of the position of the four synchronized hydrophones, the time-delay of arrival of individual sounds at each hydrophone can provide a 3D estimation of the position of vocalizing animals. In this way, individual behaviors observed in the video recordings can be accurately connected to specific sounds.
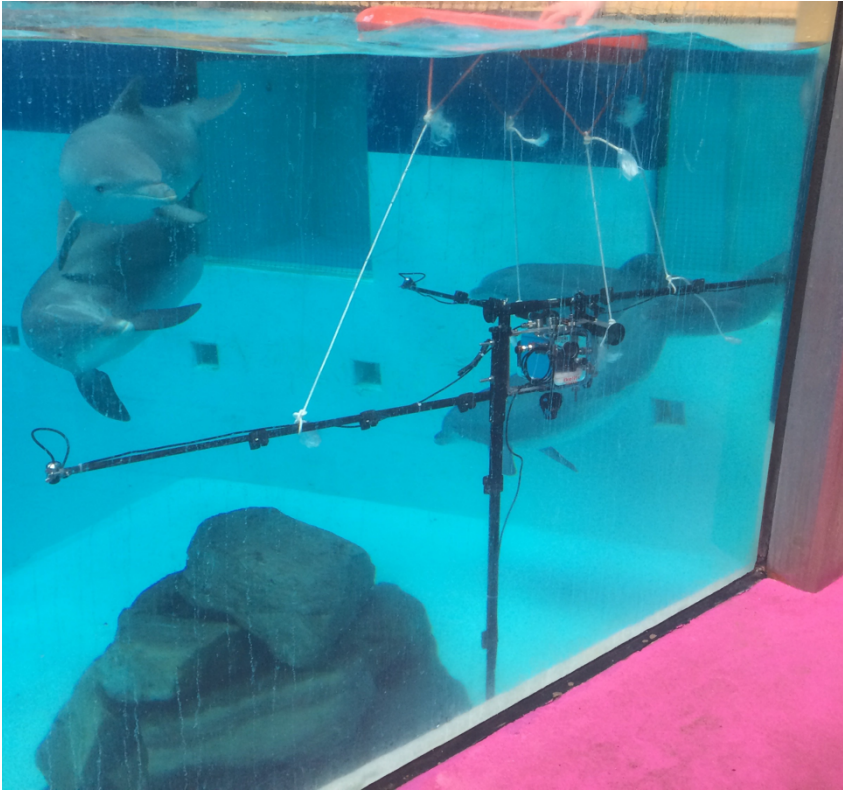

Fig. 4: *The BaBeL audio-video device at the Boudewijn Seapark*

The data that were used for the acoustic analysis presented in this master's thesis were recorded by the audio component of this device. This audio system consisted of four calibrated Aquarian H2a-XLR hydrophones coupled with a ZOOM H6 digital audio recorder. The acquisition rate for the recordings was 96 kHZ and samples were coded on 24 bits. The hydrophone array of the device was designed to have maximum aperture between the hydrophones and still allow manipulation and maintenance of the device. A waterproof case was provided to protect the recorder from water damage to depths of up to 60m.

Animal caregivers at the facility were provided with the underwater casing and hydrophone extensions 3 weeks prior to the experiment to desensitize the animals to the presence of the device in the water. The desensitization process carried out by the animal trainers 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 took the device into the water, standing on the underwater platform in the channel that connects the main show pool to the quarantine pool, holding the device in the main show pool (cf. Fig. 3). During this phase, the animals could see the device in the water, but were kept under control by other trainers. This meant the animals were rewarded for performing learned behaviors in the presence of the device but not encouraged to approach it. Thirdly, 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. For the fourth step, the device was placed in the water without a trainer while the animals were kept under control as in step two. In step five, the device was left in the water while the animals swam freely with enrichment items to distract them from showing interest in BaBeL. 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.

During the recordings, the device was suspended from a buoy and kept in place at the side of the tank by two ropes and a pole manipulated by one of the observers (Fig. 5). The location of the device during the recordings is marked by a red dot in Fig 3. Between recording sessions, the device was removed from the water and cleaned with fresh water to prevent erosion by the saline water from the tanks.
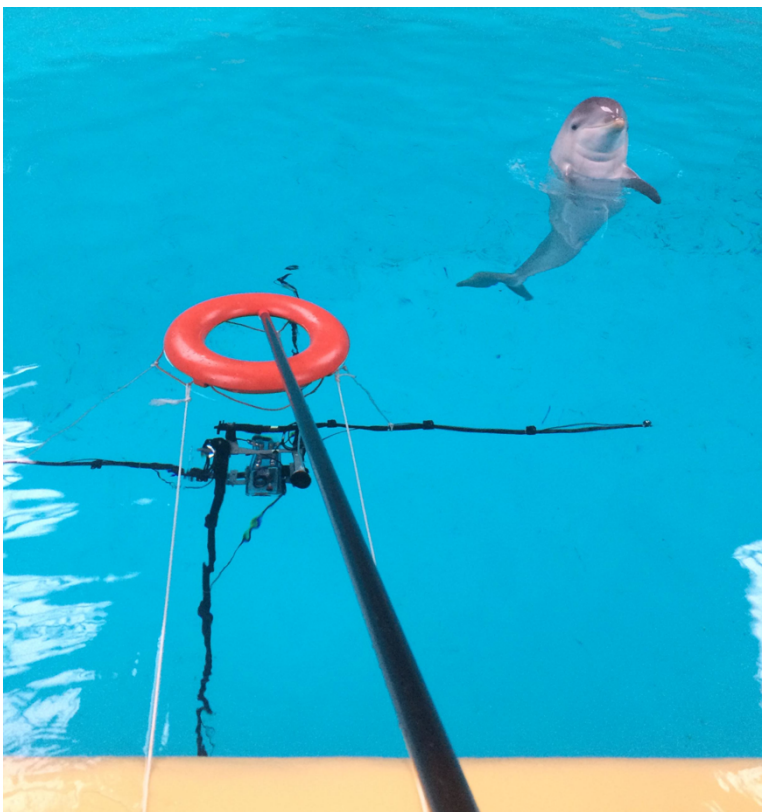

Fig. 5: The audio-video recording device was suspended from a floating buoy and kept in position by the observer by means of two attached ropes and a stick.

