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

Fall 2019

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

Course description: This CURE course engages undergraduate students in topics: marine soundscape ecology, marine animal bioacoustics, and cetacean ecology, behavior, and conservation.

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

Learning goals:

1. To offer the opportunity to make discoveries and contributions to the scientific community, policymakers, 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 which is bound to be limited by replication, sample size, 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. Students that have taken this course have gone to do internships with marine researchers abroad, some have gain competitive positions as research assistants, and others have move on to continue graduate school!

I expect that together we will create a dynamic and respectful 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 (you can invite your friends!).

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**.

Research Topics

Whether you choose to work with soundscapes or bioacoustics your project is part of a larger network of collaborative studies. Any potential manuscript will be therefore within the scope of these studies and coauthored with scientists in this network (see rules below). Furthermore, your project will contribute not only to science but to marine conservation by ensuring governments have the best available scientific information to act.

Students can develop an individual project, or in a group project (no more than 3 students). Expectations will be higher for group projects. I encourage groups projects because is a great opportunity to learn how to collaborate and communicate. Students are expected to develop questions on either of the following topics.

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.

Dolphin Acoustics and Behavior: Most 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 as this, individuals play various roles in maintaining the integrity of the overall social structure of a population. Dolphin group structure is also maintained by learning to recognize others, and this is done by using signature whistles. For example, in bottlenose dolphins signature whistles are unique to each dolphin in the group and provide information about identify, gender, and age. These whistles are like name tags and are developed early in life using the mother (in the case of baby males) or group members' whistles (in the case of baby females) as templates. Dolphins also emit variant whistles and several other type of sounds that are species and context specific, and we know very little about the acoustic characteristics of these sounds.

Baleen Whale Acoustics: Unlike dolphins, the communicative signals of baleen whales are produced at much lower frequencies and limited to specific behaviors. Also, while in dolphins both males and females emit sounds, in baleen whales only males are known to sign (there are a few exceptions though). In my lab several students are involved in studying humpback whale song activity and structure in Central America. During the first part of the year, Northern Hemisphere humpback whales from California and Oregon migrate to Central America to breed, this specific breeding population is one critically endangered. During the second half of the year the same coastal areas received Southern Hemisphere humpback whales from the Antarctica Peninsula and Chile. The song of humpback whales is quite complex, it consists of hierarchical components that are sang in the same order. Breeding populations have song components unique to them, but occasionally a new song evolves via cultural transmission. Thus, whale song structure analysis can help us study population connectivity and the role of culture in whale singing behavior.

Fish Acoustic Behavior: You will be surprise at the number of fish species that emit sounds! Fish can be quite chatty! Students in this CURE course have developed projects studying the acoustic activity of toadfish. Male toadfish are territorial and emit sounds to attract females to lay eggs in their territory. Presumably, females assess the quality of the male prior releasing their eggs. We have learned that in Bocas del Toro Panama toadfish acoustic activity is primarily during dark hours, and that they respond to boat presence in various ways. Because they are important component of marine communities, toadfish presence is used to evaluate marine community's health. The toadfish is just one of several fish species we have recorded. Discovering the identity and describing the acoustic activity of other fish species in our study sties is key in understanding the dynamics of coastal marine communities. Basics on fish bioacoustics:
https://homepage.univie.ac.at/friedrich.ladich/Ladich%202014.pdf

Students working with animal vocalizations and soundscape will be learning acoustic software such as ARBIMON, RAVEN, Adobe Acoustics, and Luscina http://rflachlan.github.io/Luscinia/. See scheduled workshop.

Recommended Sources

Basics of Sound: https://dosits.org
Some cool marine animals sounds: https://scripps.ucsd.edu/labs/sirovic/
Steps to organizing your **scientific** manuscript
https://www.elsevier.com/connect/11-steps-to-structuring-a-science-paper-editors-will-take-seriously
How to write a scientific paper: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3474301/
Read previous CURE projects: http://www.lauramay-collado.com/cure-lab.html
Statistics for Biologists: https://www.nature.com/collections/qghhqm
Acoustic Ecology: https://www.acousticecology.org/scienceprograms.html

Course Calendar

Week	Chronogram
Jan 14	**Introduction to this course and description of potential projects.** **Introduction to Web of Science Search engine** ***Sign up to discuss potential projects***
Jan. 21	**Workshops on RAVEN.** **Report due at the end of class.** ***Sign up to discuss potential projects***

Jan 28	**Research your topic of interest. Prepare** - **A 500-word summary: Background, Question, Significance** - **Least a minimum of 10 references from the reviewed literature** - **Prepare a 10 min presentation with the above information including a plan on how would you answer the proposed question.** ***Sign up to discuss potential projects and Sing up for computer use***
Feb 4	**Project proposal due (see specifics below).** **Setting up rules for data collection and computer schedule.** - **Begin Data collection Feb 4th** - **End Data collection March 31st** - **You are expected to meet up with me every Tuesday for an oral and printed Progress report.**
Mar 31	**Data visualization and analysis workshop**
Mar. 31	- **Submit *Title*, *Running title*, *Introduction*, *Keywords*, and *Material and Methods* section (follow JASA format see below)** - **Data analysis.**
Ap.7	- **Feedback** - **Continuation of Data analysis** - **Submit *Results* sections with accompanying figures and tables (follow JASA format see below)**
Ap.14	- **Feedback** - **Continuation of Data analysis** - **Submit *Discussion, Abstract,* and *Reference List* (follow JASA format see below)**
Ap. 21	**Full Research Paper Due:** - **Manuscripts that are not in the requested format will have a 20 pts Penalty.** - **Manuscripts that are not submitted on time will have a 10 pts penalty for each day after deadline.**
Ap.28	**Mini-symposium begins at 1: 30 p.m. (see specifics below)**
May 1	**Blog on your CURE experience see examples here: http://www.lauramay-collado.com/2019cure-blog**

Grading

Showing up to the lab!	100 pts
Acoustic Report due in class **Jan 21**	50 pts
500 word summary+references+ppt due **Jan 28**	50 pts
Proposal due **Feb 4**	100 pts
8 Weekly Written progress reports due on - February: 4, 11,18,25 - March:3,17,24,31	12.5 % each=100 pts
Written paper - *Title, Running Title, Introduction, Keywords, Materials & Methods* due **March 31** - *Results* **April 7** - *Discussion, Abstract, Reference List* due **April 14** - Full Manuscript due **April 21**	30 pts 40 pts 40 pts 100 pts
Mini symposium **April 28**	100 pts
Blogging about your project and experience for others to learn about CURE **May 1st**	100 pts

Total	**800 pts**

INDEPENDENT RESEARCH PROPOSAL FORMAT

The proposal must consist of the following parts.

- **Introduction** – (1 page)
  - ***Background*** to problem with citations of papers or other sources that document the information you are presenting. This background should include the observations that lead to your question or hypothesis.
  - ***Purpose and scope*** - Statement of the purpose of your paper, this may be how you are testing your hypothesis. If you use hypothesis you need to make predictions about the hypothesis. Predictions will also go here.
  - ***Significance:*** How does your project advance knowledge on this field? How does your project benefit society?
- **Materials and Methods** – (1 page) What type of data have you found and what additional data are you going to try to find? How will the data you collect be analyzed to address your objectives, questions or hypothesis? It is important to make it clear how the scientific method will be used to test or address either your hypothesis or the predictions you expect if the hypothesis is true.
- **Research Plan** – (1 page) Schedule of steps to be accomplished with deadline dates.
- **Literature Cited** –(1 page) Full reference to the papers cited in the introduction and materials and methods sections. Use format from Journal of the Acoustical Society of America. **See example https://asa.scitation.org/doi/10.1121/1.5139205**
- **You will turn in an electronic version on February 4.**

Note: I recommend doing a serious literature review of your topic of interest! The more effort you put into your proposal, the easier it will be to write your manuscript at the end of the semester.

MANUSCRIPT

We will be writing each section of the paper by parts as shown in the schedule above. Each section should be in the format of *Journal of the Acoustical Society of America*. Go to the journal and download the guidelines for authors. The final manuscript is due on **April 21**.

Here is a summary of the guidelines:
https://asa.scitation.org/pb-assets/files/publications/jas/JASA_AuthorChecklist-1508440990393.pdf

Here is an example of a JASA published manuscript:
https://asa.scitation.org/doi/10.1121/1.5139205

Other important resources

Steps to organizing your scientific manuscript
https://www.elsevier.com/connect/11-steps-to-structuring-a-science-paper-editors-will-take-seriously
How to write a scientific paper: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3474301/

Statistics for Biologists https://www.nature.com/collections/qghhqm

SYMPOSIUM APRIL 28

You will have 10 minutes, 8 minutes for your presentation, and 2 minutes for questions.

Deliver your presentations 1 day prior to the symposium.

- Please embed any videos or audio within the presentation
- Also include ALL videos & audio files in a separate folder on your thumb drive. This will enable us to correct any problems on site.

DATA USE

The data that you will using to develop your project is of my property as the PI of the projects involving the collection of this data. Some data sets are shared with collaborators that significantly contributed to data collection, and thus ownership is shared. You will sign a contract of ethical use of the data. **No sharing of data on social media or with other parties is allow without my consent. This includes photographs, acoustic fields, or any other data from my databases**. We will develop a space for outreach activities and research experience communication through a blog where you can post sound files, summaries, photographs, and updates on data processing with my approval.

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.**

2nd CURE Soundscape and Behavior Symposium

Date: December 3; Location: 124 MLS; Time: 1:15 p.m.

1:15 Introduction

Session I: Soundscapes

1:30: Factors influencing marine community diversity in Central America by *Elie Byrne and Sean O'Sullivan.*

1:45: Factors influencing marine community diversity in a shallow coral reef in Bocas del Toro, Panama by *David Sileo*

2:00: Impact of the Canal of Panama on noise levels and marine community acoustic diversity. *Kenneth Tang*

2:15-2:30 Break

Session II: Animal Communication

2:30: Toadfish call acoustic structure is affected by boat traffic by *Emma Gagne*

2:45: Singing activity and song structure of humpback whales (*Megaptera novaeangliae*) in wintering grounds off the coast of Guerrero, Mexico by *Cybele Adamcewicz and Isabel Belash*

3:00: Acoustic repertoire of bottlenose dolphins in Almirante Bay, Bocas del Toro. *Addie Weeks*

3:15: Effect of boat activity on the acoustic structure of signature and variant whistles of bottlenose Dolphins (*Tursiops truncatus*) of Bocas del Toro, Panamá by *Brennan Paradee*

3:15-3:30 Break

Session II: Behavior

3:45: Intra and interspecific whale social behavior in Guerrero, Mexico by *Quynh Vo*

4:00: Behavioral budget of the Antillean manatee in Belize by *Summer Barnes*

CURE Award ceremony

Effect of boat activity on the acoustic structure of signature and variant whistles of bottlenose dolphins (*Tursiops truncatus*) of Bocas del Toro, Panama

Brennan Paradee[1]

Department of Biology, University of Vermont, Burlington VT 05405

Bottlenose dolphins use whistles to communicate with each other in many different contexts. Signature whistles are used as a unique identifier for each dolphin, whereas variant whistles can be used at any time for general communication. It has previously been shown that whistle acoustic structure of bottlenose dolphins (Tursiops truncatus) can be affected by boat traffic depending on their behavioral state (May-Collado and Quinones-Lebron 2014). However, little is known about whether different types of boat traffic may affect signature and variant whistles differently. Whistles were recorded in the Archipelago of Bocas del Toro, Panama. Two different study sites were compared, Almirante and Bocastorito. Almirante consists of primarily transport traffic, while Bocastorito consists of primarily dolphin watching boats. This study found that variant and signature whistles were more complex, higher in frequency, and longer in duration in Bocastorito than in Almirante.

I. INTRODUCTION

Bottlenose dolphin (Tursiops truncatus) whistles have been studied extensively to better understand dolphin communication and behavior. Bottlenose dolphins produce two types of whistles- signature and variant. Signature whistles are unique to each individual dolphin and are developed while the dolphin is a calf, by incorporating modified elements from other whistles that they have heard from dolphins in their community (Janik and Sayigh 2013). Signature whistles are used for two primary reasons. The first is to introduce themselves to each other when they meet in the sea (Quick and Janik 2012). The second reason dolphins use signature whistles, and the one most relevant to this study, is to help mothers reunite with their calves after being separated (King et al 2016). When a mother and calf are separated, they will each produce their own signature whistles, which helps them locate each other again. (King et al 2016). The other type of whistle that bottlenose dolphins produce is variant whistles, which are not unique to any individual, and are used for communication in many different contexts.

It has previously been shown that whistle acoustic structure of bottlenose dolphins can be affected by boat traffic depending on their behavioral state (May-Collado and Quinones-Lebron 2014). However, little is known about whether boat traffic may affect signature and variant whistles differently. This study examines signature and variant whistles in two different locations in the Archipelago of Bocas del Toro, Panama, where dolphins reside. The first location, Bocastorito, has heavy tour boat traffic. The tour boats follow dolphins throughout the day and can often separate mothers from their calves. The second location, Almirante, has regular taxi boats in the area that do not directly follow dolphins.

Due to the different nature of signature and variant whistles, this study proposes that analyzing them independently from each other is important when looking at factors that may influence whistle acoustic structure. Since signature whistles are used in such specific behavioral contexts, they should be analyzed independently of variant whistles.

II. MATERIALS AND METHODS

A. Study site

This study took place in Dolphin Bay (9.23N/-82.24 W) and Almirante Bay (9.289N, -82.332W) in the Archipelago of Bocas del Toro, Panama. The Archipelago consist of shallow and clear waters < 20 m in depth and is home to a resident population of bottlenose dolphins (May-Collado and Wartzok 2008). The selected sites contrast on the type and intensity of boat traffic. Almirante is located in the mainland and communicates with the main island in the archipelago via boat-taxis. There are two boat-taxi companies, which operate from Almirante to various points in the Archipelago including Colon Island, the largest of all islands in the archipelago. These taxis run daily from 6 a.m. to 6 p.m. In Dolphin Bay, boat traffic is due to tourism. Everyday tour boats arrive to the bay between 9 a.m. and 2 p.m. and normally approach the animals beyond the recommended regulations. A group of dolphins in this area can be surrounded by up to 40 boats (May-Collado and Wartzok 2008; May-Collado and Wartzok 2015).

B. Recordings

Dolphin recordings were obtained using a combination of methods including passive acoustic monitoring and from boat follows. The passive acoustic recordings were obtain using two RUDAR-mK2 recorders (Sampling rate up to 96kHz -169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com). The recorders were programmed to continuously sample the soundscape in segments of 30 minutes at a sampling rate of 44 kHz. 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).

C. Whistle analysis

Recordings were processed and analyzed in RAVEN PRO 1.5 build 37 (2017; Cornell Lab of Ornithology). Whistles in a recording were classified into signature or variant. The distinction was done using Signature Identification (SIGID) method, which 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). Upon classification, the following standard parameters were extracted for high signal to noise ratio whistles of both types (May-Collado and Warzok 2008, May-Collado and Quinones-Lebron 2013): low frequency (LF), high frequency (HF), duration (D), delta frequency (DF), center frequency (CF), number of inflection points (IP), and contour type (upsweep, downsweep, sine, convex, concave, constant). Additionally, the time of day was recorded.

D. Analysis

A MANOVA test was performed to analyze the effect of site and type of boat activity (Almirante=transport or Bocastorito=tourism) and whistle type (variant or signature) on whistle acoustic structure. To determine the direction of the effect, an ANOVA test was conducted to test for differences in whistle duration, frequency, and complexity. All statistical analysis was done in JMP 14.2 (SAS, 2019).

III. RESULTS

Whistle contour diversity

The sample size of whistles analyzed was larger for dolphin's whistles from Dolphin Bay where signature and variant sine whistles were the most commonly produced followed by variant upsweeps and down sweeps. There are not major differences in the distribution of whistles contour by type in Almirante (Fig.1).

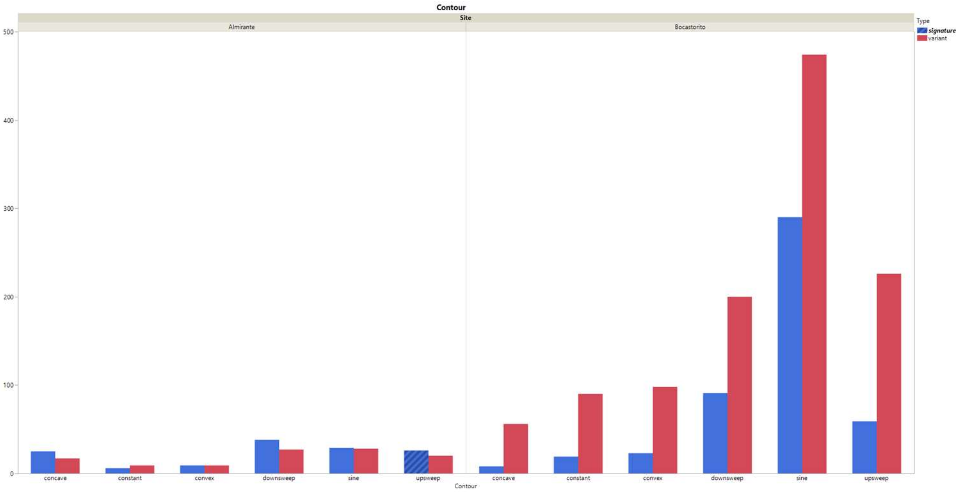


Fig. 1. Distribution of whistle contour by site and type of whistles.

Between sites whistle acoustic structure variation

Differences in whistle acoustic structure were primarily due to differences between sites and corresponding boat activity (X^2=75.7, df=15, p<0.0001, Fig.2) and to signature whistles from Almirante (X^2=6.27, p=0.0123). Signature whistles from Almirante were lower in frequency, shorter in duration, and less complex than the signature whistles recorded at Dolphin Bay (LF: X^2=6.7, df=1, p=0.0092; HF: X^2=139.9, df=1, p<0.0001; PF: X^2=62.1, df=1, p<0.0001; CF: X^2=84.9, df=1, p<0.0001; DF: X^2=175.4, df=1, p<0.0001; D: X^2=147.6, df=1, p<0.0001, IFP: X^2=292.4, df=1, p<0.0001) (Fig.3a-b). Variant whistles varied in the same directions in (HF: X^2=40.8, df=1, p<0.0001; PF: X^2=26.0, df=1, p<0.0001; CF: X^2=28.5, df=1, p<0.0001; DF:

X^2=102.6, df=1, p<0.0001; D: X^2=13.2, df=1, p=0.0003, IFP: X^2=201.7, df=1, p<0.0001) (Fig. 4a-b).

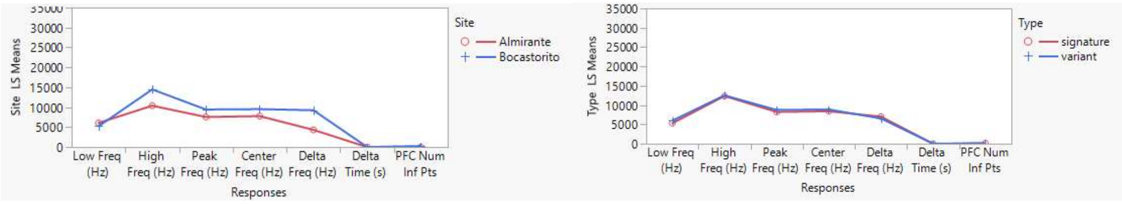
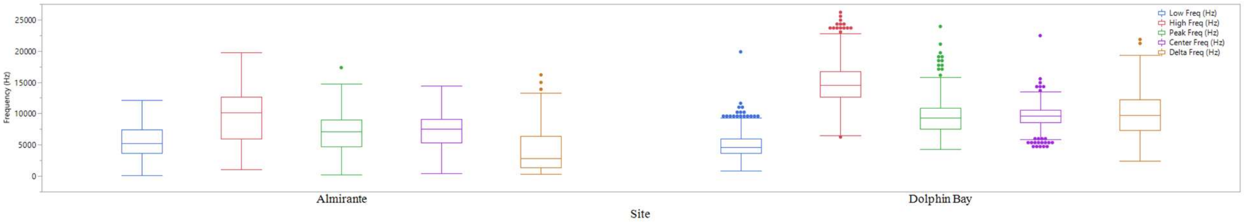


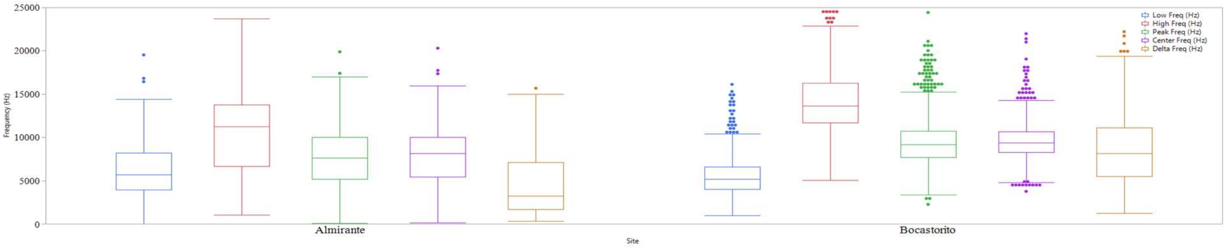
Fig. 2. Least square means for dolphin whistle type and sites (and corresponding boat activity) at Bocas del Toro, Panama.



a. frequency

b. time and complexity

Fig. 3. Distribution of signature whistle acoustic frequency, time, and complexity variables as a function of site, which corresponds to the two different types of boat traffic (Almirante: transport, Dolphin Bay: tourism).



a. frequency

b. time and complexity

Fig. 4. Distribution of variant whistle acoustic frequency, time, and complexity variables as a function of site, which corresponds to the two different types of boat traffic (Almirante: transport, Dolphin Bay: tourism).

