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[J. E. Brosseau](javascript:;) ⁽²); [V. M. Eddington](javascript:;) ⁽²)[;](https://orcid.org/0000-0002-0768-9555) [E. C. Craig;](javascript:;) [E. R. White](javascript:;) ⁽²); [L. N. Kloepper](javascript:;) ⁽²⁾

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J. E. Brosseau,¹ D V. M. Eddington,^{1,2,a)} D E. C. Craig,^{1,3} E. R. White,¹ D and L. N. Kloepper^{1,2} D ¹ Departme[nt](https://orcid.org/0009-0008-0758-9440) [o](https://orcid.org/0009-0008-0758-9440)f Biological Sciences, Univ[ersit](https://orcid.org/0000-0001-9902-3867)y of N[ew](https://orcid.org/0000-0002-0768-9555) [H](https://orcid.org/0000-0002-0768-9555)ampshire, Durham, New Hampshire 03824, USA
²Center for Acoustics Research and Education, University of New Hampshire, Durham, New Hampshire 03824 2 Center for Acoustics Research and Education, University of New Hampshire, Durham, New Hampshire 03824, USA 3 Shoals Marine Laboratory, joint program of Cornell University, Ithaca, New York 14850 and University of New Hampshire, Durham, New Hampshire 03824, USA

[joe.brosseau@unh.edu,](mailto:joe.brosseau@unh.edu) [valerie.eddington@unh.edu,](mailto:valerie.eddington@unh.edu) elizabeth.craig@unh.edu, [easton.white@unh.edu,](mailto:easton.white@unh.edu) laura.kloepper@unh.edu

Abstract: In this study, passive acoustic monitoring was used to assess the impact of investigator disturbance on the acoustic behavior of a colony of common terns. A graded antipredator response in the colony was hypothesized, which would result in an increase in acoustic energy with increased proximity to investigator disturbance. Human disturbance was found to result in a significant increase in acoustic energy within 20 meters of recorders, though not from farther distances. Our findings provide a framework for assessing the behavioral impact of disturbance on colonies and support the existence of a graded alarm call system in common terns. © 2024 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license [\(https://creativecommons.org/licenses/by/4.0/\)](https://creativecommons.org/licenses/by/4.0/).

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1. Introduction

Many animals produce antipredator or alarm vocalizations when faced with predator encounters or other stressful situa-tions, and some species adjust these vocalizations in response to perceived threat level ([Eddington](#page-6-0) et al., 2024; [Farrow](#page-6-0) et al.[, 2017;](#page-6-0) [Griesser, 2008](#page-6-0)). Graded alarm call systems are antipredator responses that use functionally referential calls, which are calls that vary depending on the type of external stimulus. These calls are used to communicate information regarding context and urgency of external threats, which can then be used by receivers to appropriately respond [\(Macedonia and Evans, 1993](#page-6-0)). Functionally referential calls used in graded alarm systems can vary by either call type, calling rate, or both [\(Seyfarth](#page-6-0) et al., 1980; [Townsend](#page-6-0) et al., 2012). Detection of graded alarm calling behavior within a population provides insight into predation and defense behaviors and can be important for informing conservation and management decisions. Observations of alarm calls through passive recordings can provide behavioral information to researchers without the need for in-person visual observation, including documenting human or predator disturbance or identifying potential threats to a population.

One class of animals that lends itself to acoustic monitoring is that of avian species. Graded alarm call systems have been documented in multiple passerine species, including Siberian jays (Perisoreus infaustus) and Japanese great tits (Parus minor), which adjust antipredator calls in response to factors like distance and type of predator [\(Griesser, 2008;](#page-6-0) [Suzuki, 2015\)](#page-6-0). The black-capped chickadee (Poecile atricapillus) has well described graded alarm calls, with call rate and syllable patterns closely correlated with the distance and urgency of a threat. ([Baker and Becker, 2002](#page-6-0)). While alarm calling behavior of non-passerine species has been understudied compared to passerine species, the herring gull (Larus argentatus) also varies the pitch and rate of calls in response to different levels of simulated threats (Shah et al.[, 2015](#page-6-0)). Although this suggests non-passerine and passerine birds may have similar alarm calling strategies, alarm call systems can be highly species specific, with even closely related species exhibiting significantly different antipredator responses to similar stimuli [\(MacLean and Bonter, 2013](#page-6-0)).

