Investigating, measuring and characterising whistles of Indian Ocean humpback dolphins *Sousa plumbea* in Richards Bay, KwaZulu Natal

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfilment of the requirements for the degree of Master of Science.

Declaration

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

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GLOSSARY

Alliance: a long-term cooperative partnership between two or more male dolphins.

Bout: brief periods of repeated whistle production separated by longer periods of silence.

Capture: when a signature whistle type has been identified/sighted in a sample, it has been captured.

Category: the three categories used to describe different whistle contours, i.e., unclassified (uncl), variant (var) and potential signature whistle (psw).

Class: the groups that potential signature whistles are assigned to during the visual classification of whistles. One class is made up of multiple whistles of the same shape.

Encounter: the sampling unit of a recording session where an individual or dolphin pod was present, near the hydrophone, in order to record dolphin whistles.

Recapture: when a signature whistle type, identified in a previous sample, is sighted in another sample, it has been recaptured.

Signature whistle type: distinct whistle types (see below) carrying identity information in their frequency modulation pattern. A signature whistle type represents one dolphin/individual because each individual produces a unique signature whistle type.

Spectrogram: a sound spectrograph showing time on the horizontal axis, frequency on the vertical axis, and intensity is indicated by varying shades of darkness of the pattern.

Stereotypy: frequent repetition of the same whistle type.

Type(s): distinctive whistles of the same shape that met the SIGnature IDentification (SIGID) criteria.

Whistle category: whistle classes determined by the whistle frequency modulation pattern and shape.

Whistle contour: a visual representation of the pattern of frequency changing over time.

Whistle: a unit of 1 continuous whistle contours or 2 or more repeated whistle contours separated by a short period of silence with a minimum frequency and duration of 0.52 kHz and 300ms, respectively.

ABSTRACT

Some dolphin species produce individually distinctive vocalisations, called signature whistles, that broadcast the signal sender's identity. Signature whistles are behaviourally important, and are used for recognition and group cohesion. Previous chance encounters and opportunistic behavioural observations of Australian humpback dolphins *Sousa sahulensis* and Indo-Pacific humpback dolphins *S. chinensis* have suggested signature whistle use in the genus but empirical evidence is absent. Signature whistles may be identified by analysing the temporal patterns of whistle production to identify bouts of stereotyped whistles separated by gaps lasting 1 – 10 s, an approach called SIGnature IDentification (SIGID). I investigated signature whistle occurrence in the endangered Indian Ocean humpback dolphins *S. plumbea* at Richards Bay, South Africa. I collected acoustic data to examine the temporal patterns of the whistles of humpback dolphins to evaluate whether similar whistle contours were repeated in quick succession, and if so, to describe the pattern of repeats. I also assessed at what rate new signature whistles were discovered and recaptured. Acoustic data were collected using a hydrophone deployed at the Richards Bay harbour mouth continuously from May 2017 to September 2019, and during March 2020. Occasions when only humpback dolphins were present were verified photographically. Spectrograms of whistle contours were categorised visually by quality and similarity in shape, in a sound window with a minimum frequency of 0 kHz and a maximum of 24 kHz. I collected 30 encounters, of which potential signature whistle types were identified in 24 encounters. In total, 19 of 22 potential signature whistle types fulfilled the SIGID criteria using a sequential bout analysis, with 26.09% repeated at intervals between 0 and 1 s, 60.53% between 1 and 10 s, 30.66% between 10 s and 1 min. The inter-whistle-interval population average was 11.4±14.19 s standard deviation (SD) and the median inter-whistle-interval was 5.30 s. There were 667 signature whistle contours occurring in bouts and 672 occurring out of bout. Daily recaptures ranged from 2 to 18 days, and annual recaptures were high with 8 signature whistle types captured in 2 years, 7 captured in 3 years, and 1 captured in all 4 years. In conclusion, I have shown that humpback dolphins in Richards Bay use signature whistle types in a natural context by visually categorising whistle types and thereafter conducting the SIGID test on potential signature whistle types using acoustic data collected from free swimming humpback dolphins. However, without identifying the whistling individual, I cannot speculate on the function of signature whistles. The high boat presence, calf presence and muddy coastal waters at the Richards Bay harbour mouth suggests that the humpback dolphin population here relies heavily on acoustic signals to maintain group cohesion. Future studies could incorporate focal follows to collect photo-identification data to investigate potential matches between signature whistle types and photo-identified dorsal fins in order to determine whether the number of photo-identified individuals in a group correspond to the number of signature whistle types classified or use acoustic tags to assess signature whistle use in *Sousa* and formulate species-specific bout-interval criteria. In the process, this could yield larger signature whistle type catalogues which could serve in mark-recapture studies to assess humpback dolphin population demography and aid in the conservation of this genus.

Keywords:

Acoustic communication, Indian Ocean humpback dolphin, signature whistle type, temporal patterns.

INTRODUCTION

Marine mammal communication

There is an array of signals, sensory systems and signalling behaviours in animals but different environments impose different constraints on effective signal transmission and thus have implications for within species communication (Endler 1992). Communication signals occur in various modalities in both terrestrial and aquatic environments, and include olfactory, tactile, acoustic, visual and electromagnetic sensory modalities, but certain sensory channels are limited in some environments and emphasised in others (Reynolds and Rommel 1999). Water is a poor medium for olfactory or visual cues due to currents, plankton blooms, depth or darkness (Anderson 1969, Reynolds and Rommel 1999). Therefore, acoustic communication modes are favoured in marine mammals due to how easily sound is transmitted through water and over long distances (Richardson et al. 1995, Dudzinski et al. 2009).

According to Bradbury and Vehrencamp (1998), communication occurs when a sender broadcasts information to a receiver followed by the receiver deciding whether to respond or how to respond to the given information. This information is exchanged between the sender and receiver by way of a signal that helps the sender and receiver in overcoming challenges such as predator defence, reproduction, foraging, maintaining social bonds, territory defence or parental care (Dudzinski et al. 2009). Over time, signals are specialised to become noteworthy, informative and adapted for optimal transmission in a specific environment (Endler 1992; Bradbury and Vehrencamp, 1998). My study focuses on acoustic communication in dolphins, which occurs when an individual produces sounds by passing pressurized air through any specialised sound-producing anatomical structures, such as pairs of phonic lips located within the nasal complex in the blowhole (Tyack 2002; Madsen et al. 2013).

Oceanic dolphin whistles

Odontocete (toothed) cetaceans produce a variety of vocalisations, such as whistles, that are suited for specific interactions in different cetacean societies and are used in different environmental and behavioural contexts (Tyack 1986). Whistles are frequency-modulated acoustic signals used for communication and can be categorized in a variety of ways (Popper 1980; McCowan and Reiss 1995, Janik and Slater 1998). Whistle production rates are influenced by the behaviour or “excitement” level of an individual (Frankel 2009) with higher whistle rates in active groups, e.g., those that are feeding and socially active, whereas resting groups produce fewer whistles (Norris et al. 1994). There are two main whistle types, (i) signature whistles, and (ii) non-signature whistles, otherwise known as variant whistles (Janik and Sayigh 2013). Signature whistles have a high degree of stereotypy and are considered the distinctive aspect of dolphin communication (Janik and Sayigh 2013). Signature whistles type frequencies often range from 1 to 30 kHz while their duration lies between 0.1 to 4 s and they are often produced in bouts separated by 1 -10 s intervals (Buckstaff 2004; Sayigh and Janik 2009; Janik et al. 2013). Whistles with a low degree of stereotypy, i.e., less stereotyped, are called variant whistles (Watwood et al. 2005). Signature whistles can be distinguished from variant whistles through the repetitive segments of signature whistles, separated by approximately 250 millisecond gaps called loops (Esch et al. 2009a). Multi-looped signature whistles can contain multiple short modulation patterns called introductory and terminal loops which usually occur as a single unit (Caldwell et al. 1990; Sayigh et al. 2007, Esch et al. 2009a). In contrast, variant whistles are highly variable whistle types, often separated by less than 1 s or more than 10 s, which is the opposite for signature whistles (Janik et al. 2013; Janik and Sayigh 2013). The proposed functional difference between signature and variant whistles is that the former encodes the individual's identity information whereas the latter could either be shared whistle types, which are whistle types produced by all the individuals in a pod, or variable whistles that are produced infrequently, when all group members are together (Janik and Sayigh 2013). However, the function of variant whistles in the dolphin communication system is not yet definitively known (Janik and Slater 1998; Fripp et al. 2005; Sayigh et al. 2017).

Signature whistles

Caldwell and Caldwell (1965) found that captive bottlenose dolphins *Tursiops* have a propensity to emit the same whistle type when isolated from conspecifics and thus hypothesized that certain features within a whistle relay the whistler's identity. This hypothesis was based on a suggestion that individual common bottlenose dolphins *T. truncatus* may use distinct whistle components which are recognizable by other individuals (Essapian 1953). Signature whistles were thus conceptually defined as the most frequently emitted whistle type produced by captive dolphins, which is unique to an individual and exhibits stereotypy in some acoustic elements (Caldwell et al. 1990). Thereafter, Janik and Slater (1998) demonstrated that signature whistles were produced only when individuals were out of visual contact, supporting the Caldwell and Caldwell (1965) hypothesis. Janik and Slater (1998) showed that a young dolphin modifies the signal structures of its signature whistle based on its experience of other individuals' whistles, a process referred to as vocal production learning (Janik and Slater 1998). The definition was then refined by Janik and Sayigh (2013), who proposed that signature whistles are individually distinctive whistle types that broadcast the signal sender's identity and develops in a dolphin's early life. This definition highlights that a signature whistle is a learnt whistle type that is modified over time and is influenced by the signature whistles of their conspecifics (Janik and Sayigh 2013).

Proposed function of signature whistles

A signature whistle appears to be an integral part of the whistle repertoire because it functions as a signal that can encode information about identity and has been functionally linked to individual recognition (Janik et al. 2006; Sayigh and Janik 2009). Playback experiments conducted on temporarily restrained common bottlenose dolphins *T. truncatus* showed that individuals responded more to playbacks of signature whistles of their kin rather than non-kin (Sayigh et al. 1999). This indicates that individuals recognize familiar whistles. In another study, allied common bottlenose dolphin males increased signature whistle production when separated from their partners, thus indicating that signature whistles could also be a sign of social stress (Watwood et al. 2005). The increased rate of signature whistle production in alliances implies that allies use signature whistles for group cohesion because they are close associates and are thus motivated to reunite after being separated from one another (Janik and Slater 1998; Watwood et al. 2005). This has been shown in groups of captive common bottlenose dolphins, which produce mostly signature whistles during separation from one another (Janik and Slater 1998). In contrast, when all group members are together, non-signature whistles are produced almost exclusively, indicating that they also function as a contact call in common bottlenose dolphins (Cook et al. 2004, Watwood et al. 2005). Dolphins can also encode additional information such as an individual's motivational state by changing whistle parameters but maintaining their fundamental frequency (Sayigh and Janik 2009). For example, during brief capture-release events, common bottlenose dolphins increased their whistling rate compared to their whistle rate when undisturbed (Watwood et al. 2005; Esch et al. 2009b). The increase in whistle rate during temporary capture-release events suggests that signature whistles can thus be used to infer short-term stress (Esch et al. 2009b).

Signature whistle development and stability

Dolphins develop signature whistles through vocal production learning which entails an individual modifying a signal that already exists in its vocal repertoire (Lily 1965). This is often a signal the learning individual has been exposed to (such as other conspecifics' signature whistles), and the individual modifies the signal until it produces a signature whistle that is unique to it (Caldwell and Caldwell 1979; Janik and Slater 2000). Vocal production learning is a key component in the development of signature whistles, which has been supported by anecdotal evidence and the studies below (Richards et al. 1984; Miksis et al. 2002). A two-month-old stranded female common bottlenose dolphin calf developed its signature whistle to resemble that of its foster mother of the same species (Tyack and Sayigh 1997). In another case, a captive male common bottlenose dolphin calf's signature whistle resembled that of a completely different species, a Pacific white-sided dolphin *Lagenorhynchus obliquidens,* with which it was housed (Caldwell and Caldwell 1979). This shows that calves develop their whistles to mirror the sounds of other dolphins that they encounter in their natal environment (Tyack 1997).

Captive common bottlenose dolphins produce variant whistles within the first 4 to 6 months of age, and within its first year, a common bottlenose dolphin calf signature whistle becomes established and the modulation pattern rarely changes thereafter (Caldwell and Caldwell 1979; Sayigh 1992). Once developed, a signature whistle appears to be unvarying; for example, signature whistles have been found to remain stable for up to 11 years in captive common bottlenose dolphins (Sayigh 1992). Although signature whistles tend to vary in duration, frequency and number of loops, they still maintain a distinct contour pattern (Caldwell et al. 1990). However, one exception is the signature whistles produced in male alliances. Alliances are social units made up of reproductive males which cooperate when competition arises with other groups of males (Connor et al. 2001). First order alliances are made up of pairs or trios. When first order alliances form cooperative associations with other pairs or trios, they form second-order alliances (Connor and Krützen 2015). Both first- and second-order alliance members can recruit alliance partners, regardless of the order, to aid in female acquisition, alliance attacks or for defence against alliance attacks (Connor et al. 1992a). Although female common bottlenose dolphin signature whistles remain the same over years, males modify the structure of their signature whistles when forming stable first-order alliances (Watwood et al. 2004; King et al. 2018). Through vocal production learning, alliance partners converge on a signature whistle as their affiliative relationship develops (Sayigh et al. 1990, 2007). Even though the males' whistles become very similar, each individual still retains specific aspects of their original signature whistle modulation pattern (Smolker and Pepper 1999; Watwood et al. 2005). However, second-order alliance members show varying preferences for different partners which leads to lack of stability in second-order alliances, resulting in males in these alliances retaining their distinct signature whistles (Connor and Krützen 2015, King et al. 2018).

Identifying signature whistles

Researchers conventionally use qualitative classification techniques to group whistles, but quantitative classification techniques are often used as an alternative to, or in conjunction with qualitative classification techniques (Janik 1999; McCowan and Reiss 2001; Quick and Janik 2012). The qualitative technique for categorising whistles involves visually categorising whistle contours into whistle classes whereas the quantitative techniques determine similarities between whistle contours by using multiple acoustic parameters, such as frequency and time parameters (Tyack 1986; Sayigh et al. 1995; Janik 1999; Bazúa-durán and Au 2002). Although computer algorithms exist to discriminate between signature whistles, they have yet to account for the subtle differences in modulation pattern, duration, and number of loops (Caldwell et al. 1990; Janik 1999). Since signature whistle types are individually distinctive, each individual's signature whistle differs from that of another. These differences can be assessed by analysing the shape of the contour or the acoustic parameters such as start, end, minimum and maximum frequency or time (Janik 1999; Deecke and Janik 2006). These measurements can be used to determine the inter-whistle-intervals and inter-loop-intervals of signature whistles because these also differ between individuals (Caldwell et al. 1990; Esch et al. 2009a; Gridley 2011).

Classifying whistle contours

1.1. Visual classification

Dolphin whistles are often classified and categorized according to their modulation patterns, which is the contour shape, i.e., a curve that tracks the whistle frequency over time (Dreher 1961). Whistles can be converted into spectrograms, which are visual representations of the spectrum of frequencies varying in time and intensity. A spectrogram is a tool often used to distinguish between different whistle types using visual classification. Identifying signature whistles is relatively easy in captive situations and when free-living dolphins are temporarily restrained. Isolating common bottlenose dolphins from their groups leads to whistle production, with signature whistles accounting for approximately 80% - 100% of the whistles and it makes it easier to identify the whistling individual (Caldwell et al. 1990). Visual classification is a qualitative technique and has been widely used to show signature whistle use in these isolation contexts (Caldwell and Caldwell 1965; Janik et al. 1994; Janik and Slater 1998; Watwood et al. 2005; Sayigh et al. 1990, 1995; Esch et al. 2009b).

1.2. McCowan Method

The McCowan method normalizes whistle duration and extracts an equal number of frequency measurements and time points, thus comparing the frequency measurements using correlation and principal components analysis (McCowan and Reiss 1995, 2001). Once a similarity measure has been calculated for pairs of sampled whistles and a cluster analysis is conducted to categorize whistle types, the whistle types are grouped based on the non-overlapping clusters. The McCowan method is therefore a quantitative technique.