Within site, whistle acoustic structure variation

Dolphins in dolphin Bay emit signature and variant whistles that are more complex, longer in duration and at higher frequency than dolphins in Almirante. These differences are largely due their response to direct interactions with tour boats. In the presence of multiple tour boats dolphins at Dolphin Bay dolphins increase their frequency variables (HF: X^2=11.9, df=1, p=0.0005; PF: X^2=69.0, df=1, p<0.0001; CF: X^2=61.2, df=1, p<0.0001) and produced more complex whistles (IFP: X^2=162.1, df=1, p<0.0001).

IV. DISCUSSION

This study found that bottlenose dolphin whistles are shorter in duration, less complex, and lower in frequency in Almirante than in Bocastorito. There were no major differences between signature and variant whistle acoustic parameters in either location, indicating the primary influence on the acoustic parameter differences is the type of boat traffic (tour vs. taxi). However, because of the difference in the nature of the boats in the two locations and the difference in the purpose of signature and variant whistles, signature and variant whistles should still be examined independently of each other. Tour boats in Bocastorito are much more likely to cause separation of mothers and calves than the taxi boats in Almirante. These separation events are one of the most important times for signature whistles to be used. Some populations of dolphins alter their acoustic patterns in response to stress, specifically by increasing the frequency, and the rate of emission during stressful events (Esch et al 2009). This is consistent with our findings. The emission rates were much higher in bocastorito than in almirante, and the frequency was higher in all but one parameter (low frequency), which indicates that bocastorito is a more stressful environment for the dolphin population. The whistles were also much longer in bocastorito, which could indicate that longer whistles may help dolphins reunite in the presence of boats.

Previous research has shown that dolphins simplify their whistles as a response to increased ambient noise levels (Fouda et al 2018). It would be valuable to investigate the ambient noise levels in almirante and bocastorito. Since the whistles were much simpler in Almirante, we would expect more ambient noise there, which would make sense because that location has larger boats. However, Fouda et al found that dolphins also increase the frequency of their whistles in response to more ambient noise, in which case bocastorito would be expected to have more ambient noise which is contradictory to the previous prediction. Perhaps the combination of noise levels and stress levels (which can be predicted with the type of boats present as we did here) would be worth researching more.

ACKNOWLEDGMENTS

Esch, H.C., Sayigh, L.S., Blum, J.E., and Wells, R.S. (**2009**). "Whistles as potential indicators of stress in bottlenose dolphins," Journal of Mammalogy **90**, 638-650.

Fouda, L., Wingfield, J.E., Fandel, A.D., Garrod, A., Hodge, K.B., Rice, A.N., Bailey, H. (**2018**). "Dolphins simplify their vocal calls in response to increased ambient noise," Biology Letters **14**.

Janik, V.M., King, S.L., Sayigh, L.S., Wells, R.S. (**2013**). "Identifying signature whistles from recordings of groups of unrestrained bottlenose dolphins (Tursiops truncatus)," Marine Mammal Science **29**, 109-122.

Janik, V.M., and Sayigh, L.S. (**2013**). "Communication in bottlenose dolphins: 50 years of signature whistle research," Journal of Comparative Physiology A **199**, 479-489.

King, S.L. et al (**2016**). "Maternal signature whistle use aids mother-calf reunions in a bottlenose dolphin, Tursiops truncates." Behavioral Processes, **126,** 64-70.

Marley, S.A., Kent, C.P.S., Erbe, C., Parnum, I.M. (**2017**). "Effects of vessel traffic and underwater noise on the movement, behavior and vocalisations of bottlenose dolphins in an urbanised estuary," Scientific Reports **7**.

May-Collado, L.J., and Quinones-Lebron, S.G. (**2014**). "Dolphin changes in whistle structure with watercraft activity depends on their behavioral state," Journal of the Acoustical Society of America **135**, 193-198.

Quick, N. J., Janik, V.M. (**2012**). "Bottlenose Dolphins exchanges signature whistles when meeting at sea," Proceedings of the Royal Society B: Biological Sciences **279**, 2539-2545.

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

Singing activity and song structure of humpback whales (*Megaptera novaeangliae*) in wintering grounds off the coast of Guerrero, Mexico

Cybele Adamcewicz[1], Isabel Belash[2]

ABSTRACT

Central American (CA) humpback whales are known to breed off of the coast of Guerrero, Mexico during the month of February. Autonomous underwater recorders were used to record the whales singing activity. It was hypothesized that there would be variation in the whales song structure over the course of the breeding season. Humpback whale singing activity was negatively correlated with boat activity in the area. A total of five phrases were identified in the humpback whale song, and were conserved throughout the entire study period. This study provides important information in understanding the small genetic unit of the Guerrero humpback whales. This could help conservation efforts for this threatened population of whales.

I. INTRODUCTION

Humpback whales (*Megaptera novaeangliae*) are common in ocean basins around the world. Humpback whales have been a particular species of interest for marine biologists due to the fact that the entire species was almost completely decimated (NMFS and NOAA 2016). Since then, many efforts have been taken to study humpback whale populations in order to monitor the population size and gain a better understanding of the species overall. In recent history, scientists have developed methods to accurately study humpback whales using acoustic data. It is known that male humpback whales repeat loud, long, complex sequences of sounds during their lifetime. These sequences are referred to as whale "song" (Darling 2019). Whale song has been recorded in winter breeding grounds, migratory routes, and summer feeding grounds (Murray et al. 2012). There are many different theories which attempt to explain the reason behind these songs. Popular proposed theories suggest that: (1) Male song plays a role in female attraction (Tyack 1981); (2) Humpback whale song is a way for males to associate with each other to create a means of reciprocity in mating (Nicklin et al. 2006).

In order for scientists to accurately study and compare whale song, consistent methodology for classifying and identifying whale songs is necessary. Leaders in the field of humpback whale acoustic analysis have established a general system for classifying the humpback whale song structure. The shortest sound is called a unit. A set of units is combined to form a phrase. Similar phrases are repeated to form a theme. The whale song is defined as the combination of multiple distinct themes (Cholewiak 2012). Using these guidelines, scientists are able to compare whale songs in different ocean regions. The highest degree of similarity (same phrases and themes) exists within a breeding area of a particular breeding season (Winn and Winn 1978). Throughout the breeding season, the humpback whale song of a particular region has been seen to change. Proposed hypotheses for the variation of whale song include: (1) Mixing of subpopulations of humpback whales during feeding migrations; (2) Mixing due to humpback whales returning to different breeding grounds; (3) Mixing that occurs due to whales visiting multiple breeding grounds within a season (Murray et. al 2012). These hypotheses suggest why there might be change in the composition of whale song from one season to the next and even from one region to another. Human activity also has a significant impact on humpback whale singing patterns. Boat traffic from human activities such as whale watching was shown to negatively correlate with instances of whale song, as less singing activity was recorded during hours of high boat traffic. The proposed mechanism of this effect was hypothesized to be; (1) Male humpback whales moving outside of the location range, (2) Males ceasing singing, or (3) A combination of 1 and 2 (Sousa-Lim et al. 2008).

The Guerrero humpback whale population is a small subset of the greater humpback whale population belonging to the Central American breeding grounds. This whale population consists of about 500 individuals, which represent a small endangered genetic unit. In 2018, scientists were able to record their song off the coast of Guerrero, Mexico. In this study, the major repeating components of the Guerrero humpback whale song were analyzed. The main goal of this study was to identify the causes of variation in the Guerrero humpback whale song. Observations from this study would be able to answer many questions such as: Is there variation in song between the Guerrero whales and other whales of the Central American breeding grounds? If so, how conserved are their songs? Has human activity had a significant impact on the Guerrero whales' singing activity?

Determining the similarity between humpback whale songs of different regions would allow us to draw conclusions about whale migration patterns. This is significant in determining if smaller whale populations (such as the Guerrero whales) are isolated by their feeding grounds, and if so, is this isolation adding to their vulnerability as an endangered population? This would lead us to further identify and define populations based on acoustic data. This study will also aid in setting defined guidelines for acoustic research that can be followed in future studies. Consistent framework used between researchers allows for sharing data and communicating findings in an easier manner. A communal voice is important to the scientific community as it allows for collaboration and further understanding of complex topics. In addition, gathering present data on current humpback song themes, components and cycles allows for comparisons to be made with future whale recordings to continue to monitor and understand the cultural changes in song experienced by these populations and individuals (Payne et al. 1971).

II. METHODS

This study took place off of the coast of Guerrero, Mexico, one of the reported breeding areas of the Central American humpback whale stock. An autonomous underwater recorder microMARS^{TM} 1.02 (Flat frequency response up to 125 kHz) with two 512 GB SD cards was used to record the soundscape. The recordings were made from February 3 - February 16, 2017. The recorder continuously captured the soundscape at a sample rate of 12.7 kHz in 30-minute files. A total of 656 sound files were analyzed representing 328 hours of acoustic data. Song presence/absence per file was recorded to estimate whale-singing activity throughout the day. Using this information, the proportion of files with songs was calculated per day and hour. In addition to whale sounds, the presence of boats, dolphins, and fish were also recorded when applicable. To determine if boat activity affected singing activity, a linear regression analysis was run in JMP 14.0 (SAS, 2019).

To study the song structure of these whales, only songs with a SNR > 6dB were selected. These songs were then manually visualized in RAVEN version 1.5.0 (Cornell Laboratory of Ornithology, NY.) with a fast Fourier transformation size of 2048 points, an overlap of 50%, and a 2000 sample Hann window. Through collaboration with other observers, the hierarchical structure of whale song into units, phrases and themes that occurred in the Guerrero whale population were classified (Cholewiak 2012). The duration of each was extracted from the spectrogram to estimate the contribution of each phrase to the overall song.

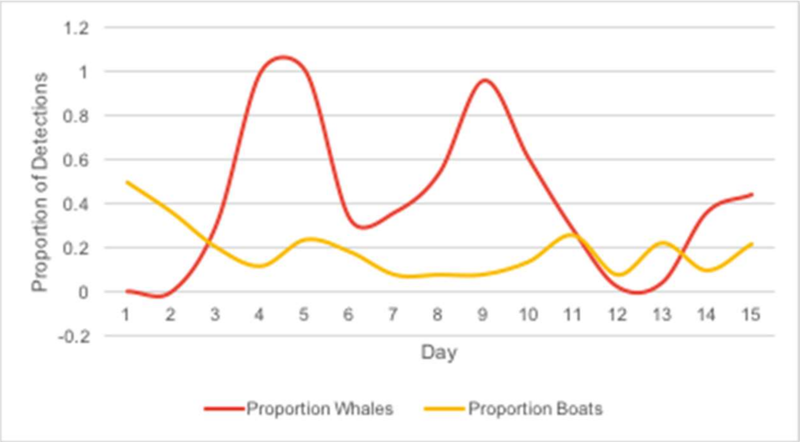
III. RESULTS

A. Singing Activity

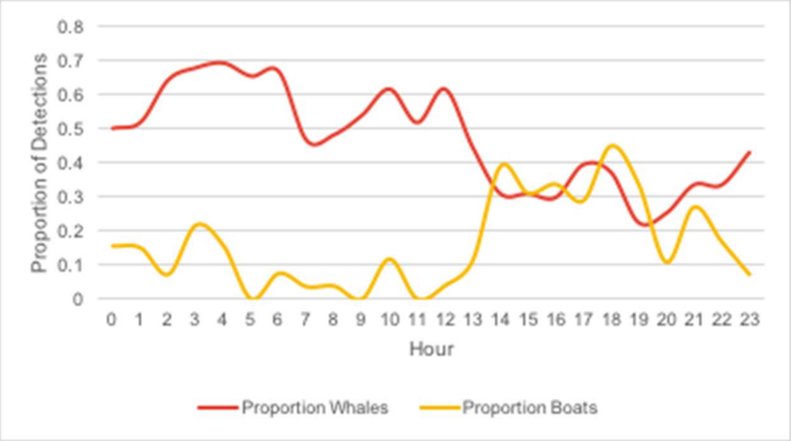
Of 656 files, a total of 306 files contained humpback whale song, representing approximately 45% of the recording time analyzed. The majority of humpback whale sound detections were found on the 5th and 6th days of the breeding season (50 songs detected on each of these days) and on the 10th day of the breeding season (47 songs detected on this day) (Fig. 1a). For the 15-day study period, boat activity remains relatively constant throughout. However, there is a slight increase in boat activity the first day of the breeding season. The peak time of day for whale song was found to be from 0000-1200 hours (Fig. 1b). There is a significant negative relationship between boat and whale song detection (R^2=0.03, p=0.002, Fig. 2), however boat presence only explains 3% of the variation in whale song activity. Overall, whale singing activity is at its lowest between the hours 1pm-7pm (Fig. 1b). Boat activity is at its lowest between the hours of 0000-1200 hours, and is at its highest from 1300-2000 hours.

B. Song structure

A total of 29 one-cycle songs were analyzed. Overall, Central American whale songs consisted of five phrases (Fig.3). The most used phrase was phrase C followed by A, B, E, and D (Table 1). The theme structure was consistent throughout the study period and the sequence of phrases in the song was as follows: A->B->C->D->E.



a.



b.

Figure 1. Proportion of whale and boat detections by day (a) and hour (b) at Guerrero, Mexico.

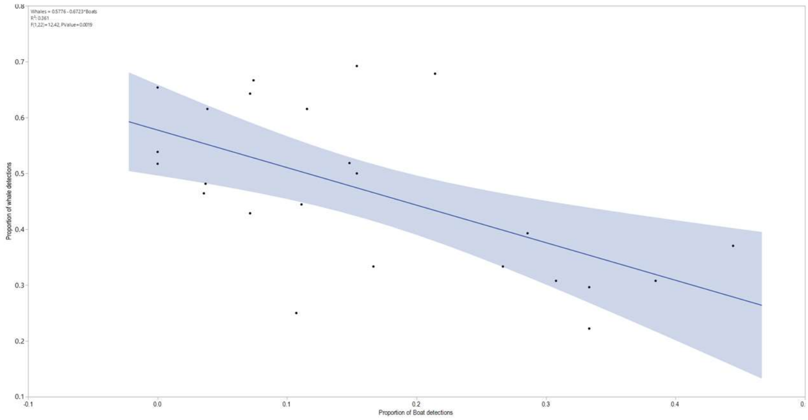
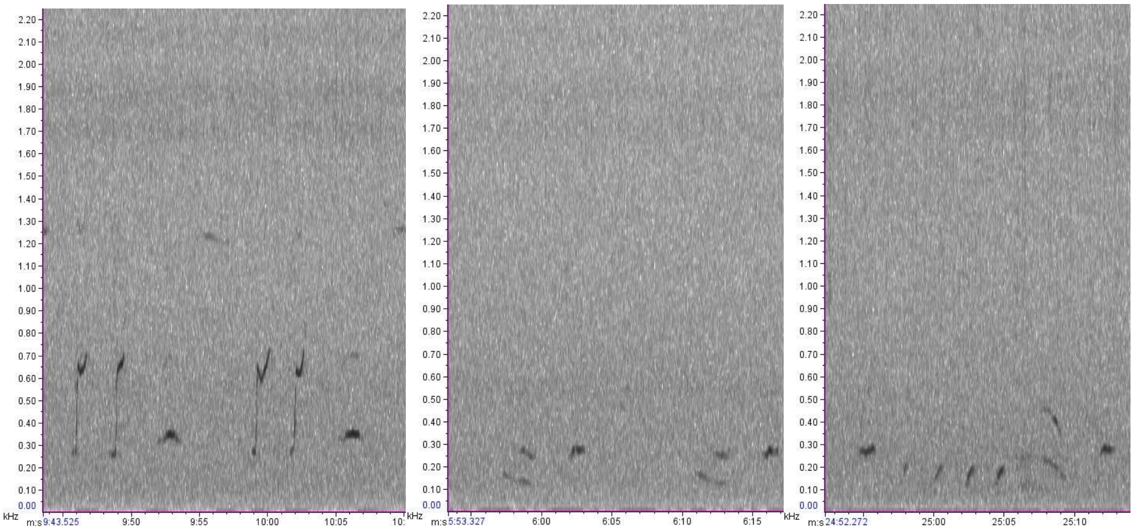


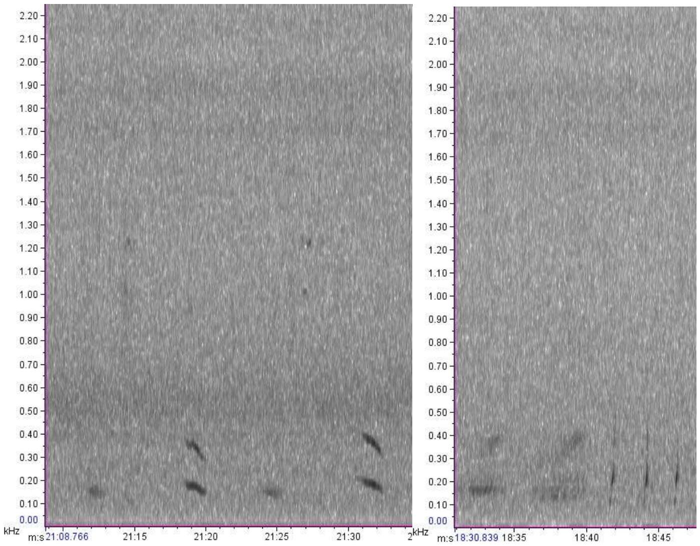
Figure 2. Linear regression between the proportion of whale and boat detections.

Table 1. Song phrases duration and contribution to the overall song.

PHRASE	DURATION (S)	% OF THEME
A	3537.918	0.226047715
B	3291.756	0.21031972
C	4850.933	0.309940005
D	1494.749	0.095503795
E	2475.887	0.158191512



Phrase A **Phrase B** **Phrase C**



Phrase D **Phrase E**

Figure 3. Central American breeding stock song structure in Guerrero, Mexico 2017.

IV. DISCUSSION

Phrases, themes and duration of humpback whale song has been seen to change over the passage of time (Darling et al. 2019). The variation has been theorized to be the result of subpopulations of humpbacks interacting during migration to feeding grounds, returning to breeding grounds, and visiting more than one breeding ground in a season (Murray et al. 2012). These variations accrue over time while different populations of humpbacks are in proximity and diverge at and after the end of the migratory season (Darling et al. 2019). This data suggested that there is little variation in humpback whale song over the span of one month in Guerrero, Mexico. The songs consisted of five phrases with little variation in the units of each phrase. These findings are in conflict with past literature, as songs recorded and analyzed from Hawaii and Mexico were composed of six distinct themes (Cericho et al. 2001). The lack of variation in the number and composition of themes in this data may be attributed to the timeframe during which the recordings were made. The month of February during which this data was collected is near the end of the humpback breeding season (Kobayashi et al. 2016), suggesting that the five phrases seen were the result of convergence of song components during the interaction of subpopulations.

The data collected showed that there are whales present in the breeding grounds around Guerrero, Mexico. Northern Guerrero humpback whale singing activity from 2017 was compared to that of Costa Rican southern humpbacks over the course of one day. On average, more instances of singing were recorded at Costa Rican sites than in the Guerrero waters with an exception noted from 0500-1200 hours. After 1200 hours, there was a decrease in the presence of Guerrero singing, but an increase in the number of recorded songs off the coast of Costa Rica. The data from the two locations seem to have an inverse relationship to one another, as the Costa Rican southern whales commonly sing from 1200 hours to around 0200 hours, while the Northern Guerrero whales most commonly sing from 0000 to 1200 hours. These data suggest a difference in the singing habits subpopulations of the humpback whales, as well as how different migration patterns and breeding grounds may influence activity.

This study also emphasized the fact that the coast of Guerrero, Mexico is a popular destination for tourist boats and fishermen. Aquatic environments are greatly impacted by this human activity. This study exemplifies that there is indeed a significant difference between the presence of humpback whales and the presence of boats ($p=0.002$). There is a significant negative relationship between the presence of boats and the presence of singing humpback whales ($R^2=0.036$). Approximately 3% of the variation in whale song activity can be accounted for by boat activity. This significantly low value could be due to the small amount of data that was analyzed during the study. The analyzed data was representative of a 15-day study period during the month of February. This is a short span of time in comparison to the humpback whale breeding season and also Guerrero's tourism season. Other studies have shown consistent results. In a study comparing Brazilian whale song activity and boat presence, data suggests that boat presence does in fact have a negative relationship with whale singing activity. Researchers found that whale song activity decreased after the boat's passage. Researchers concluded that humpback whales experience a significant amount of stress during the passage of a boat. After the boat has distanced itself from the whale, the whale will stop singing as a stress response. This could explain why the data of our study is not a direct correlation, but slightly delayed (Tsujii et al. 2018).

