

Short Note

Signature Calls in West Indian Manatee (*Trichechus manatus manatus*)?

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Studies of animal vocal communication have shown that many species exhibit high inter- and intraindividual variability in their vocal signals (e.g., birds: Naguib et al., 2001; primates: Fischer et al., 2002; marine mammals: Kerstenbaum et al., 2013). Although it is unclear whether these acoustic differences are always meaningful to the animals, there is evidence that these signals potentially carry different kinds of information about internal state or external events (e.g., predators and food absence or presence, environmental conditions) and about caller identity. Together with caller identity, these signals can convey diverse additional information such as age, sex, or social role (Lemasson et al., 2009). Thus, individually distinct vocalizations play a crucial role in animal communication, reducing the uncertainty of the external world (Seyfarth et al., 2010).

In terrestrial mammals, individual differences of vocal signals are largely reflected by physical characteristics of the vocal production system. The spectral features are determined by vocal folds and the filter function of the vocal tract (e.g., reviewed in Fitch & Hauser, 1995; Ey et al., 2007). For example, the fundamental frequency as the primary determinant of perceived pitch is controlled by vocal fold size and tension, whereby longer, thicker, and more relaxed folds produce lower pitched sounds. In contrast to terrestrial mammals, the production of underwater sounds differs in marine mammals (Tyack & Miller, 2002). For instance, manatees (*Trichechus* sp.) produce their sounds through laryngeal cartilage vibration and not through vocal folds (Ronald et al., 1978; Hartmann, 1979; see also Landrau-Giovannetti et al., 2014). Even though the link to physical characteristics is less clear in manatees as, unlike in various terrestrial living mammals, their mouths and nostrils are closed

during vocalization, vocalizations of aquatic mammals can be individually specific. This was shown for bottlenose dolphins (*Tursiops truncatus*) as the most prominent example of animals producing signature calls (whistles) (Janik, 2000; Janik & Sayigh, 2013) and even vocal learning (McCowan & Reiss, 1997). As there is no hint that manatee vocalizations are learned like the whistles of bottlenose dolphins, we denote a call as a signature call in cases in which their calls are individually distinct and the call structure is stable over a longer time period.

The first general description of West Indian manatee (*Trichechus manatus manatus*) vocalization related to sex and behavior came from Hartmann (1979). Meanwhile, we know that the vocal repertoires of all subspecies seem to be very similar (Nowacek et al., 2003). Although different studies provide different classification schemes from two to six call types, the descriptions of overall repertoire structures were very similar (reviewed in Brady et al., 2020). Brady and colleagues (2020) described their five call types as (1) squeak, (2) high squeak, (3) squeak-squeal, (4) squeal, and (5) chirp. The names of the vocal types confirm that it is possible to summarize the five vocal types in fewer categories. In addition, for the first time, a recent study has shown that manatee vocalizations vary according to their behavior (Brady et al., 2021). Further, there are two studies providing convincing evidence that manatee calls are individually distinct (Sousa-Lima et al., 2002, 2008).

Besides the communicative function, individual or group vocalizations play an increasingly important role in acoustic monitoring. Recently, and thanks to the rapid technological development of miniaturized recording devices and software programs, bioacoustic monitoring has emerged as a valuable tool in wildlife

conservation (Teixeira et al., 2019). One prerequisite is that the species is vocally active. Finding further species-level distinctiveness in their vocalizations would make it possible to get more information about home range and population size, for example. Bioacoustic monitoring has been used to estimate the abundance of species, such as harbor porpoises (*Phocoena phocoena*) and vaquita porpoises (*Phocoena sinus*), to assess biodiversity and provide information, such as population density, to determine presence or absence of individuals, and may be used to identify some species and individuals (Aide et al., 2013; Kvsn et al., 2020).

As West Indian manatees are an endangered species throughout their known range and visual observations are difficult to conduct due to reduced water visibility, acoustic monitoring could be an important tool when it comes to estimating population size, thus making a considerable contribution to the conservation of the species. For this reason, it is essential to know if a species exhibits so-called “signature calls,” which would provide the ability to estimate the exact number of subjects in a certain area and describe the spatial distribution or home range of the animals.