1.3. Cross-correlation method

Cross-correlations and cluster analyses are computer-based whistle classification methods that group whistles according to their similarity and dissimilarity respectively. The cross-correlation method determines a similarity measure using a specific formula generated by Beeman (1996) and Khanna et al. (1997), whereas the cluster analysis method calculates the absolute difference in frequency between the two whistle contours. The similarity and dissimilarity matrices are then used to determine the final whistle types using cluster analysis (Janik 1999). The cross-correlation method is therefore a quantitative technique.

1.4. ARTwarp

There is a deep learning programme available called ARTwarp. ARTwarp is a MATLAB-based programme used to automatically categorize tonal animal sounds. It has been tested successfully on common bottlenose dolphin whistles and killer whale calls (Deecke& Janik 2006), but will be applicable to any sound that can be described by frequency contours. It has also been used to verify signature whistle types after the visual categorisation process in signature whistle studies involving other species such as Indo-Pacific bottlenose dolphins (Gridley et al. 2014) and common dolphins (Fearey et al. 2019). The algorithm combines dynamic time-warping (Buck & Tyack 1993) to measure contour similarity with an Adaptive Resonance Theory (ART) neural network to group sounds into different categories. The programme provides categorisation details, as well as a reference contour representing the typical frequency shape of each category.

I chose to use the visual classification method because when the three methods were compared, computer methods (i.e., McCowan method and the cross-correlation method) differed significantly on the classification of non-signature whistles (Janik 1999). Moreover, researchers have been shown to classify whistles of the same type reliably in blind classification experiments using visual classification (Janik 1999; Sayigh et al. 2007, Kriesell et al. 2014). The success of the visual classification lies in the fact that human observers use the overall shape and weigh each part of the whistle contour equally when classifying whistles. Inaccurate classifications from computer methods could be attributed to the way they compare whistle contours. The issue with the McCowan method is that it normalizes the duration and number of frequency measurements taken from each contour, which disregards the differences in frequency and time parameters naturally present in dolphin whistles (Janik 1999, de Figueiredo and Simao 2009). The problem with the cross-correlation method is that the similarity value in the cross-correlation method is not directly dependent on the shape of the whistle contour when classifying whistles into classes (Khanna et al. 1997). The variation in signature whistle parameters requires sophisticated analyses to account for intra-individual variation, such as when bottlenose dolphins stretch and compress the overall modulation pattern of their signature whistles (Janik et al. 1994).

SIGnature IDentification (SIGID) method

It is more challenging to identify signature whistles and the identity of the whistler when individuals cannot be isolated or restrained. However, an alternative method has been devised which has facilitated identifying signature whistle use in free-swimming dolphin populations using whistle temporal parameters. The SIGnature IDentification method (referred to as SIGID throughout) was used to identify the signature whistles in unrestrained common bottlenose dolphins, recorded from a single hydrophone, by analysing the temporal pattern of whistle production (Janik et al. 2013). According to SIGID criteria (from Janik et al. 2013), if most (>75%) of the whistles in bouts of the same type are produced within 1-10 s of another whistle, it's likely a signature whistle. These timing parameters account for frequency shifts and time warping (dolphins increasing or decreasing frequency and time parameters), i.e., context-specific changes in whistle parameters, which cause the same signature whistle type to look slightly different (Buck and Tyack 1993; Janik et al. 1994). It is important to note that this approach is very conservative: Janik et al. (2013) designed it to eliminate false positives completely, and it identified only 50% of the signature whistle types in their study populations. However, it is the only method to date that can identify signature whistles in unrestrained dolphins with a single hydrophone. Janik et al. (2013) present two subtly different methods to assess the criteria. I chose the more accurate one in which sequences of whistles are analysed to identify instances in which there are at least four whistles and at least three of them meet the 1-10 s criterion.

Dolphin species with signature whistles

Signature whistle research has focused on common bottlenose dolphins *T. truncatus*, but other species have been found to use signature whistle types too. These include Indo-Pacific bottlenose dolphins *T. aduncus* (Gridley et al. 2014), common dolphins *Delphinus delphis* (Caldwell and Caldwell 1968; Fearey et al. 2019), Pacific white-sided dolphins *Lagenorhynchus obliquidens* (Caldwell and Caldwell 1970), Guiana dolphins *Sotalia guianensis* (Duarte de Figueiredo and Simão 2009; Lima and Le Pendu 2014), and possibly Australian humpback dolphins *Sousa sahulensis* and Indo-Pacific humpback dolphins *S. chinensis* (van Parijs and Corkeron 2001; Cheng et al. 2017). Guiana dolphins emit stereotypic, looped whistles which is indicative of signature whistle use. de Figueiredo and Simão (2009) classified these whistles qualitatively using the visual classification method and quantitatively using the McCowan method. 78% of the signature whistle types were recorded while the dolphins exhibited behaviours that have been functionally linked with signature whistle use, specifically travelling and foraging. Furthermore, 17 of the 27 signature whistle types had short inter-whistle-intervals, also indicative of signature whistles. Similarly, visual classification revealed that common dolphin whistles exhibit a high degree of stereotypy (Caldwell et al. 1990, Fearey et al. 2019). Additionally, Gridley et al. (2014) also established signature whistle use in Indo-Pacific bottlenose dolphins using the SIGID method. By identifying bouts and analysing the temporal patterns of stereotyped whistle production, signature whistles may be identified.

Sousa *acoustics*

There is anecdotal evidence that members of the *Sousa* genus may be using signature whistle types despite only a few acoustic studies being conducted on the *Sousa* genus compared to the *Tursiops* genus. For example, Australian humpback dolphin *S. sahulensis* vocalisation and behaviour suggests signature whistle use within the species (van Parijs and Corkeron 2001a). Through the visual classification method, van Parijs and Corkeron (2001a) identified whistle types which were predominantly used during socializing and foraging. The results also indicated an increase in whistle production as the number of mother-calf pairs increased, therefore suggesting that whistles could be used as contact calls (Van Parijs and Corkeron 2001a). However, signature whistle use could not be tested due to restraints imposed by data collection methods.

A repeated, stereotyped whistle type was recorded from an injured adult Indo-Pacific dolphin *S. chinensis* which suggested possible signature whistle use (Cheng et al. 2017). Spectrograms were visually analysed and classified into 11 whistle types using the visual classification method (Janik and Slater 1998). Whistle type 1 met the SIGID criteria with 22 out of 29 inter-whistle-intervals ranging from 1-10s, thus indicating that whistle type 1 may be a signature whistle type (Cheng et al. 2017). Similarly, van Parijs and Corkeron (2001b) recorded a stranded female Australian humpback dolphin. Using the visual classification method, they found whistle contours consisting of four variations of looped whistles. Taking into consideration the repetitive nature of this whistle type, it coincided with whistling behaviour seen in captive and temporarily restrained common bottlenose dolphins, ultimately suggesting signature whistle use (Caldwell and Caldwell 1968; Janik and Slater 1998). Furthermore, the stressful situation to which the individual was subjected suggests that the individual was producing a signature whistle type (van Parijs and Corkeron 2001b). However, systematic studies of signature whistles in the *Sousa* genus are sorely lacking. To date, there have been findings hinting towards the use of signature whistles in Australian humpback dolphins (van Parijs and Corkeron 2001b, Seekings et al. 2010), and only one confirmation of stereotyped whistles in Indo-Pacific humpback dolphins (Cheng et al. 2017).

Study species

The Indian Ocean humpback dolphin *S. plumbea* (referred to as humpback dolphin throughout) is the most threatened marine mammal within South African waters, with their regional Red List status classified as Endangered B1ab (iii,v) since 2016 (Plön et al. 2016). Their restrictive near-shore distribution, declining habitat quality and anthropogenically-related mortality are the underlying causes of their endangered status (Braulik et al. 2015; Plön et al. 2016). This species is particularly susceptible to the impacts of human activities in terrestrial and marine environments, especially since they prefer habitats located 2 km off the coastline and restricted to the 25 m deep isobath (Karczmarski et al. 2000; Plön et al. 2016). The humpback dolphin is distributed from the Bay of Bengal, Asia to False Bay, South Africa, but most parts of the species' range have yet to be surveyed (Jefferson and Rosenbaum 2014; Braulik et al. 2015). Humpback dolphins have been studied in Richards Bay, Algoa Bay, False Bay, Plettenberg Bay and Mossel Bay in South Africa. Low population numbers have been reported throughout while continuing decline in population size has been inferred (Braulik et al. 2015; Plön et al. 2016; Vermeulen et al. 2018). This highlights the need for studies that aid in the acquisition of presence data, habitat use and spatial distribution data on humpback dolphins to appropriately inform policies in managing anthropogenic threats to the species (Baldwin et al. 2004; Karczmarski et al. 1998; Weir et al. 2011).

Areas with several anthropogenic threats can exacerbate population decline and when such an area exhibits high habitat suitability for a species along with high anthropogenically-related mortality, it is considered an ecological trap (Battin 2004). Richards Bay is one of many areas in KwaZulu-Natal Province, South Africa, using shark nets in bather protection programmes with the intent to catch large sharks in order to reduce the probability of shark attacks (Dudley 1997). Humpback dolphin bycatch occurs in these shark nets, with 60% of the bycatch taking place at Richards Bay. This area comprises a mere 5% of the netting effort in the KwaZulu-Natal Province (Atkins et al. 2013, 2016). In addition, the Richards Bay harbour mouth is considered a core feeding/foraging area, making it an ecologically important site for transient and resident humpback dolphins (Keith et al. 2013; Atkins et al. 2016). As a functioning estuary, the Richards Bay harbour is a preferred feeding ground for humpback dolphins (Durham 1994; Weerts and Cyrus 2002a). However, humpback dolphins are exposed to various threats in this area, such as noise, chemical and plastic pollution, the probability of ship-strikes, urban expansion and persistent coastal development (Keith et al. 2013). The shipping lane, used by recreational and commercial vessels arriving and departing from Richards Bay, increases some of these threats (Keith et al. 2013). The combination of anthropogenic threats with the relatively high population density of humpback dolphins makes Richards Bay a zone of risk, an ecological trap, and thus an important study area for humpback dolphin conservation.

Determining movement patterns or estimating various population parameters in order to monitor threatened marine mammal populations often requires individual recognition (Hammond et al. 1990). Photo-identification (photo-ID) is a well-known method of identifying individuals using visual cues in cetaceans (Hammond 1995; Marshall and Pierce 2012). However, the humpback dolphin population of Richards Bay occurs in muddy waters and are very elusive, thus making photo-ID a difficult task.

Signature whistles encode information of individual identity (Caldwell and Caldwell 1965; Janik and Slater 1998) which can potentially be used to determine movement patterns or estimate population parameters (Hammond et al. 1990; Longden et al. 2020). Vocal signatures of individuals could be used as an alternative way to identify individuals for mark-recapture studies monitoring populations, habitat use, individual ranging patterns and population size (Terry et al. 2005; Janik et al. 2013). This could be conducted through acoustic surveys such as passive acoustic monitoring (PAM), which involves using anchored hydrophones in the water column to potentially aid in monitoring population parameters (Janik et al. 2013). This study is the first to validate signature whistle occurrence in humpback dolphins *S. plumbea*.

AIMS AND OBJECTIVES

The aim of my study is to establish whether Indian Ocean humpback dolphins *S. plumbea* in Richards Bay, KwaZulu-Natal Province produce signature whistles. There are two objectives associated with this aim.

a. Objective 1:

Collect acoustic data of Indian Ocean humpback dolphins *S. plumbea* at Richards Bay to examine the temporal production of potential signature whistle types using the SIGID method.

I. Key questions:

ii. Are some whistles with similar contours repeated in quick succession?

ii. If so, do they match the criteria used to identify signature whistles in bottlenose dolphins *T. truncatus*?

iii If not, can the pattern of repeats be described, i.e., what are the inter-whistle-intervals and inter-loop-intervals?

Prediction 1: I predicted that humpback dolphins use signature whistle types.

b. Objective 2

Determine the recapture rate of signature whistle types over the sampling period.

II. Key questions

i. Can signature whistle types be recognised over sampling years between 2017 and 2020?

ii. If so, what is the recapture rate?

iii What is the rate of discovery?

ii. How much acoustic sampling effort might be required to capture all the individuals in the population?

Prediction 2: If humpback dolphins *S. plumbea* use signature whistles, I expect the signature whistle types to remain stable over time.

Prediction 3: I expected to have recaptures of some individuals.

METHODS

Data collection

Acoustic data were collected from Richards Bay (28.808731°S, 032.089663°E) which is on the coast of KwaZulu-Natal Province, South Africa. The study area was the harbour entrance and comprised of a 900m wide harbour mouth, bordered by sandy beaches and the North and South breakwaters extending out to sea (Figure 1). The substrate is predominantly fine particles, i.e., muddy (Weerts and Cyrus 2002a, b). The two breakwaters approximately 1.2 km long act as artificial reefs that may attract humpback dolphin prey which could explain the high proportion of humpback dolphins feeding in this area (Fennessy et al. 1998; Atkins 2004). The water temperature ranges from a minimum of 17.2◦C in winter to a maximum of 27.1◦C in summer, which is considered representative of subtropical estuaries (Harris and Cyrus 1997; Vivier and Cyrus 1999; Izegaegbe et al. 2020). The average spring tide is 1.86 m and the average neap tide is 0.50 m, which categorises the Richards Bay tides as semi-diurnal (Rautenbach et al. 2019). The larger parts surrounding the study area exhibits high water turbidity and the channel at the harbour mouth is often dredged as deep as 20 m to allow exit of the coal bearing ships (Durham 1994).

Figure 1. Aerial photograph of Richards Bay harbour mouth, the sampling area, with a white box marking the location of an anchored hydrophone. Visual surveys were conducted from the distal end of the northern breakwater, to the east of the hydrophone. Google Earth version 9.142.01 (2021). *Richards Bay Harbour Mouth. 28°48’21”S 32°05’52”E.* Available through https://earth.google.com/web/search/richards+bay+harbour/. Accessed 06/08/2021.

Most of the acoustic data were recorded using a SoundTrap hydrophone (ST300STD, Ocean Instruments Inc., New Zealand) with a sampling rate of 48 kHz and a frequency response of 20 Hz to 60 kHz. The hydrophone was deployed from 2017 to 2019 and recorded acoustic data for approximately 59 minutes of every hour. A local citizen scientist surveyed the area regularly, during daylight hours, and recorded visual-survey start and stop times, dolphin sightings and species identification. He took photographs of the dolphins using a Canon EOS 1300D equipped with a 75-300 mm zoom lens. These data were supplemented with short-term data collected by SeaSearch from 14 to 20 March 2020 using a SoundTrap hydrophone (ST300HF, Ocean Instruments Inc., New Zealand) with a sampling rate of 576 kHz and a frequency response of 20Hz to 150kHz. The hydrophone was left to record acoustic data for 10 minutes, with 10-minute intervals. Both hydrophones were securely attached with cable ties to the rope of an anchored buoy approximately 300 m from the beach and 200 m from the north breakwater (Figure 1). Species presence was determined by conducting visual surveys from a Dolphin Viewing Platform by way of continuous sampling in the presence of the focal species and 5-minute point samples when individuals were submerged. To confirm species, dolphins were photographed using two cameras, a Canon EOS 50D equipped with a canon EF 100-400mm 1:4.5-5.6 lens and Canon EOS Rebel T5 equipped with a canon EFS 55-250mm macro 0.85m/2.8ft lens.

Data organisation

Acoustic files were chosen systematically to ensure recordings were of humpback dolphins only (as Indo-Pacific bottlenose dolphins *T. aduncus* are occasionally sighted in the study area). This is to ensure that Indo-Pacific bottlenose dolphin acoustics were not included in the analysis of the humpback dolphin acoustics as this would contaminate the humpback dolphin acoustic data and potentially result in Indo-Pacific bottlenose dolphin whistles being incorrectly classified as humpback dolphin whistles. Using the timestamps from the photographs captured, I identified times when only humpback dolphins were present in the study area. For those confirmed single-species sightings, I used the visual survey start and stop times to determine the acoustic sampling window. These sampling windows were extended by one hour on each side and then extended again to start and stop "on-the-hour" so that the entire length of each recording could be analysed to maximise the amount of data. The 2020 recordings started four minutes into the hour. This entire period was referred to as an encounter.