Although our data does not provide a definitive answer as to the absolute source of variation in humpback whale song, they suggest that human activity could potentially play a role in variation. Our study is limited by the lacking amount of data we had to make an accurate conclusion. For future studies, it would be useful to use a broader set of data in order to monitor whale song activity throughout the entire year including the breeding and feeding seasons. This information is important to conservation biologists and marine biologists because it would provide more information on the mysterious Guerrero humpback whale population. There is not much published data on this small cohort of whales and it is important to understand how the population is affected by its surroundings (of humans and other whale populations).

REFERENCES

Cerchio, S., Jacobsen J. K., and Norris T. F. (**2001**). "Temporal and geographical variation in songs of humpback whales, *Megaptera novaeangliae*: synchronous change in Hawaiian and Mexican breeding assemblages," Animal Behaviour **62**(2): 313-329

Cholewiak, D. M., et al. (**2012**). "Humpback whale song hierarchical structure: Historical context and discussion of current classification issues," Marine Mammal Science **29**. 312-332.

Darling, J. D. et al. (**2019**). "Convergence and divergence of songs suggests ongoing, but annually variable, mixing of humpback whale populations throughout the North Pacific," Scientific Reports **9**(1). 7002.

Kobayashi, N. et al. (**2016**) "Peak Mating and Breeding Period of the Humpback Whale – (*Megaptera novaeangliae*) in Okinawa Island, Japan," Open Journal of Animal Sciences **6**(3): 169-179

Murray, A. et al. (**2012**). "Minimal similarity in songs suggests limited exchange between humpback whales (Megaptera novaeangliae) in the southern Indian Ocean," Marine Mammal Science **28**(1), 48-57.

National Marine FiESPeries *NMFS), National Oceanic and Atmospheric Administration (NOAA), Commerce (2016). "Endangered and Threatened Species: Identification of 14 distinct Population Segments of the Humpback Whale (*Megaptera novaeangliae*) and Revision of Species-Wide Listing," Federal Register **84**, 174.

Nicklin, C. P., Darling J. D., and Jones M. E. (**2006**). "Humpback whale songs: Do they organize males during the breeding season?" Behaviour **143**, 1051–1101

Payne, R. S., and McVay, S. (**1971**). "Songs of Humpback Whales," Science **173**(3997), 585–597.

Sousa-Lim, R. S., and Clark, C. W. (**2008**). "Modeling the Effect of Boat Traffic on the Fluctuation of Humpback Whale Singing Activity in The Abrolhos National Marine Park, Brazil," Canadian Acoustical Association **36**(1), 174–181.

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

Tyack, P. (**1981**). "Interactions between singing Hawaiian humpback whales and conspecifics nearby," Behavioral Ecology and Sociobiology **8**(2), 105–116.

Winn, H. E., and Winn L. K. (**1978**). "The song of the humpback whale (*Megaptera novaeangliae*) in the West Indies," Marine Biology **47**(2): 97– 114.

SOUNDSCAPE CHARACTERISTICS AND SPECIES COMPOSITION OF A SHALLOW CORAL REEF COMMUNITY AT BOCAS DEL TORO, PANAMA.

David Sileo[1]
Department of Biology, University of Vermont, Burlington, VT 05403

The use of sound as a tool of study in marine ecosystems has grown in popularity over the years. Sound can be used to study ecosystem diversity through soundscape ecology. Factors like time, moon phase, and anthropogenic noise can play a role in the soundscapes of marine ecosystems. Changes induced by these variables can be quantified and used to explain fluctuations in the diversity of a given community. This study focused on Sharkhole, Bocas del Toro, Panama. The recordings were broken into 1 min samples per hour and analyzed for acoustic species. The diversity of this ecosystem was compared to the Acoustic Complexity Index (ACI) for the area. The data was also analyzed for any impacts due to time, lunar phase, and anthropogenic activity. The study showed results that indicate a relationship between time and the soundscape. The overall number of acoustic species detections appeared to increase at night but decrease during the day. The impact of time on individual species showed similar impacts with certain species preferring night, while others preferred the daytime. The impact of boat traffic showed no significant effect on the data while lunar phase showed some difference in detection with fluctuation in proportion of individuals by phase. The study found that there was no correlation between the ACI and the sampled results however, this may be attributed to the ability of the ACI to account for abundance. The conclusions indicate that there was evidence for time and lunar phase impacts on diversity, but that human based impacts and correlation to the ACI would require more data. Overall the study deemed substrate the main variable impacting marine soundscapes.

I. INTRODUCTION

A considerable proportion of the fauna in marine communities emit species-specific acoustic signals for communication, and prey and predator detection. Using passive acoustic monitoring researchers can translate this cacophony of sounds into a better understanding of the integrity and dynamics of marine communities with minimal disturbance to the underwater environment (Lyon 2019, Sugai 2019, Desiderà 2019, Carriço 2019). Many factors can influence the spatio-temporal dynamics of marine soundscape, including time of day, anthropogenic activity, substrate and lunar phase (Coquereau 2017, Mcwilliam 2013, Staaterman 2014).

The use of sound to understand the makeup of an underwater community (soundscape ecology) has been widely used in the field to model the dynamics of many communities and the impacts of external variables like moon phase and boat traffic. (Lillis 2018, Lindseth 2018, Mustonen 2018). Soundscape ecology allows for diversity to be analyzed with algorithms such as the Acoustic Complexity Index (ACI). The ACI quantifies variation of intensity in non-human sounds and has been used in a variety of aquatic applications (Sueur 2014, Pieretti 2011). Sharkhole, Bocas del Toro, Panama provides an area of study with varied biotic and abiotic conditions. Sharkhole along with other regions of Bocas del Toro provide a variety of marine habitats and three-dimensional space that induce a wide variety of marine organisms (Collin 2005, Mclntyre 2010).

With biodiversity loss a pressing issue, studies of ecosystem diversity are of the upmost priority. Increased human activity and changing ocean chemistry are leading a fast evolving and dynamic marine ecosystem in which sound is a major part. Such impacts include increases in sound propagation induced by decreased ocean pH (Joseph 2010). The objective of this research was to better understand the roles of different factors on biodiversity levels in a Sharkhole coral reef community. This study expects to find variation in the soundscape with relation to time of day, lunar phase and anthropogenic noises. This variation would indicate the diversity changes of acoustic species in the Sharkhole community due to the above factors.

II. METHODS

A. Study Site

The data was collected from Sharkhole (9.184N/-82.176W), Bocas del Toro, Panama. The Archipelago of Bocas del Toro consists of a collection of mangrove fringed islands bordering coral reefs and sea grass beds (Collin 2005). Sharkhole is home to one of the shallow coral reefs in the area, which is exposed to limited boat traffic.

B. Soundscape sampling

Passive acoustic recorders model RUDAR-mk (RUDAR-mK2, sampling rate up to 96kHz - 169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com) were used to sample the soundscape at Sharkhole. The recorder was programmed to continuously record the soundscape in segments of 30 minutes at a sampling rate of 48 kHz from May 3rd - 13th and 28th-31st to June 1st - 7th of 2018 and deployed at a depth of 12 m.

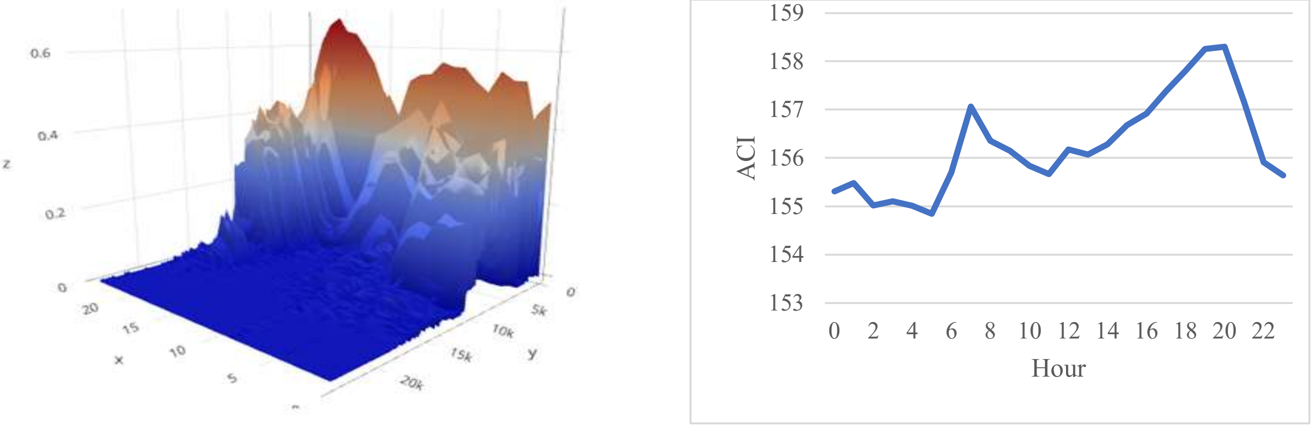
C. Data Analyses

Soundscape analyses were performed in ARBIMON (https://arbimon.sieve-analytics.com/) using a 1-min sample every 10 min per hour. Using a bin bandwidth of 86 (Hz) a matrix of acoustic events per hour of the day was calculated. This matrix was then used to generate a 3D plot of acoustic events per hour and per frequency band. In addition, the Acoustic Complex Index (ACI) (Pieretti et al. 2010) was calculated to estimate the acoustic diversity of Sharkhole. The index has been widely used to study marine communities because it filters out anthrophonies such as boat engine noise. To test the accuracy of the ACI index and to identify the most important biological contributors to the soundscape, 1-min samples for every hour of a day were analyzed to identify acoustic species based on the spectrogram, audio pattern, and repetition of a signal. To standardize the data, the proportion of detection by species was compared to the time. An ANOVA (analysis of variance) was used to compare the mean number of acoustic species by lunar phase to indicate a significant difference between them.

III. RESULTS

A. Sharkhole soundscape characteristics

The distribution of acoustic events in the soundscape for Sharkhole shows a reef community with a significant decrease in activity between 10 a.m. and 3 p.m. Most of the acoustic events occurred at frequencies below 10 kHz (Fig.1a). The diversity index ACI shows a similar pattern of increasing acoustic diversity starting at 4 p.m. and with a peak between 6- 7p.m. (Fig.1b).



a. b.

Fig. 1. Soundscape characteristics of the coral reef at Sharkhole (a) Distribution of acoustic events by time and frequency bin (b) Acoustic Complexity index per time of day.

The ACI can be used as an indicator of the community acoustic species composition as shown by the regression (Fig. 2). The regression showed a negative correlation in this instance with a low r^2.

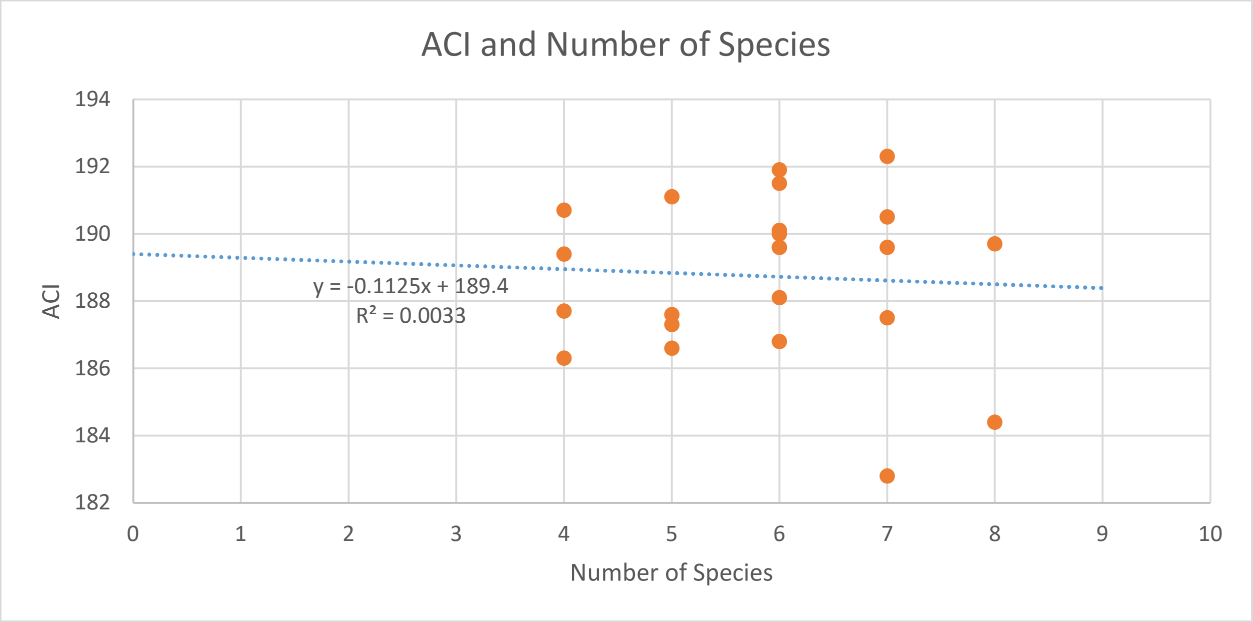


Fig. 2 Scatter plot with a regression of the number of acoustic species by hour and the ACI value by hour

B. Sharkhole marine community composition

A total of 15 audio species were identified (Table 1). The most commonly detected species were the toadfish, snapping shrimp, 'mmm species', and 'woodpecker'. The toadfish and snapping shrimp were detected throughout the day. About 40% of the total detections for both species were between 4:00 and 19:00, with a slight increase in snapping shrimp detection to 51% between 7:00 and 9:00. Snapping shrimp and toadfish decreased in detections levels to approximately 30% between 20:00 and 2:00. The "Woodpecker" detections peaked at 25.4% of detections between 1:00 and 2:00 before falling off with other detections occuring less than 11% of the detections per hour. The "woodpecker" showed a complete decrease in detections at 21:00

with no individuals heard at this time. The “mmm” species” was detected mainly at night between 20:00 and 6:00 accounting for around 15% of the dectections, while during the day it accounted for between 1.96% and 10.7% of the detections (Fig. 3).

Acoustic Species	**Detections**	**Proportion**	**True Name**
bahh_sound	1	0.08%	unknown
boop_sound	8	0.62%	unknown
boop_sound 2	4	0.31%	unknown
croak	9	0.70%	unknown
deep_grunt	3	0.23%	unknown
dolphin	2	0.15%	Dolphin-(*Tursiops*)
fish_chorus	21	1.62%	unknown
fish_chorus_2	33	2.55%	unknown
mmm_species	130	10.05%	Squirrel Fish-(*Holocentrus*)
rubbing	1	0.08%	unknown
snapping_shrimp	496	38.33%	Snapping Shrimp-(*Alpheidae*)
toadfish	484	37.40%	Toadfish (*Batrachoididae*)
woodpecker	74	5.72%	Grunt-(*Haemulon*)
woodpecker_2	27	2.09%	Grunt-(*Haemulon*)
woof_sound	1	0.08%	unknown

Table 1: Audio Species, their detection number and respective proportions of total detections (1294 individuals)

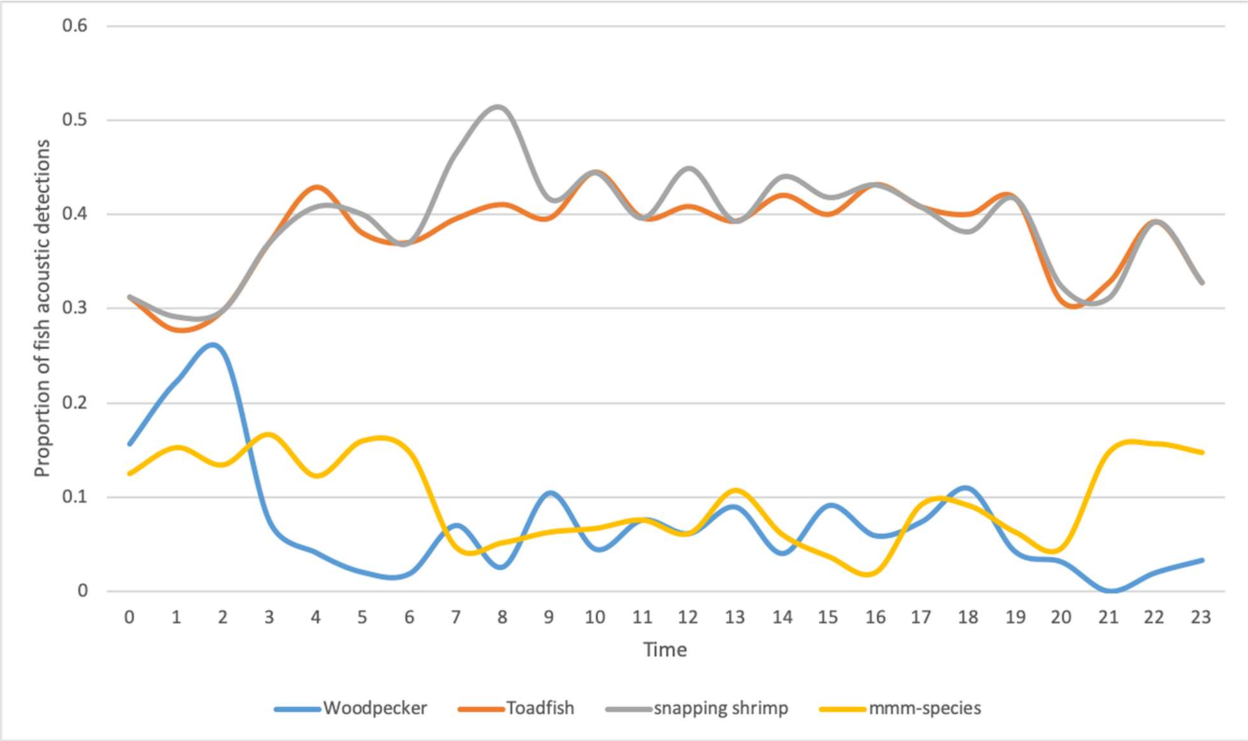


Fig. 3. Proportion of species detections by hour for the most commonly detected species at Sharkhole.

B. Lunar Phase

Overall, the mean number of fish acoustic detections was greater during the waning gibbous and last quarter (Fig. 4). Correcting for sample rate, snapping shrimp and toadfish showed their highest acoustic activity independently of moon phase, however, both show a slight increase during the waning crescent (44.8% and 41.9%). The woodpecker's highest acoustic activity was during the last quarter moon with a proportion of detection of 9.8%. Finally, the "mmm" species had the highest acoustic activity during the full moon and waning gibbous moon at 13.4% (Fig.5). The soundscape was divided by phase and an analysis of the average total entropy (H), average acoustic complexity index (ACI) and average frequency peaks (NP) was ran. Indices were displayed graphically (Fig.6).

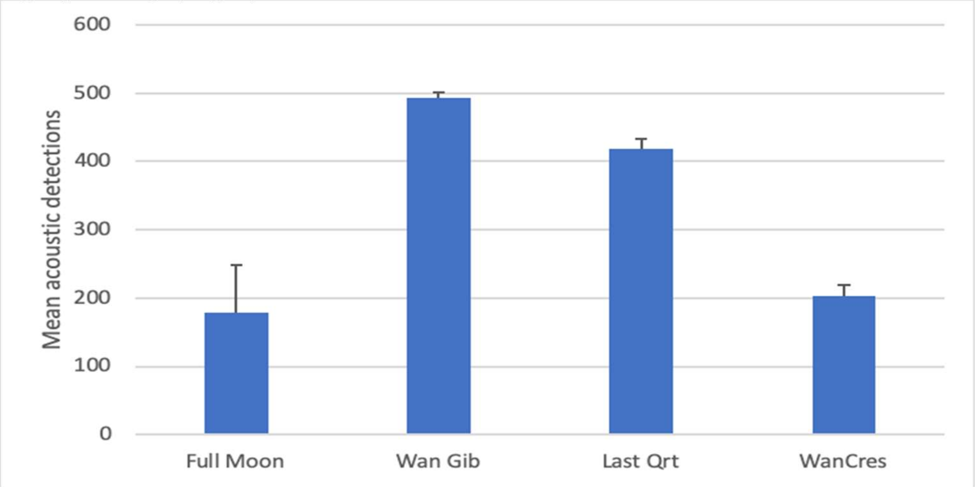


Fig. 4. Mean acoustic detection by moon phase at Sharkhole.