The common tern (Sterna hirundo) is a migratory species of colonial seabird that migrates from their wintering grounds in South America to their summer breeding grounds in North America ([Arnold](#page-6-0) et al., 2020). During the breeding season, they form large colonies reaching thousands of individuals [\(Burger and Gochfeld, 1988](#page-6-0)). Daily activity within the colonies can vary, with feeding and foraging occurring on daily and seasonal cycles [\(Arnold](#page-6-0) et al., 2020). Breeding colonies of terns are characterized by loud, overlapping vocalizations used for both intra- and inter-specific communication [\(Veen,](#page-6-0) [1987](#page-6-0)). Qualitatively, individuals make long calls to communicate information related to nesting or breeding with conspecifics across the colony ([Veen, 1987](#page-6-0)). Terns also produce calls described as attack and alarm calls, which are both

a) Author to whom correspondence should be addressed.

associated with swooping attacks and defensive circling, and often present during periods of human disturbance within the colony [\(Veen, 1987\)](#page-6-0). Given the similarity of these calls and behavior with other bird species, it is possible that terns also use a graded alarm calling system, in which acoustic characteristics such as colony-wide call amplitude or frequency could provide insight regarding potential predator threats or disturbance. Both measurements have been found to vary in the alarm calls of other species ([Baker and Becker, 2002;](#page-6-0) Shah et al.[, 2015](#page-6-0)), and could provide insight into the type of response demonstrated by the common tern. Given the use of passive acoustics for monitoring responses to anthropogenic distur-bance [\(Buxton](#page-6-0) et al., 2018), understanding the alarm calling behavior of focal species is important when considering employing passive acoustic monitoring (PAM) to study that species. By better understanding the common tern's graded alarm call system, we can help fill a gap in our knowledge of this locally threatened seabird.

Traditional methods for surveying these seabirds primarily include ground counts of nests, which inherently introduce investigator disturbance to colonies. This disturbance can adversely impact the results of ecological surveys, lower reproductive success, and in extreme scenarios, result in nest abandonment and intraspecific aggression ([Carey,](#page-6-0) [2009](#page-6-0); [Carney and Sydeman, 1999](#page-6-0)). Furthermore, visual methods are often prone to human bias and can be both time-and resource-intensive [\(P](#page-6-0)érez-Granados and Traba, 2021). Compared to traditional monitoring, PAM can reduce cost and investigator disturbance ([P](#page-6-0)é[rez-Granados and Traba, 2021;](#page-6-0) [Shonfield and Bayne, 2017](#page-6-0); Sugai et al.[, 2019](#page-6-0)), providing an alternative for monitoring colonial seabirds.

Motivated by our desire to understand how terns vocally respond to threats and to best inform colony monitoring strategies, we aimed to quantify the colony-level response to human disturbance at different distances, representing potentially different threat levels. Using PAM to monitor dense aggregations of animals with overlapping vocalizations has historically presented considerable challenges given that typical approaches require isolation of individual calls to deter-mine abundance, occupancy, and to answer behavioral questions [\(Buxton](#page-6-0) et al., 2018; Leach et al.[, 2016;](#page-6-0) [P](#page-6-0)érez-Granados [and Traba, 2021\)](#page-6-0). Recent development of acoustic indices ([Bradfer-Lawrence](#page-6-0) et al., 2023), including the use of acoustic energy integration for single-species aggregations ([Kloepper](#page-6-0) et al., 2016), can overcome some of these limitations. Building on these methods, we hypothesize that if the common tern demonstrates a graded alarm call system, we will detect colony-level changes in acoustic characteristics in response to changes in disturbance. Specifically, we hypothesize that the colony response will be louder as a disturbance threat gets closer, resulting from louder calls, more frequent calls, and/or more individuals calling, quantified through increases in acoustic energy. We also hypothesize that birds will change their primary call type as the disturbance threat gets closer, resulting in a shift in the distribution of acoustic energy across frequency bands. In total, our study aims to not only understand the alarm calling behavior of terns, but also improve the potential for the implementation of a PAM system to monitor species' behavior and disturbance responses.

