Course-based Undergraduate Research Experience: Soundscapes and Behavior Research (BIOL 188-A)

Fall 2018

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

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

E-mail: lmaycoll@uvm.edu
CURE Website: http://www.lauramay-collado.com/cure-lab.html

About CURE: Course-based Undergraduate Research Experiences (CUREs) provide early opportunities to undergrads to participate in scientific research. CUREs allow students to get hands-on experience in the process of scientific discovery, which increases students interest in science, helps them decide if they want to pursue research careers, increases confidence in their ability to do science, and promotes early participation in the scientific community through publication, conference presentation, and more. A hallmark of a CURE course is that students participate in all aspects of a research project from asking questions, proposing hypothesis, making predictions, selecting tools for visualization, data processing and statistical analysis, to communicating findings. All this while learning to navigate the messiness of the real-world data! Regardless of your ultimate career goals, participation in a CURE will help you to develop skills in interpretation of results and establishing solid arguments (Corwin et al. 2014).

For more information about CUREs, go to **CUREnet**: https://serc.carleton.edu/curenet/index.html

Course description: This course engages undergraduate students in topics I work on: marine soundscape ecology, marine animal bioacoustics, and cetacean ecology, behavior, and conservation. For this CURE, students will work on projects related to two topics. Topic I involves the use of sound as a tool to study marine communities, the vocal repertoire of marine species, and human impacts on wild populations. Topic II involves the use of traditional photo-identification methods in dolphins to study association patterns and population size (more details below).

Learning goals of this CURE course are:

1. To offer the opportunity to make discoveries and contributions to the scientific community, policy-makers, and the public.
2. Engage students in all aspects of research: literature reading and discussion, asking questions that can be answered during the semester, collecting, processing, and analyzing data, learning how to interpret analytical results and how to communicate the results.
3. Create an environment that promotes active collaboration and contributions among students and instructor during the semester through problem solving and analysis.
4. Learn that science is not about eureka moments! Good science takes time, involves failure, troubleshooting, discussions, re-evaluations, and yes frustration. Good science is always challenging at different levels, from collecting the data to its analysis.
5. Learn that there is not a single "right" way to do science! Different questions, systems, or species will require different approaches. For example, some research questions rely on well-planned experimental designs involving multiple controls. My research is field based and correlative, which is bound to be limited by replication, sample size, lack of controls (because they are impossible to have!), and logistics. However, field-based projects are essential for our understanding of our biological world and are often the spark for more controlled experimental studies.

Course expectations: During this semester, students will develop skills that will help them to further a successful career in sciences and technology. I expect that together we will create a dynamic environment for scientific communication and collaboration and a learning space for all (including myself), involving discussion of scientific papers, data processing and data analysis, establishing solid arguments supported by the data. The course will culminate with a manuscript and a mini-symposium open to the public. I expect students to actively communicate with me; I will always be available in my office except for when I am teaching. We will have our own CURE lab where you will work on your projects and find the support you need to be successful. While my policy will be an open door, I expect students to take charge of their projects, be independent and resourceful readers of scientific literature related to their projects and demonstrate initiative in learning new programs or analysis that can help them address their research questions.

Students are expected to develop questions on either of the topics described below. Students can develop an individual project, or in a group project (no more than three students per group). Expectations will be higher for group projects.

Research Topics

I. Soundscape Ecology: Biodiversity survey methods are labor-intensive and limited to a few locations and short-periods of time. This is true when it comes to study marine communities. New acoustic technology provides marine scientists the opportunity to study community dynamics using sound as a cue for biodiversity. My recent research aims to use underwater acoustic technology to study biologically important marine communities in Central America. My goal is to provide information on biodiversity, target species (i.e., fish, dolphins, whales) and human activity. Students that choose to work on this topic will be working with my acoustic file database that includes marine soundscapes from protected and non-protected areas in Belize, Costa Rica, and Panama. Some of these data needs to be processed and uploaded to my iCloud database before it can be used for analysis. Students working with animal vocalizations (whales, dolphins, or fish) will be primarily using RAVEN and ARBIMON tools, although there are other programs that can also be of use for specific analysis.

To learn more about marine soundscapes go to BB: Course Material: Soundscape Ecology; Animal Bioacoustics.

II. **Dolphin Behavior:** Bottlenose dolphins live in complex fission-fusion societies, where animals associate with different individuals in a fluid manner. The strength of these associations appears to vary across groups and over time. In a society such this, individuals play various roles in maintaining the integrity of the overall social structure of a population. The loss of an individual (through natural mortality or live-captures) to a society will depend on the role that those individuals play. Students that choose to work on this topic will be working with my decade old photo-ID data base. Students will use photographs of dolphin's dorsal fin and will identify individuals based on natural marks using my catalogue. PhotoID information can be used to learn about how dolphins associate with one another, but also to evaluate health status, follow individuals through time, estimate population size and home range. My matrix is up today to mid-2013, students working on this project will have to process data for the last 4-5 years. Students working with this database will be learning to use SOCPROG a program used to study social groups, estimate group size, and more.

To learn more about bottlenose dolphins: Course Material: Dolphin Behavior and Ecology. Also, go to my website and read the reports to the International Whaling Commission Regarding this dolphin population: *http://www.lauramay-collado.com/publications.html*

Whether you choose to work with soundscapes, bioacoustics, or dolphin behavior your work will help me and my colleagues to ensure governments responsible to protect our biodiversity have the best available scientific information to act.

Course Plan

Week	**Chronogram**
Aug. 28	Introduction to this course. This first week of classes students are expected to make appointments with me to discuss research interests.
Sept. 4	Presentation of Project ideas with feedback from instructor. **You are required to read the assigned literature during the summer and come with 5 project questions to discuss in class.**
Sept. 11	Workshops on ARBIMON and RAVEN. Documentation will be made available in BB.
Sept 18	Workshop on SOCPROG and other programs. Documentation will be made available in BB.
Sept. 25	Project proposal due on the 25th. Guidelines and rubric will be available in BB. This day we will set up a schedule for computer time and any other resource that you need.
Oct.1-Nov.13	Work on Data processing and analysis. Every Tuesday you will provide a progress report. I expect you to come to my office as often as you need to.
Dec.4	Research Paper Draft (Due on December 4th). Guidelines and rubric will be available in BB.
Dec. 11	**Mini-symposium and Final Paper Due**

Grading

Participation (progress reports, participation in discussion, communication with instructor, work ethics)	30%
Sharing your Research Experience Blog	10%
Proposal	20%
Written paper	20%
Oral presentation	20%

Data Use

The data that you will using to develop your project is primarily my property as the PI of the projects involving the collection of this data. However, other data sets are shared with collaborators that significantly contributed to data collection. You will sign a contract of ethical use of the data. No sharing of data on social media or with other parties is allowed. We will develop a space for outreach activities and research experience communication through a blog where you can post sound bites, summaries, photographs, and updates on data processing.

Publication agreement

If your project results in a publishable journal article, we will discuss co-authorship prior to the end of the semester. My policy on co-authorship on an article include three of the following five contributions: (1) contributed to the conceptualization of the research project, (2) provided funding, (3) collected the data, (4) contributed significantly in the analysis and interpretation of results, and (5) participated in the writing. Student co-authorship will depend on contributions to points 1, 4, and 5. If a student fulfills these three contributions, we will discuss order of co-authorship given that many people have been involved in data collection. I hope we get to have several publishable papers!

ACADEMIC HONESTY

Academic honesty is expected of all students. The University of Vermont has a very strict policy concerning academic honesty and plagiarism. Please see the statement on academic honesty http://www.uvm.edu/~uvmppg/ppg/student/acadintegrity.pdf.

Plagiarism constitutes a violation of Academic Honesty. Plagiarism of ANY sort will NOT be tolerated. The consequences of plagiarism or cheating range from a score of zero on the assignment, failure in the course, to filing a complaint with the University's Coordinator for Academic Honesty, which can result in expulsion from the University.

COURSE CONTENT AND DATA IS THE PROPERTY OF THE INSTRUCTOR.

Consistent with the University's policy on intellectual property rights, all teaching and curricular materials (including but not limited to classroom lectures, class notes, exams, handouts, and presentations), and **research data**, are the property of the instructor. Therefore, electronic recording and/or transmission of classes or class notes is prohibited without the express written permission of the instructor. Such permission is to be considered unique to the needs of an individual student (e.g. ADA compliance), and not a license for permanent retention or electronic dissemination to others. For more information, please see the UVM policy on Intellectual Property, sections 2.1.3 and 2.4.1

RELIGIOUS HOLIDAYS: Students should submit in writing to their instructors **by the end of the second full week of classes** their documented religious holiday schedule for the semester. Students who miss work for the purpose of religious observance will be allowed to make up this work.

STUDENT DISABILITY POLICY. In keeping with University policy, any student with a documented disability interested in utilizing accommodations should contact ACCESS, the office of Disability Services on campus. ACCESS works with students and faculty in to find reasonable and appropriate accommodations, which are communicated to faculty in an accommodation letter. Contact ACCESS: A170 Living/Learning Center; 802-656-7753; access@uvm.edu; or www.uvm.edu/access.

First CURE Soundscape and Behavior Symposium

Location: 217 MLS

Time: 2 p.m.

2:00 Introduction

Session I: Soundscapes

2:10: Diversity of Signature Whistles in Bottlenose Dolphins (*Tursiops truncatus*) of Dolphin Bay, Bocas del Toro, Panama by Rebecca Daw.

2:20: Toadfishes calling activity decreases with boat traffic by Isabel Mize

2:30: Singing patterns of humpback whales (*Megaptera novaeangliae*) in wintering grounds off the coast of Central America and potential negative effects of tour boat traffic by Danieel McAree, Kate Ziegler, and Shelby Rosten.

2:40: Effects of boat traffic on the mating call acoustic structure of the Bocon Toadfish (*Amphichthys cryptocentrus*) in the Archipelago of Bocas del Toro, Panama by Cooper Peterson.

2:50 Break

3:00: Monitoring Marine Biodiversity through Soundscape Analysis: Isla del Caño, Costa Rica by Jessa Houghton and Caroline Dunbar

3:10: The acoustic repertoire and temporal activity of Antillean Manatees (*Trichechus manatus manatus*) in Belize by Carly Sarbacker

Session II: Behavior

3:20 Female Productivity and Calf Survivorship of Bottlenose Dolphins (*Tursiops truncatus*) in Bocas, Panama by Kahlia Gonzales and Natalia Swack

3:30: Analysis of the Population Size of bottlenose dolphins (*Tursiops truncatus*) in Dolphin Bay, Bocas del Toro, Panama by Amanda Jones

2:40 Break

3:50: Spatial analysis of critical areas for bottlenose dolphin female and calves in Bocas del Toro indicates high overlap with dolphin watching activities by Hannah Hutchens

4:00 Social Structure of Bottlenose Dolphins in Bocas del Toro Panama by Erin Powell.

Analysis of the population size of bottlenose dolphins (*Tursiops truncatus)* in Dolphin Bay, Bocas del Toro, Panama

Amanda Jones, University of Vermont, Burlington, VT, USA

ABSTRACT

Due to habitat overlap, human activities can pose a threat to coastal bottlenose dolphin populations, which is like the case in Dolphin Bay in the Archipelago of Bocas del Toro, Panama. This population is genetically isolated, with both males and females showing high site fidelity. They are under pressure from intense interactions with dolphin-watching boats. Therefore, having an estimation of the population size and variation over time is important for establishing their conservation status. In this study, photo identification data and four capture-recapture models are used to estimate the population size. The mortality model, which best fits the data, indicates that the population in Dolphin Bay is between 39-48 dolphins. There is evidence for fluctuations in population size over the years. These differences could be due to variation in sampling efforts between the years, but may also indicate times of high mortality. This study shows that the dolphins in Dolphin Bay are at risk. They are especially vulnerable to activities that directly target them, such as dolphin watching, due to their small population size, isolation, and high dependence of the bay. Future research will address differences in survey efforts and increase sample size.

Keywords: capture-recapture, photo identification, conservation

INTRODUCTION

Coastal bottlenose dolphins (*Tursiops truncatus)* live in isolated populations in close proximity to human activities, often making them a focal point of tourism (Nowacek, et al. 2001, Constantine, et al. 2004). Boat-based dolphin watching is characterized by following dolphins on a regular basis, which can lead to behavioral changes such as a decrease in resting behavior and an increase in milling behavior (Constantine, et al. 2004). In bottlenose dolphins off the coast of Italy, during interaction with recreational boats, dolphins surfaced more often and did not partake in activities such as feeding or socializing as frequently (Pennino, et al. 2016). In addition, a high amount of boat activity poses an increased risk of injury to the animals (Wells & Scott 1997, Trejos and May-Collado 2015).

In the Archipelago of Bocas del Toro off the coast of Panama, a resident population of bottlenose dolphins has been under increased stress due to a high number of dolphin-watching boats in the area (May-Collado, et al. 2015). Since this population already has low genetic diversity (Barragán-Barrera, et al. 2017), the added pressures that boats put on this population put it further at risk. Boat collisions killed 10 dolphins over just a three-year span, which cannot be afforded by this small, isolated population (Trejos & May-Collado 2015). Many boats also fail to follow the whale-watching regulations, frequently get too close to the dolphins, and do not switch off their engines within the proper distance of the animals (Sitar, et al. 2016).

In order to understand the impact of these activities at the population level, photo-identification data from 2004 to 2014 will be analyzed using capture-recapture models. A previous study using photo-ID data from 2004-2012 estimated the overall population of the Archipelago to be between 70-90 dolphins, with the sub-population in Dolphin Bay consisting of 37 dolphins (May-Collado, et al. 2015). In this study, capture-recapture data from 2013 and 2014 is added, along with additional mortality information. Given the state of the population and the pressure from dolphin-watching activities, it is predicted that the population is declining.

MATERIALS AND METHODS

Study Area and Fieldwork

The Archipelago of Bocas del Toro is located in the Caribbean coast of Panama. Survey efforts covered approximately 79.2 km^2 within the inner part of the Archipelago, which is characterized by shallow and clear waters and bottom substrates consisting of sea grass, coral, and sand. The main mode of transportation between the islands and mainland is through powered boats with 50 and 150 hp engines and canoes. This study focused on Bocas Torito Bay, also known as Dolphin Bay. This is a closed bay with resident dolphins that are highly predictably attracting most of the dolphin watching operators. The bay is also considered an important nursery ground. The area was surveyed using a 10 m fiberglass boat with two engines (150 hp/4-stroke) from 7 a.m. to 6 p.m., following predetermined routes. Survey effort varied from 7 days to 4 weeks a year, depending on funding support. Once a group of dolphins was encountered, the boat approached slowly and in a parallel position to avoid dolphin disturbance (Würsig and Jefferson 1990, Resolution ADM/ARAP NO. 01, 2007). A distance of 30-50m from the group was maintained before turning the engine off to initiate data collecting and photoID. This type of approach is standard in cetacean studies because it minimizes behavioral impact on the group (e.g., Würsig and Jefferson 1990). A group was defined as “a collection of conspecifics in a limited area, often engaged in similar activities and moving in the same general direction, maintained by social factors as a unit” (Wells et al.1999). The following information was collected: group size (minimum, maximum, and best estimation), photo-ID data, geographical position using a GARMIN GPS, predominant behavioral state at the moment of the encounter and during acoustical recording sessions, presence/absence of boats other than the research boat, and acoustic recordings.