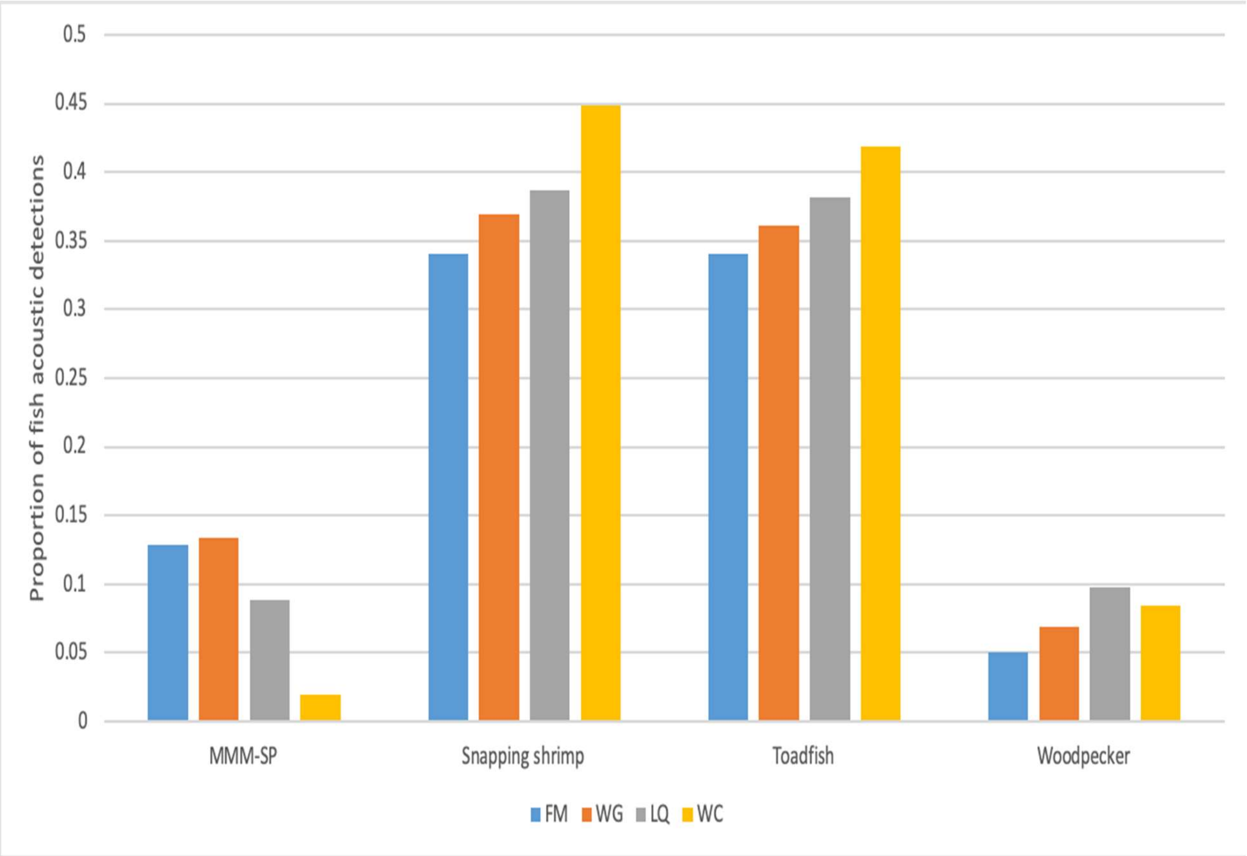
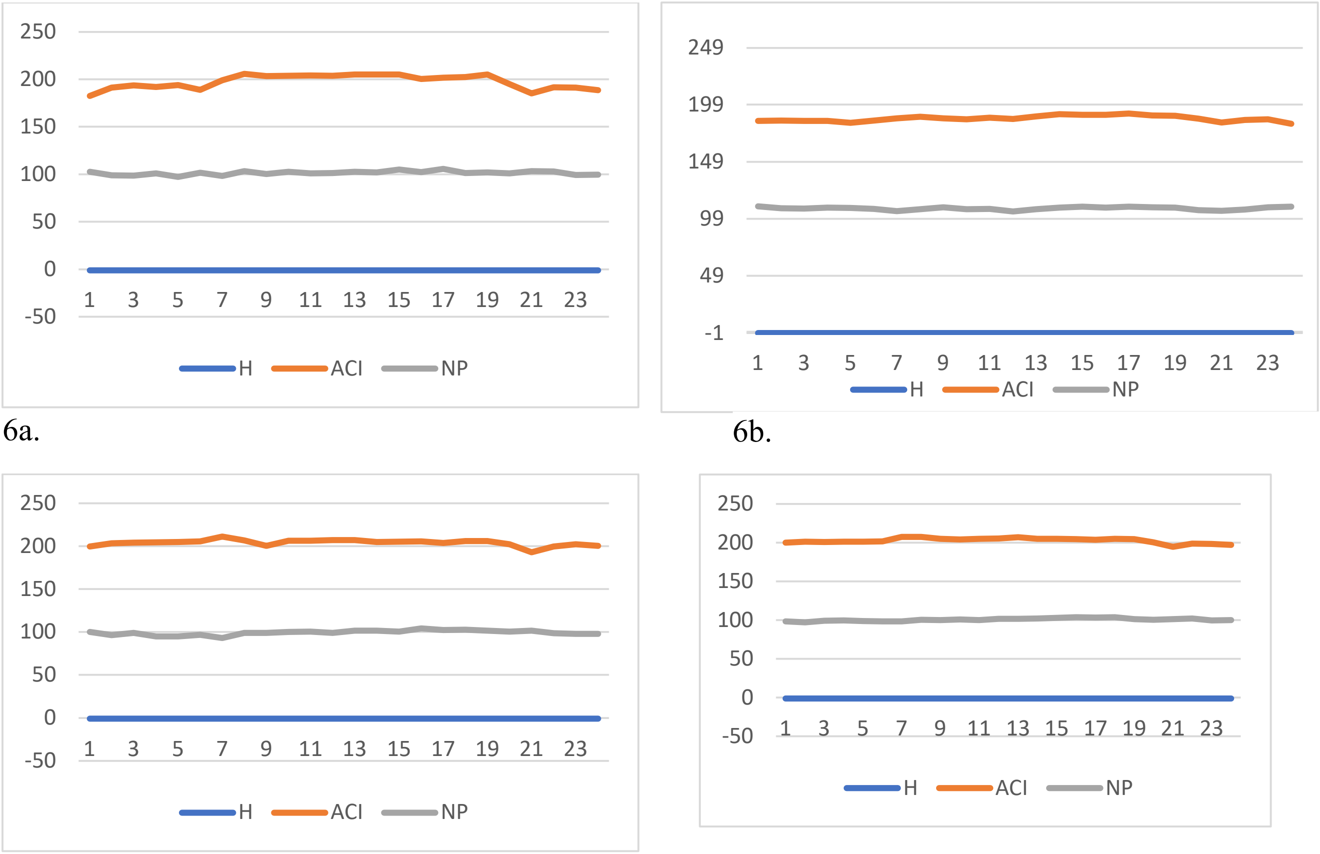


Fig. 5. Proportion of acoustic detections of the four most common acoustic species by lunar phase at Sharkhole.



6a. 6b.

6c. 6d.

Fig. 6. (a) the indices for a full moon, (b) the waning gibbous, (c) the waning crescent and (d) the last quarter where H is average entropy, ACI is the acoustic complexity index and NP is the average frequency peaks.

IV. Discussion

Using the ACI allowed for a quantification of the diversity based on the soundscape. Correlating the ACI values with the number of audio species found a weak negative correlation (slope = -0.1125, $r^2 = 0.0033$). The study attributes this to the ability for the ACI to also be able to account for abundance. Abundance was skewed due to the high number of toadfish and snapping shrimp. The presence of two dominant species produced an inaccurate representation of the soundscape through the ACI. Further studies could combat this error by omitting toadfish and snapping shrimp or adjusting the sampling in a way that would reduce the impact of abundance in the ACI calculation.

The composition of the community used the proportion of detections of each unique acoustic species to identify what sound producers are active in the region. Cross referencing between species found in Bocas del Toro (https://stri.si.edu/) and an acoustic species data base (https://www.macaulaylibrary.org/) led to the matching of a few species with the recorded sound. Toadfish and snapping shrimp were identified earlier. Others were compared to the banked sounds and likely groups of candidates for each were identified. The woodpeckers were matched to grunts (*Haemulon*), the mmm species to squirrel fish (*Holocentrus*) and dolphin sound to a dolphin (*Tursiops*). Species left as "unknown" were either an off tone of one of these and miss identified as a new acoustic species, or their sound was not found in the data base comparison. The addition of human error into the determination of what was a unique acoustic species produced a degree of bias in this study.

The main species found in the study were "mmm species" (10.05%), "snapping shrimp" (38.33%), "toadfish" (37.40%), and "woodpecker" (7.81% -both 1 and 2). Isolating these main species adjusted for any outliers, and better represented what roles the target variables played on the community. Sorting the proportion of detections by hour for the four main species showed mmm species and woodpeckers having higher activity at night while toadfish and snapping shrimp maintained a relatively stable presence. A slight drop for snapping shrimp and toadfish was noted at night for both toadfish and snapping shrimp. This was attributed not to decreases in their activity, but an increase in the presence of other nocturnal species not represented in the top four i.e. fish chorus. Such species cut down their proportion of detection and in some cases were able to drown out the audio production of these two. Accounting for the 'major minorities' would better represent these changes. Additionally, counting the number of calls per 1-min by a species would show differences in activity not represented by an overall proportion and may have better represented changes in the soundscape at a species level.

Of the other variables, lunar phase was the only one to have been found significant with a p-value less then 0.05. The mean detection showed more acoustic species heard during the waning gibbous and the last quarter compared to the waning crescent and full moon. The full moon and waning crescent were deemed to show a significant difference in mean acoustic detection while the other two produced p-values greater than 0.05 (https://www.r-project.org/). The elevated mean detection in the waning gibbous and the last quarter leading to these differences may be due to sample size. These two phases were sampled more than the later leading to inaccurate representation of the mean. Adjusting for the proportion of detection and grouping by species showed the waning crescent and the last quarter having higher detection than the other two in snapping shrimp, toadfish and the woodpecker but the inverse in mmm species. Interestingly, when placed in chronological order, the proportion of detection appears to show a trend of increase towards the new moon in snapping shrimp, toadfish and woodpecker, but decrease in mmm species. A pattern cannot be concluded at this point, but further studies could look into the soundscape by moon changes at Bocas del Toro to see if this pattern continues throughout the entire cycle. Using the generated indices from ARBIMON it was shown that the waning gibbous phase showed the lowest ACI compared to the other three phases, but the highest average frequency peaks showing lower diversity and higher activity during this phase. All in all, the moon phase and time of day showed some significance in the soundscape however, it was deemed that the substrate may still play the largest impact in the soundscape diversity of marine communities. This hypothesis could be studied by running a similar experiment in different areas of varied substrate.

V. References

Carriço R., M. A., Silva, G. M. Menezes, P. J. Fonseca‚ M. C. P. Amorim. 2019. Characterization of the acoustic community of vocal fishes in the Azores. Peer J 7:e7772

Collin, R. 2005. "Ecological Monitoring and Biodiversity Surveys at the Smithsonian Tropical Research Institute's Bocas Del Toro Research Station." Caribbean Journal of Science. 41:367–373.

Coquereau, L., J. Lossent, , J. Grall, L. Chauvaud. 2017. Marine soundscape shaped by fishing activity. Royal Society Open Science, 4:160606.

Desiderà, E., P. Guidetti, P. Panzalis, A. Navone, C-A. Valentini-Poirrier, P. Boissery, C. Gervaise, L. Di Iorio. 2019. Acoustic fish communities: sound diversity of rocky habitats reflects fish Species diversity. Marine Ecology Progress Series 608:183–197.

Joseph, J. E., C. S. Chiu. 2010. A computational assessment of the sensitivity of ambient noise level to ocean acidification. Journal of the Acoustic Society of America 128:EL144-EL149.

Lillis, A., T. A. Mooney. 2018. Snapping shrimp sound production patterns on Caribbean coral reefs: Relationships with Celestial Cycles and Environmental Variables. Coral Reefs. 37:597–607.

Lindseth, A. V., P. S. Lobel. 2018. Underwater Soundscape Monitoring and Fish Bioacoustics: A Review. Fishes. 3:36

Lyon, R. P., D. B. Eggleston, D. R. Bohnenstiehl, C. A. Layman, S. W. Ricci, J. E. Allgeier. 2019. Fish community structure, habitat complexity, and soundscape characteristics of patch reefs in a tropical, back-reef system. Marine Ecology Progress Series 609:33–48.

Mclntyre, A. 2010. Coral Reef Biodiversity. Pp. 65-67 in Life in the World's Oceans. John Wiley & Sons. West Sussex, United Kingdom.

Mcwilliam, J. N., A. D. Hawkins. 2013. A comparison of inshore marine soundscapes. Journal of Experimental Marine Biology and Ecology. 446:166–176.

Mustonen, M., A. Klauson, M. Andersson, D. Clorennec, T. Folegot, R. Koza, J. Pajala, L. Persson, J. Tegowski, J. Tougaard, M. Wahlberg, P. Sigray. 2019. Spatial and Temporal Variability of Ambient Underwater Sound in the Baltic Sea. Sci Rep. 9:13237

Pieretti, N., A. Farina, D. Morri. 2011. A new methodology to infer the singing activity of an avian community: The Acoustic Complexity Index (ACI). Ecological Indicators. 11:868-873.

Staaterman, E., C. B. Paris, H. A. DeFerrari, D. A. Mann, A. N. Rice, E. K. D'Alessandro. 2014. Celestial patterns in marine soundscapes. Marine Ecology Progress Series. 508:17–32.

Sueur, J., A. Farina, A. Gasc, N. Pieretti, S. Pavoine. 2014. Acoustic Indices for Biodiversity Assessment and Landscape Investigation. Acta Acustica United with Acustica. 100:772-781.

Sugai, L. S. M., D. Llusia. 2019. Bioacoustic time capsules: using acoustic monitoring to document biodiversity. Ecological Indicators. 99:149–152.

Factors influencing marine community diversity in Central America

Sean O’Sullivan and Elie Byrne

Abstract

The importance of monitoring marine ecosystems using various soundscape recording techniques can help identify and compare soundscape complexity of ecosystems located in different parts of the world. This study observed the effect of lunar phases on marine soundscape production in 4 locations in Panama and Costa Rica: Coiba, Tiburon, Diablo, Jardin. We hypothesized that soundscape complexity and structure will vary with lunar cycles. It was also hypothesized that soundscape activity would be significantly different between each site. It was predicted that acoustic activity, complexity, and diversity will increase during the new moon (complete darkness) for low frequency sonorous species like fish and whales. In addition, we predicted that soundscape activity would be higher in areas that were encompassed by reef ecosystems. The results indicated that there was a significant difference between soundscape complexity and activity between the four identified sites. It was also concluded that there was a significant difference between soundscape complexity at each site during different cycles of the moon phase. This study is important for understanding how marine soundscapes and complexity are affected by both environmental and anthropogenic conditions. Observing soundscape data in different marine communities can be helpful for predicting changes in behavior and overall dynamics of the ecosystem. Continuing research on how marine community dynamics are altered by various conditions will be helpful in propelling government officials to make more informed decisions on marine conservation and protection.

I. Introduction:

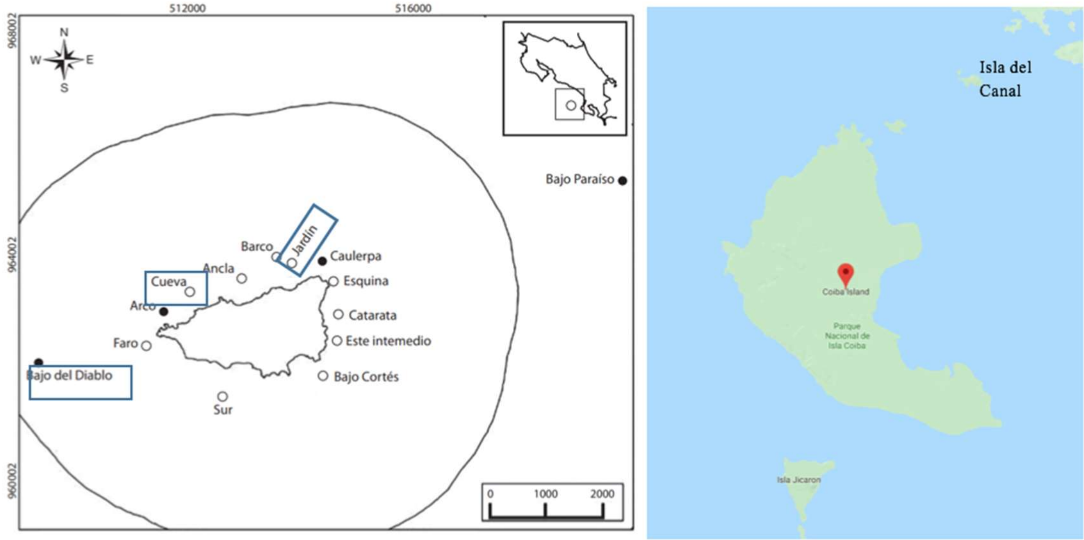
Ecosystem’s dynamics are often caused by fluctuations in abiotic (e.g., nutrient availability, temperature, tides) and biotic factors (e.g., predators, diseases) which can be intensified by climate change and/or anthropogenic activity (Fisher-Pool et al. 2015; Coquereau et al. 2017; Gordon et al. 2018). Coral reef ecosystems contain the highest amount of biodiversity on the planet and are under immense pressures from global stressors like changing sea temperatures (Smith et al. 2010). In marine communities, celestial patterns such as moon phases have been shown to promote changes in daily activity like migrations and spawnings by fish, corals, and other invertebrates (Yang et al. 2019). In addition, moon phases can affect the entire ocean’s basis by influencing tidal patterns, pressure changes, salinity, and temperature (Fritzen et al. 2019). Long-term studies of marine community composition are now possible with the use of passive acoustic monitoring and newly developed methods to estimate diversity based on sound cues. Because many animals in a marine community use sound for communication, prey and predator detection (Collin et al. 2000) signals can be used to infer community dynamics and diversity (Ruppe et al. 2015).Soundscape refers to the interrelations between sounds produced by living organisms and human activities (Farina 2014). Because soundscapes are reflections of marine communities, their diversity can also be influenced by abiotic factors. For example, moon phases can impact soundscapes by influencing when animals communicate and interact. A recent study found that in reefs acoustic activity at low frequency was higher during the new moon while high frequency acoustic events vary more in relation to other environmental factors (Staaterman et al. 2014). This infers that marine communities respond to light and dark phases of the moon. Costa Rica and Panama protect some of the most important marine communities in Central America. However, there is little understanding of the long-term dynamics of these marine communities and the factors that may influence these dynamics.

The purpose of this research was to determine if the lunar cycles affect the acoustic activity and soundscape dynamics in one site at the periphery of Coiba National Park and in three sites within Caño Island Biological Reserve. Preliminary analysis indicate that the four sites vary in their soundscape and acoustic complexity, with more complex habitats showing more diverse acoustic communities in general (Houghton et al. 2019). In that same study there was also evidence for seasonal changes within each site, here we will evaluate factors that may be contributing to that variation: lunar cycles, temperature, and tidal cycles. It was hypothesized soundscape complexity and structure will vary with lunar cycles. We predict that acoustic activity, complexity, and diversity will increase during the new moon (complete darkness) for low frequency sonorous species like fish and whales. The results from this research can provide key information on marine community dynamics and help predict changes in behaviors such as mating and spawning of important members of these communities. Together the results will help government officials to make informed decisions regarding the protection and management of these MPAs.

II. Material and Methods

A. Study Site

The study took place in two protected areas of Central America, Caño Island Biological Reserve (CIBR) in Costa Rica and Coiba National Park (CNP) in Panama (Fig.1). In CIBR underwater recorders, we deployed in three locations that vary in substrate characteristics: Jardin (8.719N/-83.863W) is characterized by sandy bottom, Cueva del Tiburon (8.715N/-83.891W) consists of a mix of sandy bottom and large boulders and Diablo (8.701N/-83.915W) is a rocky reef. Some of the local marine diversity includes humpback whales, spotted and bottlenose dolphins, and about 79 other species (Salas et al. 2014). In CNP the recorder was place at the periphery of the park called Isla del Canal (7.687N/-81.611W) where the substrate was a combination of sandy bottom and large boulders. The island is part of 38 other small islands within this secluded protected area. CNP protects about 760 species of fish, 33 species of sharks and 20 species of whales and dolphins (https://whc.unesco.org/uploads/nominations/1138rev.pdf). Both protected areas face challenges regarding ecotourism and fishing (Brown n.d.).



a. Caño Island Biological Reserve (CIBR) b. Coiba National Park (CN))

Figure 1. Study sites in Costa Rica highlighted by blue boxes and Isla del Canal, Panama (a is taken from Salas et al. 2014),

B. Moon phase data collection

Moon phases drive tidal patterns and currents that change water temperature and consequently affect an organism's ability to find mates, avoid predation and find food (Merchant et al. 2015). For this study peak moon phase data were obtained from online lunar calendar for each country (https://www.vercalendario.info/es/luna/costa_rica-mes-octubre-2016.html). Tidal data for Caño Island Biological Reserve, Costa Rica was obtained from the closest tidal station in Quepos (https://tablademareas.com/cr/costa-oceano-pacifico/quepos). For Coiba National Park, tidal data was collected through the GETESA website (http://www.hidromet.com.pa/mareas.php), and the data collected was from the pacific side.

