COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE: SOUNDSCAPES AND BEHAVIOR RESEARCH (BIOL 188-A)

Spring 2021

A special semester studying the impact of COVID-19 lockdowns on marine communities

Course Instructor Laura J May-Collado, Ph.D.

Meeting Time: Tuesday 1:15-4:15 p.m. in 217A Marsh Life Science Bldg

Website: http://www.lauramay-collado.com/cure-lab.html

Week	Where?	Chronogram
Feb. 2	Remote	• Introduction to this course Syllabus • Gala Sounds of Tropics 1: How can audio recordings improve tropical biodiversity conservation? • Gala Sounds of Tropics 2: How are frogs responding to hurricanes, droughts, and climate change • Gala Sounds of Tropics 3: Can you hear me? How dolphins in Bocas del Toro, Panama communicate in a noisy habitat? • Use these resources to think about what would like to research this semester.
Feb. 9	Remote In-person meeting by appointment (217 MLS)	• **Introduction to ARBIMON AND RAVEN** • How to Write a Research Proposal? Use Web of Science for scientific paper, other resources available incourse materials
Feb. 16	Remote In-person meeting by appointment (217 MLS)	• Q & A meeting with instructor to discuss projects and research proposal

Feb. 23- Mar.30	Remote
	In-person meeting by appointment (217 MLS)
		• Submit Research proposal in BB.
		• Begin Data Collection
		• Weekly progress reports
		  ○ March 2: upload wiki report in BB
		  ○ March 9 upload wiki report in BB
		  ○ March 16 add to your wiki report a doc file with Material and Methods Section (follow format see course materials)
		  ○ March 23: add to your wiki report a doc file with the Introduction and Key words
		  ○ March 30: add to your report a xls file with your clean dataset

Apr.6	TBD	Data visualization and analysis & Write up of Results & Key points for SRC poster/talk
Apr. 13	TBD	• Continue data analysis • How to make an awesome scientific poster? how to give a fantastic scientific talk?
Apr.?	TBD	**Student Research Conference**
Apr. 20	Remote In-person meeting by appointment (217 MLS)	Write up: Introduction, Methods, Discussion, Abstract, References
Apr. 27	Remote In-person meeting by appointment (217 MLS)	Peer Review
May 4	TBD	Submit: complete manuscript & CURE ymposium
May 11	Remote	Complete your CURE Blog

CURE Soundscape and Behavior Symposium 2021

Presentations	**Time**
Prof. May-Collado	**Introduction**
Impacts of Covid-19 Regulations on the Soundscape of Marine Communities within Coiba National Park by *Anna Hodson*	1:20
Does boat activity influence the acoustic structure of humpback whale songs? *By Brianna Heller*	1:30
Soundscape biodiversity in Marine Protected Areas versus unprotected areas in Costa Rica and Panama *by Cecilia Vichi*	1:40
A Comparative Analysis of Humpback Whale Soundscape Ecology, Before and During COVID-19 Marine Restrictions *by Chris Wilson*	1:50
How does Covid-19 lockdown impact boating and Humpback whale (*Megaptera novaeangliae*) singing activity? *By Grace Durant*	2:00
	BREAK
Panama bottlenose dolphin (*Tursiops truncatus*) whistles indicate less stress during COVID-19 pandemic *by Emma Shapera and Logan Hillger*	2:05
Ambient noise levels as an indicator of marine community health: a comparison of protected vs non-protected Areas *by Meghan Murphy*	2:15
Acoustic Structure of False Killer Whale (*Pseudorca cassidens*) Sounds off The Coast of El Salvador *by Sam Koslowsky*	2:25
Ambient noise levels of a protected marine community in Costa Rica before and during Covid-19 *by Sawyer Miller-Bottoms*	2:35
Geographical Variation of Pantropical Spotted Dolphins (*Stenella attenuata*) Whistle Acoustic Structure *by Sydney Tomaseski*	2:45
	BREAK
The impact of COVID-2019 lockdowns on toadfish calling behavior in Bocas del Toro, Panama. *By Tessa Kilmer*	2:50
Climate change and the Coquí: Temperature-driven changes to frog calls in Puerto Rico *by Kristen Werner*	3:00
	Awards

Impacts of Covid-19 Regulations on the Soundscape of Marine Communities within Coiba National Park

Authors: Anna M. Hodson
[1]niversity of Vermont, Department of Biology

ABSTRACT
Anthropogenic noise from vessels can interfere with the underwater communication of many sonorous marine species. Because boat transit and fishing restrictions were put in place due to the Covid-19 pandemic in Panama since late March 2020, we were interested in determining if these restrictions translated into a decrease of ambient noise levels. To answer these questions, we used acoustic data collected from Coiba National Park before and during the pandemic. This area is a tour destination in Panama and serves as a migratory destination of humpback whales. From the acoustic database in RFCxArbimon, we collected presence/absence data of whales and boats. RFCxArbimon soundscape tools were also used to identify changes in soundscape complexity and dBWav was employed to estimate ambient noise levels. 2020 data revealed increased boat presence in comparison to 2017 data, but overall noise levels in 2020 at Isla Contreras were found to be higher than 2017 Isla Canal noise levels. Differences in noise levels are likely due to differences in substrates, habitats, and marine communities at the two sites. Understanding the level of contribution of boat noise to marine soundscapes is necessary when designing policy and regulation of human activity in protected areas whose goal is to preserve marine biodiversity.

Key Words: ambient noise levels, anthropogenic noise pollution, biodiversity, boat traffic

1. Introduction

The loss of global biodiversity is accelerated by human activities, among them noise pollution. Many marine organisms use sound to communicate, obtain information from their environment, and locate prey (Boyd et al, 2011). Healthy marine communities are full of biological acoustic signals from sources including shrimps, fish, and marine mammals (Jones, 2019). Habitats with high levels of noise pollution from sources including shipping, coastal development, and wind farms can potentially mask important biological signals and cause temporary impacts on species hearing abilities. Together these impacts can reduce the communication range of marine organisms, recruitment of marine species that use sound as cue to locate healthy habitats (Weilgart, 2017) and cause shifts in hearing sensitivity (Wysoci et al., 2005). For example, a study found that fish exposed to different sound pressure levels showed some fish might be able to adapt to changing background noise (Wysoci et al, 2005) while in other species exposed to nearby shipping vessels, showed a decreased in their communicative space during important biological periods (Stanley et al., 2017).

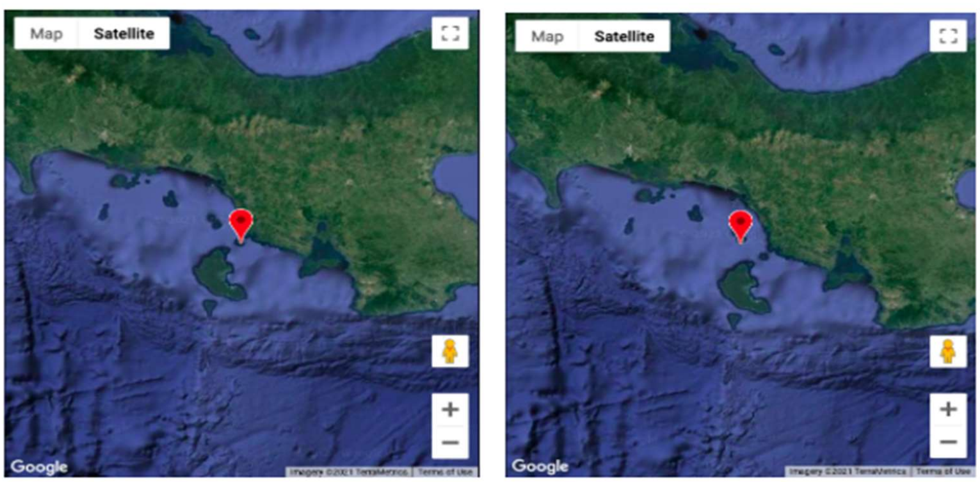
Even within protected areas, marine communities face threats from fishing pressures, tourism, and natural resource extraction. This is the case in Coiba National Park, the location of interest for this study off the southwest coast of Panama in the Gulf of Chiriquí. The 2020 IUCN World Heritage Conservation Outlook deemed Coiba under significant concern and very high threat in regard to its protection status due to unregulated tourism development and unsustainable fishing rates (IUCN, 2020). These human activities undoubtedly have a negative effect on the marine community of Coiba, but is it possible that with recent restrictions due to the coronavirus pandemic, the frequency of these activities has decreased? Limited tourism due to travel guidelines, decreased global maritime trade, and a decline in the fishing industry due to Covid-19 present the opportunity to study areas like Coiba to analyze whether there was a change in boat traffic noise as an effect of the pandemic as well.

The purpose of this study is to use acoustic monitoring data between two locations within Coiba National Park from times before and during the Covid-19 pandemic. My hypothesis is that during lockdowns there will be a change in boat presence and the soundscapes of a marine community. I expected to find that during Covid-19 lockdowns there will be a decreased boat presence and thus biological sound sources noise levels will increase. The results of this study will provide insights on how human noise pollution impacts marine communities inside and outside protected areas.

2. Materials and Methods

A. Study Site

Data for this study was collected at two sites within the Gulf of Chiriquí, a popular fishing and tour destination off the southwest coast of Panamá. These sites serve as a refuge and ecologically important habitat for hundreds of marine species including sharks, sea turtles, and several pelagic fish species (WHC, 2020). This area is also an important migratory breeding area for southern hemisphere humpback whales, which winter here from July to November (Rasmussen et al., 2007).



a. Isla Canal (7.697N, -81.611W) b. Isla Contreras (7.777N, -81.759W)

Figure 1. Locations of Soundtrap recorders in the Gulf of Chiriquí, Panama.

B. Acoustic Recordings

A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) recorder was deployed at 25 m from October to December 2017 at Isla Canal (7.697N, -81.611W), and from October to December 2020 at Islas Contreras (7.777N, -81.759W) (Figure 1). The recorder in Isla del Canal was programmed to record the soundscape for 5 minutes every 30 minutes while the recorder in Isla Contreras recorded the soundscape for 10 minutes every hour. We randomly selected 7 days from each month in each location to be analyzed (see Table 1). Due to the differences in recording cycles, we selected the first minute of each hour for inspection and scored presence (1) and absence (0) of boats for each 1-min file. To determine if there was contribution of boat traffic to the marine soundscape, we used these 1-min files and calibrated them using the software dBWav to measure noise levels as dB_{RMS} for each location. dB_{RMS} is the root mean square and measures the average power output of over the 1-min file. Data visualization and analyzes were done in JMP 14 (SAS Institute, NC, United States). We used a Least Square analysis test to affect the year, time of day, and frequency bands in dB_{RMS} noise levels.

Table1: Acoustic Recording dates and locations before and during the Covid-19 pandemic

Location	Selected dates	Recording cycle
Isla Canal (2017)	- October 1, 4, 14, 11, 27, 17, and 21 - November 2, 15, 10, 5, 26, 18, and 8 - December 28, 1, 12, 26, 16, 7, and 11	5 minutes every 30 minutes
Contreras (2020)	- October 26, 9, 28, 24, 8, 5, and 13 - November 11, 15, 9, 2, 30, 7, and 28 - December 16, 11, 9, 13, 5, 2, and 14	10 minutes every hour

3. Results

3.1. Boat Presence/Absence

A total of 42 days (1,008 1-min files) were scanned for presence/absence of boats using spectrogram data in RFCx Arbimon from 2017 and 2020. During this time only four boats were detected in the data in 2017 at the Isla de Canal site. No boat detections occurred at Isla Contreras, 2020.

3.2. Noise Levels

We found significant differences in noise levels due to site (F Ratio=1676, df=1, $p<0.0001$), frequency (F Ratio=1439.4, df=10, $p<0.0001$), and time of the day (F Ratio=54, df=1, $p<0.0001$). The pattern is that noise levels were higher in 2020 than in 2017 (Fig. 2). Noise levels were greater at all frequencies in 2020 than in 2017, especially above 5000 Hz (Fig. 3). Noise levels were also greater throughout the day in 2020 than in 2017 (Fig. 4).

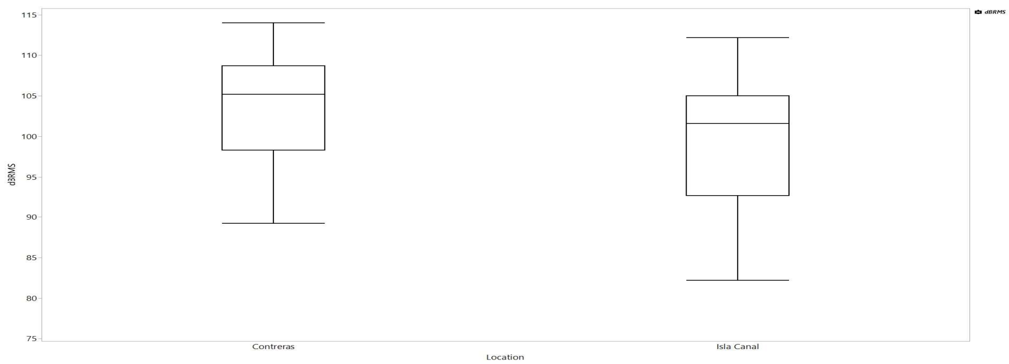


Figure 2. Overall ambient noise levels in dB_{RMS} of neighboring islands Isla Contreras (2020) and Isla Canal (2017).

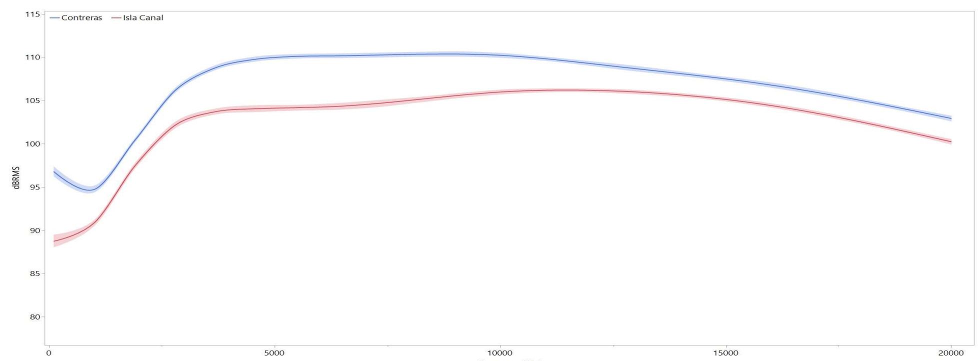


Figure 3. Overall ambient noise levels at Isla Contreras (2020) and Isla Canal (2017) in dB_{RMS} by frequency.

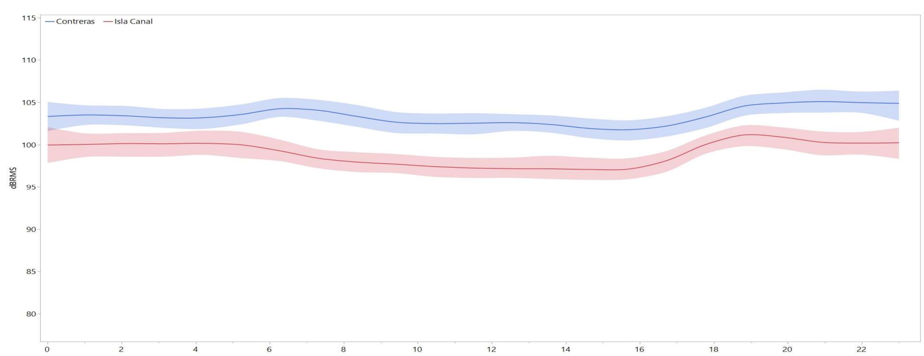


Figure 4. Ambient noise levels at Isla Contreras (2020) and Isla Canal (2017) by location and time of day (hours)

4. Discussion

Our results found that ambient noise levels were consistently greater in Isla Contreras throughout the day when location and time of day was plotted (Figure 4). Contreras data also showed frequency bands consistently over 100 Hz. Both sites revealed a slight increase in noise frequencies during late nighttime hours. As the Gulf of Chiquiri is a popular migratory destination for southern hemisphere humpback whales during the austral winter season (July-October) (Rasmussen et al, 2013), it can be inferred that humpback whale singing is largely contributing to ambient noise levels at the study sites, especially at night, as maximum song occurrences from similar populations of humpback males have been shown to occur at night (Ryan et al, 2019). Further research done at these study sites at Coiba could be done to see whether there is humpback whale presence during the suspected times in the acoustic data in RFCx Arbimon. This would add to the results of this study by showing whether the increased ambient noise levels were in fact due to the presence of humpback whale singing.

The low values of noise levels below 5000 Hz suggests relatively low boat presence at both sites independent of the regulations and lockdowns brought on as a result of Covid-19. The difference between sites and years analyzed may be due to differences in substrate and community structure as opposed to Covid-19 regulations. At this time, there is no data on these characteristics for the islands in this study; however, previous studies have shown that substrate can result in differing soundscapes as a result of the levels of biodiversity. A 2005 study done in Bocas del Toro, Panama showed a relationship between species richness and substrate structural complexity, with general species diversity increasing with habitat complexity (Dominici-Arosemena, 2005). This indicates that a difference in substrate complexity could lead to differing ambient noise levels because of differences in species richness and biodiversity between Isla Canal and Isla Contreras.

Different site substrates, depths, latitudes, and even years could additionally affect biodiversity levels at the study sites. Past studies have shown high benthic biodiversity in regions of high latitude and deeper waters (Piacenza et al., 2015), suggesting recorder depth could also be a factor influencing acoustic biodiversity levels. Ultimately, this study poses questions for further research regarding the individual study sites within the marine community of Coiba National Park and how substrate differences affect the biodiversity of the respective sites. The result of this study, and possible further studies, stimulates the exploration of these marine communities and the use of sound as a tool to assess habitat quality and biodiversity.

Acknowledgements
This study was made possible by the extensive data collection by fellow associates in Panama. Special thanks go out to Betzi Perez, Julio Jose Casas, and Fundación Panacetacea and the Ministry of the Environment, Panama. Lastly, I would like to thank Dr. Laura May-Collado, whose dedication, support, and guidance not only made this study possible, but an incredible learning experience.

References

Boyd, I.L., G. Frisk, E. Urban, P. Tyack, J. Ausubel, S. Seeyave, D. Cato, B. Southall, M. Weise, R. Andrew, T. Akamatsu, R. Dekeling, C. Erbe, D. Farmer, R. Gentry, T. Gross, A. Hawkins, F. Li, K. Metcalf, J.H. Miller, D. Moretti, C. Rodrigo, and T. Shinke. 2011. "An International Quiet Ocean Experiment." *Oceanography* 24(2):174–181, doi:10.5670/oceanog.2011.37.

Christine Erbe, Colleen Reichmuth, Kane Cunningham, Klaus Lucke, Robert Dooling, (2016) "Communication masking in marine mammals: A review and research strategy", *Marine Pollution Bulletin*, 103(1–2) Pages 15-38, doi:10.1016/j.marpolbul.2015.12.007.

Dominici-Arosemena, A., & Wolff, M. (2005). "Reef Fish Community Structure in Bocas del Toro (Caribbean, Panama): Gradients in Habitat Complexity and Exposure." *Caribbean Journal of Science*, *41*(3), 613–637.

Jones, N. (2019). “Ocean uproar: saving marine life from a barrage of noise.” *Nature*, *568*(7751), 158–161. https://doi.org/10.1038/d41586-019-01098-6

Mooney TA, Iorio LD, Lammers M, Lin T-H, Nedelec SL, Parsons M, Radford C, Urban E, Stanley J. (2020) “Listening forward: approaching marine biodiversity assessments using acoustic methods.” R. Soc. Open Sci. 7: 201287. doi:10.1098/rsos.201287

Piacenza, S. E., Barner, A. K., Benkwitt, C. E., Boersma, K. S., Cerny-Chipman, E. B., Ingeman, K. E.,. Heppell, S. S. (2015). “Patterns and variation in benthic biodiversity in a large marine ecosystem.” *PLOS ONE, 10*(8). doi:10.1371/journal.pone.0135135

Pine, M. K., Nikolich, K., Martin, B., Morris, C., & Juanes, F. (2020). “Assessing auditory masking for management of underwater anthropogenic noise.” *The Journal of the Acoustical Society of America, 147*(5), 3408-3417. doi:10.1121/10.0001218

Popper AN, Hastings MC. (2009) “The effects of anthropogenic sources of sound on fishes.” J Fish Biol;75(3):455–89. doi:10.111 1/j.1095-8649.2009.02319.x.

Rasmussen, K., Palacios, D. M., Calambokidis, J., Saborio, M. T., Dalia Rosa, L., Secchi, E. R., Steiger, G. H., Allen, J. M., and Stone, G. S. (2007). “Southern hemisphere humpback whales wintering off Central America: Insights from water temperature into the longest mammalian migration,” Bio. Lett. **3**, 302–305. https://doi.org/10.1098/rsbl.2007.0067

Rasmussen, K., & Palacios, D. M. (2013). “Highlights from a Decade of Humpback Whale Research in the Gulf of Chiriquí, Western Panama, 2002-2012.” *Panacetacea.org*.

Rayment W, Dawson S, Slooten L. (2009) “Use of T-PODs for acoustic monitoring of Cephalorhynchus dolphins: a case study with Hector’s dolphins in a marine protected area.” *Endanger. Spec. Res*. 10, 333–339. (doi:10. 3354/esr00189)

Ryan, J. P., Cline, D. E., Joseph, J. E., Margolina, T., Santora, J. A., Kudela, R. M., … Fischer, M. (2019). “Humpback whale song occurrence reflects ecosystem variability in feeding and migratory habitat of the northeast Pacific.” *PLOS ONE*, *14*(9). https://doi.org/10.1371/journal.pone.0222456

Stanley, J. A., Van Parijs, S. M., and Hatch, L. T. (2017). “ Underwater sound from vessel traffic reduces the effective communication range in Atlantic cod and haddock,” Sci. Rep. **7**, 14633. doi:10.1038/s41598-017-14743-9

Weilgart, L. (2017). “Din of the Deep: Noise in the Ocean and Its Impacts on Cetaceans.” *Marine Mammal Welfare*, 111–124. https://doi.org/10.1007/978-3-319-46994-2_7

World Heritage Outlook. (2020). “Coiba national Park and its special zone of marine protection.” Retrieved February 22, 2021, from https://worldheritageoutlook.iucn.org/explore-sites/wdpaid/902479

Wysoci, L. E. & Ladich, F. (2005) “Hearing in Fishes under Noise Conditions.” J. Assoc. es. Otolaryngol. 6, 28–36

Does boat activity influence the acoustic structure of humpback whale songs?

Briana Heller
Department of Biology, University of Vermont, Burlington, VT, United States

ABSTRACT
Every year during their breeding season, southern humpback whales migrate from the Antarctica Peninsula and Chile to the tropical waters of Central America. Due to the covid-19 pandemic, there has been less tourism in Costa Rica and thus decreased boat activity. In this experiment, we studied the effects of boat activity on the acoustic structure of humpback whale's songs. Data was taken from outside of Caño Island Biological Reserve, Costa Rica. We hypothesized that with less boat activity during the pandemic, songs in 2019 and 2020 would have different acoustic structures. We compared the phrase P-YYY10 which is distinguished by three inverse v units and one flat unit. To compare the acoustic structure, we analyzed low, central, high and delta frequencies (Hz) as well as duration (s) and modulation in songs during 2019 and 2020. Our results showed that in 2020 whales sang P-YYY10 with units that were longer in duration and at lower frequencies than in 2019. A study measuring noise levels found that boat presence and noise levels at low frequencies significantly decreased in 2020. This might explain whales lowering their frequency in 2020. Low frequency sounds propagate better and in a breeding area it may extend male's advertisement range.

Keywords: frequency, duration, tourism, migration, song units

1.Introduction

Every year during their breeding season, populations of southern humpback whales (Megaptera novaeangliae) migrate from the Antarctica Peninsula and Chile to Central America (Chereskin et al., 2019). During their breeding season, humpback whales are very vocal in their singing. Social vocalizations can be used for identification, forming a reunion or warning others (Guazzo et al., 2020). The major theory as to why humpback whales sing is to perform a display for females to win their favor to mate (Tyack, 1981). The song humpback whales produce is only made by males and can be long and intricate. Songs are made of themes, which are made of phrases; units are the syllables that make up phrases (Cholewiak et al., 2013). Multiple themes make up a song. The themes in a song can vary depending on region. Humpback whale songs are important for mating, but boat activity is posing a threat to whale communication.

Previous studies have looked at the potential risk boats and their noise pollution have on the singing of humpback whales. In a study of humpback whales during their breeding season in Hawaii, whale songs were impacted by increasing ambient noise (Guazzo et al., 2020). The source level of their song units increased when there was ambient noise, but not enough to overpower the ambient noise. A similar study in Glacier Bay National Park found that for every ambient noise increase of 1 dB, there was a source level increase for the whales of 0.81 dB (Fournet et al., 2018). In addition, a study of migrating whales going from the great barrier reef to Antarctica observed that whales changed their communication in natural vs. vessel sounds (Dunlop, 2019). The whales were able to still successfully communicate when natural sounds were present by increasing vocal source level but were not able to communicate louder than the vessels. These studies give context to the changes observed in whale songs when vessel noise is disturbing their environment.

Due to the predictable nature of whale's migration to Central America, whale watching has become a popular tourist activity. While whale watching is a way to encourage public interest in wildlife protection, when unregulated, boats can cause acoustic changes in the whale's singing. Small vessels emit broadband frequency noise that has the potential to cause acoustic masking of whale songs (Amrein, 2020). During the covid-19 pandemic, the activity of tourist boats decreased with worldwide shutdowns. Researchers studying humpback whales in Glacier Bay National Park have found that there has been a decrease in underwater noise from boats allowing whales to be less stressed (Sommer, 2020). The goal of our study was to determine if during the 2020 lockdowns whale song structure changed. I hypothesized that humpback whale's song structure will vary between 2019 and 2020 due to the changes in tour boat presence. I predicted that humpback whale song frequency and duration would reflect the changes in soundscapes due to boat presence. For example, less boats results in a decrease in noise at frequencies that overlap with whale songs. Results of this study can inform policy on tourist boats and help protect areas where humpback whale mating occurs.