2. Methods

2.1 Data collection

We conducted the current study from May 30 to June 26, 2023 at a mixed-species tern breeding colony on White and Seavey Island (42°58' N, 70°37' W) located in the Isles of Shoals archipelago, New Hampshire (NH), USA, within the Gulf of Maine. This island is NH's primary breeding colony for common, roseate (S. *dougallii*), and Arctic terns (S. paradisaea), and is the largest tern breeding colony in the Gulf of Maine. Restoration, management, and research activities have been conducted at this site since 1997 under the auspices of the NH Fish and Game Department. In 2023, the colony supported 3165 pairs of common terns and 151 pairs of roseate terns. Arctic terns, though historically recorded breeding on White and Seavey Island, did not establish any nests at this colony during the year of the current study.

Four AudioMoth (device version 1.2.0, software version 1.8.1, Southampton, England) recorders (low-gain sensi-tivity, relatively flat frequency response from 20 to 20 000 Hz) (Hill et al.[, 2019](#page-6-0); [Lapp, 2021\)](#page-6-0) were mounted approximately 25 cm above the ground inside fenced productivity-monitoring plots (Fig. [1\)](#page-3-0), erected across the colony site prior to the migratory return of the terns. The plot fences are 25 cm tall, intended to prevent tern chicks from escaping while not influencing the behavior and nesting locations of adults. Recorders were programmed to record for 10 min at the start of the hour at low-gain sensitivity with a 48 kHz sampling rate, with the recording schedule fixed, regardless of disturbance. Recording locations were selected to be in areas dominated by common terns in prior years, avoiding areas of prior roseate tern activity, to focus recordings on this focal species. Researchers confirmed the presence of common terns in typical numbers in close proximity to chosen placements. We also prioritized locations closer to the center of the island, to minimize environmental noise (e.g., wind). Data collection was limited to daylight hours (8 AM to 8 PM EDT) to align with times that investigator disturbance occurred. While the colony is off limits to the general public, investigator disturbance occurs as the result of the regular research and conservation activities by Tern Conservation Project personnel. Across the dates of this study, the colony experienced investigator disturbance nine times on eight separate days. The investigators spent time within multiple recorded plots during these visits, though they did not visit every plot on each day. Disturbances lasted between 20 min and 2 h from entering to exiting the colony. Researchers made an effort to minimize unnecessary noise and disruption to the colony, beyond the inherent disturbance of their presence. Researchers recorded location and timestamps of investigator disturbances when conducting research activities in the colony, by noting time spent within each of the eight productivity-monitoring plots distributed across the colony (half with AudioMoth

Fig. 1. (A) A low tide map of White and Seavey Island tern colony. Black stars represent the locations of the four recorder plots, and white stars represent the additional four productivity-monitoring plots. Researchers enter the tern breeding colony on Seavey Island via the land bridge from White Island, which is only accessible during low tide. Inset depicts the location of the islands within the Gulf of Maine. (B) An example spectrogram of typical audio conditions during <10 m distance disturbance. Darker colors indicate greater amplitude. (C) An example spectrogram of typical audio conditions outside of disturbance periods.

installations and half without), with relative distances measured using satellite imagery. These plots are approximately 10–17 square meters, with researchers typically staying within 1 m of the plot while performing monitoring activities. While the time spent in each plot was recorded, the exact locations of the researchers within the plots could not be recorded precisely, so the distances were binned rounding down into 10 m buckets to avoid false levels of precision and maintain adequate sample sizes. Approximately 22 of the 261 total hours of audio recordings were removed from the dataset over the course of the study due to incomplete disturbance location documentation. This also includes the time researchers spent moving between plots, as precise investigator location was not recorded.

