Assessing biological and anthropogenic drivers of spatio-temporal variation in the Galápagos marine soundscape

Glennie Ann O'Reilly LeBaron

Thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honors in Environmental Science

Institute at Brown for Environment and Society

Thesis Advisor: Jon D. Witman, Ph.D.

Second Reader: Dov Sax, Ph.D.

May 2d, 2023

Abstract

Acoustic anthropogenic activity from tourism may impact levels of biological sound production, as vocal fish species are known to produce fewer sounds at sites highly impacted by human activity, indicating changes in foraging and species interactions. A decrease in visitors to the Galápagos Islands due to the COVID-19 lockdown in March 2020 resulted in a temporary cessation of diving tourism in the region, creating a unique opportunity to assess anthropogenic impacts on benthic communities. As long-term passive acoustic monitoring captures variation on a wide range of temporal scales, hydrophones were deployed at six sites across a range of diver activity for 48+ hours biannually from October 2020 to August 2022. This sampling design aimed to assess immediate and longer-term effects of anthropogenic activity on biological sound production. Soundscape variation was analyzed in response to a hierarchy of factors impacting sound pressure levels, which in order of magnitude included diel, site-specific, and seasonal patterns in both fish vocalizations and snapping shrimp. Median sound pressure levels ranged from 112 to 129 dB re 1 μPa2 Hz−1 in the 50 to 45000 Hz frequency band, demonstrating prominent diel variation for all sites. Results suggest that the Galápagos have not surpassed the threshold of anthropogenic sound levels that cause long term impacts, but that diver and boat noise may temporarily impact reef fish.

Introduction

Context

Passive acoustic monitoring offers unique insight into the realm of marine soundscapes. Separated by classes of biophony geophony and anthropogenic drivers, acoustic ecology seeks to disentangle these drivers across the broad brand frequency window and determine overall characteristics of biological activity. Informative descriptions that are possible using comprehensive records such as soundscape offer both a challenge in disentanglement and opportunity in dynamic analysis (McKenna et al., 2021). Abiotic conditions, or geophony, represents natural environmental sources. In the Galápagos marine reserve, the most relevant geoponic sources are currents, and weather events. Biophony is the activity of individuals including vocalizations and sounds from foraging and movement. Anthropophony captures sound produced by humans such as diving, boat motors and industrial scale activity (Mooney et al., 2020). Characterizing and categorizing the different elements of a soundscape marks a first step in pursuing a diversity of ecological objectives.

Disciplinary Background

The biological components of a marine soundscape are primarily driven by vocal fish species and megafauna. Existing marine acoustic work historically has focused on cetaceans; however, a soundscape wide analysis must include vocalizations and activities of a wider range of marine species (Weiss et al., 2021). Components and responses of each regional soundscape vary with the assemblages of species present. As marine soundscape analysis has grown in capacity and relevance, efforts to catalog species vocalizations are underway. However, extensive knowledge of the classifications of vocal fish species remains underdeveloped (Parsons et al., 2022). Multiple efforts to create regional and global species sound libraries exist, though are incomplete and often opportunistic (Parsons et al., 2022). In addition to vocalizations, acoustic records capture behaviors that produce sound, for example urchins scraping a substrate (C. Radford et al., 2008). Therefore, soundscape recordings can represent entire communities and offer unique insights into the interactions and patterns of the soundscape. Additionally, changes in these patterns of activity indicate a capacity to be disturbed by human activity.

Passive acoustic monitoring accomplishes unprecedented noninvasive methods that expand upon and compliment traditional benthic experimental design. In contrast to diver conducted sampling methods, hydrophones can be mounted less invasively and offer undisturbed sampling. Using passive acoustic records originated in the terrestrial context has been largely successful in its recent transition to the marine soundscape (Mooney et al., 2020). As equipment and data storage capacity has increased, the capacity for long term passive acoustic monitoring efforts became more feasible (Merkens et al., 2021). Recent progress had allowed for collecting constant recordings on the order of multiple days rather than a few minutes per hour. The increase in battery life for recording devices, and ability to store terabytes of information easily enables this novel method (Wall et al., 2021). In a centralized effort to track long term records, NOAA maintains a library of 100 terabytes of passive acoustic audio files (Wall et al., 2021). Increasing the capacity of sampling better records how long-term cycles such as seasonal and tidal changes impact soundscapes as well as captures major disturbances or long-term shifts in ecosystem health. The desire to create a more complete picture of regional soundscapes has increased the amount of data collected. Machine learning is an increasingly vital tool to process the high volume of data now being produced in these efforts (Stowell, 2022). Capturing full frequency bands across long term deployments is vital to model the impact of long-term drivers and offers a methodology for tracking the health of an ecosystem over extended periods. Improvements in technology have increased the capacity for passive long term marine acoustic monitoring, and with this increased storage new methods for data analysis of these complex records are increasingly needed.

Anthropogenic disturbances

Separating the extent of anthropogenic disturbances on the soundscape offers insight into the impact of human activity on multiple temporal scales. This project is specifically classified as a noise impact study as it seeks to identify biological responses to anthropogenic activity. Anthropogenic activity is known to impact the ability of vocal species to communicate and forage effectively. It also exists on a wide spectrum of levels of disturbance. Common drivers of high impact localized activity include seismic surveys explosions and echosounders (Weiss et al., 2021). The World Health Organization has identified the anthropogenic acoustic impact on the marine soundscape as an area of concern (Kunc et al., 2016). Different levels of impact from anthropogenic activity have been recorded on the individual scale, including fitness costs associated with decreased forging efficiency and communication. Additionally, the physiology and anatomy of individuals consistently exposed to high levels of anthropogenic activity may alter immune responses and cause hearing loss that leads to changes in community structure and recruitment (Kunc et al., 2016). Beginning with an understanding of how acoustic monitoring can first measure health of a system, and then applying it to measure the ways anthropogenic activity changes these indicators of health, allows insight into responses to human activity at both the individual and community levels.

Uncertainty exists in the capacity to apply terrestrial methodology to marine cases, as this is a relatively novel application of existing terrestrial soundscape ecology. Acoustic records have demonstrated environmental degradation on a regional scale to varying levels of success (Mooney et al., 2020). Changes in acoustic complexity and sound production also have been used to assess the recovery of marine communities (Znidersic & Watson, 2022). Wildlife occupancy models can also be applied to acoustic records (Balantic & Donovan, 2019). Many existing analyses of biodiversity and health are metrics that originated in terrestrial ecology and are inconsistently verified through the literature. Therefore, verifying that acoustic findings are consistent with existing sampling methods is still the norm in many studies (Bertucci et al., 2016; Gabriele et al., 2018; Williams et al., 2015). Stemming from terrestrial acoustic methodology, the validity of similar ecological significance is still under consideration.

Sampling design

Still a relatively new methodology, a wide variety of experimental designs exist to measure varying ecological questions that can be approached using passive acoustic monitoring. With the goal of characterizing soundscapes for multiple sites within a region, one study analyzed 31 days of data from 8 sites during a simultaneous deployment window. This allowed inter-site characterization, as well as identifying consistent drivers for different regions. Using boat pass data as well as contextual information about the sites was relevant to draw broader conclusions (McKenna et al., 2021). To achieve a regional analysis that captured seasonal changes, duty cycle schedules were used to collect data from multi-month deployments. This specifically captured both diel and seasonal trends and recorded vocal fish species as well as vessel sound. A major goal of this study was to verify how known cetacean vocalizations were reflected in different types of acoustic analysis, and how to incorporate anthropogenic activity into these questions (Merkens et al., 2021). In addition to regional characterization efforts, pairwise design has been used to compare acoustic activity levels of protected areas as connected to community health (Bertucci et al., 2016).

Opportunistic design because of the COVID-19 pandemic exists in the passive acoustic monitoring realm. With the COVID-19 pandemic, patterns of human disturbance changed across a wide variety of environments. In New Zealand, usage of shipping lanes decreased in Hauraki Gulf. Hydrophones placed at five sites before and during lockdowns demonstrated how median sound levels decreased during lockdown. These metrics were used to estimate the changes of communication range for vocal fish species and cetaceans (Pine et al., 2021). Combining efforts to draw general characterizations of a region using multiple sites over long sampling periods, and a temporal aspect of variation in anthropogenic activity, creates a framework for assessing anthropogenic activity's impact on biological acoustic variation.

Understanding soundscape structure

Consistent elements of this soundscape follow routine patterns in both duration and pitch. By first broadly defining the elements of the soundscape and its general trends, later analysis can focus on how these specific windows react to different perturbations. Frequency, measured in kHz, represents the inverse of the time of a complete period of a sound wave. It correlates to the pitch of different sounds. Splitting the soundscape into bands of different frequency windows allows for producer level acoustic isolation. Fish reptiles and invertebrates operate in the low frequency band, consistently below 7 kHz (Mooney et al., 2020). Most fish vocalizations are limited within the very low frequency range from only two to a few 1000 Hertz as opposed to the wider range of invertebrates. Snapping shrimp energy is known to operate up to 20 kHz, representing a unique example of non-vocalization biological sound production. (Bertucci et al., 2016; Kaplan et al., 2015; Merkens et al., 2021; Mooney et al., 2020). Alternatively, species such as pinnipeds and cetaceans are known to operate in higher frequency bands (Kunc et al., 2016). The acoustic niche hypothesis describes why stratification of vocalization across a soundscape emerges (Weiss et al., 2021). If the bands of acoustic frequency are considered finite resources each biological sound source would undergo either a temporal or frequency partitioning in their vocalization and hearing. This model is often used as an underlying assumption in which more activity across a wider range of bands is correlated to higher biodiversity (Parsons et al., 2022; Weiss et al., 2021). This assumption is complicated by new anthropogenic disturbances regimes.

Anthropogenic activity similarly can be defined within specific frequency bands. Larger vessels have been correlated to approximately 4 kHz, and generally appear in low frequency bands overlapping with vocal fish species (Merkens et al., 2021). Understanding the areas of overlap and isolation for different biological and anthropogenic sources of sound production allows the soundscape to be strategically split into frequency bands of interest. Common isolations include exclusively low frequency, to exclude snapping shrimp, and high frequency bands.

Abiotic and temporal factors also must be considered as potential drivers of biological sound production variation. Diurnal trends correlated to sunrise and sunset are common globally as many fish species are identified because crepuscular and nocturnal choruses are distinct from daytime choruses due to changes in species composition. Therefore, a common practice is to divide the soundscape by period of day including daytime and overnight, with certain experimental designs including a third period for a corpuscular chorus (Bertucci et al., 2016; Merkens et al., 2021). Fish and snapping shrimp sound production are also known to change with season lunar periodicity, light levels, temperature upwellings tides and salinity (Mooney et al., 2020). Wind speed and temperature have been successfully combined with acoustic records to indicate that these have an effect on sound production (McKenna et al., 2021). An alternative approach to classification is based in the qualitative understanding of the factors that occur at sites to create context for trends in biological data (Kaplan et al., 2015). Understanding how abiotic factors influence temporal changes in biological sound production is critical to first characterize the soundscape. By first understanding how these factors influence the soundscape and then layering in anthropogenic activity, we can establish a more nuanced understanding of human impacts on sound production.

Connecting sound production to biological indicators of diversity

While machine learning offers new capacity to process long term records to a signal level specificity, without well documented and robust acoustic libraries, it remains a challenge to classify sound production to the genus or species level. Therefore, broadband metrics of complexity and richness using the acoustic niche hypothesis as an assumption often serve as alternative measures of species richness or activity.

Measures such as acoustic entropy and acoustic complexity, developed for terrestrial evaluation, are demonstrated to be inappropriate for the marine soundscape. Over 70 different indices exist attempting to capture measures of biodiversity or abundance, the majority terrestrial in origin (McKenna et al., 2021). Acoustic entropy, which evaluates the evenness of amplitude across the full frequency band, and acoustic complexity, which assesses the complexity of frequency change over time, have been found to have varying levels of correlation to fish abundance counts (Bertucci et al., 2016; Mooney et al., 2020). The majority of indices make the underlying assumption that biological sound will have distinctly predictable frequencies and times, ensuring that, as per the acoustic niche hypothesis, there is minimal frequency and temporal overlap (Bradbury & Vehrencamp, 2011). This indicates that, given the early stages of marine acoustic ecology, current best practices must include a secondary sampling method to verify whether an index is appropriate for the research objective and region. Use of acoustic indices to connect sound production to measures of biodiversity require further verification for marine soundscapes.