Recording sessions

Recording sessions were organized to take place directly before, during and after training sessions as scheduled by the Boudewijn Seapark. Pre-training and post-training times consisted of 15 minutes immediately before and after training sessions. Between pre-training and training sessions on the one hand and between training and post-training sessions on the other hand, the device was briefly removed from the water to examine the battery levels and to mark the context transitions by hand clapping. These procedures took up about 30 seconds.

Whistle analysis and categorization

In order to work towards a categorization of the whistles produced by the animals, we visualized the audio recordings into spectrograms using Audacity 2.1.2 Software (GNU General Public License, The Audacity Team, Pittsburg, PA). The created graphs covered a frequency range of 0 to 48 Hz in the y-axis and showed time intervals of 0.5 seconds in the x-axis to obtain a consistent visualization of the tonal sounds for analysis. This was done to allow a standardized comparison and categorization of the whistle frequency modulations. Whistles were individually extracted from the recordings by sight and converted into separate image and audio files. After all the whistle sounds were collected into our database, we carried out a visual categorization process to distinguish signature whistles from non-signature tonal sounds. To do so, we applied the SIGID (SIGnature Identification) method as developed by Janik et al. (2012). These researchers propose that signature whistles generally occur in bouts of whistles of the same type and that they are separated by 1 to 10 seconds, whereas non-signature whistles come about with shorter or longer intervals (Janik, 2012). Based on this finding, they set the following criteria to identify signature whistles in audio recordings: first, to qualify as signature whistles, whistles of the same signature category must occur in bouts where at least 75% of the whistles of one type occur within a 1-to-10-second whistle interval. Secondly, at least four whistles of the same type must be present in a recording session. Applying these criteria, we obtained a database with signature and non-signature whistles. Not all whistles in the recordings could be categorized, either because their signal-to-noise ratio was too low, or because they represented several overlapping whistles (Fig. 6). Low signal-to-noise ratios were usually due to interference from echolocation clicks emitted by the animals. Whistles that could not be categorized were put on record but not analyzed.
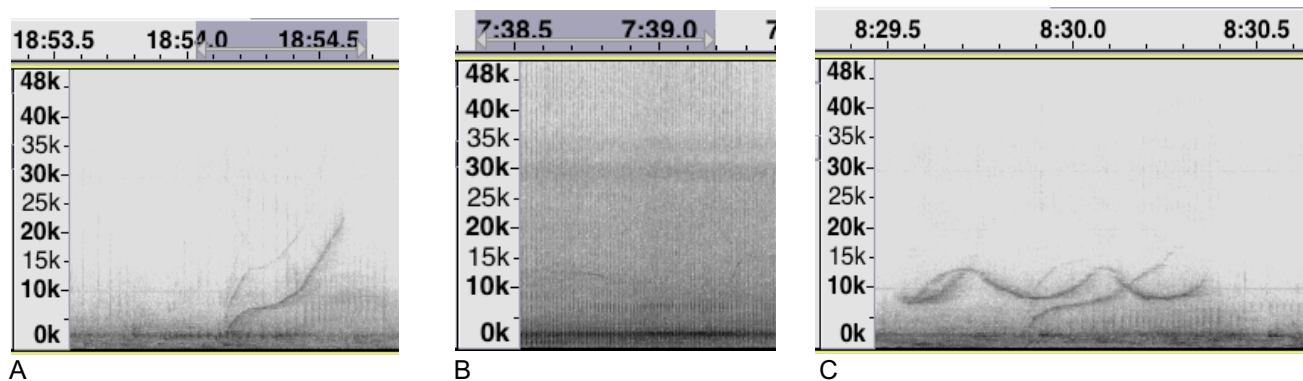

Fig. 6: Examples of a well defined whistle (A), a whistle with a too low signal-to-noise ratio (B), and overlapping whistles (C).

We subjected our recorded data to a statistical analysis to determine the influence of contexts (pre-training, training and post-training) on the emission of tonal sounds by our study subjects. In order to achieve this, we compared whistle rates for signature whistles, non-signature whistles and overall whistles in all three contexts. The overall whistle rates included both signature and non-signature whistles, but did not include those whistles which could not be categorized due to poor quality or overlap. To do this, we used freely available software for statistical analysis: R, version 3.4.1 GUI 1.70 El Capitan build (R Foundation for Statistical Computing, 2016). In order to determine whether there was a statistically relevant difference between the recording sessions for each type of whistle, we applied the Friedman Rank Test. For those comparisons that were shown to be significantly different with a significance level of $P < 0.05$, we applied the Wilcoxon signed Rank Test with Bonferroni corrections to determine between which contexts this difference was observed and was relevant.

RESULTS

Recordings

In total, 6202 whistles were extracted from the recordings. The visual quality of 691 (11.1%) of these was too low to analyze and 351 whistle images (5.7%) contained overlapping tonal sounds, leaving 5160 (83.2%) whistles to be categorized according to the SIGID signature whistle identification method (Janik 2013). Among these, 243 (4.7%) qualified as signature whistles in compliance with this method and 4917 (95.3%) as non-signature whistles (Fig. 7).
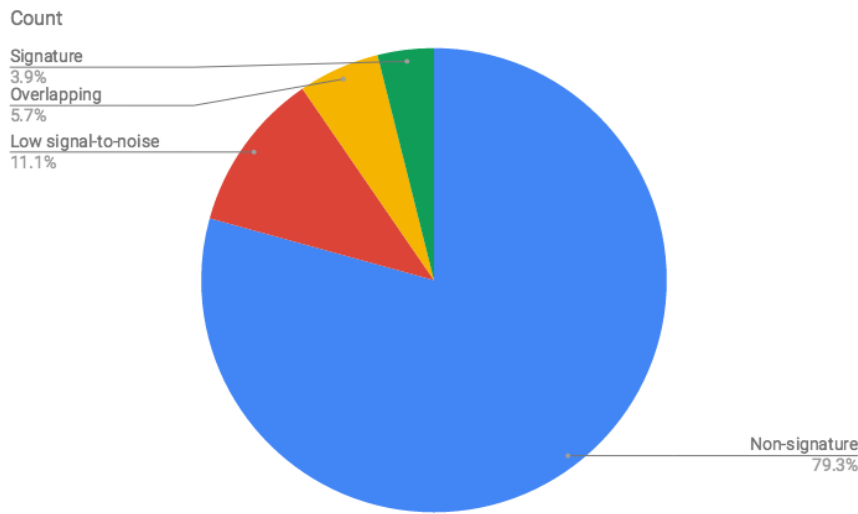