C. Acoustic recordings

At CIBR two types of recorders used were the SM2M+ (Sampling rate: 4-96 kHz -165dB re 1V/µPa) from Wildlife Acoustics (www.wildlifeacoustics.com) and the RUDAR-mK2 (Sampling rate up to 96kHz -169dB re:1V/µPa) from Cetacean Research Technology (www.cetaceanresearch.com). The recorders were deployed interchangeably to reduce field costs and maximize recording time. Recorders were programmed to continuously capture the soundscape at a sampling rate of 43-48 kHz in 30-minute files. In CNP the recorder was a Soundtrap (Sampling rate up to 250kHz -169dB re:1V/µPa). The Soundtrap was program to record every 30 min for 5 minutes.

D. Soundscape and Statistical analysis

A 1-min sample for every 10 minutes of recordings was taken from each location at CIBR and together with the 5-minute sample from CNP where uploaded to ARBIMON for cataloguing and analysis (https://arbimon.sieve-analytics.com). Playlists were made for each moon phase at each site and a soundscape analysis to estimate the acoustic complexity index (ACI) (Pieretti et al. 2011, Farina and

James 2016, Bertucci et al. 2016) was done for each moon phase. Data for each playlist was normalized and set to 43-bin bandwidth (Hz). Each playlist covered 24-hour periods of each day that correlated with a moon phase. A least square analysis was used to determine differences in ACI values among sites and moon phases using JMP 14.0 (SAS, 2019).

III. Results

The four marine communities were significantly different in their acoustic complexity (ACI values) (F=2.08, p=0.0023). The differences are primarily to differences among sites (F=68.75, df=2, p<0.0001) and due to the interaction between location and moon phase (F=7.24, df=5, p<0.0001). Diablo had the most acoustically diverse community, followed by Coiba, Tiburon, and Jardin (Fig.1). Diablo was also consistently the site with higher diversity by moon phase but primarily during the full moon. Interestingly the greatest variation in ACI values for Diablo was during the new moon (F=31.0, df=3, p<0.0001). New moon was also the moon phase with lowest ACI values in Jardin (F=12.8, df=3, p<0.0001) and Tiburon (F=73.9, df=2, p<0.0001). Fig. 2 shows the hourly variation in ACI values per community. In Diablo there is a peak of activity between 5 and 8 a.m., in the other sites the peak of activity is between 5 a.m. and 3 p.m.

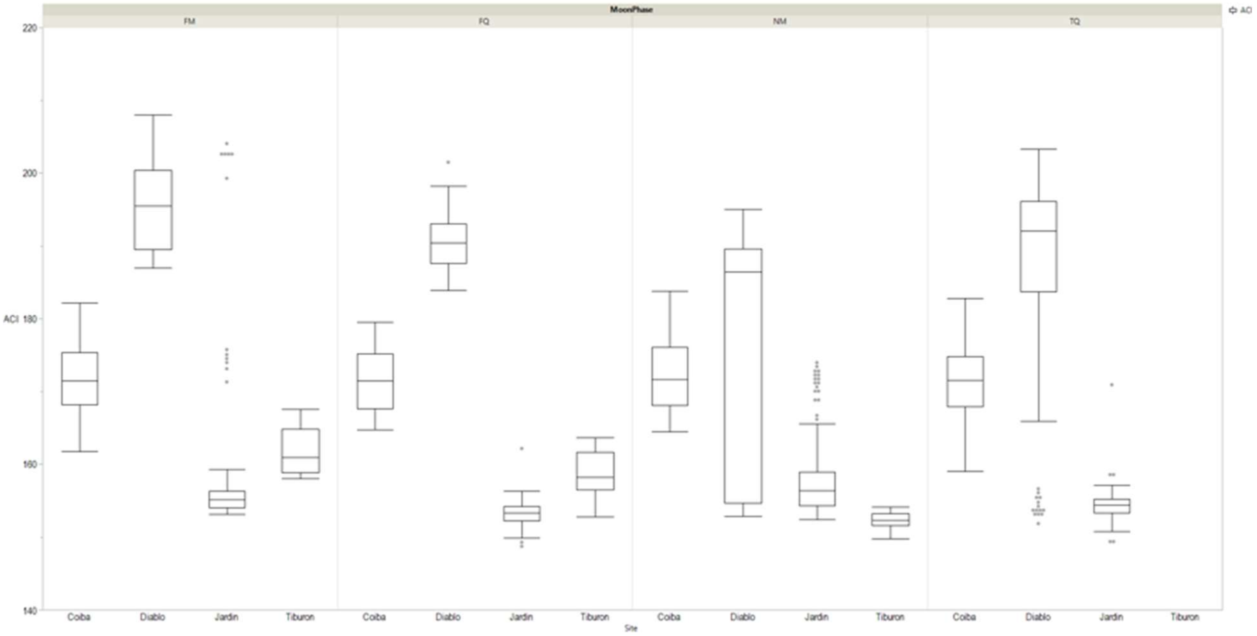


Figure 1: Mean ACI by marine community and moon phase.

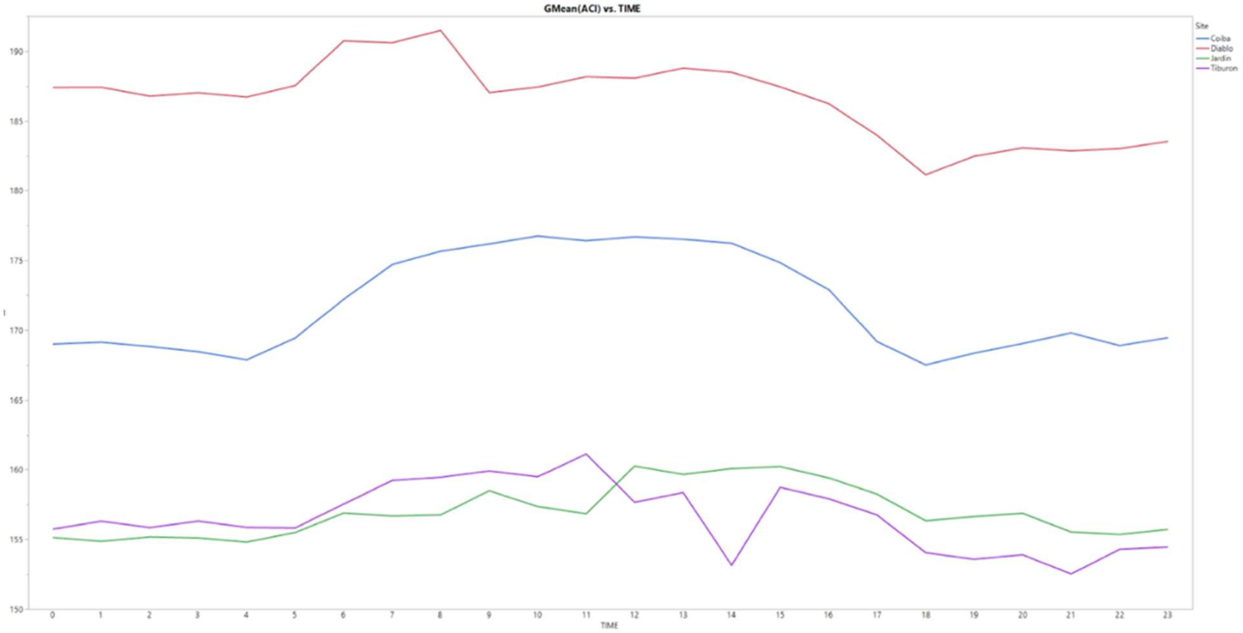


Figure 2. Hourly distribution of the ACI values per site.

IV. Discussion

The purpose of this study was to observe how marine soundscape complexity and activity in geographically different areas were affected by the lunar cycle. It was indicated that soundscape complexity was significantly different for each observed site. The data collected revealed dramatic differences in soundscape complexity between each site and also indicated that there was some variation in soundscape activity during each moon phase.

A. Variation of soundscape complexity between sites

Diablo had the highest ACI over time (between about 180 and 193 Hz). Coiba had the next highest ACI over time with between 167 and 176 Hz. These two sites are primarily reef ecosystems, while the other sites observed were not. Higher soundscape complexity in these areas indicate that there is a correlation between soundscape activity in reef ecosystems. A study found that there was a strong positive relationship between soundscape complexity and fish density and biomass (Graham et al. 2012). The positive correlation between soundscape complexity and marine biomass in reef ecosystems indicates that there must be an urgency in protecting and conserving reefs. A study found that more larval settlements took place in the environments with higher quality reef systems (Lillis et al. 2016). This study touches on the importance of keeping coral reefs healthy so that smaller marine organisms like coral algae can orchestrate an effective food web. Healthy coral provides opportunity for smaller fish to persist, which will consequently bring in larger predatory organisms. These studies support what we found and can explain why we have higher values for Coiba and Diablo. Tiburon and Jardin were both very similar to each other with the lowest ACI values, between 153 and 161 Hz. Both these locations have a mix between sand substrate and rocky boulders. This also supports a correlation between the substrate and the soundscape complexity observed at these areas as well as an increase in soundscape complexity observed in reef ecosystems.

B. Celestial Patterns

After observing differences in soundscape complexity in response to moon phases, Diablo showed higher diversity on nights of a full moon. It was also observed that Diablo had the most variation in data on nights of new moon (Figure 1). Diablo has the highest variation of 13Hz with the peak between the hours 5-8 and the low at hour 18. Coiba has a variation of 9Hz with a raised plateau between the hours 6-17. Peak activity in Diablo was displayed between 5 and 8 a.m (Figure 2), indicating that there could be a correlation between the time of day that organisms in reef ecosystems were more active compared to sites located in geographically different areas. Other sites displayed a peak of soundscape activity between 5 a.m and 3 p.m (Figure 2). The difference in the peak of soundscape activities between each of these sites indicates that there could be a correlation between the duration of sunlight that results from different moon phases. Some influencing abiotic factors that correlate strongly with variances in the moon phase that could strongly affect soundscape complexity are temperature, tidal and salinity changes. These factors have the ability to change the behavior and overall dynamic of the ecosystem. The reason why the sites like Coiba and Diablo have the highest variation in soundscape complexity during different moon phases is because these ecosystems are typically much more diverse. With so much diversification in reefs, the alterations in abiotic factors display higher volume of changes in each species present. These changes have the opportunity to affect different species in different ways. Higher water temperatures or changes in salinity are extremely detrimental for coral algae, which may become an ultimate stressor for the entire ecosystem. Referencing figure 2, the hourly variation within each site can be estimated. The median values of ACI show the highest value on full moon and the lowest value at new moon, while first and third quarter are relatively similar. Jardin and Tiburon also had the lowest ACI values for new moon. Coiba interestingly appeared to have similar values for all phases. High variability in the data could be due to changes in soundscape production differences between seasons (Staaterman et al. 2014). Because of limitations in data availability, there is variation in the seasonality in the phase data.

D. Conclusion

It was found that there is high variation of soundscape activity that occurs at each site seem to correlated to the substrate type. The lunar data showed some significant differences in diablo to the moon phases but the variation was very high in the other sites to draw a clear conclusion. A multitude of factors that may have affected the data collected including, Seasonality, water temperature, tourism months, and tidal changes. These are factors that are not accounted for in our results and may have a significant effect on the results collected. The findings of this study can be applied to the helping propel conservation and protection, understanding how to monitor soundscape production, and future experiments that could be possible including the study the effects of these abiotic factors.

Future endeavors

- Importance of study and how it is helping propel conservation and protection
- Importance of understanding how to monitor soundscape production
- Future experiments that could be possible

Literature Cited

Bertucci, F., E. Parmentier, G. Lecellier, A. D. Hawkins, and D. Lecchini. 2016. Acoustic indices provide information on the status of coral reefs: an example from Moorea Island in the South Pacific. Scientific Reports 6:33326.

Brown, M. no date. Ecotourism in Panama: 11. (https://preserve.lehigh.edu/cgi/viewcontent.cgi?article=1004&context=perspectives-v25)

Collin, S. p., N. j. Marshall, R. D. McCauley, and D. H. Cato. 2000. Patterns of fish calling in a nearshore environment in the Great Barrier Reef. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 355:1289–1293.

Coquereau, L., J. Lossent, J. Grall, and L. Chauvaud. 2017. Marine soundscape shaped by fishing activity. Royal Society Open Science 4:160606.

Farina, A. 2014. Soundscape and Landscape Ecology. Pp. 1–28 in Soundscape Ecology: Principles, Patterns, Methods and Applications (A. Farina, ed.). Springer Netherlands, Dordrecht.

Farina, A., and P. James. 2016. The acoustic communities: Definition, description and ecological role. Biosystems 147:11–20.

Fisher-Pool, P., M. Lammers, J. Gove, and K. Wong. 2015. Does Primary Productivity Turn Up the Volume? Exploring the Relationship Between Chlorophyll a and the Soundscape of Coral Reefs in the Pacific. Advances in experimental medicine and biology 875:289–293.

Fritzen, M. R., J. Cagliari, M. Candido, and E. L. C. Lavina. 2019. Tidal bar cyclicity record in the Lower Permian: The Rio Bonito Formation, Paraná Basin, southern Brazil. Sedimentary Geology 381:76–83.

Gordon, T. A. C. et al. 2018. Habitat degradation negatively affects auditory settlement behavior of coral reef fishes. Proceedings of the National Academy of Sciences of the United States of America 115:5193–5198.

Houghton, J. E., C. B. Dunbar, J. L. McMillan. 2019. Monitoring marine biodiversity through soundscape analysis.

Lillis, A., D. Bohnenstiehl, J.W. Peters, and D. Eggleston. 2016. Variation in Habitat soundscape characteristics influences settlement of reef-building coral. PeerJ 4:e2557

Merchant, N. D. et al. 2015. Measuring acoustic habitats. Methods in Ecology and Evolution 6:257–265.

Pieretti, N., A. Farina, and D. Morri. 2011. A new methodology to infer the singing activity of an avian community: The Acoustic Complexity Index (ACI). Ecological Indicators 11:868-873

Ruppé, L. et al. 2015. Environmental constraints drive the partitioning of the soundscape in fishes. Proceedings of the National Academy of Sciences of the United States of America 112.

Smith, J. E., C. L. Hunter, and C.M. Smith. 2010. The effects of top-down versus bottom-up control on benthic coral reef community structure. Oecologia 163:497-507.

Staaterman, E., C. Paris, H. DeFerrari, D. Mann, A. Rice, and E. D'Alessandro. 2014. Celestial patterns in marine soundscapes. Marine Ecology Progress Series 508:17–32.

Yang, C. et al. 2019. Diel vertical migration of zooplankton and micronekton on the northern slope of the South China Sea observed by a moored ADCP. Deep-Sea Research Part Ii-Topical Studies in Oceanography 167:93–104.

Anon. no date. Coiba National Park and its Special Zone:220.

Boat traffic in Bocas Del Toro, Panama associated with selection for lower and louder toadfish (*Amphichthys cryptocentrus)* calls.

Emma K. Gagne

University of Vermont. Department of Biology. 109 Carrigan Drive Burlington, VT 05405.

I. ABSTRACT

The toadfish is an important acoustic contributor to the local marine soundscape of Bocas del Toro. Toadfish emit mating calls to attract females to their burrows to spawn. Given that male toadfish calling behavior determines their reproductive success, boat traffic is expected to affect their acoustic behavior. This study evaluates the effects of boat traffic on toadfish call acoustic structure by comparing two sites within the Archipelago of Bocas del Toro, Panama that vary in boat traffic. The study finds the potential presence of three acoustic toadfish species that varied in call contour. One of the acoustic species, called here as 'flat species' was found in both study sites. Overall, this 'flat species' call was significantly shorter in duration, lower in frequency, and higher in amplitude in the site with high boat traffic than in the site with low boat traffic. The results suggest that noise associated with boat traffic may be selecting for lower and louder signals in noisy habitats. Given the importance of toadfish as health indicators of marine communities these results are important as they indicate how humans are changing their calls and interactions.

II. INTRODUCTION

A. Background

Male toadfish (family Batrachoididae) are an indicator species of many marine communities in North and Central America (O. Campana et al., 2003). They use acoustic communication to attract females to their burrows to spawn, and is therefore critical to their reproductive success. Their mating calls consist of low frequency "grunts" and "boops" which convey information about the male's guarding quality to females (Staaterman et al., 2017). The grunts have been examined, and appear to be used in intrasexual competition (Salas et al., 2018). Therefore, it is important both grunts and boops to be heard at a distance as to attract and outcompete other males in their vicinity (Robertson, 1983). In previous studies, toadfish calling behavior is described to increase with higher water temperatures, and can vary given the day length and tidal amplitude differences (Maruska, 2009).

B. Purpose and Scope

Through this study, I hope to compare the call structures of the Bocon toadfish, *Amphichthys cryptocentrus,* in the Archipelago of Bocas del Toro, Panama. I am asking questions about toadfish's plasticity in call frequency and time variables regarding boat traffic. A previous study by CURE students in 2018 found the calling activity of toadfish in the Archipelago varied between sites that share similarity in structure but differ on daily boat traffic patterns. In Almirante, boat traffic is scheduled and consistent between 6am-6pm. In Sharkhole, boat traffic is not permitted. One of the studies found overall patterns in which toadfish appeared to lower their frequency and signal energy during high boat traffic time. However, these patterns could be due to different toadfish species using the same spatial space. To address this limitation of potentially seeing the difference due to species' specific call structures, I will classify 'acoustic' toadfish species based on the contour of the boop and extract acoustic information during the times of the day at which calling activity is higher: dawn, midday, dusk.

I expect the toadfish call acoustic structure will vary throughout the day. I also hypothesize that if boat noise is the major driver of toadfish call structure, the above patterns should be detected in both the described acoustic species. My prediction is that toadfish calls will be emitted at lower frequency and with higher power values when boat activity is higher, regardless of 'acoustic' species.

C. Significance

This study will provide relevant information about fish responses to boat sounds, a primary source of anthropogenic noise in Bocas del Toro. Toadfish are necessary in marine communities to signify the health of the ecosystem. They can indicate to researchers what kinds of chemicals are present, and how an accumulation of those chemicals can impact other fish. This study will help us to understand how humans are changing the calls and interactions of toadfish, and give insight as to what management steps can be taken to preserve them as a health indicator and critical part of marine communities.

III. MATERIALS AND METHODS

A. Study site

The study took place at the Archipelago of Bocas del Toro, Panama. This is a significant geological area, where the islands were separated from the mainland due to sea level rise. Coral reefs, mangroves, and seagrass habitats compose integral parts of the marine environment there; coral reefs and seagrass meadows are important environmental indicators of water quality (Guzman, M. H., et al. 2005).

B. Recordings

Passive acoustic recordings were taken with RUDAR-mk (RUDAR-mK2 (Sampling rate up to 96kHz -169dB re:1V/uPa) from Cetacean Research Technology (www.cetaceanresearch.com). The recorder was programmed to continuously record the soundscape in segments of 30 minutes at a sampling rate of 48 kHz from March 28 to April 7, 2018. Recorders were deployed in two sites Almirante (9.289N, -82.332W) and Sharkhole (9.184N, 82.176W) at about 12 meters in depth and with coral reef substrate. The main differences between these sites is based on boat traffic. In Almirante, taxi-boats travel daily from mainland to the main island in the Archipelago between 6 a.m. and 6 p.m. In contrast, Sharkhole is relatively deprived of boat traffic with occasional tour and fishing boats passing by.

C. Fish call data

Toadfish calls with good signal-to-noise ratio were selected from dawn (2am-4am), midday (11am-1pm), and dusk (7pm-9pm) to analyze their frequency and temporal characteristics. These time periods were based on Maze (2018) analysis of toadfish activity in these two study sites, representing times of high (dawn, dusk) and low (midday) calling activity. These times also represent times of low (dawn, dusk) and high (midday) boat traffic. The analyses were done in Raven 1.5 (2016; Cornell Lab of Ornithology) with a Fast Fourier Transform size of 4,000 points, an overlap of 50%, and a 4096-sample Hann window. Toadfish mating calls consist of two parts boops and grunts (Staaterman et al., 2018). For each of these call components (and intergrunts, intergrunt-boops, and interboops) the following standard acoustic variables were extracted from each call: grunt duration, grunt peak frequency, grunt fundamental frequency, grunt maximum amplitude, grunt RMS amplitude, inter-grunt interval, grunt-boop interval, boop duration, boop peak frequency, boop fundamental frequency, boop maximum amplitude, boop RMS amplitude, and inter-boop interval (Staaterman et al., 2018).

D. Statistics

A Generalized regression analysis was to use to determine the contribution of acoustic species, time of day, and site to the call acoustic structure. An ANOVA test was done to determine if acoustic species found in both low and high boat traffic sites significantly vary in call acoustic structure. The statistical analyses were done using JMP Pro 14.2 (SAS, 2019)

IV. RESULTS

A. Acoustic species

Three acoustic species of toadfish were identified based on contour differences of the boop: flat, flat*, and sine. Species flat was characterized by having a boop with constant contour, species sine by a sinusoidal contour, and species flat* had a deep and mostly constant contour (Fig.1). Species flat was most commonly detected (55%) followed by species sine (25%) and flat* (20%). Only species flat was found in both study sites.