2.Materials & Methods

2.1 Study Site and Recordings

Data for this experiment was collected outside of Caño Island Biological Reserve shown in Figure 1. The reserve is 15 km off the Osa Peninsula on the southern coast of Costa Rica. Recording came from Jardin (8.719 N/-83.863W) which is 0.8 km northeast from the island (Chereskin et al. 2019). This site is characterized by a sandy ocean floor. A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) recorder was deployed 25 m deep during the month of September 2019 and 2020. In 2019 the recorder was programmed to record 5 minutes every 30 minutes and in 2020 it recorded 10 minutes every hour.

Figure 1. Caño Island Biological Reserve, Costa Rica. The red pin represents Jardin where the recordings for this experiment were taken (Sieve Analytics, 2015).

2.2 Data Analysis

To study the effects of the covid-19 lockdowns we selected the phrase P-YYY10, which was commonly used in the songs recorded from 2019 and 2020. This phrase is characterized by having a flat unit and three inverse v shaped units. Recordings with these phrases were analyzed in RAVEN Pro 1.6 (2017; Cornell Laboratory of Ornithology, New York) as seen in Figure 2. To analyze acoustic structure, we extracted the following standard acoustic variables: minimum, maximum, center, peak and delta frequency (Hz) as well as delta time (s), duration and peak frequency contour number of inflection points (PFC Num Inf Points). Once our data was collected, we used the program JMP Pro 15 (SAS, 2019) to create boxplots of our data and perform chi-squared tests.

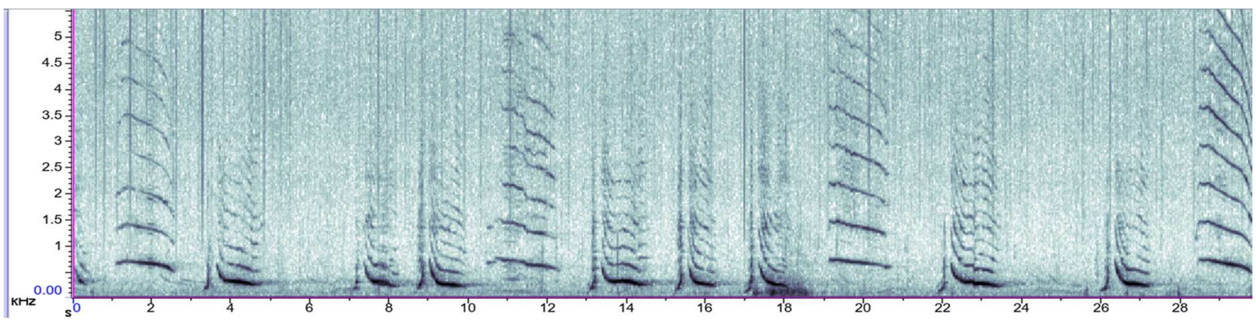


Figure 2. Spectrometer of whale theme P-YYY10 taken from September 16, 2020 and analyzed in RAVEN 1.6 (2017; Cornell Laboratory of Ornithology, New York).

3.Results

We found that units in the P-YYY10 varied significant in frequency and duration (Fig. 3). Units were lower in low frequency (X^2= 13.7, p=0.0002), high frequency, (X^2= 13.7, p=0.0002), and center frequency (X^2= 35, p<0.0001) in 2020 than in 2019. Units were also significantly longer in duration (X^2= 35.1, p<0.0001). There was no significant difference found in delta frequency (X^2=0.077, p=0.7812) or PFC Num Inf Points (X^2=1.27, p=0.2588).

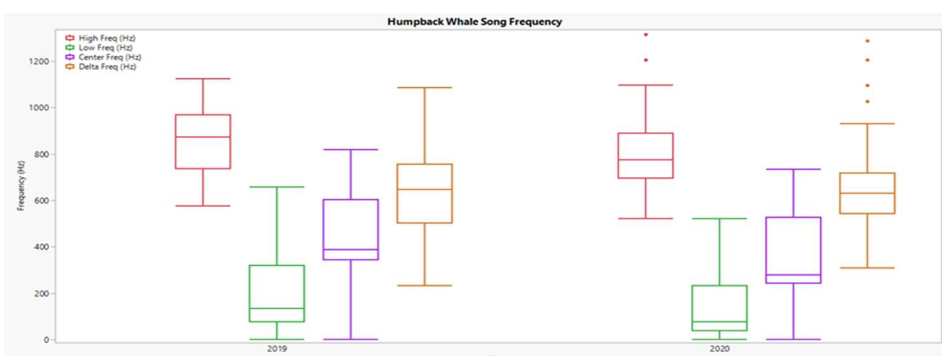


Figure 3. Boxplots of P-YYY10 unit high, low, center and delta frequency in 2019 and 2020 at Caño Island Biological Reserve, Costa Rica.

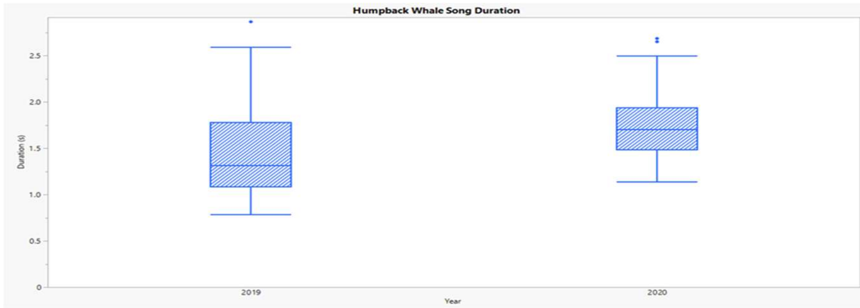


Figure 4. Boxplots of P-YYY10 unit duration (s) in 2019 and 2020 at Caño Island Biological Reserve, Costa Rica.

4.Discussion

Our results show that there were significant differences between whale song units in 2019 and 2020. Our original hypothesis was that there would be differences in acoustic structure before and during the Covid-19 lockdowns, thus, our results align with our hypothesis. Before the pandemic, whale migration attracted tourists to go whale watching. During the pandemic, worldwide shutdowns slowed boat activity. Changes in frequency and duration of whale song units during the Covid-19 lockdowns alludes to boat disturbances to whales. Boat noise is produced at low frequency bands, which can mask whale songs (Pellegrini, et al., 2021). When boats were absent, whales sang at lower frequencies so their songs could travel further distances. The longer duration of their songs units suggests that the whales no longer had to purposefully shorten their units because of boat disruption. Without boats, whales were able to dominate the acoustic space.

There are studies like ours that support our research on humpback whale's songs. In one study, they found that in the presence of boats, whale song units were shortened (Rey-Baquero et al., 2021). This may be because of the masking effect boat noise has on marine communication. This study also found that the inter-unit interval (time between units) was longer when boats were present. There was also less singing occurring in the presence of boats for these whales studied off the coast of Columbia. Furthermore, from a study in Hawaii, ambient noise caused whales song units to increase their source level (Guazzo et al., 2020). This study showed the effects of the lombard effect with anthropomorphic noise affecting whales singing. There has also been research on the presence versus absence of boats and the frequency of whales singing. In one study, the presence of boats caused whales to decrease their singing after a boat had passed (Tsujii et al. 2018). Lastly, increased boat activity and tour boats have caused changes in the acoustic structure of dolphin whistles (Perez-Ortega et al., 2021). For these dolphins, lower frequency modulation was adjusted in the presence of boat traffic based on the intensity of boat traffic. The changes in the acoustic structure of dolphin whistles is analogous to the changes seen in whale songs.

Overall, our study shows the impacts of boat noise on the acoustic structure of whale songs. Our research can be used to inform policy for whale watching vessels in Costa Rica. Reduced boat traffic and regulation on the distance boats are allowed near whales could potentially help noise interference that causes whales to adjust their acoustic structure. More research should be done into the effects boat noise can have on whale song acoustic structure.

Acknowledgements

I would like to thank Laura May-Collado for guiding me through this study and providing me with the data I used. I would also like to thank Jose David Palacios and Juan Jose Alvarado for their contribution in collecting the data I used for this experiment.

References

Amrein, A., Guzman, H., Surrey, K., Polidoro, B., Gerber, L. (2020) "Impacts of Whale Watching on the Behavior of Humpback Whales (*Megaptera novaeangliae*) in the Coast of Panama," Frontiers In Marine Science 7, 601255.

Cholewiak, D., Sousa-Lima, R., Cerchio, S., (2013) "Humpback whale song hierarchical structure: Historical context and discussion of current classification issues," Mar Mam Sci, 29: E312-E332.

Dunlop, R. 2019. "The effects of vessel noise on the communication network of humpback whales," Royal Society Open Science, 6.

Chereskin*,* E., Beck*, L.,* Gamboa-Poveda*, M.,* Palacios-Alfaro*, J.,* Monge-Arias*, R.,* Chase*, R.,* Coven*, B.,* Guzmán*, A.,* McManus*, N.,* Neuhaus*, A.,* O'Halloran*, R.,* Rosen*, S., and* May-Collado, L. (2019). "Song structure and singing activity of two separate humpback whales populations wintering off the coast of Caño Island in Costa Rica," The Journal of the Acoustical Society of America 146, EL509.

Fournet, M. E. H., Matthews, L. P., Gabriele, C. M., Haver, S., Mellinger, D. K., Klinck, H. (2018). "Humpback whales Megaptera novaeangliae alter calling behavior in response to natural sounds and vessel noise," Marine Ecology Progress Series, 607: 251-268.

Guazzo, R. A., Helble, T. A., Alongi, G.C., Durbach, I.N., Martin, C.R., Martin, S.W., Henderson, E. (2020) "The Lombard effect in singing humpback whales: Source levels increase as ambient ocean noise levels increase," Journal of the Acoustical Society of America 148, 2 542-555.

Mercado, E. (2021) "Song Morphing by Humpback Whales: Cultural or Epiphenomenal?" Frontiers in Psychology, 11.

Perez-Ortega, B., Daw, R., Paradee, B., Gimbrere, E., and May-Collado, L. (2021) "Dolphin-Watching Boats Affect Whistle Frequency Modulation in Bottlenose Dolphins," Front. Mar. Sci., 17.

Pellegrini, A., Romeu, B., Ingram, S.N., Daura-Jorge, F.G. (2021). "Boat disturbance affects the acoustic behaviour of dolphins engaged in a rare foraging cooperation with fishers,"Animal Conservation.

Rey-Baquero M. P., Huertas-Amaya L. V., Seger K. D., Botero-Acosta N., Luna-Acosta A., Perazio C. E., Boyle J. K., Rosenthal S., Vallejo A. C. (2021) "Understanding Effects of Whale-Watching Vessel Noise on Humpback Whale Song in the North Pacific Coast of Colombia With Propagation Models of Masking and Acoustic Data Observations," Frontiers in Marine Science, 8.

Sommer, L. (2020) "Whales get a break as pandemic creates quieter oceans," npr.

Tsujii, K. et al. (2018). "Change in singing behavior of humpback whales caused by shipping noise," Public Library of Science ONE 13(10)

Tyack, P. (1981) "Interactions between singing Hawaiian humpback whales and conspecifics nearby". *Behav Ecol Sociobiol* 8, 105–116.

Soundscape biodiversity in Marine Protected Areas versus unprotected areas in Costa Rica and Panama

Cecilia Vichi
University of Vermont, Department of Biology, Burlington, VT, 05405, USA

ABSTRACT
In response to the current biodiversity crisis, a global effort to protect biodiversity in the oceans has resulted in the establishment of many new Marine Protected Areas (MPA's). MPAs are designed to slow devastation of critical ecosystems that are at high risk due to rapidly changing marine climate conditions. However, in most cases after an MPA is established, there is little follow up to determine if these efforts translate into long-term successful preservation of biodiversity. In this study, we used sound recorded from passive acoustic recorders as a cue for biodiversity. We created a presence-absence matrix of boats and whales and estimated the distribution of acoustic events by time and frequency bins using RFCxArbimon soundscape tools. We hypothesize acoustic diversity to be greater in MPAs than in the non-protected area. We found a higher proportion of acoustic events in MPAs than non-protected areas. In some MPAs, Humpback whale presence is likely the main driver behind the differences in acoustic activity. The result of this study suggests MPAs are fulfilling their job protecting marine biodiversity.

Keywords: Soundscapes, Acoustic Activity, Conservation, Marine Protected Areas (MPA'S), Marine Biodiversity.

1. Introduction

In response to the current biodiversity crisis, a global effort to protect biodiversity in the oceans has resulted in the establishment of many new Marine Protected Areas (MPA's) (REFS). The presence of activities such as commercial fishing, tourism, industrial practices, and those creating environment devastation are prohibited or regulated in most marine protected areas, (REFS) although variable across the globe with some allowances. However, in most cases after an MPA is established, there is little follow up to determine if these efforts translate into long-term successful preservation of biodiversity. Given the importance of sound in the life of marine organisms (Middel *et al.,* 2017) using a passive acoustic recorder can provide a rapid assessment of biodiversity and it threats in protected and non-protected areas.

There is increasing amounts of data and concern about the effects of anthropogenic noise on fish populations. The following question when thinking in terms of marine protections is; are protections helping against the increase in anthropogenic noise? Are they preserving biodiversity and health by mitigating noise in these areas? A study involving *Diplodus sargus* explored a method of determining of protections were in fact protecting. The movement patterns of this species were observed with acoustic tags in both no-take and take MPA's. The results showed a significance in the no-take areas, providing the fish with a refuge (Belo *et al.,* 2016). This refuge was from outside anthropogenic pressures that included anthropogenic noise. While this methodology explored species movement it provided results on the efficiently of no-take vs take MPA's. the data here can be extrapolated into other studies exploring biodiversity.

In marine habitats, biodiversity heath is not an easy feat. Quantifying biodiversity in general is challenging and in marine habitats especially. It is paramount we find a successful and efficient way to measure biodiversity. Since acoustics travel well underwater, it only makes sense to explore marine habitats acoustically. Mooney *et al., 2020* suggested passive acoustic recorders as an efficient way to measure biodiversity levels within a marine environment. Providing a summary of acoustic measurement history in marine ecosystems, he suggests a cautious approach to acquire sufficient sampling and capturing of the marine soundscape (Mooney *et al.,* 2020). In other protected areas, biodiversity assessments have revealed effects of anthropogenic noise on the marine organisms that habit the ecosystems. In the Laje de Santos Marine State Park and Xixova-Japui State Park, both protected areas, data was collected on the soundscapes (Sanchez-Gendriz and Padovse, 2016). Specifically evaluating the presence of anthropogenic noise in these protected areas can shed light on to the state of the marine protections in place.

There is a huge diversity of assessments that can be performed acoustically in marine habitats, biodiversity being one of them. This sort of methodology can be used then to determine biodiversity levels within protected ecosystems versus unprotected ecosystems therefore leading to information on the success of the protections.

Soundscapes can be used to characterize different types of marine habitats. These differences can provide information on communities, structures, and biodiversity within the areas. Using passive acoustics is a great method for obtaining soundscapes (Pieretti and Danovaro, 2020). In a 2016 study, researcher explored passive acoustic measuring to obtain average sound pressure level and acoustic complexity index-based spectrum of frequency (Bertucci *et al.,* 2016). The study suggested that soundscapes can be used to accurately evaluate acoustic features within a marine environment, specifically marine protected sites. Protected sites were found to be more acoustically complex, than sites not under protection. Not only does this study parallel what we are looking to investigate, but it provides us with a baseline of information that can be used in explanation of our results.

To determine if soundscape data can provide information about how effective MPAs are, we deployed autonomous underwater recorders in 2020 during the Covid-19 lockdowns inside and outside MPAs in Costa Rica and Panama that have similar substrates. We hypothesized that biological noise levels would be higher in MPAs than outside them as a indicator of success in protecting habitat complexity and biodiversity. We expect that noise levels as indicators of health, will be higher in MPAs and that the proportion of acoustic events should be evenly distributed in by time and frequency bin in MPAs than in non-protected areas.

2. Materials and methods

2.1 Study Sites

This study took place in the three sites along the Pacific coast of Costa Rica and Panama. Two of these sites are marine protected areas (MPAs), Caño Island Wildlife Refuge (CIWR), Costa Rica, and Coiba National Park (CNP), Panama, and one non-protected area in the Archipelago of Islas Secas (AIS), Panama. CIWR has been a protected site since 1978 and is known for its abundant marine life. The protected area is approximately 2900 hectares, both marine and terrestrial. It is also a known site for coral research and recolonization. Coiba is a known refuge for many endemic species. The protected region prohibits commercial fishing, any development that isn't for park use. Because of the buffer capacity of the Gulf of Chiriquí, Coiba is not affected by the El Nino temperature fluctuations and in turn has a high degree of biodiversity, home to approximately 760 different fish species plus several sharks and cetacean species. In contrast, the un-protected Secas Archipelago is a known tourist destination and owned privately. However, because of its proximity to Coiba, the populations of migrating humpback whales are travel through the area. AIS is relatively not isolated and despite the potential for being an eco-reserve, anthropogenic factors are not monitored.

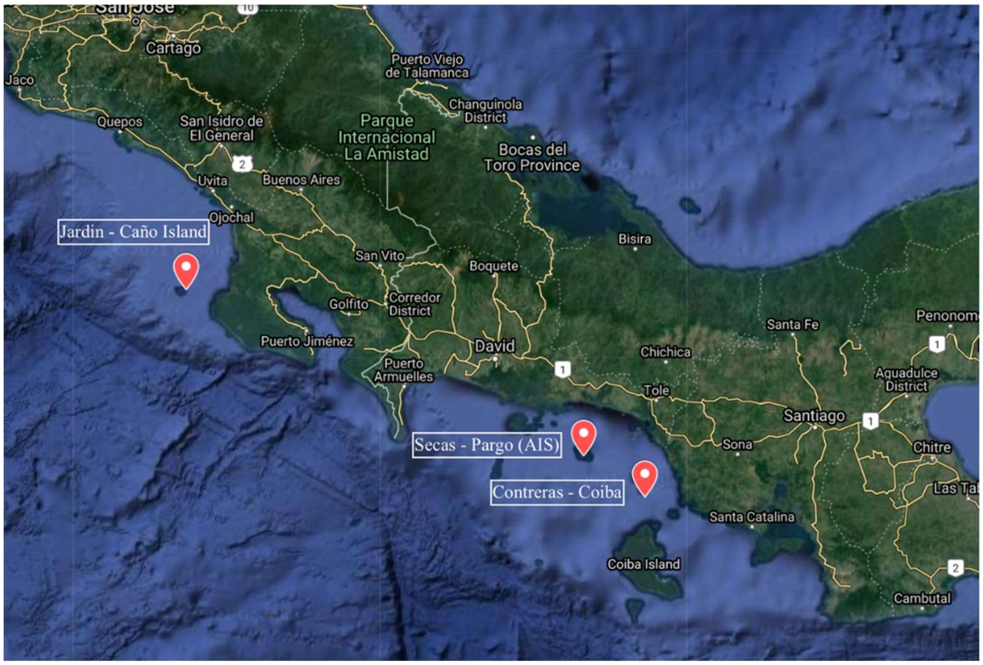


Figure 1. Represented geographically each site is marked. Jardin Caño Island (protected) in Costa Rica and Secas Pargo and Contreras, Cobia (protected) in Panama.

2.2 Recordings

Autonomous underwater recorders were deployed in sandy microhabitats in each site: Jardin in CIWR; Contreras in CNP, and Secas-Pargo in AIS during October 2020. These recorders were Soundtrap 300 SD devices (Ocean Instruments, frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) and were programed to record the soundscape in each location using different cycles. In CIWR recordings were made for 15 minutes every hour and for 10 minutes every hour at Contreras and Toro sites. Recordings were uploaded to the RCFxArbimon online platform where spectrograms were generated to inspect for presence (1) and absence (0) of boats and whales at the first 1-min of every hour recorded. Using RCFxArbimon soundscape tools, we generated a matrix of the proportion of acoustic events by time and frequency bins (86 Hz) to determine when most acoustic events happen and at what frequency bands in each location.

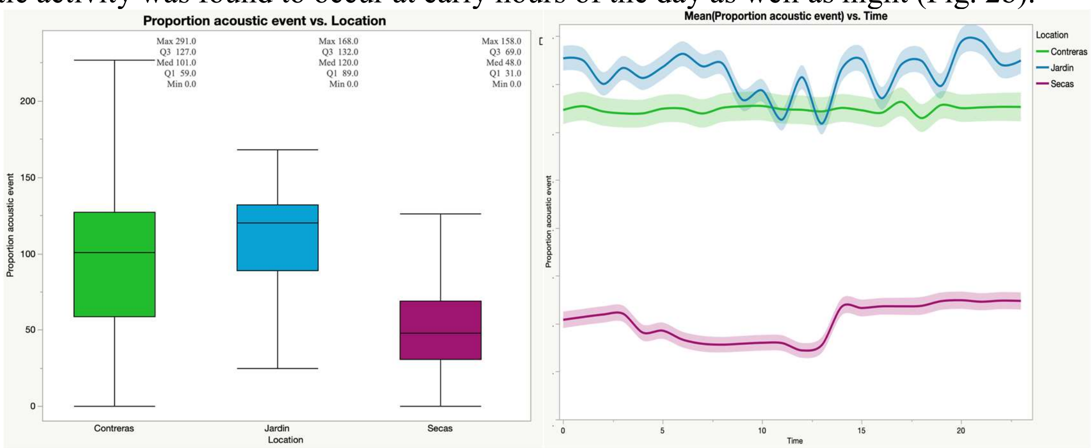
2.3 Analysis

Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, Kruskal-Wallis Test to determine if there are significant differences in the proportion of acoustic events by site and a Chi-square to determine if the distribution of acoustic events vary by time of day between sites.

3. Results

3.1 *Proportion of Acoustic Events*

We found significant differences in the proportion of acoustic events between sites, with MPAs having a higher proportion of acoustic events than non-protected areas (Kruskal-Wallis=48, df=2; p>0.0001). The MPA Jardin had the highest proportion of acoustic events compared to non-protected site in Secas-Pargo (z=-63.3; p<0.0001) and the MPA including the site Contreras (z=15; p<0.0001) (Fig. 2a). These differences are maintaining throughout the day (Fig 2b). Most acoustic activity was found to occur at early hours of the day as well as night (Fig. 2b).



a. b.

Figure 2. Proportion of acoustic events by location (a) and time of day (b).

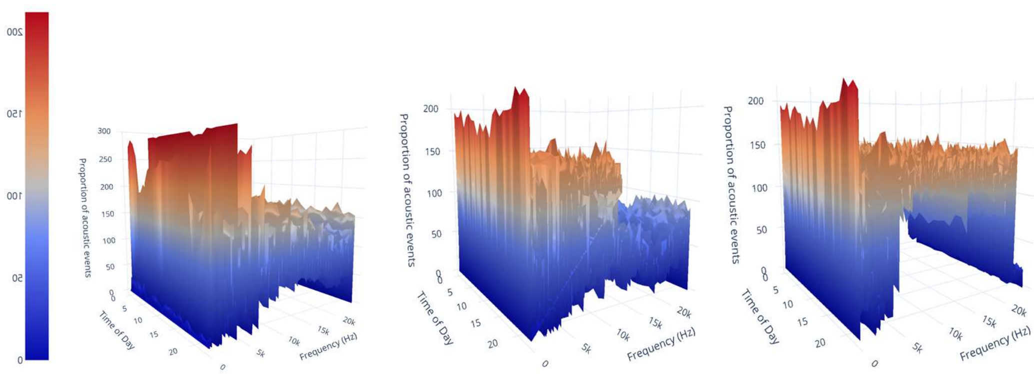


Figure 3. Proportion of acoustic events over time of day and frequency (hz) for Contreras (left), Secas (center), and Jardin (right)

Data shows variance in acoustic activity between sites (Fig. 2). In the MPA sites of Jardin and Contreras most acoustic activity was below 5 kHz throughout the day. Whereas in Secas, the non-protected area, acoustic activity was below 10 kHz. Jardin MPA showed the highest proportion of acoustic events on average, with events occurring throughout the day (Fig 3). In comparison, in the unprotected site, Secas, the acoustic activity and proportion of events ceased at frequencies above 10 kHz during the day (Fig 2).

The most influential contributors of sound during this time of year are boats and humpback whales. We found a greater number of humpback whale song detections in Jardin than in Contreras ($X2=114.4$; $df=1$; $p<0.0001$) and insignificant differences in boat presence. ($p>0.05$) (Fig. 4).

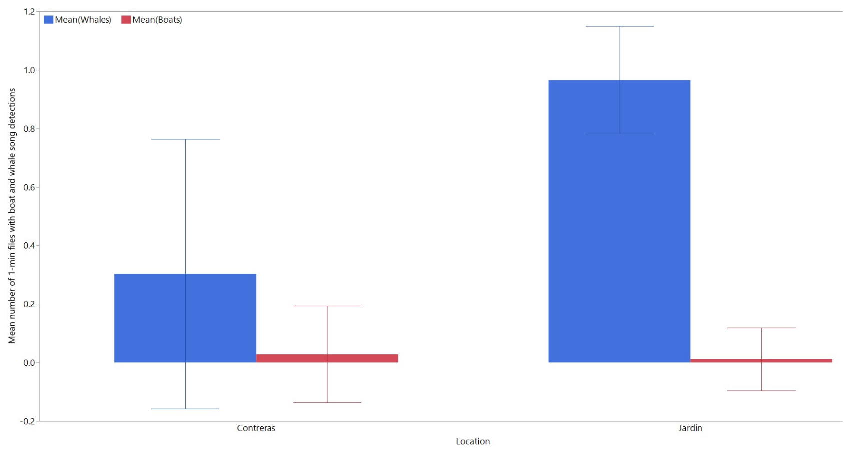


Figure 4. Boxplot of whale and boat presence in Contreras (left), and Jardin (right). Whale presence in blue and boats in red.