To answer the question whether West Indian manatees exhibit such signature calls that are stable over time and thus allow for individual manatees to be identified, we studied a group of West Indian manatees living in the Manatee House at the Nuremberg Zoo (water volume 700 m³), an

indoor enclosure which resembles a rainforest house with tropical flora and fauna (Figure 1). The subjects were three West Indian manatees, one female and two males, named “Mara” (21 y old and mother of Herbert), “Zorro” (12 y old), and “Herbert” (6 y old, not fully grown). All three animals were already sexually mature, were born in zoos (Mara and Herbert in Nuremberg Zoo; Zorro in Odense Zoo, arriving at Nuremberg Zoo in 2009), and were the only manatees living in the facility at the time of the study. They had been housed together since 2009. Regarding the social structure and based on our own and the keeper’s observations, it can be noted that Mara is the dominant animal, with Zorro taking a subordinate role. This hierarchy is clearly evident during feedings and in favored resting places. Associated with the manatees were about five different species of fish and freshwater turtles.

This environment allowed us to record and investigate the manatees’ vocalizations with two hydrophones over a period of 1 year. We were also able to precisely monitor their behavior and, more importantly, track their location and therefore the distance to the hydrophones. This setting allows an accurate assignment of a vocalization to the respective animal through comparing spectrogram intensities of both hydrophones with the location of the animals and the distance to the hydrophones, as well as the body position orienting towards or away from the hydrophones.

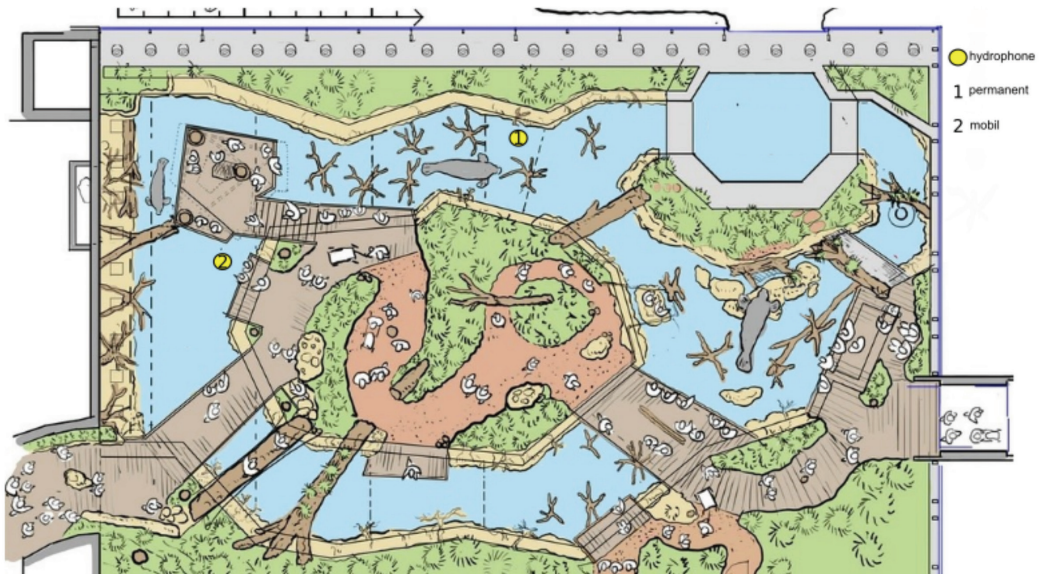


Figure 1. Drawing (top view) of the Manatee House at Nuremberg Zoo. This illustration shows to scale the pool and surrounding landscape. The yellow dots (1 and 2) indicate the position of the hydrophones in the pool.

We recorded the manatee sounds with two hydrophones: one permanently installed RESON TC4042 (receiving sensitivity: -173 dB re $1\text{V}/\mu\text{Pa}$; linear frequency range: 15 Hz to 45 kHz) connected to an electroacoustic sound system in the basement of the Manatee House, and a mobile hydrophone (RESON TC 4014; receiving sensitivity: -186 dB ± 3 dB re $1\text{V}/\mu\text{Pa}$; linear frequency range: 30 Hz to 100 kHz) placed in the feeding area ~ 50 cm below the surface. This hydrophone was connected to an Avisoft UltraSoundGate 116 (Avisoft Bioacoustics, Berlin, Germany; 96 kHz sampling frequency, 16-bit amplitude resolution). Avisoft *SAS Lab Pro*, Version 5.2, was used to visualize the underwater sounds in real time. For every vocalization, we collected behavioral data and the location of all three animals. We synchronized the audio tracks of both hydrophones (using *SAS Lab Pro*) to compare sound intensities with the animals' locations, allowing us to assign most vocalizations to the respective individual who produced the sound. In cases for which we could not make a clear assignment, we excluded the call from our analysis. Although we recorded all sounds that could be assigned to one of the animals, we focused our analysis on the chirp vocalization as defined by Miksis-Olds & Tyack (2009). These calls are harmonic sounds with at least three frequency bands (Figure 2). They build a well-distinguishable "call category" that included the highest amount of call vocalizations in this study. We used only vocalizations for which we found a sufficient signal-to-noise ratio (SNR). To investigate whether the vocalizations were stable over time, we recorded during two different time periods. The first recordings were conducted during March and April 2014. The second recordings were conducted 1 year later during February and March 2015. We made continuous acoustic recordings of both hydrophones during the daily observations.