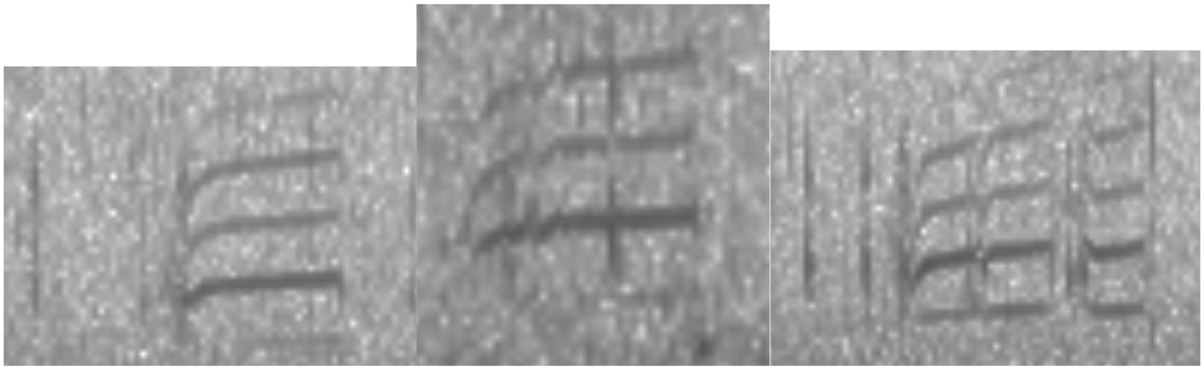


FIG 1. The spectrogram of the three acoustic species are shown. From left to right; species flat, species sine, and species flat*.

B. Factors influencing call acoustic structure

Toadfish call frequency, time, and amplitude characteristics varied in relation to species identity, time of day, and boat traffic level (site). Toadfish call low frequency and peak frequency did not varied significantly among species ($p>0.05$) but it did vary between sites (LF: F=40.4,df=2, $p<0.0001$, PK: F=361.2,df=2, $p<0.0001$) and time of day (F=3.3,df=2, p=0.0367, PK: F=3.3,df=2, p=0.0437$p<0.0001$). Call high frequency and call duration differences are attributed to site (HF: F=50.3, df=1, $p<0.0001$, D: F=246.8, df=1, $p<0.0001$), acoustic species (HF: F=12.4, df=3, $p<0.0001$, D: F=11.3, df=3, $p<0.0001$) and time period (HF: F=4.76, df=2, p=0.0086, D: F=5.4, df=2, p=0.0042). Call amplitude differences were also influenced by site (Max. Amp.: F=1710, df=1, $p<0.0001$, RMS: F=5251, df=1, $p<0.0001$) and time of day (Max. Amp.: F=10.7, df=2, $p<0.0001$. RMS: F=108, df=2, $p<0.0001$) and acoustic species (RMS: F=64, df=2, $p<0.0001$).

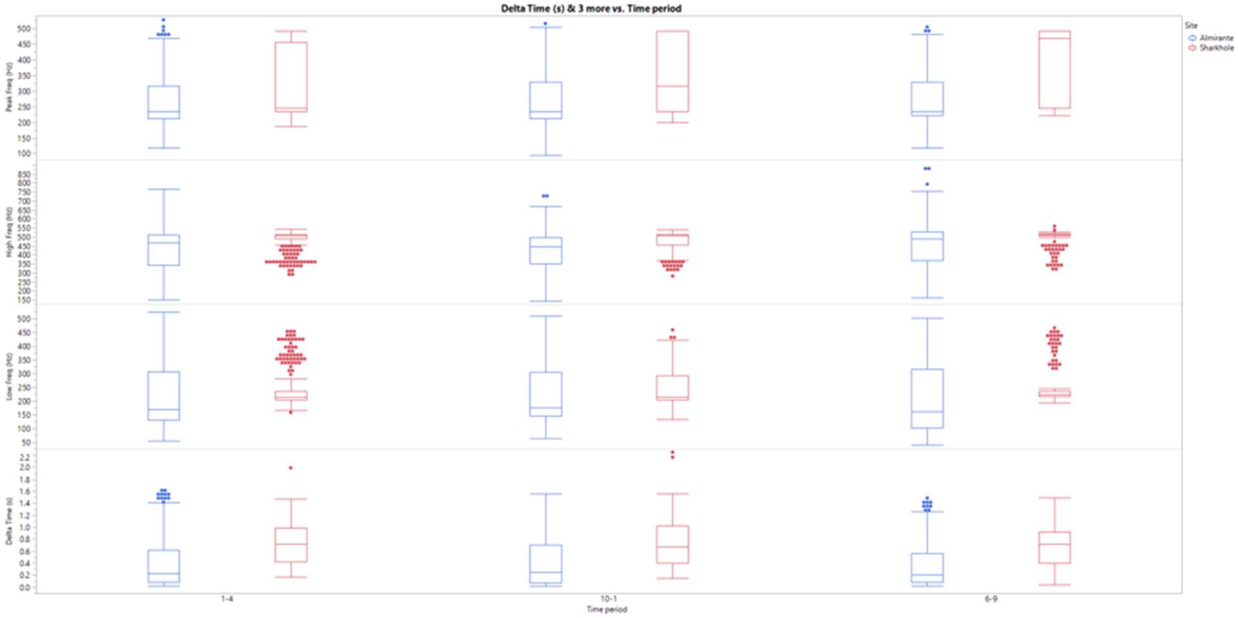


FIG. 2: Box plot comparison of call parameters between Almirante and Sharkhole throughout the day (1 am-4 am, 10 am-1 pm, and 6 pm - 9pm), . Top row shows peak frequency (Hz), second row shows High frequency (Hz), third row shows low frequency (Hz), and the bottom row shows delta time (s).

C. Flat species call acoustic structure

Toadfish flat calls vary significantly in acoustic structure. Calls were lower in frequency in the site with higher boat traffic (Almirante) than in the site with low boat traffic (Sharkhole) (LF:F=49.8, df=1, $p<0.0001$; HF: F=57, df=1, $p<0.0001$, PF: F=350.4, df=1, $p<0.0001$). Calls were also shorter (F=233.1, df=1, $p<0.0001$) and louder (F=77716, df=1, $p<0.0001$) in the site with higher boat traffic. Regarding the time of day, in the site with higher boat traffic toadfish call high frequency was significantly lower in the presence of boat traffic (10 a.m. and 1 p.m.) (x2=22.7, df=2, $p<0.0001$). In contrast, in the site with low boat traffic toadfish call frequency was higher at night (7 to 9 p.m.) (LF: x2=25.7, df=2, p<0.0001, HF: x2=36, df=2, p<0.0001, PF:). No significant differences were found in max amplitude or RMS amplitude with time of day.

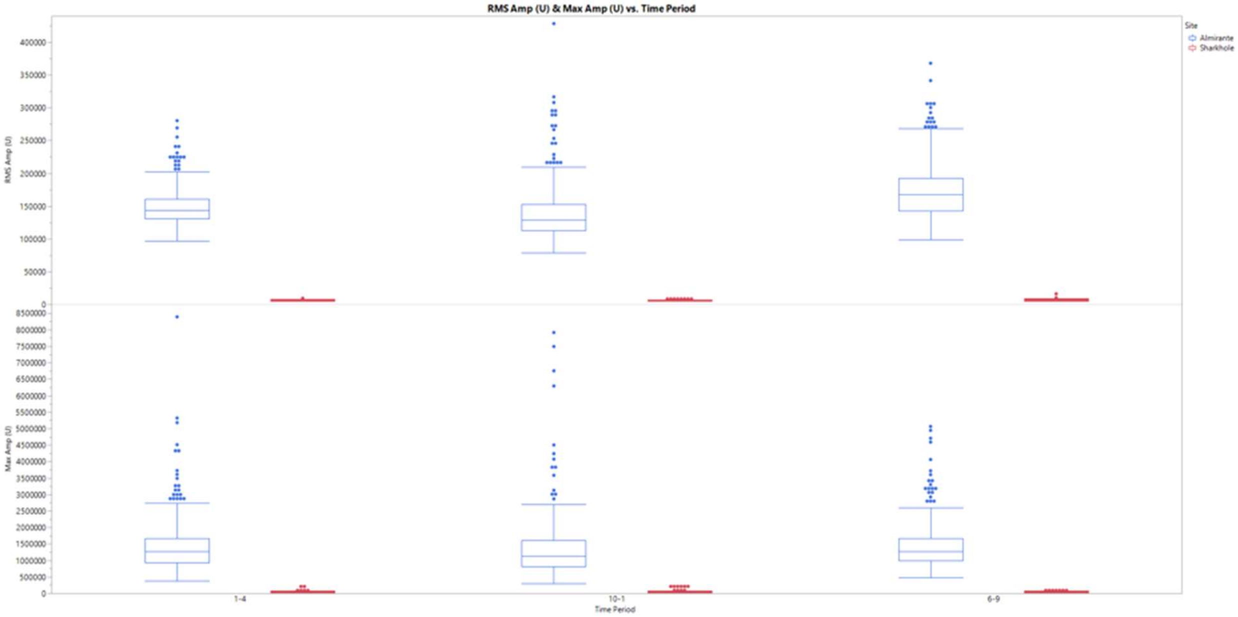


FIG. 3: Box plot comparison of call parameters between Almirante and Sharkhole throughout the day (1 am-4 am, 10 am-1 pm, and 6 pm - 9pm), . Top row shows RMS amplitude and the bottom row shows maximum amplitude.

V. DISCUSSION

In the beginning of the study, our aim was to examine differences in three acoustic toadfish species that all varied in call contour. We examined "flat," "sine," and "flat." Only the 'flat species,' however, was found in both study sites. When looking at the time of day, in the site with higher boat traffic toadfish call high frequency was significantly lower in the presence of boat traffic (10 a.m. and 1 p.m.), while the site with low boat traffic toadfish call frequency was higher at night (7 to 9 p.m.); this was expected based on previous studies by my peers. Overall, we find the 'flat species' call was significantly shorter in duration, lower in frequency, and higher in amplitude in the site with high boat traffic than in the site with low boat traffic. These results suggest that noisy habitats due to constant boat traffic select for lower and louder toadfish calls.

The "acoustic adaptation hypothesis" states that efficient communication in contexts of mate choice or attraction and territorial defense is predicted to enhance the Darwinian fitness of the individual making the call, despite potentially having adverse costs (Slater, P.J.B, 1983). Most studies on this to date have focused on birdsong and habitat structure (Boncoraglio et al., 2007), however, this study indicates a novel situation with fish and boat traffic. Sound transmission in different habitats suggest that acoustic signal spanning long-distances are likely to be degraded by a number of environmental factors; human noises are no exception (Hansen, P., 1979). Natural selection will favor calls that are able to span those long distances despite degradation from factors. Masking, or threshold change in signal level from neighboring noise (Pollack I, 1975), describes how boat noises can degrade toadfish calls. In the case of the toadfish, we see individuals capable of making lower frequency calls to avoid this. We expect this lower frequency to influence a greater survival rate, according to the acoustic adaptation hypothesis.

In order to create a lower frequency sound, there is likely an associated increase in swim bladder size. In toadfish, they have a swim bladder which is a large pocket of air located in their abdomen; sound is produced through the drumming of the sonic muscle on the swim bladder, causing contraction and expansion at high rates (“How”, 2019). Frogs and roadway traffic noise have been extensively studied; results from these studies show males used higher frequencies to avoid traffic masking, and were therefore, significantly smaller in size closer to the road (Hoskin, C.J., and Miriam, W.G., 2010).

Similarly, because toadfish lower their frequencies to avoid masking from the boats, we could speculate individuals with greater swim bladder size, and therefore greater body size, to be present in boating areas. Future studies could determine if the acoustic adaptation hypothesis holds true here, and describe a novel case of anthropogenic masking in fish. As anthropogenic modification in natural environments continues to increase, species have to adapt quickly in order to survive. Understanding this in toadfish gives insight as to how some communities are changing in response to one human factor; boat traffic and noise. Given the importance of toadfish as health indicators of marine communities these results are important as they indicate how humans are changing their calls and physiology.

REFERENCES

Boncoraglio, Giuseppe, and Nicola Saino. (**2007**). “Habitat Structure and the Evolution of Bird Song: a Meta-Analysis of the Evidence for the Acoustic Adaptation Hypothesis.” *Functional Ecology*, vol. **21**, no. 1. doi:10.1111/j.1365-2435.2006.01207.x.

Campana, O., Sarasquete, C., & Blasco, J. (**2003**). “Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish Halobatrachus didactylus.” *Ecotoxicology and Environmental Safety*, ***55***(1), 116-125.

Guzman, M. Hector., et al. (**2005)**. “A Site Description of the CARICOMP Mangrove, Seagrass and Coral Reef Sites in Bocas del Toro, Panama.” Caribbean Journal of Science, **41**:3:430-440.

Hansen, Poul. (**1979**). “Vocal Learning: Its Role in Adapting Sound Structures to Long-Distance Propagation, and a Hypothesis on Its Evolution.” *Animal Behaviour*, **27**:1270–1271., doi:10.1016/0003-3472(79)90073-3.

Hoskin, Conrad J., and Miriam W. Goosem. (**2010**). “Road Impacts on Abundance, Call Traits, and Body Size of Rainforest Frogs in Northeast Australia." *Ecology and Society*, **15**:3 doi:10.5751/es-03272-150315.

"How Do Fish Produce Sounds?" *Discovery of Sound in the Sea*, 28 Jan. (**2019**). dosits.org/animals/sound-production/how-do-fish-produce-sounds/.

Maruska, K. P. and A. F. Mensinger. (**2009**). "Acoustic characteristics and variations in grunt vocalizations in the oyster toadfish *Opsanus tau*." Environmental Biology of Fishes **84**:325–337.

Pollack, Irwin. (**1975**). "Auditory Informational Masking." *Scitation*, Acoustical Society of America, asa.scitation.org/doi/abs/10.1121/

Robertson, D. Ross., et al. (**1983**). "Foraging and reproduction of two Caribbean reef toadfishes (Batrachoididae)." Bulletin of Marine Science, **33**:919-927.

Salas, A. K., P. S. Wilson and M. J. Ryan. (**2018**). "Acoustic communication in the Bocon toadfish (*Amphichthys cryptocentrus*)." Environmental Biology of Fishes 101:1175–1193.

Slater, P.J.B. (**1983**) The study of communication. Animal Behaviour, Vol. 2 (eds T.R. Halliday & P.J.B. Slater), pp. 9–42. Blackwell Scientific, Oxford.

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

Wert, J. C. V. and A. F. Mensinger. (**2019**). "Seasonal and Daily Patterns of the Mating Calls of the Oyster Toadfish, Opsanus tau." The Biological Bulletin **236**:97–107.

Impact of Shipping Noise Levels in Marine Communities and Evaluate Future Oil Drilling Feasibility at Panama Sea Area

Kenneth Tang
University of Vermont

Abstract

Marine communities are essential to Panama sea area. Underwater noise pollution may cause huge impact on marine animals' behavior. The problem I am trying to solve in this paper is how high and low frequency underwater noise affect marine communities' diversity and evaluate possibilities of building deep water oil platform in Panama sea area. The approach I adopt to solve this problem is to analysis sound records from both protected sea area and public sea area. Record each activity of marine animals in both areas to see the variations. The results obtained in this research include acoustic events in public area have higher frequency and energy, acoustic events in protected area have much higher diversity. Peak of activities in public area was pushed back for 4 hours compared with protected area. The impact of my obtained results is low frequency under water noise significant affect marine communities in public sea area in Panama, building deep water oil platform may make current situation worse since increasing continuing offshore boat activities.

I. Introduction

Panama Canal is one of the largest sea transportation hubs in the world, providing convenient "ocean to ocean" pathway between Pacific Ocean and Atlantic Ocean (David McCullough, 2001). On 2018, 13,795 transits through Panama Canal (Panama Cancel Authority, 2019). Globally, oceans are experiencing increasing noise levels, largely from increasing shipping activity (George V.Frisk, 2012; E. Staaterman et al., 2013), and resulting in negative impacts on marine life. For example, baleen whales (Mysticeti) found suffered high physiological stress because of increasing low-frequency ocean noise (Rolland et al., 2012; Adrian Farcas et al., 2016). Sound is an essential method for dolphins and cetaceans for foraging and social activities, human activities usually oversea transportation can mask their sound (Douglas P. Nowacek et al., 2007; Enrico Pirotta et al., 2015; Clark et al., 2009) and their prey (Popper et al. 2003) to cause food shortage. The marine mammal diversity of Panama represents 36% of the species described worldwide (May-Collado et al. 2017) including migratory species like the Humpback whales and residents species like bottlenose dolphins. On August 2017, the National Energy Secretary of Panama, Víctor Urrutia, said in ION Geophysical Corporation's press that Panama will continue Deepwater oil exploration including those areas were "not considered economically viable, such as deep-water deposits and/or those that are geologically more difficult locate". Panama is assessing the possibility to move away 100% from importing oil. Together with noise from the Canal, is expected that the ocean noise in Panamanian waters will drastically change the soundscape of many marine organisms including cetaceans. The purpose of this study is to determine how noise levels may impact the acoustic space in which marine mammals communicate.

The importance of evaluating impossibilities of deep-water drilling activities is that soundwaves could been detected almost 4000km away (Nieukirk et al., 2012) which means Cobia (protected) area could also be affected. Offshore boats activities, pile riving, and Semisubmersible drilling vessel will provide continuing noise (frequency: 20-4000Hz) combined with supertankers and container ships (frequency: 6.8-70Hz) (Richardson et al., 1995).

In this paper, I summarized: (1). Shipping noise frequency was much higher than normal. (2). Marine communities' diversity was obviously decreased in public water area. (3). Marine animals such as humpback whales tend to increase their sound's frequency in public water area. (4). Marine communities tend to avoid make sound during frequent shipping noise period. (5). Under water shipping noise disturbed the pattern of marine communities' activities.

II. Materials and Methods

Passive acoustic recordings were obtained from two locations in the Panamanian Pacific coast: Coiba National Park and the Archipelago of Las Perlas. The Coiba National Park is relatively isolated from the mainland, and most boats seen within the park are for tourism or fishing. In contrast, Perlas is not protected and located is near the Canal de Panama, an area highly transited by large vessels. Recordings were made with a Soundtrap 300STD (Ocean Instruments, 20Hz-60kHz±3dB). The recorder was programed to record 5 minutes of the soundscape every 30 minutes at sampling rate of 48 kHz for 24hours between Oct 16 and 17, 2017. Data was analyzed using RAVEN Pro 1.3 (Cornell Laboratory of Ornithology, NY, USA.) with a fast Fourier transformation size of 1024 points, an overlap of 50%, and using a 3500-4045 sample Hann window. A 1-sec sample covering a frequency span of 0-1.4 kHz was selected for every hour in a 24h cycle per day in both locations. For each biological acoustic event, the following measurements were recorded: low frequency (Hz), high frequency (Hz), peak frequency (Hz), max power (dB), max amplitude (U) and average power (dB). Signals that were within. A non-parametric Wilcoxon analysis was performed in JMP Pro 14.2 (SAS Institute, Inc, NC, USA) to determine if biological sound events vary in acoustic structure between sites. Examples of signals analyzed in this study can be observed in Appendix I.

III. Results

The study finds significant differences in the acoustic marine communities of Coiba and Perlas. The acoustic events in Coiba included sources such as whales, dolphins, and fish. Overall, the acoustic events from this protected area were lower in frequency (Hz) in Coiba than in Perlas (LF: X2=112.0, df=1, $p<0.0001$, HF: X2=652.3, df=1, $p<0.0001$, PF: X2=74.3, df=1, $p<0.0001$, Fig. 1). In contrast, acoustic events had more energy in Perlas than Coiba (Max P (dB): X2=163.1, df=1, $p<0.0001$, Max Amp (U): X2=51.8, df=1, $p<0.0001$, Fig.1). Average power (dB) was no significantly different between sites ($p>0.05$). Max Amplitude also vary between sites throughout the day, with higher values in Perlas (Fig. 2). Finally, there were a higher number of acoustic events detected in Coiba. The distribution in time of these events showed two peaks of activity one between 9 and 11 p.m. and another 1 and 3 a.m.