4. Discussion

This study investigates the success of MPAs in Costa Rica and Panama and their relationship to acoustic activity and biodiversity within these marine systems. We expected a higher level of acoustic activity in protected areas versus unprotected areas due to decreased boat travel, and tourism, along with them being more, relatively isolated areas. The results show higher proportion of acoustic activity in produced in protected areas. The two MPA sites; Jardin and Contreras showed greater proportions of acoustic activity than Secas, the unprotected site. This suggests that the protections used in the MPA's at these sites are successful in their purpose of protecting biodiversity. However, the presence of humpback whales provides an explanation for the increased activity in the protected areas as well. Most activity in Jardin were Humpback whale vocalizations, occurring primarily at night, this significant presence is likely the main driver behind the drastic difference in results.

In recent studies of Caño Island, Humpback whale presence has been seen peak in the month of October, supporting the data for increased activity in Jardin (Chereskin *et al.,* 2012). Because Humpback whale songs are known to be some of the loudest marine mammals (Chen, *et al.,* 2016), their vocalizations are significantly loud and overpowering in the acoustic marine environment. Our results also showed a constant level of acoustic activity at extremely low frequencies (<5kHz). This constant level of noise could be attributed to the presence of snapping shrimp. Abundant in all locations, snapping shrimp were present in almost every recording listened to. This could account for the levels of acoustic activity in the unprotected sites where whales were found not to be present.

The influence of boat presence was found to be insignificant from this data. Presence and absence data on boat travel did not provide us with any information on differences in biodiversity levels or MPA success as it was nearly the same in both measured sites. Because it was insignificant, possible explanations could stem from the Covid-19 virus. Although there are no studies done on the matter, the insignificance of boat travel might be due to decreases in overall travel and tourism during the pandemic. Anthropogenic acoustic noise levels have been found to increase marine mammals stress, leading to responses such as decreased reproduction and accelerated sickness, or aging in behavior (Wright *et al.,* 2007).

Since the pandemic anthropogenic noise has been found to have decreased. As the virus spread more rapidly, causing a global lockdown socially, economically, and industrially, the environment benefitted (Arora, *et al.,* 2020). Insufficient data on presence/absence in Secas was obtained, therefore measure of whales and boats in this site specifically could not be determined. The soundscape yielded acoustic activity lower than protected areas, but we are unable to determine whether the sound were whale vocalizations or other marine species. Further research on this topic needs to explore the specific types of organism present in these areas and obtain sufficient and significant data on presence and absence. Future studies should also be performed when Humpback whales are not present as well as during periods of normal boat travel to determine if these patterns are maintained and so whales do not overpower the soundscape.

Since this study is the first of its kind looking into the acoustic changes in marine environments after a worldwide pandemic. Boat travel and biodiversity data collected and analyzed here will lead to new findings considering environmental changes. Findings such as these can be contributed to further research on the topic of biodiversity affected by the pandemic and lockdown as well as more information on the noise levels of these areas and how these changes affect the surrounding marine life. Acoustic patterns of marine habitats are an incredible too for gaging health, and soundscape studies such as this shed light onto the patterns in protected versus unprotected areas. From these findings, we can conclude that the marine protections in place in Caño and Coiba Islands have a higher proportion of sound than unprotected areas, so these protections must be successful in protecting the biodiversity they are designed to. Measuring and monitoring the health of MPA's is paramount and doing so acoustically provides insight into the activity below the surface that can be missed from above. The results found here can allow researchers a jumping off point when looking into MPA biodiversity levels and what are successful and unsuccessful management techniques.

References

Arora, S., Bhaukhandi, K.D., and Mishra, P.K. (2020). "Coronavirus lockdown helped the environment to bounce back," Sci Total Environ. **10**, 742-140573. doi: 10.1016/j.scitotenv.2020.140573.

Belo, A.F., Pereira, T.J., Quintella, B.R., Castro, N., Costa, J.L., and de Almeida, P.R. (2016). "Movements of Diplodus sargus (Sparidae) within a Portuguese coastal Marine Protected Area: Are they really protected?," Mar Environ Res. **114**, 80-94. doi: 10.1016/j.marenvres.2016.01.004.

Bertucci, F., Parmentier, E., Lecellier, G., Hawkins, A.D., and Lecchini, D. (2016) "Acoustic indices provide information on the status of coral reefs: an example from Moorea Island in the South Pacific," Sci Rep. **15,** 33326. doi: 10.1038/srep33326.

Chen, J., Pack, A.A., Au, W.W., and Stimpert, A.K. (2016). "Measurements of humpback whale song sound levels received by a calf in association with a singer," J Acoust. Soc. Am. **140**, 4010. doi: 10.1121/1.4967444.

Chereskin, E., Beck, L., Gamboa-Poveda, M.P., Palacios-Alfaro, J.D., Monge-Arias, R., Chase, A.R., Coven, B.M., Guzmán, A.G., McManus, N.W., Neuhaus, A.P., O'Halloran R.A., Rosen, S.G., and May-Collado, L.J. (2019) "Song structure and singing activity of two separate humpback whales populations wintering off the coast of Caño Island in Costa Rica," The Journal of the Acoustical Society of America **146**, 509-515 https://doi.org/10.1121/1.5139205

Middel, Heleen; Verones, Francesca (2017). Making Marine Noise Pollution Impacts Heard: The Case of Cetaceans in the North Sea within Life Cycle Impact Assessment. *Sustainability,* **9**, 113*8,* doi:10.3390/su9071138

Mooney, T.A., Di Iorio, L., Lammers, M., Lin, T.H., Nedelec, S.L., Parsons, M., Radford, C., Urban, E., and Stanley, J. (2020). "Listening forward: approaching marine biodiversity assessments using acoustic methods," R Soc Open Sci. **7**, 201287. doi: 10.1098/rsos.201287.

Pieretti, N. and Danovaro, R. (2020). "Acoustic indexes for marine biodiversity trends and ecosystem health," Philos Trans R Soc Lond B Biol Sci. **375**, 20190447. doi: 10.1098/rstb.2019.0447.

Sánchez-Gendriz, I., and Padovese, L.R. (2016). "Underwater soundscape of marine protected areas in the south Brazilian coast," Mar Pollut Bull. **105**, 65-72. doi: 10.1016/j.marpolbul.2016.02.055.

Wright, A., Soto, N.A., Baldwin, A., Bateson, M., Beale, C., Clark, C., Deak, T., Edwards, E., Fernández, A., Godinho, A.I., Hatch, L., Kakuschke, A., Lusseau, D., Martineau, D., Romero, M.L., Weilgart, L.S., Wintle, B., Notarbartolo-di-Sciara, G., & Martín, V. (2007) "Do Marine Mammals Experience Stress Related to Anthropogenic Noise," *International Journal of Comparative Psychology.* **20,** 274-316.

A Comparative Analysis of Humpback Whale Soundscape Ecology; Before and During COVID-19 Marine Restrictions

Christopher Wilson
University of Vermont

ABSTRACT
The novel Coronavirus' strict marine lockdowns decreased global ocean traffic density by nearly 45.0%. The focus studies the effect of Covid-19 restriction on boat traffic and humpback whale singing activity. Autonomous underwater recorders were deployed to determine changes in boat traffic and whales singing activity at two sites in the Islas Secas archipelago in Panama. I hypothesize that during Covid-19 restriction whale detections will increase. To test this hypothesis a presence-absence data for boats and whale's matrix was generated pre-covid-19 (2017). Our results included an overall increase in humpback whale song detections in 2020 which was maintained throughout the day. The results of this study provide insights on the impact of boat traffic on humpback whale breeding habitat.

Key words: humpback whale, covid-19, pre-covid, comparison, aribimon

1. Introduction

The Covid-19 pandemic is arguably the most shocking event of modern time. Human beings around the globe have suffered and governments still struggle to recover from serious issues such as food supply shortages and unemployment. Unemployment rate in the US was at 14.7% in April 2020 (Congressional Research Service Report, 2020) and 31.4% by the second quarter (Bureau of Economic Analysis, 2020). In response to these unprecedented circumstances governments from all over the world restricted human activity and encouraged the public to stay home. Reports from all around the world surfaced of animals venturing into cities vacant of human activity. As we start to get back on our feet, calls for global collaborative efforts are emerging to provide a comprehensive understanding and quantification of these effects (Bates et al., 2020).

Strict marine lockdowns the global ocean decreased traffic density by nearly 45.0% (Erbe et al., 2019). The lockdowns have provided scientists with the opportunity to study the impact of underwater noise on marine life (Isaifan 2020). For example, A study published in 2020 used humpback whales as an example stating 31% to 45% are less likely to sing when vessel noise is present in their environment versus only natural sounds (Childs et al., 2021). In this study we look at the impact of a decreased in boat traffic during the lockdowns on the singing activity of humpback whales breeding off the coast of Panama.

Humpback whales are highly vocal animals that produce a variety of social sounds with songs being the most studied and prevalent type of acoustic behavior produced at breeding areas (Dunlop et al., 2007). In these breeding areas, male humpback whales can be detected miles away making them the perfect test subject for this thesis. My hypothesis is that a decreased in boat presence will lead to an increase in humpback whale song detections. Behavioral changes from wildlife in response to newly favorable conditions indicate their inherent resilience to anthropogenic pressures (Derryberry et al. 2020). The aim of this manuscript is to provide insights on the impact of boat traffic on humpback whale breeding habitat.

2. Materials and Methods

2.1 Study site

This study took place in the archipelago of Islas Secas situated on the Gulf of Chiriquí in Panama. The Gulf of Chiriquí is known for its tropical marine climate. In August temperatures average a warm 84°F/29°C. It has a maximum width of 160 miles (250 kilometers) a maximum depth of 720 feet (220 m) being sized at around 930 square miles. drawing in whales from all over the pacific to the ideal breeding conditions. This site is also an important breeding ground for southern hemisphere humpback whales that migrate from the Antarctica Peninsula to Panama every year (Rasmussen et al., 2007). Male humpback whales are very vocal, and in breeding areas are known to sing complex songs to attract females and to compete with other males (Dunlop et al., 2007). In this breeding ground it was found that humpback whales sign mostly during the colder parts of the season yet still produce consistent data, (Cheresking et al. (2019).

2.2. Recordings

A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/μPa) recorder was deployed at 25 m in August 2017 Secas (Lat N, long W) and August 2020 at Pargo (Lat N, Long W). In Secas the recorder was programmed to record the soundscape continuously in a 24 cycle at a sampling rate of 48 kHz. From these recordings a 1-min sample was manually selected from every hour. In Pargo, the recorder was programmed to record the soundscape for 10 minutes every hour also at a sampling of 48 kHz yielding a total of 202 1-min files from (n=61 for 2020, n=152 for 2017). These 1-min were inspected in RFCxArbimon for presence (1) and absence (0) of boats and humpback whale songs. The presence-absence data matrix was used to calculate the mean number of 1-min files with humpback whale song and boat presence pre-covid-19 (2017) and during covid-19 lockdowns (2020). Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, Chi-square to determine if the distribution of whale song detections varied before and during COVID-19 lockdowns.

3. Results

The mean number of 1-min files with humpback whale song detections was significantly higher in 2020 than in 2017 (X^2=75.9, df=1, p<0.0001). In 2017 the mean number of 1-min files with humpback whale songs was 0.24±0.42, while in 2020 was 0.63±0.48 (Fig. 1). Additionally, the proportion of humpback whale songs by time of day shows that while whales were detected throughout the day in both years, the proportion of song detections was greater in 2020 (Fig.2). Interestingly, no significant differences were found in the mean number of 1-min files with boat detections between years.

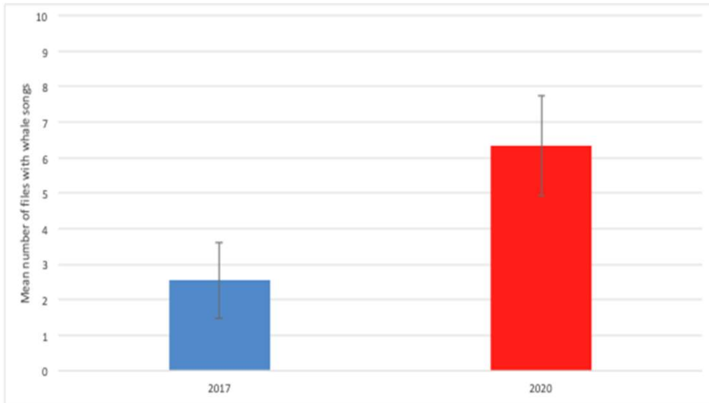


Figure 1. Mean of 1-min recordings with humpback whale sound per hour in the archipelago of Islas Secas, Panama.

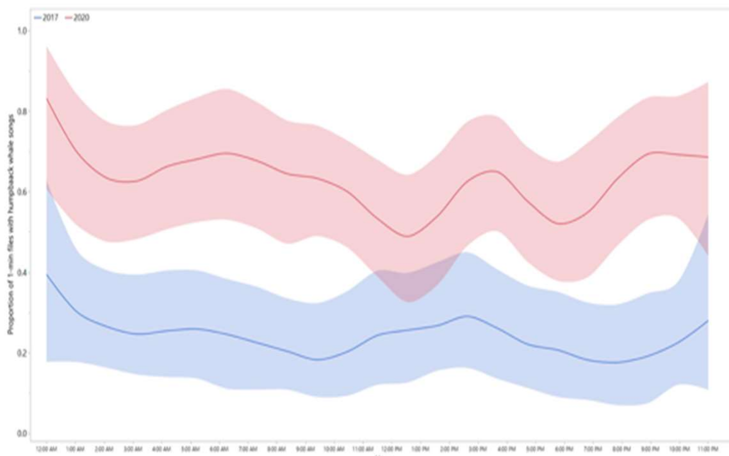


Figure 2. Proportion of 1-min recordings with humpback whale sounds per hour in the archipelago of Islas Secas, Panama.

4. Discussion

Our results support our hypothesis that humpback whale song activity increased during COVID-19 lockdowns in Islas Secas, Panama. We found a doubling of singing activity in 2020. However, that do not seem to be directly related to boat presence, given that boat presence was in general low and similar between both years. The patterns found in humpback whale singing activity is in congruence with reports on other marine animals. Within a few weeks into the pandemic, media worldwide broadcasted news related to higher water quality in watersheds, canals, lakes, bays and harbors (Braga et al. 2020) and CO2 emissions, expected to fall by a maximum of 7−8% in 2020 depending on the duration of the confinement (Le Quéré et al., 2020). This decline is roughly the equivalent of the annual emission reductions needed to limit global warming to 1.5°C above pre-industrial temperatures, in alignment with the Paris Agreements (UN Environmental Programme 2019, Le Quéré et al. 2020). There will be no long-term benefit from these lockdowns, however, this time in history has introduced an inspirational perspective for change.

Future studies based on data collected during the lockdown will provide important quantitative insights into human− wildlife interactions and the specific mechanisms behind them (Rutz et al. 2020). In the future, analyses for this location should consider other months of the reproductive season, and areas that vary in boat density. The pandemic highlights that people lack a system that allows for dynamic calibration between use and abuse of biological diversity. (Cawthorn et al., 2021). This study provides insights on the impact of human presence on humpback whale singing behavior.

References

Acevedo, J., Rasmussen, K., Felix, F. E. R. N. A. N. D. O., Castro, C., Llano, M., Secchi, E., ... & Pastene, L. A. (2007). Migratory destinations of humpback whales from the Magellan Strait feeding ground, Southeast Pacific. Marine Mammal Science, 23(2), 453-463.

Bates AE, Primack RB, Moraga P, Duarte CM (2020) COVID-19 pandemic and associated lockdown as a 'Global Human Confinement Experiment' to investigation

Braga F, Scarpa GM, Brando VE, Manfè G, Zaggia L (2020) COVID-19 lockdown measures reveal human impact on water transparency in the Venice Lagoon. Sci Total Environ 736:139612

Cawthorn, D. M., Kennaugh, A., & Ferreira, S. M. (2021). The future of sustainability in the context of COVID-19. Ambio, 50(4), 812-821.

Childs, J. W. (2021) "Coronavirus Pandemic Brought Quieter Ocean Waters for Humpback Whales, Other Marine Life"

Coll M, Libralato S, Pitcher T, Solidoro C, Tudela S (2013) Sustainability implications of honouring the code of conduct for responsible fisheries. Glob Environ Change 23:157−166

Derryberry, E. P., Phillips, J. N., Derryberry, G. E., Blum, M. J., & Luther, D. (2020). Singing in a silent spring: Birds respond to a half-century soundscape reversion during the COVID-19 shutdown. Science (New York, N.Y.), 370(6516), 575–579.

Dunlop, R. A., Noad, M. J., Cato, D. H., and Stokes, D. (2007). " The social vocalization repertoire of east Australian migrating humpback whales (Megaptera novaeangliae)," J. Acoust. Soc. Am. 122, 2893–2905.

Erbe, C and Marley, SA (2019) " The Effects of Ship Noise on Marine Mammals - A Review" FRONTIERS MEDIA SA, AVENUE DU TRIBUNAL FEDERAL 34, LAUSANNE, CH-1015, SWITZERLAND

Le Quéré C, Jackson RB, Jones MW, Smith AJ and others (2020) Temporary reduction in daily global CO2 emissions during the COVID-19 forced confinement. Nat Clim Chang 10:647−653

Manenti, R.; Mori, E.; Di Canio, V.; Mercurio, S.; Picone, M.; Caffi, M.; Brambilla, M.; Ficetola, G.F.; Rubolini, D. The good, the bad and the ugly of

McNeely, J. A. (2021). Nature and COVID-19: The pandemic, the environment, and the way ahead. Ambio, 1-15.

Rutz C, Loretto MC, Bates AE, Davidson SC and others (2020) COVID-19 lockdown allows researchers to quantify the effects of human activity on wildlife. Nat Ecol Evol 4:1156–1159

Sala, O.E., F.S. Chapin, J.J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L.F. Huenneke, et al. 2000. Science 287: 1770–1774.

UN (2019) The Sustainable Development Goals Report. United Nations publication issued by the Department of Economic and Social Affairs (DESA), New York. http:// unstats.un.org/sdgs/

How does Covid-19 lockdown impact boating and Humpback whale (*Megaptera novaeangliae*) singing activity?

Grace Durant
Department of Biology, University of Vermont, Marsh Life Science, 109 Carrigan Drive, Burlington, Vermont 05405, USA

ABSTRACT

The Wildlife Refuge of Caño Island in Costa Rica is an important breeding ground for southern Humpback whales (Megaptera novaeangliae). The predictable occurrence of these whales has stimulated the growth of boat-based whale watching in this area. Male humpback whales sing complex songs to attract mates and compete with other males. Previous studies have found a potential negative relationship between boat occurrence and whale signing activity. However, such relationships are difficult to test without adequate controls. The Covid-19 lockdowns provide a rare opportunity to test this observation. We used autonomous underwater recorders to study boat and whale singing activity. We generated presence-absence data for whales and boats from September 2019 and 2020. Our results show that boat presence decreased, and whale song detection increase during 2020. The results provide a unique glimpse at the effects of boat activity on male humpback whales singing activity and can lead to policy that regulate more effectively tour-boats in their breeding area.

1. Introduction

Humpback whales (*Megaptera novaeangliae*) produce a variety of sounds, with songs being the most common. Humpback singing has been recorded largely in coincidence with breeding behaviors by males of the species (Herman et al. 2013). The duration and frequency of Humpback whale songs can be an indicator of the health of both the whale and its ecosystem (Ryan et.al. 2019). However, the oceans have become noisier than ever before due to increasing human shipping activity and the growing tourism industry, and testing and expanding naval fleets for exploration and research (Peng et al. 2015). For the Humpback whales in feeding and breeding grounds, human noise levels and increased boat traffic have negative consequences. Firstly, in the presence of increased boat activity, Humpback whales seem to practice avoidance behavior, indicating that boats are a disturbance to normal activity (Amrein et.al. 2020). Secondly, a correlation between increased boat sounds and a decrease in frequency of whale song (Clark & Sousa-Lima 2008) but an increase in intensity of vocals has been found (Guazzo et.al. 2020).

While anthropogenic noise in the ocean has increased over the past decade, in the last year the noise levels declined during the Covid-19 pandemic. This is likely due to decreased boat traffic related to decreased economic activity (Barclay & Thomson 2020). Additionally, boat noise from tourism also significantly declined. An area popular for tourism of Humpback Whales can be found near Jardin, Isla del Caño, Costa Rica. Isla del Caño is a biological reserve frequented by Humpbacks (Bahaía Aventuras 2020) . It is also a site of snorkeling and scuba diving adventures, which contribute to anthropogenic noise pollution in the water. During the Covid-19 pandemic, tours were restricted, leading to a decrease in boat noise disturbance in the waters of Jardin. Given this information we hypothesized that with decreased anthropogenic noise, there will be an increase in Humpback whale vocalization frequency in 2020 compared to 2019 in Jardin, Isla del Caño, Costa Rica. This research can be used to determine the health of the ecosystem in

Jardin, Isla del Caño, Costa Rica based on the change in vocalization pattern in Humpback whales. It can also be used to study Humpback songs and sounds with less anthropogenic noise interruption. Finally, the data collected from this project could be used to inform tourism guidelines around whale watches in and around Isla del Caño.

2. Materials and Methods

2.1 Study Site

The study took place in the protected marine area of the Isla del Caño, Wildlife Refuge in Costa Rica (8.719N/-83.863W). This site is 0.8 km away from the mainland and is an important tourist destination. Several tourism companies visit the refuge daily for snorkeling, scuba diving, and whale watching. The area is also an important breeding area for southern hemisphere Humpbacks that migrate every year from the Antarctica Peninsula (Rasmussen et al. 2007). In this breeding ground, male humpback whales singing activity is nearly constant; however, most singing activity is at night (Chereskin et al. 2019).

2.2 Recordings

A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) recorder was deployed at 25 m in September 2019 and September 2020 at Jardin a location with a sandy ocean floor . The recordings were taken at a sampling rate of 48kHz and 16bit. In 2019, the recorder was programmed to record for 5 minutes every 30 minute throughout the day and in 2020 for 15 minutes every hour. These recordings were then uploaded to the Arbimon RFCx online platform for remote access. Using Arbimon RFCx analytical tools, a spectrogram was generated for manual inspection of presence (1) and absence (0) of whales' songs and boats for each minute.

2.3 Analysis

The proportion of humpback whale song and boat presence pre-covid-19 (2019) and during covid-19 lockdowns (2020) was calculated by dividing the number of 1-min files with songs by the total number of 1-min files per day for each year. To determine if singing activity increased and boat presence decreased before and during Covid-19 lockdowns, JMP statistical software was used. Mean number of boats and mean number of whales present each day was determined, as well as mean number of boats and whales at each hour for September of 2019 and 2020. Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, a Chi-square to determine if the distribution of whale and boat detections vary by year.

3. Results

Approximately 124 hours were analyzed in September 2019 and about 186 hours in September 2020 for the presence or absence of boats and whales. The presence of boats in September 2019 was significantly greater (X^2=28.8; df=2; p<0.0001) than in 2020 (Fig.1.), and the presence of Humpback whale song throughout the day in September 2020 was significantly greater (X^2=59.5; df=2; p<0.0001) than in 2019 (Fig.2.). Boats were most frequently present between 7 a.m. and 5 p.m., particularly in 2019 (Fig.2). In 2019, Humpback whale songs were least frequently detected during the hours of 5 a.m. to 6 p.m., correlating to a greater presence of boat noise during those hours (Fig.3). In 2020, the presence of Humpback whale song was mostly uniform and constant throughout the day, independent of time.