Photo Identification Processing

Photo identification (photoID) is a form of capture-recapture data collection that does not involve physical tagging of animals. Capture-recapture data assumes that the population is steady, the tags (in this case, photographs) are correctly recorded, and that animals act independently (Amstrup, McDonald, & Manly 2010). PhotoID was used to identify individual dolphins based on high-quality photographs of their dorsal fins. New photos of dorsal fins were compared to an existing catalogue of dorsal fin photos of known dolphins in the population. All parties had to agree upon the identity of each dolphin before it was recorded as being “present” during a certain sighting. Once a dolphin had its identity verified, a photo of its fin was saved and recorded as present in a presence/absence matrix. If a dolphin was seen multiple times during the same sighting, its presence was only marked once in the matrix. In some of the photos, the dolphin was unable to be identified either because of the quality of the photo or the angle of the animal such that its identity could not be confirmed. Those photos were marked as "unknown" and were not included in this study.

Population Analyses

Population analyses were performed using the software SOCPROG, which utilizes several population models. The following models were included in the analysis: Closed Petersen, Closed Schnabel, Jolly-Seber, and Mortality. The Closed Petersen model assumes a closed population between each pair of consecutive sampling intervals. The Closed Schnabel also assumes a closed population, whose size is estimated by maximum likelihood. The Jolly-Seber model accounts for variation in mortality/emigration rates and birth/immigration rates. The Mortality model assumes a population of constant size, where mortality and emigration are balanced by birth and immigration. Both mortality and population size are estimated using maximum likelihood (Whitehead 2017). The matrix used for the estimations consisted of 50 individuals, all of which had above 10 recaptures over time. These dolphins represent the core population of Dolphin Bay, as they have been seen the most in that area. While many other dolphins were identified during the photoID process, they were not included in this study so that an estimate of the core population could be obtained.

RESULTS

All population size estimates indicate the Dolphin Bay population is small, ranging from 39 to 48 animals. Table 1 shows the estimates from the four different models, and highlights the Mortality model as the model best fitted to the data. The Dolphin Bay population appears to fluctuate between years, as shown by the Closed Petersen model (Fig. 1), where the mean population is shown for each year.

Table 1: Population estimates of the core population of Dolphin Bay

Model	Population Size	Bootsrap 95% c.i.	AIC
Closed (Petersen)	40.2-44.5	39.92-45.07	---
Closed (Schnabel)	45.97	44.4-48.0	355.22
Jolly-Seber	39.7-43.9	---	---
Mortality	43	39-48	**315.87**

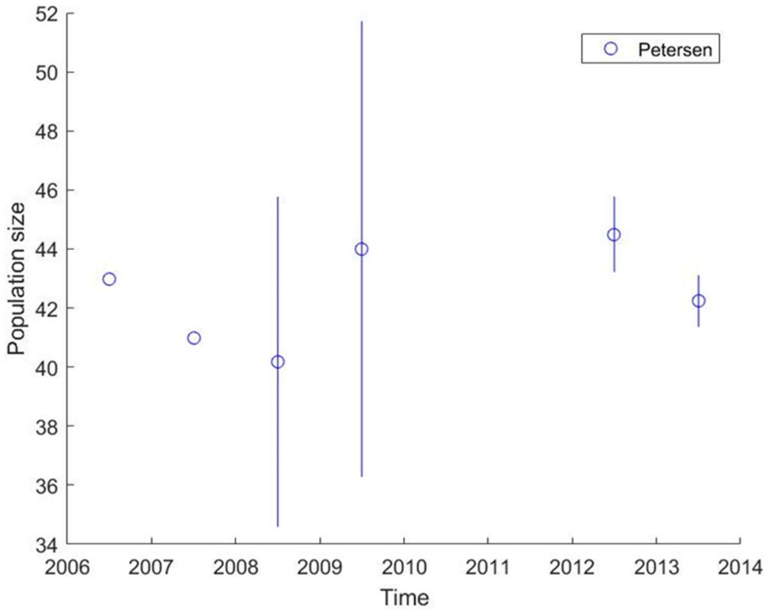

Figure 1: Closed Petersen population estimates over time of the core population of Dolphin Bay

DISCUSSION

The results indicate the Dolphin Bay bottlenose dolphin population is small and fluctuates over time, with no evidence of decline, contrary to expectations. The model that best fit the data was the one accounting for mortality, indicating that mortality is a driving factor for population changes in this area.

While there are other dolphins in the overall population of the Archipelago, the sub-population that interacts daily with the boats is small and vulnerable. May-Collado et al. (2012) found that a group of dolphins could interact with up to 40 boats in one hour during the low tourism season, and up to 100 boats per hour at peak tourist times. This high boat traffic in the bay has severely impacted the resident dolphins' behavior and habitat use (May-Collado, et al. 2015), but particularly of the females, which are the main contributors to population growth (Kassamali-Fox et al. 2015). In addition, the exponential growth of tour boats using the bay has led to collision, resulting in severe injuries and death (Trejos & May-Collado 2015). In an ongoing study by graduate student Betzi Perez, she finds that there is evidence of increasing stress hormones in these dolphins during times of high boat activity, which increases concerns of the impact of these hormones on immune system function and reproductive success (e.g. female calving cycles, calf survivorship).

Previous research has found that reducing the impact of boats to only two dolphin-watching boats at a time should be allowed to interact with a group of dolphins, with a 30-minute resting period between interactions (May-Collado, et al. 2014). The government of Panama does have regulations in place for dolphin-watching boats, to ensure safety of the dolphins. However, there is no one to enforce these rules on site. Future management strategies to protect this dolphin population should include a monitoring system of boat-dolphin interactions and control the number of boats that are in the bay at one time. Minimizing the number of boats will result in fewer interruptions during key biological behaviors such as foraging and reproduction (Kassamali-Fox, et al. 2015), and reduce the potential negative impacts of a stressed population.

There are four more years of photo-ID data that needs to be included to generate a better understanding of long-term changes in population size in the Archipelago. Therefore, all dolphins in the Archipelago will be included and not just those in Dolphin Bay, which will help determine if the population is indeed divided into communities (sub-populations). Information on the sex of dolphins, reproductive status, and mortality will also be included in order to gain a better representation of the population trends.

In conclusion, the core population of Dolphin Bay is small, isolated, stressed, and at risk of decline. This study helps provide some insight into their population status and trends.

ACKNOWLEDGEMENTS

Thanks to Dr. Laura J. May-Collado for the data and the assistance throughout this entire scientific process. Thank you also to Erin Powell, Kahlia Gonzales, Natalia Swack, and Hannah Hutchens for their assistance and knowledge during the photoID process.

RESUMEN

Porque de la superposición de hábitat, las actividades de humanos pueden ser una amenaza a las poblaciones costeras de los delfines nariz de botella, que es la situación en Bocas Torito en el archipiélago de Bocas del Toro, Panamá. Esta populación es aislada genéticamente, con los machos y las hembras demuestran fidelidad de sitia alta. Están sufriendo la presión de interacciones intensas con los botes que ven los delfines. Por lo tanto, tener una estimación de la tamaña de la populación y la variación encima del tiempo es importante para establecer su estado de conservación. En este estudio, la identificación de las fotos y cuatro modelos de captura y reconquista utilizan para estimar la tamaña de la populación. La modela de mortalidad ajuste los datos mejores y indica que la populación en Bocas Torito es entre 39-48 delfines. Hay evidencia para las fluctuaciones en la tamaña de la populación durante los años. Estas diferencias pueden estar de la variación en las esfuerzas de muestra, pero también indica tiempo de mortalidad alta. Este estudio demuestra que los delfines en Bocas Torito están en riesgo. Están vulnerable a las actividades que los elige como blanco, como ver de los delfines, porque de su populación pequeña, aislamiento, y dependencia de la bahía. En el futuro, la investigación tratará con las diferencias en las esfuerzas de muestra e incrementará la magnitud de la muestra.

Palabras clave: identificación de las fotos, captura y reconquista, conservación

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Diversity of Signature Whistles in Bottlenose Dolphins (*Tursiops truncatus)* of Dolphin Bay, Bocas del Toro, Panama

Rebecca Daw
University of Vermont, College of Arts and Sciences, Department of Biology, Burlington, VT 05405, USA
Correspondent rdaw@uvm.edu

Bottlenose dolphin whistles play an important role in their communication. Their whistles are often classified as (1) variant whistles, which are not individual specific and are produced in the context of group cohesion when traveling, foraging and socializing; and (2) signature whistles, which are individual specific and used to maintain contact between specific group members (e.g. mother-calf). Given their importance in communication, here I analyze the diversity and presence of signature whistles the in Bocas del Toro dolphin population. Using recordings obtained from passive acoustic monitoring and boat follows, a total of 84 signature whistle occurrences were seen, with 40 different types being identified. The number of signature whistles demonstrated the diversity of signature whistles as well as their complexity. Additionally, the majority of whistles were emitted during social behaviors and while tour boats were present, supporting signature whistles' use as contact calls. Future studies should continue to look at tour boat impact on signature whistle emission as well as attempt to pinpoint individual dolphins to record in order to match individuals with their unique signature whistle. Doing so would allow a better understanding of signature whistles and allow passive recorders to be used more to study underwater dolphin behavior.

Keywords: acoustics, communication, marine behavior, passive monitoring

I. INTRODUCTION

A vital aspect of bottlenose dolphin behavior is their communication. The most common signals they emit are whistles, which are narrow banded, frequency-modulated sounds (May-Collado and Wartzok 2008). Bottlenose dolphins are known to produce two types: (1) variant whistles that are used by all dolphins for group cohesion when traveling, foraging and socializing and (2) signature whistles, which are unique to each individual dolphin and used as contact calls (May-Collado and Wartzok 2008; Janik and King 2013; Hiley et al. 2016).

Most of dolphin whistle research has focused on the importance of signature whistles as contact calls. Unlike variant whistles, which change due to habitats and behavioral contexts, signature whistles are developed through vocal learning. Young dolphins listen to signature whistles being emitted early in their lives and develop their own signature whistles often similar in contour to those emitted around them. This is usually their mother's, other close relatives, or close members of their group. (King et al. 2013; Janik and Sayigh 2013; Janik et al. 2006). Research has shown that mother dolphins will increase the frequency they emit signature whistles during the first few weeks of their calf's life, potentially helping them in their vocal learning (Erb et al. 2016). Once developed, a dolphin's signature whistle will remain stable for the rest of the dolphin's life. Though dolphins have the capability of copying each other's signature whistles, only those with close relationships do this and even then, the copy is slightly modified (King et al. 2013; Janik and Slater 1998). This allows signature whistles to act as unique identifiers for dolphins assisting in group cohesion, mother-calf interactions, and male-male alliances (Erb et al. 2016; Janik and Slater 1998; Mann et al. 1993; Watwood et al. 2004).

Previous research done has indicated that signature whistles rely on the contour, or shape of the whistle in order to convey the identity information encoded within it. When dolphins were played back signature whistles with altered vocal characters, the whistles were still recognizable to the dolphin. This indicates that signature whistles are independent of vocal cues, most likely due to the fact they can be used by other dolphins to refer to an individual, and thus should not rely on vocal features, as well as the fact that living in water presents an unreliable environment for vocal cues such as frequency (Janik et al. 2006, Janik et al. 2017).

In this study I analyzed acoustic data of bottlenose dolphins from a resident population of 72-87 dolphins in the Archipelago of Bocas del Toro, to identify signature whistles and determine the context at which they are produced. This research will hope to catalog as many signature whistles as possible from this population in the hopes of matching signature whistles to their corresponding dolphin. By examining recordings previously gathered from a research boat that also photo identified the dolphins around the boat at that time, it is predicted that a signature whistle catalogue can be made for this population as well as several individuals matched to their whistles. Additionally, once individuals are matched, passive recordings containing signature whistles will then be examined for individual dolphins, in the hopes that whistles can be identified as belonging to certain individuals and a greater understanding of dolphin behavior without a boat present can be obtained.

II. MATERIALS AND METHODS

A. Study Site

This study took place in Dolphin Bay also known as Boscastorito (9.23N/-82.24 W) in the Archipelago of Bocas del Toro, Panama. This site has shallow, clear waters with the bottom covered in a variety of surfaces including mud, coral, sea grass, and mangroves. Only bottlenose dolphins are found in this area and dolphin watching is highly prominent. (May-Collado and Wartzok 2008; May-Collado and Wartzok 2015).

B. Recordings

The whistles recordings were obtained from passive acoustic monitoring and from boat follows. The passive recorders were deployed in four sites: Dolphin Bay, Almirante, Tierra Oscura and Sharkhole. The passive acoustic recorders were model RUDAR-mK2 (Sampling rate up to 96kHz -169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com) and programmed to continuously record the soundscape in segments of 30 minutes at sampling rate of 44 kHz and 16 bits. Only bottlenose dolphins are found in this area (May-Collado and Wartzok 2008). Recordings from boat follows were done using a broadband recording system consisting of a RESON hydrophone 4033 (203 dB re 1 V/lPa, 1 Hz to 140 kHz; RESON Inc., Goleta, California) connected to an AVISOFT recorder and Ultra Sound Gate 116 (sampling rate 400–500 kHz, 16 bit; Avisoft Bioacoustics, Berlin, Germany) that sent the signals to a laptop computer (May-Collado and Wartzok 2008).

Recordings were analyzed in RAVEN PRO 1.5 build 37 (2017; Cornell Lab of Ornithology). For each whistle found in a file, it was determined to be signature or variant using the Signature Identification (SIGID) method. This method states that individual whistles of the same type that occur more than once within a 1-10s interval between them can be classified as a signature whistle (Janik and King 2013). As signature whistles were emitted multiple times, only one whistle was selected for each round of emissions. Signature whistle types were only selected more than once if they occurred after a significant time lapse or in another file. In some cases, whistles would be emitted both in and out of the designated interval of 1-10s. These whistles were still considered signature whistles and this occurrence was noted.

In order to attempt to match dolphins with their corresponding signature whistles, photo ID analysis previously done on the Dolphin Bay bottlenose dolphin population was compared to which whistles were emitted when (May-Collado et al. 2015). To understand the context at which signature whistles are emitted, corresponding sighting information on behavior and presence of tour boats was used.

III. RESULTS

A total of 84 signature whistles occurrences were seen. Of these, 59 signature whistles were extracted from the recordings done with boat follows and 25 were extracted from passive recordings. Of the total occurrences, 40 different signature whistles contours were determined (Fig. 1) and 77% were sine shaped. The most emitted signature whistle was type DA, which was observed on eleven separate occasions (Fig. 2).