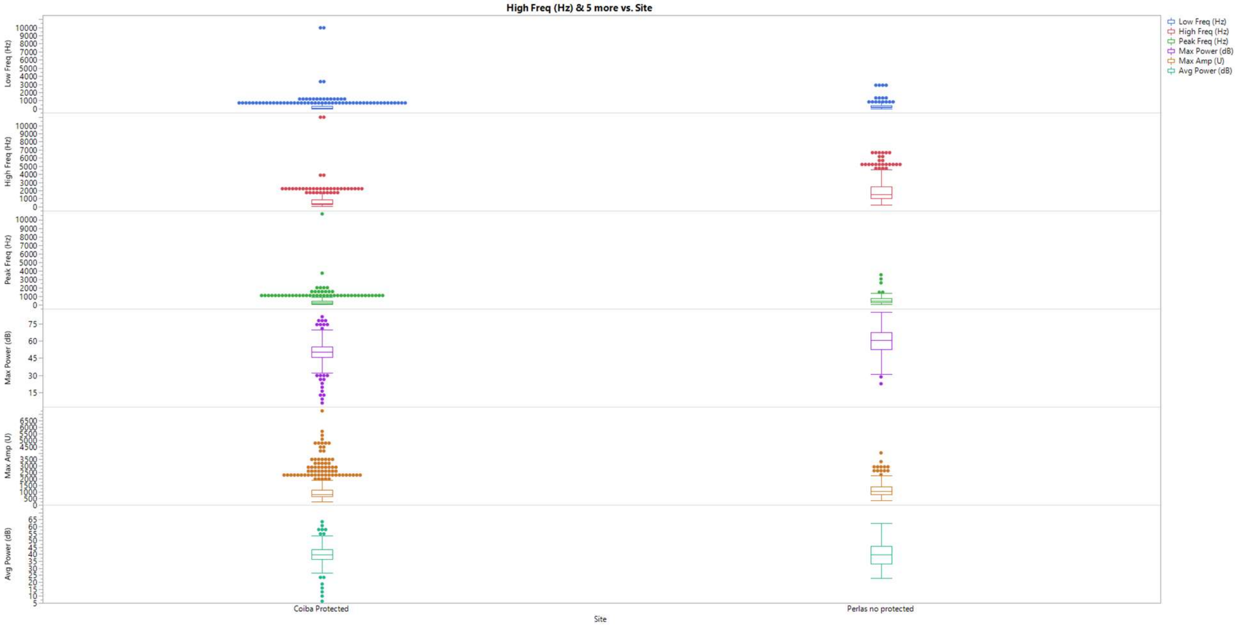


Fig. 1. Frequency, time, and power box plots for Coiba and Perlas, Panama.

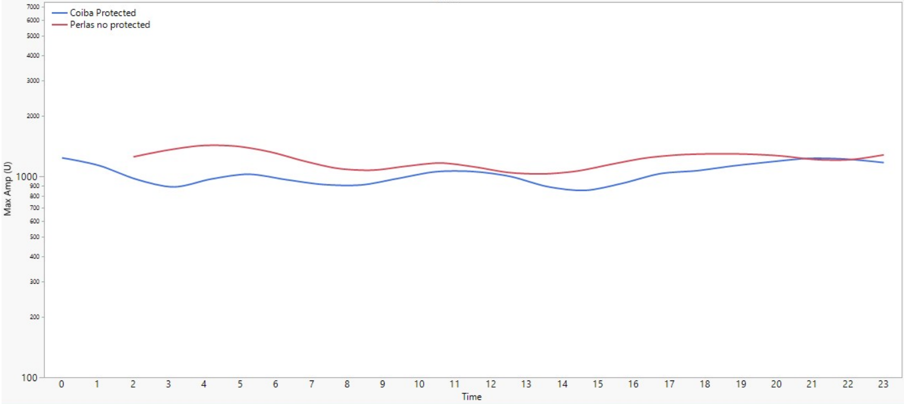


Fig. 2. Maximum amplitude levels (U) throughout the day in Coiba and Perlas, Panama.

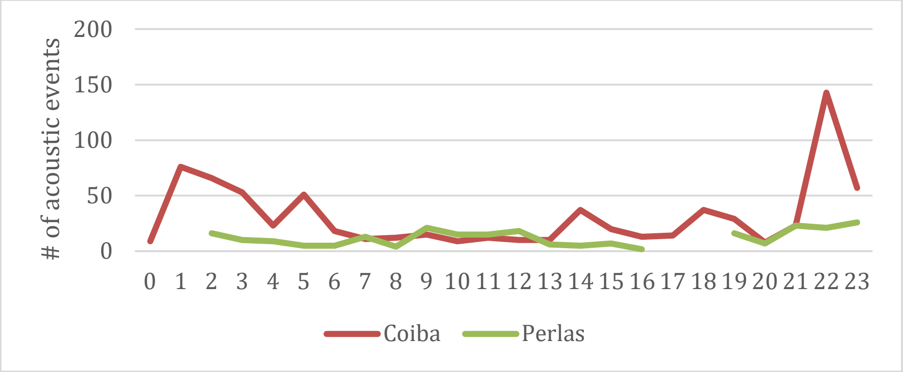
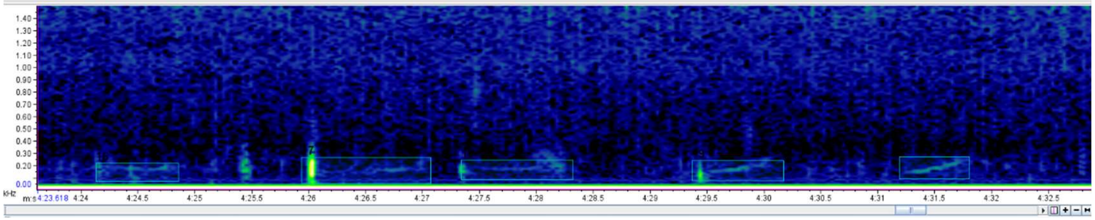
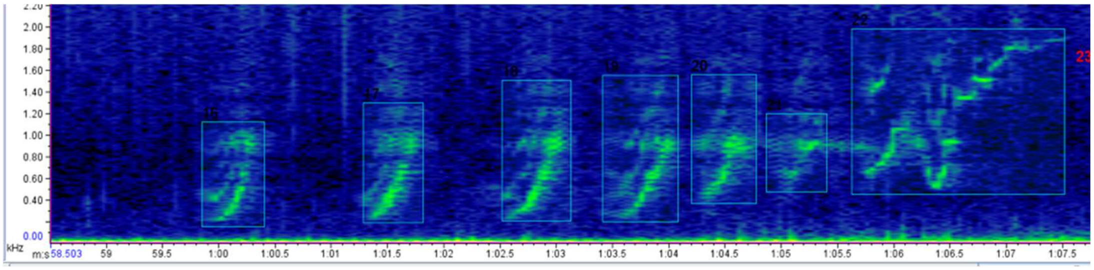


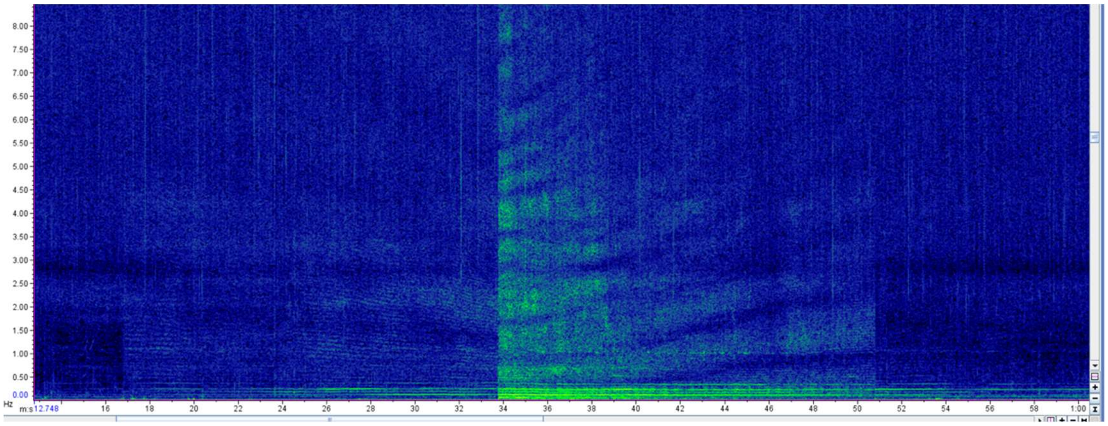
Fig. 3. Number of acoustic events documented for Coiba and Perlas, Panama throughout the day.



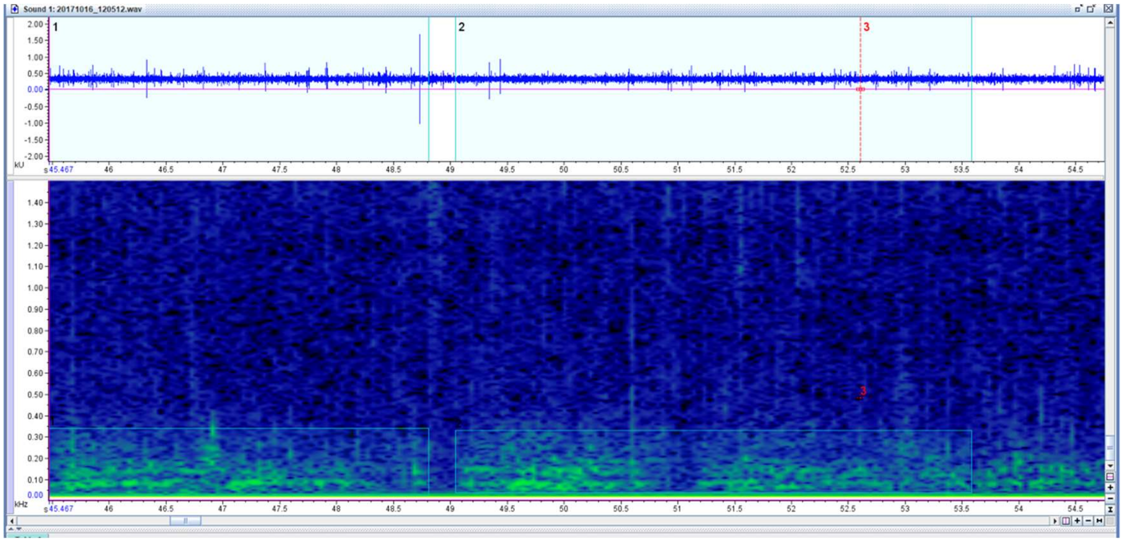
a. Low-frequency Whale's Sound in Coiba



b. High-amplitude Whale's Sound in Perlas



c. Noise Sound from Large Cargo Ship in Perlas Area between 3:30pm to 6pm



d. Long and low-frequency Potential Noise from Small Boats in Coiba

Appendix I. Examples signals detected during this study.

IV. Discussion

This research directly shows marine communities' diversity was extremely negatively affected by underwater noise pollution. Panama government's future oil drilling plan will continuing decrease local marine communities' diversity and may affect protected sea area. Compared with Coiba area, peak of marine communities' activities in Perlas island have been pushed back for 4 hours to avoid and adapt noise sound from large cargo ships between 3:30pm to 6pm. Future oil platform will produce continuing noise from small and large ship and drilling which are unavoidable. Recorded noise sound from large cargo shows high frequency (above 8000Hz) which away higher than normal. Rolland et al., 2012; Adrian Farcas et al., 2016 already proved that continuing long-frequency underwater noise will have negative impact on marine animal's psychological health then cause metabolism and immune system function issues.

Because of time limitation and data incomplete, I cannot recognize and track specific specie's activities except whales and dolphins. The general data shows a big view of noise impact on marine communities. For specific species, such as small fishes, more data and research need to be collected and done to figure out how noise would affect whole marine food chain.

Acknowledgement

I thank Professor May-Collado provide me this special research opportunity, data, lab and improved this paper.

Reference

Adrian Farcas, Paul M. Thompson, Nathan D. Merchant, Underwater noise modelling for environmental impact assessment, Environmental Impact Assessment Review, Volume 57, 2016, Pages 114-122, ISSN 0195-9255, https://doi.org/10.1016/j.eiar.2015.11.012

Clark CW, Ellison WT, Southall BL, Hatch L, Van Parijs SM, Frankel A, Ponirakis D (2009) Acoustic masking in marine ecosystems: intuitions, analysis, and implication. Mar Ecol Prog Ser 395:201-222. https://doi.org/10.3354/meps08402

David McCullough. (2001). The Path Between the Seas: The Creation of the Panama Canal, 1870-1914. Simon and Schuster, 2001

Enrico Pirotta, Nathan D. Merchant, Paul M. Thompson, Tim R. Barton, David Lusseau, Quantifying the effect of boat disturbance on bottlenose dolphin foraging activity, Biological Conservation, Volume 181, 2015, Pages 82-89, ISSN 0006-3207, https://doi.org/10.1016/j.biocon.2014.11.003.

George V. Frisk. (2012). Noiseonomics: The relationship between ambient noise levels in the sea and global economic trends. Scientific Reports. https://www.nature.com/articles/srep00437

Kight, C. R. and Swaddle, J. P. (2011), How and why environmental noise impacts animals: an integrative, mechanistic review. Ecology Letters, 14: 1052-1061. doi:10.1111/j.1461-0248.2011.01664.x

Nieukirk, S. L., Mellinger, D. K., Moore, S. E., Klinck, K., Dziak, R. P., and Goslin, J. (2012). Sounds from airguns and fin whales recorded in the mid-Atlantic Ocean, 1999–2009. J. Acoust. Soc. Am. 131, 1102–1112. doi: 10.1121/1.3672648

Nowacek, D. P., Thorne, L. H., Johnston, D. W. and Tyack, P. L. (2007), Responses of cetaceans to anthropogenic noise. Mammal Review, 37: 81-115. doi:10.1111/j.1365-2907.2007.00104.x

Panama Cancel Authority. (2019). Oceangoing Commercial Traffic through the Panama Canal by Month. https://www.pancanal.com/eng/op/transit-stats/2018/Table-01.pdf

Popper, Arthur N. et al. (2003) Anthropogenic Sound: Effects on the Behavior and Physiology of Fishes. Marine Technology Society Journal, Volume 37, Number 4, Winter 2003, pp. 35-40(6)
May-Collado et al,. (2017) Ecology and Conservation of Cetaceans of Costa Rica and Panama. Advances in Marine Vertebrate Research in Latin America. Springer, p.293-p.319

Richardson W.J., Finley K.J., Miller G.W., Davis R.A., Koski W.R. Feeding, social and migration behavior of bowhead whales, Balaena mysticetus, in Baffin Bay vs. the Beaufort Sea—Regions with different amount of human activity. Mar. Mammal Sci. 1995;11:1–45. doi: 10.1111/j.1748-7692.1995.tb00272.x.

Rolland Rosalind M., Parks Susan E., Hunt Kathleen E., Castellote Manuel, Corkeron Peter J., Nowacek Douglas P., Wasser Samuel K. and Kraus Scott D. Evidence that ship noise increases stress in right whales279Proc. R. Soc. B http://doi.org/10.1098/rspb.2011.2429

Staaterman, E., Rice, A.N., Mann, D.A. et al. Coral Reefs (2013) 32: 553. https://doi.org/10.1007/s00338-012-1007-8

BEHAVIROAL BUDGET OF ANTILLEAN MANATEE (*TRICHEUS MANATUS MANATUS) OF BELIZE*

Summer Barnes
University of Vermont
Department of Biology, Marsh Life Science
109 Carrigan Drive
Burlington, Vermont 05405

The Antillean manatee (*Trichechus manatus manatus)* is one of two subspecies of the West Indian manatee. A resident population exists in Saint Georges Cayes, Belize, a relatively diverse habitat. This area is characterized by sea grass patches and sink holes. Current understanding of the behavioral budget of this population is fairly limited. In addition, little is known on their habitat use. In this study, the behaviors of individual manatees and mother-calf pairs were recorded across multiple UAV obtained observational flights, around this area. The behavioral states were recorded, incorporating rate of occurrence of each behavior, and relative time spent in each behavior. It was assumed that there would be a variation in behavior budget of individual manatees and mother calf pairs. Additionally, it was assumed that the primary behaviors would be feeding and traveling, due to the manatee's high food consumption needs. A relative time budget of the entire population, for just mother-calf pairs, and just individuals, was made. There was no statistically significant difference found between behavior occurrence and time in behavior across subjects. Feeding, milling and traveling were the highest recorded behavior occurrence, and feeding seemed to be the longest lasting behavior within observations. These results are limited due to the small sample size of 33 observations. In addition, no information was able to be gathered on variable habitat use, and social interactions. However, they provide relevant information on how this population of manatees is spending its time. Understanding the behavioral budget will help to ensure protection of areas of importance, as well as aid in developing better behavioral sampling methods for marine mammals. Upon further analysis of more data, more significant results may be found.

KEY WORDS: ethogram, habitat, individual, mother-calf, UAV survey, West Indian

INTRODUCTION

In recent years, the invasion of the aquatic habitat of marine mammals has increased. Studies have shown that marine mammals are especially susceptible to negative effects caused by a greater human presence (Schipper et al. 2008). They are consistently bombarded with boat traffic, rising noise levels, overfishing, and most apparently pollution. One species of marine mammal, that exists in a hazardous habitat, is the Antillean manatee (*Trichechus manatus manatus)*. The Antillean manatee inhabits regions of Central America and the Caribbean, but these populations are at risk. Due to more illegal hunting and a larger abundance of plastic entering the ocean, specifically from banana plantation bags, along with more boater traffic, there is a decreasing population number (Reynolds II et al. 1995). In addition, the entire combined population of Antillean manatee have been labeled endangered on more than one platform, most notably the ICUN red list and the Endangered Species Act (Deutsh et al. 2008). Such a classification is a call for greater conservation measures. However, the Antillean manatee population of Belize, is not as at risk compared to other populations of the region. The populations found in Belize and Quintana Roo, Mexico, are among the more successful (Reynols et al. 2009). Despite their apparent health, there is still a need for study.

As an herbivore with a large body size, manatees must consume an abundant amount of vegetation on a daily basis (Montgomery et al. 1981). Therefore, they spend a majority of time-consuming food and looking for feeding opportunities. In order to reduce time spent looking for seagrass, their home ranges will typically incorporate large underwater fields of sea grass (Castelblanco-Martinez et al. 2009). However, other activities do makeup the remainder of their daily behavioral budget. Due to their environment, they remain somewhat elusive to researchers, leaving a lot unknown about their behavior. It has been determined that manatees spend a large portion of time engaging in feeding and foraging behaviors (Montgomery et al. 1981). Although, their home range incorporates food sources, they will still need to travel on a daily basis for foraging purposes and social interactions. Despite the relative difficulty of tracking and determining their home range, some studies have found that despite their seemingly widespread range, they will mostly be found in condensed areas (Rodas-Trejo et al. 2008). Males specifically will dedicate time to finding possible mates (Castelblanco-Martinez et al. 2012). The commonly held belief of manatee social structure, is primarily individual manatees with groups mostly being mother and calf pairs. There has been little study completed on the social structure of the Antillean manatee, but Florida manatees have demonstrated a social structure that somewhat resembles a fission-fusion society (Perrin et al. 200).

Despite the lack of knowledge to date on Antillean manatees and social interactions, there have been sightings of these manatees in large groups, typically for the purpose of breeding (Ramospers.comm. 2019). The male encounters are typically aggressive, a stark contrast to the tight knit interactions of mother and calf (Reynolds et al. 2009) and (Rammos per.comm 2009). Mother and calf pairs are characteristic of many marine mammals. As seen in other species, this pair exhibits a tight bond between the individuals. Manatee mother and calf pairs will actually stay together for 1-2 years, during which the mother will introduce the calf to important habitat characteristics; such as, areas to feed, how to travel between these areas and where to obtain freshwater (Reynolds et al. 2009). Aside from seagrass, another important habitat characteristic of manatee home range is sink holes, characterized by a lower current then surrounding areas (Bacchu et al. 2009). These holes are often used for resting behavior because of the lower current (Bacchu et al. 2009). Upon viewing of video of Saint Georges Cayes, Belize, Antillean manatees, obtained with an unmanned aerial vehicle (UAV), there seems to be use of the sink holes as a place for social interaction. From the years of 2016-2018, there are UAV videos of Saint George Cayes, Belize, of multiple sink holes. Therefore, it is hypothesized that these manatees may be using areas of their habitat in different ways then previously thought. Sink holes may be providing an additional opportunity for social interaction. Specifically, with the requirements placed on mother-calf pairs, it is hypothesized that the daily behavioral budgets of individual manatees will differ significantly from that of mother-calves. Through analyzation of the drone videos, a relative behavior budget for each subject type can be made and compared. This will likely show how the nutritional and behavioral requirements of each group, are affecting the behavior. In addition, it is hoped that we will reveal differences in the use of sink holes, as well as deviations from the normal use of habitats in this area. This was done in order to develop a better understanding of the behavioral trends of the manatees of Saint George Cayes Belize.

METHODS

Study Area and Aerial Surveys

This study took place at Saint Georges Cayes, Belize and area characterized by shallow, warm and relatively clear waters (Ramos et al. 2018). Manatees were observed using an unmanned aerial vehicle (UAV) (DJIP3&4) from 2016 to 2018. UAV flights were opportunistic using land base platforms around the island. Flight effort was primarily focused on sea grass fields and sinkholes, as these are sites well used by local manatees. The sinkholes are typically surrounded by the fields of sea grass. The holes themselves are relatively bare, and are located around the entirety of the Cayes. A total number of 48 flights were done in 2016, for a total 15-20 minutes, taken multiple times a day. These flights were taken in a manner that reduced the negative response to the UAV, as it has been found that this population is affected by its presence (Ramos et al. 2018). However, videos where manatees responded with a fleeing behavior were noted, as these are not part of the natural behavior patterns. Only videos with good quality were included in the analysis. Good quality was measured based on visibility of manatees in the water, ability to differentiate individuals, sun glare, water disturbance, and relative depth, and a minimum time in which the individual was in frame. As result of this a total of 16857.744 minutes were included in the analysis.