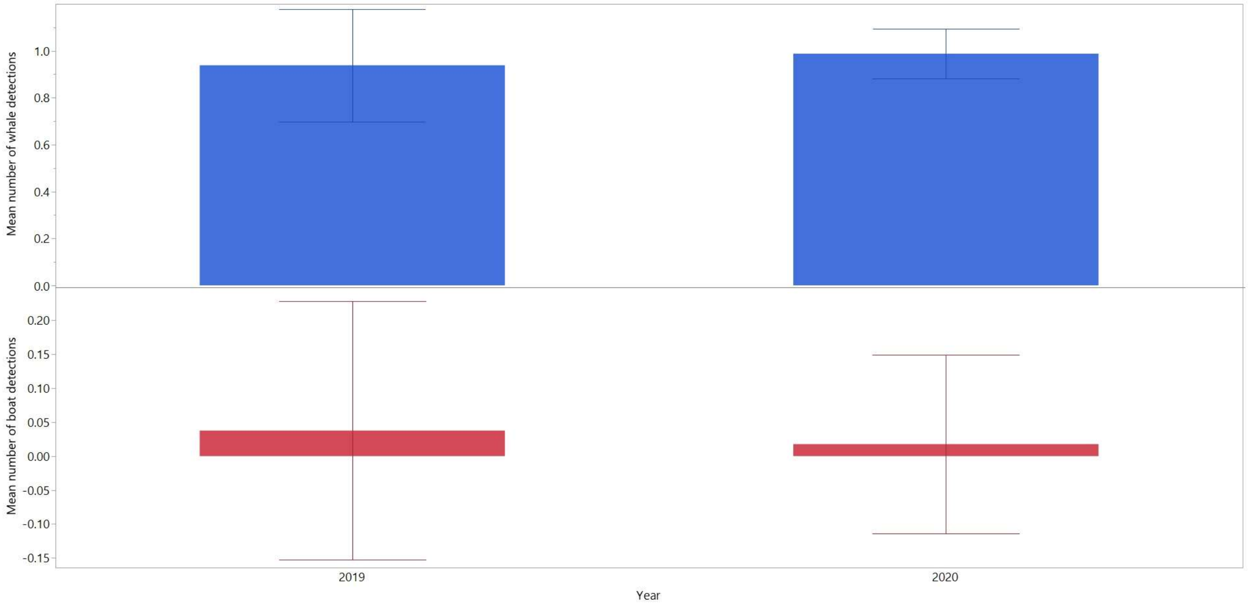


Fig. 1. Overall mean number of whale and boat detections (left: 2019, right: 2020, top: whales, bottom: boats)

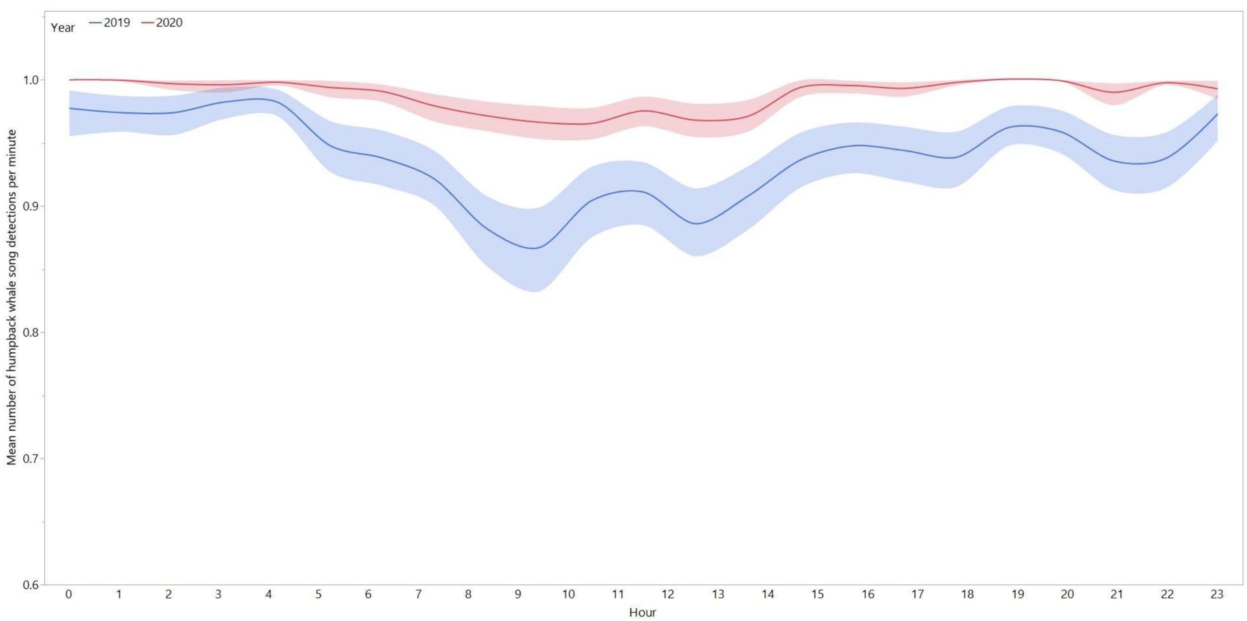


Fig. 2. Mean boat presence throughout the day (red: Sept. 2019, blue: Sept 2020)

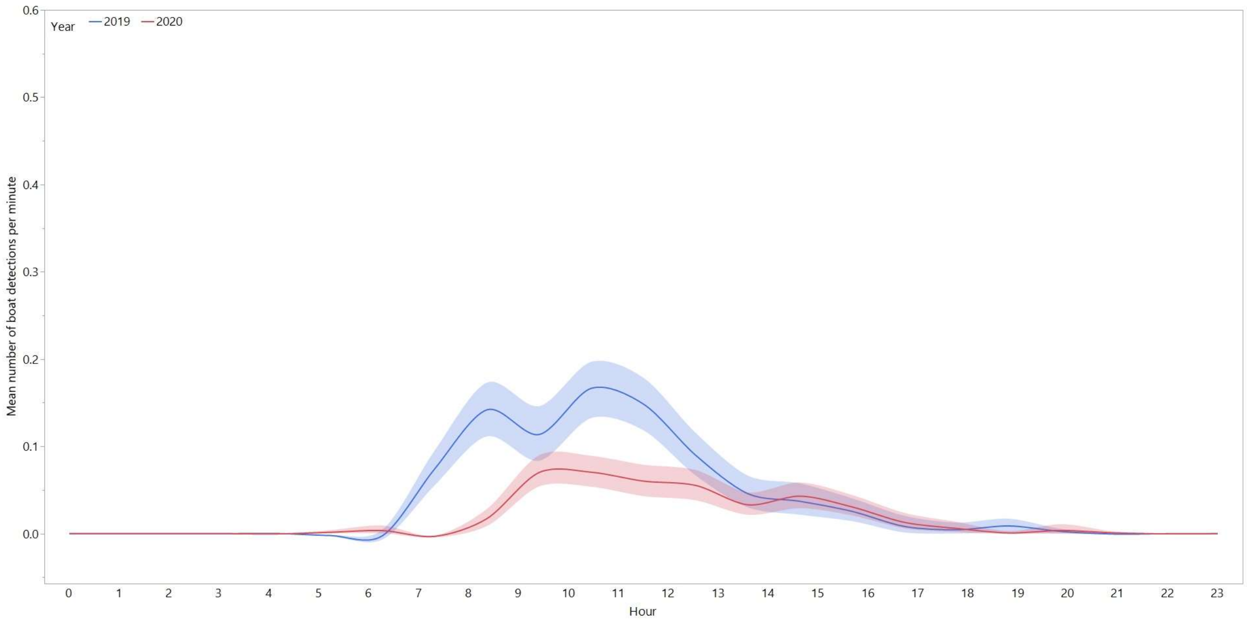


Fig. 3. Mean Humpback whale song presence throughout the day (red: Sept. 2020, blue: Sept. 2019)

4. Discussion

The purpose of this study was to determine the impact of anthropogenic noise on humpback whale vocalization frequency. During the unique time period of less frequent boat presence off Caño Island, Costa Rica in 2020 due to the Covid-19 pandemic, the difference in whale song presence was analyzed between September 2019 and September 2020. Humpback whale song is an important breeding behavior in males and is therefore necessary in a breeding ground such as that of Caño Island (Herman et.al. 2013). The study found that during periods of the day with high boat presence, there was lower frequency of humpback whale songs detected, which indicates human noise has a negative impact on Humpback whale communication. During the period in 2019 when boats were most frequently present (7 a.m. to 5 p.m.), whales sang the least and sang the most at night. However, with restrictions on tourism and fewer boats, whales sang consistently throughout the day in September 2020. Only at 3 a.m. was there an overlap between mean whale song presence between 2019 and 2020; otherwise, average whale song presence was greater in 2020 than in 2019 for every other time. This is concurrent with other studies showing that Humpback whales sing less or not at all when anthropogenic noise is present near (within 200 km or less) Humpbacks (Risch et.al. 2012; Rey-Baquero et.al. 2021). The presence of boats in Jardin is a clear disruption of Humpback whale song and could therefore harm the breeding activity of these marine mammals.

This research is significant for both understanding the natural acoustic behavior of Humpback whales in Jardin, Caño Island, Costa Rica and for informing boating policy. Anthropogenic noise has an impact on whales, and unregulated whale watching is a documented problem in Costa Rica, leading to negative effects on whale populations (Collado et.al. 2017). Stricter policies and greater water patrol may be required to protect the natural soundscape of Caño Island, so it remains a breeding ground for Humpback whales. Further studies may be needed to visually analyze the change in sound structure of whales in the presence of anthropogenic noise during this time in Jardin. Additionally, data could be analyzed from more months throughout 2019 and

2020 to get a better understanding of the relationship between boat noise and whale song during the Covid-19 pandemic.

References

Amrein, A. M., Guzman, H. M., Surrey, K. C., Polidoro, B., & Gerber, L. R. (2020). "Impacts of Whale Watching on the Behavior of Humpback Whales (Megaptera novaeangliae) in the Coast of Panama", *Frontiers of Marine Science, 7*. https://doi.org/10.3389/fmars.2020.601277

Avila, I.C., Correa, L. M., Parsons, E. C. M. (2015) "Whale-watching activity in Bahía Málaga, on the Pacific Coast of Colombia and its effect on Humpback whale (*Megaptera Novaeangliae)* behavior". *Tourism in Marine Environments 11(1)* 19-32(14). https://doi.org/10.3727/154427315X14398263718394

Bahía Aventuras. (2020). *Bahía Aventuras.* http://www.bahiaaventuras.com/national-parks/cano-island/

Guazzo R.A., Helble T.A., Alongi G.C., Durbach I.N., Martin C.R., Martin S.W., Henderson E.E. (2020). "The Lombard effect in singing humpback whales: Source levels increase as ambient ocean noise levels increase", *J Acoust Soc Am. 48(2*) 542. doi: 10.1121/10.0001669

Herman, Louis M., Pack, Adam A., Spitz, Scott S., Herman, Elia K.Y., Rose, Kathryn, Hakala, Siri, & Deakos, Mark H.. (2013). "Humpback whale song: who sings?", *Behav Ecol Sociobiol 67*, 1653–1663. https://doi.org/10.1007/s00265-013-1576-8

May-Collado, L.J., Amador-Caballero, M, Casas, J.J.,Gamboa-Poveda, M.P.,Garita-Alpízar, F., Gerrodette, T., González-Barrientos, R., Hernández-Mora, G., Palacios, D.M., Palacios-Alfaro, J.D, Pérez, B., Rasmussen, K., Trejos-Lasso, L., Rodríguez-Fonseca, J. (2018) "Ecology and Conservation of Cetaceans of Costa Rica and Panama". *Advances in Marine Vertebrate Research in Latin America. Coastal Research Library, vol 22.* https://doi.org/10.1007/978-3-319-56985-7_12

Peng, C., Zhao, X., & Liu, G. (2015). "Noise in the Sea and Its Impacts on Marine Organisms", *International journal of environmental research and public health, 12*(10), 12304–12323.https://doi.org/10.3390/ijerph121012304

Ryan, John P., Cline, Danelle E., Joseph John E., Margolina, Tetyana, Santora, Jarrod A., Kudela, Raphael M., et al. (2019). "Humpback whale song occurrence reflects ecosystem variability in feeding and migratory habitat of the northeast Pacific", *PLoS ONE 14*(9): e0222456. https://doi.org/10.1371/journal.pone.0222456

Rasmussen, K., Palacios, D. M., Calambokidis, J., Saborio, M. T., Dalia Rosa, L., Secchi, E. R., Steiger, G. H., Allen, J. M., and Stone, G. S. (2007). "Southern hemisphere humpback whales wintering off Central America: Insights from water temperature into the longest mammalian migration," *Bio. Lett. 3*, 302–305. https://doi.org/10.1098/rsbl.2007.0067

Rey-Baquero MP, Huertas-Amaya LV, Seger KD, Botero-Acosta N, Luna-Acosta A, Perazio CE, Boyle JK, Rosenthal S and Vallejo AC (2021) "Understanding Effects of Whale-Watching Vessel Noise on Humpback Whale Song in the North Pacific Coast of Colombia With Propagation Models of Masking and Acoustic Data Observations". *Front. Mar. Sci. 8:623724*. https://doi: 10.3389/fmars.2021.623724

Risch D, Corkeron PJ, Ellison WT, Van Parijs SM (2012) "Changes in Humpback Whale Song Occurrence in Response to an Acoustic Source 200 km Away" *PLoS ONE 7(1): e29741*. https://doi.org/10.1371/journal.pone.0029741

Sousa-Lima, R. S., & Clark, C. W. (2008). "Modeling the effect of boat traffic on the fluctuation of humpback whale singing activity in the Abrolhos National Marine Park, Brazil", *Canadian Acoustics, 36*(1), 174-181. Retrieved from https://jcaa.caa-aca.ca/index.php/jcaa/article/view/2008

Thomson, Dugald J.M. & Barclay, David R. (2020). "Real-time observations of the impact of COVID-19 on underwater noise". *The Journal of the Acoustical Society of America 147*(5). https://doi.org/10.1121/10.0001271

Panama bottlenose dolphin (*Tursiops truncatus*) whistles indicate less stress during COVID-19 pandemic.

Emma Shapera and Logan Hillger
University of Vermont, Department of Biology, Burlington, VT, 05405, USA

ABSTRACT
A sub-population of bottlenose dolphins in the archipelago of Bocas del Toro is regularly exposed to tour-boat traffic. A recent study characterized their habitat as 'stressful' due to the high number of tour-boat/dolphin interactions and lack of compliance of dolphin-watching regulations. An increase in whistle frequency modulation is considered an indicator of stress in the Bottlenose Dolphin, thus we test the hypothesis that during COVID-19 lockdowns, dolphin whistle modulation should decrease. We extracted dolphin whistles from passive acoustic monitoring data collected before and during the lockdowns, to test this hypothesis. We found that boat presence did not vary before and after COVID-19 lockdowns, rather the change was the type of boat traffic. In 2020, no tour-boats were present. As result, we observed a decrease in dolphin whistle modulation, supporting our hypothesis. Our study provides key information on dolphin communication and results can be translated into mitigation strategies to reduce the impact of tour-boats on Dolphin Bay's dolphins.

Key words: signature, variant, modulation, frequency, boat traffic

1. Introduction

Dolphin Bay, at the archipelago of Bocas del Toro sustains one of the largest dolphin watching industry of Panama. Here dolphins interact with tour boats on daily basis. These interactions are often intense due to boat-captains not following whale-watching regulations (May-Collado et al., 2017). Previous research has found that during these encounters, key biological behaviors of dolphins such as foraging and social activities are interrupted, and the animals are unlikely to return to these activities after the interruption (Kassamali-Fox et al., 2020). Betzi et al. (2021) argued that such interactions make Dolphin Bay a stressful habitat for these dolphins.

Dolphins produce signature whistles that encode individual information (Caldwell et al., 1992) and dolphin use these signals to maintain contact among themselves when separated. In captivity, when bottlenose dolphin mothers and calves are separated during physical examinations, researchers found that their whistles showed an increased in modulation (Esch et al. 2009). Betzi et al., (2020) found the same pattern, in Dolphin Bay when dolphins were interacted with tour-boats they produced highly modulated whistles than when they were recorded only with the research boat or in other locations of the archipelago where boat traffic is primarily for transport.

Due to the COVID-19 virus outbreak, boat traffic decreased globally during the lockdown (March et al., 2020). In Panama, the government shut down tourism from the end of March 2020 to January 2021. This setting provides a unique opportunity to test the hypothesis that whistle modulation is an indicator of stress in this dolphin population. We expected that during the COVID-19 lockdowns whistle modulation will decrease given the absence of tour boats.

2. Materials and methods

2.1 Study Site

This study took place in Dolphin Bay located in the archipelago of Bocas del Toro, Panama. is This semi-closed bay is about 6.3x3.1 km (Kassamali-Fox et al., 2020). The bay is home to a resident population of bottlenose dolphins (*Tursiops truncatus)*. These dolphins belong to the "inshore" ecotype and has an effective population of about 73 individuals as of 2021 (Barragan-Barrera et al. 2017). The Bay is regularly visited by various tour-boat companies, from 9 a.m. to 3 p.m. Although boats are expected to maintain a distance of 100 m from the dolphins and to reduce overcrowding (Resolution N° Dm-0530-2017, 2017) it is common to find up to 40 boats following the same group of dolphins at the same time (May-Collado et al. 2017).

2.2 Recordings

Recordings of dolphins and boats were made using the µRUDAR-mK2 autonomous recorders (−169 dB re:1 V/µPa, 1–96 kHz) in 2018 and a Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) in July and from September to November 2020. The µRUDAR was scheduled to, simultaneously and continuously, record the acoustic environment in a 24-h cycle and Soundtrap was scheduled to record for 10 minutes every hour at a sampling rate. Both recorders recorded at a sampling rate of 48 kHz and 24 bits.

Acoustic recordings were opened in RAVEN PRO 1.5 build 37 (Center for Conservation Bioacoustics, 2014) and a spectrogram was opened with a fast Fourier transform (FFT) size of 1,024 points, an overlap of 50%, and using a 1,024-sample Hann window. It is important to note that many of the dolphin whistles found are very light, and sometimes broken up on the spectrogram. Only dolphin whistles that were dark on the spectrogram and had a clear structure and connection were used. Once dolphin whistles were found we proceeded to classify dolphin whistles into signature and variants using the Signature Identification (SIGID) method, which classifies signature whistles as groups of whistles within 10 seconds of one another that have the same contour (Janik et al., 2013) and to extract standard acoustic measurement for each whistle as described by Perez et al. (2021): Low frequency (LF) (measures the frequency in the lowest point in the contour), high frequency (HF) (measures the frequency at the highest point in the contour), duration (D), delta frequency (DF) (this is the difference between HF and LF), center frequency (CF) (represents the midpoint frequency between the lower and upper cutoff frequencies), peak frequency (PF) (frequency where the maximum amplitude occurred), and peak frequency contour number of inflection points (PFC Num Inf Pts) (measures the number of times the slope changes sign in peak frequency contour slope).

2.3 Analysis

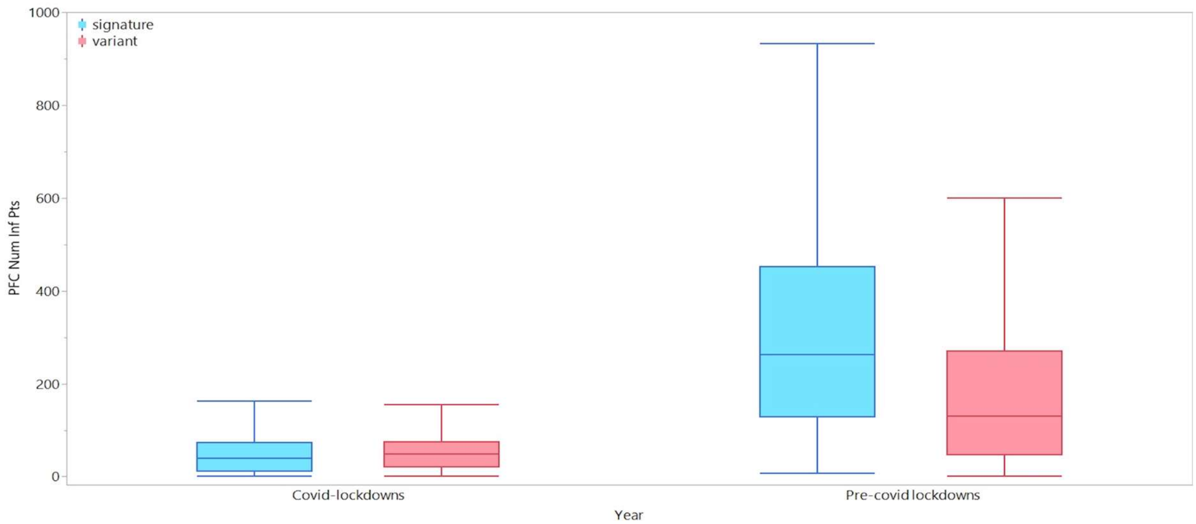
Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a Fit Least Square analysis to test the effect of year (covid-lockdowns) and type of whistle for modulation measured as PCF Num Inf Pts and Delta Frequency.

3. Results

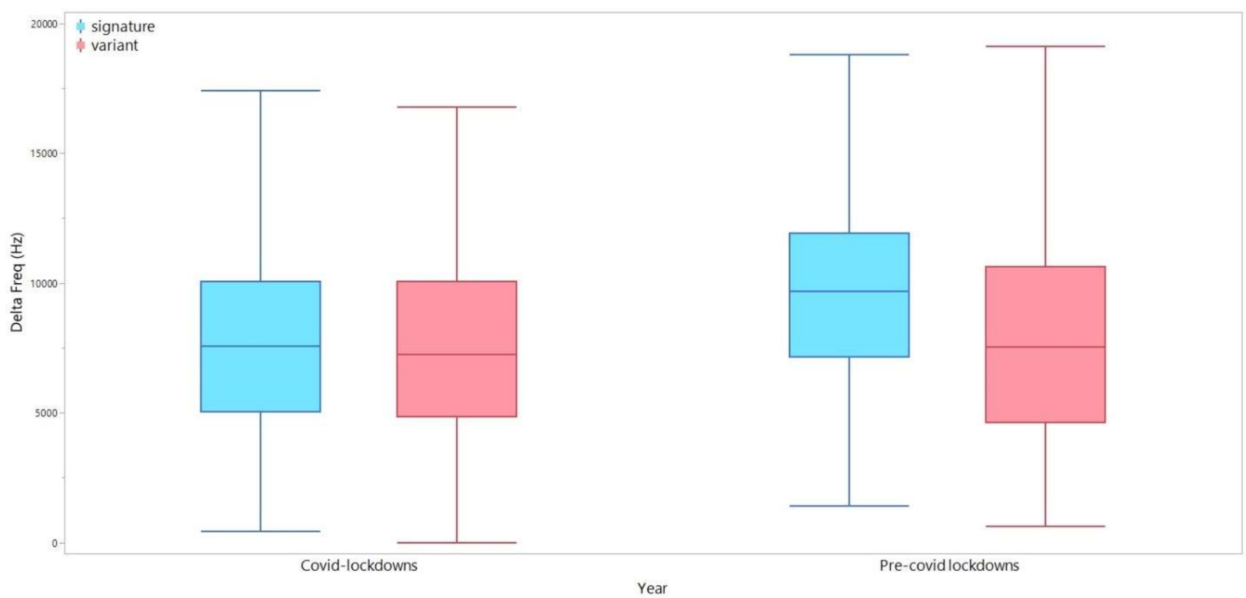
A total of 2,653 dolphin whistles were analyzed (n= 1755 before COVID-19, and n= 898 during Covid-19). Table 1 summarizes the acoustic structure of signature and variant whistles before and during lockdowns. (Table 1). We found that dolphin whistle modulation for signature and variant whistles did significantly decreased during Covid-19 lockdowns (PCF Num Inf Pts-F Ratio=71.4, df=1, p<0.0001, Fig.1a; Delta Frequency-F Ratio=29.9, df=1, p<0.0001; Fig.1b). Boat presence decreased in 2020, and there was a shift in the type of boat activity within the bay from tour-boats to transport boats (F Ratio=62, df=2, p<0.0001, Fig. 2).

Table 1. Summary descriptive statistics for all acoustic characteristics of signature and variant whistles pre and during covid-19 lockdowns (mean±SD**)**.

	Low Freq (Hz)	**High Freq (Hz)**	**Center Freq (Hz)**	**Delta Freq (Hz)**	**Delta Time (s)**	**Peak Freq (Hz)**	**PFC Num Inf Pts**
Covid Overall	6326.2 ±2295.6	13861.9± 3426.5	9803.2± 1917.0	7535.7± 3594.8	1.0± 0.6	9330.4± 2559.2	51.8± 42.1
Signature	6217.8 ± 2046.2	13838.1± 3409.6	9733.6± 1761.7	7620.4± 3546.6	1.0± 0.7	9251.9± 2339.4	49.5± 44.0
Variant	6394.8 ± 2451.4	13884.0± 3443.3	9850.0± 2024.1	7489.1± 3619.9	0.9± 0.6	9382.0± 2705.8	53.5± 40.6
Pre-covid overall	5355.4 ± 2030.0	13684.8± 3793.6	9258.6± 2123.8	8329.4± 4079.4	1.0± 0.7	9198.6± 2858.1	233.5± 211.4
Signature	4973.7 ± 1865.6	14702.8± 3793.6	9441.2± 1726.3	9729.1± 3757.6	1.3± 0.6	9313.5± 2703.3	327.1± 256.3
Variant	5504.7 ± 2072.4	13286.8± 3888.3	9187.2± 2256.8	7782.2± 4071.0	0.8± 0.7	9153.6± 2916.1	196.9± 211.4



a. PFC Number of Inflection points



b. Delta frequency

Figure 1. Boxplots of PFC Number of Inflection points and delta frequency as indicators of dolphin whistle modulation by whistle type before and during Covid-19 lockdowns.

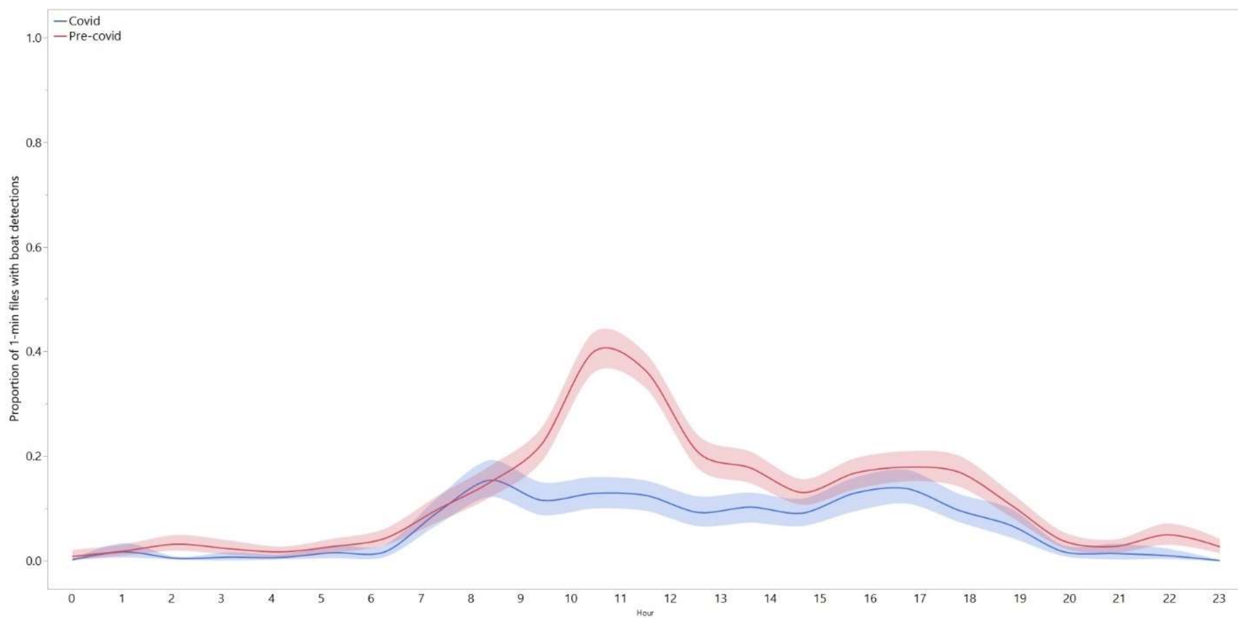


Figure 2. Proportion of tour-boats by time of day in Dolphin Bay before and during Covid-19 lockdowns.

