Bottlenose dolphin (*Tursiops truncatus*) whistles indicate less stress during COVID-19 pandemic in Bocas del Toro Archipelago, Panama.

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Previous studies indicate that the bottlenose dolphin population of Dolphin Bay in Bocas del Toro, Panama increase modulation in their whistles when exposed to dolphin-watching boats as an indicator of stress. The COVID-19 pandemic created a unique situation in which their typically popular tourist industry was shut down, and the dolphin-watching boat activity ceased. By examining dolphin whistles in Dolphin Bay before and during the COVID-19 pandemic, we expect to see less modulation during COVID-19 acoustic recordings due to the absence of tourist boats. Using passive acoustic monitoring and *Raven* software we were able to analyze 139 whistles during COVID and compare them to previously obtained data on whistles before the pandemic. Mann-Whitney tests revealed that there was significantly less modulation in COVID whistles as compared to pre-COVID whistles. Understanding how stress negatively impacts the resident bottlenose dolphins of Bocas del Toro, Panama contributes to previous understanding of boat impacts on this population and provides more evidence for the need to enforce regulations in the region.

Key Words: bottlenose dolphin, bioacoustics, animal communication, stress, COVID-19

INTRODUCTION

Background. - Bottlenose dolphins (*Tursiops truncatus*) have been known as vocal animals since the mid 20th century (McBride and Hebb 1948). Delphinid communication is complex; including whistles, clicks, and pulsed sounds that are shaped by vocal learning and include great plasticity (Janik 2009). They utilize high-frequency, individually distinctive calls to communicate information about individual identity (Aubin et al. 2013). These calls are known as signature whistles, and they are developed using a learned whistle from their environment as a model and modifying it to create a novel one. Signature whistles are typically used when dolphins are separated from conspecifics, as they are most commonly noted when individuals are in isolation (Caldwell et al. 1992). Signature whistles are often produced within 1-10 seconds of each other, and if not, they are known as non-signature or variant whistles (Aubin et al. 1970). Whistles can be highly variable in their frequency, altitude, and contour. The alterations in these parameters is most useful in studying the impacts of a variety of environmental and anthropogenic factors.

Ecotourism in Bocas del Toro, Panama is not only an integral part of their economy, but it can also be a way of raising awareness for conservation to a certain extent. However, the constant presence of tour boats that follow dolphins around the archipelago can be highly stressful for the cetaceans. There are approximately 100-150 individual dolphins that inhabit Dolphin Bay in Bocas del Toro, Panama year-round. Their high sight fidelity has made them important study organisms for the impact of boats on marine mammals in that area (May-Collado et al. 2012).

Stress is one way in which bottlenose dolphin call parameters and behavior can be impacted (Perez et al. 2020 and Kassamali-Fox et al. 2020). At the location of this study,

Dolphin Bay, tour boats that targeted bottlenose dolphins caused them to socialize less and travel more, also reducing the amount of time they forage. The alterations in energetic budget for this population is likely to be energetically costly in addition to the stress of being chased by the boats. Additionally, the presence of tour boats is found to alter call parameters; dolphins in boat-watching areas increase the frequency, duration, and modulation of their calls (Perez at al. 2020).

Purpose and Scope. - The presence of boats in Bocas del Toro, Panama is highly correlated with stress in dolphins that inhabit the area. The high site fidelity of the resident dolphins in Dolphin Bay make them a consistent population to study changes in. Based on previous studies, the presence of tour boats has been found to be associated with greater modulation in bottlenose dolphin whistles. During COVID-19, no tourists were allowed into Panama, and therefore, the activity of tour boats was significantly reduced. Using call data from July 10th to July 14th, 2020 we can expect to see less stress, and therefore less modulation, lower frequency, and lower duration calls from the resident dolphins as compared to pre-covid times associated with boat presence. The ability to identify boat presence as a significant source of stress in bottlenose dolphins in Dolphin Bay is critical in impacting governmental and conservation decisions there.

MATERIALS AND METHODS

Study site. - Bocas del Toro is an archipelago located in the western part of Panama (Guzman et al. 2005). This is a significant geological area, where the islands were separated from the mainland due to sea level rise. The marine environments there are defined mainly by seagrass meadows, mangroves, and coral reefs. Resident dolphins are known to be impacted by continuous boat traffic (May-Collado and Wartzok 2008).