Alternative uses of acoustic indices include sound pressure densities and sound pressure levels for isolated frequency bands. By separating sound pressure levels into high and low frequency bands increases in sound production have correlated with the health of coral (Bertucci et al., 2016). Similar summary statistics that assess the overall sound production of the soundscape include cumulative dynamic range which computes variability using power spectral density for increasing sample sizes (Mooney et al., 2020). As a full departure from variability metrics, analyzing sound level metrics assesses the level of sound production for a given frequency band. This computation of mean or median sound pressure levels allows for the isolation of known activity bands and characterizes a general measure of sound production. While it does not capture information regarding statistical variance in a soundscape, it also does not require assumptions of complexity and distribution of sound production across a frequency band.

Further demonstrating issues with the acoustic niche hypothesis, masking effects, in which signals overlap and obscure one another, call into question the efficacy of acoustic indices for measuring biodiversity. Especially relevant to disturbance acoustic ecology, major disturbances in the soundscape create a masking effect. A biological example includes fish choruses and other large-scale phenomena that may reduce the detectability of other signals which may still be occurring. For temporal and frequency co-occurring signals, it is challenging to separate by discrete producer, thereby creating a masking effect. Masking effects are also common with anthropogenic activity. When considering the impacts of boat noise and other major disturbances, it is feasible that anthropogenic sound production masks biological sound production that is still occurring (McKenna et al., 2021; Parsons et al., 2022).

This makes ecological implications challenging to understand as it is unclear if masked biological signals retain the capacity to fulfill their functions. This effect introduces challenges for methodology, especially when assessing biological characteristics in environments with high anthropogenic and environmental disturbance levels. Even if the acoustic niche hypothesis became verified in the marine soundscape, the introduction of anthropogenic activity which does not follow this assumption calls into question the use of variance-based indices to assess biological parameters.

Assigning drivers of variation

A multitude of factors inform the measured levels of biological sound production. By introducing multiple factors as well as holistic understandings of site differences, drivers can be contextualized for an entire region. Use of long-term spectrogram analysis (LTSA) compress and assist in visualization of long-term records. Targeting median or percentile values that correlate to specific events can offer unique analysis of continuous sounds, including environmental sounds or biological choruses (Mooney et al., 2020). Specifically isolating low frequency bands isolates biological (Kaplan et al., 2015; Merkens et al., 2021). By first characterizing which frequency bands contain events of interest and then isolating those bands in LTSA analysis, effect level targeting is feasible. Similarly, soundscape metrics can be integrated to create values that are less sensitive to undesired effects. Clustering may offer unique insight for long term deployments (Mooney et al., 2020). An example of a commonly cited natural driver of changes to the biological soundscape is changes in sunlight (diel trends). Sunrise and sunset are known to trigger chorus events, and therefore splitting data into overnight and daytime periods can be a useful way to subset data by known activity level (Kaplan et al., 2015; Merchant et al., 2015; Mooney et al., 2020; C. A. Radford et al., 2008).

An additional critical consideration is non biologically driven site differences. Examples include the topography and landscape of a site, or predominant currents and weather events that occur nearby. Temperature is also known to impact sound pressure levels. These types of differences exemplify the difference between biological sound production and measured sound pressure levels. While topography and temperature may not alter the amount of biological sound production at a site, they impact the ability of sound to travel and reflect through water and can impact sound pressure levels. This is why holistic site characterization can be valuable when connecting sound pressure levels to conclusions regarding sound biological sound production (McKenna et al., 2021). Not all site characterization parameters may be accounted for in analysis. While general characterization can be useful, combining acoustic data with existing datasets can be vital in determining the role drivers of variation play in biological sound production. Examples include AIS data, wind speed data, tidal flow data, and temperature loggers. Additionally, to establish relationships between sound pressure level and biological sound production, visual data such as fish surveys or video data is often correlated to acoustic data to determine validity (Bertucci et al., 2016).

Anthropogenic soundscape impacts

The capacity for humans to alter the structure and availability of the soundscape for biological sound production is extensive and varied. Human activity, ranging from individual divers to large scale long-term shipping and drilling projects, is known to cause changes in biological sound production (Archer et al., 2018; Weng et al., 2023). Ecological responses occur in both short and long term and can indicate a variety of health impacts (Ziegenhorn et al., 2022). These varied responses are thought to result from two opposing possibilities. The first is that biological sound production may show an immediate drop followed by an increase despite the continuation of a disturbance. This desensitization to the disturbance may indicate that there is biological recovery after an initial disruption (Williams et al., 2015). Alternatively, introducing anthropogenic activity may cause a decrease in biological sound production overtime, indicating that there are habituation, relocation, or abundance effects. Additionally, an increase in biological sound production does not inherently indicate a positive change in the health of a system. For example, species may vocalize louder in order to be heard over anthropogenic activity (Holt et al., 2009). While using total sound production to determine the anthropogenic effects on biological sound production is an important first step, contextually understanding target species dictates the capacity of interpretation.

Anthropogenic disturbance can be understood as both a binary state and a continuous variable. A period can be defined as disturbed or undisturbed in a binary classification (Fournet et al., 2022). This is particularly common in contexts which use AIS data to define disturbance states. Rather than identifying where in the acoustic record anthropogenic activity occurs, which could offer a continuous measurement, AIS data can at most offer a count for number of boats in an area. Using a binary classifier, biological response can be measured before and after a disturbance state (Kok et al., 2021).

One of the current challenges with this field is determining exactly where and how much anthropogenic activity is occurring. Vessel passes occur at a similar frequency to most common biological activity, and therefore cannot be band isolated. Two existing methods attempt to extract anthropogenic activity from a complete soundscape. The first is by hand, in which a trained user visually assesses periods of data and identifies whether target disturbance is occurring (Fournet et al., 2022; Kaplan et al., 2015; McKenna et al., 2021; Mooney et al., 2020). The user may either identify a consistently sized window as disturbed or select the exact timestamp and frequency band for which the target disturbance occurs. An alternative, which is gaining favor as datasets grow and machine learning becomes more accessible, is automated methods (Merkens et al., 2021). Some models use machine learning to identify patterns that are consistent with target disturbances. This is best for consistent and extreme disturbances. For example, regions in which boat activity appears for consistent durations at consistent intensities are better suited to this type of processing than regions which may include many different types of vessels or high variation in their purpose which may cause different engine patterns. Machine learning classifiers such as neural nets can be used to identify more variable boat traffic (Pine et al., 2021). An important caveat is that automated classifiers still require approximately 1/3 of data to be user identified in order to create training and validation data sets (Stowell, 2022). The varying methods designed to extract anthropogenic activity from long term acoustic records continues to grow even as standards for best practice remain inconsistent.

<u>Methods for Long term Acoustic Record Processing</u>

Passive acoustic monitoring introduces unique issues for sound analysis primarily due to the high volume of data. Traditional packages using Rstudio, such as WarbleR, are insufficient for the high volume of data required to be processed. Early in the discipline of passive acoustic monitoring, sub sampling was often used as a method to narrow data sets into more manageable sizes. Practices to accommodate the limitations of RStudio included subsampling deployments to only include several minutes per hour for longer time periods (Bertucci et al., 2016; C. Radford et al., 2008). A major concern is that subsampling will often lose transient events that may be quite indicative in the long term (Mooney et al., 2020). Additionally, complete records are necessary for measuring overall health and creating the capacity for regional comparison (Znidersic & Watson, 2022). Therefore, given the capacity of newer technologies that can accommodate large datasets and are user-friendly, avoiding sub sampling has become an emerging best practice.

Use of sound pressure levels give a general indication of sound production and can be strategically isolated by frequency to answer varying questions regarding biological health and disturbance effects. Specifically isolating frequency bands into a low frequency band including most known fish vocalizations, a mid-level band which includes snapping shrimp, and a broadband for overall analysis is common (Bertucci et al., 2016; Merkens et al., 2021). Computing root mean squares of sound pressure levels, medians or means can create a single metric for an entire frequency band. Given that distribution of sound production is often skewed across a frequency band, median is the most common metric (Archer et al., 2018; McKenna et al., 2021; Mooney et al., 2020; Wall et al., 2021). Additionally, percentiles can be used to identify major sound production incidents (Wall et al., 2021). By determining whether low gain or high gain filters are appropriate background noise can sometimes be removed (Archer et al., 2018). This basic structure can be used to assess changes for a targeted frequency as well as over different time scales. Tailoring bins on the order of seconds to hours as well with target bands and defined resolution specific properties makes sound pressure level analysis an incredibly versatile tool.

Use of GLMM models for acoustic sampling

Generalized linear mixed models serve as an alternative to traditional pairwise significance tests for particularly complex data types. Specifically, interdependent multi-factor cases can be better modeled using GLMMs in which the relative strengths of factors can be weighed. This model additionally does not rely on independence and can be used for varying distributions of data. With both binary and count result variables, this model offers flexibility and rigor to assess different outcomes. It also allows for observations to be nested within grouped categories.

Purpose, goals, and hypothesis

As the first long term passive acoustic monitoring effort in the Galápagos region, the primary goal of this thesis was first to establish appropriate processing protocol, and then determine general patterns that exist in the Galápagos soundscape. This required deciding on methods that were appropriate for long term multi-site records and enabled the eventual goal of signal separation to the producer level. This meant that sub-sampling was not appropriate for this project indicating that more novel methodologies were required for processing large data sets. Once methodology and general context was established, our research goal of understanding how anthropogenic activity impacts the biological soundscape in the Galápagos Marine Reserve (GMR) could be assessed. This enables qualitative comparison between the Galápagos marine reserve and other established monitoring sites seeking to understand anthropogenic disturbance. This study specifically sought to determine if there were long term effects of changes in anthropogenic activity due to the COVID-19 lockdown. Secondarily, anthropogenic disturbance levels were placed into the context of other factors influencing biological sound production, ultimately creating a hierarchy of influence.

This thesis had three central questions. First: as a result of the drop in tourism due to the COVID-19 lockdown, was there an increase in anthropogenic disturbance events specifically at high diver sites over the duration of the sampling efforts? Would any increase in anthropogenic events to correlate with a decrease in biological sound production, implying that anthropogenic activity is causing a dampening effect to the biological community? Finally, for low diver sites, would no clear trend in anthropogenic disturbance events or biological sound production emerge?

Methods:

Study site

The Galápagos Marine Reserve (GMR), a multi-use marine protected area, covers 138,000 square kilometers approximately 1,000 kilometers from the coast of mainland Ecuador (Castrejón & Charles, 2020). Known for unique and complex marine communities (Witman, 2010), ecotourism continues to grow in the area as the primary economic sector (Mestanza-Ramón et al., 2019). Understanding how increases in these activities impact the biologic communities remains vital to striking a balance between economic vitality, long-term planning, and overall ecosystem health. The Galápagos Islands attract a wide variety of tourists and are known to have some of the most popular recreational diving sites in the world (*Shark Diving: The 12 Best Shark Diving Sites in the World*, n.d.). To best capture changes in tourist activity level, three pairs of sites were selected for the duration of the study. Within each pair, one site was identified as a commonly visited tourist site or high diver site, and the other was a less commonly visited tourist site. The pairs were selected based on over a decade of observation (*personal observation Jon Witman*). Sites have variation in levels of biologic activity, topography, and proximity to higher traffic boat routes. All hydrophone deployments occurred simultaneously by site pairs.

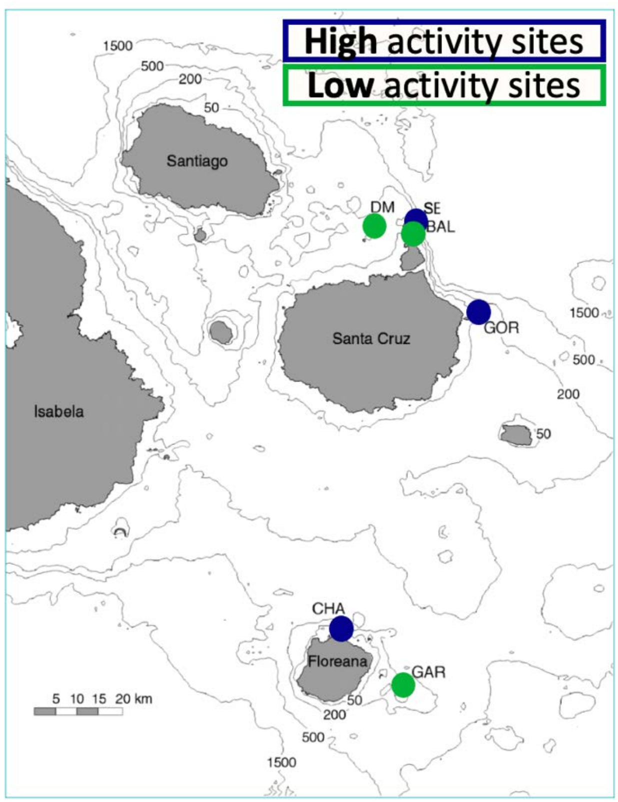

Figure 1: Site selection for hydrophone deployment showing high and low disturbance sites.

