



Effects of vessel sound on oyster toadfish *Opsanus tau* calling behavior

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ABSTRACT: In coastal waters, anthropogenic activity and its associated sound have been shown to negatively impact aquatic taxa that rely on sound signaling and reception for navigation, prey location, and intraspecific communication. The oyster toadfish *Opsanus tau* depends on acoustic communication for reproductive success, as males produce 'boatwhistle' calls to attract females to their nesting sites. However, it is unknown if *in situ* vessel sound impacts intraspecific communication in this species. Passive acoustic monitoring using a 4-hydrophone linear array was conducted in Eel Pond, a small harbor in Woods Hole, MA, USA, to monitor the calling behavior of male toadfish. The number of calls pre- and post-exposure to vessel sound was compared. Individual toadfish were localized, and their approximate sound level exposure was predicted using sound mapping. Following exposure to vessel sound, the number of calls significantly decreased compared to the number of calls pre-exposure, with vessel sound overlapping the frequency range of male toadfish boatwhistles. This study provides support that anthropogenic sound can negatively affect intraspecific communication and suggests that *in situ* vessel sound has the ability to mask boatwhistles and change the calling behavior of male toadfish. Masking could lead to a reduction in intraspecific communication and lower reproductive efficiency within the Eel Pond toadfish population.

KEY WORDS: Anthropogenic sound · Communication · Passive acoustic monitoring · Toadfish

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1. INTRODUCTION

Anthropogenic sound is increasing throughout the world's oceans (Slabbekoorn et al. 2010), and its impact on marine organisms is just beginning to be understood (NRC 2003, Popper 2003, Hastings 2008, Richardson et al. 2013). Underwater sound travels at high speeds and long distances with low attenuation, and the detection and localization of acoustic signals and cues of interest, such as those produced by prey or conspecifics, play a crucial role in the lives of many aquatic organisms (Hawkins 1986). However, anthropogenic sound can disrupt intraspecific communication by decreasing hearing sensitivity of fishes (Scholik & Yan 2002, Smith et al. 2004, Popper et al. 2005, Rogers et al. 2020), and may also overlap with

frequencies of biologically and environmentally relevant sounds (Amorim 2006, Kasumyan 2008, Ladich 2008, Popper & Hastings 2009). Auditory masking occurs whereby an individual cannot detect, discriminate, or recognize sounds of interest (Slabbekoorn et al. 2010). For example, boat engine sounds overlap the frequency range of courtship sounds produced by silver perch *Bairdiella chrysoura*, black drum *Pogonias cromis*, oyster toadfish *Opsanus tau*, spotted seatrout *Cynoscion nebulosus*, and red drum *Sciaenops ocellatus* in the May River estuary, South Carolina (USA) (Smott et al. 2018), potentially posing a risk to reproductive success.

Acoustic communication is critical for the reproductive success of many fishes (Myrberg & Lugli 2006). For example, male oyster toadfish establish

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nests in shallow estuarine and coastal waters in late spring (Gray & Winn 1961, Price & Mensinger 1999) and use the sonic muscles attached to their swim-bladder to produce mating calls, termed 'boatwhistles,' to acoustically attract females to their nests for spawning. The male guards the eggs while continuing to produce boatwhistles to attract additional females (Fish 1972, Winn 1972). Boatwhistles have an initial broadband grunt-like segment (30–50 ms) followed by a longer tonal portion (200–650 ms), with the fundamental frequency (120–250 Hz) correlated with water temperature (Tavolga 1958, Winn 1972, Edds-Walton et al. 2002, Maruska & Mensinger 2009). Each male produces a unique signal that varies in fundamental frequency, amplitude, waveform, and duration that allows for identification of individuals (Fish 1972, Edds-Walton et al. 2002, Amorim & Vasconcelos 2008, Putland et al. 2018). Boatwhistles and individual calling rate may also contain additional information regarding fitness, providing an opportunity for females to choose mates based on acoustic signals that reflect the size and age of a male (Amorim et al. 2015).