Recordings. - Passive acoustic recordings were taken with RUDAR-mk (RUDAR-mK2 (Sampling rate up to 96kHz -169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com). The recorder was programmed to continuously record the soundscape in segments of three hours at a sampling rate of 48 kHz from July 10th to July 14th, 2020. Recorders were also deployed before COVID-19 and data had previously been extracted. Recorders were deployed in Dolphin Bay (Bocas torito).

Signature Whistle Data. - Whistles were identified and used if there was a good signal-to-noise ratio. Each whistle was isolated and analyzed for a variety of parameters as well as characterizations. The analyses were done in Raven 1.5 (2016; Cornell Lab of Ornithology) with a Fast Fourier Transform size of 4,000 points, an overlap of 50%, and a 4096-sample Hann window. For each whistle, the following call components were obtained; begin time (s), end time (s), low frequency (Hz), high frequency (Hz), delta frequency (Hz), delta time (s), peak frequency (Hz) and peak frequency contour number of inflection points (PFC Num Inf Pts). Whistles were manually characterized based on the contour of the whistle, whether it was a variant or signature whistle, and if there was a boat present or absent. A whistle was considered a signature whistle if it occurred more than once within 10 seconds.

Statistics.- Mann-Whitney tests were used to analyze differences in the call parameters on *Graphpad Prism*. Separate tests were run on frequency parameters, time parameters, and peak frequency contour number of inflection points. These parameters were compared from the recordings during COVID-19 to the same parameters obtained from whistles that occurred before the COVID-19 pandemic started at the same location.

RESULTS

A total of 139 whistles were analyzed from the 2020 data (during COVID-19), and 797 whistles were analyzed from (before COVID-19). The nonparametric Mann-Whitney tests were run because they can test the likelihood samples are from the same population. The multiple Mann-Whitney tests for frequency parameters revealed only low frequency was significantly different between pre-COVID and COVID whistles ($p<0.000001$, $q<0.000001$) [Fig. 2]. Low frequency tended to be higher during COVID times as compared to pre-COVID times [Fig. 2]. Mann-Whitney tests on whistle duration and peak frequency contour (PFC) number of inflection points revealed that only PFC number of inflection points differed significantly ($p<0.0001$) [Fig. 1, 3]. Modulation indicated by PFC number of inflection points appeared to be significantly lower during COVID with the absence of boat traffic, as well as less variant [Fig. 3].

DISCUSSION

By comparing bottlenose dolphin whistle characteristics before and during COVID using passive acoustic monitoring, we were able to come to conclusions about the impact of boat traffic on dolphin acoustic communication. The results of our statistical analyses indicate that, in the absence of boat traffic, bottlenose dolphins in the archipelago of Bocas del Toro, Panama tend to increase low frequency and decrease modulation. The frequency trends seen in this study are not consistent with those seen in other studies (Perez et al. 2020). However, the results seen from PFC number of inflection points, also referred to as modulation, support our initial hypothesis; we expected that in the absence of boat traffic the resident dolphins would not exhibit as much modulation. This was hypothesized based on previous studies that found an increase in modulation with an increase in boat activity.

Understanding the impacts of anthropogenic factors on marine mammal communication is an understudied yet essential aspect of science. The dolphin watching industry in Bocas del Toro is the largest in Panama (May-Collado et al. 2014). The tourism industry there has grown massively in a short amount of time, with little management to go along with it. As a result, it has been described that it has significant negative impacts on the resident bottlenose dolphins. The constant presence of dolphin watching boats appear to indicate the bottlenose dolphins are stressed by their presence. Stress, especially chronic stress, can have long-lasting effects; marine mammals have been found to exhibit an exaggerated stress response that causes severe deterioration and even death (Clark et al. 2006).

As the number of dolphin watching boats that interact with dolphins continues to rise, it is critical that we first examine the impacts of the current dolphin watching boats to aid in decision-making for the future (May-Collado et al. 2014). A previous review has recommended that only two dolphin watching boats interact with dolphins at a time, with at least a 30 minute rest period in between. The results of this study provide all the more reason to support this management action (May-Collado et al. 2014).