Classifying whistle types

Data analysis was undertaken through visual classification on a personal computer, as it is common practise in bioacoustic research and has been successfully used to classify whistle types in various delphinid species (Janik 1999; Sayigh et al. 2007). Multiple phases of visual spectrographic analysis were conducted using Raven Pro 1.6 software (Cornell Lab of Ornithology, Ithaca, NY). The appropriate settings, i.e., Fast Fourier Transform (FFT) level and smoothing window, were identified during the spectrogram analysis by comparing different window functions in order to obtain a good frequency resolution and reduced spectral leakage which resulted in an FFT of 512, contrast of 66 and a brightness of 34 using the Plasma colour map.

Step 1 – assessing whistle quality

Whistle contours in the dataset were assessed and graded based on the quality rating as follows: Q1- an indistinct signal but visible on the spectrogram, i.e., the contour shape is very faint with breaks in the shape; Q2- a clear and unambiguous signal, i.e., the overall shape is visible with some parts appearing slightly fainter than others; and Q3- a prominent signal, a contour that is clearly visible with no breaks in shape. Whistle clarity was further graded based on (i) masking caused by whistle overlap which occurs when multiple individuals whistle simultaneously; and (ii) ambient noise, e.g., boat or ship generated noise, crashing waves or rattling equipment. Selection boxes were drawn around whistle contours and automatically generated time and frequency measurements.

Step 2 – visual categorisation of potential signature whistle types

I inspected all the whistle contours and placed them into one of three categories: unclassified, variant or potential signature whistle types. Whistles with a quality rating of 1 and those that were not clear due to noise or masking could not be classified confidently, i.e., unclassified. Whistles that were not repeated or stereotyped were classified as variant whistles. Whistles that exhibited stereotypy and were recognised as unique whistles were grouped into unique classes. I used these classes to generate a catalogue of potential signature whistles (psw), i.e., a reference collection of whistle types. The approach was largely descriptive, similar to Kriesell et al. (2014) and Fearey et al. (2019), using visual classification to place whistle contours into categories and signature whistle type classes, thereafter performing a verification task to confirm the classes they created. Kriesell et al. (2014) conducted a visual classification task with 6 judges to verify the signature whistle type classes they compiled and Fearey et al. (2019) verified their signature whistle type classes using ARTwarp, a MATLAB-based script. In my study, I was the sole observer analysing the recordings and placing potential signature whistles into distinct classes. Thereafter, two experts, Dr. Tess Gridley and Ms. Sasha Dines, also analysed the classes independently and made informed suggestions with regards to the distinctiveness of each class until there was complete agreement between myself and the experts with regard to the classes in the potential signature whistle type category.

Step 3 – creating the potential signature whistle type catalogue

All potential signature whistle classes were allocated a unique identification number, regardless of the quality rating. This collection of whistle types is known as a catalogue. In cases where two potential signature whistle classes had a very similar shape, I decided to group them. This occurred on two occasions, where four signature whistle classes were merged and reduced to two classes, following suggestions made by the experts during catalogue verification. This may potentially inflate the recapture rate. The entire dataset was re-examined for whistle contours that matched a potential signature whistle class but were not produced in bouts, or were of poor quality but recognisable. In other words, I compared all variant and unclassified whistle contours to the potential signature whistle classes to avoid inaccurately categorising the potential signature whistles as variant or unclassified whistles. Following this process of re-examination, the updated catalogue and the quality ratings of all the contours were verified once again by the two experts to ultimately confirm that each potential signature whistle class was distinctive and unique.

Step 4 – conducting the SIGID test on potential signature whistle types

Once the potential signature whistles had been classified, the next step was to test whether they met the criteria for identifying signature whistles. Janik et al. (2013) present two subtly different methods to assess the criteria. I chose the more accurate one - the sequential bout analysis - in which sequences of whistles are analysed to identify instances in which there are at least four whistles and at least three of them meet the 1-10 s criterion. Using the measurements taken in step 1, the inter-whistle-intervals were calculated by subtracting the end time of the first whistle from the begin time of the subsequent whistle which were generated by Raven Pro 1.6 software. This was repeated for all whistles of the same class in each encounter. I used a NESTED IF formula to identify instances in which there were four whistles and at least three of the four (75%) whistles occurred within 1-10 s of the others. Potential signature whistle classes that did not have (i) at least one encounter with four whistles or (ii) had four or more whistles but did not meet the 1–10 s criteria were thus omitted from the signature whistle type catalogue. Whistles with a quality rating of 1 were included when calculating the inter-whistle-intervals in order to prevent the overestimation of the inter-whistle-interval measurements. If poor quality whistles are omitted from the analysis, inter-whistle intervals will be inflated. There is a dilemma because using whistles of poorer quality reduces the certainty of the results, but omitting them is likely to inflate the measurements of inter-whistle interval. However, subsets of the data were created to demonstrate the effect quality ratings would have on inter-whistle-intervals.

Step 5: Creating subsets of the data according to whistle quality ratings

Subsets of the data were created according to whistle quality ratings, resulting in the SIGID method being applied to three separate datasets: (i) the full dataset, (ii) good quality whistles, and (iii) good quality sequences. The full dataset contained potential signature whistle classes with all quality ratings, i.e., 1-3, from all 30 encounters (i.e., sampling windows). The first subset comprised of potential signature whistle classes of good quality whistles only, i.e., whistles with a quality rating of 2 or 3 from 30 encounters, whistles with a quality rating 1 were not included. The negative aspect of using this approach is inflated inter-whistle intervals. Good quality sequences were systematically selected in the following way. Firstly, the proportion of good quality potential signature whistle classes (rating 2 and 3) to bad quality whistles (rating 1) was calculated for each encounter. Encounters with 75% or more good quality potential signature whistle classes were selected for analysis. Secondly, for encounters that had a lower proportion of good quality potential signature whistle classes, each was scanned for a single sequence in which 75% of the potential signature whistles were of quality 2-3. Encounters that did not meet the quality criterion were excluded from the analysis since these could not be tested against SIGID criteria. Good quality sequences were obtained from 17 encounters.

Investigating loop structure

The signature whistle types were classified according to their loop structure, categorizing each as either a single element, continuous multi-loop or disconnected multi-loop whistle. A single continuous whistle was classified as either a single element or continuous multi-loop whistle, whereas whistles with multiple successive contours, with short breaks between the contours (less than 0.25s) were classified as disconnected multi-loop whistles. The successive contours are referred to as terminal loops, and the first contour in the disconnected multi-loop was the introductory loop. Loop temporal production, i.e., inter-loop intervals, was analysed for all disconnected multi-loops by subtracting the end time of the introductory loop from the begin time of the following loop, and so forth for all subsequent terminal loops (Janik et al. 2013).

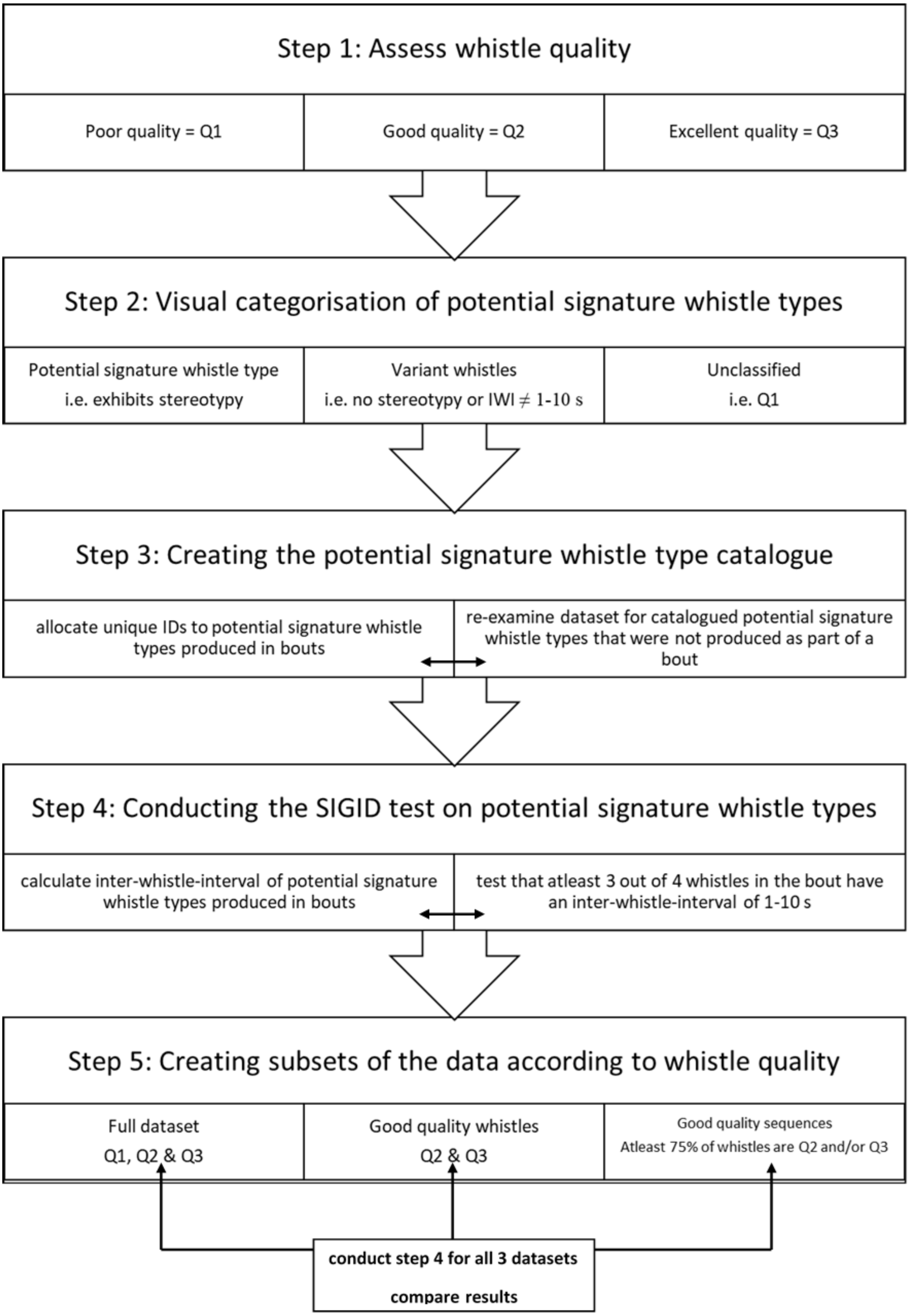


Figure 2: A schematic diagram outlining five steps (Step 1 to Step 5) of the whistle classifying process used to classify the signature whistle types of humpback dolphins *Sousa plumbea* in this study. Below each step heading, the main categories have been outlined. The double-ended arrows represent iterative processes. For example, in Step 3 the process of calculating inter-whistle-intervals was systematically followed by checking the number of whistles in bouts, and these processes were repeated interchangeably.

Signature whistle type recapture over time

Discovery curve

Discovery curves were plotted to ascertain: (i) the rate of discovery of new signature whistle types; and (ii) how much acoustic sampling effort is required to 'capture' all the individual humpback dolphins in the Richards Bay population. I used rarefaction curves instead of species accumulation curves in order to account for biases associated with sampling techniques which often influence the shape of species accumulation curves, i.e., sampling biases associated with differences in sampling methods similar to the varying number of sampling days in each year throughout my sampling period (Colwell 2000a; Gotelli and Colwell 2001; Work et al. 2005). Accumulation curves plot the total number of individuals sampled in the order they were sampled during data collection, whereas rarefaction curves are created by randomly resampling a pool of individuals repeatedly, and plotting the average number of individuals encountered at each sampling unit, ultimately generating the observed number of individuals (Gotelli and Colwell 2001). Once I have established that humpback dolphins use signature whistles, I can assume that a unique signature whistle type is a unique individual. Using the number of signature whistles captured and recaptured over the 4-year sampling period, the rarefaction curve plotted the averages of repeatedly re-sampled individuals in the Richards Bay humpback dolphin population. As a result, the curve represents what could be the observed individuals in an accumulation curve as estimated averages of the observed individuals, referred to as S(est) values (Gotelli and Colwell 2001; Colwell et al. 2004, 2012).

I plotted the cumulative signature whistle type count to the rarefaction curve to aid in discerning how rate of capture of individuals over the study period coincided with the curve. In addition, rarefaction curves were plotted for each sampling year on a single axis, which aided in visualising differences in sampling effort because the number of sampling units, i.e., encounter days, varied between each sampling year (Cantor et al. 2012). The sample-based rarefaction helped ascertain whether the final estimated number of 'captured' individuals were near the asymptote of the curve, specifically using the EstimateS diversity statistic Chao2 mean $\hat{S}_{est\,Chao2} = \hat{S}_{obs} + \hat{Q}_0 + \hat{Q}_{0\,Chao2}$ (Work et al. 2005; Cantor et al. 2012). Chao2 and the associated descriptive statistics simplified interpretation, which allowed me to avoid interpretation problems associated with rarefaction curves by using the Chao2 mean to determine whether the curve is asymptotic (Gotelli and Colwell 2001). If the Chao2 mean is not approximate to the observed number of signature whistle types, this would indicate an inadequate sampling effort. Considering the small population size, i.e., 19 individuals, I only used the full dataset when plotting the discovery curve and investigating the number of individuals (i.e., signature whistle types) recaptured.

RESULTS

Encounter data

Data were analysed from 30 encounters of humpback dolphins *S. plumbea* at Richards Bay Harbour. These encounters took place over 6 days in 2017, 10 days in both 2018 and 2019, and 4 days in 2020. The recording time per encounter ranged from 59 to 480 min (mean= 229.53±85.09 min), with 93% of encounters having more than 120 min of acoustic survey effort, i.e., acoustic recordings. A total of 114 hrs 46m 04 s of recordings were collected and analysed for potential signature whistles (Table 1).

Table 1. The length of acoustic recordings, encounter information, the number of whistle contours in each quality rating category (i.e., Q1, Q2, Q3) and the total number of whistle contours of the Richards Bay humpback dolphin *S. plumbea* population.

Sampling year	Encounter ID	Recording length (hh:mm:ss)	Q1	Q2	Q3	Total number of whistles
2017	170520_1	03:56:00	7	0	0	7
	170523_2	02:57:00	134	56	0	190
	170524_3	03:56:00	9	2	0	11
	170629_4	03:56:00	25	7	0	32
	170704_5	02:57:00	8	3	0	11
	171009_6	02:57:00	3	2	0	5
2018	180209_7	01:58:00	84	78	16	178
	180224_8	04:55:00	91	45	0	136
	180228_9	00:59:00	14	1	0	15
	180303_10	02:57:00	50	57	18	125
	180318_11	03:56:00	109	64	22	195
	180321_12	03:56:00	62	59	4	125
	180324_13	02:57:00	2	0	0	2
	180329_14	02:57:00	71	30	0	101
	180406_15	02:57:00	71	33	2	106
	180526_16	03:56:00	127	93	1	221
2019	190109_17	03:56:00	61	48	8	117
	190122_18	06:53:00	182	392	157	731

	190131_19	02:57:00	94	166	29	289
	190202_20	02:57:00	0	0	0	0
	190214_21	03:56:00	62	56	11	129
	190522_22	05:00:00	182	85	12	279
	190602_23	06:00:01	131	6	0	137
	190706_24	04:00:00	27	30	1	58
	190719_25	08:00:01	48	18	5	71
	190815_26	02:00:00	8	34	1	43
2020	200314_27	05:00:01	85	42	7	134
	200315_28	05:00:01	57	47	20	124
	200318_29	04:00:00	52	16	5	73
	200320_30	03:00:00	15	34	24	73

Classified whistles

Whistle quality and whistle categories

In total, 3718 contours were categorised (Table 1). The analyses were conducted on the full dataset and two subsets of the data: good quality whistles only; and good quality sequences (Table 2 and 3). During the classification process of the full dataset, 1339 contours were categorised as potential signature whistles, 799 as variant contours, 1580 were unclassified. 77.45% of the potential signature whistles were good or excellent quality. The “good quality only” dataset had 1037 whistles categorised as potential signature whistles, resulting in a loss of 302 whistles and the added risk of reporting inflated inter-whistle-intervals. The “good quality sequences” dataset had 1076 contours categorised as potential signature whistles, with 86.62% of the potential signature whistles being good or excellent quality. This method left 17.74% less data compared to the full dataset. However, I could assume that the inter-whistle-intervals would be more accurate because there were no gaps when poorer quality whistle contours were excluded and because good quality sequences had a higher proportion of good quality whistles compared to the full dataset.