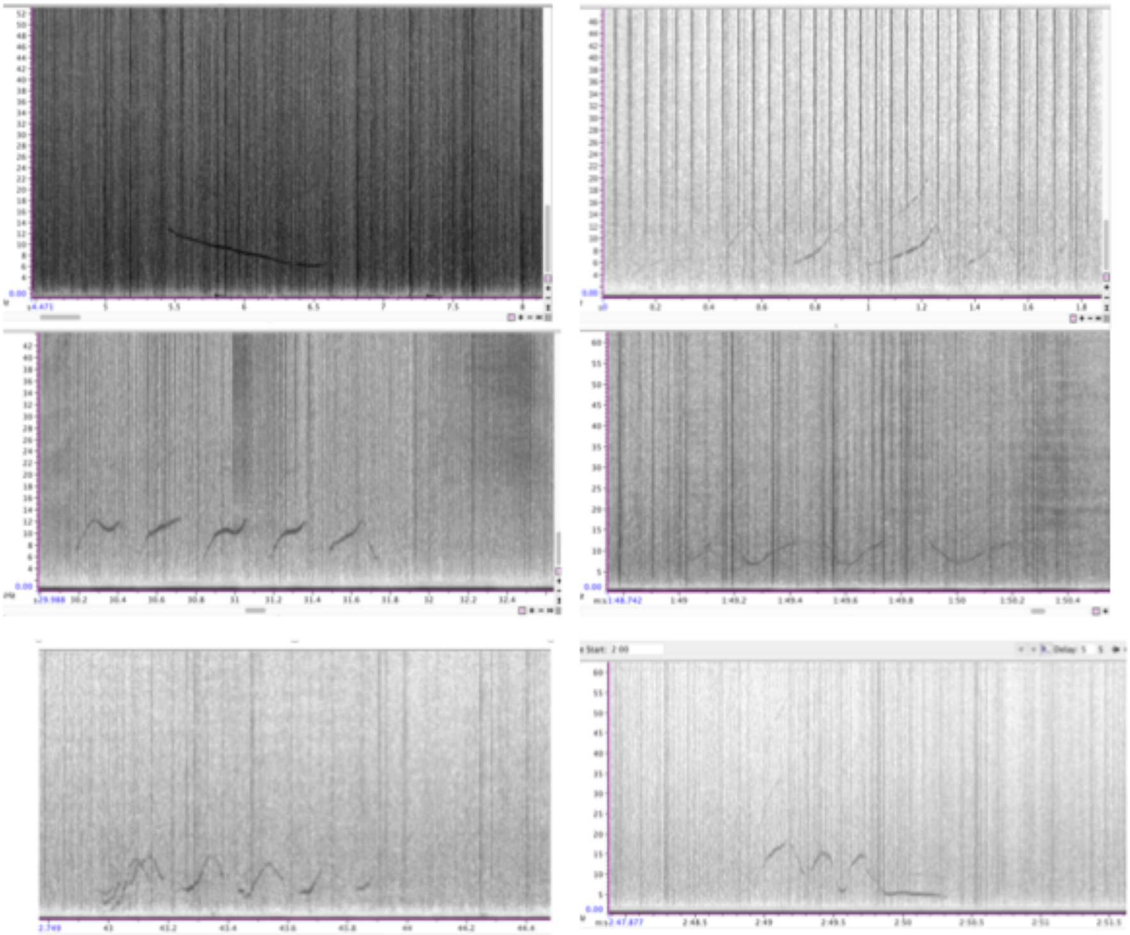

FIG. 1: Images of the six most common signature whistle contours found in the Bocas Del Toro bottlenose dolphin (*Tursiops truncatus*) population.

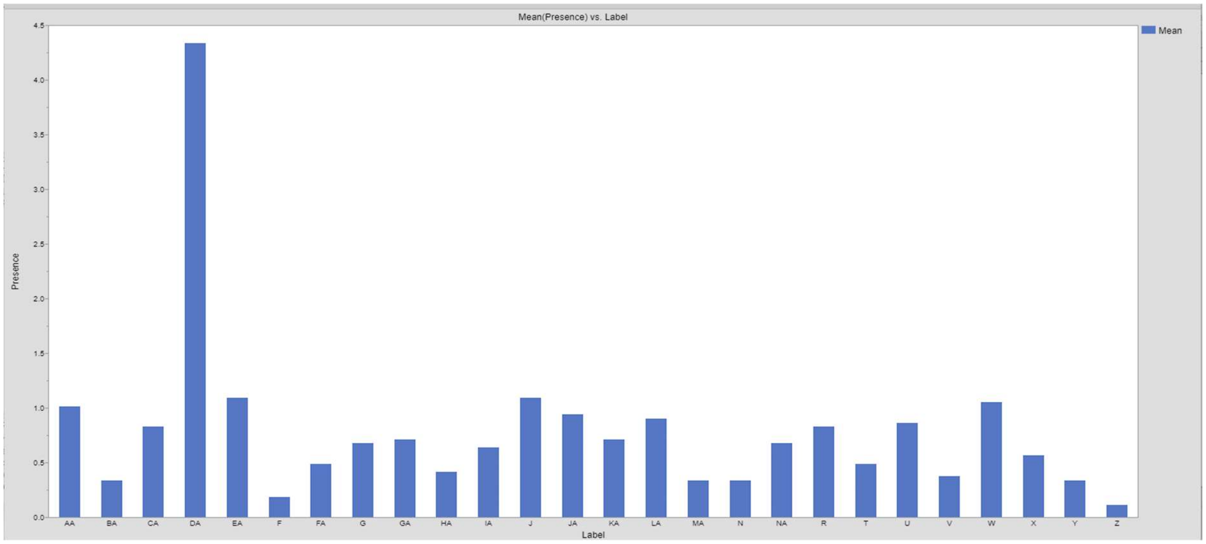

FIG. 2: Mean presence of each signature whistle contour found in the Bocas Del Toro bottlenose dolphin (*Tursiops truncatus*) population.

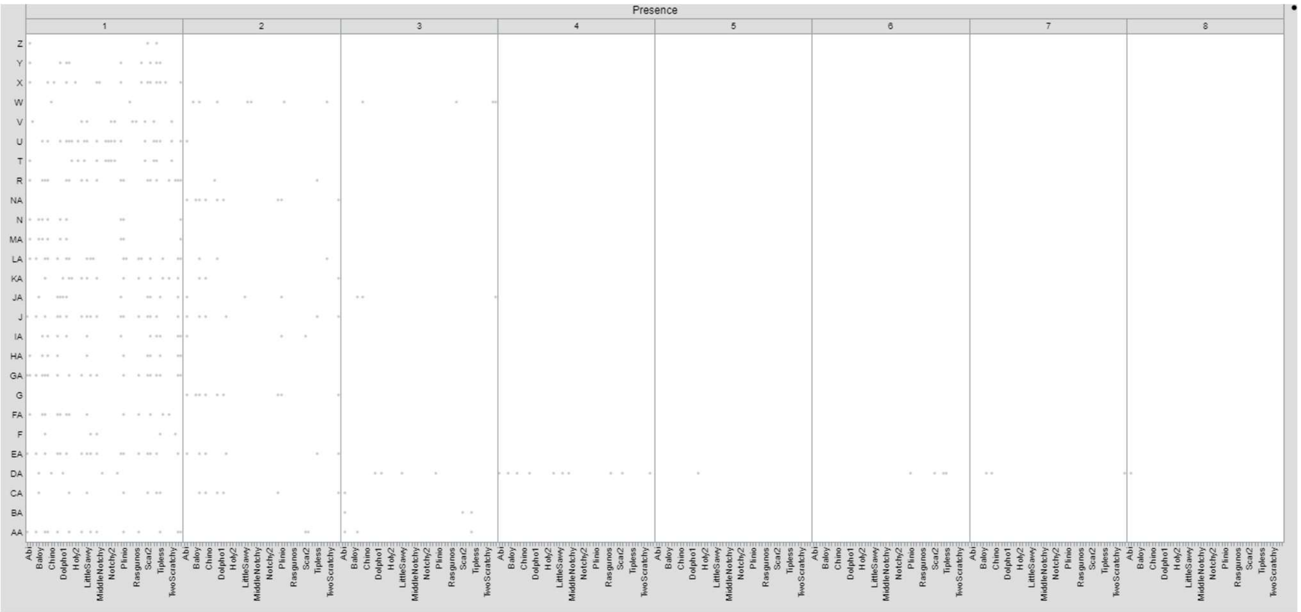

FIG. 3: Total number of presences of each bottlenose dolphin (*Tursiops truncatus)* individual seen in Bocas del Toro when each whistle contour was seen.

In terms of context, about 62% (n=59) of the whistles were emitted when the dolphins were engaged in social behaviors and the remaining when the dolphins were traveling (21%), foraging (13%) and milling (4%). Signature whistles were primarily (72%) produced when tour boats were present. No dolphins could be matched to a signature whistle; however, some individuals were present more often others when signature whistles were produced (Fig.3).

IV. DISCUSSION

In this study, we showed the Bocas dolphins produce signature whistles depending on the context and presence of tour boats. Previous research has shown that individual recognition from signature whistles is not embedded in the frequency and temporal characteristics of the whistle but in their contour (Janik et al. 2006). This means while the frequencies and other vocal characteristics can differ, bottlenose dolphin populations must have a diverse number of contours for each to serve as an individual identifier. In this study, most signature whistles were sine shaped; these complex contours are responsible for transmitting information (Janik et al. 2006).

That signature whistles were predominantly produced during social behaviors supports previous work that signature whistles promote group cohesion and are often used when groups meet each other in the wild (Janik and Sayigh 2013). Another interesting result is that over 70% of the signature whistles were emitted when the dolphins were in the presence of tour boats. May-Collado and Wartzok (2008) have shown that Bocas dolphin shift frequency and duration during these encounters presumably to avoid signal masking. This is the first time we see a shift in whistle type emission as a result of interactions with boats. It is important to note that in Bocas, dolphin-watching activities are intense and often lead to the separation of group members including mother-calf pairs (May-Collado et al. 2015, Kassamali-Fox et al. 2015). Previous work has shown that signature whistle are produced when animals are isolated from their groups (Janik and Sayigh 2013), thus it is not surprising that emission and repetition of signature whistles increases during such encounters.

Because the Bocas dolphin population is small, and animals overlap in their home range, it became impossible to match the signature whistles to specific dolphins. However, we were able to narrow down the individuals to a small group of residents. With an established signature whistle catalogue, the next step is to individually record dolphins in the field. Assigning dolphins to whistles will be of significant use to continue monitoring the population using more cost-effective methods such as acoustic passive monitoring. As signature whistles are unique to the dolphins, signature whistles can be used to identify dolphins, how they individually respond to specific conditions (like boat presence), and to generate new insights about their natural history.

V. CONCLUSIONS

This study demonstrates the complexity and diversity of signature whistles, most likely due to the information they must convey to the receiver. As so much diversity in contour was found, this supports the theory that the individual information they are conveying is encoded in the contour of the whistle, rather than the frequency or other vocal characteristics. The data also supports some of the already known functions of signature whistles primarily their use in group cohesion and when dolphins are isolated. Understanding when dolphins use their signature whistles is important for understanding their behavior and vital in creating better conservation strategies for them. As not enough data was found in order to match dolphins to their unique signature whistle, more research should be gathered to do so. Not only would this provide greater insight on the Bocas del Toro dolphin population, but also it would allow passive recordings to be used more in dolphin behavior monitoring.

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Effects of boat traffic on the mating call acoustic structure of the Bocon Toadfish (*Amphichthys cryptocentrus*) in the Archipelago of Bocas del Toro, Panama.

Cooper Peterson,[1a]

[1] University of Vermont and State Agricultural College, Burlington, Vermont 05401, USA

Abstract. Increasing urbanization of coastal areas have resulted in an increase of noise levels associated to boat traffic. Because sonorous fish are important component of the marine communities, they have become important indicators of habitat quality. The Bocon Toadfish (*Amphichthys cryptocentrus)* is a common Batrachoidids species found in the Archipelago of Bocas del Toro, known for is mating calls, boatwhistle. In this study I study the acoustic structure of these signals and the potential impact of boat traffic on their structure. The results indicate that the bocones in the site with high boat activity emit mating calls that are lower in frequency, short in duration, and louder than those emitted by the bocones in the low boat activity site. This suggest that bocones in high traffic areas are under natural and sexual selection. Potential for signal masking may have led to evolve signals that have less attenuation and that can be detect by females in a noisy space.

Keywords: acoustic analysis, soundscape activity, descriptive variable, anthropogenic disturban

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I Introduction

In the past decade there has been an increased focus on determining marine ecosystem health via soundscape analysis (Linseth 2018). The emerged patterns are that noisy soundscapes correspond with more diverse and healthy communities (Lobel 2013). A common contributor to the soundscape of tropical marine communities is the toadfish (Batrachoidiae) (McIver 2014). The toadfish males are territorial and emit mating calls, boatwhistle to attract females (Amorim 2006). Toadfish create nesting dens in less than 1-meter depths and primarily reside there while conducting mating calls at a 200 Hz sound, and the strength and distance of mating calls can be varied based on the substrate of the sea floor (Fine and Lendhardt 1983). Longer-duration calls tend to be more attractive to females (Staaterman et, al. 2018). Mating calls are longer-duration 'boatwhistles' at 250 to 650ms (Mensinger 2014) during which competing males may pulse agonistic 'grunts' over just 100ms (McIver 2014; Lindseth 2018; Balshine 2018). However, it is possible that females can miss calls and be unable to localize far-field signals (Fine and Lendhardt 1983). Because male calls are short and rapid (Lobel 1992, Lindseth 2018) they will often send multiple calls rapidly as a form of redundancy to ensure signal transmission to mates (Fine and Lendhardt 1983). Since Toadfish also have control over the temporal axis of their sound production (Barimo and Fine 1998) they can stop calls while disturbed.

In the Caribbean Sea the Bocon Toadfish (*Amphichthys cryptocentrus*) is a common species whose behaviors and acoustic activity remain relatively unknown (Staaterman 2018). Although the acoustic activity of the Batrachoidids has been previously studied, these studies have largely focused on the mechanism by which toadfish produce these iconic sounds rather than focusing on the importance of the call acoustic structure in relation to the habitat or behavioral context (Amorim 2016). As noted by Lobel (2013), it is important to understand the factors that influence Bocon acoustic behavior as the species fish can be used as an indicator of habitat health in tropical marine ecosystems. Increasing urbanization of coastal communities is translating in increasing levels of noise that are negatively transforming the acoustic space of many sonorous organisms. The impact of man-made noise can be detrimental for interspecies communication and could negatively impact fish reproduction success (Lobel 2018). In this study, I describe the acoustic structure of the Bocones mating call ‘boatwhistle’ in two sites within the Archipelago of Bocas del Toro in Panama that vary in boat traffic activity. My prediction that individuals call acoustic structure will reflect the noise conditions of each site. Toadfish tonal boops suffer from transmission loss over long distances more so than broadband grunts do (Staaterman 2018) therefore in noisy conditions toadfish are expected to use acoustic traits that optimize signal remission.

II Methods

1. Study site

The Bocon is a new-world toadfish species distributed on the southern Caribbean Sea (Staaterman 2018). This study took place in two locations within the Bocas del Toro Archipelago of Panama; Almirante (9º17.34' N, 82º 19.92' W) and Sharkhole (9º 11.04' N, 82º 10.56' W). Both sites are characterized by having coral reef substrates which rigidity allows acoustic signals to propagate farther (Barimo and Fine 1983). While similar in seafloor and habitat structure, the sites differ in boat traffic (and associated noise levels). Almirante is a port from which boat-taxis exit and enter the main island, Colon, on schedule every day between 6 a.m. and 6 p.m. Sharkhole is located away from boat transit routes and thus experience less boat activity throughout the day. Recordings were obtained from March and early April 2017, which coincides with the mating season of the Bocon (Amorim, 2006).