Despite governmental efforts, it has been made clear that boat operators Bocas del Toro do not typically comply with regulations anyway (Sitar et al. 2016). Not only do they keep their engines on, but they get much closer to dolphins than is permitted by current regulations. The dolphin population will continue to face negative impacts such as less socialization, decreased foraging, and increased energy costs due to avoiding boats physically or avoiding their masking (Kassamali-Fox et al. 2020 and Perez et al. 2020). This study contributes to the idea that bottlenose dolphins must change their call parameters in order to avoid masking, and increase energetic costs as a result of stress. Besides indirect decreases in fitness, boats also often strike

the dolphins, ultimately resulting in their death (Sitar et al. 2016). The constant threats exacerbated by boat presence can have long-term impacts on lifespan of the dolphin population.

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FIGURE LEGENDS

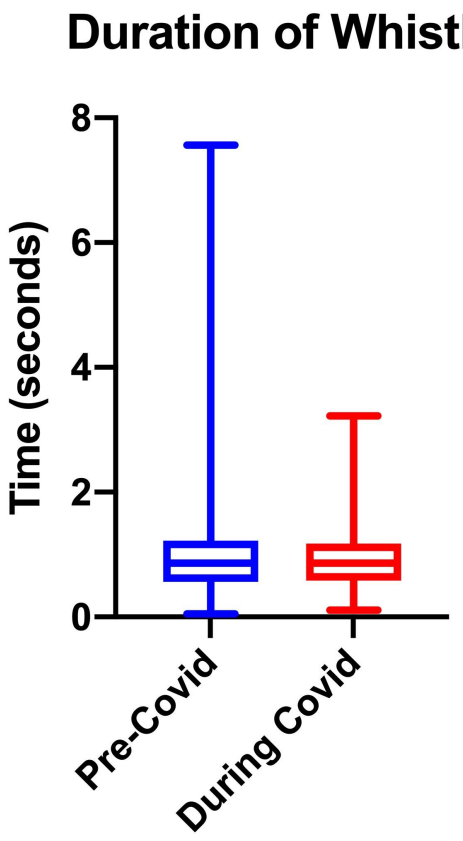

Fig. 1. - Box plot comparison of whistle duration, in terms of seconds, of pre-covid dolphin whistles and whistles during covid. Error bars reflect standard deviation. Differences in whistle duration depending on time relation to covid were not significant (P = 0.8822), according to Mann-Whitney test.