2.2 Data analysis

All acoustic and statistical analysis took place in R Statistical Software (version 4.3.1, Vienna, Austria) [\(R Core Team,](#page-6-0) [2023](#page-6-0)). A high pass filter was applied at 1000 Hz using the seewave package (version 2.2.1, Paris, France) [\(Sueur](#page-6-0) et al., [2008](#page-6-0)), as wind noise was prominent below 1000 Hz but tern vocalizations rarely subceeded 1000 Hz. Root mean square (RMS) amplitude [relative decibels (dB)] measurements were extracted every 10 s using the tuneR package (version 1.4.4, Dortmund, Germany) (Ligges et al.[, 2023\)](#page-6-0). Because Audiomoth units were not calibrated, we report our RMS amplitudes relative to the quietest and loudest signals recorded across our analysis. The values were sorted by location and time of day, and a Kruskal–Wallis test using the stats package (version 3.6.2) ([R Core Team, 2023\)](#page-6-0) was performed on both variables to assess the potential impact of these factors on colony-wide call amplitude across all recordings taken. To detect colony-level changes in acoustic characteristics in response to human disturbance, the median RMS amplitude at each location and time of day, while there were no documented disturbances, served as baseline measurements. The difference between the RMS amplitude during a disturbance compared to the baseline at the same time of day and location was calculated and reported as adjusted decibels. The resulting adjusted values were compared across distances between the disturbance and recorder at 0–9 m, 10–19, 20–29, 30–39, 40–49, and >60 m. Data were not collected with a disturbance distance between 50 and 60 m. A Kruskal–Wallis test using the stats package (version 3.6.2, Vienna, Austria) ([R Core Team,](#page-6-0) [2023](#page-6-0)) followed by a Dunn test using the dunn.test package (version 1.3.5, Portland, OR) [\(Dinno, 2017\)](#page-6-0) were performed to examine differences in RMS amplitude across and between groups at different distances from investigator disturbance.

To determine whether the terns changed call types or call pitch in response to disturbance, which could indicate a shift in call types, we separated our recordings into 1 kHz bands from 1–8 kHz using a bandpass Butterworth filter (Sueur et al.[, 2008](#page-6-0)), then calculated the relative RMS amplitude for each frequency band. This range was chosen based on the frequency range of a subset $(n = 58)$ of common tern vocalizations analyzed using RavenPro (version 1.6.4, Cornell Lab of Ornithology, Ithaca, NY). A Kolmogorov Smirnov test using the stats package (version 3.6.2) ([R Core Team, 2023\)](#page-6-0) was then used to determine whether there were differences in energy distribution across frequency bands while disturbances were 0 m away, which might indicate a change in the dominant call type.

3. Results

A Kruskal–Wallis test found significant difference in RMS amplitude between hours of the day [Kruskal–Wallis rank sum, $H(11) = 815.663$, $p < 0.001$], and a second Kruskal–Wallis test found significant difference in RMS amplitude (relative dB) between recording locations [Kruskal–Wallis rank sum, $H(3) = 7700.143$, $p < 0.001$] (Fig. [2](#page-4-0)).

Due to the significant variations in amplitude across sites and time of day, for all further analysis, we controlled for location and time of day, as described in the methods. Disturbances at distances of 0–9 and 10–19 m resulted in

Fig. 2. (A) Variation in RMS amplitude (relative dB) (see Methods 2.2, Data analysis) measured at a tern breeding colony across different times of day. (B) Variation in RMS amplitude (relative dB) across four locations of audio recorder deployment within the breeding colony. Median values are represented by bold lines, boxes represent the interquartile range, whiskers represent the first and fourth quartiles, and statistical outliers are indicated by open dots.

significant increase in acoustic energy compared to baseline [0–9 m having a median value of 39.076 adjusted dB; 10–19 m having a median value of 23.567 adjusted dB, Kruskal–Wallis rank sum, $H(5) = 386.722$, $p < 0.001$. Disturbances 20 m or greater from the recorder did not result in significant increases in colony acoustic energy compared to baseline. A Dunn test revealed a significant ($p < 0.05$) difference between disturbances less than 10 m and those between 10 and 19 m, significant ($p < 0.001$) differences between disturbance within 10 m and all other groups, and significant ($p < 0.05$) differences between disturbances from 10 to 19 m and all further disturbances (Fig. 3).

A Kolmogorov Smirnov test found no significant difference in acoustic energy distribution across 1000 Hz frequency bins between 1000 and 8000 Hz in response to the presence of investigator disturbance at a distance of 0 m, compared to the distribution of energy across frequencies without disturbance present (Fig. [4\)](#page-5-0).