Passive acoustic monitoring (PAM) is a non-invasive technique that can detect and localize soniferous species (Wall et al. 2013, Ricci et al. 2017). A resident oyster toadfish population in Eel Pond, Woods Hole, Massachusetts (USA), has been monitored since 2015 using PAM, and individual male fish have been localized (Putland et al. 2018). The population within the detection range of the PAM array contained 10 to 12 calling males with an unknown number of females in 2017 (Putland et al. 2018), and a similar population size has been estimated for the area each summer between 2018 and 2020 (R. Putland pers. obs.). Oyster toadfish display both seasonal and daily variation in calling, with boatwhistles initiated in mid-May, peaking in June and July, and then ceasing in mid-August (Van Wert & Mensinger 2019). The daily calling pattern is characterized by relatively infrequent calls during midday, a sharp increase at sunset, a peak midway through the night, and gradual tapering off after sunrise (Van Wert & Mensinger 2019). These natural variations in calling patterns are important to consider when evaluating the effects of vessel sound on intraspecific communication of toadfish.

The oyster toadfish population in Eel Pond presented a unique opportunity to study the effects of vessel sound on male calling behavior *in situ*, as male toadfish exhibit high site fidelity throughout the reproductive season (May–August). While other fishes would typically avoid aversive sound stimuli (Popper 2003), male toadfish remain sedentary in their nests,

thereby providing a good model to study the effects of anthropogenic sound on calling behavior. Motorized watercraft activity is common during the summer months in Eel Pond and consequently may increase the amplitude of the soundscape during the toadfish mating season. While previous studies have found that toadfish calling rates are significantly reduced in response to vessel sound (Krahforst et al. 2016), these studies were done using a speaker playback method. Thus, there is a need to investigate *in situ* vessel sound. In this study, we focused on the effects of sound produced by a research vessel (RV) operated by the Marine Biological Laboratory (MBL). We hypothesized that the sound produced by the RV on operational days would negatively impact the calling rate of male toadfish and result in a decreased number of calls following vessel departure. The soundscape in Eel Pond was measured to assess the increase in sound pressure level (SPL) at different locations during vessel operation. Individual fish were localized in an attempt to correlate sound exposure with the change in call rate, as the nests of males varied in distance from the sound source of the vessel and experienced different sound levels. Overall, the aim of this study was to use PAM to investigate the effect of *in situ* vessel sound on the calling behavior of oyster toadfish in Eel Pond.

2. MATERIALS AND METHODS

2.1. Study site and passive acoustic monitoring of toadfish

To monitor the effect of vessel sound on a resident oyster toadfish *Opsanus tau* population, PAM was conducted in Eel Pond (41.53° N, 70.67° W), a small salt-water harbor (approximately 300 m wide) that is frequented by motorized watercraft. The MBL borders the southwest shore of Eel Pond with a research dock extending approximately 50 m into the harbor, which provided a platform to deploy the PAM array in the vicinity of 10 to 12 actively vocalizing male toadfish (Fig. 1). The PAM array consisted of 4 hydrophones (h1, h2, h3, h4; HTI 96 min, High Tech) that were deployed in a linear arrangement under the dock (Fig. 1). The hydrophones were deployed 23.2 m (h1), 28.4 m (h2), 32.1 m (h3), and 39.6 m (h4) from the mean high tide mark on the shoreline. Hydrophone locations were chosen based on the positions of hatches on the dock that provided access for deployment and retrieval. A previous study conducted in Eel Pond using this linear array found that boat-