Table 2. The number of whistles in each quality rating (i.e., Q1, Q2 and Q3) for each humpback dolphin *Sousa plumbea* whistle category: UNCL: unclassified, PSW: potential signature whistle, VAR: variant whistle.

	UNCL			**PSW**			**VAR**			**Total**
Encounter ID	**Q1**	**Q2**	**Q3**	**Q1**	**Q2**	**Q3**	**Q1**	**Q2**	**Q3**	
170520_1	7	0	0	0	0	0	0	0	0	7
170523_2	103	1	0	31	51	0	0	4	0	190
170524_3	8	0	0	1	0	0	0	2	0	11
170629_4	24	0	0	1	0	0	0	7	0	32
170704_5	7	0	0	1	2	0	0	1	0	11
171009_6	3	0	0	0	1	0	0	1	0	5
180209_7	80	0	0	4	53	13	0	25	3	178
180224_8	63	0	0	28	36	0	0	9	0	136
180228_9	14	0	0	0	0	0	0	1	0	15
180303_10	46	1	0	4	45	18	0	11	0	125
180318_11	101	1	0	8	44	19	0	19	3	195
180321_12	48	0	0	14	49	3	0	10	1	125
180324_13	2	0	0	0	0	0	0	0	0	2
180329_14	54	0	0	17	25	0	0	5	0	101
180406_15	60	0	0	11	23	1	0	10	1	106
180526_16	74	0	0	53	74	0	0	19	1	221
190109_17	54	0	0	7	21	5	0	27	3	117
190122_18	167	1	0	15	199	90	0	192	67	731
190131_19	85	1	0	9	69	11	0	96	18	289
190202_20	53	1	0	0	0	0	0	0	0	0
190214_21	0	0	0	9	15	9	0	40	2	129
190522_22	162	2	0	20	25	3	0	58	9	279
190602_23	105	1	0	26	5	0	0	0	0	137
190706_24	8	0	0	19	17	1	0	13	0	58
190719_25	41	0	0	7	7	4	0	11	1	71
190815_26	5	0	0	3	14	0	0	20	1	43
200314_27	83	0	0	2	9	3	0	33	4	134
200315_28	51	0	0	6	16	16	0	31	4	124

200318_29	49	0	0	3	4	4	0	12	1	73
200320_30	12	2	0	3	17	16	0	15	8	73
Grand Total	1569	11	0	302	821	216	0	672	127	3718

Table 3. The number of whistles in each quality rating (Q1, Q2 and Q3) for each humpback dolphin *S. plumbea* whistle category, UNCL: unclassified, PSW: potential signature whistle, VAR: variant whistle. These whistles form part of good quality sequences, where at least 75% of the potential signature whistle had a quality rating of 2 or 3.

	UNCL			**PSW**			**VAR**			**Total**
Encounter ID	**Q1**	**Q2**	**Q3**	**Q1**	**Q2**	**Q3**	**Q1**	**Q2**	**Q3**	
180209_7	80	0	0	4	53	13	0	25	3	178
180303_10	46	1	0	4	45	18	0	11	0	125
180318_11	30	0	0	5	39	19	0	1	3	97
180321_12	48	0	0	14	49	3	0	10	1	125
180329_14	3	0	0	4	14	0	0	0	0	21
180406_15	45	0	0	7	22	1	0	10	1	86
190109_17	54	0	0	7	21	5	0	27	3	117
190122_18	167	1	0	15	199	90	0	192	67	731
190131_19	85	1	0	9	69	11	0	96	18	289
190214_21	25	0	0	6	14	9	0	22	1	77
190522_22	45	2	0	4	13	3	0	15	0	82
190719_25	20	0	0	3	6	4	0	6	1	40
190815_26	5	0	0	3	14	0	0	20	1	43
200314_27	83	0	0	2	9	3	0	33	4	134
200315_28	51	0	0	6	16	16	0	31	4	124
200318_29	3	0	0	0	1	4	0	1	1	10
200320_30	12	2	0	3	17	16	0	15	8	73
Grand Total	802	7	0	96	601	215	0	515	116	2352

SIGID – did the potential signature whistles meet the criteria?

The potential signature whistle catalogue comprised of a total of 22 potential signature whistle classes (Table 4). The catalogue grew progressively smaller as the potential signature whistles were tested against three main criteria: whistle quality, inter-whistle-interval and number of whistles in a bout.

Potential signature whistles occurring in bouts, where at least 75% of the whistles occurred within 1-10 s of subsequent whistles, were thus confirmed as signature whistle types. The full dataset, containing whistles with all the quality ratings (Q1-3), had three potential signature whistle classes that failed to meet the SIGID criteria and were omitted from the catalogue. The good quality whistles generated the same results as the full dataset, omitting the same three potential signature whistle classes. When applying the SIGID method to good quality sequences, four potential signature whistle classes were discarded from the catalogue since they failed to meet the bout-interval criterion and were of poor quality. The potential signature whistle classes discarded from the catalogue (n = 4) were thus categorised as "unfulfilled" whistle contours because they did not qualify as signature whistle types despite exhibiting stereotypy.

The unfulfilled potential signature whistle classes in the full dataset and the good quality only whistles were psw32b, psw53 and psw66 and the good quality sequences of unfulfilled potential signature whistle were psw32b, psw50, psw53 and psw66 (Table 3). This was largely because they did not occur in bouts of four whistles and the inter-whistle-intervals being less than the 1 s lower limit (psw53 and psw66) or being higher than the 10 s upper limit (psw32b). Additionally, when selecting for good quality sequences, 13 encounters were removed since these had less than 75% good quality whistles in sequence. This resulted in psw50 and psw53 being omitted from analysis because they were present only in encounter eight which only had 73% good quality whistles in sequence (Table 2).

The inter-whistle-intervals of the omitted potential signature whistle classes showed considerable variation. For psw32b, the inter-whistle-intervals ranged from 1.69 to 960.07 s in all three datasets, with a median of 36.91 s (mean±SD = 191.66±378.52 s) in the full dataset, a median of 15.66 s (mean±SD = 208.66±420.63 s) in good quality whistles and good quality sequences. In the full dataset, psw53 ranged from 0.11 to 6410.04 s with a median of 0.35 s (mean±SD = 277.34±1306.97 s), whereas psw53 had a range of 0.28 to 21.39 s with a median of 0.33 s (mean±SD = 4.35±8.44 s) in the good quality whistles only. Psw66 had a range of 0.88 to 1939.05 s with a median of 0.97 s (mean±SD = 485.47±969.06 s) in all three datasets, i.e., full dataset, good quality whistles only and good quality sequences. The omitted potential signature whistle classes had high standard deviation from the mean which indicates high variation between inter-whistle-intervals, regardless of the whistle quality rating. This could be attributed to each class having relatively low numbers of whistle contours. In the full dataset psw32b had 16 whistles, psw53 had 27 whistles, and psw66 had 5 whistles in all the datasets. Psw32b had 13 whistles in the good quality whistles only and good quality sequences (Table 4).

Bout structure and signature whistle type diversity

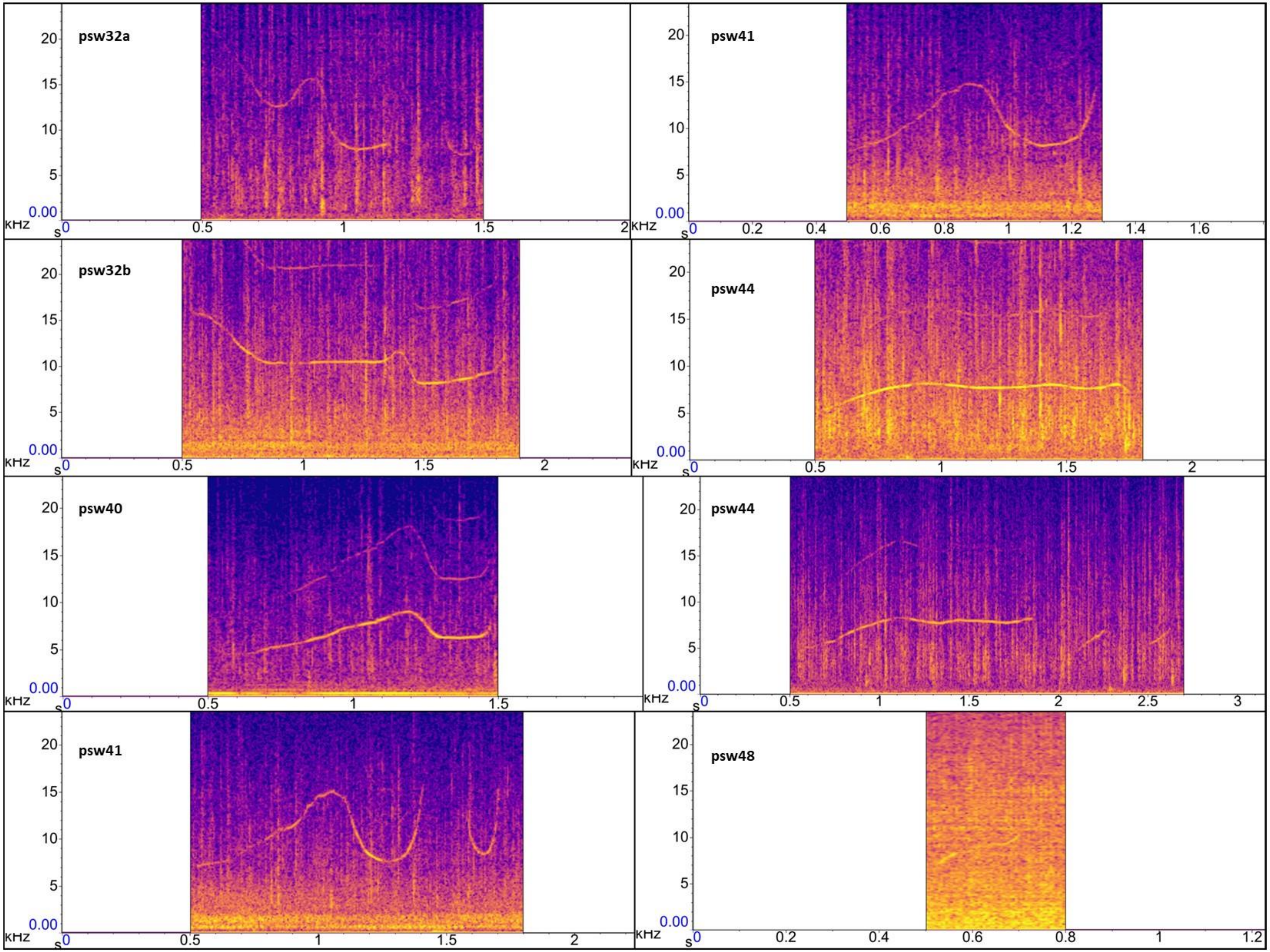
Overall, the full dataset (n = 1339) and good quality whistles only (n = 1037) had 19 signature whistle types in the final signature whistle type catalogue since they fulfilled the SIGID criteria specified by Janik et al. (2013). Good quality sequences (n= 912) had 18 signature whistle types (Figure 3).

In terms of bout structure, the full dataset had 667 signature whistle types that occurred in bouts and 672 occurring out of bout (Table 4). The number of whistle repeats in each signature whistle type ranged from 3 to 94 with an average of 9.01±11.90 (SD) for the entire population. The signature whistle type with the most whistle repeats in a single bout was swt56 with 94 repeats. Good quality whistles had 486 signature whistle types in bouts whereas 551 were out of bout, with whistle repeats that ranged from 4 to 39 with a population average of 8.84±7 (SD) and psw44 had the most whistle repeats in a single bout (n = 39). In good quality sequences, 443 signature whistle types occurred in bouts and 469 out of bout. Overall, the whistle repeats in the population ranged from 4 to 42 with an average of 7.86±6.66 (SD), with psw44 producing the most whistle repeats, i.e., 42, in a single bout.

Signature whistle occurrence was high for all three datasets. Signature whistle types were identified in 80.00% of the encounters (n = 30) in the full dataset. The number of signature whistle types identified, i.e., signature whistle diversity, varied between encounters. The average number of signature whistle types detected per encounter was 3.73±2.85 (SD), with a maximum of 12 different types identified during a single encounter. Signature whistle type diversity was relatively low, with 56.67% of encounters containing between 1 and 3 signature whistle types, accounting for a maximum of 15.79% of the signature whistle type catalogue, and 43.33% of encounters had more than 3 distinct types (4 to 12). Good quality whistles only had signature whistle types present in 77.00% of the encounters (n = 30). Signature whistle type diversity was still relatively low, with the average number of signature whistle types detected per encounter being 3.14±8.51 (SD), and a maximum of 12 different types identified during a single encounter. Of the 30 encounters, 57.00% of encounters had 1 to 3 signature whistle types and 43.00% encounters had more than 3 distinct types. Good quality sequences comprised of signature whistle types in 94.00% of the encounters (n = 17). The average number of signature whistle types detected per encounter was 4.41±2.81 (SD), with a maximum of 12 different types identified during a single encounter. Signature whistle type diversity was slightly higher, with 65.00% encounters made up of more than 3 distinct types and 35.00% of encounters containing at least 1 to 3 signature whistle types. The highest proportion of signature whistle types in a single encounter was 71.00% for all three datasets.

Table 4. The total number of whistle contours, whistles in a bout, whistles not in a bout, and the number of bouts for each potential signature whistle class. Each catalogue was compiled using the full dataset including all quality ratings, good quality ratings only and good quality sequences of the Richards Bay humpback dolphin *S. plumbea* population. Potential signature whistle classes that did not qualify as signature whistle types are highlighted in red. The full dataset had the highest whistle count whereas good quality sequences had the least.