Fig. 7: *Whistle types from the recordings. 79.3% of whistles were qualified as non-signature, 3.9% as signature whistles. For 11.1% of whistles, the signal-to-noise ratio was too low to be categorized. 5.7% of whistles contained overlapping whistles in the same image, making categorization impossible.*

Whistle rates

Overall whistle rates

The average overall whistle rate in our recordings, taking all categorized whistles, sessions and recording contexts into account, was 2.83 whistles per minute. Before training sessions, the overall whistle rate was 2.15 ± 2.20 whistles per minute. During training sessions, this rate increased to 5.55 ± 3.32 whistles per minute. After training sessions, this rate went down to 0.77 ± 1.03 whistles per minute. This variation in rates was determined to be statistically significant when the Friedman test was applied. (Friedman Rank Sum Test X^2 = 43.009, df = 2, P = 4.579×10^{-10} with a significance level of P < 0,05)

Implementing the Wilcoxon signed Rank Test, we found that differences between rates were significant when comparing pre-training with training sessions (Wilcoxon signed Rank Test: V = 1, P = 7.451×10^{-9} with a Bonferroni corrected significance level of P < 0,0167), pre-training with post-training sessions (Wilcoxon signed Rank Test: V = 343, P = 0,00149, with a Bonferroni corrected significance level of P < 0,0167) and

training with post-training sessions (Wilcoxon signed Rank Test: V = 435, P = 3.725 x $10^{=9}$, with a Bonferroni corrected significance level of P < 0,0167). A visual representation of overall whistle rate ratios per recording context is provided in Fig. 8.
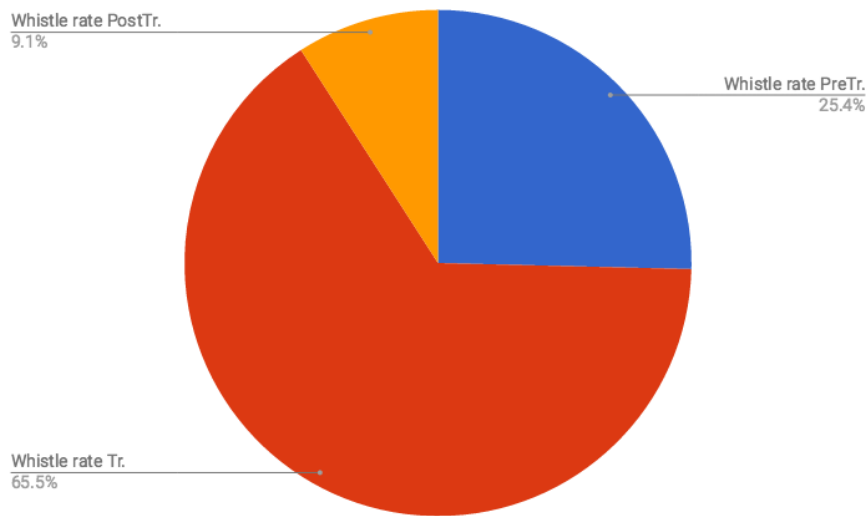

Fig. 8: *Relative whistle emission (signature and non-signature whistles) during pre-training, training and post-training recordings, based on whistle rates per minute.*

Signature whistle rates

Like overall whistle emission rates, signature whistle emission rates also varied significantly between contexts. The average signature whistle emission rate over all recording moments was 0.12 signature whistles per minute. During pre-training sessions, this rate was 0.02 ± 0.10 signature whistles per minute. Training sessions showed an increased signature whistle rate of 0.32 ± 0.41 signature whistles per minute. After training sessions, signature whistle rates were reduced again to 0.02 ± 0.09 signature whistles per minute. The Friedman test demonstrated that this variation was statistically significant. (Friedman Rank Sum Test X^2 = 23.825, df = 2, P = 6.708 x 10^{-6} with a significance level of P < 0,05)

Statistically significant variation was determined to exist between pre-training and training sessions on the one hand (Wilcoxon signed Rank Test: V = 4, P = 0.0004187 with a Bonferroni corrected significance level of P < 0,0167), and between training and post-training sessions on the other hand (Wilcoxon signed Rank Test: V = 168, P = 0.000349 with a Bonferroni corrected significance level of P < 0,0167). Giving a significance level of P > 0.0167, the Wilcoxon test showed no significant difference in signature whistle rates between pre-training and post-training sessions. (Wilcoxon signed Rank Test: V = 3, P = 1). A visual representation of signature whistle rate ratios per recording context is provided in Fig. 9.
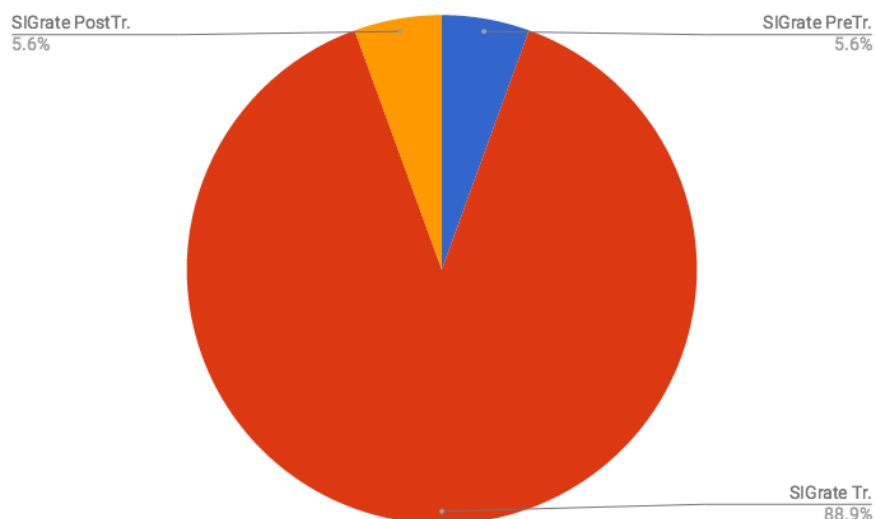
Fig. 9: Relative signature whistle emission during pre-training, training and post-training recordings, based on whistle rates per minute.