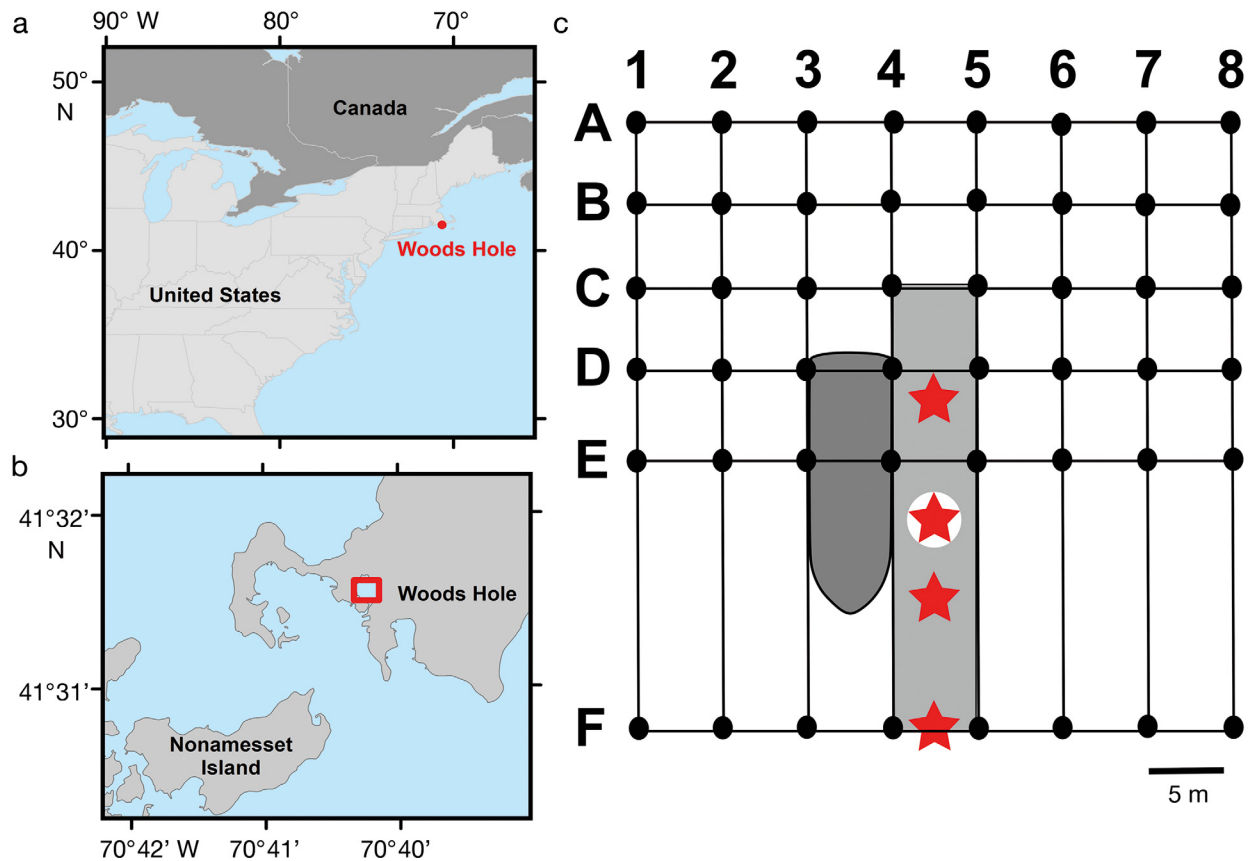


Fig. 1. (a) US east coast showing the location of Woods Hole, MA. (b) Enlargement of the Woods Hole area, with Eel Pond outlined in the red box. (c) Enlarged image of the research area in Eel Pond, showing the location of the 4-hydrophone linear array (red stars, from bottom to top: h1, h2, h3 and h4), the sound mapping grid, and the RV (dark grey) to the left of the dock (light grey). The black dots indicate the locations where the hydrophone was placed for sound mapping and the large white circle shows the underwater speaker position (at h3)

whistles (root mean square [RMS] received level >80 dB re $1 \mu\text{Pa}$) recorded by a minimum of 3 of the 4 hydrophones can be localized within a 35 m radius of the dock (Putland et al. 2018). All 4 hydrophones were mounted 1 m above the substrate (soft sediment interspersed with rocks), and the overall water depth ranged from 2.4 to 3.4 m, depending on tidal conditions. Underwater sound was continuously recorded from 15 June to 22 July 2017 and from 10 June to 21 July 2018 using a 4-channel digital acoustic recorder (SoundTrap ST4300, Ocean Instruments) with the 4-hydrophone linear array attached. All acoustic data were sampled at 24 kHz, which allowed analysis up to the Nyquist frequency of 12 kHz. The hydrophones had a flat -3 dB re $1 \mu\text{Pa}$ frequency response between 0.002 and 30 kHz and had sensitivities ranging from 164.9 to 165.4 dB re $1 \text{ V}/\mu\text{Pa}$ (h1: 165.4, h2: 165.0, h3: 165.1, and h4: 164.9).