Sampling methods

Deployments ranged from two to eight days. Each site had a minimum of one deployment for five sampling periods approximately six months apart from October 2020 through August 2022. This entire sampling effort was designed to coincide with the COVID-19 lockdown, in which numbers of visitors to the islands ceased before returning to normal over the following two years. Sampling periods broadly coincided with three periods during the cool season, and two periods during the warm season.

For each deployment, a Soundtrap300 was attached to a temporary metal stand using a pair of metal rings. Members of the Witman lab dive team placed stands at approximately 15-meter depths on ledges near walls, anchored using concrete blocks. The long-term monitoring stand locations were generally consistent across periods. This setup was also used for long-term video monitoring, and therefore acoustic records may also capture members of the research team adjusting the stand to retrieve cameras during acoustic deployments. Sampling rate was set to 98 times per second to capture individual vocalization events. Broadband frequency sampling rates were captured with usable range between 50 to 48,000 Htz.

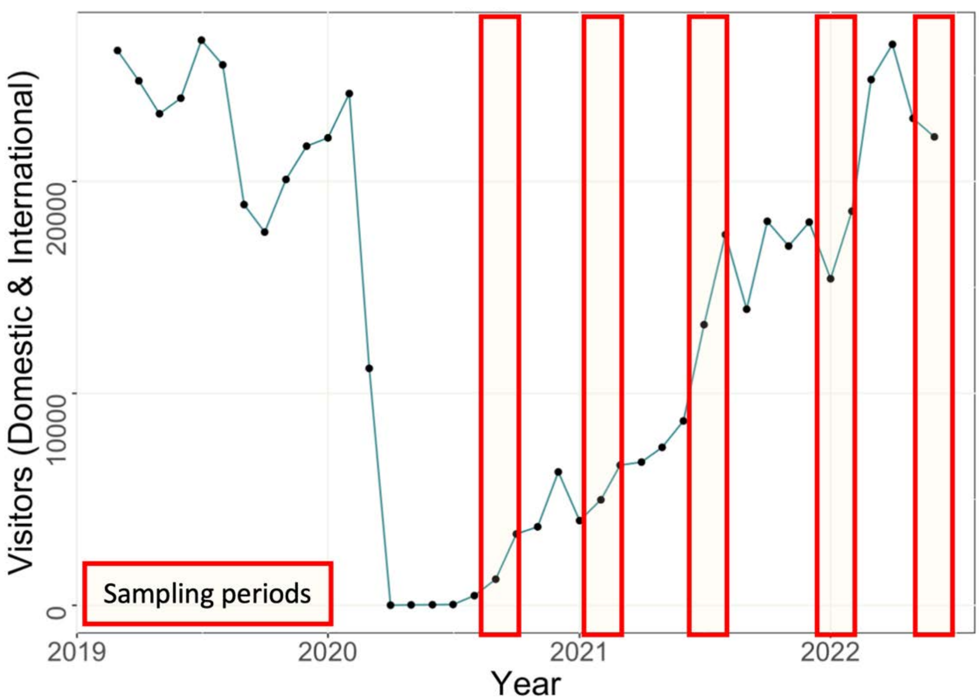

Figure 2: Change in visitors to Galápagos National Park during sampling period. Data sourced from Galápagos National Park, Nico Moity FCD

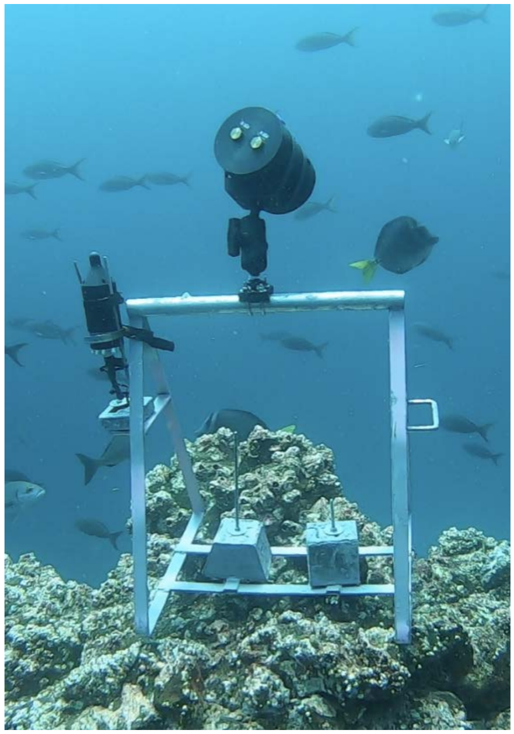

Figure 3: Hydrophone (left) and long-term video camera (right) on stand

Data cleaning & processing

Approximately 2000 hours of underwater deployment records, including setup and breakdown of the monitoring stands, were captured during the sampling effort. To process these 800 gigabytes of data without using a sub-sampling technique, an emerging methodology for long-term passive acoustic monitoring was used. Cleaning primarily occurred using the acoustic application Raven, which was designed for terrestrial acoustic record visualization (Raven Manual). This software was used to compile common anthropogenic activity visual signatures including hydrophone setup, boat noise, and near diver noise. I looked for these visual cues in the beginnings and ends of deployments to identify where setup and breakdown of hydrophone stands occurred. I cropped data sets to exclude any dives where one of the primary objectives was setup or breakdown of the long-term monitoring stand. The usable deployment record is 1875 hours, or 1.3 terabytes,

Long-term acoustic records are often visualized as spectrograms, which plot the distribution of sound production across frequency and time scale. Once deployment records were cleaned, converting WAV files into both visual and statistical metrics required the use of Matlab based acoustic processing package Triton. This package originated from SCRIPPS Oceanographic Lab in order to aid in their long-term passive acoustic monitoring computations period (*Scripps Whale Acoustics Laboratory | Technologies: Triton*, n.d.). Both the standard package and soundscape metrics remora were utilized to convert WAV files into full spectrogram files. Data from single deployment can contain over 80 gigabytes of information, meaning that visualizing a long-term record directly from WAV files is too cumbersome for most computer processors. The Triton package allows long-term deployments to be converted into a single long-term spectrogram analysis file, allowing users to easily page through deployments, and visualize multi-day deployments in a single image using five second, 20htz windows.

Soundscape metric long-term spectrogram analysis is a separate type of information packaging that, once created for each deployment, can be easily used for processing soundscape metrics, including power spectral density and mean/median sound pressure levels. In addition to computing long-term spectrogram analysis for visualization, this workflow allows for easy subdivision by frequency band, use of varying time averaging bin sizes, and calibrating to our hydrophones for absolute sound pressure levels. We computed sound pressure levels for the full broadband and low frequency band to isolate fish vocalization (Kaplan et al., 2018; Merkens et al., 2021). Mean and median sound metrics were computed in both frequency bands at 15-minute and 30 second intervals. Median sound pressure levels across the broadband using 15-minute intervals offers a general coarse grain profile of the soundscape. This process created 15,800 usable sampling windows total.

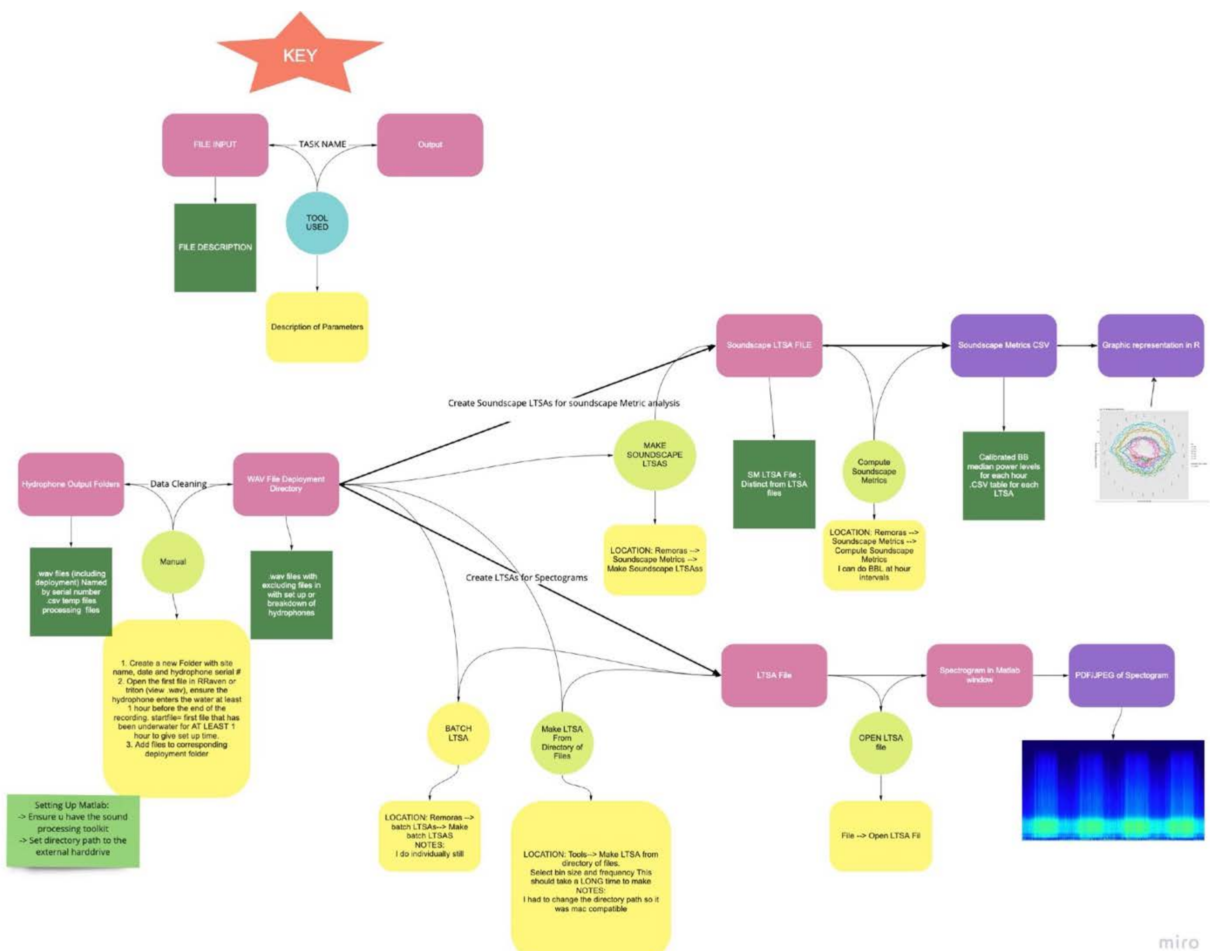

Figure 4: Workflow to convert deployment data into spectrograms and SPLs

<u>Binary disturbance identification</u>

Anthropogenic and biologic sound production events occur at overlapping frequency bands (Ellison et al., 2012). To isolate an exclusively biological acoustic record, any period including a major anthropogenic event must be excluded. To exclude major anthropogenic events, as well as setup disruption, I compiled a guide of visual indicators of anthropogenic or set-up disturbance for 15-minute windows (Appendix). Trained lab members used spectrogram visualization from LTSA files to identify which 15-minute bins included obvious disturbance events. These disturbance events were then removed from the data set for statistical modeling of exclusively biological sound production.

Statistical modeling for factor hierarchy construction (GLMMS)

Once sound pressure levels were computed using the MATLAB package Triton, output CSVs were compiled and analyzed using Rstudio. Each sound pressure level was identified by date and time, site, season, diel (day/night), and type of analysis (broadband or low frequency and mean or median). Given the GMR's proximity to the equator, sunrise and sunset varied by at most 10 minutes per year, therefore 6:00 PM and 6:00 AM were used as approximate estimates for sunrise and sunset throughout the sampling effort. This allowed for data visualization based on potential factors of influence, as well as the construction of a generalized linear mixed model. GLMMs were constructed using the GLMMR r package. Multiple models were constructed for the disturbance excluded data using different combinations of the four factors period (capturing the long-term anthropause question), site, diurnal state, and season in order to assess their relative influence to the biological sound production. Data were defined as clustered by deployment and no random factors were included. AIC values were compared to determine the best pairwise and tri-factor models. P-values for each element of the best models were used to assess individual elements of the model.

