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Short Note

Stereotyped Whistles May Be First Evidence to Suggest the Possibility of Signature Whistles in an Injured Indo-Pacific Humpback Dolphin (*Sousa chinensis*)

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Delphinid vocalizations are traditionally characterized as a combination of whistles, burst pulses, and echolocation clicks (Janik, 2009). Whistles and burst pulses are predominantly used in social contexts, while echolocation clicks are mainly used for foraging and navigating (Au & Hastings, 2008; Janik, 2009). More recently, researchers have focused on better understanding how members of any given group are able to identify individual conspecifics. First described in bottlenose dolphins (*Tursiops truncatus*) (Lilly, 1963; Caldwell, 1965; Caldwell & Caldwell, 1968), signature whistles are calls with a frequency contour unique to an individual; it is the predominant call produced when isolated (Caldwell et al., 1990; Janik & Slater, 1998; Sayigh et al., 2007). Signature whistles are thought to serve as a cohesion call and can provide information about identity, stress, social relationships, and context-related information (Janik et al., 1994, 2006; Sayigh et al., 1995, 1999; Janik & Slater, 1998; Esch et al., 2009). Signature whistles are learned within the first year of life (Caldwell & Caldwell, 1979); and once developed, individual dolphins maintain their signature whistles for long periods of time. Individuals have been observed to maintain signature whistles for up to at least 12 y, but they are suspected to be maintained throughout an individual's lifetime (Sayigh et al., 1990). Signature whistles have also been described within other delphinids such as Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Gridley et al., 2014) and Guiana dolphins (*Sotalia guianensis*) (de Figueiredo & Simão, 2009; Lima & Le, 2014).

There are four species of humpback dolphin: (1) the Indo-Pacific humpback dolphin (*Sousa chinensis*), (2) the Atlantic humpback dolphin (*S. teuszii*), (3) the Indian humpback dolphin (*S. plumbea*), and (4) the Australian humpback dolphin (*S. sahulensis*) (Jefferson & Rosenbaum, 2014). Previous studies that have focused on the characterization of whistles produced by these four humpback dolphin species reveal considerable interspecific differences between whistle durations, start and end frequencies, and the overall bandwidths (Zbinden et al., 1977; Parijis & Corkeron, 2001a; Weir, 2010; Wang et al., 2013; Hoffman et al., 2015). For example, Atlantic humpback dolphin whistles are of longer duration (by approximately 400 ms) and larger bandwidths (by approximately 0.78 kHz) compared to the Indo-Pacific humpback dolphin, while the minimum frequency of Indo-Pacific humpback dolphin whistles has been found to be higher compared to their Atlantic cousins (Weir, 2010; Wang et al., 2013). However, when a single female Australian humpback dolphin was stranded, all recorded whistles showed similar spectral contours, thereby suggesting a signature whistle (Parijis & Corkeron, 2001b). Currently, no information on signature whistles in Indo-Pacific humpback dolphins has been found. In this study, we describe a new distinct whistle type in an injured adult Indo-Pacific humpback dolphin that may suggest the possibility of a signature whistle. Acoustic data were collected from an injured dolphin in Sanniang Bay, China (Figure 1), as part of ongoing research program investigating whether