To assess the prevalence of toadfish and vessel acoustic activity, recordings were divided into 10 min

files and inspected both aurally and visually using scrolling spectrograms (Hanning window, FFT = 1024) in RavenPro (version 1.50) to identify toadfish vocalizations and vessel sound (presence or absence). A total of 11 232 recordings (10 min each) were analyzed, and the percentage of the recordings that contained these sounds was then calculated. Additionally, to understand temporal fluctuations in sound level recorded, the RMS SPL was calculated between 0.01 and 12 kHz for each 10 min file using MATLAB (version 2015a). A previous study found that water temperature is correlated with calling activity, as male toadfish only produced mating vocalizations when the temperature was between 15 and 26°C (Van Wert & Mensinger 2019). Therefore, water temperature was recorded every minute using a temperature logger (Hobo® Pendant $\pm 0.1^\circ\text{C}$) at the h4 location and positioned 1 m above the substrate. The water temperature ranged from 17 to 26°C during the recording periods in 2017 and 2018 (Fig. S1 in

the Supplement at www.int-res.com/articles/suppl/m622p115_supp.pdf). RMS SPL was compared to corresponding water temperature (mean of the 10 temperature measurements recorded over the corresponding 10 min time period) using a Pearson's correlation coefficient to assess whether there was a relationship between temperature and amplitude. Time of day and lunar rhythms were monitored, which have been shown to influence calling behavior of toadfish (Monczak et al. 2017), with sunrise occurring between 05:08 and 05:27 h and the moon passing through approximately 1.5 lunar phase cycles (new moon, first quarter, full moon, and third quarter) during each year of the study.

2.2. Change in calling behavior and vessel sound exposure

To investigate the effect of vessel sound activity on toadfish calling, the number of calls was compared pre- and post-exposure to engine sound from an RV. Eel Pond boat access is regulated by a drawbridge that opens upon request on the hour and half hour between 06:00 and 19:00 h (15 May–14 June) or 21:00 h (15 June–15 September). The MBL operates a 15 m RV that is positioned parallel to and near the terminus of the MBL dock with its stern facing towards the center of the pond. The RV is started at approximately the same time (between 06:00 and 07:00 h) 3 or 4 times per week and idles at the dock before exiting via the drawbridge-controlled channel.

During the course of this study, the RV idled between 5.8 to 33.0 min before leaving the dock; therefore, a 10 min time window was chosen to analyze toadfish calls pre- and post-exposure to coincide with the mean idling duration (11.8 min). The number of boatwhistles from the toadfish population within the pond soundscape was counted over a 10 min period pre- and post-exposure to the engine start on 10 d during both 2017 and 2018, for a total of 20 d. The sound level of frequencies matching those of the boatwhistles increased when the RV was operational (Fig. 2), which inhibited the detection of toadfish calls using the PAM array. Therefore, calls could not be monitored during the RV idling period. The post-exposure period started once the RV had left the dock and background sound returned to ambient levels. Control days ($n = 20$), when the RV was not operational, were adjacent to the experimental days to minimize the influence of water temperature and lunar phase. For control days, the calls were counted during the same 10 min periods that were analyzed

during the preceding or succeeding experimental day. Acoustic recordings were analyzed in Raven Pro 1.5.0, using a scrolling spectrogram display of 10 s windows (Hanning window, FFT length = 512 with 50% overlap, providing a frequency resolution of 46.8 Hz and a time resolution of 0.4 ms).

The term 'call change' was used to assess the change in the number of calls before and after RV exposure on experimental and control days (Eq. 1). Therefore, values >0 indicate an increase, and values <0 indicate a decrease in calling post-exposure to the vessel sound.

$$\text{Call change (\%)} = \left(\frac{\text{no. of post exposure calls}}{\text{no. of pre exposure calls}} \times 100 \right) - 100 \quad (1)$$

Statistical analysis was conducted using SigmaPlot (version 13). The call data failed a Shapiro-Wilk test for normality; therefore, a non-parametric Wilcoxon signed-rank test was used to test the significance of differences in call change on experimental and control days using a p-value of 0.05.