Machine learning training set protocol

Another element of this thesis was to support efforts to establish machine learning workflows to identify anthropogenic activity at finer scale frequency and time bands. Given the size of the acoustic data set, disturbance-level specific classification was not feasible by hand. Machine learning methodology, once established, can greatly increase the data processing capacity. To establish effective workflows requires substantial training sets, so a protocol was employed for trained individuals to identify diver breathing, boat motor revving, and set-up sound at the disturbance level using Raven software packages. Users created selection tables that defined the type of sound production, and exact time and frequency bands that the disturbance occurred on. This workflow produced both training sets and verification sets for ongoing machine learning efforts.

Results:

<u>Regional soundscape profile</u>

Generic characteristics can be drawn from overall patterns in the soundscape. Median biologic sound pressure levels ranged from approximately 112 to 129 dB re 1 μPa2 Hz−1 in the 50 to 45000 Hz frequency band. More than 15,000 15-minute windows were processed as part of the cleaned median sound pressure levels. Of these, approximately 1,000 contained either set-up or clearly anthropogenic disturbance events. Disturbance events were detected at all six sites for all sampling periods. A low diver disturbance site at night consistently had the highest broadband sound production. The lowest biologic sound pressure levels were from a different low diver disturbance site during the daytime. This is approximately the difference between a motorcycle and a jet engine (*Hearing Center of Excellence*, n.d.). The low frequency band specifically demonstrates a range of approximately 90 to 125 dB re 1 μPa2 Hz−1 in the 50 to 1200 Hertz frequency band. This drastic change is largely due to excluding effects caused by snapping shrimp. The distribution of sound pressure levels appears to follow non-normal distributions and SPLs for a single 15-minute window are highly correlated to the sound pressure levels immediately before and after.

<u>Disturbance events</u>

Frequency of daytime disturbance events doubles over the course of the sampling effort, consistent with trends showing an increase in visitors to the island through the sampling effort. As expected, 15-minute bins that contained either setup or anthropogenic disturbance events had higher median broadband levels than non-disturbed bins. This confirms the need to exclude those bins from records that exclusively seek to understand biological sound production.

The range in frequency of daytime disturbance levels was 2.1% to 35%. The two lowest impact deployments occurred in October 2020 during the anthropause at two high impact sites. One of these high impact sites demonstrated the highest disturbance level in the following Jan 2021 period. Of the high disturbance sites, two show clear trends of an increase in disturbance level, with one including the highest levels of variation across sites. Overall trends demonstrate a more than double increase in disturbance events from the start of sampling to the most recent sampling, with the minimum and maximum overall disturbance levels occurring in October 2020 and August 2022, respectively.

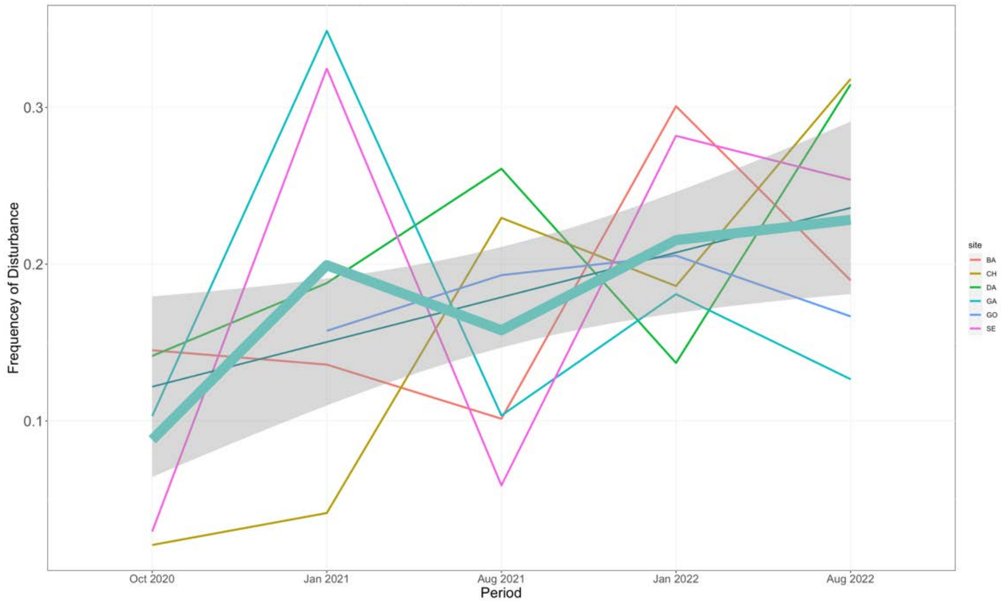

Figure 4: Changes in the frequency of daytime disturbance events during the sampling effort. Overall (thick teal) and by site included

<u>Soundscape signatures</u>

Key elements of the soundscape can be identified, and their frequency band verified in the literature. Using Raven, fish vocalizations were found at all six sites occurring between the 50 - 1500 Htz frequency band. These vocalizations appear as equidistant multi-frequency pulsing events often occur for two to five seconds, were found more often in night periods, and appeared clustered. Sea lion barks operated in a slightly wider band between 100 to 2000 Hertz, and similarly were often clustered. Unlike fish vocalizations, snapping shrimp appear as a constant presence in all acoustic records with varying levels of strength. Across all sites snapping shrimp snaps occurred at approximately 2,000 to 10,000 Hertz.

As most boats operating in the Galápagos marine reserve are small scale, short-term infrequent boat cavitation appeared more commonly in the visual record. Given the operating procedures for dive boats to drop divers off at sites, as boats neared hydrophone stands, engine cavitations became inconsistent, making it easy to define in the acoustic record. Depending on the strength and distance of the cavitation, boat engines could span the full broadband record of 0 to 48,000 Hertz. Dive boats were often accompanied by scuba divers who showed the clearest trace in the acoustic record during underwater breaths. These breaths were clear enough to be attributed to specific divers, including exact regulator and equipment usage that emitted different high frequency signatures. Given the level of overlap in anthropogenic and biologic sound production, we found that, in general, when anthropogenic activity was present, it was significantly stronger than any biologic signals, so that even if biologic signals were present, they were effectively masked by anthropogenic activity.

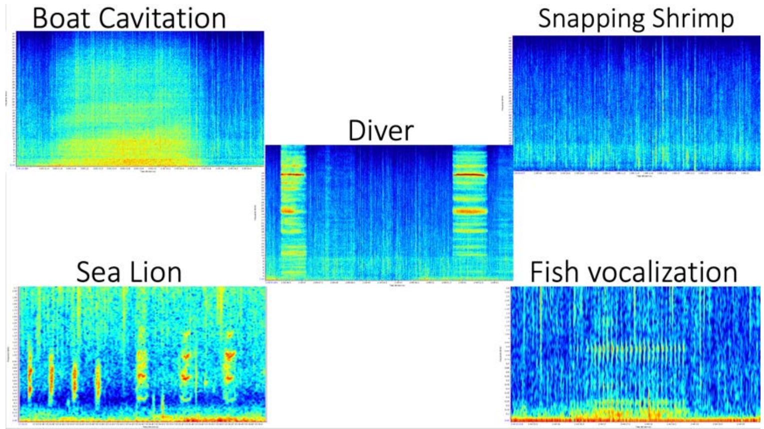

Figure 5: Examples of clear signals from anthropogenic and biological target sounds producers

Diel effect

Diel trends demonstrate a source of consistency across all sites. Sunrise and sunset have such a distinct impact on broadband biologic sound production that one can identify diel shift simply by looking at multi-day spectrogram records. Especially for high biological activity sites, a crepuscular pulse can be identified at approximately 6:00 AM and 6:00 PM within the 30 minutes of diel shift. However, when analyzing the distribution of day, night, and crepuscular classes, the hour of sound pressure levels around diel shift did not appear visually different from night events. Given the significantly smaller sampling size, crepuscular chorus was combined with night data for further data analysis modeling. The site with the highest overnight median sound pressure level also showed the largest change between median daytime SPLs and median nighttime SPLs.

Strong diel signaling indicates the likelihood that the acoustic record is primarily biologic sound production. Abiotic factors, such as topography, temperature, and weather events would not show such striking diel patterns. While diel changes are visually identifiable for all six sites, they are far clearer for Gardner, with a 3.9 dB re 1 μPa2 Hz−1increase in median SPL between overnight and daytime, as compared to a 1.5 dB re 1 μPa2 Hz−1 increase at Seymour.

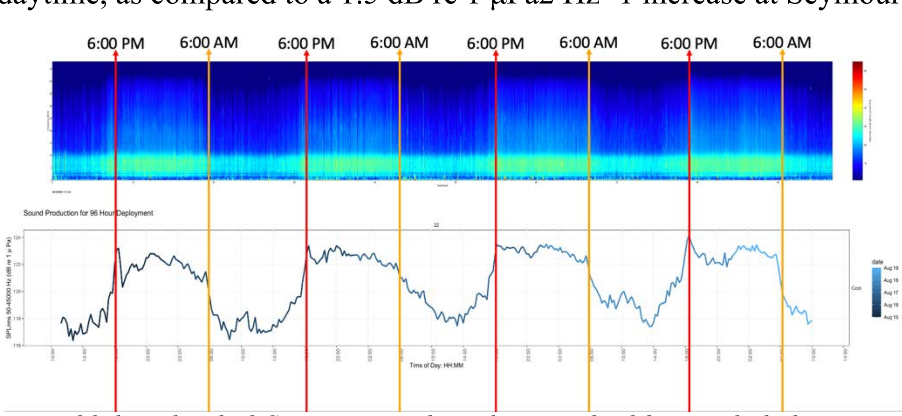

Figure 6: Demonstration of diel trends in both Spectrogram and sound pressure level for a single deployment at the loudest site. Sunrise and sunset are indicated by the vertical lines.

Site profiles

Qualitative and quantitative site-specific characteristics demonstrate the need for local-level analysis. Use of multi-day spectrograms allow users to analyze overall patterns occurring over the span of days. Understanding the context of each sampling location is important for further interpretation of findings (Mooney et al., 2020). All six sites show consistent distribution of snapping shrimp sounds as well as a 2 1/2 kHz ban that can be associated with biological activity. Baltra demonstrates consistent crepuscular pulsing, with less obvious midday and midnight differences through the broadband. The low frequency band does not show clear changes between day and night. Champion demonstrates strong overnight chorusing due to snapping shrimp as well as low frequency fish chorusing events demonstrated over two nights of January 2022. This same low frequency fish chorusing can be seen in Seymour, in which an approximately 1 kHz frequency event begins at sunset and occurs for the first several hours of the night. This is only seen in the January 2022 record. Seymour appears to have one of the stronger crepuscular pulses of all sites. Gordon, a high visitation site, has visually identifiable crepuscular choruses that seem to end during the night before resuming shortly before dawn. This overnight cessation of biological sound production is particularly clear for low frequency bands at this site. This noticeable lack of low frequency activity is also reflected in the Daphne record. Daphne demonstrates a consistent striping effect in the acoustic record and shows breaks in snapping shrimp sound production. The loudest biological site, Gardner, reveals the most intensive snapping shrimp activity overnight. Potentially as a factor of more snapping shrimp masking fish vocalizations, extremely low frequency activity appears less common overnight than during the day for some periods.

Additionally, by assessing the distribution and values of sound pressure levels, consistent site trends can be assessed. Site order of median biological SPLs does not change between day and night, except for two mid-level sites that have similar distributions in both day and night. Two low diver activity sites have the highest night SPLs at the broadband level. The third low diver activity site has the lowest overnight SPLs. Range of overnight median sound pressure levels for sites ranges from approximately 112 to 133 dB re 1 μPa2 Hz−1. Polar plots by time of day provide a key way to assess how changes in time of day vary across sites.

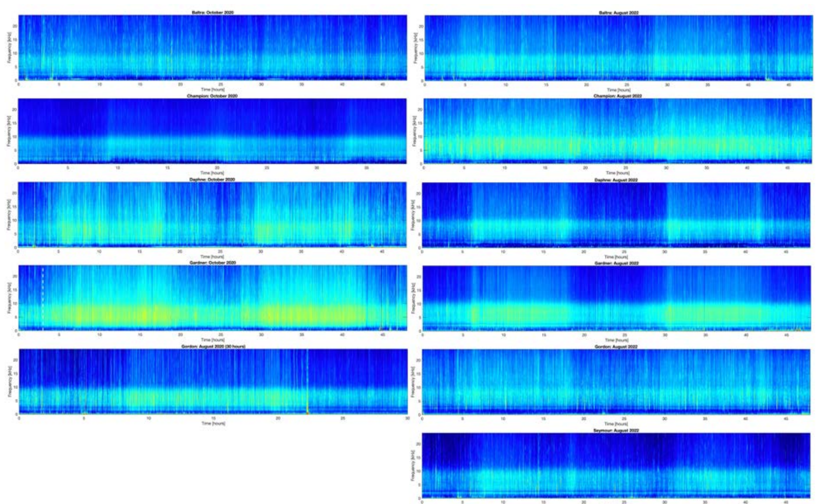

Figure 7: Illustration of 48-hour site characterization spectrogram. Profiles of sites remain consistent across periods. October 2020 and August 2022 used as examples.