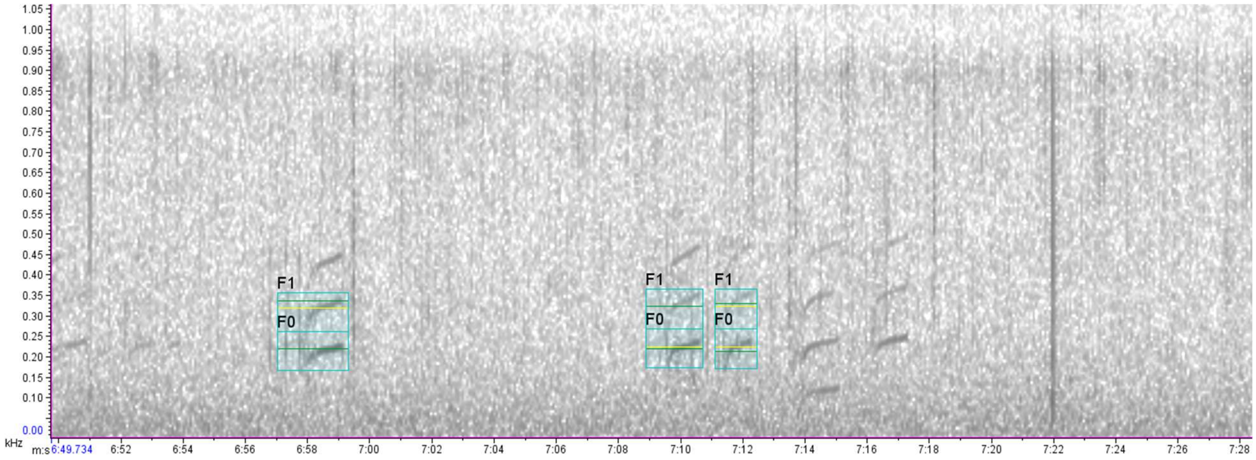

FIG. 1 Examples of redundant boatwhistles over 40 seconds emitted by Toadfish from Bocas del Toro, Panama. Calls highlighted (light blue; fundamental frequency F0 and first harmonic F1) with Center Frequency (yellow) and Peak Frequency (green) overlaid.

2. *Boatwhistle recordings and analysis*

Toadfish boatwhistle mating calls were recorded using a RUDAR-mK2 (sampling rate up to 96kHz – 169dB re: 1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com). Recorders were programmed to sample the soundscape continuously in 30-minute segments with a sampling rate of 48 kHz and 16 bits. A 1-minute sample for every 5 minutes of recordings were taken and uploaded to the online analytical platform of ARBIMON II (https://arbimon.sieve-analytics.com) for cataloguing and inspection. Selected recordings were then retrieved and analyzed from the raw data.

High-quality toadfish boatwhistles were manually selected and analyzed using the program RAVEN 1.5 (Cornell Laboratory of Ornithology, New York) using a spectrogram a Hann window set with a Fournier resolution of 7000 Hz, with contrast set to 65 and brightness to 55. A frequency reference frame was set from 0 to 1050 Hz, and a 512 and power reference frame from 90 to 140 dB. The scale of time was set at 2-second intervals.

High quality calls were chosen for multiple harmonic bands with dark coloring (see Fig. 2). A boatwhistle was identified as distinct without heavy masking from interference and identified as redundant when a near-identical harmonic banding pattern was seen multiple times within a two-minute segment. Selection of boatwhistles was kept standard by capturing the entire frequency range and duration of each individual harmonic and tagging a set of stacked harmonics as one individual's mating call. The following standard acoustic variables (Lobel 1992) were measured on six standards for analysis of harmonic frequency: low frequency (LF), high frequency (HF), center frequency (CF), peak frequency (PF) time duration, and max power.

3. Statistical Analyses

The statistical software JMP Pro 14 (SAS Institute Inc.) was used for statistical analyses. Descriptive statistics were performed to procure mean, standard deviation, frequency ranges and coefficient of variation values for boatwhistles. Six frequency variables were Box-Cox transformed to normalize the data distribution. Multivariate analyses of variance (MANOVA) were performed to determine whether frequencies (by LF, HF, CF, PF), duration, and maximum power varied between individuals. Box student *t*-tests were used to evaluate variation between variables. MANOVA analyses were adjusted by a Bonferroni procedure to correct significance ($p = 0.0008$) to account for type I error. A scatterplot of low frequencies against high frequencies was made to visualize any variation between harmonic frequencies. Box plots were created to compare harmonic structure across individuals.

III Results

Toadfish emitted boatwhistles with two to four harmonics, with most recordings showing three or four harmonic bands (Fig. 2). Table 1 shows the descriptive statistics for each of the acoustic variables per site. There were significant differences in boatwhistle acoustic structure between the sites, with toadfish emitting boatwhistles that were significantly lower in low, high, peak and center frequencies (Confidence: 0.95, Student's *t*: $p < .0001$), shorter in duration (Confidence: 0.95, Student's *t*: $p < .0001$) and louder (Confidence: 0.95, Student's *t*: $p < .0001$) in the high traffic site (Almirante) than the boatwhistles emitted by toadfish in the low boat traffic site (Sharkhole) (Fig.3). The overall frequency range shown in figure 4 suggest the possible documentation of two species of toadfish in the stud sites. Regardless the acoustic patterns based on boat activity are the same as described above.

TABLE I. Descriptive statistics for Toadfish boatwhistle frequencies. Means given with standard deviation (SD), Range and Coefficient of Variation (CV) values provided.

Location	Stats	Low Freq. (Hz)	High Freq. (Hz)	Center Freq. (Hz)	Duration (s)	Peak Freq. (Hz)	Max Power (dB)

Almirante, N=230	Mean ± SD	153.289±63.386	216.866±77.452	181.522±70.134	1.1652±0.4796	181.373±68.269	123.428±4.539
	Range	414.500	477.100	474.600	1.964	474.600	27.100
	CV	41.351	35.714	38.639	41.164	37.640	3.678
Sharkhole, N=126	Mean ± SD	346.676±124.579	406.678±125.452	381.00±125.051	1.794±0.543	328.048±124.421	99.684±3.690
	Range	276.200	264.500	269.500	2.321	275.300	17.500
	CV	35.935	30.848	32.822	30.266	32.567	3.702

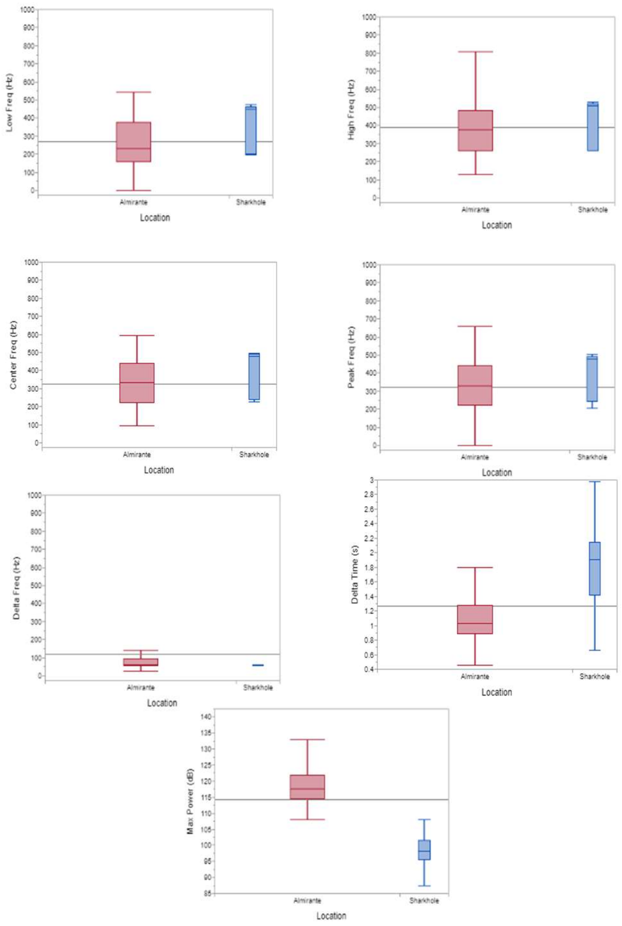

FIG. 4 Boatwhistles call acoustic structure differences in sites with high boat traffic (Almirante, n=230) and low boat traffic area (Sharkhole= 126)

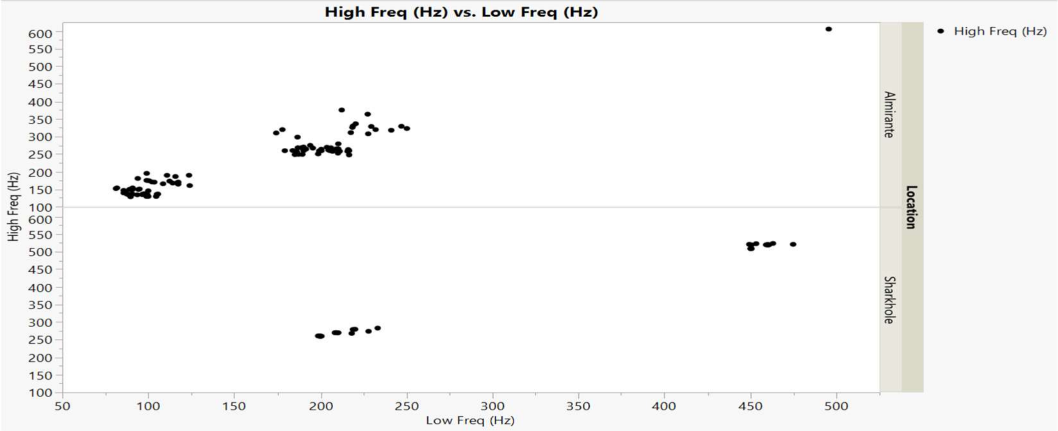

FIG. 4 Frequency range for toadfish mating calls in the high boat traffic area (Almirante: n=230) and low boat

IV . Discussion

In this study we find that the Bocon emits low frequency, short duration, and loud mating calls in the site with higher boat activity (Almirante). Body size is one of the most important morphological factors believed to influence animal signal frequency (Marquet and Taper 1998). Broadly, body size and the size of sound producing organs correlate (Fletcher 1992) and size of vocal tract places physiological constraints on signal production. For example, small body sizes (small sound producing organs) limit animals to the production of relatively high-frequency signals, which are more subject to sound attenuation and degradation, limiting the range at which animals can communicate. In Batrachoidids sounds production has been shown to be proportional to the size of their swim bladders (Amorim et al. 1983). Given the advantages of low frequency sounds, we suspect that increasing noise levels in Almirante have favor the evolution of low frequency mating calls in a relatively short period of time. The boat whistles of toadfish are known for have low propagation ranges. In addition, shortened calls are less attractive to females and have an increased chance of being missed altogether (Staaterman 2018; Fine and Lendhardt 1983). In a location with high noise levels females are more likely to miss male calls and unable to localize far-field males, thus louder and lower frequency sounds may compensate for short signals and allow males to overcome masking and reach nearby females.

Previous research has shown that in other toadfish species longer-duration calls tend to be more attractive to females (Gerhardt 1991; Shaw and Herlihy 2000). Longer call durations in the low boat traffic site may allow for more competition among territorial males. Mize (2018) found that in this same site calling rate is significantly higher than in Almirante, suggesting that there are more competitive interactions among males at this low-noise location.

IV Conclusion

A comparison between two sites within the Bocas del Toro Archipelago revealed insights into the potential impacts of anthropogenic disturbance upon the acoustic behavior of the Bocon Toadfish *Amphichthys cryptocentrus*. The effect of continuous disturbance has created a noisier environment in Almirante, leading to various negative impacts upon the behavior of the Bocon.

The toadfish produce louder and shorter calls in order to compensate for a noisier environment. Toadfish in Sharkhole, by contrast, appear to produce longer calls that may be indicative of males being more ably competing for females. The increasing water traffic in the Bocas del Toro Archipelago may be causing selective pressures towards lower frequency mating calls, as noted by scattered overall frequency ranges in these data. These impacts should not be taken lightly, as they may be indicative of a similar trajectory for other species within Panama's waters. Future studies should dedicate resources to close-range monitoring of toadfish for more accurate acoustic recordings without masking from the environment (such as scuba-based hydrophones or fabricated burrows in a shallow pool). Other studies could also pursue translocating fish from one population to another, and examining whether or not Bocon will change their boatwhistles in response to a change in soundscape

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Social Structure of Bottlenose Dolphins in Bocas del Toro Panama

ERIN POWELL UVM Zoology Department ecpowell@uvm.edu

ABSTRACT

Bottlenose dolphins live in fission-fusion societies, in which individuals associate in small groups that change composition often on daily or hourly basis. Because these associations are strongly dependent on context, we can gain insights on residency patterns of individual dolphins and identify key members of the population that are at the center of their society. This study uses long-term photo-ID data (2004-2014) to study the association patterns of 50 of the resident bottlenose dolphins in the Archipelago of Bocas del Toro in Panama using the Half Weight Association Index (HWI). The results indicate the possible presence of two dolphin communities within the Archipelago one outside Dolphin Bay (n=8) with low association values and another resident to Dolphin Bay and nearby areas (n=42) with multiple associations above 0.8. Dolphin Bay is an important habitat for these dolphins it provides safety from predators and abundance food resources. Therefore, the observed relationships may be the result of home-range overlap. Future studies should include information on sex, kinship, behavior, and home range to better identify the factor driving these relationships. This is important, as Dolphin Bay dolphins are frequently targeted by dolphin watching boats, in which encounters have resulted in deadly collisions.

Key words: fusion-fission society, *Tursiops truncatus*, association patterns, community structure

INTRODUCTION

Understanding an animal species social structures is an integral part of understanding the species (Lusseau et al 2006). Bottlenose dolphins live in fission-fusion societies where individuals associate in small groups that change composition often on daily or hourly basis and where some individuals will consistently associate with one another (REFS). These associations can be based on multiple factors. For example, dolphins near Grand Bahama Island, Bahamas, form closest associations with individuals of the same sex 74% of the time (Rossbach and Herzing 1999). In Shannon Estuary, Ireland, dolphins association preferences are on the basis of age, with juvenile dolphins associating together and adult dolphins associating together (Baker et al. 2017), contrasting the dolphins in Sado Estuary Portugal, where association patterns show no preference for sex or age (Augusto et al. 2011).

The bottlenose dolphin population of Bocas del Toro is small, genetically isolated from other Caribbean populations, and how high levels of site fidelity (May-Collado et al. 2015, Barragan-Barrera et al. 2017). Previous research on this area indicates the population is under considerable pressure by local dolphin watching industry. These dolphins respond to tour boats by shifting from biological important behaviors to scape behaviors including swimming and diving (May-Collado et al. 2017), by changing their communicative signals to avoid signal masking (May-Collado and Wartzok 2008, May-Collado and Quinones 2014). Ongoing research is finding that stress hormone levels also increase during times of the year when dolphin watching activities are high. All these factors may affect how and when animals associate and may result in the deterioration of important associations between mature animals and young ones.