2.3. Sound mapping

To understand the received level of vessel sound for resident toadfish in Eel Pond, the 35×35 m area encompassing both sides of the MBL dock and the RV was sound mapped to predict RMS SPL (100–500 Hz) at the approximate location of individual fish when the RV was present and absent (Fig. 1). This frequency range was used to match the fundamental frequency range of toadfish vocalizations (Edds-Walton et al. 2002) and the predicted frequency range of best auditory sensitivity (Yan et al. 2000). Point measurements used to model the sound field were taken with a single hydrophone (SoundTrap ST300, Ocean Instruments) attached to a rope with a weight at the bottom and a float at the top. The hydrophone was deployed for 3 min at each of 48 stations (Fig. 1) at 1 m water depth to be planar to the depth of the RV engine. Hydrophone deployments were made using a kayak to avoid introducing extraneous sound into recordings, and measurement locations were chosen to form a grid while avoiding obstructions and moored boats. The kayak was allowed to drift after the hydrophone was placed into position and then used to retrieve it after the 3 min recording period.

Ambient sound map measurements were conducted on 29 May and 13 July 2018, when no vessel traffic was present in the harbor, and were used as

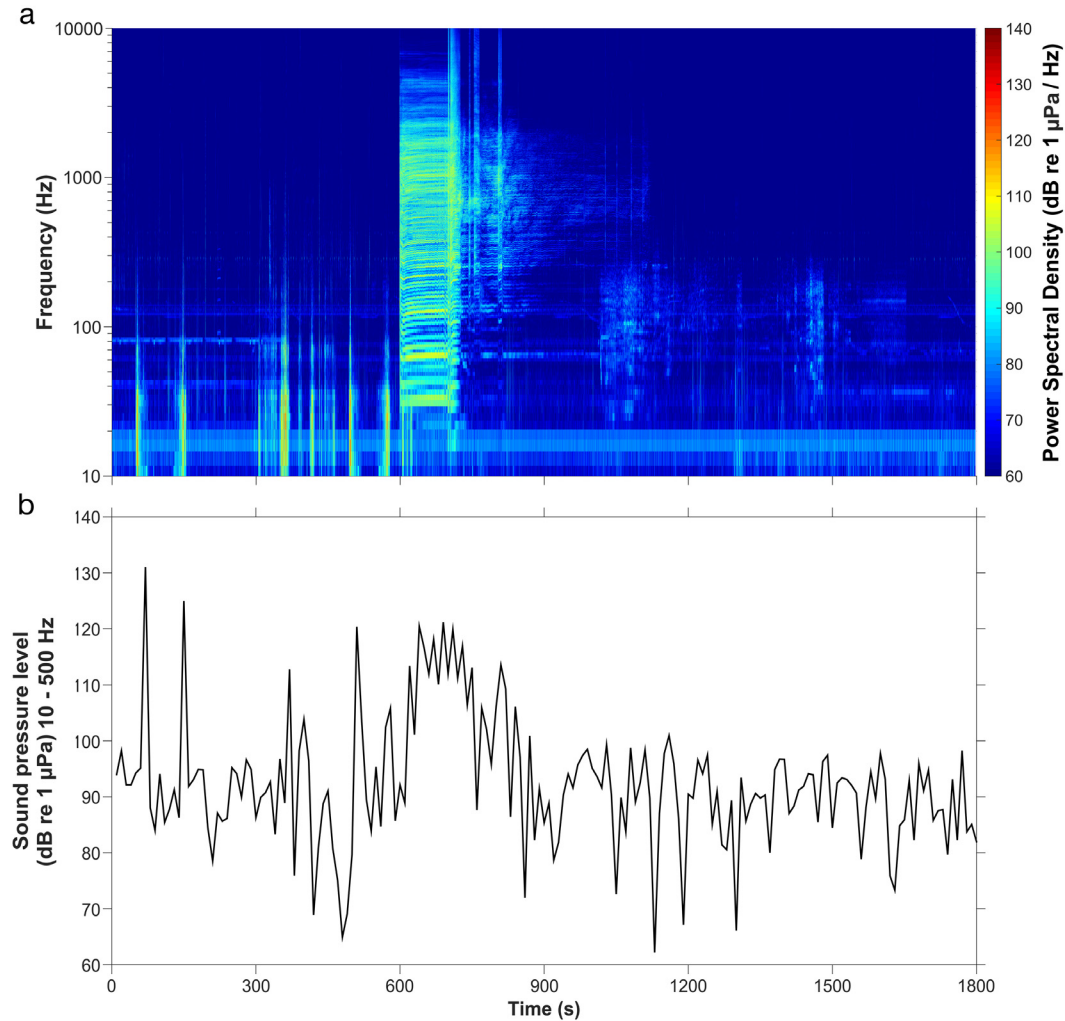


Fig. 2. (a) Spectrogram and (b) root mean square sound pressure level between 100 and 500 Hz during pre-exposure (0–600 s), research vessel idling (600–1200 s), and post-exposure (1200–1800 s) time periods recorded from h3

proxies for ‘control’ days. Vessel sound map measurements were conducted on 19 June and 15 July 2018. It was not possible to record at the 48 locations during the mean engine idling time of the RV (11.8 min) to predict the sound field with the RV engine sound present. Therefore, the sound map was created using acoustic measurements made at the same 48 locations while an underwater speaker (University Sound UW-30, Lubell Labs) broadcasted a 3 min idling RV recording (source level: 130 dB re 1 μ Pa between 0.01 and 12 kHz at 1 m). The playback clip was created by measuring the RV for 3 min using a single hydrophone (SoundTrap ST202, Ocean Instruments) positioned 1 m away horizontally from the RV (1 m below the water surface in 3.4 m water depth) while the engine was idling on 5 random days during 2018. The RMS SPL was calculated for each of the recordings