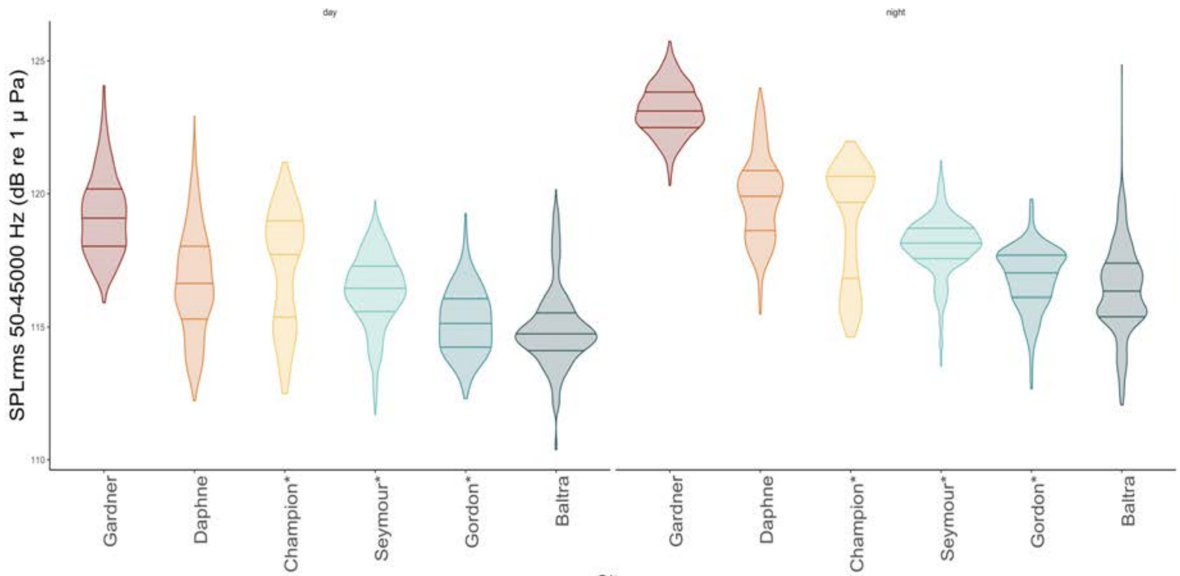

Figure 8: Distribution of median sound pressure levels per site.

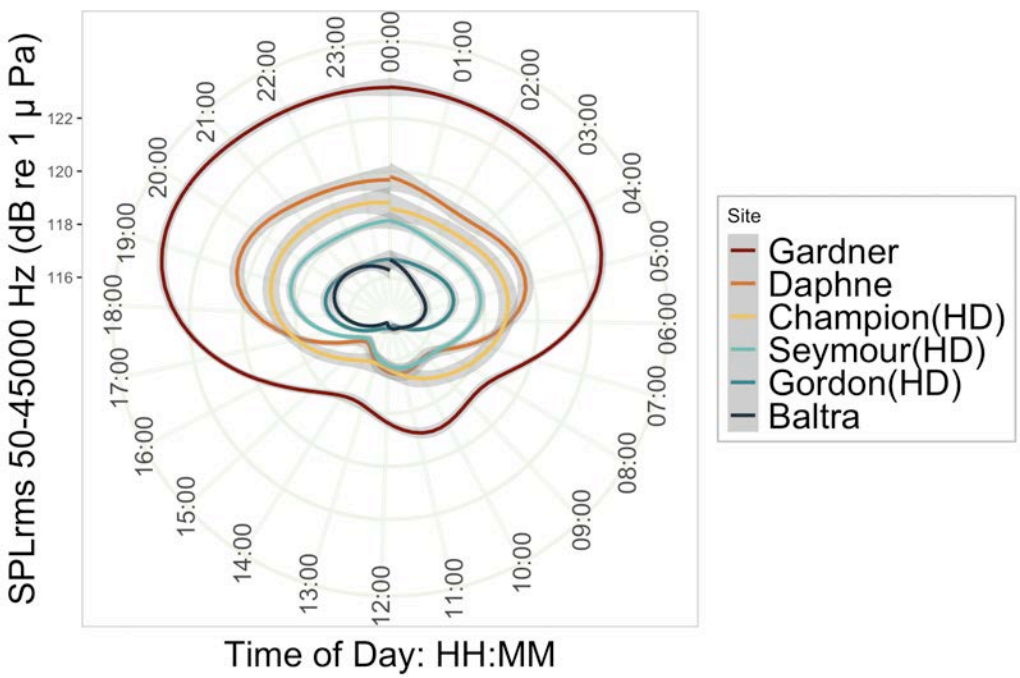

Figure 9: Use of polar plot as method to identify consistent site profiles.

<u>Season and additional factors:</u>

Season does not reveal obvious changes in biologic sound production. By splitting sites into warm and cool periods, median sound pressure levels still appear more closely clustered by site or time of day than by season. Season was defined as the warm season in January and cool season in July through October.

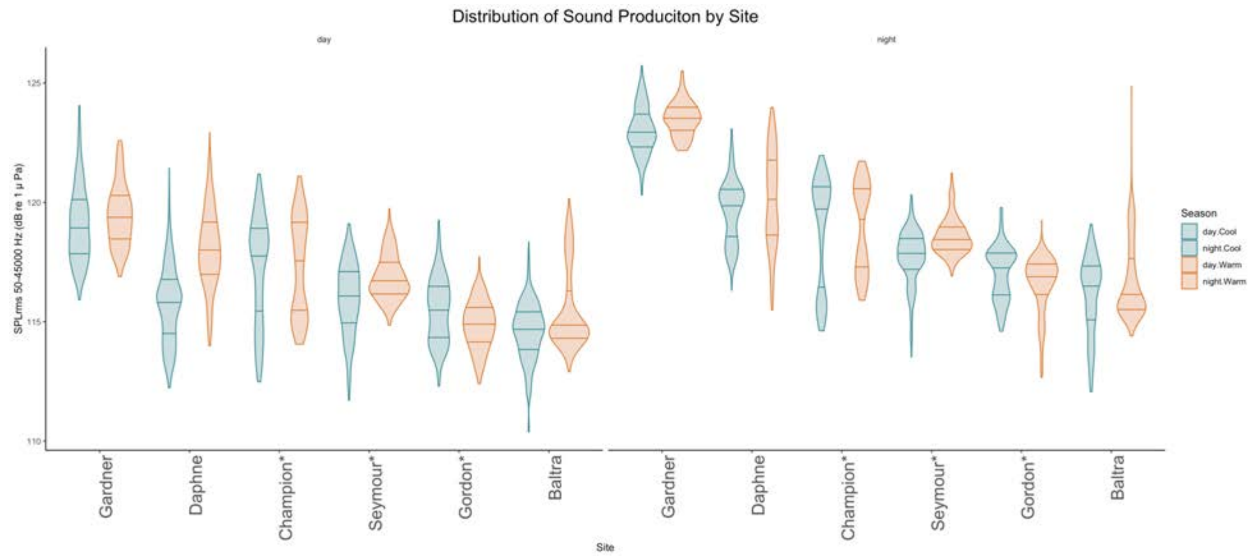

Figure 10: Dividing SPLs by season shows little change in distributions

<u>Assessing anthropause as driver of long-term biological response</u>

Overall, time series trends do not demonstrate obvious biologic recovery effects using the 15-minute bins at the broad band frequency. There is an approximately 3 dB re 1 μPa2 Hz−1 change in the median sound pressure level for each period. Biologic sound production was lowest in January 2021, and highest in January 2022. Cool season data also shows an increase in biologic sound production with the lowest occurring in October 2020 and the highest occurring in August 2022. These trends are consistent when the record is SPLit into day and night data, with all night SPLs above day SPLs by period. However, separating by site demonstrates differences in trends through the study period. Two out of three high activity sites demonstrated an increase in biological sound pressure through the sampling, while one showed a decrease in biologic sound production. Low-activity sites generally showed less extreme and inconsistent trends. Using these 15-minute windows to construct sound pressure levels for each deployment and then separating the COVID-19 record by relevant nested factors demonstrates that changes in sound pressure level may be highly localized. No clear trends emerge for discrete disturbance events over 15-minute windows.

Site-wise analysis demonstrates potential local scale effects. Baltra shows a general increase in disturbance level events over the sampling period with the highest number of disturbances correlating with the highest levels of biologic sound production. Daphne demonstrates the opposite effect in which the highest level of disturbances occurred in the same period as the lowest biologic sound production, and the least disturbed periods occurred when biologic sound production was highest. Gardner demonstrates almost no response to disturbance levels, despite having one of the highest disturbed periods overall and the loudest biological SPLs. This could indicate that Gardner is a robust site and is particularly insensitive to single disturbance events. Of the disturbed sites, Gordon shows a consistent increase in biologic sound pressure level with the increase of disturbance events. Champion similarly shows the most extreme increase in disturbance events throughout the five sampling periods. This is interesting as Champion has the clearest increase in biologic sound pressure levels. Finally, Seymour shows the highest variation in daytime disturbance frequency, and therefore it is interesting that it has a relatively consistent biological sound pressure level.

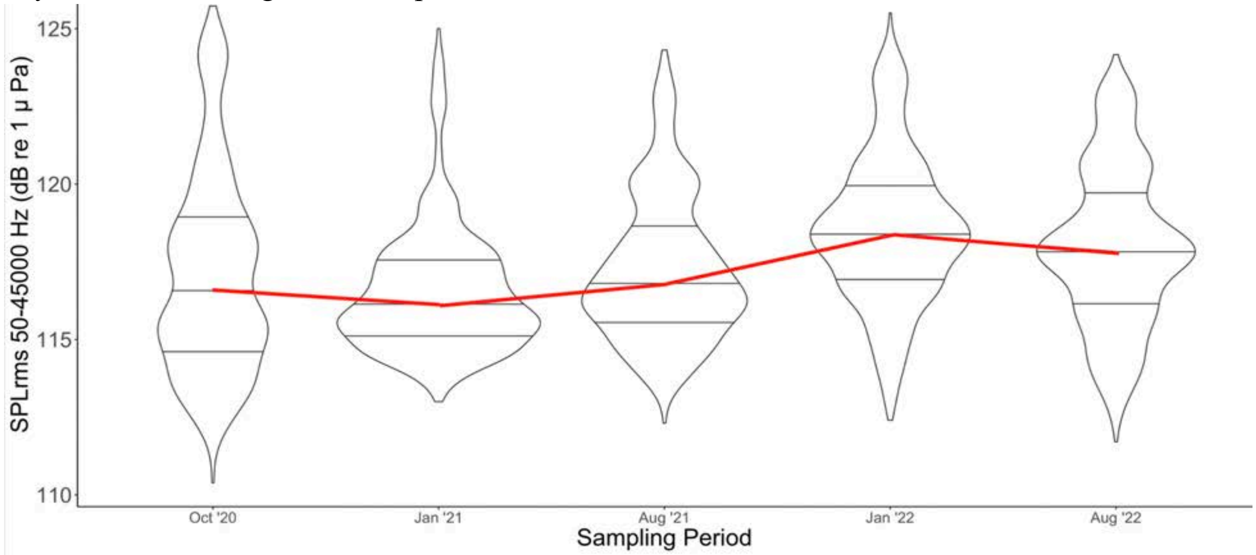

Figure 12: Overall trend in median sound pressure levels through the sampling effort.