4. Discussion

Our results show, that during the COVID-19 lockdowns dolphins in Dolphin Bay showed significantly decrease in the modulation of both signature and variant whistles, suggesting a decline in stress levels. As proposed by Esch et al. (2019) and supported by Perez-Ortega et al. (2021) less modulation in the context of separation, indicates less stress, which is important for the overall health and lifestyle of the dolphins because the whistles are largely indicative of the needs and activities of the dolphins. The patterns were particularly evident in the signature whistles, which are essential for individual identification and group cohesion (Janik and Slater, 1998; Janik 2009).

The results also mean that limiting the number of boats and in situ regulation of dolphin-watching norm of conduct will significantly improve the quality of life of these dolphins. This is particularly important for population growth, as Dolphin Bay is a nursery ground minimizing the separation of mom and their calves and reducing the interruption of key behaviors is key in calf survival and female reproductive success (Kassamali-Fox, et al. 2019, Smolker et al., 1993). Without regulation, the short-term behavioral changes in i.e., habitat use, energy budgets, can lead to long-term cumulative effects on this dolphin population (Lusseau 2004, Williams et al. 2006, Christiansen et al. 2013, Symons et al. 2014).

The results of these study can be translated in easily implemented solutions. If boat captains behave more like transport boat captains, by minimizing the interaction with dolphins in numbers and time, this can lead to a reduction in stress levels. Such implementations must happen before tourism is back to normal.

References

Anderson, M.J. (2001). “A new method for non-parametric multivariate analysis of variance”. Austral Ecology 26, 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Barragan-Barrera, D.C., May-Collado, L.J., Tezanos-Pinto, G., Islas-Villanueva, V., Correa-Cardenas, C.A., Caballero, S. (2017). “High genetic structure and low mitochondrial diversity in bottlenose dolphins of the Archipelago of Bocas del Toro, Panama: a population at risk?”. PLOS ONE. https://doi.org/10.1371/journal.pone.0189370

Caldwell, M.C., Caldwell, D.K., Tyack, P.L. (1990). “10 – Review of the signature-whistle hypothesis for the Atlantic Bottlenose Dolphin”. The Bottlenose Dolphin, 199-234. https://doi.org/10.1016/B978-0-12-440280-5.50014-7

Center for Conservation Bioacoustics (2014). “Raven Pro: Interactive sound analysis software (Version 1.5)”. Cornell Lab of Ornithology.

Esch, H.C., Sayigh, L.S., Blum, J.E., Wells, R.S. (2009). “Whistles as potential indicators of stress in the Bottlenose Dolphin (*Tursiops truncatus*)”. Journal of Mammalogy 90, 638-650. https://doi.org/10.1644/08-MAMM-A-069R.1

Janik, V. (2009). “Acoustic communication in Delphinids”. Elsevier 40, 123-157. https://doi.org/10.1016/S0065-3454(09)40004-4

Janik, V.M, Slater Peter J.B, (2002) “Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls” Elsevier 56, 829-838. https://doi.org/10.1006/anbe.1998.0881

Janik, V.M, King, S.L., Sayigh, L.S., Wells, R.S. (2013). “Identifying signature whistles from recordings of groups of unrestrained bottlenose dolphins (*Tursiops truncates*)”. Marine Mammal Science 29, 109-122. https://doi.org/10.1111/j.1748-7692.2011.00549.x

Kassamali-Fox, A., Christiansen, F., May-Collado, L.J., Ramos, E.A., Kaplin, B.A. (2020). “Tour boats affect the activity patterns of bottlenose dolphins (*Tursiops truncates*) in Bocas del Toro, Panama”. PeerJ. 10.7717/peerj.8804

Lusseau, D., Christiansen, F., Williams, R., (2016). “Meta-analyses of whale-watching impact studies: comparisons of cetacean responses to disturbance” MEPS 545: 251-263 http://doi.org/10.3354/meps11497

Mann, H.B., Whitney, D.R. (1947). “On a test of whether one of two random variables is stochastically larger than the other” The Annals of Mathematical Statistics 18. https://www.jstor.org/stable/i312749

March, D., Metcalfe, K., Tintore, J., Godley, B.J. (2020). "Tracking the global reduction of marine traffic during the COVID-19 pandemic". ResearchGate. https://doi.org/10.21203/rs.3.rs-47243/v1

May-Collado, L.J., Wartzok, D. (2008). "A comparison of Bottlenose Dolphin Whistles in the Atlantic Ocean: factors promoting whistle variation". Journal of Mammalogy 89, 1229-1240. https://doi.org/10.1644/07-MAMM-A-310.1

Perez-Ortega, B., Daw, R., Paradee, B., Gimbrere, E., May-Collado, L.J. (2021). "Dolphin-watching boats affect whistle frequency modulation in Bottlenose Dolphins". Frontiers in Marine Science 8, 618420. https://doi.org/10.3389/fmars.2021.618420.

Smolker, R.A., Mann, J., Smuts, B.B. (1993) "Use of signature whistles during separations and reunions by wild bottlenose dolphin mothers and infants." Behav Ecol Sociobiol 33, 393-402. https://doi.org/10.1007/BF00170254

Ambient noise levels as an indicator of marine community health: a comparison of protected vs non-protected Areas

Meghan Murphy
Department of Biology, University of Vermont

ABSTRACT
Noise generated by healthy marine communities appear to influence larval settlement and adult habitat selection in fish, lobsters, and several species of crustaceans. This relationship between natural noise and habitat healthiness provides a straightforward approach to evaluate Marine Protected Areas success in preserving biodiversity. Here, we used an acoustic network of underwater recorders deployed in Costa Rica and Panama between 2019 and 2020 inside marine protected and non-protected areas. We used RFCxARBIMON soundscape tools to calculate the distribution of acoustic events by time of day and frequency bin and use the program dBWav to calculate nose levels (dB Root-Mean-Square). Our results found that biological noise levels were greater outside protected areas, raising concerns about their success in preserving marine biodiversity. This study provides the first assessment of this nature for the area and can have important implications in mitigating human activities in MPAs.

Key Words: Marine Protected Areas, soundscape, noise levels, community health

1. Introduction

Sound is utilized by many different animals across a variety of taxa for communication and navigation (Slabbekoorn and Bouton, 2008). The sum of biological, geological, and anthropogenic sounds is what is referred to as the soundscape of a habitat (Pijanowski *et al.,* 2011). Studying the soundscape can provide an efficient method for determining the species composition and biodiversity of a specific region (Suer and Farina, 2015; Radford *et al.,* 2010). This is especially true for regions that are difficult to access, such as marine ecosystems (Pieretti *et al.,* 2017; Harris *et al.,* 2015). Assessing the soundscapes of marine ecosystems can also provide insight into the overall health of that region by providing information about habitat quality through the presence of specific indicator species (Butler *et al.,* 2017), assessing human impact on a region (Coquereau *et al.,* 2017; Butler *et al.,* 2016), and providing insight into species composition and interactions (Slabbekoorn and Bouton, 2008). Therefore, the soundscape of a region should be considered an indicator for specific conservation measures (Dumyahn and Pijanowski, 2011).

Analyzing a marine soundscape could therefore be beneficial in determining the effectiveness of marine protected areas by comparing the health of marine communities in protected areas to non-protected areas. A higher species composition would indicate greater biodiversity and a healthier marine community. In addition, this comparison could provide a replicable framework for utilizing soundscapes to assess community health in other areas of the world by utilizing soundscapes. Assessing biodiversity and community health through a soundscape analysis could promote necessary conservation action and mitigation of human activity in regions that would otherwise be difficult to assess. This study aimed to analyze the success of Marine Protected Areas (MPA) in Panama in preserving biodiversity by comparing noise levels to those of a non-protected area in Panama. We hypothesize that greater levels of acoustic activity and noise levels will be found in the MPAs if they are adequately preserving biodiversity.

2. Materials and Methods

2.1 *Study Site*

This study took place in protected and non-protected areas in Panama. These included the non-protected archipelago Islas Secas and the protected area of Coiba National Park. Marias and Pargo are two islands in the Islas Secas archipelago, each with an environment consisting mostly of sandy sea floors. Islas Secas hosts several hundred species of fish including whale sharks, giant mantas, and marlin, and attracts various marine mammal species including bottlenose dolphins and humpback whales (Islas Secas, n.d.). Playa Blanca and Granito de Oro of the Coiba Islands host a sandy sea floor environment and attracts a diversity of marine life including several species of sharks, sea turtles, humpback whales, dolphins, and orcas (Panama Vacations, n.d.).

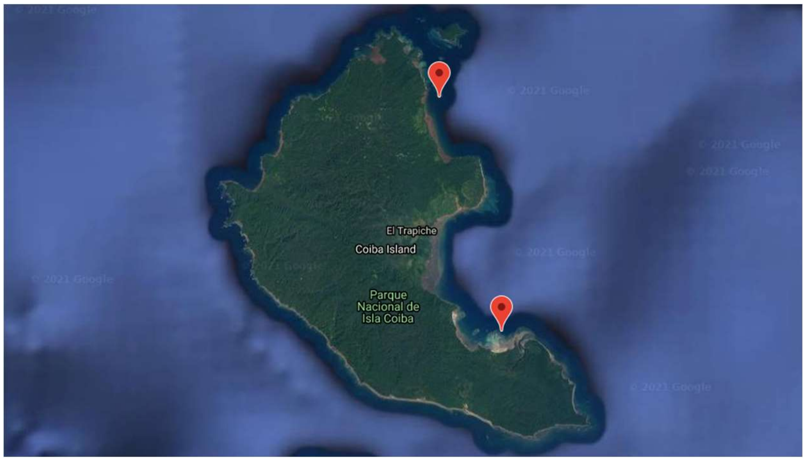


Figure 1. Deployment sites in the Coiba Islands, including Granito de Oro (top pin) and Playa Blanca (lower pin).

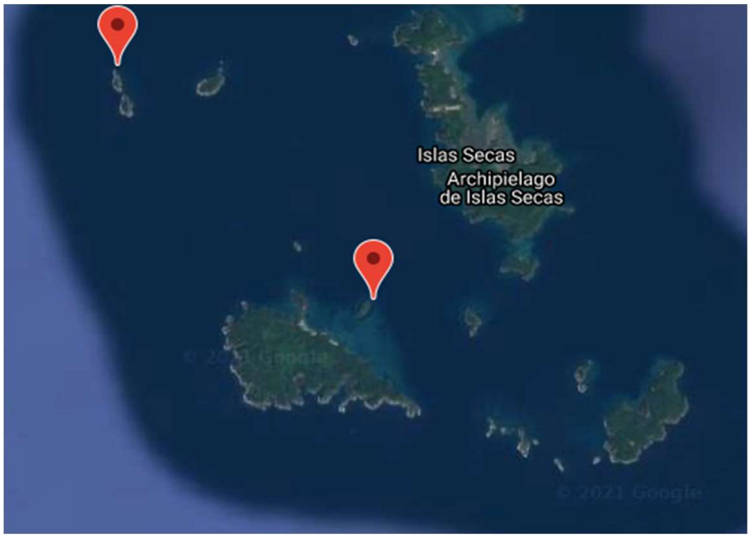


Figure 2. Deployment sites in Islas Secas, including Las Marias (top pin) and Pargo (lower pin).

2.2 *Recordings*
A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) recorder was deployed in each site at about 25 m depth in 2020 to record the soundscape at a sampling rate of 48 kHz. Table 1 shows the locations, selected dates for analysis and recording cycle. These recordings were then uploaded to the Arbimon RFCx online platform for remote access. Using Arbimon RFCx analytical tools, a playlist of 1-min files were generated for each site and used to generate a soundscape analysis by calculating the proportion of acoustic events by frequency, using a frequency bin of 86 Hz, and time of day. These files were then calibrated in dBWav (dBWave, 2014) to estimate noise levels.

Table 1: Coordinates, dates, and locations of Soundtrap deployments.

Location	MPA	Lat/Lon	Selected dates	Substrate	Recording cycle
Coiba-Granito	Yes	7.592, -81.713	Oct-20, 25, 30	Sandy bottom	10 minutes every hour
Coiba-Playa Blanca	Yes	7.403, -81.662	Sept-15, 20, 25, 30	Sandy bottom	10 minutes every hour
Isla Secas-Marias (2020)	No	7.993, -82.071	Oct-20, 25, 30	Mostly Sandy bottom	10 minutes every hour
Islas Secas-Pargo (2020)	No	7.964, -82.042	Sept-15, 20, 25, 30	Mostly Sandy bottom	10 minutes every hour

2.3 *Analysis*
Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, a Kruskal-Wallis Test to determine there are differences in noise levels and acoustic activity between MPA and non-protected sites.

3. Results

3.1 *Noise levels*
Noise levels were significantly higher in the non-protected sites than the MPA sites (F-Ratio= 314, df=3, $p<0.0001$ (Fig.3). These differences are due to level of protection (F-Ratio= 420, df=1, $p<0.0001$) and variation in of noise levels by time of day (F-Ratio= 4.40, df=23, $p<0.0001$), and the interaction between these factors (F-Ratio= 4.34, df=23, $p<0.0001$) (Fig. 4). MPAs and non-protected areas also varied in frequency bands where noise levels (F-Ratio= 60.2, df=28, $p<0.0001$) with MPAs experiencing higher noise levels above 500 Hz and in protected areas below 500 Hz.

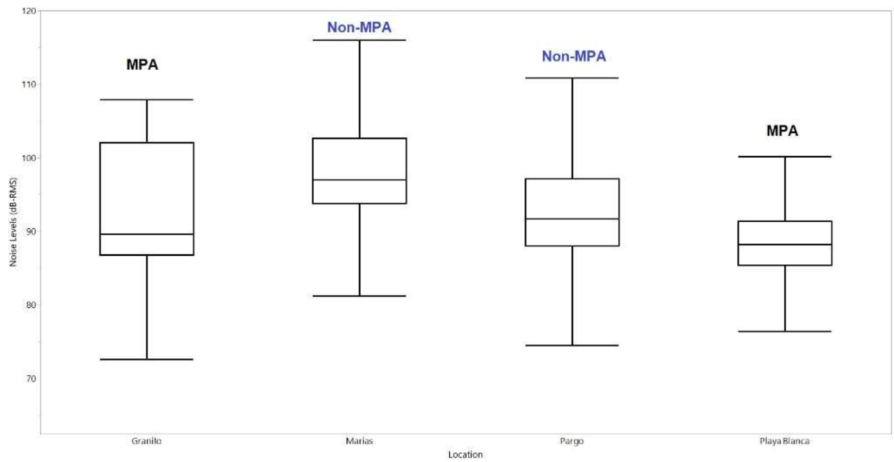


Figure 3. Boxplots of noise levels in dB RMS by level of protection for each site.

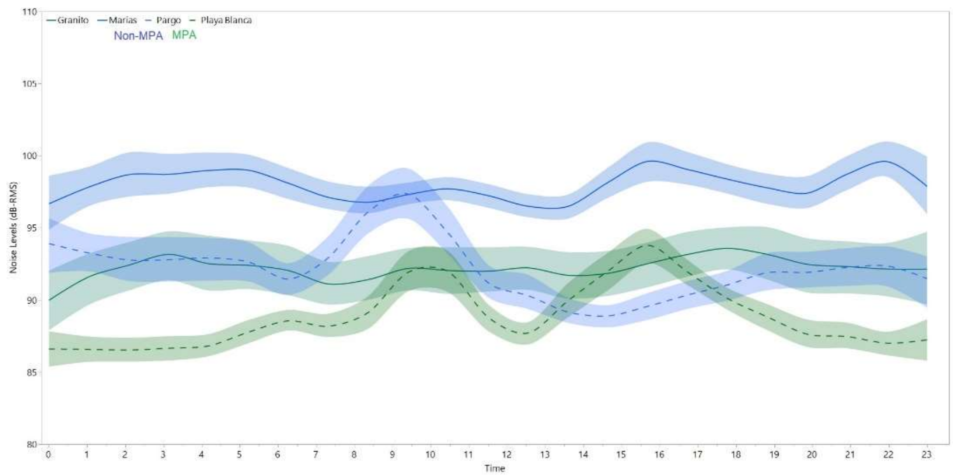


Figure 4. Diel variation of noise levels (dB RMS) by time of day and level of protection.

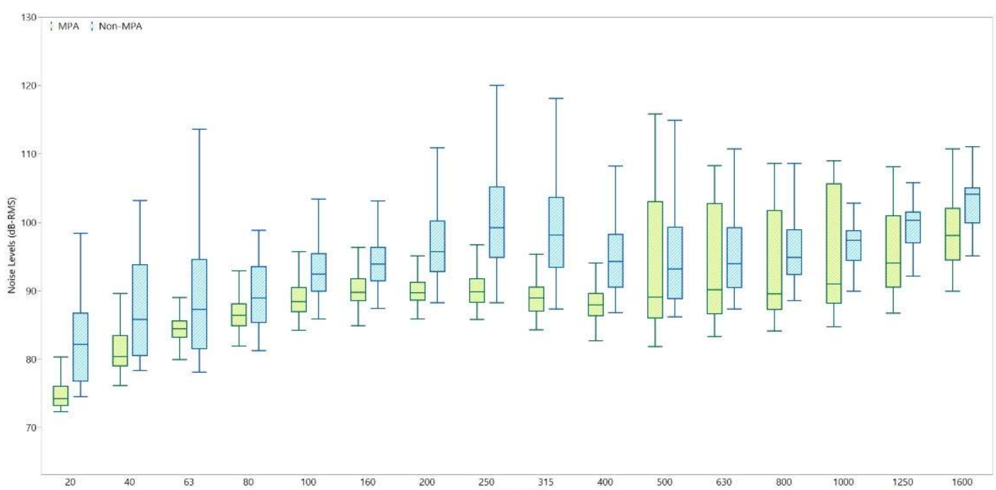


Figure 5. Boxplot of noise level in dB RMS by frequency bin and protection level.

4. Discussion

Our results find that noise levels tend to be higher in the non-protected areas than the protected areas. This is contrary to the expectation that marine protected areas would have greater biological noise levels than non-protected areas due to a greater abundance of biodiversity (Dumyahn and Pijanowski, 2011). These recordings were taken in 2020 during the Covid-19 pandemic, a time when quarantine mandates resulted in increased documentation of wildlife in areas typically occupied by humans (Zellmer, *et al.,* 2020). Costa Rica and Panama implemented strict bans on outdoor recreation and travel, greatly reducing the human activity near marine habitats (International Monetary Fund, 2020). These restrictions and the subsequent lack of anthropogenic disturbance could have led to an increase in biological activity within non-protected areas. Further analysis would be helpful in determining whether the greater noise level in non-protected areas was due to boat use, but the restriction on outdoor recreational activity makes this unlikely. Humpback whales do migrate through both areas (Islas Secas, n.d.; Panama Vacations, n.d.) indicating that high biological noise levels could be attributed to the vocalizations of these whales. The impacts of illegal and underreported fishing activity on biological activity in protected areas should be considered. Panama has previously been issued sanctions by the European Union for failing to satisfactorily combat illegal fishing in the country (The Panama Perspective, 2019). Costa Rica has recently agreed to make its vessel tracking data available to the Global Fishing Watch, which will aid in the identification of unusual fishing vessels that could be linked to illegal harvesting (Global Fishing Watch, 2020).

This study provides the first comparison of biological noise levels by protection level in Panama and Costa Rica. The results contribute to the efforts of Granito and Playa Blanca to preserve their unique marine life. The recordings were taken during the Covid-19 pandemic, suggesting that the decrease in human activity in non-protected areas allowed more biological activity to occur. These results implicate the need for further investigation to determine whether human activities should be further mitigated in Marine Protected Areas.

Acknowledgements

Thanks to the University of Vermont Course-based Undergraduate Research Experience program for providing the opportunity for collaboration on this research.

References

Butler, J., Butler, M. J., Stanley, J. (2016). "Underwater soundscapes in near-shore tropical habitats and the effects of environmental degradation and habitat restoration," J. Exp. Mar. Biol. Ecol. 479, 89-96.

Butler, J., Butler, M. J., Gaff, H. (2017). "Snap, crackle, and pop: Acoustic-based model estimation of snapping shrimp populations in healthy and degraded hard-bottom habitats," Ecol. Indic. 77, 377-385.

Coquereau, L., Lossent, J., Grall, J., Chauvad, L. (2017). "Marine soundscape shaped by fishing activity," RSOS. 4.

dBwave.i. Acoustic Engineering (2014). *dBWave* (Version 4.2011.8) [Computer Software]. https://www.dbwave.pt

Dumyahn, S.L., Pijanowski, B.C. (2011). "Soundscape conservation," Landscape Ecol. 26, 1327.

Global Fishing Watch. Kimberly Vosburgh, (2020). https://globalfishingwatch.org/press-release/costa-rica-mou/ (Last viewed 4/27/21).

Harris, S.A., Shears, N. T., Radford, C. A. (2015). "Ecoacoustic indices as proxies for biodiversity on temperate reefs," Ecol. Evol. 7, 713-723.

International Monetary Fund. (2020). https://www.imf.org/en/Topics/imf-and-covid19/Policy-Responses-to-COVID-19#top (Last viewed 4/26/20).

Islas Secas. (n.d.). https://islassecas.com/discover-islas-secas/ (Last viewed 3/26/21).

Panama Vacations. (n.d.). https://www.vacationtopanama.com/travel-guide/coiba-island-national-park/ (Last viewed 3/26/21).

Pieretti, N., Lo Martire, M., Farina, A., Danovaro, R. (2017). "Marine soundscape as an additional biodiversity monitoring tool: A case study from the Adriatic Sea (Mediterranean Sea)," Ecol. Indic. 83, 13-20.

Pijanowski, B., Villanueva-Rivera, L., Dumyahn, S., Farina, A., Krause, B., Napoletano, B., Gage, S., Pieretti, N. (2011). "Soundscape Ecology: The Science of Sound in the Landscape," *BioScience*. 61, 203–216.

Radford, C., Stanley, J., Tindle, C., Montgomery, J. C., Jeffs, A. G. (2010). "Localised coastal habitats have distinct underwater sound signatures," Mar. Ecol. Prog. Ser. 401, 21-29.

Acoustic Structure of False Killer Whale (*Pseudorca cassidens*) Sounds off The Coast of El Salvador

Samuel A. Koslowsky
University of Vermont

ABSTRACT
The False Killer Whale (*Pseudorca crassidens*) is a dolphin found primarily in tropical waters. They are gregarious and have strong social bonds. Like other species of dolphins, false killer whales rely on sound for navigation, foraging, and form social bonding. However, there is little information about their acoustic behavior throughout their distribution. This study identified three types of social sounds: whistles, burst whistles, and continuous burst whistles. These three types of social sounds were found to be distinct in their frequency and duration characteristics. While whistle and grades sounds have been described in other populations of false killer whales, this study provides the first description of the acoustic repertoire of false killer whales in El Salvador.

Key Words: Cetaceans, Bioacoustics, Pelagic species, El Salvador, Pacific Ocean

1. Introduction

The False Killer Whale (*Pseudorca crassidens*) is one of the large species of dolphins found in primarily in tropical waters (International Whaling Commission, 2020). Due to their pelagic habits, the False Killer whale is poorly studied, and knowledge about the species comes primarily from captive individuals and beaching incidents (Baird, 2009). Consequently, data related to social acoustic signals and social behavior is lacking. A study by Murray et al. (1998) described the production of graded sounds in false killer whales in which the signal started as a pulse trains and continue as a whistle.

The purpose of this study is to document the acoustic structure of social signals produced by false killer whales in El Salvador using opportunistic recordings of the species. The results of this study can be harnessed in ongoing efforts to utilize acoustic monitoring to study marine mammals in Central America. The data collected can inform algorithms to distinguish this dolphin species from others inhabiting the waters of Central American.

2. Materials and Methods

2.1 Study Site and Recording

Data for this study was sourced from opportunistic observations of False Killer Whales (*Pseudorca crassidens*) off the coast of El Salvador between 2018 and 2019. Animals were observed from a research boat and recorded over-the-side using a Zoom digital recorder and a SQ26-H1 hydrophone at a 48 kHz sampling rate.

2.2. Data collection

Acoustic recordings were opened in RAVEN PRO 1.5 build 37 (Center for Conservation Bioacoustics, 2014) and a spectrogram was opened with a fast Fourier transform (FFT) size of 1,024 points, an overlap of 50%, and using a 1024-sample Hann window. All false killer whales' sounds were manually detected and classified into whistles and biphonal sounds. Whistles are defined as narrow banded frequency modulated sounds (May-Collado and Wartzok 2008) and biphonal sounds are a combination of simultaneous emission of whistles and pulsed sounds (Kaplan et al. 2018). Biphonal sounds included burst-pulsed-whistles, continuous burst-pulsed-whistles, and burst-pulses. Whistles were a clear discrete sound that included upsweeps or constant contours. Burst-pulsed whistles were defined by burst-pulses at the beginning of the sound, generally proceeding through a portion of the discrete whistle. Continuous burst-whistles were distinct in that the entire whistle would contain burst pulses. For each sound, the following standard acoustic measurements (May-Collado and Wartzok 2008) were extracted from: start Time, end Time, low, high, peak, and delta frequency, and Peak Frequency Contour (PFC). These parameters were extracted for sounds that had a good signal-to-noise ratio meaning that their contours were dark and not excessively compromised by background noise or the noise from the engine of the research boat.