between 0.01 and 12 kHz by applying a bandpass filter using MATLAB software (version 2015a). The approximate source level was then calculated by taking the mean of the RMS values. The speaker was powered by an amplifier (20 W Talk-Back Mobile Amplifier, Speco Technologies) and deployed at 1 m water depth next to h3 (Fig. 1). Recordings of the speaker playback and RV were compared using power spectra and found to be similar between 100 and 500 Hz (Fig. S2).

For both ambient and RV playback sound measurements, the RMS SPL between 100 and 500 Hz was calculated for the 3 min recording taken at each hydrophone location by applying a bandpass filter, and a sound map was created using the ‘contourf’ function in MATLAB. This function displays isolines calculated from a gridded matrix (in this study, the received SPL recorded at each of

the 48 locations) and fills the areas between the isolines using constant colors represented by the color bar in the graph (Mathworks 2020).

2.4. Toadfish localization

Individual toadfish were localized and their approximate location was superimposed on the generated sound maps using MATLAB to estimate the vessel sound potentially impacting each toadfish. Individual males were identified and characterized based on boatwhistle parameters, which included a combination of waveform shape, frequency composition, and relative amplitude (Putland et al. 2018). The calls from 4 toadfish were of sufficient fidelity (≥ 3 dB above the ambient soundscape between 0.01 and 12 kHz) to be localized within Eel Pond to predict how sound exposure may affect calling patterns. Localization (± 2 m) of individual toadfish was done using the time difference of arrival (TDOA) method established by Watkins & Schevill (1972), developed by Speisberger & Fristrup (1990), and adapted for toadfish by Putland et al. (2018). In brief, the waveform of each boatwhistle call was analyzed in LabChart (version 8) using a scrolling display of 1 s windows (Hanning window, FFT length = 4096 with 50 % overlap, providing a frequency resolution of 5.9 Hz and a time resolution of 0.04 ms) to identify the time of arrival (TOA) at each hydrophone. The TDOA was determined to be the difference in the TOA that the boatwhistle reached h2, h3, and h4 relative to the TOA of h1.