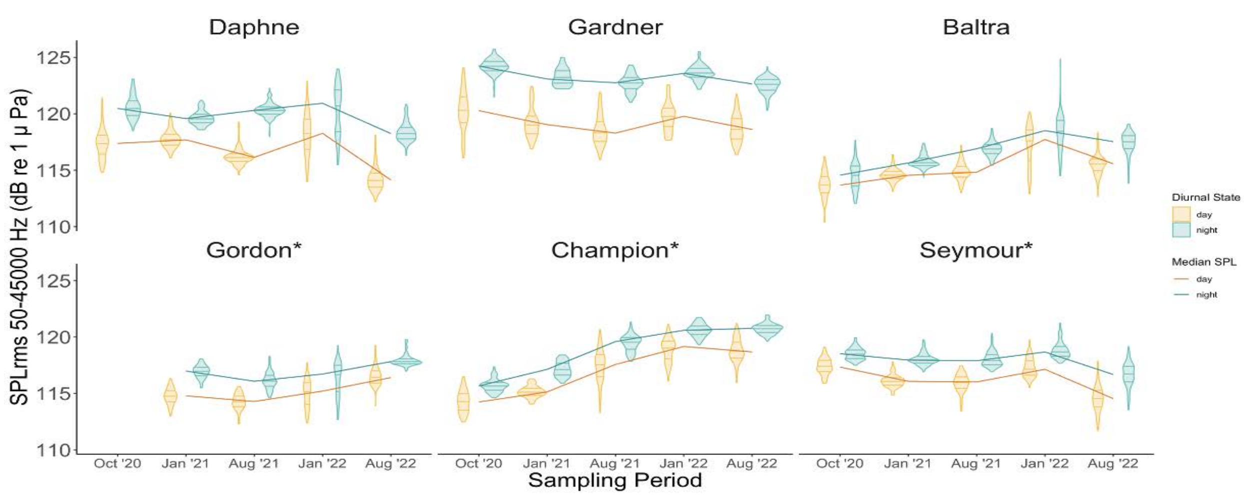

Figure 13: Changing sound pressure level separated by overnight and site. Columns represent paired deployments. Top row is low activity sites, bottom row is high activity sites.

<u>Assessing the immediate effect of disturbance events using 15-minute windows</u>

The relationship of SPL 30 minutes and 15-minutes before a disturbance event are tightly correlated, with an R-squared of 0.9588, indicating that under standard conditions, each SPL is clearly not independent of the next one. This makes sense as the time step before should influence the time step after regardless of disturbance state. If anthropogenic disturbance caused either a dampening or compensation effect in the biologic soundscape, the slope plotting SPL of the 15-minutes before the disturbance and 15-minutes after a disturbance event should have a slope of non-one. The slope of this relationship is 0.92 with an R-squared of 0.826. This shows a slight biologic dampening effect but is unlikely to be significant. A recovery effect on the order of 45 minutes was assessed by looking at the two bins directly after an identified disturbance event. Differences between the 15- and 45-minute post disturbance bins have an R-squared of approximately 0.9, demonstrating that there is unlikely to be a disturbance effect that impacts the biologic soundscape on the order of around an hour. Additionally, it appears that the size of the disturbance may not have a detectable impact on the change in biological sound production before and after a disturbance using these 15-minute bins. The relationship between median SPL during disturbance periods and the change in biologic sound production before and after an event does not demonstrate a clear trend.

<u>Constructing a final hierarchy</u>

GLMMs suggest that while period may be relevant, it is not a primary driver for changes in biological sound production. Six pairwise GLMMs were constructed to ascertain which factors had the clearest signaling. Diel state was the only factor to converge to a model for all three combinations. Site was able to converge with one (diel) factor. The two-factor GLMM with the lowest AIC used diel and site, excluding season and period since COVID-19. Assessing a tri-factor GLMM, all models converged except for the one excluding diel state, and the best fitting tri-factor model excluded season, but was not significantly different from the tri factor model excluding period since COVID-19. A four-factor model did not converge. Assessing the individual elements of the tri-factor model excluding season, identifies strong significance for three out of the five included sites. Diel state is significant, and no period emerges as significant. Using the findings from the pairwise model AICs, tri factor model AICs, and P-values of the best fitting tri-factor model we can begin to approximate a hierarchy of effect. In order of significance, we can suggest that for the Galápagos region, diel, site, period, and finally season are the order of importance these factors play in influencing biological sound production.

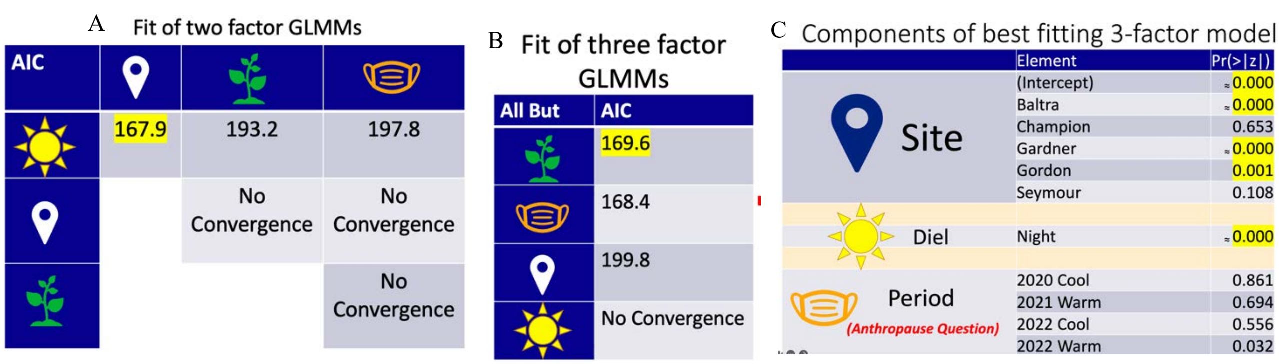

Figure 14: Assessing the fit of various two (a) and three (b) factor GLMMs. Summary of components for the best fitting tri factor GLMM (c).

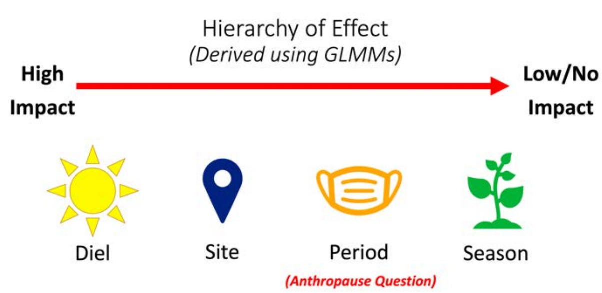

Figure 15: Ultimate hierarchy of effect for GMR region.

Discussion

Interpreting the GLMM hierarchy

Use of GLMM modeling is a novel application to consider factors that are consistently analyzed separately or qualitatively in acoustic ecology (Archer et al., 2018; Gabriele et al., 2018; Mooney et al., 2020; Pine et al., 2021; Weng et al., 2023). This model has not been applied to SPLs in the active literature. However, it is appropriate as the distribution of data is non normal and includes nested factors that are clustered by deployment. SPLs can be thought of as a pseudo count allowing for Poisson family modeling. Assessing the fits of various models, moves towards the capacity to weigh the relative importance of sound production influences. This novel methodology is especially useful as a challenge of this emerging discipline is identifying cross region trends. Soundscapes tend to be highly localized in both the types of sound production and complexity of influencing factors. Therefore, creating a new model that does not require sub sampling to assess biologic sound production by factor could expand the discipline beyond regional assessments.

The specific outcome of this model suggests that diel trends show the greatest influence in sound production levels. This is likely due to the strong changes in snapping shrimp levels in the mid-frequency band. GLMMs conducted on low frequency models will likely confirm this as diel trends become less critical factors. For future publications, data will be randomly subset into a model selection group and a model evaluation group, to avoid P-hacking. It is also relevant to consider that these models consider the period since COVID-19 as categorical rather than a time series, improving this model to instead consider the period since COVID-19 as a numerical value could more appropriately assess the time series element, especially as this project continues to grow and include more periods. The addition of diel and site factors consistently improved the fit of both two factor and tri-factor models, whereas the period since COVID-19 generally did not improve models. However, period did not significantly worsen models as the addition of season did. This indicates that as categorized, period since COVID-19 may introduce some explanatory power, though it is unlikely to be the strong biological response due to the increase of visitors through the sampling effort as hypothesized.

Given the factor most relevant to our goals of anthropogenic disturbance was the post anthropause effect, results of this model can be used to assess how best to separate and analyze trends in biological sound production to isolate this research question. As site and diel trends are relevant to biological sound production, separating SPLs by both diel and site and then assessing overall trends places the research question in the context of relevant regional factors. While existing studies often subset sound pressure levels by known influencing factors, this provides a unique and universal methodology that is grounded in statistical analysis.

Interpreting SPLs before and after disturbance events

Comparing neighbors before disturbance events reveals a slope of 0.983 with an R-squared of 0.96. This indicates that on the 15-minute bins, biological sound production is highly correlated to its neighboring bins. This makes sense as individuals present in one bin are likely to be present in the next, as well as having consistent influence factors. This reinforces that traditional statistical tests such as ANOVA are inappropriate for this type of data unless significant random sub-sampling is done.

Comparison of neighboring bins before, during, and after disturbances reveals information on both the size of disturbance level effects, as well as demonstrating the need for statistical modeling that considers highly correlated data. Evidence of a medium-term damping or compensation effect would be seen in changes in the biological soundscape between the bin directly before and directly after a disturbance. 15-minute bins that were processed for overall characterization were used, as disturbances were feasible to be identified by individuals by hand. Changes in the linear regression between before and after a disturbance event would demonstrate if there was biological dampening due to the disturbance event activity (slope>1), or compensation effect, in which individuals produce sound at higher volumes to be heard over disturbances (slope<1). Given that the linear regression in SPLs for the 15-minute bins before and after a disturbance did not significantly differ from one, it seems unlikely that discrete disturbance events are impacting the biologic soundscape beyond immediate flight responses. This makes sense when considered alongside long-term video monitoring analyzing disturbance responses. For discrete events, disturbance responses operate on the order of seconds rather than minutes to hours (Ward-Diorio, unpublished). This establishes disturbance level analysis must occur on a much finer scale than current methodologies.

<u>Assessing the long-term anthropause question</u>

To assess if there are long-term impacts because of anthropogenic activity, first disturbance level trends must be confirmed to change with the increase in visitors throughout the sampling effort, then, changes in biologics sound pressure level can be compared to the increase in disturbance events. While there was a clear increase in the frequency of daytime disturbance events through the sampling, the scale and type of disturbance levels remain low. There was a two-fold increase in disturbance events, which likely reflects an increase in scuba diving tourist activity, captured by the acoustic record. This overall trend is not seen consistently across all six sites. Individual assessment of site level disturbance and sound pressure level trends through the sampling effort does not reveal consistent trends between the two. Disturbance levels at high activity sites generally seem similar to low diver sites throughout the sampling effort. Low activity sites were expected to have relatively flat disturbance levels.

Biologic overnight sound pressure levels in January 2021 and August 2022 are very similar yet have a two-fold difference in daytime disturbance frequency. Low disturbance sites overall show slight correlations between number of disturbance events and biological SPLs. In contrast, high disturbance sites either show an increase in sound pressure level with disturbance events, or no response to disturbance events. Overall, these patterns demonstrate highly localized, relatively small-scale effects in which there may be simultaneous biologic compensation, dampening, and non-responsive effects. It is particularly interesting to note that for two non-simultaneously deployed sites that showed significant variation in daytime disturbance frequencies, there appeared no biological response. This is particularly interesting as one site was selected for being high disturbance and one was selected for being low disturbance. The site with the clearest increase in disturbance levels, did show an increase in biological sound production, demonstrating the possibility of biologic compensation on a localized scale. Overall, this indicates that disturbance events may be impacting site level biologic sound production, though trends are incredibly inconsistent. This may be a result of differing sound producers having different responses, as different elements of the biological soundscape are known to respond differently to disturbance events. Species composition varies by site, and this could be an effect of these differences. By working to record the number of species-specific vocalizations, this project can assess whether there are different species level responses to long-term changes in disturbance level.

Diel trends

Diel trends emerge as the strongest factor influencing the soundscape, exemplifying the capacity of the biological soundscape to have strong responses in the acoustic record. This also confirms reports from other regions that use the strength of diel trends to verify that the majority of biologic activity is occurring overnight (Bertucci et al., 2016). Given the clear, consistent biological response due to diel changes, this serves as an example for clear signaling, implying that other factors such as periods since COVID-19 are less important drivers of biological sound production. A reason for this clear trend is that snapping shrimp are incredibly sensitive to diel shifts (Dias et al., 2021). As one of the primary sound producers across all six sites in a very wide frequency band, this makes sense that they would have a high influence on biological SPLs on the broadband. As future studies attempt to isolate specifically soniferous fish and megafauna, analysis isolated for the low frequency band will become necessary. Preliminary inspection of video data provides observational support for the strong diel shifts, with an increase in individuals in the frame near sunset (Witman, personal observation).