We subjected the signature whistles to a visual categorization based on their contours and frequencies, leading to the identification of 6 whistle types as represented in Fig. 10. Two of these types occurred at notably higher frequencies than the other 4 types (Fig. 11). All signature whistle types could be found at some point during training sessions, but only Type 4 occurred during pre-training sessions and only Type 3 and Type 6 were recorded during post-training sessions. However, none of our identified signature whistle types were present in all three recordings contexts, making it impossible to compare the influence of these contexts between all whistle types. Signature whistle rates were consistently higher during training sessions than during other recording contexts for all signature whistle types except Type 3, which showed a slightly higher rate for post-training sessions (Fig. 12).
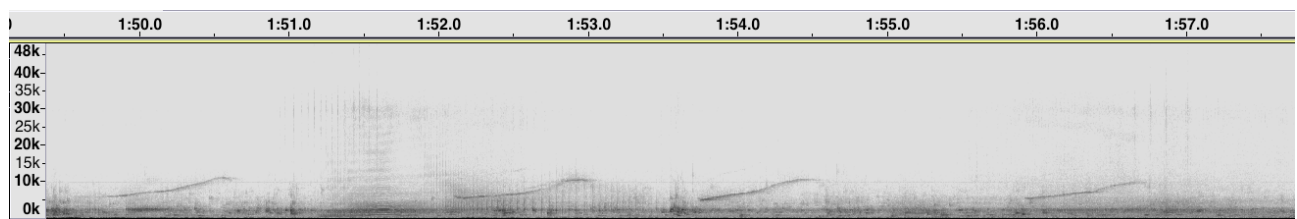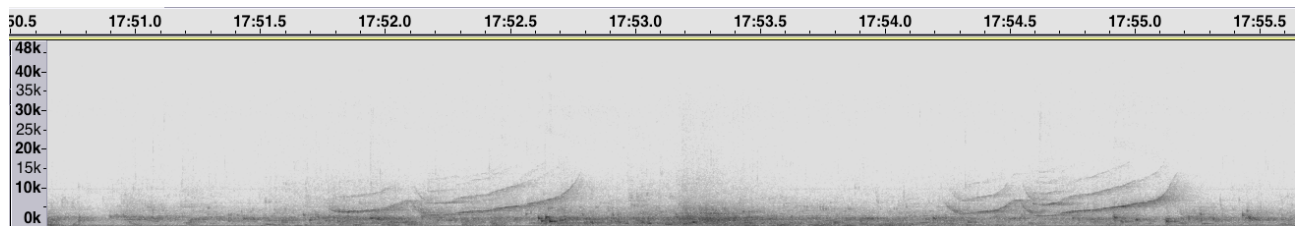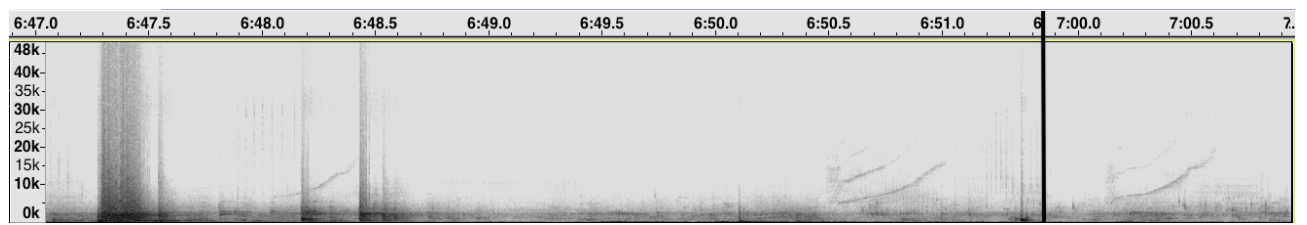
Type 1:
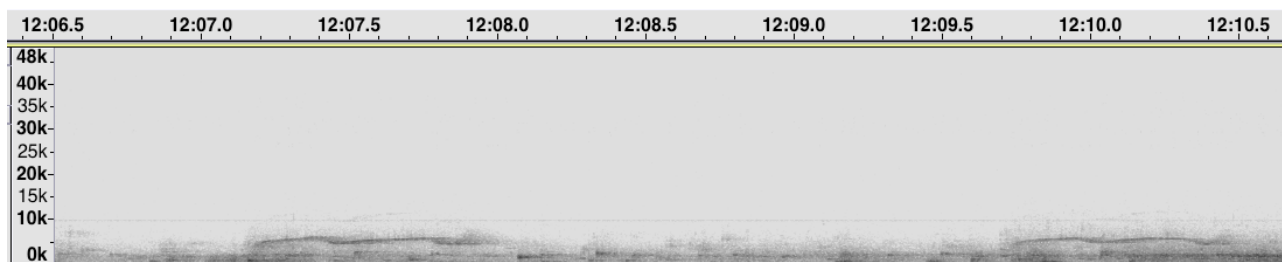
Type 2:
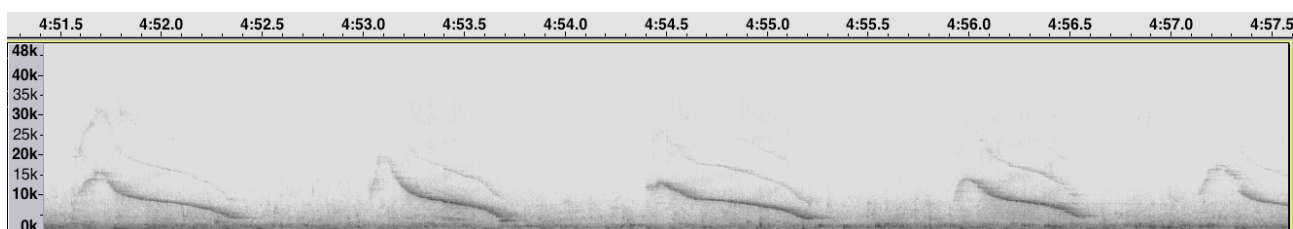
Type 3:
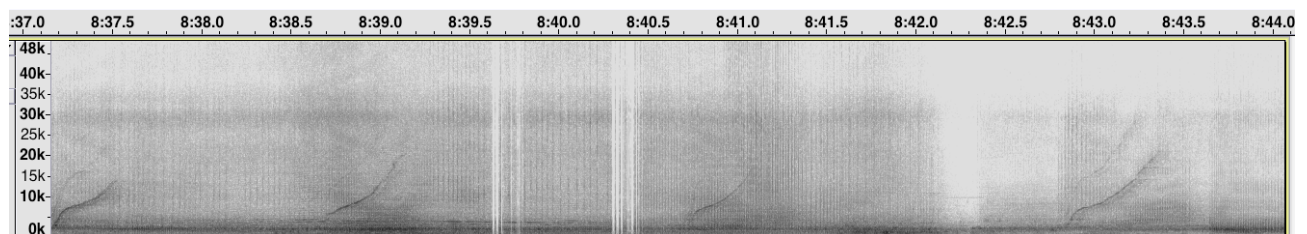