Full dataset					**Good quality whistles only**					**Good quality sequences**				
Class	**Whistle count**	**Whistles not in a bout of 4**	**Whistles in a bout of 4**	**Number of Bouts**	**Class**	**Whistle count**	**Whistles not in a bout of 4**	**Whistles in a bout of 4**	**Number of Bouts**	**Class**	**Whistle count**	**Whistles not in a bout of 4**	**Whistles in a bout of 4**	**Number of Bouts**
psw32a	10	6	4	1	**psw32a**	8	4	4	1	**psw32a**	7	3	4	1
psw32b	16	16	0	0	**psw32b**	13	13	0	0	**psw32b**	11	11	0	0
psw40	44	36	8	1	**psw40**	40	34	6	1	**psw40**	36	28	8	1
psw41	52	44	8	1	**psw41**	47	39	8	1	**psw41**	50	42	8	1
psw44	109	48	61	5	**psw44**	89	35	54	4	**psw44**	93	36	57	4
psw48	35	22	13	3	**psw48**	20	16	4	1	**psw48**	14	9	5	1
psw49	39	29	10	1	**psw49**	33	23	10	1	**psw49**	39	29	10	1
psw50	21	14	7	1	**psw50**	16	10	6	1	**psw50**	0	0	0	0
psw51	30	19	11	2	**psw51**	20	13	7	1	**psw51**	17	10	7	1
psw53	27	27	0	0	**psw53**	9	9	0	0	**psw53**	0	0	0	0
psw56	248	42	206	11	**psw56**	154	35	119	8	**psw56**	40	10	30	2
psw58	35	9	26	3	**psw58**	26	7	19	2	**psw58**	27	7	20	2
psw60	74	40	34	7	**psw60**	61	37	24	5	**psw60**	72	39	33	7
psw61	294	155	139	19	**psw61**	272	145	127	16	**psw61**	287	148	139	19
psw62	161	107	54	10	**psw62**	106	79	27	4	**psw62**	99	55	44	8
psw63	11	3	8	1	**psw63**	11	3	8	1	**psw63**	11	3	8	1
psw64	39	14	25	1	**psw64**	33	13	20	1	**psw64**	36	11	25	1
psw65	16	6	10	2	**psw65**	15	5	10	2	**psw65**	14	4	10	2
psw66	5	5	0	0	**psw66**	5	5	0	0	**psw66**	5	5	0	0
psw67	36	11	25	3	**psw67**	22	7	15	3	**psw67**	17	0	17	1
psw68	19	15	4	1	**psw68**	19	15	4	1	**psw68**	19	15	4	1
psw69	18	4	14	1	**psw69**	18	4	14	1	**psw69**	18	4	14	1
Total	1339	672	667	74	**Total**	1037	551	486	55	**Total**	912	469	443	55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psw41
psw32b
psw44
psw40
psw44
psw41
psw48
kHz
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10
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20
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2
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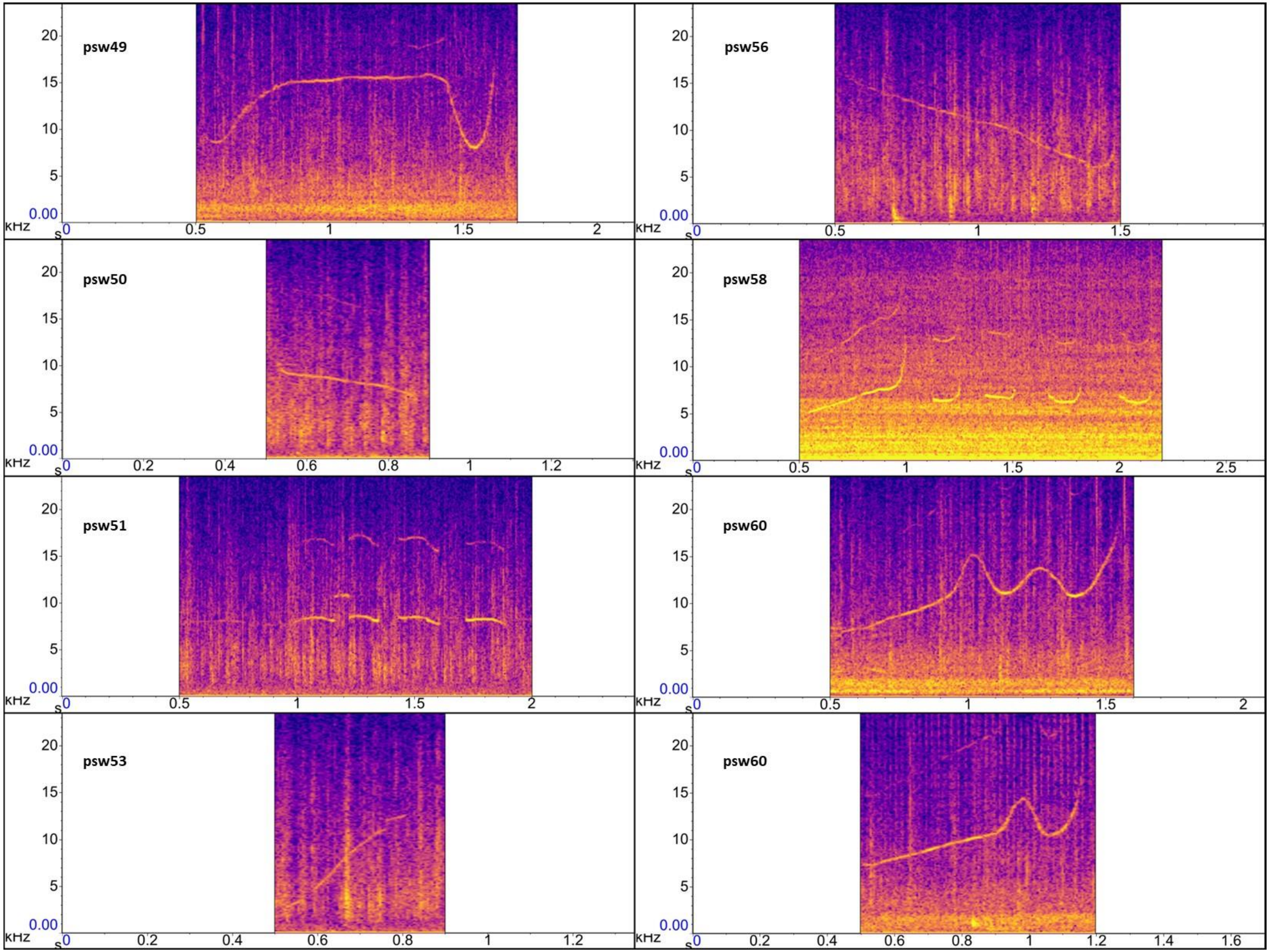
3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psw50
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0.6
0.8
1
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psw58
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psw51
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psw60
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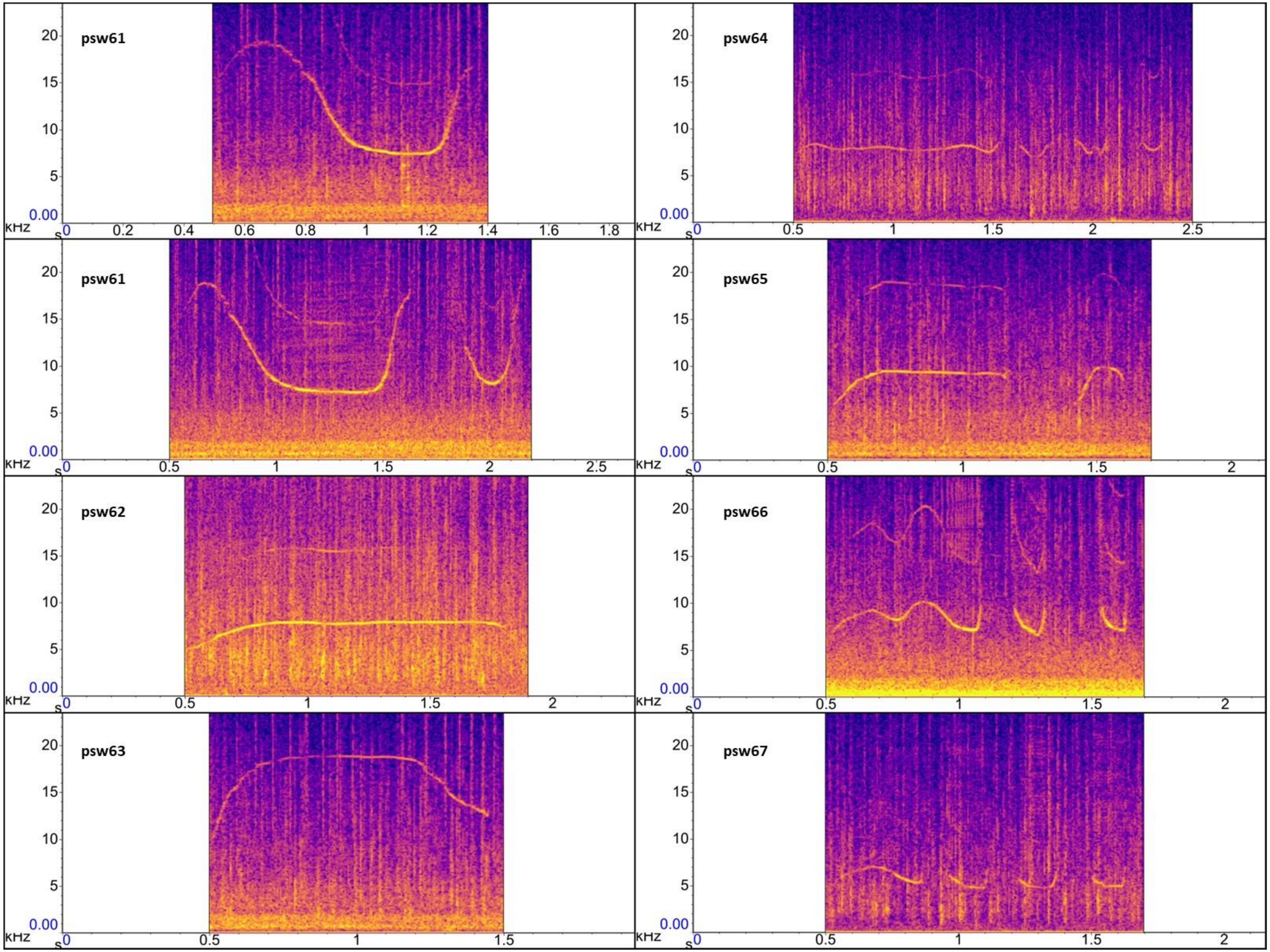
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1.6
1.8
2
2.5

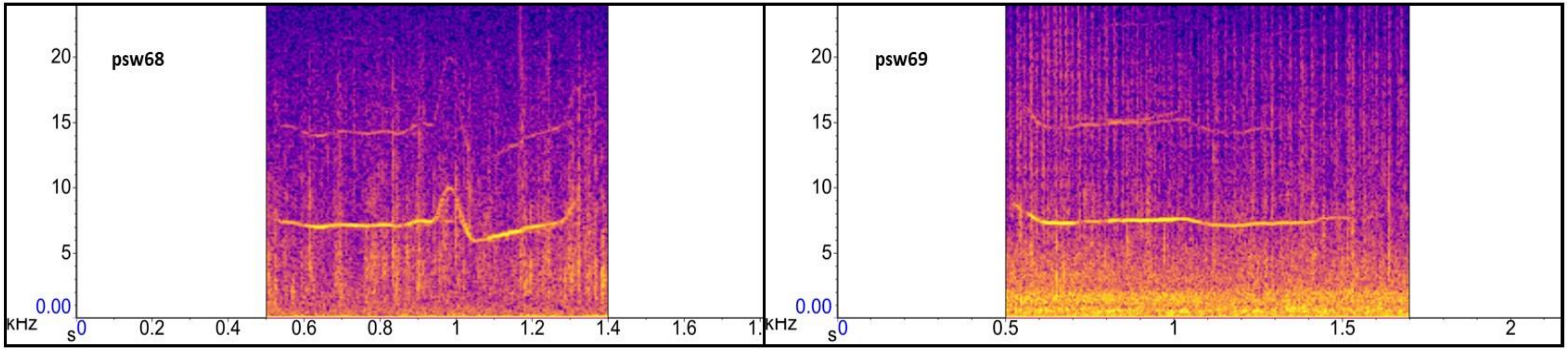
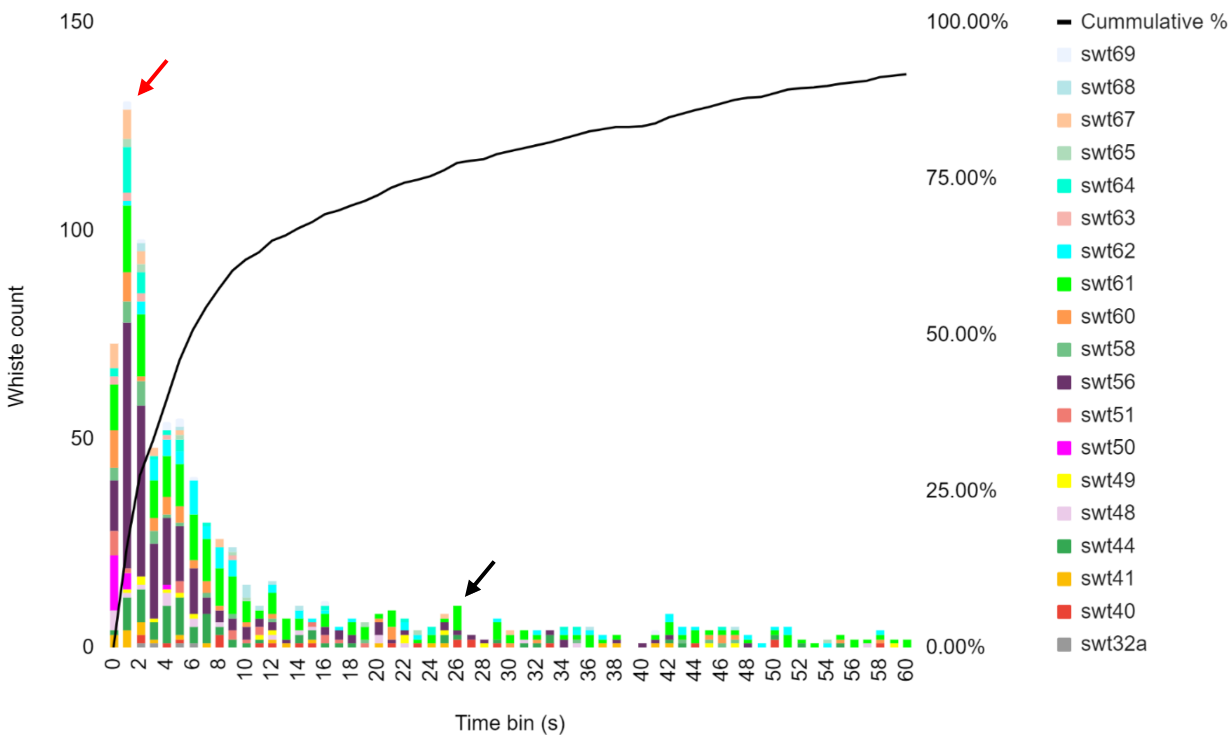


Figure 3. Spectrograms of the potential signature whistle classes of humpback dolphins *S. plumbea* identified during visual classification, with time (s) on the x-axis and frequency (kHz) on the y-axis. The whistles are depicted in the middle of the sound window and the loop structure is evident on each spectrogram. Whistle contours with a bright orange appearance are of high whistle quality. However, the more orange the background the more background noise during that recording, which often results in whistle masking as seen in psw48. Some whistles are produced in two variations, and are thus represented twice i.e., psw41, psw44, psw60 and psw61.

Inter-whistle-interval distribution

When assessing signature whistle types with an inter-whistle-interval of less than 1 min (n = 874) from the full dataset, 26.09% were repeated at intervals from 0 to 1 s, 60.53% from 1 to 10 s, 30.66% from 10 s to 1 min (Figure 4a). Whereas the good quality only whistles (n = 653) differed slightly in that 23.43% signature whistle types were repeated at intervals from 0 to 1 s, 59.72% from 1 to 10 s, 33.54% from 10 s to 1 min (Figure 4b). The signature whistle types from the good quality sequences (n = 576) comprised of 18.96% inter-whistle-intervals repeated from 0 to 1 s, 59.38% from 1 to 10 s, 35.94% from 10 s to 1 min (Figure 4c). The average for inter-whistle-intervals below 1 min was 11.4±14.19 s SD (median 5.30 s) and for the full dataset was 11.90±2.58 s SD (median 5.90 s) for good quality whistles only, and 12.80±14.80 s SD (median 6.40 s) for good quality sequences. These results indicate that the inter-whistle-intervals were often greater than the 10 s cut-off defined by SIGID.

a)