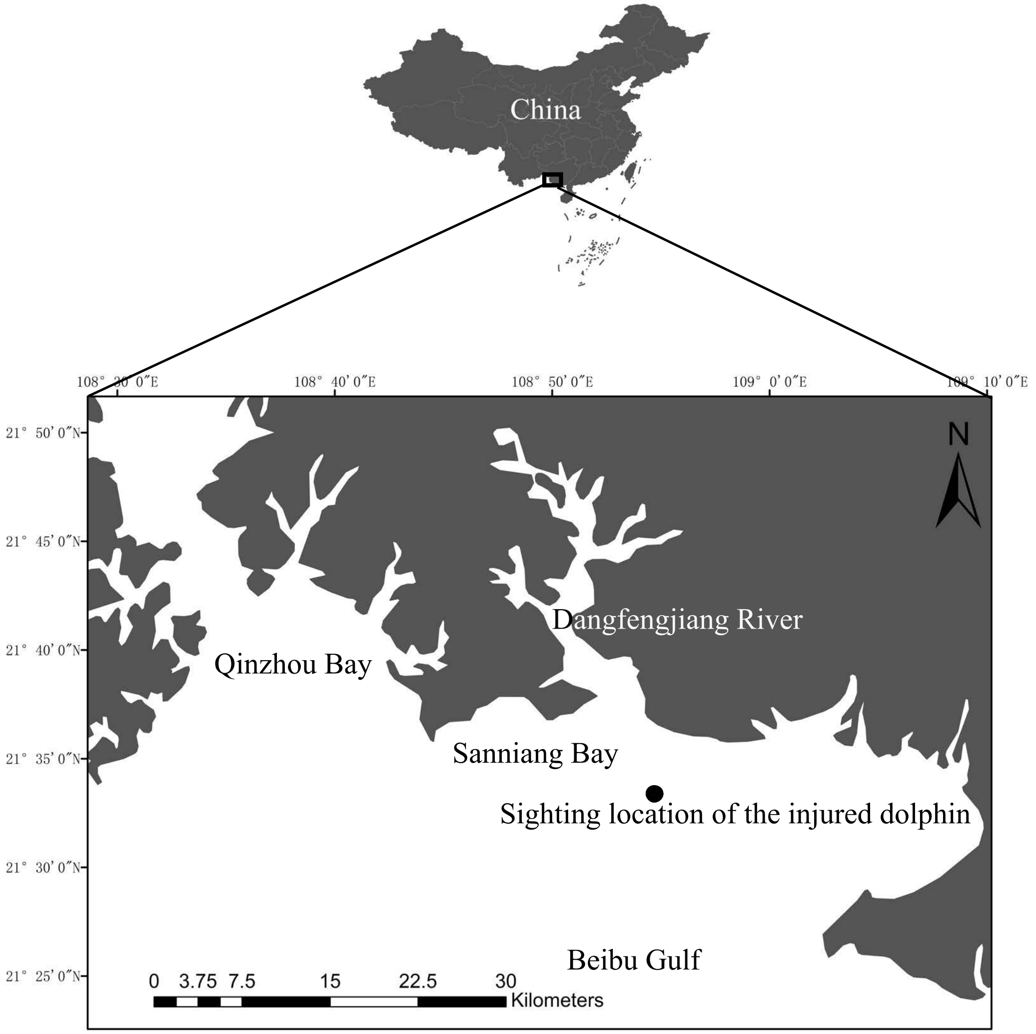

Figure 1. Map of Sanniang Bay, China, showing the location of the injured Indo-Pacific humpback dolphin and the survey course taken

or not Indo-Pacific humpback dolphins in the area produce signature whistles.

The current study was conducted from a 7.2-m vessel equipped with a four-stroke 60-hp Mercury outboard engine. A Canon EOS-1D MARK IV digital camera with a 100 to 400 mm zoom lens was used to take photographs. Acoustic recordings were carried out using a CRT C55 hydrophone (Cetacean Research Technology, Seattle, WA, USA; frequency response ranging from 9 Hz to 100 kHz +3/-12 dB; -165 dB re 1 V/µPa sensitivity). Received signals were conditioned by a 1-MHz bandwidth EC6080 voltage preamplifier (Model VP2000; Reson, Slangerup, Denmark) with high-pass filter at 100 Hz to reduce system and flow noise, and low-pass filter at 100 kHz to prevent aliasing. The system was also equipped with a Fostex FR-2 field memory recorder (24-bit resolution and 192-kHz sample rate, providing a Nyquist frequency of 96 kHz) with a flat frequency response between 20 Hz and 80 kHz +/-3 dB.

On 20 January 2015, a group of more than 20 individuals, including calves, juveniles, and adults, were sighted travelling at 1424 h. An adult was observed lagging behind the group with obvious difficulty. This injured dolphin had been identified in April 2014 (ID# BG-SA-65) as part of an ongoing photo identification effort of the humpback dolphin in the northern Beibu Gulf (Haiping Wu, unpub. data). Upon initial inspection at an approximate distance of 30 m, a white styrofoam floater (approximately 40 × 20 × 20 mm and typical of those used by local fishermen) was entangled around the dolphin's body (Figure 2). Further inspection of the photographs revealed wounds and bleeding (Figure 2c). The local fishery administration was informed and provided permission to undertake observation and take