Type 4:

Type 5:

Type 6:

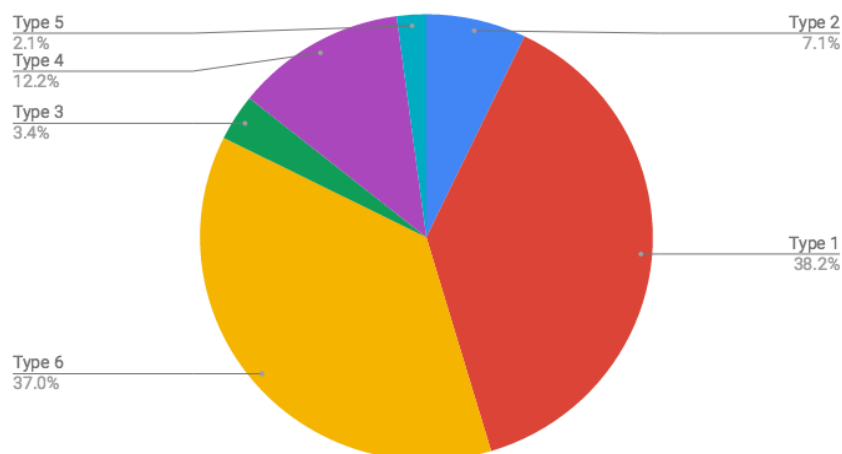**Fig. 10:** *The whistles we identified fit into 6 different categories.*

Fig. 11: *Relative frequencies of the different signature whistle types identified from our recordings.*
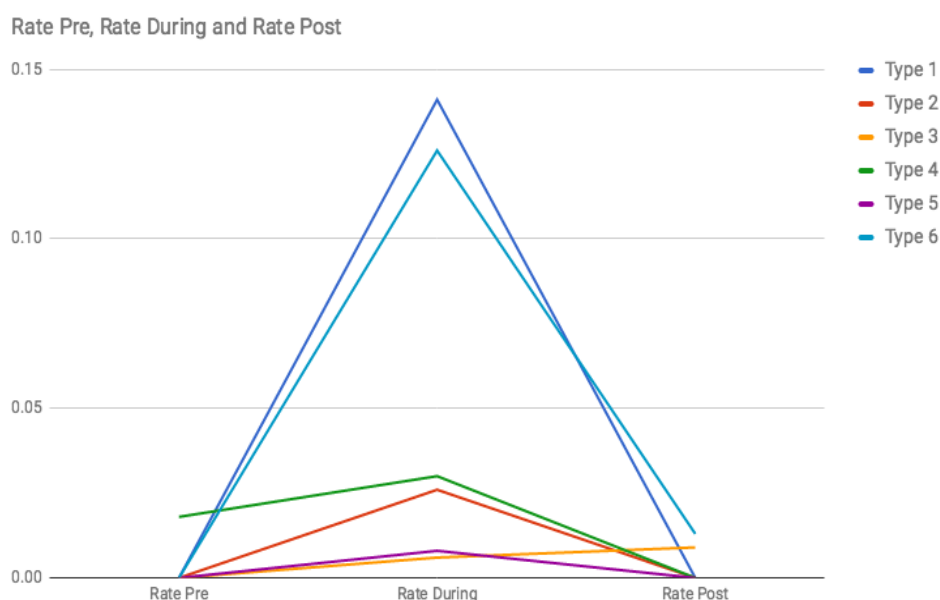

Fig. 12: *Relative signature whistle rates per context.*

Non-signature whistle rates

Non-signature whistle rates averaged 2.68 per minute over all our recording sessions. Pre-training sessions had an average non-signature whistle rate of 2.13 ± 2.20 whistles per minute. This rate increased significantly to 5.16 ± 3.21 whistles per minute during training sessions and dropped to 0.74 ± 1.02 non-signature whistles per minute after training sessions were over. This variation was demonstrated to be significant based on our statistical analysis in R. (Friedman Rank Sum Test X² = 44.619, df = 2, P = 2.046 x 10^{-10} with a significance level of P < 0,05)

The Wilcoxon tests revealed significant differences for non-signature whistle rates between all contexts. The highest significance levels were observed when comparing pre-training sessions to training sessions (Wilcoxon signed Rank Test: V = 7, P = 5.599 x 10^{-6} with a Bonferroni corrected significance level of P < 0,0167) and training sessions to post-training sessions (Wilcoxon signed Rank Test: V = 435, P =