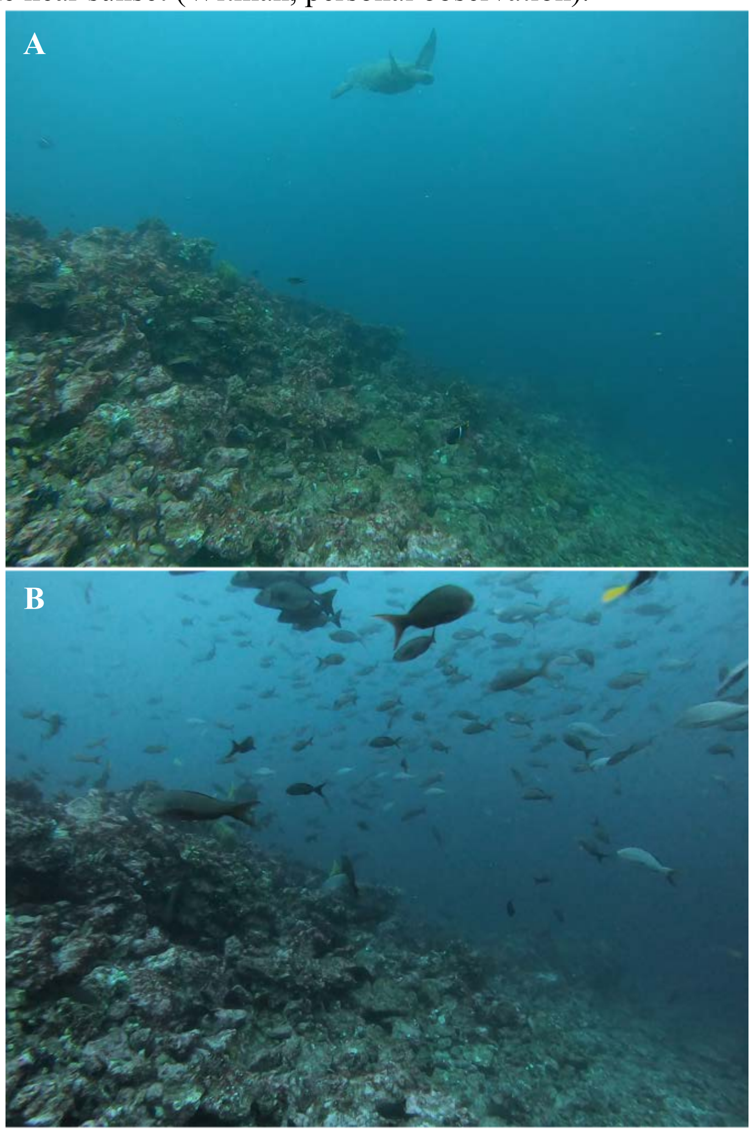

Figure 16: Difference in activity level from midday (a) to 30 minutes before sunset (b) at the site with the strongest diel trend

<u>Site trends</u>

Just as species composition can be highly localized, the spectrogram profiles demonstrate a high level of localized specificity. This indicates that sites have their own clear acoustic identity, and further builds on the hypothesis that long-term acoustic responses may be on a highly localized level. Specifically considering the data set that excludes major disturbance events, possible reasons for this include differences in abiotic and biotic factors affecting the site. A major example is the topography of different sites. Sites such as Gordon crater are bowl shaped and would be expected to reflect sound more effectively into the hydrophone. Alternatively, sites that were ledges and walls would be expected to have less acoustic reflection and generally lower biologic sound production for every equivalent unit of biological activity. While topographic differences remain constant within sites, other abiotic effects may fluctuate over periods. For example, temperature and currents are known to affect both the acoustic records as well as impacting the capacity of the hydrophone to capture biological sound production. This is the sort of change that could change on the order of seasons, years, or days. While it's challenging to assess exactly which additional abiotic drivers may be influencing the different sites, by looking at the relative changes in biological sound production for each of the sites across the five time periods, it's feasible to extract how large these time series sound pressure level shifts are in the context of how distinct each of the sites are.

Given that the interquartile range of the loudest site sits significantly above the interquartile range of several other sites for every single period demonstrates that site characteristics are relatively consistent regardless of disturbance level. This is yet another line of reasoning to conclude that disturbance activity may simply not be intensive enough to cause long-term changes to the acoustic record, as the hypothesis would have predicted that the relative biological sound production between sites change as disturbance increased non-uniformly.

These clear identities offer an opportunity for further exploration, both by exclusively analyzing the acoustic records as well as using differences in species composition from daytime video data to assess if changes in fish species composition are driving these site differences. This additionally indicates that biological vocalizations and behaviors are able to significantly alter the long-term record. While this initial study sought to characterize the entire 1.8 TB acoustic record, using our understanding of site variation could allow for experimental design that assessed soniferous fish calls or disturbances across the sites at a single period. This could be a way to scale sampling efforts down to a feasible processing capacity.

<u>Season</u>

Seasonal trends indicate varying responses across sites, with relatively small differences between the warm and cool season. This indicates that the two-season model is not demonstrating large patterns of effect. As season did not appear significant in GLMMs it is unreasonable to bisect the soundscape between warm and cool periods. This makes sense as seasonal trends may be unrelated to marine communities in which other factors such as El Nino and upwelling strength are more important drivers of changes to biological activity. This also indicates that if there are spawning events, which are known to be detectable on acoustic records, they are not captured in the warm/cool conceptualization. This factor appears less consequential than the COVID-19 period question, demonstrating almost no explanatory power. In fact, the majority of models that included season as a potential factor did not converge, indicating that season is an unhelpful indicator of biological sound production. This lack of strong biannual differences is a relevant finding as seasonal trends are known to exist in other acoustic communities (Higgs & Humphrey, 2020; Merkens et al., 2021). Seasonal trends have been known to exist especially in the context of mating cycles, but can depend on the seasonal cycles and changes for different regions. It is relevant to note that most sampling periods were during January and August, with one sampling period during October. This could cause the lack of strong trends emerging due to the classification of warm and cool seasonal binary.

Other factors of influence

Multiple factors remain to be explored and connected as potential drivers of sound production in the GMR. These include water temperature, which is known to alter both sound production and the detectability of acoustic signals. Additionally lunar cycles are known to play a significant role in both spawning events as well as fish coursing changes. Finally, upwelling events that alter resource availability as well as water temperature may be important drivers of activity that are captured in the acoustic record.

Long-term anthropause recovery effect

While lack of long-term consistent changes in the biological sound production for either low or high disturbance sites points towards a lack of effect from the return of tourists to the GMR, there still may be local level effects for each of the sites. Recalling that site and diel trends are relevant factors of biological sound production, analysis of the COVID-19 trend must be done for data that is first separated by diel trend and site. Including the period since the COVID-19 pandemic as a categorical variable does not decrease the AIC's of GLMMs. However, it does not significantly improve them. We would expect the fit of the GLMM model including only high impact sites to show more extreme improvement than when incorporated into the full site model.

For two of the high disturbance sites, there is a clear increase in biological sound production. This is a direct counter to the hypothesis, in which a biological dampening effect due to anthropogenic activity was expected as disturbances would cause a decrease in vocalization and biological sound production. This alternative pattern supports a biological compensation hypothesis, in which biological sound production occurs at higher power in order to be heard over disturbances. This might be evidence for a slight compensation effect in which an increase in disturbance events is causing biologic communities to vocalize at higher rates in order to be heard over disturbances. This could also indicate an increase in biological sound production during disturbance events due to flight responses. Both represent possible explanations of responses to acoustic masking, in which vocalizations are covered up by conspecifics or anthropogenic disturbances operating in the same frequency band (Gabriele et al., 2018). An unresolved issue is whether an increase in acoustic sound production exclusively demonstrates ecosystem health or can also indicate increased levels of stress (Kok et al., 2021).

Confirming non-independence

Assessing the interdependence of SPLs for 15-minute bins over a period that included a disturbance permitted coarse grain analysis of disturbance effects on the order of minutes to hours. By demonstrating that the correlation between windows before a disturbance event is nearly one, and that this correlation remains consistent even with a disturbance event between, indicates that if there are disturbance level effects they are not measurable using the 15-minute window size. This indicates a call to move to disturbance level modeling, as well as further verifying that responses are generally too small to be measurable using current methodology.

Galápagos as an acoustic refuge

Lack of strong biological sound production responses may indicate that under standard conditions, anthropogenic activity is insufficient to drive changes in the marine biological soundscape, offering the potential for the Galápagos marine reserve as an acoustic refuge. Existing studies that have measured major changes in the biological soundscape as a response to changes in anthropogenic activity either spatially or due to the COVID-19 pandemic may be operating on a far different scale of disturbance than the GMR region (Archer et al., 2018; Kaplan et al., 2018; Pine et al., 2021; Weng et al., 2023). The smaller and more varied responses may be indicative of the increased health of the GMR. For example, a strong COVID-19 effect was found in a site that saw thousands of visitors per day. This is a stark contrast to this experiment's high disturbance sites which see just a few divers in a day.

Placing findings into the context of the broader acoustic disturbance discussion allows for two conclusions about the GMR soundscape. The first is that this may be a relatively intact soundscape that is not highly impacted by anthropogenic activity overall. Therefore, even though two times change in activity level was detected, even at capacity this may still be orders of magnitude below the threshold necessary to cause long-term damage. This offers the possibility of the GMR soundscape as an acoustic refuge, indicating that it is uniquely intact compared to other measured regions.

This understanding of the GMR as a low impact region indicates that responses to disturbance events are not routine or intensive enough to cause long-term changes to the biological soundscape, but that there may be short-term stress responses to disturbance events. This necessitates moving to a far more detailed disturbance level analysis, requiring methodology that includes isolating exactly when disturbance events such as boat motors and diver breathing occurs in the soundscape, and computing fish vocalizations before and after these events. These responses in an ecosystem that has under-saturated anthropogenic effects may offer unique insights into short- and long-term stress responses for communities.

Conclusion

This senior thesis produced its goal of effective, appropriate workflows for the first long-term passive acoustic monitoring effort in the GMR. Through data analysis the need and usage of preliminary novel modeling applications to understand contextual effects of biological sound production levels in marine soundscapes is demonstrated. Applying the workflow to a sampling effort that includes significant differences in anthropogenic disturbance allows preliminary analysis on whether there are long-term effects of human disturbance on biological soundscapes in the region. While changes in disturbance events does not seem to be a major driver of broadband biological sound production, especially in the context of other more relevant drivers of biological sound production, there may be localized effects and short-term disturbance effects not captured by the large bin size used for long-term analysis. This introduces the potential for the GMR as an acoustic refuge, in which anthropogenic activity does not operate at a significant enough level to have a major impact on the biological marine soundscape.

Evaluating this new hypothesis will require future analysis of both acoustic and video long-term records. Excluding disturbance events and running GLMM models for low frequency sound pressure levels that have already been created will eliminate one of the most prolific and obvious drivers of acoustic variation, the snapping shrimp. Additionally, incorporating factors that are known to be relevant to the region will improve the modeling capacity for the GMR. Potential new factors include temperature, lunar phase, upwelling state, and El Niño phase. Additionally, running this existing methodology with 30 second bins may offer higher resolution information and demonstrate a clearer overall trend.

Acoustic deployments were purposefully in tandem with long-term video monitoring efforts to eventually establish how acoustic trends are correlated to visual data, and then expand to determine changes in species assemblages. Video information verification is especially common for establishing new long-term acoustic monitoring efforts (Gibb et al., 2019; Kaplan et al., 2018). Use of species richness indices, fish surveys, and biodiversity could all be useful to verify that acoustic outputs consistently correlate to biodiversity. Additionally, this type of information could be used to verify the validity of acoustic indices in our region.

This preliminary study calls for more exacting isolation of acoustic signals. Specifically, isolating fish vocalizations and discrete disturbance events to the regulator and boat cavitation level is a necessary next step. This region of the Pacific has almost no known acoustic species-specific libraries, and by identifying various vocalizations and determining the species of origin efforts can begin to create the first. In doing this, these isolated signals can be used as training and validation sets for machine learning processes. These machine learning processes will be vital in ensuring that sub-sampling is not a necessary component of signal specific analysis.

This project and data set has implications beyond the GMR. A long-term goal of this project may include submitting data to NOAA passive acoustic data set, which builds universal libraries and coordinates efforts to measure anthropogenic impact and global changes to marine soundscapes (*SanctSound | Sound Monitoring | Office of National Marine Sanctuaries*, n.d.).

With both methodological and ecological goals, this project has sought to conduct preliminary analysis to assess the overall impact that human disturbance may have on the GMR. This research question necessitated intensive methodological workflows as well as applications of novel data analysis for the acoustic discipline. Ultimately this offered preliminary characterization of the soundscape, demonstrating the site level specificity of responses to increased disturbance events. Further research can explore the potential for the GMR as an acoustic refuge in which the soundscape offers a unique and rich characterization of a relatively undisturbed ecosystem.