Here I study the social structure of the Bocas del Toro dolphins. Previous research has found that dolphins that live in isolated coastal populations tend to form strong and long-term associations than dolphins that live in open populations (Lusseau et al. 2003). Thus, I predict that the association values found in this population will be similar to those reported for other coastal populations. Area has been found to be an important factor in other dolphin populations (Baker et al. 2017). Dolphins have been observed practicing site fidelity for up to 17 years (Wells et al 1987) In addition, given the high site fidelity these dolphins show to some areas (May-Collado et al. 2015), I expect that area use will be a key factor in establishing long term associations.

METHODS

Study Site:

The Archipelago of Bocas del Toro is located in the Caribbean coast of Panama. The Archipelago is characterized by shallow and clear waters and bottom substrates consisting of seagrass, coral, and sand. This research is was collected within approximately 79.2 km2 of the inner part of the archipelago, with a focus on Bocas Torito Bay. Also known as Dolphin Bay, Bocas Torito Bay is a closed bay with a predictable resident dolphin population that attracts tourists on dolphin watching boats.

Data Collection:

Data were collected every year from 2004 to 2014 except 2005. The months data were collected in and how often depended on the amount of funding received that year, but the majority was collected in the summer. Outings occurred between 7am and 6pm, with the majority being in the morning.

The main mode of transportation between the islands and mainland was with powered boats with 50 and 150 hp engines, as well as canoes. Areas were surveyed using a 10 m fiberglass boat with two engines (150 hp/4-stroke) following predetermined routes. Once a group of dolphins was encountered the boat was SC/64/WW2 2 approached slowly and in a parallel position to avoid dolphin disturbance (Würsig and Jefferson 1990, Resolution ADM/ARAP NO. 01, 2007). A distance of 30-50 m distance to the group was maintained before turning the engine off to initiate data collecting and photo-ID. Photos were taken with a digital camera Canon EOS 10D, 6.3 Megapixel SLR and a digital Canon Rebel, both coupled with a 75-300 mm zoom lens.

Data Analysis:

Dolphins were identified using photo-ID. For each outing photographs of the dolphin's dorsal fins were matched to a catalogue of known fins. The highest quality photo from each dolphin in the outing was cropped, saved, and recorded into a matrix. They were identified by fin shape, notches, scratches, and any other identifiable mark (for example one dolphin was always identified by the bump on his back). If a dolphin was not recognized as any of the existing, but had a clear distinguishable fin, it was named and added to the catalogue.

A dolphin catalog of 142 dorsal fins from 2004-2014 was pruned to 50 animals that had a minimum of 10 recaptures to ensure sufficient individual representation for the analysis. Data were analyzed in SOCPROG 2.8 (uncompiled) using a Half Weight Association Index (HWI). The HWI ($HWI = 2N_T/(N_A + N_B)$, where N_T is the number of times two individuals (*A* and *B*) are seen in the same sighting, N_A is the number of times individual *A* was seen without individual *B*, and N_B represent number of times individual *B* is seen without individual *A*. The HWI ranges from 0 (animals never seen together) to 1 (animals always seen together) (Cairns and Schwager 1987).

Hierarchical cluster analyses were run at HWI levels of 0.4 and 0.6 to assess moderate and strong associations. Cluster analysis modulated for gregariousness were performed at both levels and graphed. These were done to assess the average summed association rate of individuals and to produce clusters of individuals with high mutual association rates. A sociogram was also created to visualize the social network.

RESULTS

The total of 50 dolphins analyzed were all sighted a total 1,311 times. The results indicate that regardless of HWI threshold the dolphins of Bocas del Toro appear to be organized into two communities, a small community with eight dolphins and a large one with 42 dolphins. The 0.4 HWI association threshold indicates the small community consist of a single social group, while the large community consist of three major social groups one of which is primarily found in Dolphin bay (light blue) (Fig.1). However, under this scenario the most social groups had associations values between 0.3 and 0.5 (Fig. 1). The 0.6 HWI identified two social units within the small community and nine within the large community (Fig. 2) where the most social groups have association values of 0.7 (Fig. 2). Social groups with HWI values above 0.8 consisted of couples and triads; one exclusively male (Sway3 and Curvy), one exclusively female (Valdez and Cristal), two mixed sex (e.g., Scar2 and Messy, Tipless2 and Almostclean) couples, and three mix sex triads (Dolpho3, Piquito, and Supermessy, and Bity, Topnotchy and Panama). Figure 3 shows a sociogram of the social connections between individuals where thicker lines connect individuals that socialize more often.

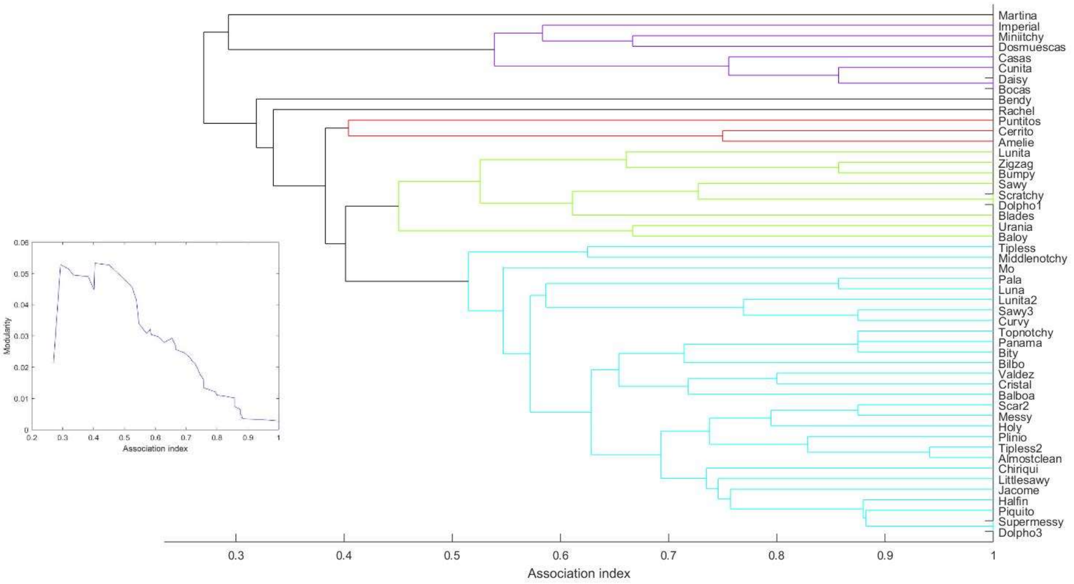

Figure 1: Hierarchical cluster analysis of the 50 most sighted dolphins in Bocas del Toro with a HWI threshold of 0.4. with modularity of expected proportions of association given the summed association rates between individuals in the Bocas del Toro bottlenose dolphin population with a HWI threshold of 0.4.

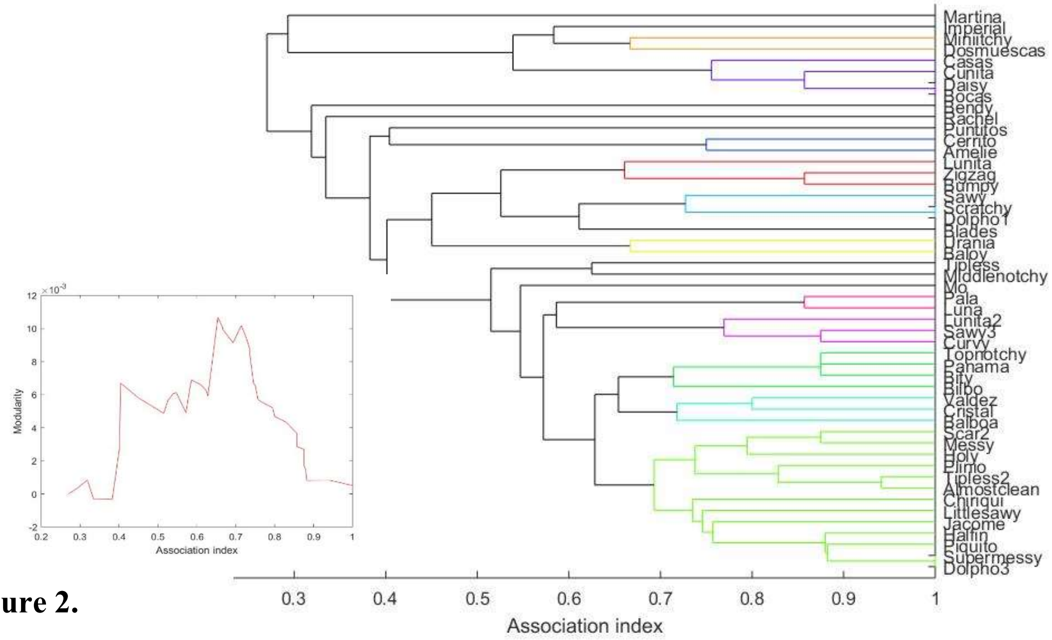

Figure 2.

Hierarchical cluster analysis of the 50 most sighted dolphins in Bocas del Toro with a HWI threshold of 0.6. Modulation by gregariousness of expected proportions of association given the summed association rates between individuals in the Bocas del Toro bottlenose dolphin population with a HWI threshold of 0.6

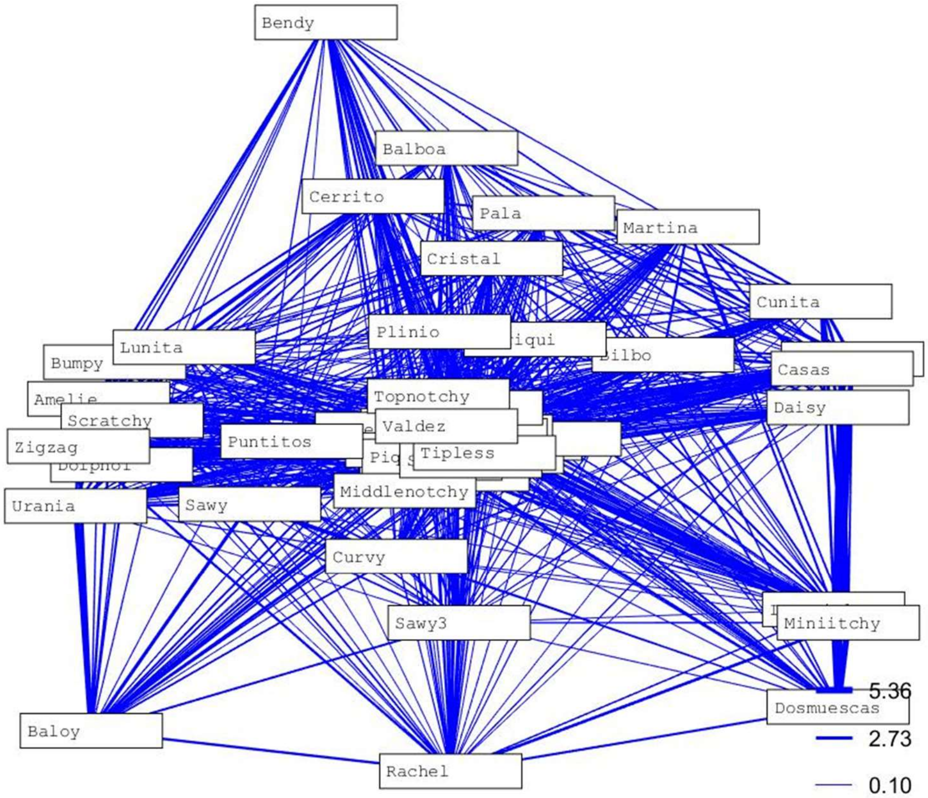

Figure 3. Sociogram illustrating the social network of bottlenose dolphins (n=50). Thickness of the line indicates strength of the association. The names within the nodes are the individual's names.

DISCUSSION

In this study we find that the study 50 dolphins associate in two communities within. Each individual interacts relatively frequency in loose groups (HWI <0.5) with a few long-term association (HWI >0.6). Social groups of all sizes appear to consist of dolphins of both sexes. In other studied populations bottlenose dolphins have been observed practicing long term site fidelity for up to 17 years (Wells et al 1987), for Bocas we know some of these animals are over 20 years old, and given their small home ranges and high overlap is expected that some of these animals social regularly.

The average HWI for most bottlenose dolphin populations is between 0.1-0.3 (Smolker et al.1992; Felix 1997; Connor et al. 2000; Quintana-Rizzo & Wells,2001; Chilvers & Corkeron 2002; Eisfeld & Robinson 2004). Although it has been observed as low as 0.01 in the Indian River Lagoon (a large lagoon where groups face physical separation) (Titcomb et al. 2015) or as high as 0.45 in Sado Estuary (very small geographically isolated population) (Augusto et al. 2011). Therefore, the average HWI for these dolphins between 0.3-0.5 is slightly higher than average, which makes sense with it being a smaller isolated population. The maximum HWI's for some of the groups within this population were very high. With there being six groups with HWIs over 0.8. Shannon Estuary in Ireland, a population I believe is similar, has a max HWI of 0.72 (Baker et al. 2017). This shows that although the HWI for the majority of the population is not unexpected for bottlenose dolphins, there are some surprisingly long term and close associations.

Dolphin Bay was the main site for most of the dolphins in this study, with 42 of the 50 dolphins belonging to the large Dolphin Bay social group. Dolphin Bay is both the main location of this research, and most sighting took place within the bay. The higher number of sighting of each dolphin gives more confidence in the associations between them. It is possible to have further research into just the dolphins within or outside Dolphin Bay to contrast the social structures. If this research was preformed, I would predict the outer population would have much looser associations. To do this we would need more data for the outer dolphin populations.

All three of the dolphins that did not belong to one of the four main clusters (Bendy, Martina, and Rachel) barely hit the minimum of 10 sightings. This can help to explain why they were observed having lower associations. In addition to the low number of sightings, two of the three (Rachel and Bendy) have very distinct dorsal fins and the third (Martina) lives outside Dolphin Bay. Because of this they are some of the easier dolphins to identify. These two dolphins can be identified with greater certainty in lower quality photos than many other dolphins that exist on the outskirts of the social network. Ease of fin identification did play a part in which dolphins were included in this analysis. The two dolphins seen the most times, Bity (n=57) and Supermessy (n=58), have two of the easiest fins to identify. Although this not the sole reason these two dolphins were identified the most (both live primarily in Dolphin Bay, were some of the earliest identifications, and are both part of highly associated triads), every photo that includes Bity or Supermessy can be identified.

All data over the 10-year period was pooled together. We know a few of the dolphins have died during this period, but they were still included if they were seen over 10 times regardless of mortality. Looking at the change in social structure before and after these individuals' deaths would be an interesting way of visualizing how social structure can change over time. Being able to look at how a population's social structure changes due to the loss of one individual would be an interesting way of seeing how important an individual is to the whole. One of the dolphins that has died is Dolpho3, had one of the highest HWIs with Piquito and Supermessy. Seeing how what associations Piquito and Supermessy have made post Dolpho3's death, as well as how the network has changed as a whole is something that can now be done. If the social structure of Bocas del Toro is significantly disturbed by the death of an individual, there is significant evidence in favor of protecting the whole population.