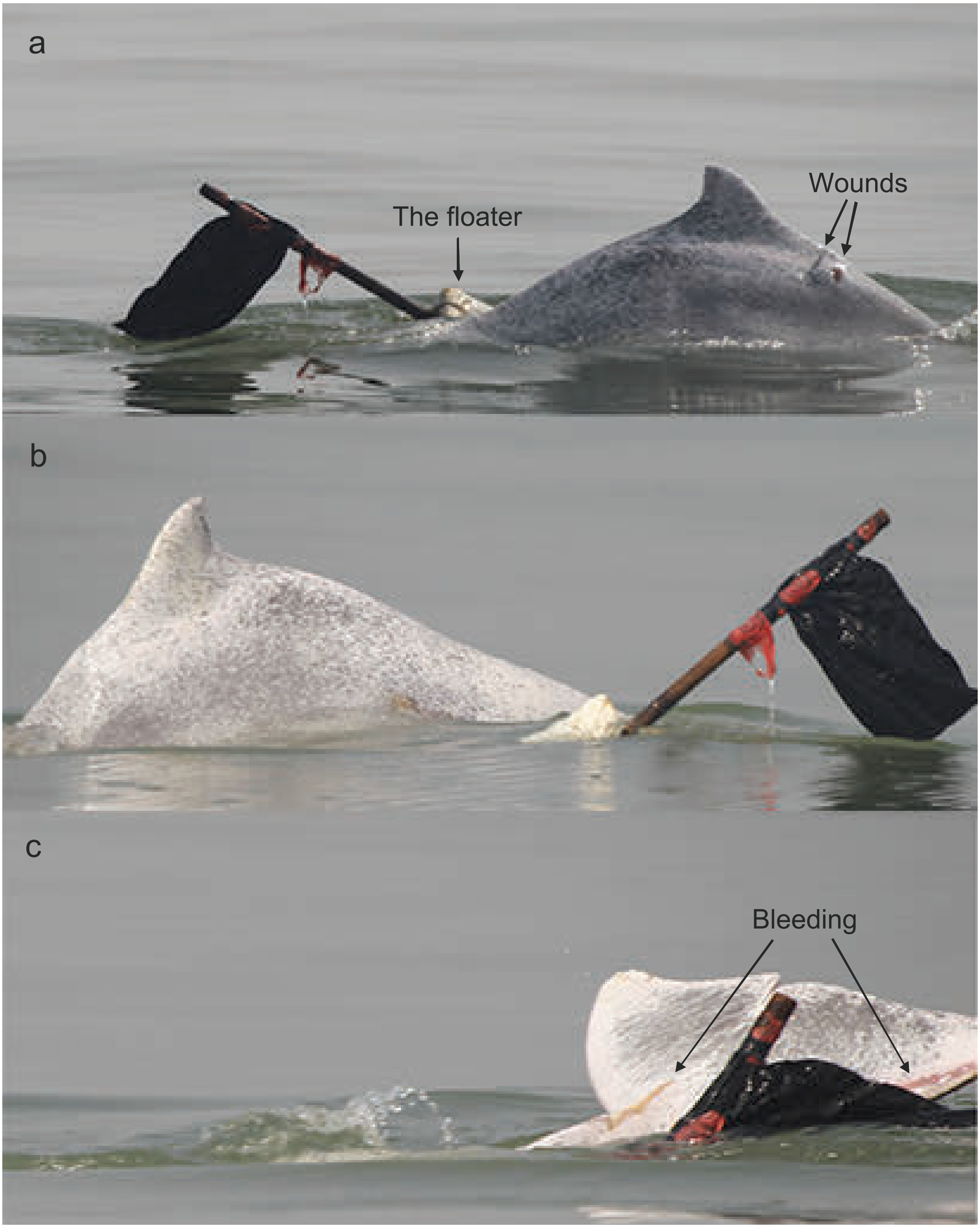

Figure 2. (a) The right side of the injured *Sousa chinensis* individual, showing a fishing floater attached to the animal's body and two wounds in front of the dorsal fin; (b) the left side of the dolphin; and (c) when the injured animal dove, bleeding was observed along its fluke.

photographs of the animal before it was rescued. After 11 min from the point of the initial sighting, the remaining members of the pod had moved beyond our visual range, while we continued to follow the injured dolphin at a distance between 30 and 100 m. Before deploying the hydrophone, the vessel's engine was turned off. The hydrophone was deployed at 1 m depth to record the dolphin's vocalizations and was recovered after the dolphin moved 100 m away. Observations lasted approximately 2 h and resulted in 56 min of recordings. The local fishery administration tried to get close to the dolphin and remove the entangled floater but were unsuccessful, and the dolphin has not been sighted since (Haiping Wu, unpub. data, June 2016).