Acknowledgments

I'm incredibly grateful to my advisor Jon Witman, who trusted me to take on this project and then mentored me through the ups and downs of the past year and a half. I am truly an example of your dedication to ensuring students experience the full scientific cycle including experimental design, data collection at 15 meters underwater, late nights in the lab, and presenting findings to our scientific community. My additional advisors Robert Lamb and Franzino Smith ensured that this project continued to move forward in data collection and analysis. Thank you to my second reader Dov Sax. The Voss Fellowship at IBES ensured I was able to join the Witman lab sampling efforts in August 2022 as well as providing critical mentorship while I wrote my thesis. These results would have been impossible without the hard work of Witman lab members willing to take on major data processing. Special thanks to Rebecca Ward-Diorio for her camaraderie and organization in the Galápagos and here in Providence. Finally thank you to Gemma Brand-Wolf, Galen Windsor, Hannah LeBaron and Maxine Schulte for your copy edits and support.

Works Cited:

Archer, S. K., Halliday, W. D., Riera, A., Mouy, X., Pine, M. K., Chu, J. W., Dunham, A., & Juanes, F. (2018). First description of a glass sponge reef soundscape reveals fish calls and elevated sound pressure levels. *Marine Ecology Progress Series*, *595*, 245–252.

Balantic, C., & Donovan, T. (2019). Dynamic wildlife occupancy models using automated acoustic monitoring data. *Ecological Applications*, *29*(3), e01854.

Bertucci, F., Parmentier, E., Lecellier, G., Hawkins, A. D., & Lecchini, D. (2016). Acoustic indices provide information on the status of coral reefs: An example from Moorea Island in the South Pacific. *Scientific Reports*, *6*(1), 1–9.

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication* (2nd ed). Sinauer Associates. http://books.google.com/books?isbn=9780878930456

Bruce Martin, S., Gaudet, B. J., Klinck, H., Dugan, P. J., Miksis-Olds, J. L., Mellinger, D. K., Mann, D. A., Boebel, O., Wilson, C. C., & Ponirakis, D. W. (2021). Erratum: Hybrid millidecade spectra: A practical format for exchange of long-term ambient sound data [JASA Express Lett. 1 (1), 011203 (2021)]. *JASA Express Letters*, *1*(8), 011203.

Castrejón, M., & Charles, A. (2020). Human and climatic drivers affect spatial fishing patterns in a multiple-use marine protected area: The Galápagos Marine Reserve. *PLOS ONE*, *15*(1), e0228094. https://doi.org/10.1371/journal.pone.0228094

Dias, F. F., Pedrini, H., & Minghim, R. (2021). Soundscape segregation based on visual analysis and discriminating features. *Ecological Informatics*, *61*, 101184. https://doi.org/10.1016/j.ecoinf.2020.101184

Fournet, M. EH., Stabenau, E., Madhusudhana, S., & Rice, A. N. (2022). Altered acoustic community structure indicates delayed recovery following ecosystem perturbations. *Estuarine, Coastal and Shelf Science*, *274*, 107948. https://doi.org/10.1016/j.ecss.2022.107948

Gabriele, C. M., Ponirakis, D. W., Clark, C. W., Womble, J. N., & Vanselow, P. B. S. (2018). Underwater Acoustic Ecology Metrics in an Alaska Marine Protected Area Reveal Marine Mammal Communication Masking and Management Alternatives. *Frontiers in Marine Science*, *5*. https://www.frontiersin.org/articles/10.3389/fmars.2018.00270

Gibb, R., Browning, E., Glover-Kapfer, P., & Jones, K. E. (2019). Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods in Ecology and Evolution*, *10*(2), 169–185. https://doi.org/10.1111/2041-210X.13101

Hearing Center of Excellence. (n.d.). Retrieved April 23, 2023, from https://hearing.health.mil

Higgs, D. M., & Humphrey, S. R. (2020). Passive acoustic monitoring shows no effect of anthropogenic noise on acoustic communication in the invasive round goby (Neogobius melanostomus). *Freshwater Biology*, *65*(1), 66–74. https://doi.org/10.1111/fwb.13392

Holt, M. M., Noren, D. P., Veirs, V., Emmons, C. K., & Veirs, S. (2009). Speaking up: Killer whales (Orcinus orca) increase their call amplitude in response to vessel noise. *The Journal of the Acoustical Society of America*, *125*(1), EL27–EL32. https://doi.org/10.1121/1.3040028

Huveneers, C., Jaine, F. R. A., Barnett, A., Butcher, P. A., Clarke, T. M., Currey-Randall, L. M., Dwyer, R. G., Ferreira, L. C., Gleiss, A. C., Hoenner, X., Ierodiaconou, D., Lédée, E. J. I., Meekan, M. G., Pederson, H., Rizzari, J. R., van Ruth, P. D., Semmens, J. M., Taylor, M. D., Udyawer, V., … Harcourt, R. (2021). The power of national acoustic tracking networks to assess the impacts of human activity on marine organisms during the COVID-19 pandemic. *Biological Conservation*, *256*, 108995. https://doi.org/10.1016/j.biocon.2021.108995

Kaplan, M. B., Lammers, M. O., Zang, E., & Aran Mooney, T. (2018). Acoustic and biological trends on coral reefs off Maui, Hawaii. *Coral Reefs*, *37*(1), 121–133.

Kaplan, M. B., Mooney, T. A., Partan, J., & Solow, A. R. (2015). Coral reef species assemblages are associated with ambient soundscapes. *Marine Ecology Progress Series*, *533*, 93–107.

Kok, A. C. M., van Hulten, D., Timmerman, K. H., Lankhorst, J., Visser, F., & Slabbekoorn, H. (2021). Interacting effects of short-term and long-term noise exposure on antipredator behaviour in sand gobies. *Animal Behaviour*, *172*, 93–102. https://doi.org/10.1016/j.anbehav.2020.12.001

Kunc, H. P., McLaughlin, K. E., & Schmidt, R. (2016). Aquatic noise pollution: Implications for individuals, populations, and ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1836), 20160839.

Lossent, J., Iorio, L. D., Valentini-Poirier, C. A., Boissery, P., & Gervaise, C. (2017). Mapping the diversity of spectral shapes discriminates between adjacent benthic biophonies. *Marine Ecology Progress Series*, *585*, 31–48. https://doi.org/10.3354/meps12370

McKenna, M. F., Baumann-Pickering, S., Kok, A., Oestreich, W. K., Adams, J. D., Barkowski, J., Fristrup, K. M., Goldbogen, J. A., Joseph, J., & Kim, E. B. (2021). *Advancing the interpretation of shallow water marine soundscapes*.

Merchant, N. D., Fristrup, K. M., Johnson, M. P., Tyack, P. L., Witt, M. J., Blondel, P., & Parks, S. E. (2015). Measuring acoustic habitats. *Methods in Ecology and Evolution*, *6*(3), 257–265. https://doi.org/10.1111/2041-210X.12330

Merkens, K., Baumann-Pickering, S., Ziegenhorn, M. A., Trickey, J. S., Allen, A. N., & Oleson, E. M. (2021). Characterizing the Long-Term, Wide-Band and Deep-Water Soundscape Off Hawai'i. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.752231

Mestanza-Ramón, C., Capa, M. S., Saavedra, H. F., & Paredes, J. R. (2019). Integrated Coastal Zone Management in Continental Ecuador and Galápagos Islands: Challenges and Opportunities in a Changing Tourism and Economic Context. *Sustainability*, *11*(22), Article 22. https://doi.org/10.3390/su11226386

Mooney, T. A., Di Iorio, L., Lammers, M., Lin, T.-H., Nedelec, S. L., Parsons, M., Radford, C., Urban, E., & Stanley, J. (2020). Listening forward: Approaching marine biodiversity assessments using acoustic methods. *Royal Society Open Science*, *7*(8), 201287.

Parsons, M. J., Lin, T.-H., Mooney, T. A., Erbe, C., Juanes, F., Lammers, M., Li, S., Linke, S., Looby, A., & Nedelec, S. L. (2022). Sounding the Call for a Global Library of Underwater Biological Sounds. *Frontiers in Ecology and Evolution*, 39.

Petrizzo, A., Barbanti, A., Barfucci, G., Bastianini, M., Biagiotti, I., Bosi, S., Centurelli, M., Chavanne, R., Codarin, A., Costantini, I., Cukrov Car, M., Dadić, V., Falcieri, F. M., Falkner, R., Farella, G., Felli, M., Ferrarin, C., Folegot, T., Gallou, R., … Madricardo, F. (2023). First assessment of underwater sound levels in the Northern Adriatic Sea at the basin scale. *Scientific Data*, *10*(1), Article 1. https://doi.org/10.1038/s41597-023-02033-1

Pine, M. K., Wilson, L., Jeffs, A. G., McWhinnie, L., Juanes, F., Scuderi, A., & Radford, C. A. (2021). A Gulf in lockdown: How an enforced ban on recreational vessels increased dolphin and fish communication ranges. *Global Change Biology*, *27*(19), 4839–4848. https://doi.org/10.1111/gcb.15798

Radford, C. A., Jeffs, A. G., Tindle, C. T., & Montgomery, J. C. (2008). Temporal patterns in ambient noise of biological origin from a shallow water temperate reef. *Oecologia*, *156*(4), 921–929.

Radford, C., Jeffs, A., Tindle, C., & Montgomery, J. C. (2008). Resonating sea urchin skeletons create coastal choruses. *Marine Ecology Progress Series*, *362*, 37–43.

SanctSound | Sound Monitoring | Office of National Marine Sanctuaries. (n.d.). Retrieved April 29, 2023, from https://sanctuaries.noaa.gov/science/monitoring/sound/

Scripps Whale Acoustics Laboratory | Technologies: Triton. (n.d.). Retrieved April 28, 2023, from https://www.cetus.ucsd.edu/technologies_triton.html

Shark Diving: The 12 best shark diving sites in the world. (n.d.). Retrieved April 28, 2023, from https://blog.padi.com/shark-diving-the-10-best-dive-sites-in-the-world/

Solsona-Berga, A., Frasier, K. E., Baumann-Pickering, S., Wiggins, S. M., & Hildebrand, J. A. (2020). DetEdit: A graphical user interface for annotating and editing events detected in long-term acoustic monitoring data. *PLOS Computational Biology*, *16*(1), e1007598. https://doi.org/10.1371/journal.pcbi.1007598

Stowell, D. (2022). Computational bioacoustics with deep learning: A review and roadmap. *PeerJ*, *10*, e13152.

Wall, C. C., Haver, S. M., Hatch, L. T., Miksis-Olds, J., Bochenek, R., Dziak, R. P., & Gedamke, J. (2021). The next wave of passive acoustic data management: How centralized access can enhance science. *Frontiers in Marine Science*, 873.

Weiss, S. G., Cholewiak, D., Frasier, K. E., Trickey, J. S., Baumann-Pickering, S., Hildebrand, J. A., & Van Parijs, S. M. (2021). Monitoring the acoustic ecology of the shelf break of Georges Bank, Northwestern Atlantic Ocean: New approaches to visualizing complex acoustic data. *Marine Policy*, *130*, 104570. https://doi.org/10.1016/j.marpol.2021.104570

Weng, K. C., Friedlander, A. M., Gajdzik, L., Goodell, W., & Sparks, R. T. (2023). Decreased tourism during the COVID-19 pandemic positively affects reef fish in a high use marine protected area. *Plos One*, *18*(4), e0283683.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., Hammond, P. S., Merchant, N. D., O'Hara, P. D., Purser, J., Radford, A. N., Simpson, S. D., Thomas, L., & Wale, M. A. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. *Ocean & Coastal Management*, *115*, 17–24. https://doi.org/10.1016/j.ocecoaman.2015.05.021

Witman, J. D., Brandt, M., & Smith, F. (2010). Coupling between subtidal prey and consumers along a mesoscale upwelling gradient in the Galápagos Islands. *Ecological Monographs*, *80*(1), 153–177.

Ziegenhorn, M. A. (2022). *Some Like It Cool: A Study of Odontocete Ecology in the Hawaiian Islands Using Passive Acoustic Monitoring* [Ph.D., University of California, San Diego]. https://www.proquest.com/docview/2713949076/abstract/C94BAF6F0DAB4DB0PQ/1

Ziegenhorn, M. A., Frasier, K. E., Hildebrand, J. A., Oleson, E. M., Baird, R. W., Wiggins, S. M., & Baumann-Pickering, S. (2022). Discriminating and classifying odontocete echolocation clicks in the Hawaiian Islands using machine learning methods. *PLOS ONE*, *17*(4), e0266424. https://doi.org/10.1371/journal.pone.0266424

Znidersic, E., & Watson, D. M. (2022). Acoustic restoration: Using soundscapes to benchmark and fast-track recovery of ecological communities. *Ecology Letters*, *25*(7), 1597–1603. https://doi.org/10.1111/ele.14015