3. RESULTS

3.1. Passive acoustic monitoring

The received RMS SPL at the 4 hydrophones ranged between 82 and 123 dB re 1 μ Pa (0.01–12 kHz) during the 2017 and 2018 deployments (Fig. S1). There were daily fluctuations in received sound level, with higher broadband SPL during the day than at night. There was no significant difference in broadband SPL throughout June–July, and there was no correlation between broadband SPL and water temperature (Pearson's correlation test; 2017: $r = -0.185$, $p < 0.001$; 2018: $r = 0.036$, $p = 0.080$). The 2 main contributors to the soundscape of Eel Pond were toadfish boatwhistles (detected in 100% of recordings) and boat sound (detected in 7.2% of recordings, not including the RV).

3.2. Change in calling behavior and vessel sound exposure

The median number of post-exposure calls on experimental days significantly decreased from 41 to 29 compared to pre-exposure calls (Wilcoxon signed-rank test, $p < 0.001$; Fig. 3). Control days showed no significant difference between pre-exposure (21) and post-exposure (22.5) median call number (Wilcoxon signed-rank test, $p = 0.087$; Fig. 3). The mean call change was -31.6% on experimental days and -4.5% on control days (Fig. 4), indicating that toadfish calling had a greater decrease following exposure to vessel sound.

3.3. Sound mapping and toadfish localization

Based on the constructed sound maps, RMS SPLs were greater in the mapped area during RV playback conditions compared to the ambient conditions. During ambient conditions, the SPL ranged from 85 to 99 dB re 1 μ Pa (100–500 Hz; Fig. 5a). During RV playback conditions, the SPL ranged between 106 and 126 dB re 1 μ Pa (100–500 Hz; Fig. 5b).

Eight male toadfish (TF) were identified in 2018 using their call signatures and, of those identified, 4 (TF A–D) were of sufficient fidelity for localization. Call duration for the 4 individuals localized ranged from 300 to 660 ms, and maximum received sound level ranged from 75 to 100 dB re 1 μ Pa. All 4 individ-

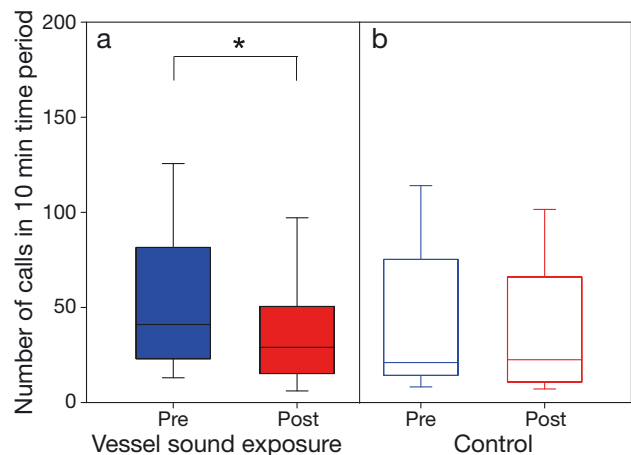


Fig. 3. Number of boatwhistle calls recorded during pre- (blue) and post-exposure (red) time periods on (a) experimental days (filled) and (b) control days (open). Bottom and top boundaries of the box indicate the 25th and 75th percentiles, respectively; the line within the box marks the median; and whiskers above and below the box indicate the 90th and 10th percentiles, respectively. The asterisk indicates a significant difference (Wilcoxon signed-rank test, $p < 0.001$)