Acoustic data were aurally and spectrographically inspected in *Raven Pro 1.4* software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) with a 4,048 sample Hanning window. A total of 125 whistles (assumed to be emitted by the injured dolphin based on the fact that it was the only dolphin in the vicinity) were counted, of which 47 whistles exceeded a signal-to-noise ratio (SNR) threshold of 10 dB. A 10-dB SNR threshold was used to guarantee reliable characterization of the frequency contours. Further analysis was carried out on those 47 whistles.

Those 47 whistles were classified into 11 whistle types based on the shape of the frequency contours and bandwidths (Table 1; Figure 3). Descriptions of each type are provided in Table 1, and representative examples are provided in Figure 3. The Cohen's Kappa statistic was used to test inter-rater reliability from which a value of 0.85 was calculated, indicating a high agreement between two individual raters. The most predominant whistle type was Type 1 (*n* = 33, 70.21% of whistles classified), followed by Type 2 (*n* = 4, 8.51%) and Type 3 (*n* = 2, 4.26%). Ten parameters—start frequency (SF), end frequency (EF), minimum frequency (MinF), maximum frequency (MaxF), duration, start sweep (SS, down = -1, flat = 0, rise = 1), end sweep (ES, down =- 1, flat = 0, rise = 1), number of inflection points (NoIP), number of gaps (NoG), and number of stairs (NoS)—were used to characterize each whistle type. The results are summarized in Table 2.

Analyses of time intervals between individual Type 1 whistles revealed 22 intervals (75.86%, mean [± SD] of 2.10 s ± 2.01 s) to be within the range of 1 to 10 s, two intervals (6.90%, mean [± SD] of 0.91 s ± 0.06 s) within the range of 0 to 1 s, and five intervals (19.16%, mean [± SD] of 28.18 s ± 20.65 s) greater than 10 s.

Previous studies focusing on bottlenose dolphins have described two methods used to identify signature whistles. The first, more widely used method is to identify the most commonly produced whistle type in isolation (Janik & Sayigh, 2013) since some dolphin species produce signature whistles when isolated. For example, free-ranging bottlenose dolphins vocalize almost exclusively using signature whistles when temporarily captured (Sayigh et al., 2007), and captive bottlenose and Risso's dolphins (*Grampus griseus*) also primarily used individually distinctive signature whistles when swimming alone (Favaro et al., 2016). Within the current study, the most commonly produced whistle type from the isolated dolphin was Type 1 with 33 whistles (comprising 70.21% of all whistles analyzed). The other method, called *SIGnature Identification* (*SIGID*), relies on the fact that signature whistles

Table 1. Whistle classification standards of the defined 11 types

Type	Description
1	Frequency contour follows that of a sine wave but with an extra down part followed (Figure 3, Type 1).
2	Frequency contour follows a V pattern (Figure 3, Type 2).
3	Frequency contour follows a V pattern with a break at the bottom and a greater, larger bandwidth than Type 2 (Figure 3, Type 3).
4	Downsweep (Figure 3, Type 4).
5	Downsweep proceeds a plateau (Figure 3, Type 5).
6	Frequency contour follows that of a sine wave but with a larger bandwidth than Type 10 (Figure 3, Type 6).
7	Frequency contour follows that of a sine wave with a sharp upsweep at the end (Figure 3, Type 7).
8	Downsweep of shorter duration and lower frequency than Type 4 (Figure 3, Type 8).
9	A sharp rise in frequency at the beginning followed by a steady decrease (Figure 3, Type 9).
10	Frequency contour follows that of a sine wave and does not meet the criteria for Types 1, 6, or 7 (Figure 3, Type 10).
11	Frequency contour follows that of a half sine wave cycle followed by a plateau.