This study just looks at dolphins seen over 10 times from 2004-2014, however data from later 2014 up to 2018 exists waiting to be processed. The addition of these next four years could allow an inclusion of multiple dolphins that were close to reaching the minimum requirement of sightings. The inclusion of more data will create a more accurate depiction of the social network in Bocas del Toro, both inside and outside Dolphin Bay.

The only information included in this study are the sightings of the dolphins. Adding supplemental data like the known sex of dolphins or approximate age would improve understanding of the social patterns exhibited by these dolphins. Previous bottlenose dolphin social structure research have been able to incorporate this data, and it would be beneficial for us to as well.

Conclusion

The bottlenose dolphin population in Bocas del Toro's social associations are similar to many other small isolated populations throughout the world. They have main social groups, dolphins within Dolphin Bay, and dolphins outside. Within the Dolphin Bay large group, many smaller clusters form. Many of these clusters show long term close associations, that are slightly above what other dolphin populations create. There is no sign of sex-based associations both in the larger groups and the smaller. Groups of all levels consisted of both sexes, and the majority of HWIs over 0.8 belonged to mixed sex pairs or triads. The high associations between this population make it more vulnerable to boat disturbance. The increase in dolphin-based tourism puts the social structure at risk of being damaged by altering the dolphin's behavior through stress, and puts the dolphins at risk of being hurt of even killed through collisions.

Acknowledgments

Thank you to Laura May-Collado for all the data and mentorship. Thanks to everyone who collected and analyzed data in past years. And thanks to Amanda Jones, Hannah Hutchens, Kahlia Gonzales, and Natalia Swack for photo-IDing with me. RIP Zigzag.

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Singing patterns of humpback whales (*Megaptera novaeangliae*) in wintering grounds off the coast of Central America and potential negative effects of tour boat traffic.

Danielle McAree[1], Kate Ziegler[1], Shelby Rosten[1]
[1]*Department of Biology, University of Vermont, Burlington, VT, USA*

ABSTRACT

The waters off of the Pacific coast of Central America are important wintering grounds for humpback whales from the Northern and Southern Hemispheres. Predictable humpback presence in these tropical areas has sparked a growing whale-watching industry, generating concerns surrounding potential communication masking from boat noise. This study investigates the daily song occurrence of humpback whales and the impact of low-frequency engine noise on those patterns. Passive acoustic monitoring was used to record activity of whales and boats in one wintering ground of Costa Rica, Isla del Cano, and two areas in Panama: Coiba National Park and Islas Secas between September and November 2016 and 2017, respectively. Humpback whale singing activity and boat detections were higher in Costa Rica than in the Panama. In Costa Rica, male humpbacks sang throughout the day with a decrease during mid-day hours. This decrease in singing activity between 8 a.m. and 4 p.m. was correlated with an increase in boat activity. In contrast, humpback whales off the coast of Panama showed more variability in their singing patterns and did not appear to be influenced by boat presence. The discrepancies in humpback singing patterns may be attributed to the designation of Coiba and Secas as transit areas rather than singing grounds for the males. Differences could also be due to the time of the year in which the data was collected. Our study provides the first assessment of humpback whale singing activity for both countries which can aid in informing conservation and management decisions.

RÉSUMÉS

Les eaux de la côte Pacifique d'Amérique centrale sont des aires d'hivernage importantes pour les baleines à bosse des hémisphères nord et sud. La présence prédicable des baleines dans ces zones tropiques avait déclenché une industrie croissante d'observation des baleines, générer préoccupations concernant le masquage potentiel de la communication par le bruit des bateaux. Cette étude examine les chants quotidiens des baleines et l'impact du bruit basse fréquence sur ces modèles. La surveillance acoustique passive avait été utilisée pour enregistrer l'activité des baleines et des bateaux en Parc National de Coiba et Îles Secas entre septembre et novembre 2017. Contrairement à la recherche précédente, nos résultats ne montrent pas de motif diurne distinct dans le chant des baleines, malgré une hausse d'activité des bateaux entre 0600 and 1400h. Ces résultats sont significativement différents que les observées a Île Cano en Costa Rica, qui ont des instances plus hautes de trafic de bateau de jour et moins de chant des baleines pendant cette période. Les contradictions en modèles acoustiques des baleines sont dues aux différences de la période de l'année et la proportion de bateaux à chaque site. Notre étude fournit une assissent acoustique d'un zone important pour la reproduction et l'allaitement des baleines et l'écotourisme panaméen. Cette information peut aider à informer les décisions de conservation et de gestion.

Keywords: autonomous recorders, behavior, ecotourism, migration, noise pollution

INTRODUCTION

Humpback whales (*Megaptera novaeangliae)* are a widely distributed species of mysticete whales that are found in all of the world's oceans. This species follows one of the longest annual mammalian migration routes from high-latitude summer feeding areas to low-latitude winter breeding areas (Herman et al. 2002). While in tropical lower-latitude breeding waters, males produce songs composed of specific parts that can last ten to fifteen minutes, and will repeat the entire song several times. The function of these songs is still not fully understood, but a major theory suggests that male humpback whales "display" for females in the form of song and compete with other males for territory and access to female mates (Oña J. et al. 2016). Therefore, song plays a crucial role in reproductive success and fitness levels in humpback whale populations.

New methods of acoustic monitoring and technological developments have allowed further insight into the breeding song structures and acoustic tendencies of marine species. The occurrence of humpback whale songs is variable over days and seasons. In the Corcovado Gulf feeding ground off of southern Chile, humpbacks were observed to sing more during twilight and at night (Español-Jiménez 2018). A similar trend was also observed in feeding grounds off of Nova Scotia (Kowarski et al. 2018). Whales wintering in Kauai and Maui also tend to sing more at night (Helweg and Herman 1994; Au et al. 2000; Baird et al. 2000). In feeding grounds, the trends in song activity have been attributed to reduced hunting efficiency at night, which leaves more time for singing (Baird et al. 2000).

The ease with which sound travels underwater has raised concerns about potential communication masking and noise pollution produced from anthropogenic sources, particularly due to the increasing shift away from the hunting and exploitation of whales to ecotourism in the form of whale-watching. Human-caused sounds have become the most significant source of low-frequency sounds in the ocean, with shipping traffic producing noise that falls into the same frequency categories as various components of the humpback whale's song (Ross 1976). Scientists in Brazil found that on recordings where boat noise was present but was not loud enough to mask whale signals on the recording, boat acoustics had a negative effect on singing activity (Sousa-Lima and Clark 2008).

The objective of this study is to analyze the singing activity of Southern Hemisphere humpback whales and boat presence in wintering grounds off the Pacific coast of Central America, including Isla del Cano, Costa Rica, and Islas Secas and Coiba National Park, Panama. Boat presence, including fishing vessels and whale watching boats, vary between each area of study. The two focal research questions for this study are (a) when humpback whales are singing more actively throughout the day, and (b) if (and how) boat traffic affects their singing activity. We hypothesized that the humpback singing activity varies throughout the day and predicted that there would be higher occurrences of singing at night and at dusk (Au et al. 2000). We also predicted that engine noise from boats has a negative correlation with singing among humpback whales, either because the whale stops singing, moves away from the sound, or a combination of the two (Sousa-Lima and Clark 2008). Data such as these are key to generating a broader scientific understanding of marine soundscapes and providing valuable information that may be used to implement adequate maintenance and conservation efforts of valuable migratory marine species.

MATERIALS AND METHODS

Study Site: Humpback whale recordings were gathered from two locations in Panama and one in Costa Rica, respectively: Islas Secas (8º00’00.0” N 82º01’19.2” W), Coiba National Park (7°41'13.2"N 81°36'39.6"W), and Isla del Cano (8°42’19.20″ N, 83°52’42.12″ W) (Fig. 1). Islas Secas is a small island archipelago located in the Gulf of Chiriqui. Coiba is also located in the Gulf of Chiriqui east of Islas Secas, and is a marine reserve area (Fig.1). High numbers of calves have been sighted in the Gulf of Chiriqui, including Secas Island, indicating that Western Panama is an important nursery ground for humpbacks migrating from both the Northern and the Southern hemispheres (Rasmussen 2018). In Costa Rica, 64% of humpback whale sightings have a calf, suggesting this is an important area for nursing moms (Palacios-Alfaro et al. 2016).

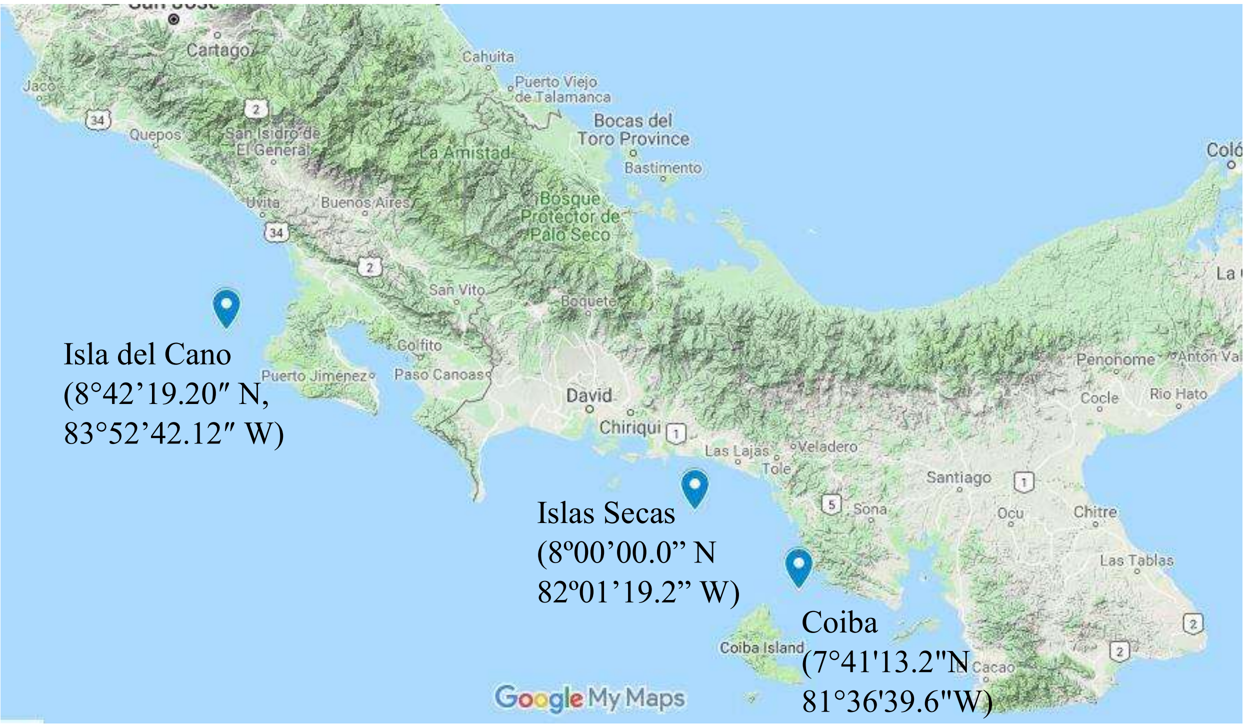

Figure 1: Map displaying the three locations, Islas Secas, Coiba National Park, and Isla del Cano, where passive recorders were deployed in September and October-November 2017 and September to November 2016, respectively.

Recording and Analysis: Autonomous recorders were deployed in the three locations. The recorder used for Islas Secas was SM2M+ (Sampling rate: 4-96 kHz -165dB re: 1V/uPa) from Wildlife Acoustics (www.wildlifeacoustics.com). For Coiba singing activity was recorded using Soundtrap recorder (flat frequency response: 20 Hz to 100 kHz [± 2 dB], clip level: 172 dB re 1μPa). For Cano Island we used a combination of SM2M+ and a RUDAR-mK2 (Sampling rate up to 96kHz -169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com). With the exception of the Soundtrap, all recorders were programmed to continuously record the soundscape in segments of 30 minutes at sampling rate of 44 kHz and 16 bits programmed from September to November in Cano, and August 2017 in Secas. The Soundtrap was programmed to record the soundscape for 5-min every 30-minutes from August 5–September 1 in 2017. For the continuous data we selected a 1-minute sample every five minutes, and for Soundtrap data the full 5-minute recordings were uploaded to the ARBIMON II platform for cataloguing and inspection (https://arbimon.sieve-analytics.com). Recording efforts for each location are summarized in Table 1.

Table 1. Total number of minutes recorded and analyzed per site and recorder model.

Site	Recorder	Recording dates	Total time recorded	No. 1-min samples analyzed
Islas Secas	SM2M+	August 5 to September 1, 2017	38,535	7,707
Coiba National Park	Soundtrap	September 30 to November 20, 2017	12,320	12,320
		November 22 to November 30, 2017	2,140	2,140
Isla del Cano		September 19 to November 5, 2016	89,910	16,881

Each 1-minute recording from both locations was inspected both visually and audibly on the spectrograms, and matrices were created by scoring presence of whales/boats as one and their absence as zero. All song detections, including those that were distant, were included in the matrix. We ran two Wilcoxon Ranked Tests to test for statistical differences in both whale and boat sounds between the two Panamanian sites and the Costa Rican site. A regression analysis was also run to determine if the proportion of deleted songs was dependent on boat detections throughout the day for the three areas. These statistical analyses were done in JMP statistical software.

RESULTS

Our results show that the occurrence of humpback song is significantly different in all three sites compared (x^2=62.24, df=2, p<0.0001). In Isla del Cano, song activity is higher than Coiba and Secas combined, and singing activity occurs primarily at night and early morning (Fig. 2). In Coiba National Park, song occurrence was highest at 02:00, however, there was no clear period of prolonged increase or decrease in activity. At Islas Secas the proportion of recordings with songs was higher than in Coiba. Even the minimum proportion observed at Islas Secas (09:00-10:00) was higher than the maximum in Coiba. The peak in song activity at Islas Secas occurred at 14:00. At this site there was a period of reduced activity between 18:00 and midnight

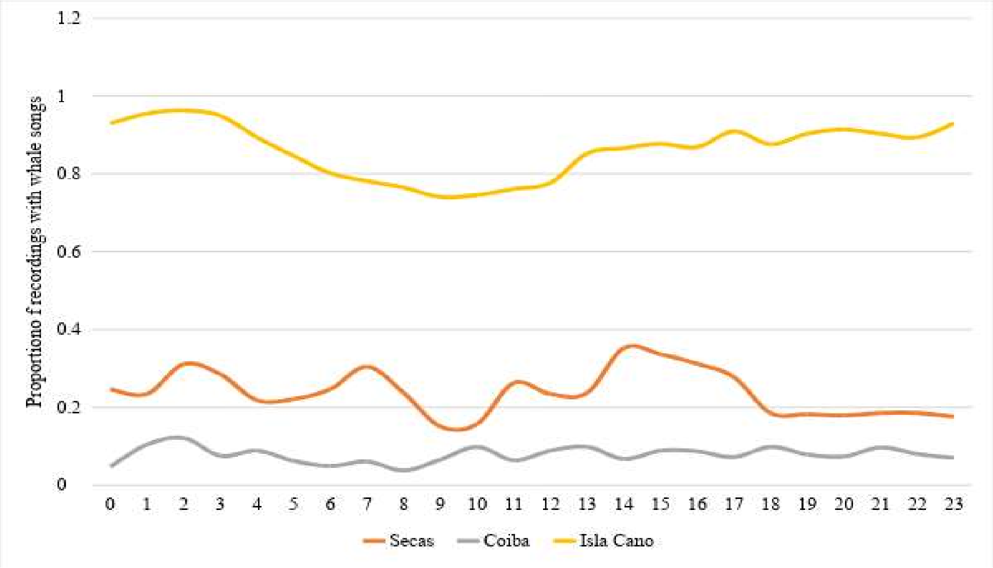

Figure 2. Proportion of recordings with whale song detection throughout the day for Coiba National Park between 30 September and 30 November 2017, Islas Secas between 30 August and 30 September 2017, and Isla del Cano (Chereskin et al. 2018).