4. Discussion

Our results indicate that common terns exhibit a predictable, graded alarm response that varies with proximity to investigator disturbance. Although the many overlapping calls in a dense colony make it difficult to distinguish between increased call rate and call amplitude; here, we observed an overall increase in acoustic energy as proximity to investigator disturbance increased. These findings are similar to what has been documented in other larid species and passerine species, suggesting that common terns employ a graded alarm call system. This provides further evidence for the usage of such a system in a non-passerine species. By measuring the acoustic energy produced by the colony, we were able to determine both an overall range of disturbance impact, as well as a graded response within that range, without the need for

Fig. 3. RMS amplitude (adjusted dB, see Methods 2.2, Data analysis) measured at a common tern breeding colony during disturbances at different distances. Median values at each distance are represented by the bold lines, boxes represent the interquartile range, whiskers represent the first and fourth quartiles, and statistical outliers are indicated by open dots. Letters indicate groups of significant difference. Groups A and C were statistically significant at the $p < 0.01$ level, while groups A and B and B and C were statistically significant at the $p < 0.05$ level.

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Fig. 4. Median RMS amplitude (relative dB) values across frequencies during times of tern colony disturbance (red) and undisturbed times (blue) for all sites between 8 AM to 8 PM EDT. The shaded ribbons represent 50th percentile distributions of data.

individual call analysis. An increase in acoustic energy was detectable for disturbances within 20 m, with an even more pronounced effect for disturbances within 10 m.

Furthermore, our findings demonstrate a clear example of a graded alarm call system in an acoustically understudied, non-passerine species. The observed increase in overall call amplitude with proximity to disturbance could contribute in several ways to the effectiveness of this coordinated alarm response, including through intra- or inter-specific communication to warn nearby terns of potential predator threats and threat deterrence [\(Isbell and Bidner, 2016](#page-6-0)). In turn, this acoustic response can disrupt potential predation activity, improving colony-level survival [\(Baker and Becker, 2002\)](#page-6-0). Although beneficial for warning conspecifics of a threat, the increase in colony-level acoustic energy during threat periods may have harmful consequences. This alarm call response is a significant behavioral event occurring over a sizable portion of the colony. If alarm periods persist long enough, and habituation does not occur, the behavioral response may impact reproductive success or increase intraspecific aggression ([Carney and Sydeman, 1999\)](#page-6-0). Considering these potential effects, future monitoring of these birds can include acoustic energy measurements as a metric to assess impacts of survey activities on focal populations and adjust methods to reduce these effects.

Our findings do not support our hypothesis that energy distribution across frequency bands shifts in response to disturbance, which could have indicated an alarm response of producing calls in different frequency ranges compared to nondisturbance behavior. Though there was change in amplitude during different disturbance conditions, the relative pitches remained comparable. It is possible that the peak frequency of non-alarm and alarm calls may be too similar to see differences using 1 kHz bins. More research is needed to quantify the call parameters of the different call types to assess this, and finer scale analysis should be done to study changes in call type production in antipredator responses of tern colonies. Although we did not detect a shift in acoustic energy across frequencies in response to human disturbance, it is still possible that terns produce different call types in response to proximity of an external threat, with those calls remaining in the same frequency range as non-threat calls. Previous research has found qualitative differences in calls used in response to disturbance but did not include a quantitative change in fre-quency [\(Veen, 1987\)](#page-6-0). Our results align with prior attempts to quantify disturbance vocalization in terns, but future work exploring parameters of individual calls might reveal shifts in time-frequency characteristics during alarm behavior.

Our results have implications for monitoring and management of common terns. In many cases, human monitoring of seabirds can cause disturbance, influencing behavior and the colony soundscape [\(Carey, 2009\)](#page-6-0). With a PAM approach, we can quantify the effect of human presence on the colony, which can lead to guidelines on best practices to reduce investigator disturbance while still allowing for visual monitoring. These practices could include guidelines for distance and colony intrusion time, informed by acoustic observations of the focal populations. By continuing to pair visual monitoring with PAM, we can ground-truth acoustic recordings to population size and behavior, and work toward a framework for monitoring seabirds solely with PAM.

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Author Declarations

Conflict of Interest

The authors have no conflicts to disclose. Tern colony fieldwork was permitted under protocol #160403 approved by University of New Hampshire's Institutional Animal Care and Use Committee, and permit #06543 under the U.S. Geological Survey's Bird Banding Laboratory.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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