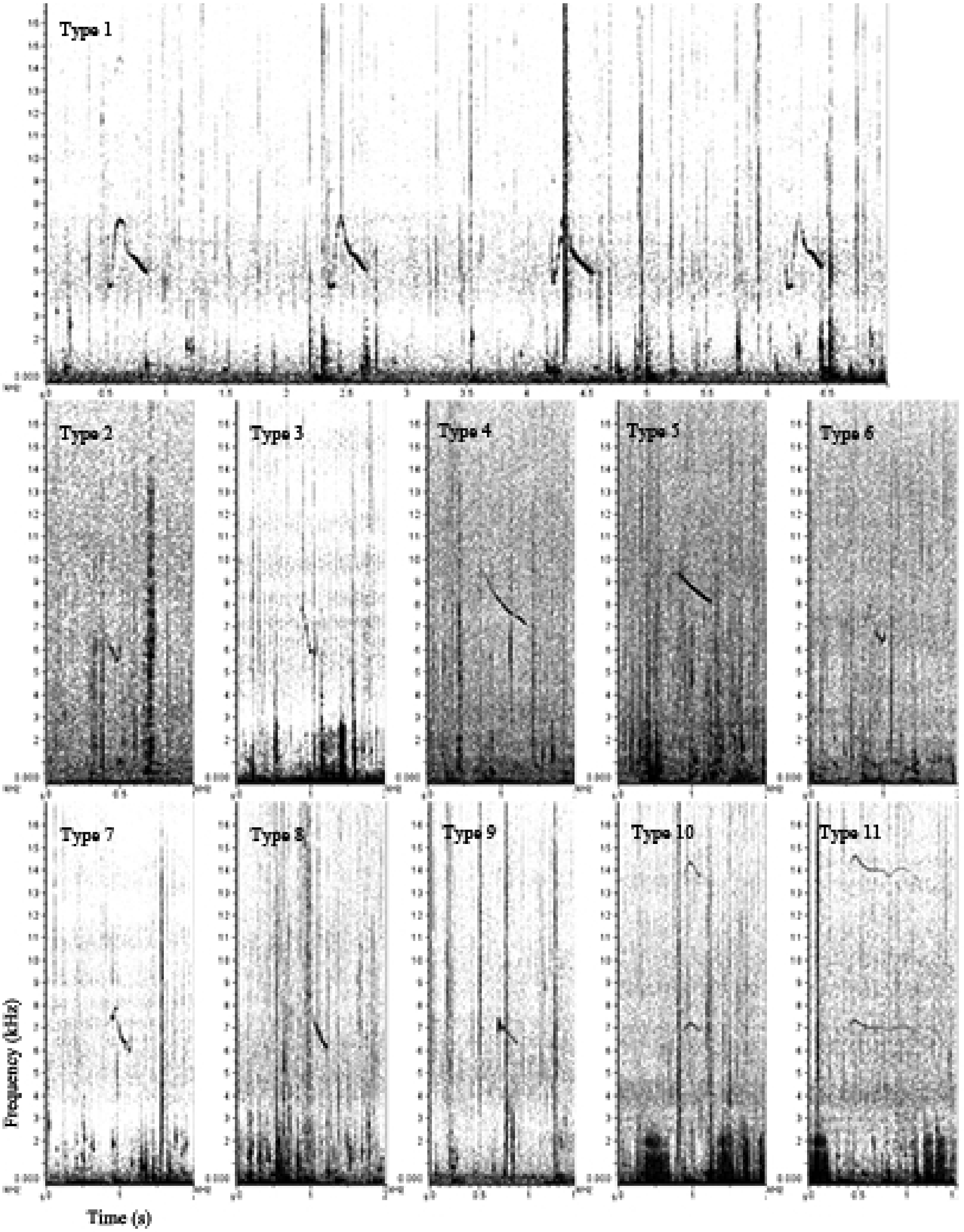

Figure 3. Spectrogram of 11 whistle types produced by a solitary injured adult *Sousa chinensis*, visualized using *Raven Pro 1.4* software (Window type: Hanning, Window size: 4,048, 3-dB filter bandwidth: 68.2Hz, Time grid overlap: 50%, Hop size: 10.5 ms, Frequency grid DFT size: 4,096, Grid spacing: 46.9Hz)

tend to be emitted in bouts, with whistles of the same type occurring within 1 to 10 s of each other (Janik et al., 2013). Thus, if 75% or more of the same whistle type is emitted in bouts within 1 to 10 s intervals, that whistle type may be a signature whistle (Janik et al., 2013). Within the current

Table 2. Descriptive statistics of the mean ($\bar{x}$) and standard deviation (SD) for time and frequency parameters (start frequency [SF], end frequency [ES], minimum frequency [MinF], maximum frequency [MaxF], and duration), start sweep (SS, down = -1, flat = 0, rise = 1), end sweep (ES, down = -1, flat = 0, rise = 1), number of inflection points (NoIP), number of gaps (NoG), number of stairs (NoS), number of harmonics (NoH), and the maximum frequency of harmonics (MHF) from the solitary injured adult *Sousa chinensis*