2.3. Statistical analysis

Data visualization and analyzes were done in in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, a Chi-square Test to determine there are differences in the acoustic variables of the different types of signals.

3. Results

A total of 383 vocalizations were extracted from recorded vocalizations. Of these vocalizations two were discarded due to excessively limited sample size. In total, there were 110 recorded burst-pulse whistles, 256 whistles, and 15 continuous burst-pulse whistles. Table 1 summarizes the descriptive statistics of these whistles. The three type of sounds were significantly different in low (X^2=38.5; df=2; p<0.0001), and high (X^2=22.7; df=2; p<0.0001), and delta (X^2=75.5; df=2; p<0.0001), in duration (X^2=57.8; df=2; p<0.0001) and modulation measured as PFC Num Inf Pts (X^2=31.4; df=2; p<0.0001).

Table 1. Summary of descriptive statistics of false killer whales' social sounds from El Salvador.

	Whistles	**Burst whistles**	**Continuous burst whistles**
Low Frequency	5316.7±1212.8 Range of 6512.6 Hz CV 22.8%	4141.0±1824.2 Hz Range of 9111.2 Hz CV 44.1%	5340.5±1008.2 Hz Range of 3669 Hz CV 18.9%
High Frequency	7581.0±1789.8 Range of 18782.9 Hz CV 23.6%	8016.4±1165.3 Hz Range of 7517.8 Hz CV 14.5%	8277.8±1630.1 Hz Range of 4405.2 Hz CV 19.7%
Delta Frequency	2264.3±1597.5 Hz Range of 12925.3 Hz CV 70.6%	3875.4±1638.5 Hz Range of 7276.1 Hz CV 42.3%	2937.3±1044 Hz Range of 3148 Hz CV 35.5%
Center Frequency	6293.6±1210.9 Hz Range of 10422.1 Hz CV of 19.2%	6038.3±990.5 Hz Range of 6416.9 Hz CV of 16.4%	6359.5±1460.6 Hz Range of 4823.5 Hz CV of 23.0%
Duration	.438±.292 s Range of 1.790 s CV 66.7%	.646±.232 s Range of 1.393 s CV 35.9%	.396±.190 s Range of 0.65 s CV 48%

4. Discussion

This study identified a multitude of strong differences between whistle types in the El Salvadorian false killer whale population. This included low, high, and delta frequencies as well as duration. Of interest was that there was no significant difference in center frequency, peak frequency, or peak number of inflection points between whistles and burst-pulse whistles. Despite the relatively small sample size of this study, this study found incredibly strong statistically significant differences enough to maintain confidence in the validity of data. Sample data was inherently limited due to the nature of the false killer whale as an oceanic species and the opportunistic nature of the recording of them. During the analysis of these recordings, it was noted that several such recordings coincided with humpback whales (*Megaptera novaeangliae*) audibly in the vicinity of the recorded pods of false killer whales *(Pseudorca crassidens*). Questions have subsequently been raised whether this may have impact on vocalizations of these false killer whales, while this was outside the scope of this study and unable to be definitively tested due to an already small sample size, future studies may be required to examine this possibility. This study opens the possibility of comparative analysis of vocalization differences between the Hawaiian, El Salvadorian, and captive populations of false killer whales *(Pseudorca crassidens)* as well, which may directly impact the reproductive viability of this species if significant differences exist between individual offshore pods.

Acknowledgements

This study would not have been possible without the efforts of the Aquatic Megafauna Research Unit of Murdoch University and the willingness of researchers in Central Americas willingness to go above and beyond their own needs of research to acquire these recordings. Furthermore, this study would never have been conducted without the tireless efforts of Professor Laura J. May-Collado in running the University of Vermont Soundscapes and Behavior Laboratory as part of the innovative Course-Based Undergraduate Research Experience (CURE) education model.

References

Baird, R. W. (2009). False killer whale: Pseudorca crassidens. In *Encyclopedia of marine mammals* (pp. 405-406). Academic Press.

International Whaling Commission. (2020, April 15). False killer whale. https://iwc.int/false-killer-whale.

Kaplan, J. D., Melillo-Sweeting, K., & Reiss, D. (2018). Biphonal calls in Atlantic spotted dolphins (Stenella frontalis): bitonal and burst-pulse whistles. *Bioacoustics*, *27*(2), 145-164.

May-Collado, L. J., & Wartzok, D. (2008). A comparison of bottlenose dolphin whistles in the Atlantic Ocean: factors promoting whistle variation. *Journal of Mammalogy*, *89*(5), 1229-1240.

Murray, S. O., Mercado, E., & Roitblat, H. L. (1998). Characterizing the graded structure of false killer whale (Pseudorca crassidens) vocalizations. *The Journal of the Acoustical Society of America*, 104(3), 1679-1688.

Richardson, W. J., Greene Jr, C. R., Malme, C. I., & Thomson, D. H. (2013). *Marine mammals and noise*. Academic press.

Zaeschmar, J. R., Dwyer, S. L., & Stockin, K. A. (2013). Rare observations of false killer whales (Pseudorca crassidens) cooperatively feeding with common bottlenose dolphins (Tursiops truncatus) in the Hauraki Gulf, New Zealand. *Marine Mammal Science,* 29(3), 555-562

Ambient noise levels of a protected marine community in Costa Rica before and during Covid-19.

Sawyer Miller-Bottoms
Department of Biology, University of Vermont, Burlington, VT 05405

ABSTRACT

Due to the Covid-19 pandemic lockdowns, anthropogenic noise levels over industrialized areas are at some of their lowest levels in decades. Similar patterns have been described in the ocean. Sound propagates faster and farther in water than in air, making it the primary way many marine animals communicate and orient themselves over long distances. In this study, we used acoustic data from autonomous underwater recorders deployed in September 2019 and 2020 at Caño Biological Reserve in Costa Rica to study the contribution of boat and humpback whales sound levels to the overall soundscape. Our results showed a significant decrease in noise levels (dB_{RMS}) during Covid-19 lockdowns, and an increase in the contribution of humpback whale songs to the noise levels in 2020. This study provides insight into the impact of human activities on the soundscape of marine habitats.

Key words: decibels, Covid-19, anthropogenic noise, whales

1. Introduction

The Covid-19 pandemic has had a major impact on the world economy and human behavior. Widespread mandatory government lockdowns and stay-at-home orders have severely limited human mobility and decreased world trade. New research suggests the global pandemic and decreased human activity may be something of a "blessing in disguise" for the environment and many of the world's most crucial ecosystems. Air pollution levels over the world's most industrialized nations have been reduced by near 30% (Muhammad et al., 2020). In addition to cleaner air, environmental noise generated by anthropogenic activities has also seen a steep decline since the Covid-19 shutdown (Zambrano-Monserrate et al., 2020).

The link between economic activity and ambient noise levels in the world's oceans has been well confirmed (Frisk et al., 2012). Sound travels more than 4 times faster in seawater than in air, and attenuates slowly, allowing marine species to communicate over long distances (Britannica, 2019). With a sharp decrease in global trade, coupled with reduced human mobility and willingness to travel, anthropogenic noise levels in some ocean ecosystems have declined (Thomson et al., 2020). In more tropical areas of the world, reduced tourism may have also had a significant impact on the noise levels of marine communities.

Humpback whales (*Megaptera novaeangliae)* are large and highly vocal marine mammals that migrate seasonally between feeding areas and breeding grounds. On the Pacific coast of Costa Rica, the protected waters of Caño Island Biological Reserve are important breeding grounds for Humpback whale populations (Oviedo et al., 2008). During the breeding season, male humpback whales sing for hours, and become major contributors to the underwater soundscape of Caño

Island. Aside from humpbacks, anthropogenic noise from small tour boats and medium-sized fishing boats are also important contributors to the underwater soundscape (Chereskin et al., 2019).

This study analyzes the impact of the Covid-19 lockdowns on ambient noise levels of a Caño Island Biological Reserve, a protected tropical marine community in Costa Rica, by comparing noise levels from pre-covid (September 2019) and during covid lockdowns (September 2020). We hypothesize that with reduced travel, tourism, and maritime transport as a consequence of the pandemic, noise levels in 2020 will be significantly lower than in 2019. Given that boat noise produces most of its sound at low frequencies, the reduction of boat presence will increase the communicative space of humpback whales in 2020. Sound pressure levels are an important component of soundscape ecology and a common and quantifiable measure of acoustic wave strength and ambient marine noise levels (Hawkins et al., 2017). The results of this study will provide insight on the impact of boat traffic in an important breeding area for migratory humpback whales.

2. Materials and methods

2.1 Study site

This study was conducted using data collected from the protected waters of Caño Island Biological Reserve, located 15 km off the Osa Penisula on the Pacific coast of Costa Rica. As a biological reserve, human presence at Cano Island is limited and regulated by the Costa Rican government. While regulated, the waters around the island are a popular snorkeling and scuba diving spot for tour boats. The waters around the island are home to many species of sharks, dolphins, sea turtles, stingrays, moray eels, large snapper and grouper, and migratory populations of humpback whales that use the protected waters as a breeding ground in the fall months.

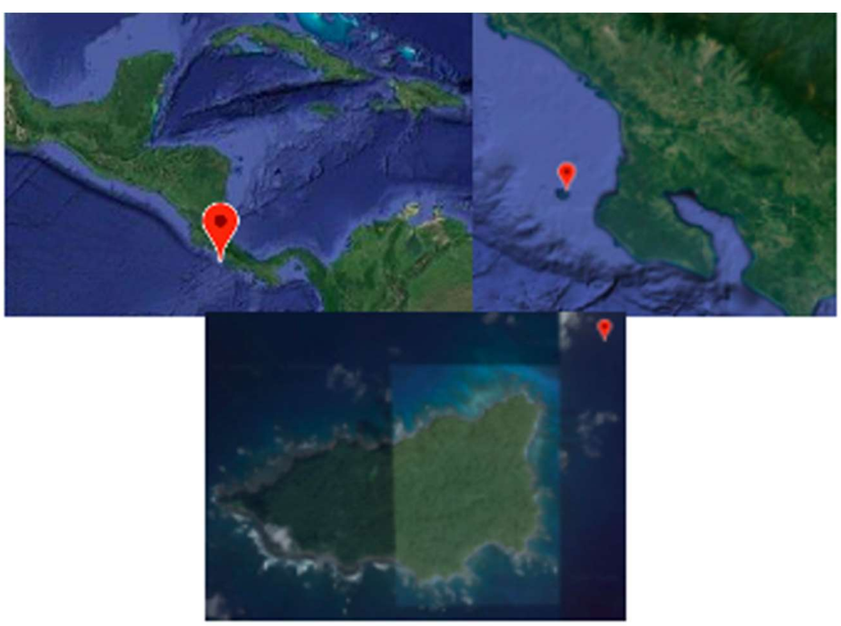


Figure 1. Location of Jardin recording site relative to Cano Island Biological Reserve.

2.1 Recordings

A Soundtrap 300 SD (Ocean Instruments; frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) was deployed at a depth of 25m at a site known as Jardin (8.719N/-83.863W) which consist primarily of sandy bottom. The recorder was programmed to record the soundscape at a sampling rate of 48kHz for 5 minutes every half hour from September 3rd - 31st in 2019, and for 15 minutes every hour from September 3rd - 31st in 2020. Data was uploaded to the RFCx Arbimon for storage and spectrogram analysis of the following days of September 2019 and 2020: 4th, 10th, 16th, 22nd, and 28th. For each day, every first minute of the hour was inspected for presence (1) and absence (0) of humpback whale songs and boat presence, resulting in a total of 120 1-min files for each year. The same 1-minute files were also analyzed in dBWav (Marshall Day Acoustics, 2021) to calculate noise levels. Sound recordings from 2019 were calibrated to 172.5dB and recordings from 2020 were calibrated to 177dB, as per the Ocean Instruments calibration recommendations.

2.3 Statistical analysis

A total of five days from September 2019 and September 2020 were selected to measure noise levels using the program dBWav. The days chosen were the 4th, 10th, 16, 22nd, and 28th as a representation of the month. Raw sound files were trimmed to the first 1-minute of each hour for analysis. The noise levels in dBRMS were estimated using an unweighted approach for a total of 120 recordings each from 2019 and 2020. Data visualization and analysis was performed in JMP 14 (SAS Institute, NC, United States). A non-parametric analysis, chi-square test was used to determine if there were differences in noise levels between pre-covid and covid-lockdown years.

3. Results

3.1 Sound Pressure Level Data

Overall, noise levels at Jardin, Cano Island were lower during Covid-19 lockdowns (2020) than before Covid-19 regulations (2019) (X^2=12, df=1, p=0.0003, Fig.2). Interestingly, during Covid-19 lockdowns, noise levels at frequencies between 1kHz and 10kHz increased (Fig.3), while noise levels at frequencies less than 1kHz decreased (Fig. 4).

3.2 Presence of Humpback Whales and Boats

Boat presence was significantly higher in 2019 (X^2=1, df=1, p=0.005, Fig.5). In 2019, boat noise was present in 14.17% of recordings, compared to 3.33% in 2020. In contrast, whale song detections were significantly greater in 2020 (X^2=203, df=1, p<0.0001, Fig.5). Whale songs were detected in 95.00% of the analyzed recordings before Covid-19 regulations, increasing to 100.00% of recordings during lockdowns.

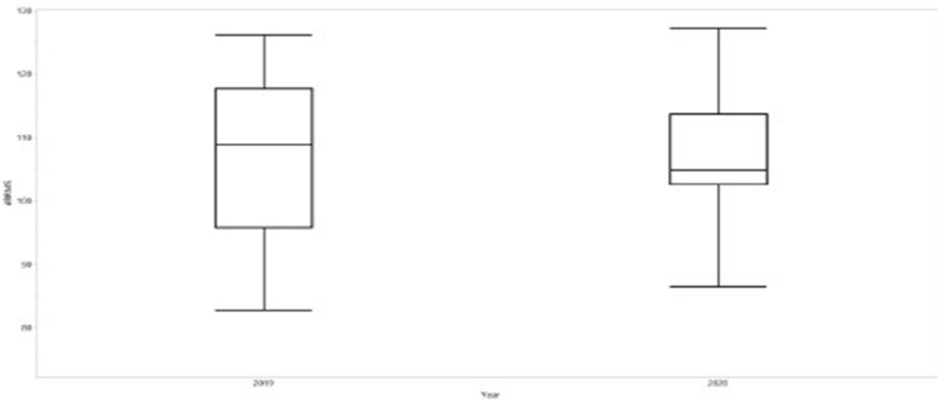


Figure 2. Boxplots showing mean noise levels in dB_{RMS} in September 2019 and 2020.

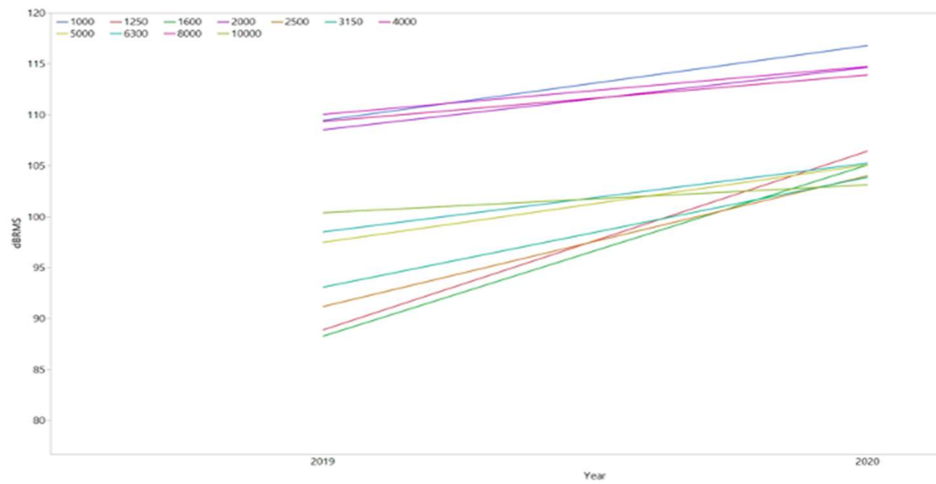


Figure 3. Noise levels in dB_{RMS} at frequencies above 1kHz in September 2019 and 2020.

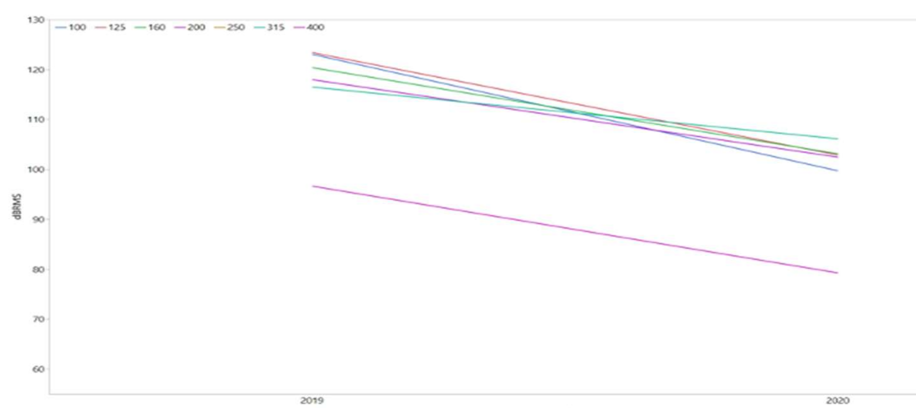


Figure 4. Noise levels in dB_{RMS} at frequencies below 1kHz in September 2019 and 2020.

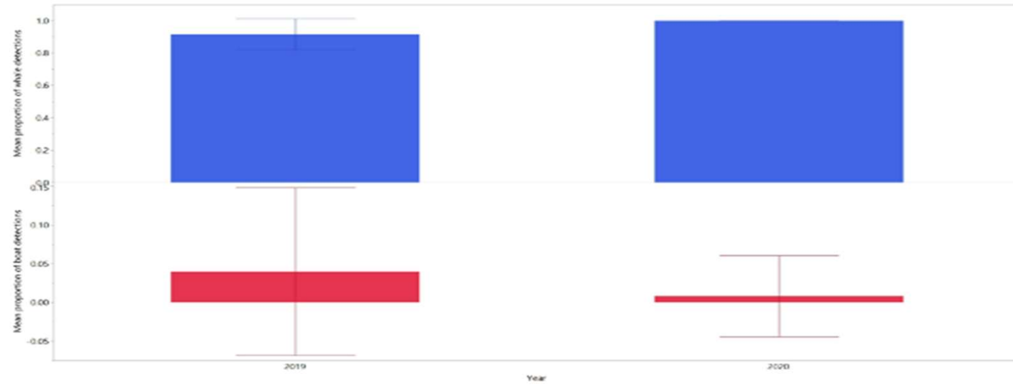


Figure 5. Mean proportion of whale and boat detections in 2019 and 2020.

4. Discussion

Anthropogenic noise levels in the world's oceans have been increasing at an alarming rate. In some marine communities, noise levels have doubled every decade for the past 60 years (Animal Welfare Institute, 2018; Jones, 2019). Within the scientific community, there is increasing concern over the threat of noise pollution on marine ecosystems, and marine mammals in particular.

The Covid-19 pandemic and resulting lockdowns of the past year have offered the unique opportunity of studying natural communities with relatively minimal human presence. Our results find that Covid-19 regulations had a significant impact on the underwater soundscape of a protected tropical marine community at Caño Island Biological Reserve. Overall, mean noise levels at Caño Island decreased in September 2020 when compared to September 2019, consistent with observed reductions in noise levels in other marine communities around the world (Thomson and Barclay, 2020). These results support the predictions of many marine biologists that Covid-19 will quiet the world's oceans (Rutz et al, 2020). An interesting pattern emerged with further visualization and stratification of noise levels by frequency bands.
We observed a significant increase in noise levels at frequencies higher than 1kHz in 2020 (Fig. 3). This result is significant because it is consistent with the reported mean peak singing frequency of 1684.1 ± 756.6 Hz of the Breeding Stock-G (BSG) population of humpback whales (Chereskin et al., 2019). The waters of the Osa peninsula and Caño Island are known to be important breeding grounds for humpback populations, and the presence of the BSG humpback population has been well documented during the months of August and September (Acevedo-Gutierrez and Smultea, 1995; Palacios-Alfaro et al., 2012; Rasmussen et al., 2007). Both the presence of the BSG population of humpback whales during the month of September and the mean peak frequency of their songs supports the conclusion that the increase in noise levels above 1kHz can be attributed to an increase in humpback whale singing activity. This explanation is also consistent with our findings of an increase in the mean proportion of humpback whale detections in 2020 (Fig. 5).

Inversely, our study found noise levels at frequencies below 1 kHz decreased significantly in 2020 (Fig. 4). In the U.S Virgin Islands National Park, it was found that small vessel boat noise contributed to significantly greater noise levels at frequencies below 1 kHz, with peak frequencies in the range of 100 to 500Hz (Kaplan and Mooney, 2015). As a protected area, the only boats that frequent Caño Island are fishing and tour boats, both of which fall into the studies classification of small vessels. An additional study on small vessels in the waters of Canada and Western Australia found underwater noise levels for the boats peaked between 70 and 400Hz, consistent with the findings at U.S. Virgin Islands National Park (Erbe et al., 2016). We attribute our decrease in noise levels at frequencies below 1 kHz to fewer boats at Caño Island and an overall reduced human presence. This explanation is consistent and further supported by the sharp decline observed in our mean proportion of boat detections in 2020 (Fig. 5).

Our findings support the conclusion that with the soundscape experiencing less human noise pollution due to Covid-19 lockdowns and increased governmental regulations, humpback whales were able to increase their communicative space within the soundscape. This study emphasized the impacts of tour boats and fishing vessels on the soundscape of a protected tropical marine community. Our work shows that small tour boats and medium-sized fishing boats contribute significantly to underwater ambient noise at low frequencies at Caño Island Biological Reserve, and supports similar results found in other tropical marine communities (Kaplan and Mooney, 2015).

Our research suggests current regulations at the Caño Island site, prior to Covid-19 lockdowns, are not sufficient to minimize the contribution of humans on the marine soundscape. This pattern of boats as major contributors to underwater soundscape appears to repeat across Central American and the Carribean, in both protected and non-protected areas (Heenehan et al., 2019). This study provides perhaps the first insight into the effect of Covid-19 lockdowns on a protected marine community in the tropics. Additional and more complete noise level data has yet to be analyzed at the Caño Island site and may provide useful results to additional research. This study can assist ongoing efforts to regulate the number of boats that visit the Caño Island protected area, and can inform policy surrounding protected marine communities throughout Central America and the Caribbean.

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References

Britannica. (2019). “Seawater: acoustic properties.” https://www.britannica.com/science/seawater/Acoustic-properties (Last viewed 4/27/21).

Chereskin, E., Beck, L., Gamboa-Poveda, M., Palacios-Alfaro, J., Monge-Arias, R., Chase, A., Coven, B., Guzman, A., McManus, N., Neuhaus, A., O’Halloran, R., Rosen, S., & May-Collado, L. J. (2019). “Song structure and singing activity of two separate humpback whales populations wintering off the coast of Caño Island in Costa Rica.” J. Acoust. Soc. Am. 146, 6. https://doi.org/10.1121/1.513920

Frisk, G. (2012). “Noiseonomics: The relationship between ambient noise levels in the sea and global economic trends.” *Sci Rep* 2, 437. https://doi.org/10.1038/srep00437

Hawkins, A., Popper, A. (2016). “A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates” ICES Journal of Marine Science. 74, 635-651. https://doi.org/10.1093/icesjms/fsw205

Heenehan, H., Stanistreet, J., Corkeron, P., Bouveret, L., Chalifour, J., Davis, G., Henriquez, A., Kiszka, J., Kline, L., Reed, C., Shamir-Reynoso, O., Vedie, F., De Wolf, W., Hoetjes, P. & Van Parijs, S. (2019). “Caribbean Sea Soundscapes: Monitoring Humpback Whales, Biological Sounds, Geological Events, and Anthropogenic Impacts of Vessel Noise.” Front. Mar. Sci. https://doi.org/10.3389/fmars.2019.00347

Muhammad, S., Long, X., & Salman, M. (2020). “COVID-19 pandemic and environmental pollution: A blessing in disguise?,” Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2020.138820

Oviedo, L. & Solis, M. (2008). “Underwater topography determines critical breeding habitat for humpback whales near Osa Peninsula, Costa Rica: implications for Marine Protected Areas.” Rev. Biol. Trop. 56, 591-602. https://doi.org/10.15517/rbt.v56i2.5610

Thomson, D. & Barclay, D. (2020) “Real-time observations of the impact of Covid-19 on underwater noise.” J. Acoust. Soc. Am. 147, 3390. https://doi.org/10.1121/10.0001271

Zambrano-Monserrate, M., Ruano, M., & Sanchez-Alcalde, L. (2020). “Indirect effects of covid-19 on the environment.” Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2020.138813

Geographical Variation of Pantropical Spotted Dolphins (*Stenella attenuata)* Whistle Acoustic Structure

Sydney Tomaseski[1]
University of Vermont. Department of Biology. Burlington, VT 05403

ABSTRACT
Dolphins produce narrow banded frequency modulated sounds called whistles that are used in several social contexts and to convey information about the environment. Understanding the factors that shape dolphin whistle's acoustic structure can provide key information about population connectivity. Here we present the first comparative study of Pantropical spotted dolphin's whistles from Central America, including data from México, El Salvador, Nicaragua, Panamá, and Costa Rica. Dolphins were recorded using bottom-mounted hydrophones and from the research boat using an over-the-side hydrophone at a frequency rate of 48 kHz. Frequency and time variables were extracted from whistle contours using RAVEN and comparisons were made considering distance between recording sites. We hypothesize distanced populations to be significantly different from adjacent populations. Our results did not support our hypothesis, spotted dolphin populations varied in their whistle structure regardless of distance. Our research provides insight on the complex vocal repertoire of pantropical spotted dolphin whistles and how they vary based on geographical location.