Behavior Data Collection

Video footage was analyzed using the program BORIS (7.8) (Friard & Gamba, 2016) to continuously log behaviors. For each video, a manatee was identified in the video and followed by identifying subjects; drone, manatee individual (individuals 1-5), and mother-calf pairs, categories of behavior; inactive, active, other, behavioral states; larger scale behaviors that occur for duration of time, and behavioral events; short term behaviors that are not able to be measured with time. The videos were then analyzed, using a focal follow methodology. The animal had to be continually observed for a duration of at least 4 minutes to be considered for a focal follow. Additionally, in situations where there was more than one manatee present in the video or frame, each individual was followed and observed for the entire duration separately. The manatees must also be clearly distinguishable in order for the individual to have been considered for a focal follow. The videos themselves were scored on quality and visibility. If there is a case where the manatees could not be clearly seen or distinguished from one another, due to sun glare, and water visibility, then the video was not incorporated into the observational data. These conditions helped reduce bias and assumptions made on behavior. The pre-identified behaviors were scored, along with the recording for the duration of the behavioral states. The duration of behavior was determined by noting the beginning and end of the behavior in the program.

Behavior Data Analysis

Using the scored behaviors and time duration of behaviors, an estimate of the overall behavior time budget and ethogram of female-calf pairs, single adults, and time of day was calculated. The analysis software provided by BORIS (7.8) was used to create a comprehensive behavioral budget of the Saint Georges Cayes manatees.

Table 1: Ethogram used to evaluate manatee behavior in UAV observations. Subject included single manatees (numbered 1-5 for cases where more than one manatee is present per video) and mother-calf pairs.

Behavior	Description	Category

EVENTS		
feeding	Categorized by sea grass present at the surface and rising to the surface, and/or sea grass in manatees’ mouth. Manatee typically causing sea floor disturbance.	active
resting	Characterized by little to no movement at the sea floor, and rising periodically to surface.	inactive
floating	Characterized by little to no movement at or near surface.	inactive
socializing	Any interaction between manatees that are not maternal.	active
traveling	Manatee moving straight and at a decent pace in one direction. Differs from milling, as it is more random movement.	active
Unknown/not visible	Any behavior that could not easily be determined, or when manatee was temporarily out of view. These behaviors were subtracted from total observation time.	other
milling	Manatee moving in no specific direction and not at a fast pace. The manatee may periodically pause.	inactive
flee	This is a fast-paced movement away from initial locating, usually in response to a drone.	active
maternal	Any interaction between mother and calf pairs that are social or caring in nature.	active
nursing	Calf is present at the flipper of the mother for a duration of time.	active
STATES		
surfacing	Any movement to the surface for respiratory pruposes.	active

A contingency test was performed to determine if there was an association between behavior duration and type of group (single vs mother-calf pairs) in JMP 14.2 (SAS, 2019). For further visual analysis a bar plot showing mean occurrence of each behavior was created, along with a bar plot comparing total behavior occurrence for each behavioral category between individual manatee and mother-calf pairs. This allowed for a visual comparison of the difference in behavioral occurrences, as well as a display of any differences between subject types. Lastly, a boxplot of duration of time spent in each behavior was constructed. This showed the variation in behavioral category duration. All analysis was done in hopes of determining a significant difference in duration of time spent in specific behaviors, in order to determine an accurate view of the manatee behavior is Saint Georges Cayes, Belize.

RESULTS

A total of 33 manatee observations were included from 48 flights taken in June of 2016, totalizing 16,857.744 of observation and analysis. Because it was difficult to identify individual manatees, individuals in each flight were assumed to be independent observations for a total of 13 manatees (6 individual and 7 mother-calf pairs). The contingency analysis indicates that group type was not associated with the duration of specific behaviors ($p>0.05$). When group behaviors were separated into passive and active, there is a non-significant tendency for mother-calves to be engaged in more active behaviors. In addition, there was a non-significant trend in spending more time in feeding and traveling behaviors (Fig.1). However, the feeding behavior category had a relatively greater variation in time spent in that, than other behaviors. Regarding behavioral occurrences, surfacing had the highest occurrence (Fig.2) and was observed only in mother-calf pairs, as individual manatees surfacing were not recorded for this video set. The rest of the behavioral behaviors had a higher occurrence in individual manatees (Fig. 3).

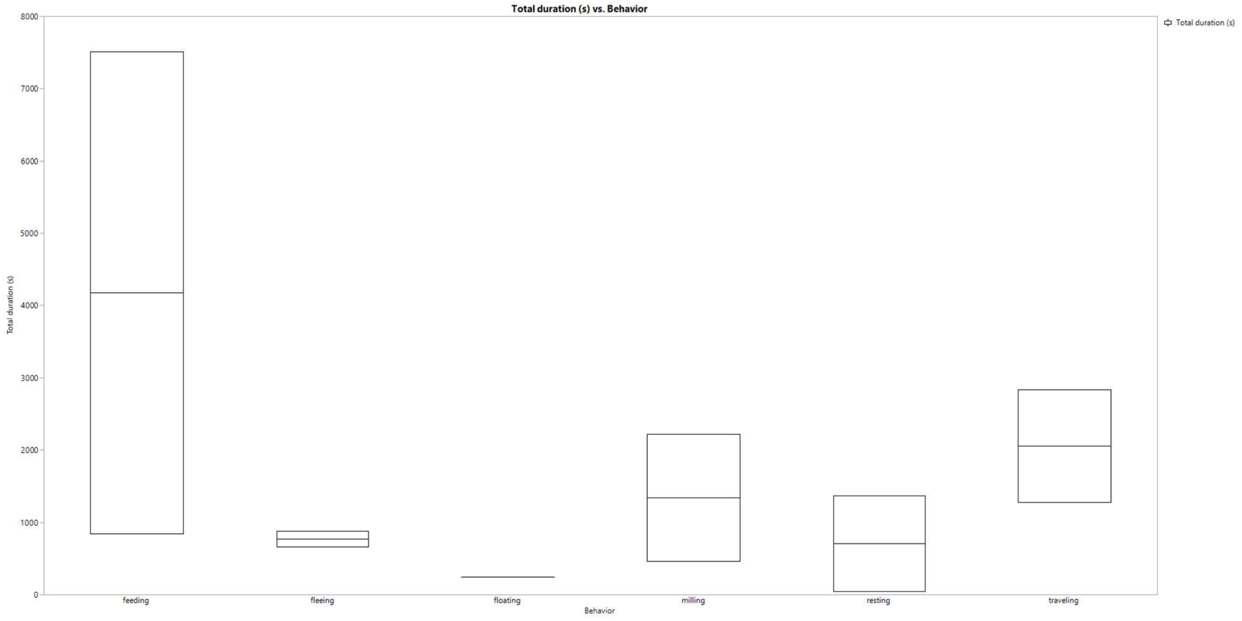


Figure 1: Comparison of mean duration and variance in time of each behavioral event, across all observations (n=33) of the West Indian manatees (n=13).

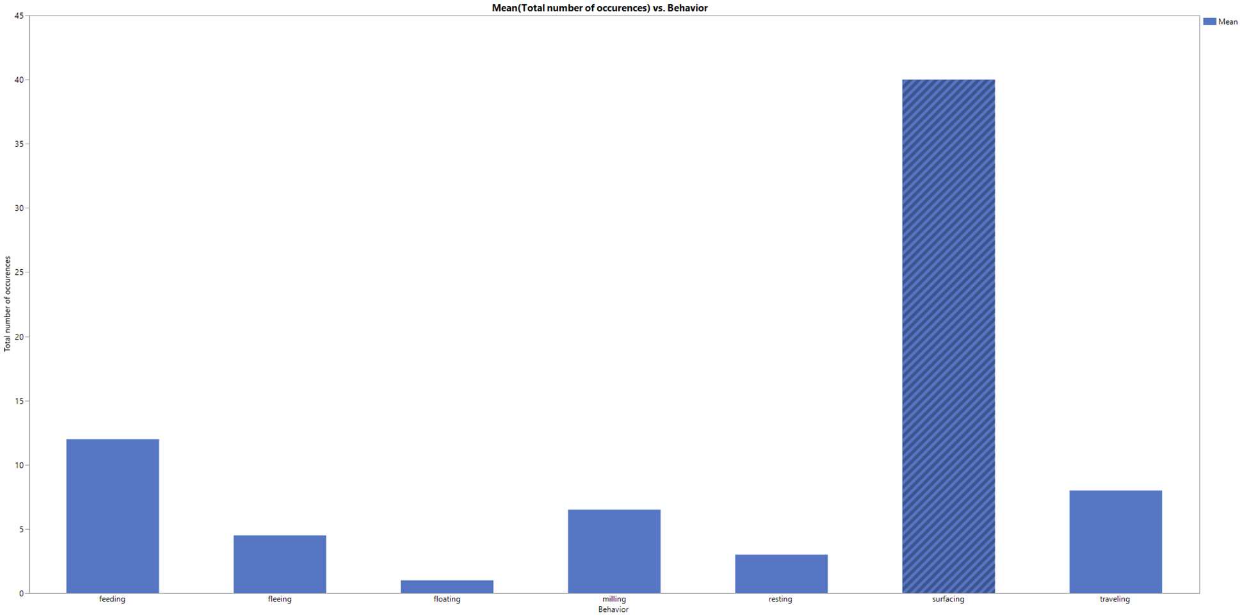


Figure 2: Depiction of differences of total occurrences of each behavior state for all manatees (n=13), for all observations(n=33).

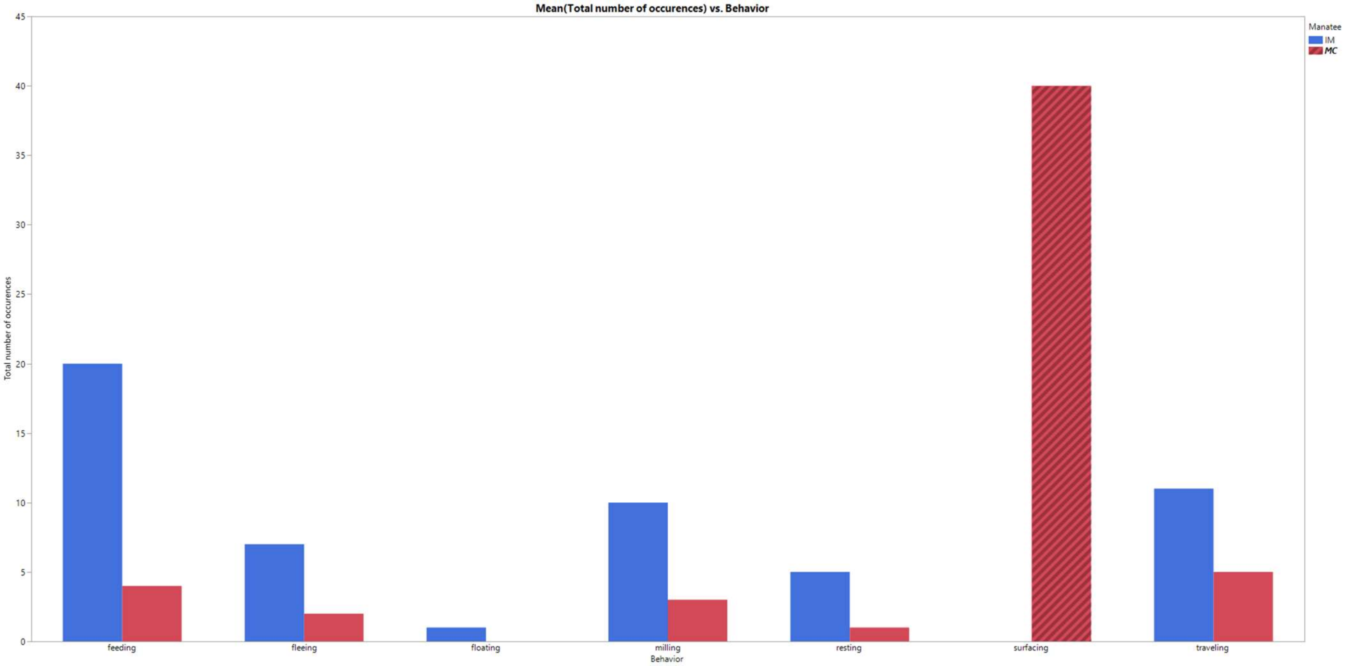


Figure 3: A comparison of the total number of occurrences of each behavioral event for both mother-calf pairs (n=7) and individual manatees (n=7), across all observation (n=33).

DISCUSSION

With the use of the UAV footage across the Saint Georges, Cayes of Belize, a rough estimate of the West Indian manatees' behavioral budget was able to be made. In addition to creating a rough behavioral estimate for the entire population, a comparison of the budgets for individuals and mother calf pairs was made. However, any noted differences in mother-calf pair and individuals' behavior time allocation were deemed statistically insignificant ($p > 0.05$). The lack of significance may be due in large to the limited data, only 33 observations were incorporated into this analysis. The lack of significance of differences between these two subjects is slightly surprising, as the time when mother and calf are together is a vital learning period for the calf. In this time window, the mother spends time showing the calf important locations and behaviors. The calf must learn where feeding grounds are located, areas of warm water influx and any fresh water sources (Reynolds et al. 2009). Therefore, it would seem that mother and calf subject would spend more time in varying behaviors, in order for the calf to learn as much of its environment and lifestyle as possible. Additionally, adult manatees must spend an extensive amount of time in feeding behavior, as the animals must ingest anywhere from 4-9% of the individuals body weight per day (Bengtson 1983). Therefore, it was surprising to find that feeding was not significantly the predominate behavior observed. In total, and across subjects, the major behaviors were actually feeding, milling and traveling. The observed manatees were spending a relatively similar amount of time in milling and traveling behaviors, which was only slightly lower in occurrence than feeding. Additionally, it was interesting to not see a difference in time feeding for individuals versus time feeding for mother calf pairs, as lactation requires an even greater caloric intake (Bengtson 1983). As previously stated, the lack of statistically significant results is majorly a resultant of the small sample size and limited observation window. Despite relatively similar rates of occurrence for feeding, traveling, and milling events, it seems that the actual time spent in feeding behavior in a given observation window, is greater than that for any other observed behavioral state. This more closely aligns with the results of other behavioral budget studies on the West Indian manatee (Montgomery et al. 1981) and (Bengtson 1983). Travel is likely another predominate behavior as manatees exist in relatively large home ranges (Berger-Tal et al. 2011). Within these large home ranges there are more central spots, such as feeding grounds, where the manatees will concentrate (Berger-Tal et al. 2011). Time traveling will, therefore, be greater in order to allow individuals to disperse, and move throughout their larger area, to places of relative importance. With further sampling of behavior of this population, a clearer picture of the behavioral budget may be revealed. This will be done by following the same process above for several more year worth of data, at the same time of year as this initial data sample.

In addition to examining the relative time and rate of behavioral events, an additional interest of the study was looking at social interactions, and use of sink holes in the habitat. This data was not quantified due to the limited amount of information from this short supply of observations. However, observational recordings were made. Through this time period, no true social interactions were really observed, this does not include mother-calf interactions. Adult social interactions, or intergroup mother-calf pair interactions, were not observed. This made quantification of the social portion of the manatee time budget impossible. In several observations, multiple manatees were spotted in the same location, but no true interaction ensued. These were most likely aggregations, individuals using the same area in the same way, rather than social gatherings. Mother-calf pairs remained the only true groupings seen in any of the 33 used observations. This is likely due to the fact, that manatee mother-calf pairs are the predominate social groups, outside of the main breeding season. (Reynolds et al. 2009) and (Perrin et al. 2000). Therefore, it is important to regard the time of year, as well as the location of the observation, when reviewing the relative budget. Similarly, the initial intent to obtain better understanding on the use of habitat, in correlation with behavioral state, was not possible. Due to the predominant behaviors being feeding and traveling, the manatees were most commonly in areas with sea grass or moving through areas; which does not allow for a specific habitat to be determined. This made any analysis, or even observational recordings, on different use of habitats impossible. However, further analysis of other years of UAV obtained observational flights may expand the possibilities of analysis in this area.

Conclusions:

Despite the lack of significance of the results, this analysis has revealed some insight into the behavioral budget of the Antillean manatee population of Saint George Cayes Belize. The limited data that was incorporated into this analysis may be at fault for the results. Developing a better understanding of the behavioral budget of these individuals will not only aid in their conservation but could also benefit in terms of a new method of behavioral study for marine mammals. In the past there has been limited research on behavior of the aquatic based life forms, as their environment makes unbiased behavioral studies difficult. Aerial studies reduce the amount of guesswork incorporated. In addition, these manatees have previously been lacking in terms of our detailed understanding of their behavior. For future analysis, a larger sample size will be incorporated, by including several more years of UAV observations. Additionally, a more detailed record of the behavior of mother-calf pairs will be obtained, by recording relative age of calf. Expanding the number of observations will likely provide a more inclusive view of these individuals behavior, and perhaps increase the rate of other observed behaviors. It will be interesting to determine if there is a difference in behavioral budget across subjects with the inclusion of more data. With an expansion of the data, hopefully more accurate and statistically significant results will be found which will hopefully aid in the conservation of the manatees of Belize, as they face increasing human presence.

ACKNOLWEDGMENTS

Thank you to Laura May-Collado for providing assistance in analyzing and developing data protocol. Thank you to Erica Angel Ramos for the compiled drone data and for assistance on understanding and interpreting the data and behavior. Thank you to the University of Vermont biology department for providing the technology and space to analyze and develop the data.

REFERENCES

Bacchu, Marie-Lys C. and Dunba, Stephen G. and Self-Sulliva, Caryn (2009) *Characterization of Resting Holes and Their Use by the Antillean Manatee (Trichechus manatus manatus) in the Drowned Cayes, Belize.* Aquatic Mmmals, 35, 62-71.

Bengtson, J.L. (1983). Estimating Food Consumption of Free-Ranging Manatees in Florida. *Journal of Wildlife Management*, 47, 1186-1192.

Berger-Tal, O., Polak, T., Oron, A., Lubin, Y., Kotler, B.P., Saltz, D. (2011). Integrating animal behavior and conservation biology: a conceptual framework. *Behavioral Ecology, 22*, 236-239. https://doi.org/10.1093/beheco/arq224

Castelblanco-Martinez, N., Hernandez-Arana, H.A., Morales-Vela, B., & Padilla, J. (2009) Diet of manatees*Trichechus manatus manatus* in Chetumal Bay, Mexico. *Latin American Journal of Aquatic Mammals, 7,* 39-46. 10.5597/lajam00132.

Castelblanco-Martinez, D.N., Padilla-Saldivar, J., Hernandez-Arana, H.A., Slone, D.H., Reid, J.P., & Morales-Vela, B. (2012). Movement patterns of Antillean manatees in Chetumal Bay (Mexico) and costal Belize: A challenge for regional conservation. *Marine Mammal Science, 29,* 166-182. **https://doi.org/10.1111/j.1748-7692.2012.00602.x**

Deutsch, C.J., Self-Sullivan, C. & Mignucci-Giannoni, A. 2008. *Trichechus manatus*. *The IUCN Red List of Threatened Species* 2008: e.T22103A9356917. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T22103A9356917.en. Downloaded on 16 September 2019

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio oding and live observations. *Methods in Ecology and Evolution*, 7.

Montgomery, G.G., Best, R.C., & Yamakoshi, M. (1981). A radio-tracking study of the Amazonian manatee *Trichechus inuguis* (Mammalia: Sirenia). *Biotropica,* 13, 81-85. DOI: 10.2307/2387708

Perrin, W.F., Wursig, B., & Thewissen, J.G.M. (2009). Manatees. In *Encyclopedica of Marine Mammals.* (1, 688). Burlington, Massachusetts: Elsevier.

Ramos, E.A., Maloney, B., Magnasco, M.O., & Reiss, D. (2018). Bottlenose Dolphins and Antillean Manatees Respond to Small Multi-Rotor Unmanned Aerial Systems. *Frontiers in Marine Science.* https://doi.org/10.3389/fmars.2018.00316

Reynolds, J. E., III , J. A. Powell, Jr., and C. A. Taylor. 2009. The manatees--Family Trichechidae (*Trichechus manatus, T. senegalensis, and T. inunguis*). IN: W. F. Perrin, B. Würsig, and H. Thewissen (eds.), Encyclopedia of Marine Mammals, 2nd Edition. Academic Press, San Diego, CA.

Reynolds III, J.E., Powell, J.A., Keith Diagne, L.W., Barton, S.L., & Scolardi, K.M. (2018). Manatees: *Trichechus manatus, T. senegalensis, and T. inunguis*. *Encyclopedia of Marine Mammals, third edition.* 558-566. https://doi.org/10.1016/B978-0-12-804327-1.00165-5

Reynolds III, J.E., Szelisttowski, W.A., & Leon, M.A. (1995). Status and conservation of manatees *Trichechus manatus manatus* in Costa Rica. *Biological Conservation* 71: 193-196.

Rondas-Trejo, J., Romero-Berny, E.I., & Estrada, A. (2008). Distribution and Conservation of the West Indian Manatee (*Trichechus manatus Manatus)* in the Catazaja Wetlands of Northeast Chiapas, Mexico. *Sage Journals.*

Schipper J, Chanson JS, Chiozza F, Cox NA, Hoffmann M, et al. (2008) The status of the World's land and marine mammals: diversity, threat, and knowledge. Science 322: 225–230.