	Type 1 $\bar{x}$ ± SD	Type 2 $\bar{x}$ ± SD	Type 3 $\bar{x}$	Type 4 $\bar{x}$	Type 5 $\bar{x}$	Type 6 $\bar{x}$	Type 7 $\bar{x}$	Type 8 $\bar{x}$	Type 9 $\bar{x}$	Type 10 $\bar{x}$	Type 11 $\bar{x}$
n	31	3	1	1	1	1	1	1	1	1	1
SF (kHz)	5.37±0.61	6.46±0.20	7.77	9.31	9.35	7.27	7.41	7.54	6.85	6.92	7.17
EF (kHz)	5.11±0.16	6.52±0.33	6.96	7.02	8.08	7.06	7.59	6.09	6.30	7.13	6.99
MinF (kHz)	4.7 ±0.36	5.62±0.19	5.73	7.02	8.08	6.32	5.90	6.09	6.30	6.82	6.99
MaxF (kHz)	7.35±0.22	6.56±0.27	7.77	9.31	9.35	7.91	7.84	7.54	7.39	7.20	7.31
Duration (ms)	336.71± 53.18	115.67± 31.07	213	583	547	267	276	179	208	233	641
SS	-0.52±0.71	-1±0.00	-1	-1	0	1	1	-1	1	1	1
ES	-1.00±0.00	1± 000	1	-1	-1	1	1	-1	0	1	0
NoIP	1.55±0.56	1±0.00	0	0	0	2	0	0	1	2	1
NoG	0.68±1.28	0	1	0	0	1	1	0	0	0	0
NoS	0.13±0.34	0	0	0	0	0	1	0	0	0	0

study, Type 1 whistles were emitted in a sequence, with at least 75% of whistles within 1 to 10 s on several occasions, thereby meeting the *SIGID* requirement. However, while this may provide a basis for suggesting that Type 1 whistles within the current study may represent a signature whistle from the injured dolphin, a greater sample size is required to determine whether these whistles are in fact a signature whistle as opposed to a stereotyped whistle transmitted during a time of stress and injury. Notwithstanding, the repetitive nature of the Type 1 whistle may indicate that Indo-Pacific humpback dolphins emit stereotyped whistles with an interwhistle interval commensurate with the bottlenose dolphin.

Whistles produced by a stranded humpback dolphin in Australia were recorded when the animal was transferred to a man-made pool or handled by people. Whistles from that dolphin shared a similar frequency contour and exhibited four variations: one looped (*n* = 121), two looped (*n* = 57), three looped (*n* = 29), and four looped (*n* = 7) (Parijs & Corkeron, 2001b). In comparison, 11 whistle types were categorized within the current study, but no looped structure whistle was observed for the free-ranging injured dolphin. In captive bottlenose dolphins, signature whistles accounted for 31.8 to 91.7% (Janik & Slater, 1998) or even 100% (Caldwell et al., 1990) of all emitted whistles emitted while the animal was in isolation. Numbers of signature whistle loops were also greater during capture-release events compared to times when the animals were left undisturbed (Esch et al., 2009). Further research is required to better understand the discrepancies between the stranded humpback dolphin in Australia and the injured Indo-Pacific humpback dolphin observed within the current study. However, the differences may be attributed to the different environmental conditions or simply because these are different species with distinct whistle characteristics.

Caldwell et al. (1990) mentioned that signature whistles of bottlenose dolphins may vary in duration, frequency, number of loops, etc., while maintaining a highly distinctive loop contour pattern. Within the current study, the coefficient of variation (CV) value for time and frequency parameters of Type 1 whistles were calculated to see which parameters of humpback dolphin vocalizations were more variable. Of all the acoustic parameters analyzed, the duration (with a CV of 15.79) and start frequency (CV of 11.36) had the highest CV values. In comparison with the stranded humpback dolphin in Australia (Parijs & Corkeron, 2001b), the end frequency (CV of 41.03) and duration (CV of 26.67) of whistles were the two most variable parameters.

Despite being based on a single individual, the findings within the current study have shown that an Indo-Pacific humpback dolphin had a unique call type when in distress. Based on two common methods used to identify signature whistles, these stereotyped whistles may suggest the possibility of this species producing signature whistles. However, to determine if the species does in fact produce signature whistles, further research focused on the vocalizations from other Indo-Pacific humpback dolphins under similar contexts is required.

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