There is also a significant difference in boat detections between the study sites (x^2=8.65, df=2, p=0.0132). This difference is accounted for by detection of significantly more boats in Isla Caño than in Islas Secas (z=-2.73, p=0.0062). In both Coiba and Islas Secas, boat activity begins to increase at 06:00 and decreases at 14:00. In Caño, boat activity starts to increase at 07:00 and steadily decreases from a peak at noon until 15:00. There is also a large peak in boat activity at 18:00 in Caño (Fig. 3).

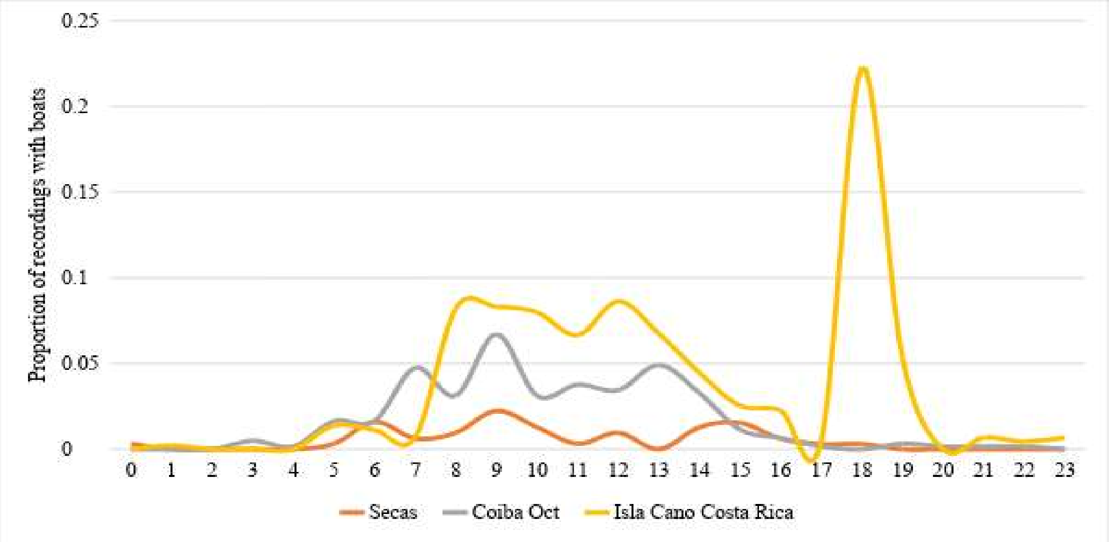

Figure 3. Proportion of recordings with boat detections mapped throughout the day at Coiba National Park between 30 September and 30 November 2017, Islas Secas between 30 August and 30 September 2017, and Isla del Cano (Chereskin et al. 2018).

The only site in which boats had a significant correlation with the proportion of whale songs was Isla Caño (R2=0.58, F=27.4, p<0.001) (Fig. 4).

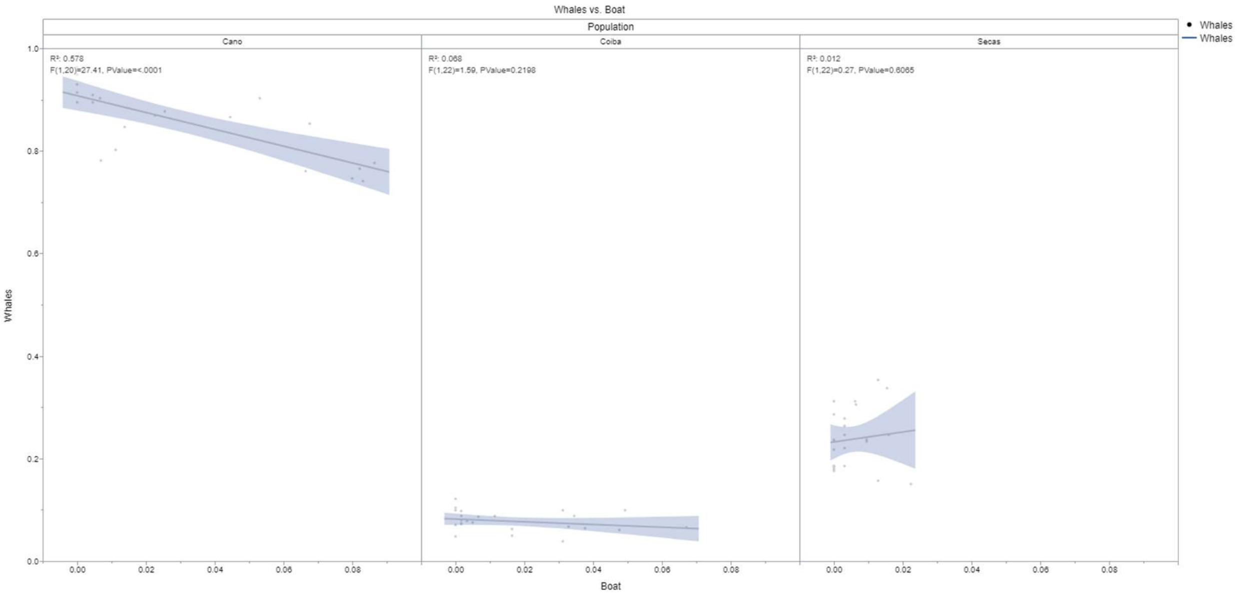

Figure 4. Regression models depicting linear relationships between boat detections and whale song detections for each location. A significant correlation was only found at Isla del Cano. Models are for Isla del Cano (left), Coiba National Park (middle) and Islas Secas (right).

DISCUSSION

The results of our study show that singing and boat activity varies between wintering grounds in Costa Rica and Panama. There are higher occurrences of both humpback whale singing and boat activity in Costa Rica than in Islas Secas and Coiba National Park combined. Singing in Costa Rica appears to follow a clear pattern where activity begins to decline at 05:00 and increase again around 13:00. Activity is highest in Costa Rica in the very early morning and late at night, which is consistent with the findings of Au et al. (2000). Data obtained from Panama follows a less-defined pattern, and singing fluctuates throughout the day. In Islas Secas, singing is lowest from 10:00 to 11:00, whereas Coiba experiences a peak in activity around this same time. Peak singing activity for Secas appears to be from 15:00-16:00 and 08:00-09:00. Therefore, our hypothesis that singing would be highest at dusk and late at night in Panama is refuted by this data.

There is a significant difference in the levels of singing activity between the Panamanian and Costa Rican sites (p<0.0001). The relatively low levels of singing activity in Panama when compared to Costa Rica does not necessarily indicate fewer whales in these sites, but may indicate that there are fewer males present in those areas. An Australian study found that several breeding grounds along coastal migratory routes in the southern Pacific had female-biased sex ratios, where the population was mainly comprised of mature females with calves and very few mature males (Franklin et al. 2017). It is possible that the Panamanian breeding grounds in Islas Secas and Coiba National Park also experience such bias. The Gulf of Chiriqui is known to be a prominent calving ground for humpback whales. Satellite and photo identification data provides evidence of many whales present in this region (Guzman et al. 2014). Therefore, it is likely that the population of whales observed is predominantly females with calves, with few singing males due to the lack of available female mates.

Au et al. (2000) found a distinct diurnal pattern in sound pressure levels (SPL) in waters off of Maui. They placed a compact acoustic probe data acquisition instrumentation package approximately 0.8 km off shore at a depth of 13 meters. Using SPL, they identified a peak in song activity between the last week of February and the third week of March. This corresponds with the middle of the boreal winter (December to April). During this seasonal peak, SPL increased one half hour before sunset, peaked at midnight, and decreased one hour after sunrise. They suggested that these patterns reflect a decrease in intraspecific interactions at night which leaves acoustic signals as the only alternative way for males to advertise their fitness. This also leads them to identify daylight and vision as important factors in these interactions. In January and April, the overall SPL were lower than in the peak season and they saw the opposite diurnal pattern. SPL were minimum at midnight and maximum at noon. They also only observed diurnal variations when songs increased above 110 dB.

Our results reflect the activity of whales at the end of their breeding season. This suggests that there are fewer whales during this time which may be contributing to the low proportion of whales observed. Based on our results and those found by Au et al., diurnal patterns in whale song behavior seem to be less rigid outside of the peak season. If the increase of singing at night reflects an alternative to intraspecific interactions that take place during the day, perhaps fewer of these interactions are taking place in general as the breeding season comes to a close.

Boat activity was similar in Islas Secas and Coiba. Most traffic is due to tourism (eg. whale watching and scuba diving). In Isla del Cano, day time traffic can also be attributed to tourism. The peak of boat activity between 18:00 and 20:00 is likely due to fishing boats. The lack of a pattern in singing for whales wintering in Panama could be due to a lack of boats in the area. In regions where boat traffic is high, whales are found to sing more at night when traffic is at a minimum (Chereskin et al., in review). The impacts of boat noise on the acoustic activity of humpbacks has been well documented. Previous studies have shown that underwater explosives can injure the inner ear of whales too close to the blast (Clapham, 2000). Humpbacks have also been observed to move to a new area in response to increased noise levels (Clapham, 2000). If previous suggestions that whales are singing at night on feeding grounds in order to avoid periods of increased ambient noise, it is possible that in the absence of acoustic pressure whales would have a different singing behavior.

For future work in this field, we would advise that recorders be deployed year-round to examine which months contain the most acoustic activity and periods throughout the year in which different migratory populations can be found. In order to deduce which migratory populations are actually present in the target months, the sonic structures must be analyzed, as humpback songs change over time and differ between populations. Additionally, exploring usage of photo ID practices in these areas could help solidify whether these breeding grounds are experiencing decreased singing occurrences due to biased sex ratios, and whether the presence of whales is primarily mothers and calves.

There is no defined diurnal singing pattern in Islas Secas or Coiba, Panama, and instances of boat noise are significantly less compared to Isla del Cano in Costa Rica. We propose that the decreased song patterns and occurrences in these two Panamanian sites is due to less pressure to alter singing times to avoid engine noise, and a greater prevalence of nursing females than singing males. To conserve these valuable areas, boat noise should continue to be kept at a minimum so as not to disturb humpbacks' natural migratory and breeding patterns. While ecotourism brings attention to this species and may help bring awareness to conservation efforts, it is important to understand the impacts of human activity and manage the tourism industry accordingly.

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THE ACOUSTIC REPERTOIRE AND TEMPORAL ACTIVITY OF ANTILLEAN MANATEES (*TRICHECHUS MANATUS MANATUS*) IN BELIZE

Carly Sarbacker
University of Vermont

Like many other species with weakly social bonds, the Antillean manatee (*Trichechus manatus manatus*) is underrepresented in acoustic and behavioral studies. This lack of information makes it difficult to determine the diversity of their signal repertoire or examine potential geographic or temporal patterns in signal structure and emission rate. In this study I described the acoustic structure of manatees in a large resting hole in Belize. A total of 239 manatee calls were detected over three full days of sampling effort. Typical vocalizations ranged from tonal sounds with minimal modulations to noisy and nonlinear signals. Belize manatees are more vocal at mid-day and their calls range in frequency from 5.2-15 kHz. A great degree of intraspecific variation was identified, but the acoustic parameters, most notably duration and peak frequency, were shown to be consistent across several studies. This study therefore corroborates previous research on the patterns of acoustic behavior and signal structure of the Antillean manatee.

I. INRODUCTION

The Antillean Manatee (*Trichechus manatus manatus*) is a subspecies of the West Indian manatee found throughout the Caribbean Sea and the northwestern coast of South America. Unlike other marine mammals, little is known about their vocal behavior, largely because all manatees (*Trichechus spp.*) are considered solitary to weakly social (Umeed *et al*., 2017). This lack of sociality may imply that the vocal repertoire of manatees is less complex than other more highly social animals, but research has indicated that their vocalizations still carry important information about individual identity, such as age and sex class (Sousa-Lima *et al*., 2008; Umeed *et al*., 2017). Therefore, their vocalizations may play an important role in kinship and mate recognition, especially considering manatees are often isolated, have poor visual acuity, and live in frequently turbid waters (Umeed *et al*., 2017).

Throughout studies done on the acoustic repertoire of the West Indian manatee or the Antillean manatee subspecies specifically, the nomenclature and categorization of unique signal types and their features is inconsistent. Anywhere from two to nine unique signal types have been identified based on their vocal structure and parameters (Sousa-Lima *et al*., 2008; Steel, 1982). Several non-linear phenomena have also been observed in Antillean manatee sounds, further making the sounds difficult to identify and describe (Mann *et al*., 2006). One study has indicated that most West Indian manatee vocalizations have a typical pattern, duration range, and frequency range, but little research on patterns of intraspecific, geographic, or temporal variation has been done (Niezrecki *et al*., 2003).

Besides filling gaps in current behavioral knowledge, studying the vocalizations of the Antillean manatee can also play an important role in conservation. The West Indian manatee as a whole is considered vulnerable, but the Antillean manatee subspecies specifically is listed as endangered by the IUCN (Deutsch *et al*., 2008). Belize, where this dataset was recorded, has one of the largest recorded populations of Antillean manatees worldwide, consisting of approximately 700-900 individuals (Galvez *et al.,* 2013). At this location, the greatest threat to manatees is injury or death from boat collisions. One solution to this problem is developing a warning system for boaters that detects manatee presence by their vocalizations (Niezrecki *et al.,* 2003). Implementation of such a system, however, would require a more thorough understanding of manatee's vocal characteristics and activity.

The purpose of this study is to (1) describe the vocal repertoire of a specific population of Antillean manatees in Belize, (2) describe temporal patterns in emission rate of manatee signals at this location, and (3) compare this population's vocal parameters and emission rate to previously studied populations. The scope will be limited to vocalizations made in a specific resting hole of Antillean manatees within Belize. This study intends to contribute to scientific understanding of manatee social behavior and ecology by providing comparison of manatee vocal parameters and behavior over time and across environments.

II. METHODS

A. Study site

Belize has the largest population of Antillean manatees in the Caribbean, with an estimated population size of 700-900 individuals (Galvez *et al.,* 2013). Recordings of wild manatees were taken at a large resting hole 1.5 km east of St. George's Caye (17.559837 °N, W -88.085865°W). St. George's Caye is a small island located near the Belize Barrier Reef, approximately 9.5 km east of the mainland. In a previous study by Ramos *et al.* (2018), 143 manatees of varying age and sex classes were sighted in the greater St. George's Caye area. Recordings at this site took place from 10 Jan 2018 to 21 Jan 2018.