3.725 x 10^{-9} with a Bonferroni corrected significance level of P < 0,0167). Even though the significance levels were lower when comparing pre-training to post-training sessions, they were still smaller than the 0,0167 significance level and therefore also showed a significant difference between these settings. A visual representation of signature whistle rate ratios per training phase is provided in Fig. 13.
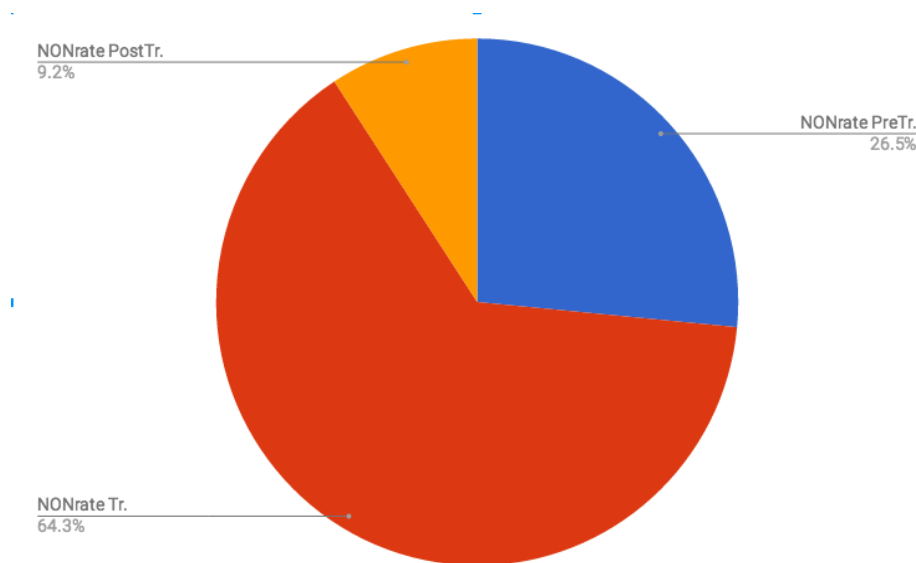

Fig. 13: Relative non-signature whistle emission during pre-training, training and post-training recordings, based on non-signature whistle rates per minute.

In summary, our recordings showed significantly higher whistle rates of all kinds during training sessions when compared to pre-training sessions and post-training sessions. Pre-training and post-training sessions showed significant differences when compared to each other for overall whistle rates and non-signature whistle rates, with higher whistle occurrences before training sessions than after training session. For signature whistle rates, no statistically significant difference between pre-training and post-training sessions was observed.

DISCUSSION

Overall whistle rates and non-signature whistle rates

Our findings show that the overall whistle rate, which took into account both signature and non-signature whistles, changed significantly before, during and after training sessions. Likewise, our statistical analysis of non-signature whistle rates showed similar differences between all three recording sessions.

First, looking at group size, several studies on whistle rates in wild dolphins indeed report changes in whistle emissions according to the number of animals present in the recorded pod: Jones and Saying (2002) report that whistle emissions are positively correlated with group size and can rise either linearly or exponentially with increasing pod members (Jones et Sayigh, 2002). Exponential increases demonstrate that individual animals tend to vocalize more in larger groups and higher whistle rates cannot always merely be attributed to the presence of more vocalizing animals. These findings were corroborated in several subsequent studies on wild bottlenose dolphins (e.g. Cook et al. 2004; Quick and Janik, 2008). Because our study subjects were split into smaller groups during training sessions, we could have expected fewer whistle emissions during

these recordings. However, our results demonstrate the opposite, since more signature and non-signature whistles were recorded during training sessions than during any other contexts. This may be attributed to the fact that all animals (both those participating in training and those in repose in the holing pens) remained within auditory reach of one another during the training sessions, so that no real difference in group size was made in the auditory sense. Quick and Janik (2008) report increased whistling not only when group sizes increased, but also when animals of existing groups were more dispersed, as could be considered the case for the dolphins at the Boudewijn Seapark during training sessions.

Similarly, the second factor influencing signature whistles, group composition, is equally unlikely to account for the differences in sound productions during the different recording contexts. In a similar etho-acoustical study done by Lopez-Marulanda et al. 2016, differences in overall whistle rates between recording periods were partially attributed to the introduction of two young individuals at the facility between the two recording phases of the project. These researchers found higher whistle rates when more young animals were present. They ascribe these increased vocalizations to an increased number of affiliative-, play- and disciplinary related behaviors in the presence of young animals (Lopez-Marulanda et al., 2016). During our experiment, the group composition changed during training sessions when animals were split up into smaller groups. Again, it should be noted that the animals stayed within acoustic reach of one another at all times, so that the group composition did not change to the extent it did in the above mentioned study where the group change entailed the introduction of new animals into the group. In addition, at the Boudewijn Seapark, closely affiliated animals such as mothers and offspring or otherwise tightly bonded individuals were usually kept in the same subgroups during training. Be that as it may, if these relative changes in group compositions did influence the overall whistle rates in our recordings, we would have expected most whistles to occur when all animals swam with the two calves – in other words before and after training sessions. Again, our results show the contrary, with most whistle emissions occurring when at least some of the animals were separated from the calves. It is therefore unlikely that either group size or group composition could account for the changes in the whistle rates we observed.

Finally, the third factor affecting whistle rates, the behavioral context, is more likely to provide an explanation for the variation in overall whistle rates. Contrary to Lopez-Marulanda et al., who found no significant differences in overall whistle rates before, during and after training sessions, we found statistically relevant variation between all three recording settings. This corroborates previous studies where human influences on the acoustic behavior of captive bottlenose dolphins were evaluated (e.g. Therien et al., 2012; Akiyama and Ohta, 2007). Therien et al. (2012) and Sekiguchi and Kohshima (2003) show that diel underwater whistle patterns of captive *Tursiops truncatus* groups change along with human interactions, with the highest whistle rates occurring during human interactions. Akiyama and Ohta. (2007) evaluated 5 behavioral context settings which were common at the facility in question: the time before feeding (1), the time during feeding (2), the instances when humans were present in the water (3) or on top of a float in the water (4) and free time without the presence of trainers or aquarium visitors (5) (Akiyama and Ohta, 2007). These researchers found that most whistles were emitted before feeding times and the least whistles were emitted during free time recording sessions. In addition, they found that more whistles were emitted when human interactions took place than when no humans were present at the facility (Akiyama and Ohta, 2007). Lopez-Marulanda et al., like the present study, made recordings before, during and after training sessions (2016). These authors point out that Akiyama's recordings before feeding times (1) correspond to our pre-training recordings, recording sessions during human interactions (2, 3 and 4) correspond to our training sessions and recordings during the