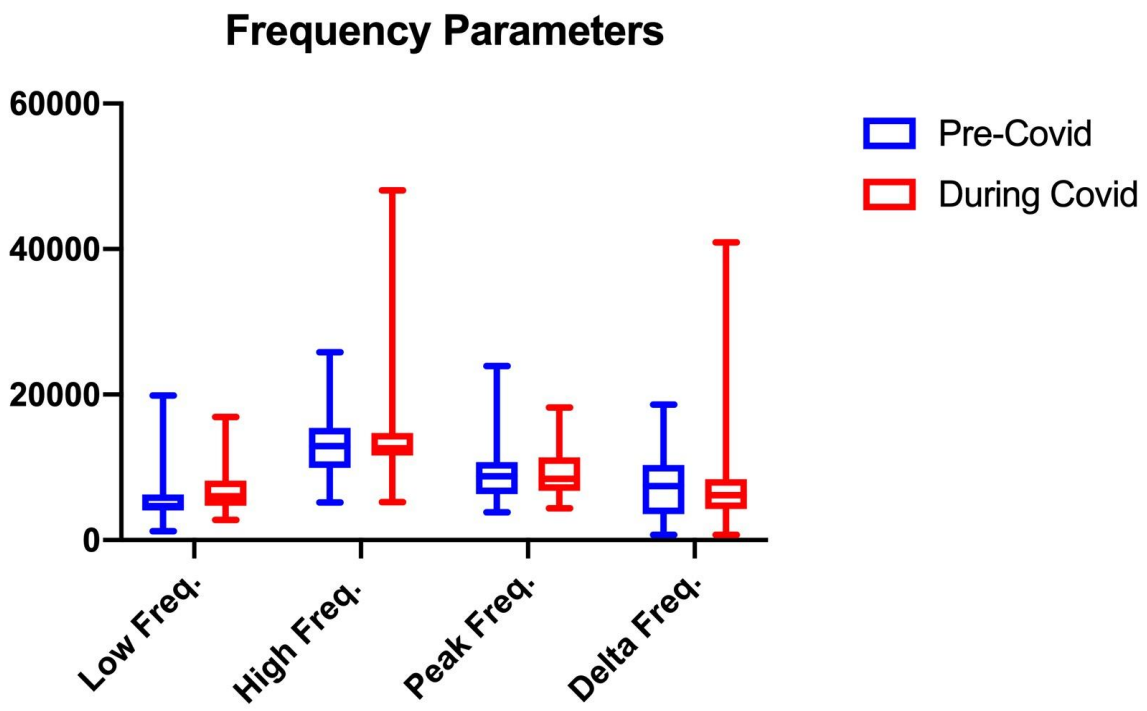

Fig. 2. - Box plot comparison of a variety of frequency parameters of dolphin whistles pre-covid and during covid. From left to right, frequency parameters include low frequency, high frequency, peak frequency, and delta frequency. Error bars represent standard deviation. Using Mann-Whitney test, the only significant parameter was in low frequency, ($P < 0.00001$).

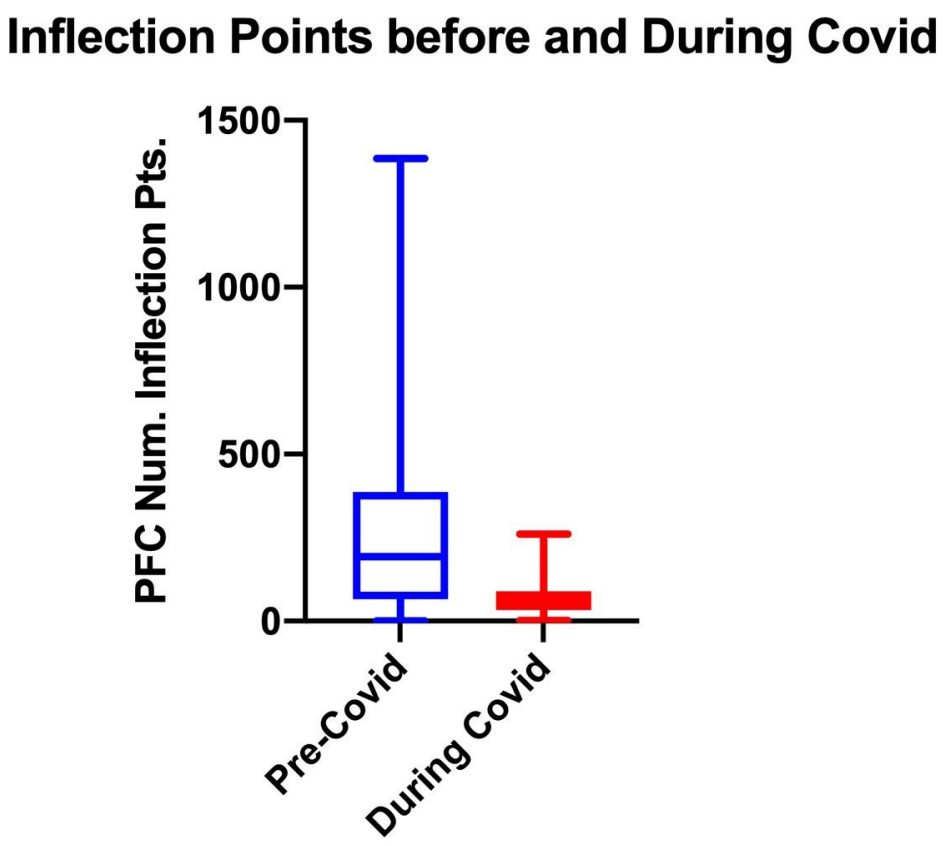

Fig. 3. - Box plot comparison of peak frequency contour number of inflection points in dolphin whistles before and during covid. Error bars reflect standard deviation. Pre-covid inflection points were significantly greater than during covid ($P < 0.0001$) based on Mann-Whitney test.