Key words: communication, behavior, coastal dolphins, acoustic behavior

1. Introduction

Dolphins produce narrow banded frequency modulated sounds called whistles that are used in several social contexts and to convey information about the environment (Oswald et al., 2007, May-Collado and Wartzok 2008). Most of our understanding on the acoustic structure and function of these sounds comes from captive and wild populations of bottlenose dolphins (Wang et al., 1995b, Morisaka et al., 2005a; ; Azevedo et al., 2007). Dolphins produce two types of whistles, variants which are generally used in a broad social context and signature whistles, which are known as contact calls because they endonce information about the individual (Janik & Sayigh, 2013).

Like bottlenose dolphins, pantropical spotted dolphins, also produce whistles (Oswald et al., 2007, Gruden et al., 2015). These coastal dolphins travel in groups ranging from solarity individuals to groups of fifty (May-Collado & Ramirez, 2004). Atlantic spotted dolphins, a relative of the pantropical spotted dolphin, is known to produce whistles in a variety of contexts including mating, warning, social associations, and coordination of activities (Dudzinski, 1996). However, to this date it is not known if like bottlenose dolphins, these species of dolphin produce whistles that convey individual identity.

In Central America, the Pantropical spotted dolphin is the most commonly found dolphin species (May-Collado et al., 2017). There are two subspecies in the region, the oceanic (*Stenella attenuata attenuata*) and coastal (*Stenella attenuata graffmani*). (Perry et al., 1987). Escorza-Trenino et al., (2005) found that the coastal subspecies genetic structure suggests independent populations. The goal of this study is to determine if the coastal spotted dolphin proposed independent populations are reflected in their whistle acoustic structure. Here we compiled recording data from southern México, El Salvador, Nicaragua, Costa Rica, and Panamá to determine geographical variation in whistle structure and to test if distance between populations explain this variation, as shown in the oceanic subspecies by Ansmann et al., (2007). The results of this study will provide important information about spotted population connectivity of coastal pantropical spotted dolphins in Central America.

2. Materials and Methods

2.1 Study Area

Pantropical spotted dolphin's whistles were recorded from multiple locations in Central America including México, El Salvador, Nicaragua, Costa Rica, and Panamá. The dolphins observed for this study were the Central America subspecies of pantropical spotted dolphin. The recorders were approximately 200 miles from the shore in shallow waters. Whistles were extracted specifically from Chiriquí, Panama, Northern and Southern Nicaragua, Cano Island, Costa Rica, Guerrero, Mexico, and El Salvador. Table I summarizes the sources and recording systems used in each location which range in sampling rate between 44 and 48 kHz.

2.2 Recordings and Analysis

Recordings were opened in RAVEN PRO 1.5 build 37 (Center for Conservation Bioacoustics, 2014) and a spectrogram was opened with a fast Fourier transform (FFT) size of 1,024 points, an overlap of 50%, and using a 512-sample Hann window. Only whistles with a clear and dark contour from start to end and below 24 kHz were selected for analysis (Silva et al., 2016) (see Table 1). Whistles with gaps less than 200 milliseconds were considered the same whistle. Overlapping whistles that could not be easily identified were excluded from analysis. For each of these whistles the following standard acoustic variables were extracted: low frequency (LF) (measures the frequency in the lowest point in the contour), high frequency (HF) (measures the frequency at the highest point in the contour), duration (D), delta frequency (DF) (this is the difference between HF and LF), center frequency (CF) (represents the midpoint frequency between the lower and upper cutoff frequencies), peak frequency (PF) (frequency where the maximum amplitude occurred), and peak frequency contour number of inflection points (PFC Num Inf Pts) (measures the number of times the slope changes sign in peak frequency contour slope) (e.g., Perez et al., 2021; May-Collado and Wartzok, 2008). By utilizing these acoustic parameters, the variation between locations was surveyed through statistical comparison. Whistles with each acoustic variable were compiled into one table. Data visualization and analysis were done in JMP 14. (SAS Institute, NC, United States). We used a non-parametric analysis, Kruskal-Wallis Test to determine if spotted dolphin's whistle acoustic structure varies among populations and a pairwise Wilcoxon comparison test was used to determine which populations varied most.

Table 1. The source, recording system and number of whistles selected for the five locations.

Location	Source and Recording System	Number of whistles selected
El Salvador	Nicole Ransome: Zoom recorder Jose David: Palacios-Zoom recorder	~265
Mexico	Eric Ramos: Soundtrap 300SD	~168
Costa Rica	Laura May-Collado: Passive acoustic monitoring: SM2M+, Rudarmk2; Soundtrap 300 SD	~339
Panama	Laura May-Collado Passive acoustic monitoring: SM2M+	~1481
Nicaragua	Joelle de Weerdt: Zoom recorder	~1761

3. Results

Pantropical spotted dolphins vary significantly in their whistle acoustic structure (Fig.1). Dolphin whistles varied in low (X^2= 351.119, df=4, p= <.0001(1), high (X^2=2254.268 df=4 p=<.0001), delta (X^2= 1933.1994, df=4, p= <.0001) and peak frequencies ($X^2$2= 668.2503, df=4, p= <.0001). Table 2 shows the results of the pairwise comparisons of whistle frequency between countries. The results of these comparisons suggest that the whistles of spotted dolphins from Mexico and El Salvador, and El Salvador and Nicaragua tend to be more similar than those from other countries.

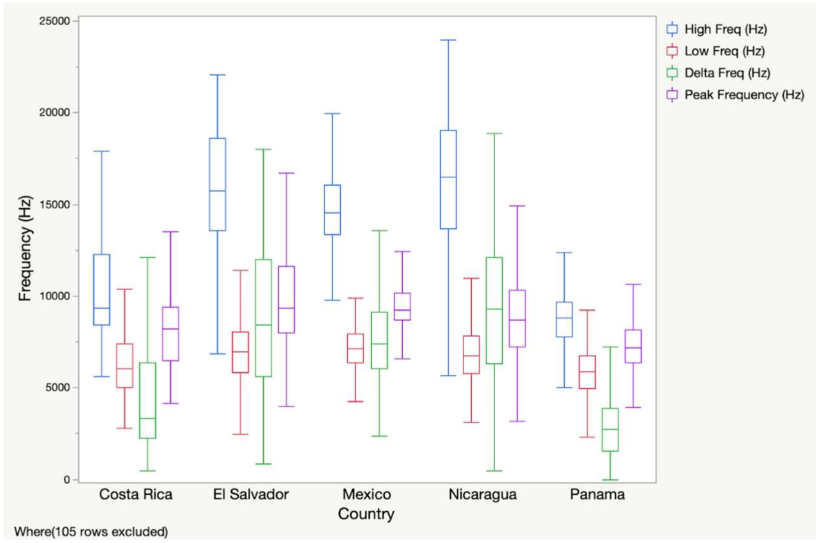

Figure 1. Box plots of spotted dolphin whistle acoustic frequency variables for each country.

Table 2. Summary of pairwise comparisons of spotted dolphin's whistle frequency (Hz) and duration (s).

Countries	LowF	HighF	DeltaF	PeakF	Duration
México-El Salvador	NS	p=0.0003	p=0.0185	p=0.8318	p<0.001
Mexico-Nicaragua	p=0.0037	p<0.001	p<0.001	p<0.001	p<0.001
Mexico-Costa Rica	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Mexico-Panama	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Nicaragua-El Salvador	NS	NS	p=0.0399	p<0.001	p=0.7389
Nicaragua-Costa Rica	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Nicaragua-Panama	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

El Salvador-Costa Rica	p<0.001	p<0.001	p<0.001	p<0.001	p=0.0178
El Salvador-Panamá	p<0.001	p<0.001	p<0.001	p<0.001	p=0.1965
Costa Rica-Panama	p=0.017	p<0.001	p<0.001	p<0.001	p<0.001

Spotted dolphins from all the countries also vary significantly in their whistle duration (X^2= 139.9124, df=4, p= <.0001, Fig.2). Spotted dolphins from Mexico produced the longest whistles (0.906 s ± 0.309 s) and those from Costa Rica produced the shortest whistles (0.587 s ± 0.274 s) (Fig.2).

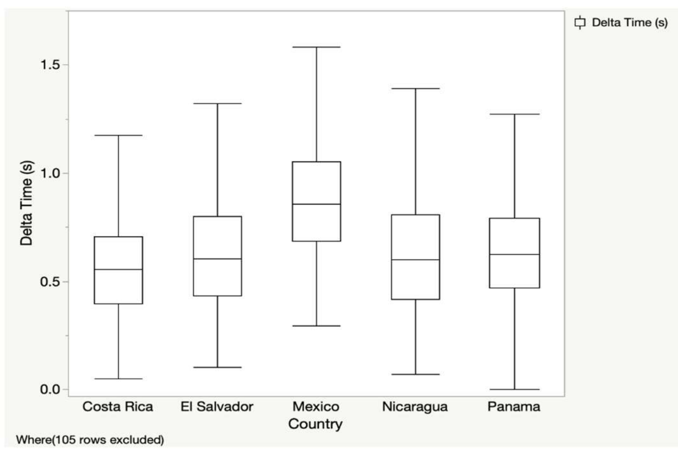

Figure 2. Box plots of spotted dolphin whistle duration in seconds for each country.

4. Discussion

This study found significant variation in pantropical spotted dolphin whistle acoustic structure between locations. The two countries with the greatest amount of variation were Nicaragua and Costa Rica. This result was unexpected considering their proximity to each other. Therefore, indicating low connectivity between them. In contrast, the countries with the least amount of differences in their whistles were Nicaragua and Panama, which suggested connectivity among their populations. Currently, the reasons for the variation between locations is unknown. Although, it is predicted that the variation in coastal spotted dolphin whistles may signify differences in acoustic environments and/or behavioral states during the time of recordings as well as group sizes. In the future, more data will be gathered from all sites and context information to determine what factors may contribute to the differences in whistle acoustic structure. Additionally, a neural network analysis will be used to observe contour similarity. Before conducting further research considering the variations among populations, previous studies that provide evidence on factors that impact whistle structure are considered.

According to preceding research, whistle variation can be a result of geographical, anthropogenic, social, and behavioral factors. La Manna et al., (2020) conducted a study on common bottlenose dolphins living in two different locations. This study evaluated the previously mentioned factors. The results of this research indicated that both geographic distant and isolated populations of dolphins had variability in their whistles. In the same study it was concluded that the group size was another factor that contributed to the differences in whistles between locations. Therefore, revealing that social and behavioral aspects impact whistle acoustic structure among isolated dolphin populations (La Manna et al., 2020).

In a similar study Ansmann et al., (2007), researched short-beaked common dolphins. They found that the whistle acoustic structure between the two populations differed significantly. Their research concluded that the frequency parameters with one population were notably higher than the other. They predicted that a possible reason for the raised frequencies in that specific population was due to high levels of boat traffic in that region (Ansmann et al., 2007). Due to both studies, it is predicted that the variation of coastal pantropical spotted dolphin whistles is a result of social, behavioral, geographical variables as well as human interference.

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References

Ansmann, I., Goold, J., Evans, P., Simmonds, M., & Keith, S. (2007). Variation in the whistle characteristics of short-beaked common dolphins, Delphinus delphis, at two locations around the British Isles. Journal of the Marine Biological Association of the United Kingdom. 87(1). 19-26.

Danaher-Garcia, N. A., Melillo-Sweeting, K., & Dudzinski, K. M. (2019). Social structure of Atlantic spotted dolphins (Stenella frontalis) off Bimini, The Bahamas (2003–2016): alternate reasons for preferential association in delphinids. acta ethol 23, 9–21.

Dudzinski, K. M. (1996). Communication and behavior in the Atlantic spotted dolphins (Stenella frontalis): relationships between vocal and behavioral activities. Texas A&M University. Pages 218.

Escorza-Trevino, S., Archer, F.I., Rosales, M., Lang, A., & Dizon, A.E. (2005). Genetic differentiation and intraspecific structure of Eastern Tropical Pacific spotted dolphins, Stenella attenuata, revealed by DNA analyses. Conservation Genetics 6, 587–600.

Gruden, P., White, P.R., Oswald, J.N., Barkley, Y., Cerchio, S., Lammers, M., & Baumann-Pickering, S. (2015). Differences in oscillatory whistles produced by spinner (*Stenella longirostris)* and pantropical spotted (*Stenella attenuata*) dolphins. Marine Mammal Science 32(2), 520-534.

Janik, V.M., Sayigh, L.S. (2013). Communication in bottlenose dolphins: 50 years of signature whistle research. J Comp Physiol A 199, 479–489.

Kaplan, J., & Reiss, D. (2017). Whistle Acoustic Parameters in Atlantic Spotted Dolphins (Stenella frontalis) and Bottlenose Dolphins (Tursiops truncatus) in Two Locations in The Bahamas and Comparisons with Other Populations. Aquatic Mammals, *43*(4). 364-377.

La Manna, G., Rako-Gospic, N., Sara, G., Gatti, F., Bonizzoni, S., & Ceccherelli, G. (2019). Whistle variation in Mediterranean common bottlenose dolphin: The role of geographical, anthropogenic, social, and behavioral factors. Ecol Evol. 2020;10:1971–1987.

May-Collado, L. J., & Wartzok, D. (2008). A comparison of bottlenose dolphin whistles in the Atlantic Ocean: factors promoting whistle variation. Journal of Mammalogy, 89(5):1229–1240.

May-Collado, L., & Morales Ramírez, A. (2005). Presencia y patrones de comportamiento del delfín manchado costero, Stenella attenuata (Cetacea: Delphinidae) en el Golfo de Papagayo, Costa Rica. Revista de Biología Tropical, 53(1-2), 265-276.

Oswald, J., Rankin, S., Barlow, J., & Lammers, M. (2007). A tool for real-time acoustic species identification of delphinid whistles. The Journal of the Acoustical Society of America 122(1). 587-595.

Perrin, W. F., Mitchell, E. D., Mead, J. G., Caldwell, D. K., Caldwell, M. C., Bree, P. J. H., et al. (1987). Revision of the spotted dolphins, Stenella spp. Mar. Mamm. Sci. 3, 99–170.

Perez-Ortega, B., Daw, R., Paradee, B.,Gimbrere, E., & May-Collado, L.J. (2021). Dolphin-Watching Boats Affect Whistle Frequency Modulation in Bottlenose Dolphins. Front. Mar. Sci. 8:618420.

Quérouil, S., Freitas, L., Cascão, I. *et al.* (2010). Molecular insight into the population structure of common and spotted dolphins inhabiting the pelagic waters of the Northeast Atlantic. Mar Biol 157, 2567–2580.

The impact of COVID-2019 lockdowns on toadfish calling behavior in Bocas del Toro, Panama.

Tessa Kilmer
Department of Biology, University of Vermont, Burlington, Vermont 05405, USA

ABSTRACT
In many species of fish, males rely on sound for mating and competition during the spawning season. The toadfish is one example of an important sonorous fish found in near-shore communities that uses vocalization for sexual reproduction and communication. The near constant, low-intensity sounds of boat traffic in populated shallow environments can correspond to the frequency of the mating calls male toadfish use to attract females to their nests. A previous CURE study found that toadfish in Bocas del Toro seem to respond to boat traffic by lowering their call frequency and increasing its amplitude, strategies implemented by other animals to cope with noisy environments. The COVID-19 global pandemic presents a unique opportunity to determine how calling behavior of toadfish alters in response to lockdowns in Bocas del Toro in July 2020. We found that overall noise levels decreased during the COVID-19 lockdown, resulting in significant changes to toadfish call acoustic structure. While there was no change to call duration, the resulting calls displayed a wider degree of variation, indicating that the quieter soundscape allowed for more diverse vocalizations.

Keywords: acoustic behavior, anthropogenic noise, stress, bioacoustics

1.Introduction
Analyzing aquatic soundscapes is increasingly being seen as an effective way to determine the health of marine ecosystems and monitor biological changes over extended periods of time (Lammers and Munger, 2016). This practice is becoming more important as the effects of anthropogenic noise on aquatic species that rely on sound for communication, prey detection, and sexual reproduction become clearer. The near-constant, low-intensity sounds of boat traffic in populated shallow environments can often correspond to the frequency at which organisms communicate, leading to either a change in the organism's behavior or the masking of biologically important calls (Butler and Maruska, 2020; Popper and Hawkins, 2019).

Members of the Bratrachoididae family, specifically toadfish, can serve as useful indicator species in soundscape analyses as they have an extensive range, occupy benthic, near-shore habitats, and have well-studied acoustic structures (Greenfield et al., 2008; Salas, Wilson, and Ryan, 2018). Male toadfish produce calls known as "boat whistles" that consist of "boops" and "grunts" and are used to defend their territories and attract females to their dens for reproduction (Pyć et al., 2021; Gray and Winn, 1961). A typical boat whistle will consist of 1-3 grunts followed by 1-2 boops (Staaterman et al., 2018). These boat whistles are produced by the contraction of muscles surrounding the swim bladder and can therefore vary depending on factors such as temperature (Maruska, 2009; Staaterman et al., 2018). Male calling behavior has been linked to reproductive success and acts as an indicator to females about the fitness of their potential mates, making it especially important for these calls to be overheard (Vasconcelos et al., 2012). Motorboat traffic produces noise levels within the same bandwidth as toadfish vocalizations, making them susceptible to acoustic masking, reduced communication (and therefore, reproductive) success, and interfere with detecting prey and predators (Pyć et al., 2021; Popper and Hawkins, 2019).

The archipelago of Bocas del Toro has many sites that experience a high frequency of boat traffic. In previous years, Almirante Bay experienced regular boat traffic to and from the mainland between the hours of 6 am and 6 pm. Since the outbreak of COVID-19 and the onset of a global pandemic, however, it is possible that boat traffic in Almirante will have significantly decreased as tourism and commuting became high-risk activities. Toadfish vocalizations at Almirante Bay in previous years have been found to be shorter in duration, lower in frequency, and higher in amplitude than at sites that experience less boat traffic (Gagne, 2019). This suggests that toadfish at sites with constant boat traffic are compensating for the increased noise by altering their calling behavior. The COVID-19 pandemic presents a unique opportunity to further explore the extent of boat whistle plasticity to determine if the calling behavior of toadfish during the pandemic will be significantly different than before the lockdown started. I predict that the toadfish calling behavior during the pandemic at this previously trafficked site will increase in duration and frequency.

Tourism, especially dolphin-watching, is an important aspect of Bocas del Toro's economy and many people rely on it as a source of income. For this industry to remain fruitful and attract people from all over the world, it is vital that the local marine ecosystem does not significantly decrease in quality. Monitoring long-term toadfish responses to spatial and temporal changes as well as human-induced impacts to the ecosystem can act as a cost-effective indicator of community health. The extent of the effect of anthropogenic noise on toadfish vocal plasticity is important to understand, as it poses threats to reproductive success of the species and may even be acting as a selection pressure on male toadfish.

2.Materials and methods

2.1 Study Location and Design:

The study took place in Almirante Bay (9.289N, -82.332W) in the archipelago of Bocas del Toro, in the Caribbean coast of Panama. Almirante is composed of mangrove, seagrass, and coral reef near-shore habitats (Guzman et al., 2005). In Almirante Bay, transport boats are the main type of boat activity, with boat-taxis running every 30 min each way between the mainland to the islands from 6 a.m. to 6 p.m, (Perez et al., 2021).

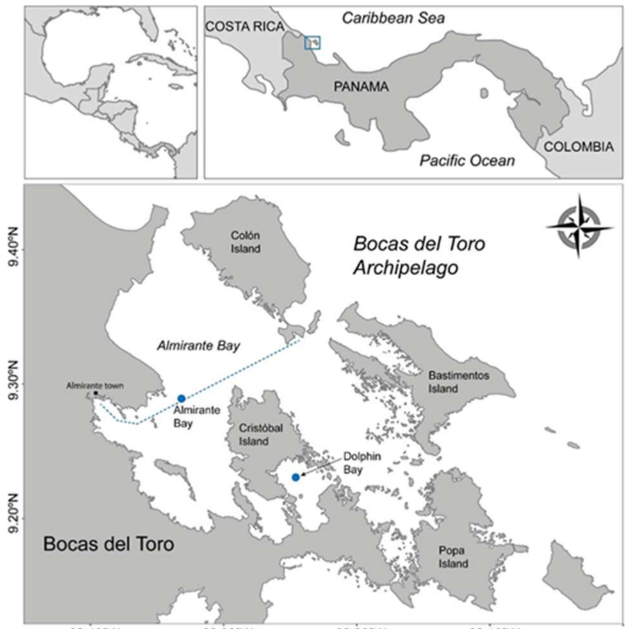

Figure 1. Almirante Bay study site where recorders were deployed (Perez et al. 2021).

2.2. Recordings

A Soundtrap 300 SD (frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) recorder was deployed at 12 m depth in Almirante (9.289N, -82.332 W). Recordings were made for 1min every 10 minutes from April 25th through the 29th in 2019 and for 10 minutes every hour during the COVID-19 lockdown from July 9th through the 13th in 2020.

2.3. Data collection

To determine if Covid-19 lockdowns resulted in an acoustic change of toadfish mating call's acoustic structure we selected recordings from 5-7 am (dawn), 11am-1 pm (midday), and 7 pm-9 pm (dusk) in both years. These times were selected due to the presence of both toadfish calls and boat traffic noise at varying levels depending on the time of day, with toadfish being most prominent at dawn and dusk and boat traffic being most prominent around midday (Gagne 2019). Clear calls with good dark contours were selected from these time periods and analyzed using Raven 1.5 (2016; Cornell Lab of Ornithology). A spectrogram was open with a fast Fourier transform (FFT) size of 1,024 points, an overlap of 50%, and using 4,096-sample Hann window.

Following Gagne (2019) nomenclature, each toadfish call analyzed was broken down into its components: grunts, boops, interboops (the period between boops), intergrunts (the period between grunts), and the grunt-boop interval (the period between the end of a grunt and the beginning of a boop). For the grunt and boop components of each call, the following acoustic measurements were collected: duration, low frequency, high frequency, peak frequency, fundamental frequency, maximum amplitude, and RMS amplitude (Staaterman et al. 2018; Gagne 2019). The duration of each intergrunt, interboop, and grunt-boop interval was also recorded. The presence of boat traffic was also noted if a boat whistle occurred while the boat could be heard or if it took place within 30 seconds of the boat's passing.

2.4 Data Analysis

An ANOVA test of variance was used to determine if the effects of COVID-19 significantly influenced call acoustic structure. All statistical analysis were conducted using JMP Pro 14.2 (SAS, 2019).

3. Results

Noise levels from 2019 and 2020 were found to vary significantly, with 2019 levels being greater overall (F=901.78, df=1, p<0.0001, Fig.2), however noise levels did not differ significantly based on time of day (morning or afternoon), regardless of year (p>0.05).

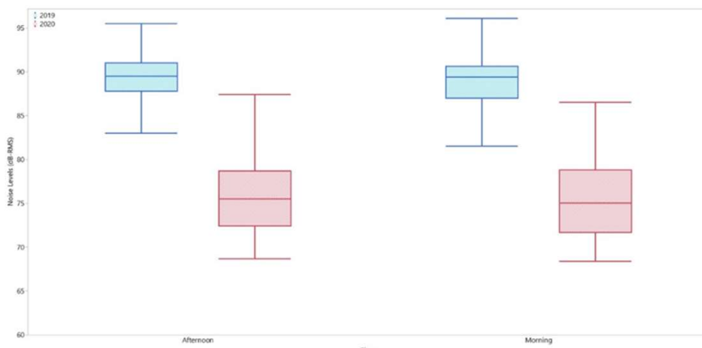

Figure 2. Noise levels measure as dB_{RMS} for each year at Almirante Bay.

Toad fish calls vary significantly in low, high, and peak frequencies by year. While duration of boat whistles was found to be independent of noise levels (p>0.05), low frequencies, high frequencies, and peak frequencies were found to vary significantly depending on the year (LF: F=88.71, df=1, p<0.0001, HF: F=23.75, df=1, p<0.0001, PF: F=53.23, df=1, p<0.0001, Fig. 3). In 2019, low frequency was lowest at night when compared to afternoon or morning (F=10.73, df=2, p<0.0001), while there was no significant difference in 2020 (F=2.39, df=2, p=0.09). High frequency was also significantly higher at night in 2019 (F=13.83, df=2, p<0.0001), but significantly lower at night in 2020 (F=28.29, df=2, p<0.0001). Duration was also significantly lower at night in 2020 (F=8.73, df=2, p<0.0002), while no difference occurred in 2019 (F=1.16, df=2, p>0.05). Peak frequency was significantly higher in the afternoon of 2019 (F=5.35, df=2, p<0.0051), while there was no significant difference in 2020 (F=1.35, df=2, p>0.05, Fig. 3).

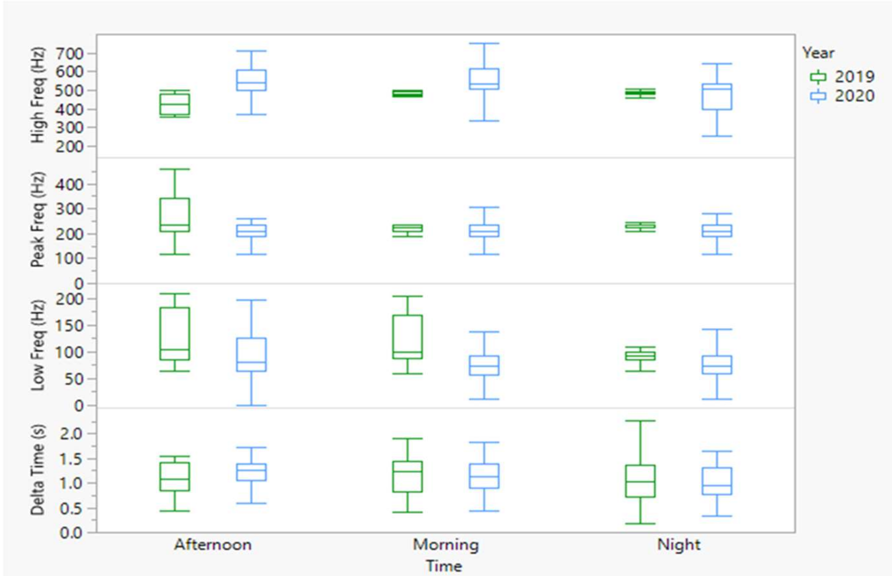

Figure 2. Box plot comparisons of toadfish boatwhistle parameters from 2019 and 2020 categorized by time of day: Morning (5-7 am), Afternoon (11am-1pm), and Night (7-9 pm).