animals free (5) time can be compared to our post-training sessions (Lopez-Marulanda et al., 2016). However, they found results that differed from Akiyama's study since they observed the highest whistle emission rates during post-training sessions rather than before and during training. Like Akiyama et al., our findings show higher rates during pre-training and training sessions. Lopez-Marulanda et al. (2016) observed that, since training sessions reoccur around the same time every day, the animals could display anticipatory behaviors before training sessions start, influencing their acoustic behavior. Indeed, Akiyama and Ohta (2007) attribute the increase of whistles before interactions with humans to the animals' excitement at the prospect of receiving food and interacting with trainers, which, according to Laule and Desmond (1998), in itself can be considered rewarding for the animals. In our study subjects, the higher whistle rate before training sessions when compared to post-training sessions could be explained by an increased arousal expressed in physical and acoustic anticipatory behaviors. The video analysis of our recordings could potentially corroborate this theory if corresponding physical behaviors are found to be exhibited by vocalizing animals (Lopez-Marulanda et al., forthcoming). It should be noted that the dolphins at the Boudewijn Seapark were usually split up into smaller groups during trainings sessions and that not all animals performed exercises at the same time. Since our audio recordings also recorded the sounds produced by those animals that were temporarily kept in the holding pens while other animals were being trained, the high whistle rates recorded during training sessions could still reflect anticipatory behaviors even after trainings started if these were exhibited by the animals waiting to interact with trainers. In the study by Lopez-Marulanda et al. (2016), the animals were all performing exercises at the same time during training sessions, which could provide an explanation for the difference in overall whistle rates between our studies. Even though non-signature whistles have not yet been unambiguously described as contact calls, it is also possible that the higher overall whistle rates during training sessions could be emitted by the animals to stay connected to one another when out of one another's sight.

After training sessions, the animals usually fell silent and were presumed to be resting after feeding and performing physical activity during the training. The upcoming behavioral analysis will possibly confirm this assumption (Lopez-Marulanda et al., forthcoming).

Signature whistle rates

Our results showed signature whistles which could be visually divided into 6 types, two of which were particularly prominent in the recordings (Fig. 7 and Fig. 11). This means the signature whistles of two animals could not be identified by using the SIGID identification method since all 8 study subjects were within acoustic reach throughout all of our recordings. This is not unexpected, since the SIGID method has been described as being very conservative in order to eliminate false positives (Lopez-Marulanda et al., 2016) and certain signature whistles might have been missed. In addition, signature whistle production in captivity can be as low as 1% of whistle emissions, so that certain signature whistles might have not been present in our recordings (Janik & Slater, 1998). The developer of this method, Vincent Janik, estimates that the SIGID method allows researchers to identify about 50% of signature whistles present in a recorded sample (Janik, 2013). A more efficient method to identify signature whistles would therefore be to make recordings of all individual animals in forced isolation (e.g. Caldwell and Caldwell, 1965; Esch et al., 2009). This would, for example, allow us to verify if indeed the signature whistle of the male calf in our study group resembles that of its mother and whether the signature whistle of the female calf does not, as in the case of wild *Tursiops truncatus* (Sayigh et al., 1990). Nevertheless, because the objective of the larger project of which this acoustic analysis is part was

to gain insight into spontaneous vocalizations under circumstances other than isolation, we chose to stay away from such invasive techniques. We did, however, make audio recordings during two medical check-ups which happened to take place in the months surrounding our data collection. These interventions involved two individuals in turn being briefly restrained on a mattress by the side of the pool while the facility's veterinarian performed diagnostic procedures. Because imposed separations from group members have been correlated to the emission of signature whistles (e.g. Caldwell and Caldwell, 1996; Sayigh et al., 1990; Esch et al., 2009), we expected the isolated animals to repeatedly emit one whistle type of stereotyped contours while undergoing this procedure. For one animal, this was indeed the case: this individual emitted an upsweep signature whistle with contours that strongly resembled Signature Whistle Type 6 which we identified using the SIGID method (Fig. 14). It is therefore most likely that this animal coincides with the individual that corresponds with Signature Whistle Type 6 from our recordings.
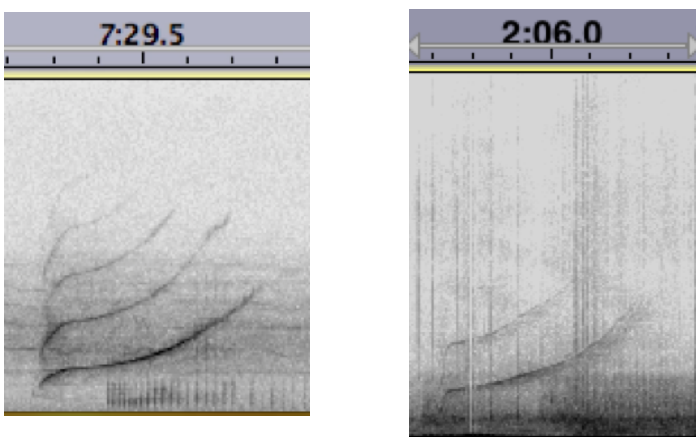

Fig. 14: *Whistle recorded in isolation (left), Signature Whistle Type 6 as identified by the SIGID method (right).*

Even though the other animal did not whistle while being restrained, her most closely affiliated podmate almost continuously emitted whistles of the same contours from inside the pool during the time she was out of the water. Since bottlenose dolphins are presumed to be able to copy each other's signature whistles when addressing one another (King et al., 2013), this whistle type could correspond either to the emitter's own signature whistle or to that of the isolated animal. This whistle did not, however, correspond with any of the signature whistle types we identified using the SIGID method. Caldwell et al. (1990) found that older animals tend to produce signature whistles containing several loops per whistle. In spite of the presence of one animal over the age of 50 and one animal over the age of 40, none of the signature whistle bouts we observed contained multiloop whistles. It is possible, however, that these animals were the two animals whose signature whistles could not be identified with our methodology. After all, it is possible and even likely that all signature whistles were indeed present in our almost 27 hours of recordings but that some did not fulfill the SIGID criteria to be identified as such and were counted as non-signature whistles. Consequently, and in accordance with previous studies applying this method (e.g. Lopez-Marulanda et al., 2016), our reported non-signature whistle rate was likely influenced by the presence of false negative signature whistles. Because of the scarce occurrence of signature whistles in captivity (Janik and Slater, 1998), however, this influence is predicted to be very limited.