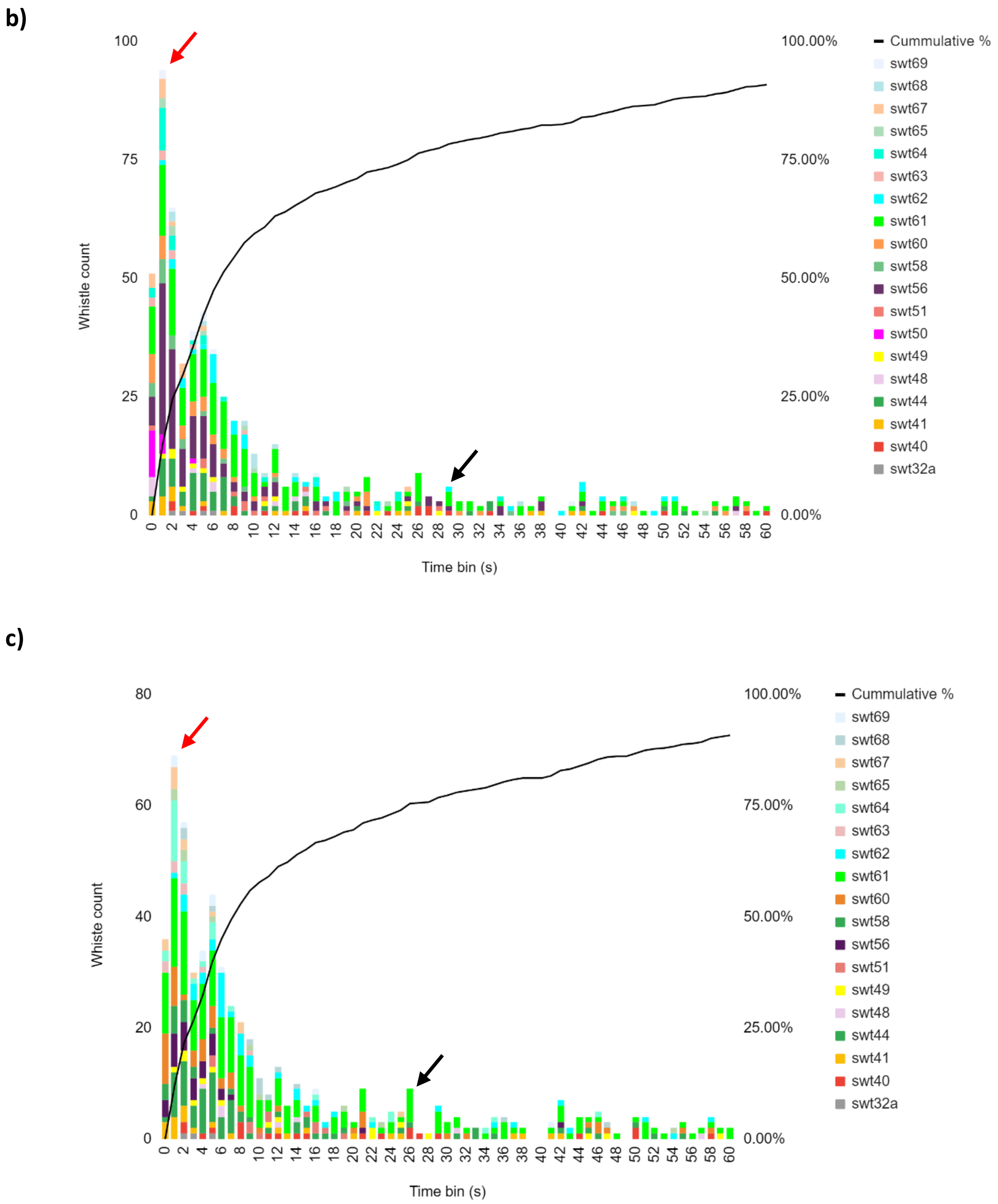


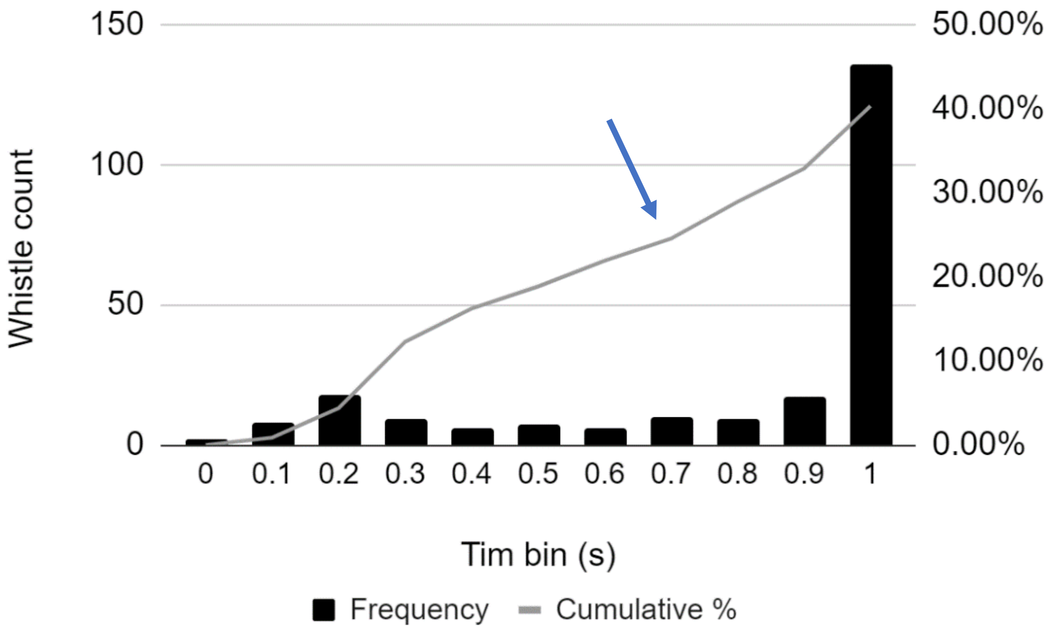
Figure 4. The distribution of inter-whistle-intervals under 1 min for a) the full dataset (n = 874) b) good quality whistles only (n = 653) and c) good quality sequences (n=576). Data are skewed with most inter-whistle-intervals under 26s (black arrow) and the highest proportion (red arrow) under 1s. Assuming each humpback dolphin *S. plumbea* signature whistle type represents an individual, individuals are represented by the colours indicated in the legend. swt56 had the highest proportion of whistles in the full dataset, swt61 had the highest proportion of the 'good quality whistles only' and 'good quality sequences'.

Possible alternative criteria for Sousa

Considering that the 0s time-bin had the third largest whistle count, I investigated the inter-whistle-interval proportion below 0s (Figure 5). The inter-whistle-intervals appeared to increase steadily from 0.7 s in the full dataset and good quality only whistles, and a lower 0.6 s for good quality sequences, suggesting that it was necessary to alter the lower limit for this population of humpback dolphins to better describe the temporal production. When assessing signature whistle types with an inter-whistle-interval of less than 1 s (n = 228) from the full dataset, 28.95% were repeated at intervals from 0 to 0.7 s and 75.44% from 1 to 10 s (Figure 5a). Good quality whistles only (n = 145) had 24.14 % repeated from 0 to 0.7 s and 80.69% from 0.7 to 1 s (Figure 5b). The good quality only whistles (n = 105) had 20.00% repeated at intervals between 0 to 0.7 s and 83.81% between 0.7 to 1 s (Figure 5c).

Altering the 1 s lower limit to 0.7 s resulted in two individuals, psw53 and psw66, qualifying as signature whistle types in all three datasets. In addition, the inter-whistle-intervals for the entire population ranged from 0.1 s to 4.43 hours, with the longest inter-whistle-interval belonging to swt61 in encounter 18. The full dataset had a mean of 244.40±994.12 s SD (median 10.70 s), good quality only whistles had a mean of 11.90±14.31 s SD (median 12.60 s). Good quality sequences whistles had a mean of 231.27±978.03 s SD (median 14.50 s). These results indicated that the population produced inter-whistle-intervals longer than the 10s defined by SIGID. However, increasing the upper limit to 12 s, 13 s and 15 s did not alter the signature whistle type catalogue in any way, i.e., the signature whistle type catalogue remained unchanged.

a)



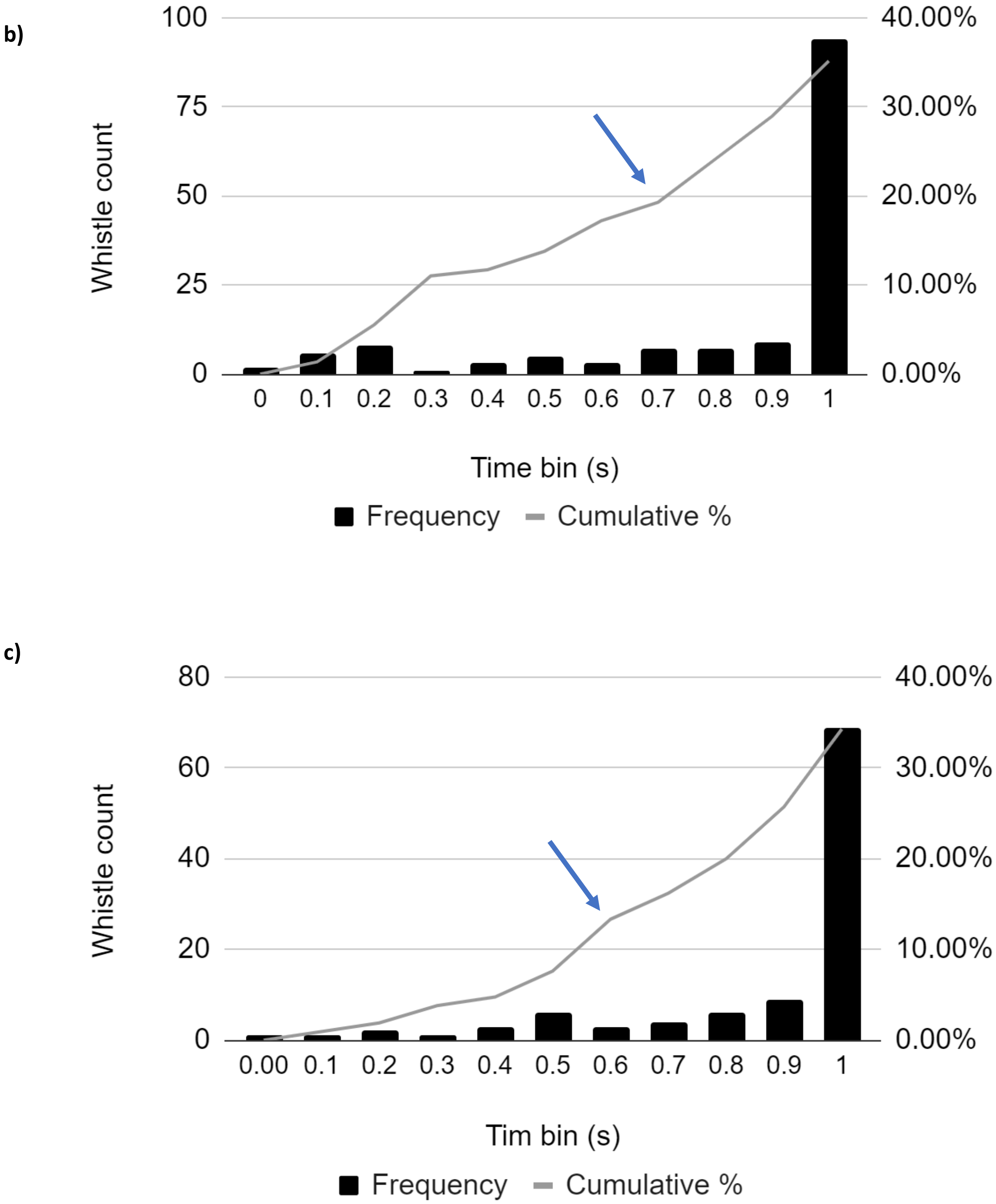


Figure 5. The distribution of humpback dolphin *S. plumbea* inter-whistle intervals between 0s and 1s for a) the full dataset (n = 228), b) good quality whistles only (n = 145) and c) good quality sequences (n = 105). The blue arrows indicate the time when inter-whistle-intervals began to increase steadily, which was 0.7s for the full dataset and good quality whistles only, and 0.6s for good quality sequences.

Loop structure and time parameters

Of all the classified signature whistle types (n = 1291), single element contours were the predominantly produced loop structure, i.e., 72.81%, whereas 26.18% were disconnected multi-loops (DCML). The loop structure is visible on the spectrograms (Figure 3). Only one signature whistle type (swt65) was produced as a connected multi-loop (CML), i.e., 1.01% (Table 5). The overall number of terminal loops produced in the population of humpback dolphins, was a minimum of 1 terminal loop for swt49 and swt50 each, and a maximum of 140 for swt44, and a population average of 42.27+42.76 SD for all 19 individuals. For the number of terminal loops for each individual DCML whistle, swt48 had the most loops, with a total of 9 terminal loops in a single whistle type (Figure 6). Due to the difficulty associated with confidently selecting poor quality terminal loops, inter-loop-intervals were calculated using all whistle quality ratings (Q1-3), then compared to inter-loop-intervals calculated using disconnected multi-loops with a quality rating of 2 or 3. This resulted in 973 introductory and terminal loops being assessed from the full dataset and 756 from good quality only whistles. In the full dataset, the inter-loop-intervals ranged from 0.001 to 1.01 s with a population average of 0.16+0.09 s SD, whereas good quality only whistles had inter-whistle-intervals ranging from 0.002 to 1.01 s with a population average of 0.16+0.09 s SD. These show that whistle quality did not have a significant effect on inter-loop-intervals since there were no differences in the range, mean and standard deviation.

Table 5. The percentages of the signature whistle type loop structure of humpback dolphin's *S. plumbea*, with single element whistles being the dominant loop structure, followed by disconnected multi-loop whistles.

Unique ID	Single Element	Disconnected Multi-loop	Continuous Multi-loop
swt32a	40.00%	60.00%	0.00%
swt40	86.36%	13.64%	0.00%
swt41	65.38%	34.62%	0.00%
swt44	30.28%	69.72%	0.00%
swt48	54.29%	45.71%	0.00%
swt49	97.44%	2.56%	0.00%
swt50	95.24%	4.76%	0.00%
swt51	0.00%	96.77%	0.00%
swt56	100.00%	0.00%	0.00%
swt58	2.86%	97.14%	0.00%

swt60	86.49%	13.51%	0.00%
swt61	78.23%	21.77%	0.00%
swt62	100.00%	0.00%	0.00%
swt63	100.00%	0.00%	0.00%
swt64	2.56%	64.10%	33.33%
swt65	31.25%	68.75%	0.00%
swt67	0.00%	100.00%	0.00%
swt68	78.95%	21.05%	0.00%
swt69	100.00%	0.00%	0.00%
Grand Total	72.81%	26.18%	1.01%

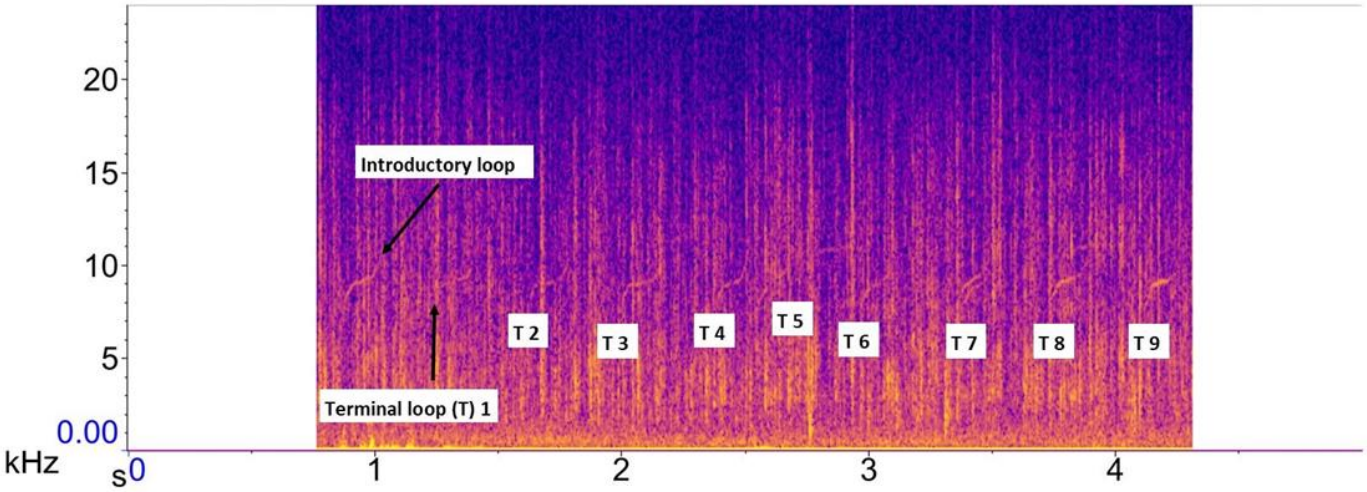


Figure 6. An image of a humpback dolphin (*S. plumbea*) individual, i.e., swt48, which is a disconnected multi-loop whistle (DCML) produced by the individual. The introductory and terminal loops have been labelled. This individual, i.e. swt48, produces varying numbers of terminal loops. In this instance, swt48 has produced 9 terminal loops.

Signature whistle type recaptures over time

Discovery curve

The discovery curve representing the entire study period (Figure 7), i.e., 2017-2020, had narrow confidence intervals which showed that the sampling effort sufficed with 114 h 46 min 04 s of sampling effort. EstimateS yielded a Chao2 mean of 19.24 (+ 0.72 SD). Chao2 was equal to S(obs), i.e., n = 19, indicating the curve was asymptotic. During the study period, sampling effort varied between years, with greater sampling effort during 2018 (31 h 28 min) and 2019 (45 h 39 min 02 s) compared to 2017 (20 h 39 m) and 2020 (17 h 02 s). When I deconstructed the discovery curve, the number of signature whistle types captured each year did not stabilize for 2017, 2018 and 2019, which had very wide confidence intervals (Figure 8; Table 6). In contrast, 2020 reached an asymptote with narrow confidence intervals, which could be attributed to 2020 having 3 h 38 min 58 s fewer hours of effort than 2017 or because there were only six days between the first and last encounter, compared to at least a couple of months in other years. Similarly, the number of signature whistle type captures were similar for 2018, 2019 and the study period, but neither 2018 nor 2019 had sufficient sampling effort compared to the whole study. This could be attributed to the difference in hours of effort once again. The whole study had 114 h 46 min 04 s of survey effort, whereas 2018 and 2019 accounted for 27.42% and 39.78% of the survey effort, respectively. Despite this, the cumulative signature whistle type count showed that I sampled a large enough part of the humpback dolphin population.