B. Audio recordings and analysis

A SoundTrap 300 HF (Ocean Instruments, New Zealand) that sampled sounds continuously at a 288-kHz sample rate in 16-bit resolution (flat frequency response: 20 Hz to 100 kHz [± 2 dB], clip level: 172 dB re 1µPa) was used to collect acoustic recordings of manatees. The SoundTrap was suspended in the water column with rope and anchored to the seafloor with a cinderblock at a depth of 1.5 m at the edge of a seagrass bed in a human-dredged channel. A large hole often used by manatees as a resting hole was located near this channel. Resting holes are deep depressions in the substrate in which manatees appear to rest, protected from strong water currents (Bacchus *et al.*, 2009).

Acoustic files from 10 Jan 2018 to 13 Jan 2018 were analyzed manually in RAVEN 1.3 with a sampling window of 1024. For each file, every high-quality signal was selected. High quality signals were defined as those with clear beginning and end points, as well as clear high and low frequency limits. In order to include nonlinear signals within the acoustic repertoire and analysis for this population, clear and visible signals were selected in their entirety, including any harmonics or non-linear phenomenon. The acoustic parameters of low frequency, high frequency, delta frequency, delta time, and peak frequency were extracted from the selections. Nonlinear signal dynamics will be identified visually using the definitions set by Mann *et al*, 2006. Acoustic parameters and their statistics were then summarized using Excel.

C. Emission rate

In order to study trends in acoustic activity throughout the day, files were classified into times of day based on their starting time. The categories were as follows: early morning (hours 1-4), morning (hours 5-10), mid-day (hours 11-15), evening (hours 16-20), and late night (hours 21-0). To calculate the emission rate considering sampling effort, the total number of high-quality selections for each time of day was calculated and divided by the total number of hours analyzed for that time period.

III. RESULTS

A total of 239 high quality sounds were extracted from three full days of recordings. The acoustic repertoire ranged from simple tonal sounds with clear harmonic structures to complex atonal sounds with a variety of nonlinear phenomena. Subharmonics and bitonations typically occurred at the beginning and/or end of vocalizations, while noise occurred in the middle or throughout the entire vocalization. No confident identifications of biphonations were made (Fig. 1).

Frequencies of vocalizations ranged from 5.2 ± 2.9 kHz to 15.4 ± 5.5 kHz. The mean frequency range of individual vocalizations was 10.2 ± 6.3 kHz. There were no trends within the frequency ranges of this population (Fig. 3). Mean duration was 177.4 ± 81.3 ms. The mean peak frequency for this population was 7.2 ± 3.6 kHz (Table 1).

In terms of acoustic activity, when sampling effort was considered, emission rate (signals/hour sampled) was the highest during mid-day at 16.92 signals/hour sampled, followed by late night at 4.25 vocalizations/hour sampled (Fig. 3). The average emission rate is 5.69 vocalization/hour sampled.

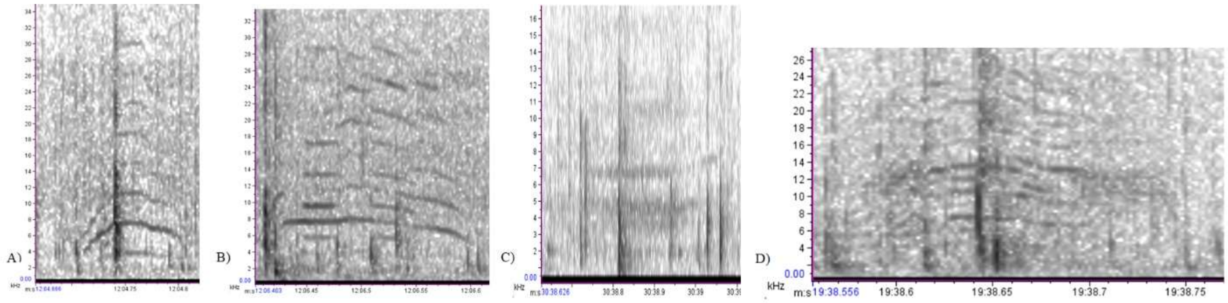

Figure 1: Example manatee signal spectrograms (x axis = time (ms), y-axis = frequency (kHz) displaying A) typical tonal structure and harmonics, B) subharmonics, C) noise, and D) bifurcations.

Table 1: Summary of descriptive statistics of acoustic parameters for Antillean manatees in Belize compared to previous studies.

	LF (kHz)	HF (kHz)	DF (kHz)	Dt (ms)	PP(dB)	PF (kHz)
This Study (n=239)						
Mean	5.32	15.41	10.19	177.36	47.57	7.36
SD	2.86	5.48	6.34	81.32	10.67	3.53
Range	0.29 - 13.74	4.04 - 33.78	0.79 - 32.61	51 – 454	18.30 - 82.10	1.41 - 21.38
CV	53.68	35.54	62.16	45.85	22.43	47.92
Sousa-Lima *et al.* 2008[a]						
Mean	.....	.....	0.97	353	......	2.45
SD	.....	.....	0.5	78	......	0.5
Range	.....	.....	.....	180 - 480	......	3.7 - 5.7
CV	.....	.....	.....	.....	......	......
Umeed *et al.* 2017 (n=110)[a,b]						
Mean	1.42 (± 0.17) - 4.56 (± 0.45)	2.90 (± 0.20) - 5.23 (± 0.53)	0.62 (± 0.09) - 2.41 (± 0.70)	140.10 (± 13.32) - 237.20 (±17.12)	......	2.19 (± 0.13) - 4.94 (± 0.48)
SD	......	......	......	......	......	......
Range	......	......	......	......	......	......
CV	......	......	......	......	......	......
Nowacek *et al.* 2003[a]						
Mean	…..	…..	…..	32 (±17) – 217 (± 98)	92.5 (± 6.6) – 100.0 (± 47)	3.18 (± 0.73) – 7.08 (± 2.21)
SD	…..	…..	…..	…..	…..	…..
Range	…..	…..	…..	…..	…..	…..
CV	…..	…..	…..	…..	…..	…..

[a] Selections made from fundamental frequency (defined as the first harmonic) only; non-linear signals excluded.

[b] Acoustic parameters divided between age class, sex class, and signal type. Values reported represent the range of mean values and their associated standard deviations for adult manatees only.

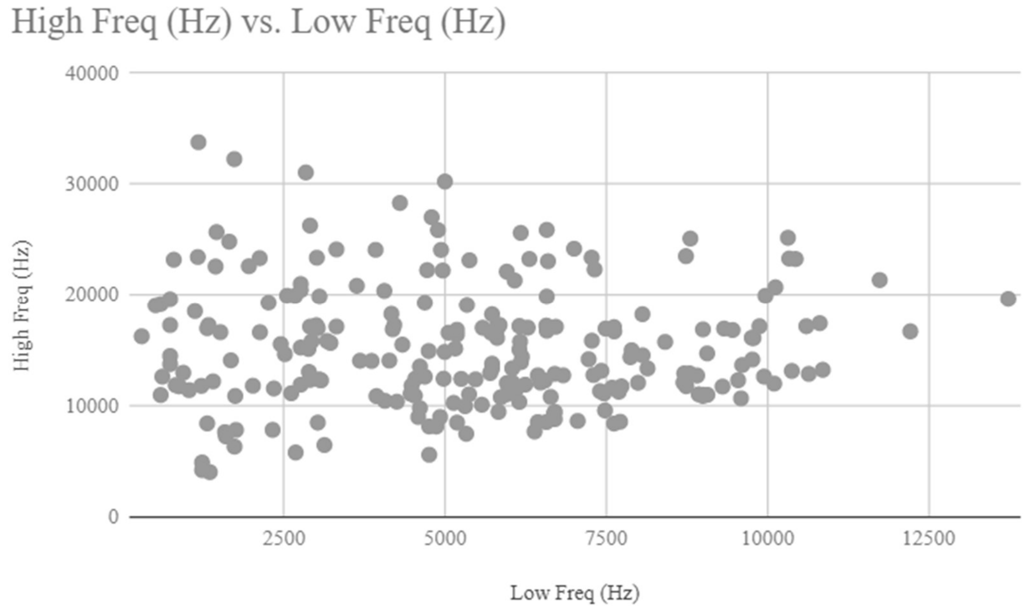

Figure 2: Cluster plot comparing high and low frequencies of signals extracted from passive acoustic monitoring of Antillean manatees

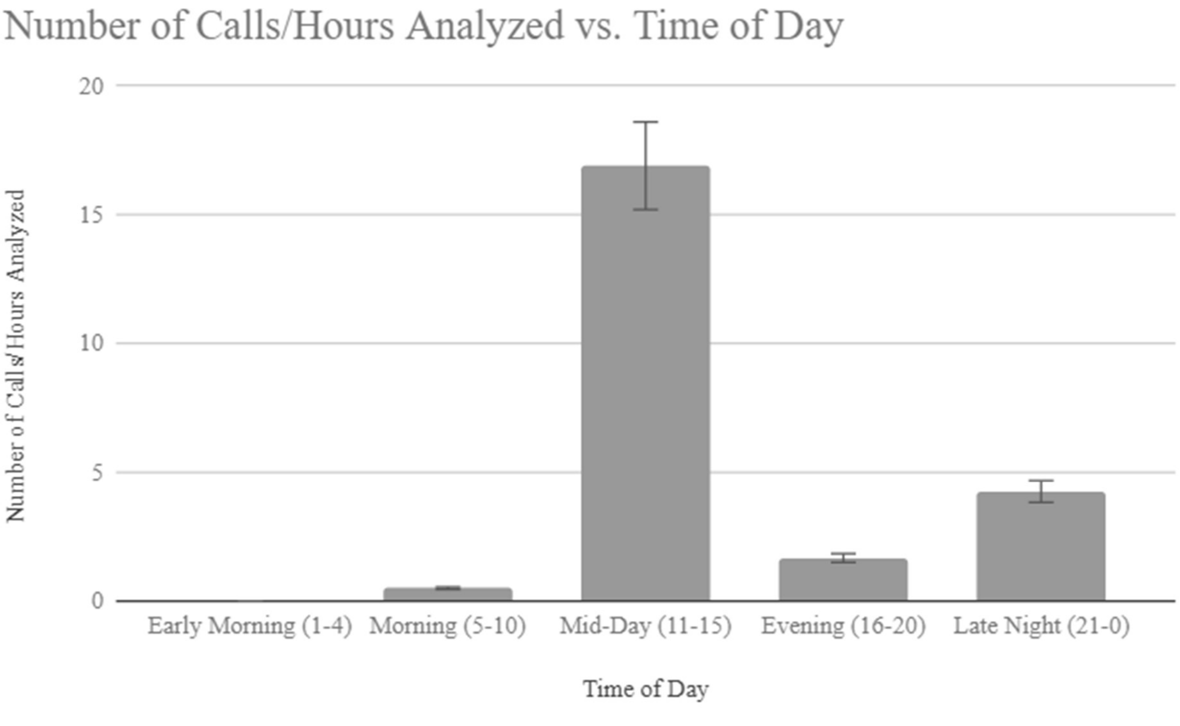

Figure 3: Bar graph of emission rate (number of calls/hours analyzed) with error bars for Antillean Manatees in one resting hole in Belize.

IV. DISCUSSION

This study provides evidence that this Belizean population of Antillean manatees has a vocal repertoire consistent with what is currently known about the subspecies. Due to the nature of the selection process, the parameters of duration and peak frequency are most easily comparable across studies. Mean duration in this study was similar to those found in other studies, although Sousa-Lima *et al*. (2008) reported a longer mean duration (353 ms) and smaller duration range (180 ms – 480 ms) than observed in this population. These parameters are likely different because Sousa-Lima *et al.* studied isolation vocalizations specifically. Mean peak frequency is somewhat higher in this study (7.36 kHz), likely due to the inclusion of harmonics; other studies, while acknowledging that typically the second or third harmonic has the most energy, only selected the first harmonic (Umeed *et al*., 2017; Sousa-Lima *et al*., 2008; Nowacek *et al*., 2003).

High coefficients of variation for all extracted parameters (22.43-62.16) provide evidence that there is a great degree of intraspecific acoustic variation. Without data on the estimated number of individuals present during the recordings, it is difficult to account for the representation of individual animals in the sample and therefore we cannot compare our degrees of variation with those of other studies. If such data were available, it would be interesting to compare acoustic diversity as a metric for population health in order to monitor populations and guide conservation action.

Variation in duration specifically could imply there are a variety of different contexts for signals. This possibility is supported by previous observations of vocalization types specific to age and sex classes (Umeed *et al.*, 2017). More data on population specifics and behavior is necessary to investigate whether or not previously identified signal types and contexts are present in this population, and if so, in what proportion. To elaborate on manatee vocalization diversity, the relative frequency of nonlinear phenomena could be investigated in comparison to previous studies (Mann *et al.*, 2006).

The results for emission rate indicate higher vocal detection during mid-day, which is supported by previous research that has observed manatees using resting holes more often during this time (Bacchus *et al.*, 2009). Due to uncertainty in the recorder's ability to detect manatee signals from any location in the large resting hole, it is possible these data are more a reflection of manatee use of certain areas of the resting hole during the day, rather than a representation of activity throughout the entire day and across other locations. Average emission rate (5.69 vocalization/hour sampled/0.095 vocalizations/minute sampled) is consistent with Nowacek *et al.*'s findings of 0.09-0.75 vocalizations/minute for manatees in Belize (2003). This knowledge of acoustic activity could have important conservation implications by comparing activity to other factors such as season or anthropogenic activity

While the entirety of a given signal was selected in order to represent nonlinear signals in the acoustic repertoire, there is still the possibility that non-linear signals were underrepresented in this sample due to their often unclear frequency boundaries. This methodology strategy also limits the potential for this research to be compared to other studies. The presence and number of harmonics is highly dependent on the direction and proximity of the animal to the recorder, but without accounting for the number of harmonics in a selection, you cannot accurately compare mean high frequency and delta frequency between studies with different methodology. Future research, especially into geographic acoustic variation, should consider the functionality of this selection methodology for future studies.

The quantity of selections was limited by the number of days chosen for analysis out of the recordings available. The placement of the recorder at the edge of the resting hole may also have limited the detection of manatees and their signals, although the existing data already demonstrates a high degree of intraspecific variation in signal parameters. Future research could investigate the functionality of acoustic monitoring at this site, especially in comparison to other techniques of detection.

V. CONCLUSIONS

This study reinforces previous studies of the acoustic repertoire of the Antillean manatee in Belize. Despite being solitary to weakly social, the acoustic structure of these animals' vocalizations varies widely from simple harmonic signals to complex, non-linear signals. In spite of this variation in signal structure, comparisons between acoustic studies of Antillean manatees suggests some stereotypy in duration, peak frequency, and emission rate. Furthermore, high degree of variation in these parameters suggests greater intraspecific variation and social contexts than previously attributed to manatees. Knowing these features as well as their increased vocal activity during mid-day and evening will assist in future research into the social function and contexts of these signals. Better understanding of the sociality and communication of these animals will assist in understanding and monitoring population dynamics of the endangered manatee.

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