For those signature whistles that were unambiguously identified as such by our identification method, our statistical analysis showed significant differences in emission rates between pre-training and training sessions

and between training and post-training sessions, with significantly higher signature whistle rates during training sessions. During training sessions, our hydrophones recorded sounds emitted both by the animals performing exercises and the animals in the side pools waiting to partake in training. It is therefore impossible for us to evaluate the effect of the training exercises themselves on signature whistle emissions without the impending behavioral analysis, since not all animals performed exercises at the same time and recorded whistles could also be produced by non-performing animals. For the time being, we are therefore limited to evaluating whether the broader circumstances of the training session could explain these differences.

The literature review at the beginning of this text showed that signature whistles can be correlated to contexts of regrouping, stress and isolation. Since our study subjects were either performing exercises or were already divided into smaller groups during the training sessions, regrouping behavior cannot explain the significantly higher signature whistle rates during these recordings. Lopez-Marulanda et al. (2016) found the highest signature whistle rates during post-training sessions. They proposed that the animals emit these signature whistles as cohesion calls when regrouping after the group division had been changed by human intervention. Even though the post-training situation at the Boudewijn Seapark is comparable to the one described in the above-mentioned study, our study subjects do not appear to rely on signature whistles to regroup with pod members in this context. Not only is the signature whistle rate in our recordings very low after training sessions, but in addition, only whistles of two out of six types (Signature Whistle Type 3 and Signature Whistle Type 6) could be identified in this context. This discrepancy between our study and Lopez-Marulanda et al. 2016 could, however, be related to differences between the way training sessions were organized at the two facilities. The animals in Lopez-Marulanda's study all performed exercises at the same time during training sessions whereas our study subjects performed exercises in sequence of one another, with one subgroup performing with a trainer and one or two subgroups waiting in the holding pens. We therefore suggest that the increased signature whistles at the Boudewijn Seapark during training sessions could serve as contact calls for the members of this group to remain connected with each other while separated into the side pools. This theory is in accordance with previous research done on the use of signature whistles which observed these sounds in isolation (e.g. Janik et al., 1994) and describe them as contact calls to maintain group cohesion (Janik and Slater, 1998). Smolker (1993) proposes that signature whistles not only broadcast an individual's identity but also their location to group members. If group cohesion was maintained by the animals in such a way and if their relative locations were communicated to each other during training sessions, there would be less of a necessity for them to restore the status quo group dynamic once the trainings were ended.

Methodological considerations

The analyses presented above were based on data that were not explicitly collected to evaluate our research question of whether human interactions influence the acoustic behavior of bottlenose dolphins in captivity. Rather, our methodology was organized around the research goals of the larger etho-acoustical project of which this acoustic analysis is part and which in turn depended on the daily schedule and practical limitations of protocols at the Boudewijn Seapark. As such, our pre-training and post-training contexts entailed 15 minutes each. Each whistle within those phases was considered as either 'pre-training' or 'post-training' and analyzed relative to those training sessions, regardless of whether it was produced one or 15 minutes before the trainings took place. Our division into pre-training, training and post-training contexts could therefore be argued to be on the rough side in the sense that we did not provide any information on how whistle rates evolved within

those phases**.** It is possible that anticipatory whistles were much higher during the last few minutes before training sessions than earlier in the pre-training recordings, influencing our conclusions for pre-training sessions on the whole. Although we did not observe any striking increases or decreases in whistle rates at particular moments when extracting and categorizing acoustical data, we would need to include a statistical analysis taking progressive whistle times into consideration to evaluate this in a scientific way. However, because the device was briefly removed from the water between the phases to examine the battery levels, we could not have provided reliable information on how the transitions between the phases influenced the animals' vocal behavior.

Another limitation of our study is that no unambiguous distinction could be made between pre-training and training contexts when discussing anticipatory behaviors since not all animals took part in training sessions at the same time at this particular facility. The increased amount of non-signature whistles during training sessions were therefore argued to be anticipatory vocalizations by the animals waiting to interact with trainers in the side pools which could not be acoustically isolated. These site-bound circumstances make it difficult to extrapolate our conclusions about the influence of human interactions to other *Tursiops* populations.

A final factor to be mentioned here is that the recording device was quite substantial and even though the animals went through a desensitization period, systematically placing the device into the water before training sessions could have influenced their behavior and vocalizations. If our research question was the only one addressed in this project, it would therefore have been preferable to make use of a more discrete hydrophone placed out of the animals' sight, rather than the BaBeL audio-video device.

CONCLUSION

Our recordings showed a significantly higher rate of whistles of all types during training sessions when compared to recordings made before and after training sessions. In addition, non-signature whistles were significantly higher before training sessions compared to after training sessions. These findings show that husbandry interactions significantly influence the acoustical behavior of *Tursiops truncatus* under human care and are in accordance with our expectations for this species. We argue the increased non-signature whistle rates before and during training sessions are likely due to anticipatory behavior and excitement at the prospect of receiving food and interacting with caregivers. We believe these anticipatory acoustic behaviors continued during training sessions because the animals did not partake in these sessions at the same time, but rather in sequence of one another. We propose this setting also provides the explanation for the increased signature whistle rates during training sessions: since some of the animals were held in the side pools during training and were out of each other's sight, we suggest these signature whistles were emitted as contact calls to maintain group cohesion while visually separated.

We succeeded in identifying signature whistles of six types, meaning we found 75% of the signature whistle types present in the study group of eight individuals. We suspect that the two remaining signature whistle types remained concealed due to the low emission rate of signature whistles by bottlenose dolphins in captivity, the conservativeness of the SIGID identification method and the limited duration of recordings.

Our study provided an acoustical analysis that will be correlated to a forthcoming behavioral analysis of simultaneously collected visual data. Linking up one with the other will hopefully enable us to localize the signature whistles we described in the present study to the individual animals that produced them, hence

allowing us to further evaluate and explain the tentative findings discussed above and venture a more robust ethological analysis of the communication and behavior of our study group.

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