Table 6. Descriptive statistics from the EstimateS sample-based rarefaction computation. Chao2, standard deviation (SD) and the 95% Confidence Interval (CI) upper and lower limits tabulated below help with interpreting the rarefaction curve in order to determine whether it is asymptotic. The rarefaction curves are used to establish whether the Richards Bay humpback dolphin *S. plumbea* population was adequately sampled.

Year	n	95% CI Lower limit	Chao2 mean	SD	95% CI Upper limit
2017	5	5.03	10.00	5.88	13.18
2018	16	16.25	17.80	2.37	28.82
2019	18	19.20	24.30	6.46	51.18
2020	5	5.54	5.00	0.13	6.11
2017-2020	19	19.02	19.24	0.72	23.68

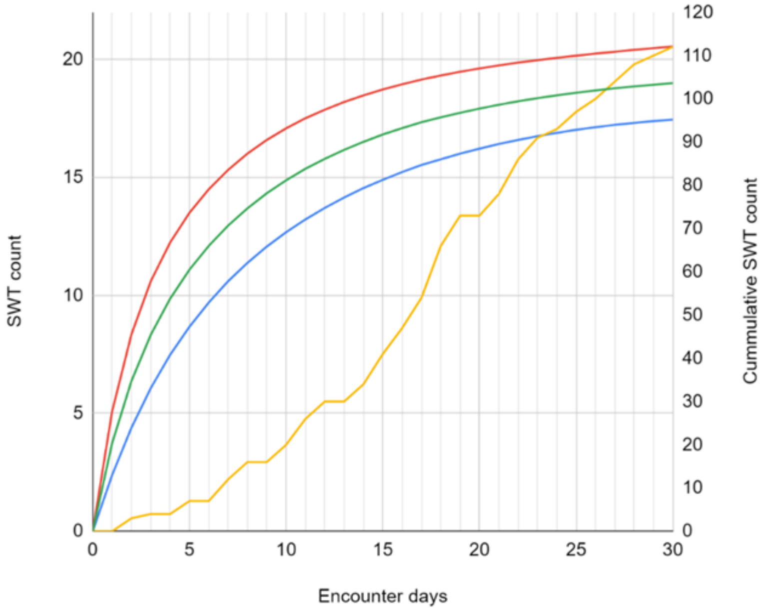


Figure 7. Discovery curve with 95% confidence intervals of humpback dolphin *S. plumbea* signature whistle types captured using the 4-year sampling period. Effort is expressed as the number of encounter days. An encounter day is a day when samples were collected, although encounters did not take place consecutively. The cumulative number of signature whistle types captured at Richards Bay is represented by the yellow line and the rarefied number of signature whistle types captured is represented by the green line, with confidence limits in red and blue.

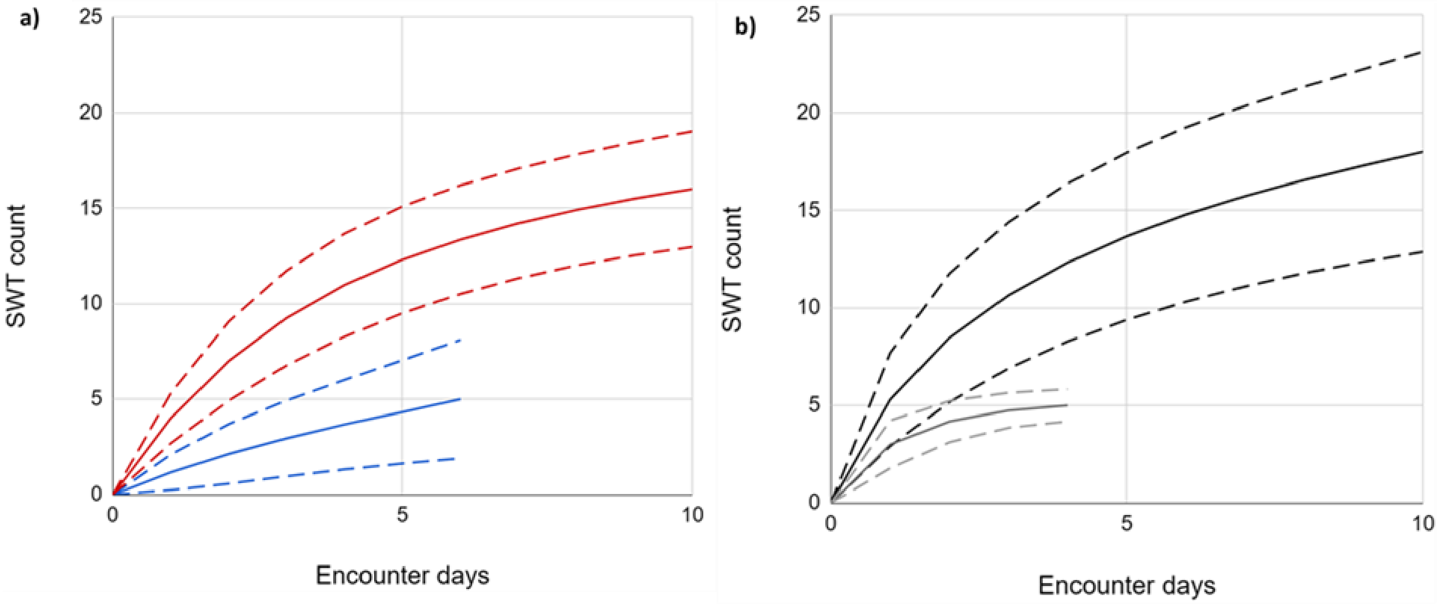


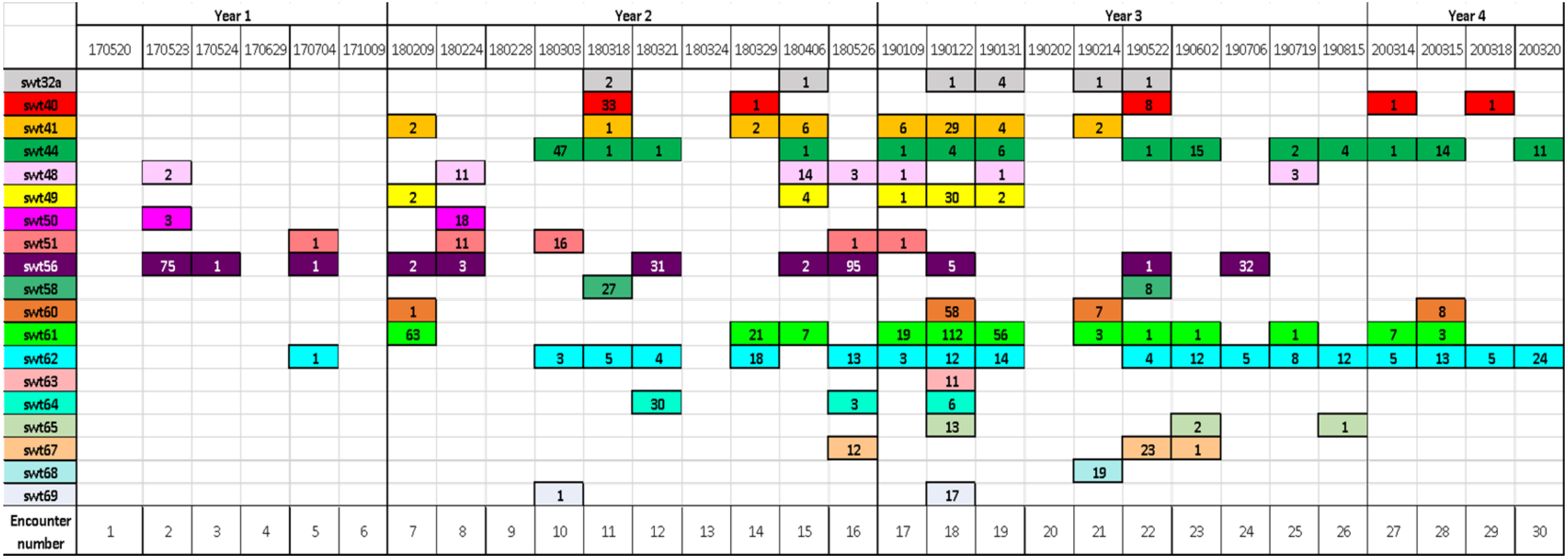
Figure 8. Two graphs plotting four sample-based rarefaction curves of humpback dolphin *S. plumbea* signature whistle types in Richards Bay. The curves represent the estimated number of signature whistle type captured during different encounter days in a) 2017 and 2018, and b) 2019 and 2020.

Dark blue represents 2017 with 5 encounters, dark red and black represent 2018 and 2019 respectively, with 10 encounters and grey represents 2020 with 4 encounters. The dotted lines represent the 95% confidence intervals.

Signature whistle type recaptures

The number of new signature whistle types captured differed each year, with 5 signature whistle types in 2017 and 2020 to 16 in 2018 and 18 in 2019 (Figure 7). 16 signature whistle types were common in the 2 well-sampled years, i.e., 2018 and 2019. Signature whistle types were recaptured over multiple days within each sampling year and between years (Table 7). For the recaptured signature whistle types, the number of days between the first and last capture ranged between 205 days (6.74 months) and 990 days (2.71 years) with sightings ranging from 2 to 18. The number of days that elapsed between captures and recaptures was between 277 to 990 days. Of the five individuals captured in 2017, there were only 2 to 18 sightings of those individuals between 2017 and 2020 (Table 8). Of the 30 encounters, 16 of the 19 signature whistle types were captured 2 or more times, with a maximum of 12 signature whistle types captured in a single encounter. Of the 19 signature whistle types, 3 were captured in one year, 8 were captured in 2 years, 7 were captured in 3 years, and 1 was captured in 4 years (Table 9). All 5 (100%) of the signature whistle types captured in 2017 were recaptured in 2018, 80% in 2019 and 20.00% in 2020. Of the 11 new captures in 2018, 83.33% were recaptured in 2019 and 36.36% in 2020. There were 3 new captures in 2019, but no recaptures.

Table 7. Recapture calendar showing capture-recapture of 19 humpback dolphin *S. plumbea* signature whistle types identified using the full dataset collected from the Richards Bay humpback dolphin population from 2017 to 2020. The colour coded boxes contain the number of whistles captured on the corresponding day.



	Year 1						Year 2										Year 3										Year 4			
	170520	170523	170524	170629	170704	171009	180209	180224	180228	180303	180318	180321	180324	180329	180406	180526	190109	190122	190131	190202	190214	190522	190602	190706	190719	190815	200314	200315	200318	200320
swt32a											2				1			1	4		1	1								
swt40											33			1								8					1		1	
swt41							2				1			2	6		6	29	4		2									
swt44										47	1	1			1		1	4	6			1	15		2	4	1	14		11
swt48		2						11							14	3	1		1						3					
swt49							2								4		1	30	2											
swt50		3						18																						
swt51					1			11		16						1	1													
swt56		75	1		1		2	3				31			2	95		5				1		32						
swt58											27											8								
swt60							1											58			7							8		
swt61							63							21	7		19	112	56		3	1	1		1		7	3		
swt62					1					3	5	4		18		13	3	12	14			4	12	5	8	12	5	13	5	24
swt63																		11												
swt64												30				3		6												
swt65																		13					2			1				
swt67																12						23	1							
swt68																					19									
swt69										1								17												
Encounter number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Table 8. The number of days that elapsed between the first and last time each humpback dolphin *S. plumbea* signature whistle type was encountered and the number of times each signature whistle type occurred during that period.

Unique ID	First Encounter date	Last Encounter Date	Number of days elapsed	Number of sightings
swt32a	2018-03-18	2019-05-22	430	6
swt40	2018-03-18	2020-03-18	731	5
swt41	2018-02-09	2019-02-14	370	8
swt44	2018-03-03	2020-03-20	748	14
swt48	2017-05-23	2019-07-19	787	7
swt49	2018-02-09	2019-01-31	356	5
swt50	2017-05-23	2018-02-24	277	2
swt51	2017-07-04	2019-01-09	554	5
swt56	2017-05-23	2019-07-06	774	11
swt58	2018-03-18	2019-05-22	430	2
swt60	2018-02-09	2020-03-15	765	4
swt61	2018-02-09	2020-03-15	765	12
swt62	2017-07-04	2020-03-20	990	18
swt63	2019-01-22	2019-01-22	0	1
swt64	2018-03-21	2019-01-22	307	3
swt65	2019-01-22	2019-08-15	205	3
swt67	2018-05-26	2019-06-02	372	3
swt68	2019-02-14	2019-02-14	0	1
swt69	2018-03-03	2019-01-22	325	2

Table 9. The ‘capture-recapture’ of 19 humpback dolphin *S. plumbea* signature whistle types identified in each sampling year from the Richards Bay humpback dolphin population from 2017 to 2020. Only a few signature whistle types were captured once, namely swt50, swt58, swt63, swt65, swt67, and swt68.

	2017	**2018**	**2019**	**2020**
swt32a		**1**	**1**	
swt40		**1**	**1**	**1**
swt41		**1**	**1**	
swt44		**1**	**1**	**1**
swt48	**1**	**1**	**1**	
swt49		**1**	**1**	
swt50	**1**	**1**		
swt51	**1**	**1**	**1**	
swt56	**1**	**1**	**1**	
swt58		**1**	**1**	
swt60		**1**	**1**	**1**
swt61		**1**	**1**	**1**
swt62	**1**	**1**	**1**	**1**
swt63			**1**	
swt64		**1**	**1**	
swt65			**1**	
swt67		**1**	**1**	
swt68			**1**	
swt69		**1**	**1**	

DISCUSSION

Classified whistle types

The aim of my study was to establish whether Indian Ocean humpback dolphins *S. plumbea* in Richards Bay, KwaZulu-Natal Province produce signature whistle types. This study has presented evidence that humpback dolphins emit stereotyped whistles with temporal patterns that match the criteria used to identify signature whistles in the common bottlenose dolphin *T. truncatus*. With multiple studies using the visual classification method to confidently identify stereotyped whistles, this technique is often paired with a verification step to ensure accurate classification of whistle categories. Visual classification is often verified by either conducting a visual classification task using multiple judges to agree on the potential signature whistle categories or sequenced analysis in ARTwarp to determine contour similarity (Quick and Janik 2012; Gridley et al. 2014; Kriesell et al. 2014; Lima and Le Pendu 2014; Fearey et al. 2019). I solicited the opinions of two experts for the visual classification task. Complete agreement between the observer and the two experts with regard to the classes in the potential signature whistle catalogue was required before conducting quantitative methods. In order to test if potential signature whistle classes match the description for signature whistles, the whistle repeat pattern of each class has to follow a particular temporal pattern. Each potential signature whistle class was analysed to identify sequences in which four or more whistle contours, of which at least 75% are within 1-10s of the others, occur at least once in an acoustic recording, a method referred to as SIGID (Janik et al. 2013). Once this temporal pattern was established, the class was presumed a signature whistle type.