4. Discussion

The purpose of this study was to evaluate how the decrease of boat traffic in a previously heavily trafficked area influenced the vocalizations of soniferous toadfish. Previous studies have compared this site to others that experience significantly less boat traffic, but the onset of the COVID-19 pandemic presented a unique opportunity to analyze sound recordings from the same study area with significantly less anthropogenic sound. The results of our analysis indicate that the toadfish present in Almirante are not significantly adjusting the duration of their boat whistles with the absence of boat traffic. These findings are intriguing given that toadfish in Almirante were found to have calls that were shorter in duration than those recorded at the less noisy Sharkhole site (Gagne, 2019). Female toadfish also likely prefer higher call rates that are longer in duration, as this could indicate individuals of higher fitness (Staaterman et al., 2018). Anthropogenic noise could therefore be acting as an opposing selection pressure to sexual selection by females.

Our study also determined that there was significant variation in the low, high, and peak frequencies dependent on year. In 2020, low frequency was found to be significantly lower on average, while high frequency was significantly higher on average. This indicates that the individuals recorded in 2020 had more acoustic space to vocalize within, allowing for more variation in call structure, while those individuals subject to heavy boat traffic had a smaller window to avoid masking. Since it is likely that male toadfish have signature whistles that convey individual information and distinguish them from other males, it is important that the acoustic environment allows for variation in call structure (Staaterman et al., 2018). The differences observed in acoustic structure of calls recorded in the morning, afternoon, or night of each year also indicates that toadfish are actively modifying their calls in the presence of boat traffic.

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References

Beak, D., Willatzen, M., and Jensen, J. A. (2019). “Parameter sensitivity study of a Field II multilayer transducer model on a convex transducer," Proc.-IEEE Untrason. Symp. 135, in press (2021).

Christian, R. S., Davies, R. E., Tubis, A., and Anderson, C. A. (1984). “Effects of air loading on tympani membrane vibrations,” J. Acoust. Soc. Am. 76, 1336–1345.

Gray, G. A., & Winn, H. E. (1961). “Reproductive ecology and sound production of the toadfish, Opsanus tau.” Ecology. 42, 274–282.

Guzman, M. Hector., et al. (2005). “A Site Description of the CARICOMP Mangrove, Seagrass and Coral Reef Sites in Bocas del Toro, Panama.” Car. J. of Sci. 41, 430-440.

Julie M. Butler, Karen P. Maruska (2020). “Underwater noise impairs social communication during aggressive and reproductive encounters,” An. Behav. 64, 9-23.

Lammers MO, Munger LM (2016). “From shrimp to whales: biological applications of passive acoustic monitoring on a remote Pacific coral reef.” Au WWL, Lammers MO (eds) Lis. oc. Spr. NY, pp 61–81.

Maruska, K. P. and A. F. Mensinger. (2009). “Acoustic characteristics and variations in grunt vocalizations in the oyster toadfish Opsanus tau,” Env. Bio. Fis. 84, 325–337.

Perez-Ortega, B., Daw, R., Paradee, B., Gimbrere, E., and May-Collado Laura J (2021). “Dolphin-Watching Boats Affect Whistle Frequency Modulation in Bottlenose Dolphins,” Fron. Mar. Sc. 8, 102.

Popper, AN, Hawkins, AD (2019). “An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes,” J. Fish. Biol. 94, 692– 713.

Pyć, CD, Vallarta, J, Rice, AN, Zeddies, DG, Maxner, EE, Denes, SL (2021). “Vocal behavior of the endangered splendid toadfish and potential masking by anthropogenic noise,” Con. Sc. Pr. e352.

Staaterman, E., S. J. Brandl, M. Hauer, J. M. Casey, A. J. Gallagher and A. N. Rice. (2018). "Individual voices in a cluttered soundscape: acoustic ecology of the Bocon toadfish, Amphichthys cryptocentrus." Env. Bio. Fish. 101, 979–995.

Vasconcelos, R. O., Carriço, R., Ramos, A., Modesto, T., Fonseca, P. J., & Amorim, M. C. P. (2012). "Vocal behavior predicts reproductive success in a teleost fish," Beh. Eco. 23, 375–383.

Climate change and the Coquí: Temperature-driven changes to frog calls in Puerto Rico

Kristen Werner
Department of Biology, University of Vermont

ABSTRACT
The Coquí frog of Puerto Rico has a distinctive vocalization with the syllables "co" and "qui". Previous studies have found that both syllables have increased in frequency and decreased in total call duration 1986 to 2006. These changes are indicative of a shrinking body size, which has been identified in many species as a physiological symptom of increasing temperature from climate change. To assess whether these trends are continuing, audio recordings from El Verde Research Station in Puerto Rico were analyzed for peak frequency of each syllable and total call duration. Climate data including temperature and precipitation were also analyzed to compare with frequency and duration results. A significant increase in peak frequency of the "co" syllable was noted, while no significant change in "Qui" frequency or total call duration was found. The increase in "co" frequency peaks in 2012, then begins to decrease likely in response to a sharp drop in temperature and precipitation in the region from 2009-2012. This study supports the hypothesis that anuran body size is highly correlated with changes in climate, and this can be monitored through vocalization analysis.

Key Words: Eleutherodactylus coqui, climate change, peak frequency, acoustic evolution

1.Introduction
The Coquí frog (*Eleutherodactylus coqui)* is an amphibian species native to Puerto Rico. These frogs are beloved by Puerto Ricans and hold a special cultural value; The name Coquí comes from the mating call produced by males that has two distinct syllables (pronounced ko- kee). These calls are mostly produced at dusk, but can can be heard well into the night (National Wildlife Federation n.d.). This species plays an integral role in the food web of Puerto Rico's rainforest by controlling insect populations as well as sustaining many species of predators.

Frogs, like all amphibians, are "indicator species" meaning that they reflect an ecosystems overall health and stability. One reason for this label is that they have permeable skin, so they are very sensitive to changes to air or water quality (Waddle 2006). They are also ectotherms meaning they get heat from their environment instead of their metabolism, so their internal temperature is primarily dependent on the ambient temperature. Consequently, changes in average temperature can result in developmental abnormalities that can impact all aspects of a frog's ability to function (Narins and Meenderink 2014).

Over the past few decades, a myriad of studies has emerged identifying a trend pervasive in the animal kingdom of shrinking body size with increasing temperatures due to climate change. There are two main factors involved in this reduction of biomass; First, increased temperatures have caused reduced water and nutrient availability across many ecosystem types, and this has led to decreased plant growth in turn reducing the size of consumers over time. The other factor is metabolism, which for ectotherms directly correlates with temperature. An increase in metabolic rate associated with global warming will reduce the body size of ectotherms unless they can significantly increase their normal food intake (Sheridan and Bickford 2011). To study these climactic phenomena in organisms, scientists can analyze their vocalizations. One reason for this is that in general, an animal's body size has an inverse relationship with its vocal frequency. Thus, one can examine the physiological traits of a species by analyzing their vocal patterns.

A study from the by Narins and Meenderink (2014) used Coquí calls to determine how the amphibians are being impacted by climate change. The study noted a significant increase in frequency (pitch) and decrease in duration of the Coquí frog calls from 1983 to 2006 coinciding with an average temperature increase of 0.37º C during the same period. The study suggested that the frog's body size, dependent on calling-site temperature and altitude, determines the frequency of its call as well as its inner ear sensitivity (Narins and Meenderink 2014).

In this study, I revisit and reevaluate this physiological trend with more recent climate and auditory data. I predict that Coquí call frequencies have continued to increase and call duration has continued to decrease since 2006, and at a faster rate. This project provides an update to ecologically relevant trends in the Coquí, which will benefit researchers studying and protecting frog species through the changing climate in the future.

2. Materials and Methods

2.1 Study site

This study uses passive acoustic monitoring data from El Verde Research Station in the El Yunque National Forest, Luquillo Mountains of Northeast Puerto Rico. El Yunque is a rainforest of around 30,000 acres with several rivers and high levels of biodiversity (USDA Forest Service n.d.). El Verde is a very remote research station located within the northwest quadrant of El Yunque at an altitude of 473 meters (Figure 1).

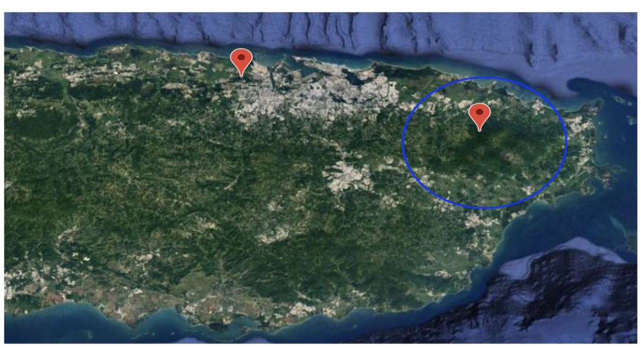

Figure 1. Satellite map of Puerto Rico showing the location of El Verde research station within the El Yunque National Forest (circled in blue).

The Coquí frog (*Eleutherodactylus coqui)* is an endemic species widely distributed throughout this area. The males are known for their mating calls that consist of the two syllables "co" and "qui". Both males and females produce an aggressive call to ward off intruders, which has a combination of repetitions of the syllable such as "co-qui-qui-qui" or "co-co-qui-qui". The males' "co-qui" mating call is the most prominent as choruses of males sing for hours without stopping. These calls can be heard throughout the day and night but generally peak from 8 pm to 12 am; this vocal mating behavior peaks in July (Stewart and Rand 1991).

2.2 Recordings

Audio recordings from El Verde were collected from the database available on the website RFCx Arbimon, https://arbimon.rfcx.org/home (Rainforest Connection 2021). Recordings were made every 10 minutes every day using an omnidirectional stationary microphone on the ground near the El Verde field station. For this study, we selected recordings from July for peak breeding activity. To determine if changes in ambient temperature influence the Coqui frog's call, we selected recordings from years 2009, 2011, 2012 and 2015. A recording from each night of July was collected for each of the aforementioned years. Then the call of one presumed individual was isolated and inspected in Raven software (Center for Conservation Bioacoustics 2019). For each call, the following variables were extracted:

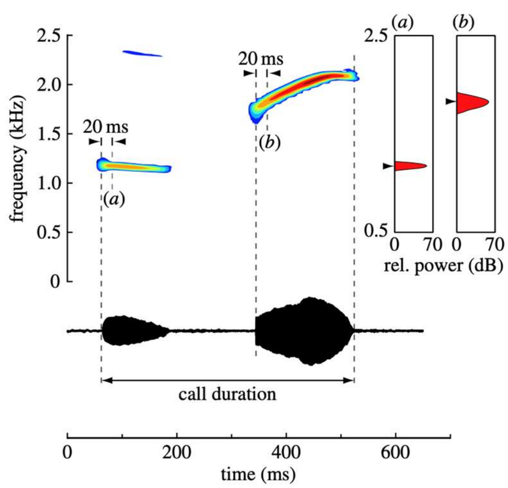

Figure 2. Diagram showing the acoustic structure of a Coquí call. The duration (s), frequency (kHz), and relative power (dB) of the co and qui notes of a typical call is represented (Narins and Meenderink 2014).

peak frequency of each syllable ("co" and "qui") and the total duration of the call (Narins and Meenderink Figure 2).

30 recordings of Coquí calls were downloaded from 2014. 13 recordings from 2012, 9 recordings from 2011, and 25 from 2009 for a total of 77 calls used in this analysis. The following acoustic variables were extracted from the co and qui notes: peak frequency (Hz) and duration (s) for the entire call. Data visualization and analyses were done in JMP 14 (SAS Institue, NC, United States). We used a non-parametic analysis of a Kruskall-Wallis Test to determine if there were changes in note frequency and call duration with year.

2.3 Climate data

Datasets from the El Verde Field Station website provided hourly temperature data and precipitation values. Average July temperature values for the years 2009, 2011, 2012 and 2015 were obtained by averaging hourly temperature readings from 7 pm – 12 am for each day of July for each year studied. Average monthly precipitation levels were obtained by averaging daily precipitation readings (Peréz-Rivera, 2021).

3. Results

3.1 Coquí calls

After removing outliers, we found that the Co note's peak frequency significantly increased from 2009-2012 and we noted a decrease in 2015 (Kruskal-Wallis Test, = 9.66 df = 3 p = 0.0216). We also found a greater variability in peak frequency in 2011 (Fig.3). The Qui syllable trend was opposite, showing a decrease in frequency from 2009-2012, and then an increase in 2015 (Fig. 4). However, this trend is not significant (Kruskal-Wallis test, $p > 0.05$). There were not significant differences in call duration with year (Kruskal-Wallis test, $p > 0.05$, Fig. 5).

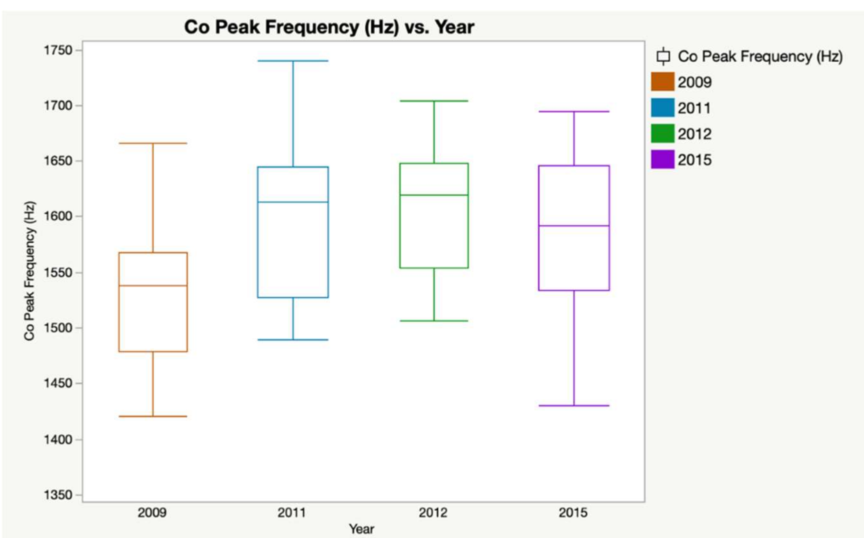

Figure 3. Boxplot showing peak frequency values of the "Co" syllable of Coquí frog vocalizations from the month of July from the years 2009 (orange), 2011 (blue), 2012 (green), and 2015 (purple) respectively.

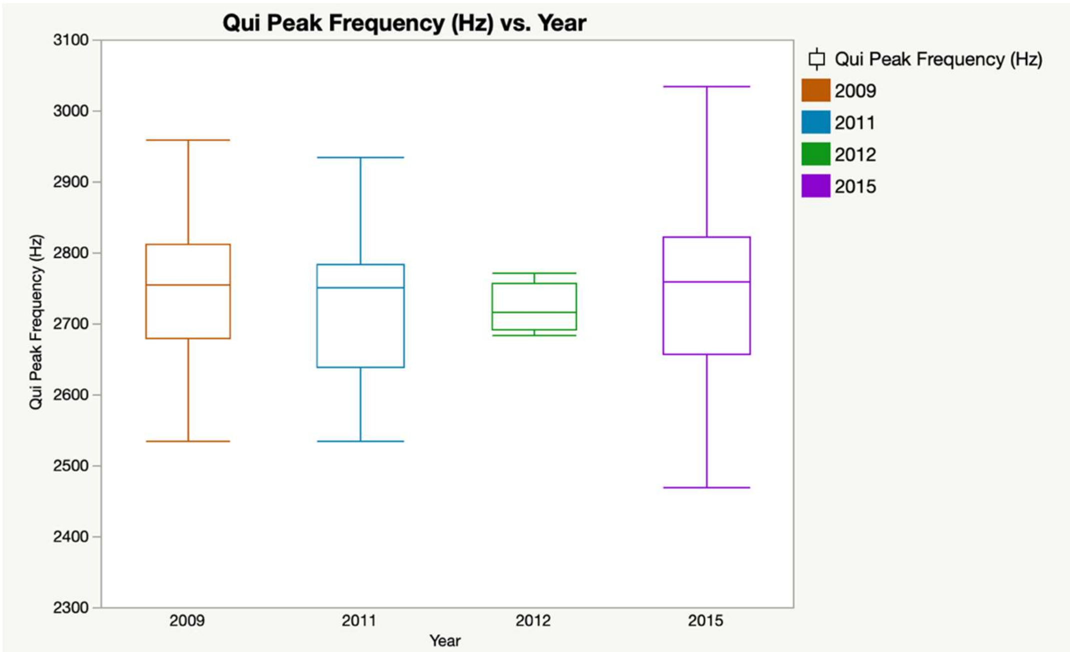

Figure 4. Boxplot showing peak frequency values from the "Qui" syllaboe of Coquí frog vocalizations from the month of July from the years 2009 (orange), 2011 (blue), 2012 (green), and 2015 (purple) respectively.

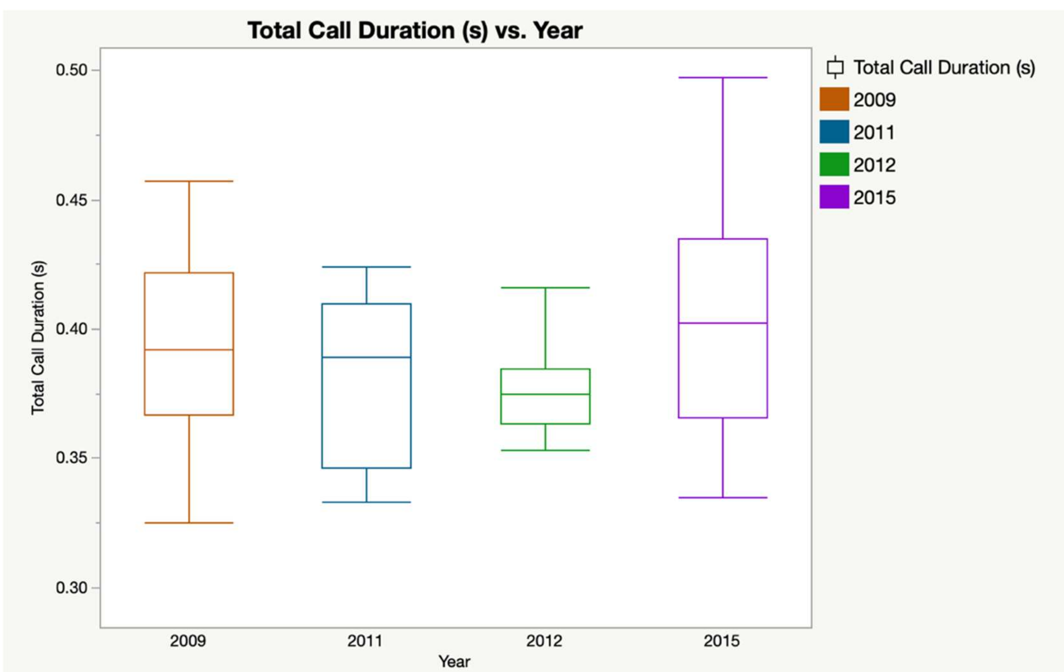

Figure 5. Boxplot showing total call duration values of Coquí frog vocalizations from the month of July from the years 2009 (orange), 2011 (blue), 2012 (green), and 2015 (purple) respectively.

3.2 Temperature Trends

July average temperature and precipitation both decreased in the region where El Verde is located from 2009-2015. Average July temperature had a moderate decrease from around 23ºC in 2009 to 21.5 ºC in 2012 and decreasing further to just over 20ºC in 2015 (Figure 4). Average July precipitation had a steeper drop from about 20 mm in 2009 to just 5 mm in 2012/2015. (Figure 5).

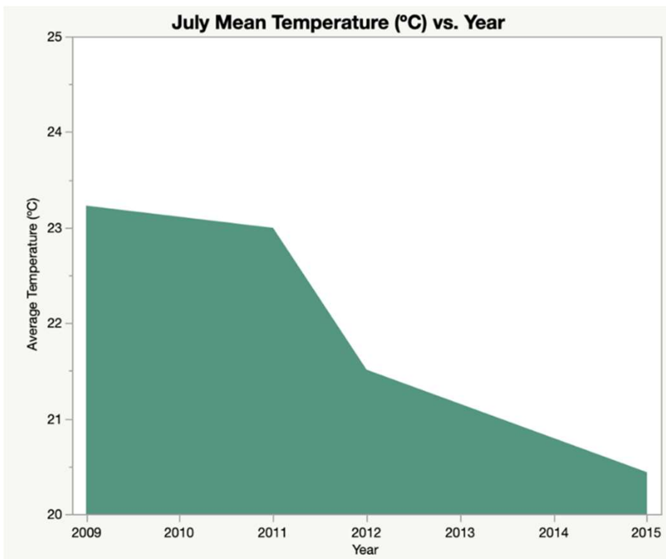

Figure 4. Average temperature of July (ºC) at the El Verde Research Station for the years 2009, 2011, 2012, and 2015.

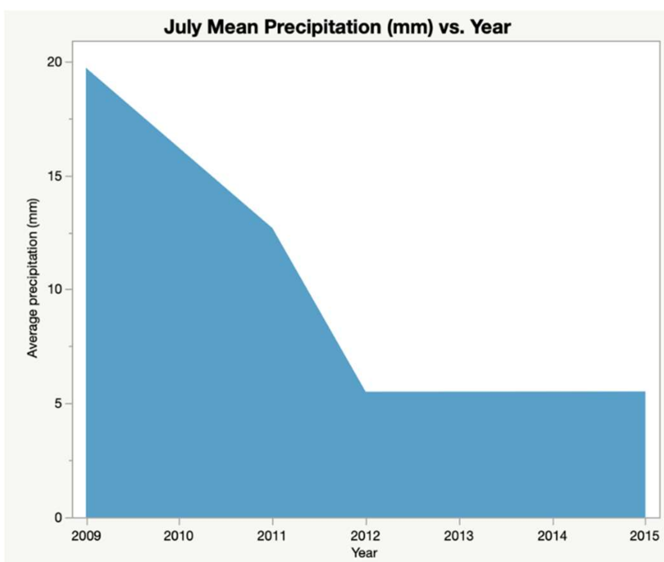

Figure 5. Average precipitation from July (mm) at the El Verde Research Station for the years 2009, 2011, 2012, and 2015.

4. Discussion

There was no significant change in either the “qui” syllable frequency nor in call duration from the years 2009-2015. Thus, I will focus on the “co” syllable results because they were significant and follow a previously identified trend (Narins and Meenderink 2014). The rate of increase in frequency decreases from 2009-2012 until the average frequency turns to decrease in 2015. A decreasing body size is a physiological reason to support why the vocalizations increased in frequency. These results are consistent with previous studies that find anuran body size is highly dependent on a trade-off between heat and water balance (Amado et. al 2018). Both precipitation and average temperature had a sharp decline from 2009 on, and I think that the frequency curve is in response to this decline. There is a slight lag as it would take a few generations for body size While the Narins and Meenderink study found a correlation between frequency increase and a temperature increase, this study found a correlation between frequency decrease and temperature and precipitation decrease (Narins and Meenderink 2014). Both studies support the same hypothesis that frog body size decreases in response to more heat, and increases in response to less heat, as measured through vocalization frequency.

This study was limited by the small sample size of vocalizations used (77 total) simply due to the availability of data on RFCx Arbimon. This lack of data may be the reason why only the “co” syllable frequency data showed significant results, while “qui” frequency and duration did not. This study could be expanded upon to include vocalization and climate data throughout other parts of the year, as well as to include data from more years in addition to 2009, 2011, 2012 and 2015. Getting a more comprehensive view of how vocalizations are changing would allow for closer analysis in the way the Coquí vocalizations respond to climate. This study provides a simple yet legitimate update to the trends in Coquí vocalizations identified by Narins and Meenderick in their 2014 study (Narins and Meenderink 2014). Studying anuran physiological responses to climate is important as temperature and precipitation anomalies become more and more frequent with climate change.

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References

Center for Conservation Bioacoustics. **(2019)**. Raven Pro: Interactive Sound Analysis Software (Version 1.6.1) [Computer software]. Ithica, NY: The Cornell Lab of Ornithology. Available from http://ravensoundsoftware.com/.

Amado, T. F., Bidau, J.C., and Olalla-Tárraga, M.A. **(2018).** “Geographic variation of body size in New World anurans: energy and water in a balance”. Ecography, **42**, 456-66.

Francisco J. Pérez-Rivera. **(2021).** El Verde Field Station. http://evfs.ites.upr.edu/.

Narins, P.M. and Meenderink, S.W.F. **(2014)**. "Climate change and frog calls: long-term correlations along a tropical altitudinal gradient," Proc. R. Soc. B. 281.

Rainforest Connection **(2021)**. *RFCx Arbimon: Bio-Acoustic Analysis Platform. [Web-Based Platform]*. Available online at: https://arbimon.rfcx.org/ (accessed 2021).

Sheridan J.A., Bickford D. "Shrinking body size as an ecological response to climate change". **(2011)** Nat Clim Change **1**, 401-406.

USDA Forest Service **(n.d.)**. "Nature and Science" El Yunque National Forest, https://www.fs.usda.gov/detail/elyunque/learning/nature-science/?cid=stelprdb5208524

Stewart, M. M. and Rand, A.S. **(1991).** "Vocalizations and the Defense of Retreat Sites by Male and Female Frogs, Eleutherodactylus coqui," American Society of Ichthyologists and Herpetologists. **4.**