Individually assigned manatee calls with a sufficient SNR were analyzed using *SAS Lab Pro* (FFT: $1,024$ pt; sampling frequency: 32 kHz; overlap of 87.5% ; time resolution: 4 ms). To determine the acoustic parameters, we used the interactive harmonic cursor tool in the acoustic analysis software, *LMA 2014* (Fischer et al., 2013), which assesses the tonality of a sound based on an autocorrelation function and estimates the F0 values for tonal parts of the sound. Manatee chirps are predominantly tonal sounds with a fundamental frequency (F0) between 1.5 and 3 kHz. The duration of sound of the three manatees' vocalizations was between 150 and 300 ms on average. For the analysis, we used 14 acoustic parameters which can be used to describe the call structure such as frequency (F0), level and modulation of peak frequency, degree of

tonality, amplitude ration between F0 and first harmonic, and frequency range.

To test whether the individual vocalizations were sufficiently different to classify them in three groups, each corresponding to one animal, we conducted a stepwise discriminant function analysis (DFA). The stepwise procedure removes highly correlated variables and, thus, takes care of issues associated with collinearity. The selection criterion for an acoustic parameter to be entered was $p = 0.05$, and $p = 0.1$ was removed from the analysis. We cross-validated the assignment quality with the leave-one-out method, which involves omitting one case per turn, calculating the functions based on the remaining $n-1$ cases and then classifying the left-out case. To estimate the similarity of calls between the 2 years (2014 & 2015), we built six categories (3 individuals from 2014 and the same 3 individuals from 2015) and again ran a stepwise DFA. We used the F values of the stepwise DFA to describe the pairwise similarity of the six categories. This approach is a proven and useful way to express structural similarity in multiparametric approaches in one single value (Thinh et al., 2011; Hammerschmidt & Fischer, 2019). Low F values express high similarity. All statistical tests were done with IBM *SPSS Statistics*, Version 25.

The stepwise DFA of the 2014 recordings selected four out of the 14 acoustic parameters—(1) duration, (2) tonality, (3) mean F0, and (4) amplitude ration—to distinguish between the three manatees. Of the 54 calls, 88.9% could be correctly classified (cross-validated = 87% ; change level = 33%). For the 2015 recordings, we found a correct classification of 100% (cross-validated = 96.6% ; chance level = 33% ; 30 calls). In this case, the analysis selected only two parameters: (1) tonality and (2) amplitude ration.

Figure 2 shows the results of the DFA demonstrating the grouping of the individual call assignments from 2014 and 2015, indicating the animals by different colors. In addition, an example of a typical spectrogram for each manatee is depicted in the rectangles of each respective color.

The pairwise comparison of calls produced in 2014 vs 2015 shown for Mara (blue) and Herbert (red) had a very high similarity (low F values; F value: Mara/Mara = 2.5 ; Herbert/Herbert = 1.2). For Zorro (green), we found a lower similarity (Zorro/Zorro = 18). Nevertheless, all between-subject comparisons showed a lower similarity (higher values) than the intraindividual comparisons (F value: Mara/others = 16.5 ± 7.7 ; Herbert/others = 23.4 ± 11.7 ; Zorro/others = 24.7 ± 10.4 mean \pm SD).

The DFA revealed highly significant individual differences in the acoustic structure of the chirp