A total of 19 signature whistle types were identified from 30 encounters over a 4-year sampling period. These findings serve as the first record of signature whistle use in a humpback dolphin population in South Africa. It is important to note that when Janik et al. (2013) developed the SIGID method, it was designed to be very conservative to eliminate any chance of false positives. This resulted in only a few signature whistle types being correctly identified. Therefore, it is not unexpected that some potential signature whistle classes were omitted from the final catalogue of signature whistle types. My findings were similar to those of the *Tursiops* genus (Gridley et al. 2014, Kriesell et al. 2014) and the common dolphin *Delphinus delphis* (Fearey et al. 2019). A Namibian population of free-swimming common bottlenose dolphins *T. truncatus* were found to use signature whistle types (Kriesell et al. 2014). 28 out of 43 common bottlenose dolphin potential signature whistles were confirmed as signature whistle types using visual classification and the SIGID method (Kriesell et al. 2014). Similarly, a study conducted in three different locations identified a total of 34 signature whistle types in Indo-Pacific bottlenose dolphins *T. aduncus* (Gridley et al. 2014). The signature whistle type catalogues were slightly smaller than those I compiled when deconstructed into the respective localities, with 10 signature whistle types from Jervis Bay, 12 from Plettenberg Bay, and 12 from Mikura Island (Gridley et al. 2014). Fearey et al. (2019) had the most similar findings to that of my humpback dolphin population. Acoustic data collected over a three-year period, of three different common dolphin populations, yielded 29 potential signature whistle classes, of which 25 met the SIGID criteria (Fearey et al. 2019). However, the number of signature whistle types identified in each location was not specified. With the existence of signature whistle types shown in various delphinids, it is apparent that signature whistle types are an integral part of the whistle repertoire (Sayigh and Janik 2009). It has been shown that signature whistles are produced when dolphin pods meet, thus allowing individuals to identify their conspecifics during encounters occurring in a natural context (Quick and Janik 2012). This suggests that signature whistle types could be a common mode of communication among delphinids, which is evident in their presence in the repertoire of different dolphin species.

It is important to note that the frequency range of the sampling equipment could impact the detection of whistles if the sampling rate is too low. The sampling rate determines sound frequency range and the maximum bandwidth of a sampled vocalisation is determined by the sampling rate. The maximum frequency representable is called the Nyquist frequency, which is equal to half the sampling rate (Charif et al. 2010). In this study, the long-term acoustic data were recorded using a SoundTrap hydrophone (ST300STD, Ocean Instruments Inc., New Zealand) with a sampling rate of 48 kHz therefore, the maximum frequency representable was 24 kHz. Before analysing the recordings in Raven Pro, the sampling rate of the short-term acoustic data collected by SeaSearch was lowered from 576 kHz to 48 kHz in Adobe Audition CS5.5 (Adobe Systems Inc., San Jose, CA) to ensure that all the files analysed had the same properties to allow for batch data processing in Raven Pro. The chosen settings and equipment used in this study did not interfere with the detection of whistles, despite the possibility of encountering humpback dolphin whistles above the Nyquist frequency, i.e., 24 kHz (Hoffman et al. 2015, Bopadikar et al. 2018, Wang et al. 2013, 2019). A population of Indian Ocean humpback dolphins, sampled by a Sensor Technology SQ26-08 hydrophone with a sampling rate of 96 kHz a maximum frequency of 48 kHz, off the Sindhudurg coast of Maharashtra, India, produced whistles with a frequency range between 2.3 - 33 kHz (Bopardikar et al. 2018). High frequency whistles have also been found in Indo-Pacific humpback dolphins, with reports of whistles produced with a maximum frequency of 27.12 kHz in the Langkawi archipelago. These whistles were recorded using a Burns Electronics hydrophone with a sampling rate of 192 kHz and a maximum frequency of 96 kHz (Hoffman et al. 2015). These high frequency whistles are usually single whistles that do not exhibit stereotypy. In addition, these high frequency whistles are partially visible below 24 kHz, making them detectable on spectrograms. However, during Step 1 of the whistle classification process, I did not observe any partially clipped whistles thus implying that the chosen equipment and sampling rate was acceptable. Previous studies conducted on other Sousa spp. support this assumption as the maximum frequency recorded was often below 24 kHz (Van Parijs et al. 2001b, Weir 2010, Dong et al 2019, 2021). Indo-Pacific humpback dolphins recorded in Zhanjiang, China produced whistles with a maximum frequency of 21.68 kHz using an icListen Smart hydrophone with a sampling rate of 128 kHz and maximum frequency of 64 kHz (Dong et al. 2019). Similarly, the Atlantic humpback dolphin *S. tueszii* has been recorded to produce whistles with a frequency of 23.4 kHz. These whistles were recorded in southern Angola using a Sensor Technology SQ26-08 hydrophone with a sampling rate of 96 kHz and a maximum frequency of 46 kHz (Weir 2010). This suggests that although the sampling rate was relatively low, it is unlikely that any whistles were left undetected due to the equipment's frequency range as all whistles observed were below 24 kHz.

Considering that recordings were collected over multiple years, my findings are comparable to those of other studies investigating signature whistle use in dolphins. Two studies recorded signature whistles in a stranded Australian humpback dolphin *S. sahulensis* and an injured Indo-Pacific humpback dolphin *S. chinensis*, both studies comprising of a sample size of one individual (Van Parijs and Corkeron 2001c; Cheng et al. 2017). Van Parijs and Corkeron (2001c) used visual classification to identify signature whistle types, stating that the stranded individual produced variants of one whistle type in a repetitive manner, resembling the behaviour of captive and restrained bottlenose dolphins when producing signature whistle types. The identification process was largely rooted in the observation that captive bottlenose dolphins emit the same whistle type when isolated from their conspecifics (Caldwell and Caldwell 1965). It was later found that whistle production differs between restrained dolphins and free-swimming dolphins, i.e., stressed individuals and undisturbed individuals (Watwood et al. 2005, Esch et al. 2009a). Free-swimming common bottlenose dolphins were found to produce half of their whistle repertoire while they were restrained, with 76% of those whistles produced being signature whistle types (Watwood et al. 2005). However, some whistle parameters differed during capture-release events compared to when individuals were undisturbed. It has been found that whistle rate and number of loops were greater during capture-release events, thus showing that certain parameters, such as whistle production rate and number of loops, of signature whistle types can be used as a non-invasive stress indicator (Esch et al. 2009a). These studies therefore supported Van Parijs and Corkeron's (2001c) deductions made from the injured Indo-Pacific humpback dolphin recordings because they collected the acoustic data while the individual was in a condition that would induce stress. Cheng et al. (2017) used a combination of visual classification and the SIGID method to show that an injured dolphin encountered at sea was producing a signature whistle type. Once they conducted the SIGID test, Cheng and colleagues (2017) found that Type 1 whistles had 75.86% of the inter-whistle-intervals ranging from 1s and 10s. The signature whistle types produced by the Richards Bay humpback dolphin population is similar to this anecdotal evidence in that they produced one whistle type in a repetitive manner similar to captive and restrained bottlenose dolphins, and these whistle types met the 1 – 10s SIGID criteria.

SIGID has since been commonly used as a method to test signature whistle use in the natural context (Janik et al. 2013; King and Janik 2013; Kriesell et al. 2014; Luís et al. 2016). However, there are multiple studies in which some criteria were altered to better represent the focal species (Gridley et al. 2014; Fearey et al. 2019). The first account of signature whistle use in free-swimming Indo-Pacific bottlenose dolphins *T. aduncus* was established using the SIGID method (Gridley et al. 2014). Gridley and colleagues (2014) took a more conservative approach since they used the SIGID method to show signature whistle presence in the Indo-Pacific bottlenose dolphin. They altered the bout-interval criteria, defining a signature whistle type as a whistle type with a minimum of 4 out of 5 whistles repeated in succession, within 1 – 10 s of another whistle in the bout. Alternatively, Fearey and colleagues (2019) took a more inclusive approach when they employed the SIGID method to classify common dolphin *D. delphis* signature whistle types. They found that the inter-whistle-intervals for this species peaked at 0.2 – 1 s, thus highlighting that the common dolphins' temporal production differed from that of common bottlenose dolphins (Fearey et al. 2019). The inter-whistle-interval criteria was then adapted to account for the proportion of inter-whistle-intervals below 1 s, by describing the common dolphin bout-interval criterion as 0.2 – 10 s. This indicates that developing species-specific signature whistle identification parameters is important, if not necessary. My findings were similar in that I deduced possible alternative criteria for humpback dolphins *S. plumbea*, thus leading to alternative bout-interval criteria of 75% of whistles occurring in a bout, produced in 0.7 to 10 s of one another. This is different to what we see in common bottlenose dolphins (Janik et al. 2013). Altering the bout-interval criteria added two more potential signature whistle classes to the humpback dolphin catalogue. This implies that the bout-interval criteria for *Sousa* might differ from that of *Tursiops*.

The SIGID bout-criteria may appear to be arbitrary, however they were specifically chosen to ensure that there are no false identifications of signature whistle types when using the SIGID method. I was tasked with merely identifying the presence of potential signature whistle types and assessing their temporal patterns of emission. The SIGID method is sufficient for the scope of my study and the signature whistle type classifications made are a great starting point for an endangered species.

However, it is important for researchers to note that the number of signature whistle types identified are probably under-represented in the population. False identifications are almost impossible with SIGID in common bottlenose dolphins; therefore, I can be sure of the signature whistle types I have identified. Janik et al. (2013) made sure of this because they knew exactly which whistles were signature whistles *a priori*. Using 8 focal follows to develop the SIGID method and 4 more to test the method, Janik and colleagues (2013) boxed 11 specific signature whistles in order to see the patterns of signature whistle emission in the wild. Using Slater and Lester's (1982) method, they quantitatively showed that signature whistle types are emitted in bouts. They plotted the log survivorship curve of inter-whistle-intervals and drew intersecting lines using their eyes, which pinpointed 15 s as the cut-off. They tried different cut-offs to determine how to maximise the number of signature whistle types identified by their method while ensuring that only signature whistle types made it through, but not non-signature whistles. Hence, the 1-10 s, 75% cut-offs.

It is possible that the bout-criteria have biological meaning. Janik (1999) demonstrated that people can sort whistles produced in specific contexts into categories through visual classification, meaning that people can recognise stereotypy. Biologically, this could mean that dolphins also use stereotypy in these specific contexts and can therefore recognise it in other dolphins i.e., whistling individuals. Knowing the context under which the whistles were emitted and the individuals emitting the whistles, Janik (1999) understood that the sounds are biologically meaningful to common bottlenose dolphins. However, in this study the context of whistle emission and the whistling individuals are unknowns. Therefore, we cannot infer the function of signature whistle types in the Richards Bay humpback dolphin population. This is evident in the fact that killer whales (Riesch et al. 2006, Reisch and Deecke 2011; Kremers et al. 2012) use signature whistle types at a group level to distinguish between matrilineal pods whereas common bottlenose dolphins use them at an individual level to distinguish between individuals whether in a pod or in alliances (Janik et al. 2006; Sayigh and Janik 2009, Connor and Krützen 2015, King et al. 2018). By noting the context under which dolphin vocalisations are recorded, i.e., taking note of behaviour exhibited when collecting acoustic data (socializing, foraging, milling or travelling) as well as the condition or situation when recording i.e., free swimming, captive, injured or restrained. One can then measure variables such as whistle rate, frequency and duration. These variables can then be compared between contexts to infer the motivation for signature whistle emission.

The differences in inter-whistle intervals could be attributed to species-specific differences in whistling behaviour or environmental influences. High boat-presence could be influencing the whistling behaviour of the Richards Bay humpback dolphin *S. plumbea* population. For example, Australian humpback dolphins *S. sahulensis* have been found to increase their whistling rate once boats have passed by a focal group (Van Parijs & Corkeron 2001a). Boat presence could also have an influence on signature whistle type production in these humpback dolphins because of high boat presence at the Richards Bay Harbour mouth. The inter-whistle-intervals below 1s could be an indication of the effect of boat presence on their whistling behaviour. Common bottlenose dolphins *T. truncatus* whistle significantly more when approached by boats and they decrease their whistle production rate during and after the approaches (Buckstaff 2004). The Richards Bay Harbour shipping lane is used by recreational and commercial vessels (Keith et al. 2013). This high boat presence could have an effect on the humpback dolphins utilising the area, causing them to increase the production of signature whistle types. The increased temporal production of signature whistle types could be a response to high boat presence as a means of reducing signal degradation in a noisy environment or to increase the chances of whistle detection by conspecifics before the signal is completely masked by boat noise (Richardson et al. 1995, cited in Buckstaff 2004). Moreover, pods with calves have been found to increase their whistle production rates in order to maintain group cohesion in the presence of tour boats, possibly to re-establish cohesion between mother-calf pairs or pods (Scarpaci et al. 2000; Van Parijs and Corkeron 2001a; Guerra et al. 2014). During my observations in 2020, each group we encountered had at least one calf present. This suggests that the Richards Bay humpback dolphin population relies heavily on acoustic signals to maintain group cohesion due to high boat presence, calf presence and the muddy coastal waters at the study site (Popper 1980; Van Parijs and Corkeron 2001a; Weerts and Cyrus 2002a, b; Guerra et al. 2014).

Signature whistle type recapture

The discovery curve indicated that the acoustic survey effort was adequate over the 4-year study period and signature whistle types, i.e., individuals, were recognised between 2017 and 2020. I predicted that if humpback dolphins *S. plumbea* use signature whistles, the signature whistle types would remain stable over time and to have recaptures of some individuals. Therefore, the recaptures support signature whistle use in humpback dolphins as signature whistle types are expected to remain stable over multiple years (Sayigh 1992). Over the whole study period, the sampling effort was adequate as the discovery curve reached an asymptote. However, the singular discovery curves constructed for each sampling year (2017-2020) shows that increased sampling effort could have yielded more captures of individuals (Cantor et al. 2012). The difference in sampling effort influences the capture-recapture of individuals, which is clearly evident as 2017 and 2020 had the least encounter days and the least number of new captures. The difference in the number of recaptures could show that some individuals were captured many times whereas others were captured only a few times. Heterogeneity in recaptures is common in mark-recapture studies of free-swimming dolphins (Smith et al. 2013; Elwen et al. 2019; Longden et al. 2020). The Richards Bay humpback dolphin population is made up of resident dolphins accounting for 8% of the population and transients of 81% (Atkins et al. 2016). Therefore, variation in individual site fidelity could influence my results since individuals temporarily immigrate to and emigrate from Richards Bay (Atkins et al. 2016). Alternatively, variation in recaptures in each sampling year could be attributed to the higher likelihood of humpback dolphins at Richards Bay being resighted one to three days following the first sighting. The lagged identification rate of humpback dolphins is high within the first day of sighting, decreases by 50% approximately 3 days later and rises once again in a week, remaining stable for 12 months (Atkins et al. 2016). This is evident in my study as 2020 has more recaptures than 2017 (2017 had 6 encounters over a 4-month, 20-day period, whereas 2020 had 4 encounters over a 6-day period).

Conclusion

I have shown that humpback dolphins in Richards Bay use signature whistle types in a natural context i.e., free-swimming individuals have been found to produce signature whistles outside of stress-inducing conditions such as temporarily restraining dolphins in nature or keeping dolphins in captivity. 22 potential signature whistle classes were identified through visual categorisation supported by expert verification. Thereafter, 19 signature whistle types were confirmed using the SIGID method (Janik et al. 2013). However, without the ability to confidently identify the whistling individual in my study population, I cannot conclusively prove signature whistle use (Caldwell and Caldwell 1968). Nonetheless, the high signature whistle diversity in the dataset and the recaptures of signature whistle types supports my prediction of humpback dolphins emitting signature whistle types. Future studies could incorporate focal follows to collect photo-identification data to investigate potential matches between signature whistle types and photo-identified dorsal fins in order to determine whether the number of photo-identified individuals in a group correspond to the number signature whistle types classified or use acoustic tags to gain a better understanding of the species-specific variation in bout-interval criteria. Increasing the sampling effort could result in a larger signature whistle type catalogue which could in-turn serve in mark-recapture studies which are of importance with regards to the endangered humpback dolphin. The threat of by-catch in shark-nets continues to affect dolphin populations, and therefore using signature whistle types as acoustic tags to monitor susceptible populations and understand behaviour in risky areas could be of importance with regards to mitigating the effect of by-catch on humpback dolphins (Atkins et al. 2016). Determining movement patterns or estimating various population parameters in order to monitor threatened marine mammal populations often requires individual recognition (Hammond et al. 1990), which has been demonstrated in a study conducted by Longden et al. (2020) where signature whistle types were used as proxies for population parameters such as individual occurrence and abundance estimation. Signature whistles encode information of individual identity (Caldwell and Caldwell 1965; Janik and Slater 1998) which can potentially be used to determine movement patterns or estimate population parameters (Hammond et al. 1990; Longden et al. 2020). Vocal signatures of individuals could be used as an alternative way to identify individuals for mark-recapture studies monitoring populations, habitat use, individual ranging patterns and population size (Terry et al. 2005; Janik et al. 2013). Using individually distinctive acoustic cues that are produced naturally, scientists could incorporate signature whistle types in mark-recapture analyses.

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