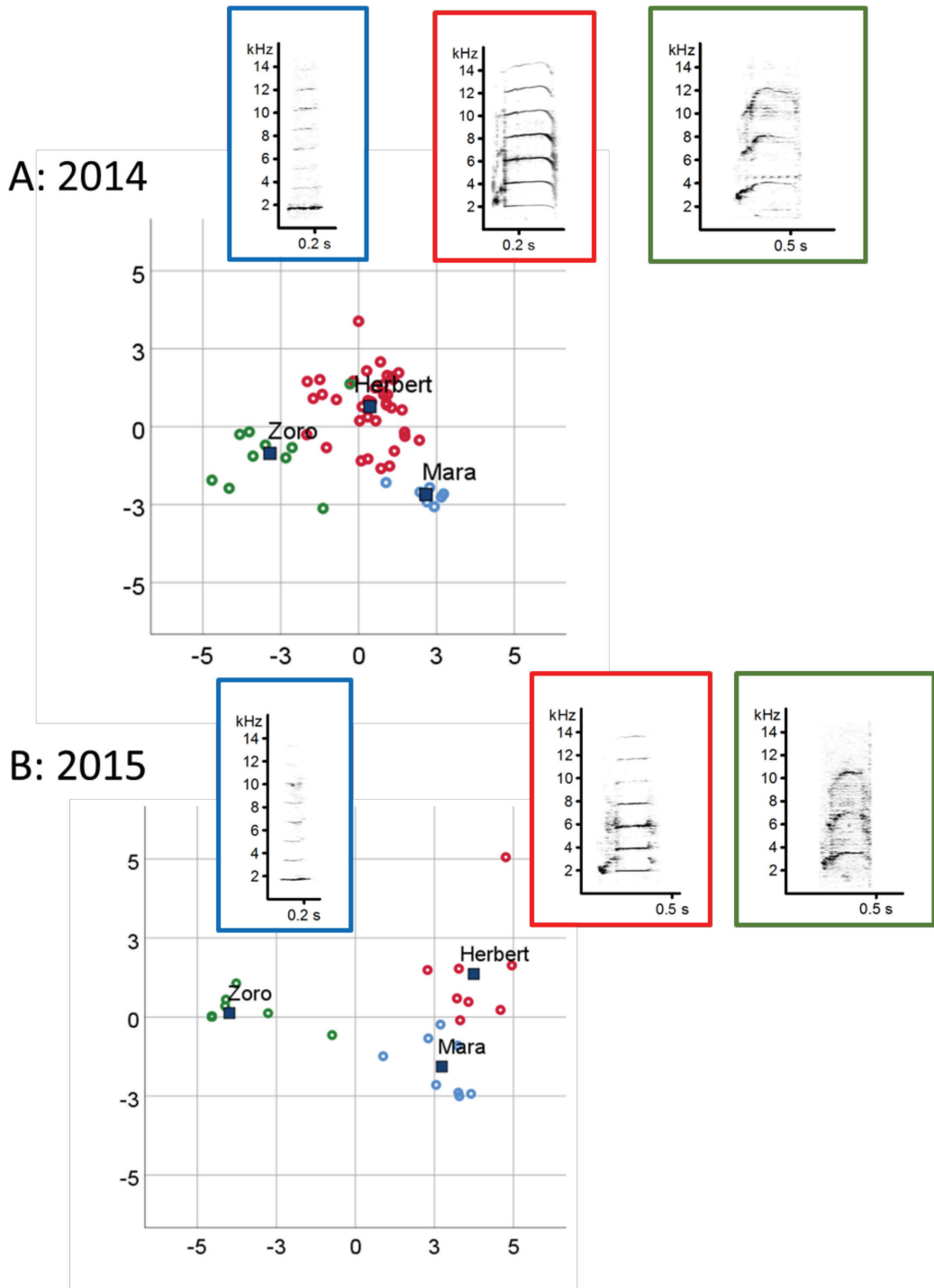


Figure 2. Scattergrams show the results of the discriminant function analysis separately for 2014 and 2015. Spectrograms present a typical call exemplar for every individual manatee (*Trichechus manatus manatus*) for the years 2014 (A) and 2015 (B). Blue refers to Mara, red to Herbert, and green to Zorro.

calls and high intraindividual similarities from year to year. Because Zorro was at 12 years of age a fully adult male, the most likely explanation for the relatively low intraindividual similarity value of Zorro's calls might be the relatively lower number of recorded calls. Zorro produced fewer calls compared to the other animals. Since a solid estimation of F values depends on enough vocalizations, lower numbers of vocalizations might lead to an overestimation of the true variation. The other possibility is that subdominant manatees like Zorro not only call less often but also do not produce comparable prominent signature calls. To which degree the production of such calls is influenced by social conditions, such as hierarchy, would need to be answered by further studies; however, Marsh et al. (1978) have suggested this in previous work.

In summary, at least two of our three manatees showed a high stability in their chirp calls over a period of 1 year. Despite our small sample size, we conclude that the chirp calls of manatees are highly individually distinct and, therefore, classifiable. This confirms the results of an earlier study, which found evidence of individual recognition based on vocal parameters in Amazonian manatees (*Trichechus inunguis*; Sousa-Lima et al., 2002).

We argue that manatees' calls can be considered signature calls because they are individually distinct and stable over a longer period, allowing for individual classification and, therefore, animal recognition. These findings provide valuable information about the acoustic communication of manatees and give rise to manifold possibilities of relatively cheap and easy acoustic monitoring of manatees in the wild, thus contributing to their conservation.

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