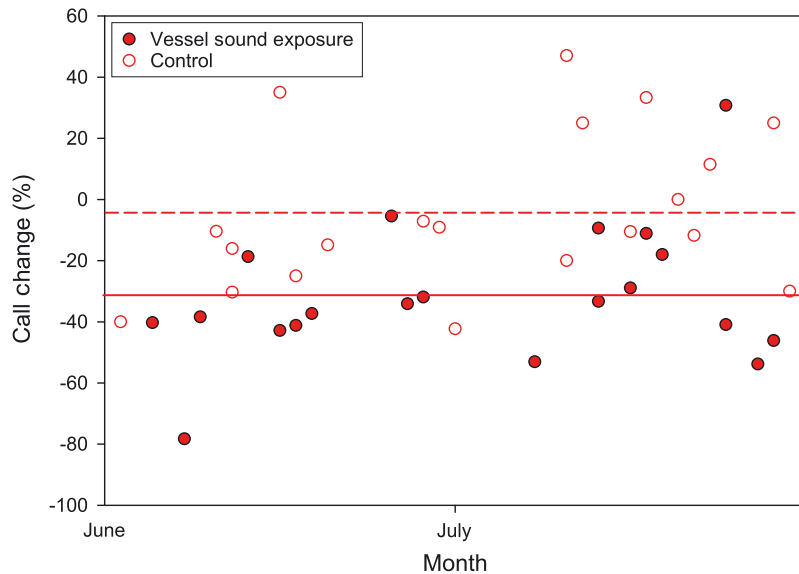


Fig. 4. Oyster toadfish call change over the course of the study. The call change for sound exposure and controls is plotted versus calendar day for 20 d (2017: $n = 10$, 2018: $n = 10$). Lines indicate the mean call change for sound exposure (solid) and control (dashed) days

uals decreased the number of boatwhistles produced after exposure to vessel sound with the call changes ranging from -13 to -63% . On control days, the call change of the 4 individuals varied from $+20$ to -40% . The distance of the localized toadfish from the RV motor ranged from 10.1 to 16.3 m (TF A: 12.8 m, TF B: 11.9 m, TF C: 16.3 m, and TF D: 10.1 m). Therefore, based on the localization of individual fish and the predicted sound levels from the vessel sound map, TF A was exposed to the highest levels of vessel sound at approximately 121 dB re 1 μPa (Fig. 5). TF B, C, and D were exposed to levels of 119, 117, and 117 dB re 1 μPa , respectively (Fig. 5).

4. DISCUSSION

We assessed the effects of *in situ* vessel sound on oyster toadfish calling behavior and found that the number of calls from the male toadfish significantly decreased following exposure to the sound of an idling RV. Intermittent operation of the RV created the opportunity to compare male toadfish calling in the presence of sound exposure to adjacent control days when there was no vessel sound. We found that call change was greater on days when the RV was operational and there was vessel sound present compared to control days. Individual male toadfish were localized and distance from the RV sound source was

assessed. However, due to low sample numbers and behavioral calling variation, a direct correlation of call change with sound exposure at the individual level could not be determined. Despite this, these results show that the presence of vessel sound, even for relatively short periods of time, can induce changes in calling behavior, and determining how exposure to different predicted sound levels influence individual fish through long-term monitoring is warranted for future studies.

The frequencies of the RV sound overlapped with the frequencies of both the auditory range of toadfish hearing and the male calls. The RV produced a mean SPL of 130 dB re 1 μPa between 0.01 and 12 kHz and toadfish hearing ranges between 0.1 and 1 kHz, with peak sensitivity at 120 Hz (Yan et al. 2000, Rogers et al. 2020). These results indicate that the

vessel sound could lead to acoustic masking, which may lead to a reduction in the ability of females to locate potential males and a subsequent reduction in reproductive efficiency (Slabbekoorn et al. 2010). Additionally, masking could also affect complex acoustic male–male conspecific interactions, as male oyster toadfish listen to each other and alternate their production of boatwhistles to avoid call overlap, and can also exhibit stealth signaling whereby one male attempts to disrupt the boatwhistle of a conspecific by producing a grunt (Mensinger 2014). A previous study found that toadfish increase their average call power in noisy conditions (Luczkovich et al. 2016), which may be an attempt to overcome masking. Motorized watercraft activity in Eel Pond increases during the summer months for recreational purposes and coincides with peak mating season for toadfish; thus it is possible that the toadfish population may increase its call power to mitigate masking. Although the RV only operates for a short time during mornings (5.8–33.0 min), other motorized watercraft are present in Eel Pond, with boat sound detected in 7.2% of the sound recordings, warranting concerns about the effects of anthropogenic sound. Daily and seasonal anthropogenic activity may pose a threat to many soniferous fishes, and sound restrictions during sensitive times could help mitigate the potential impacts on mate finding and reproductive success, and conspecific communication.

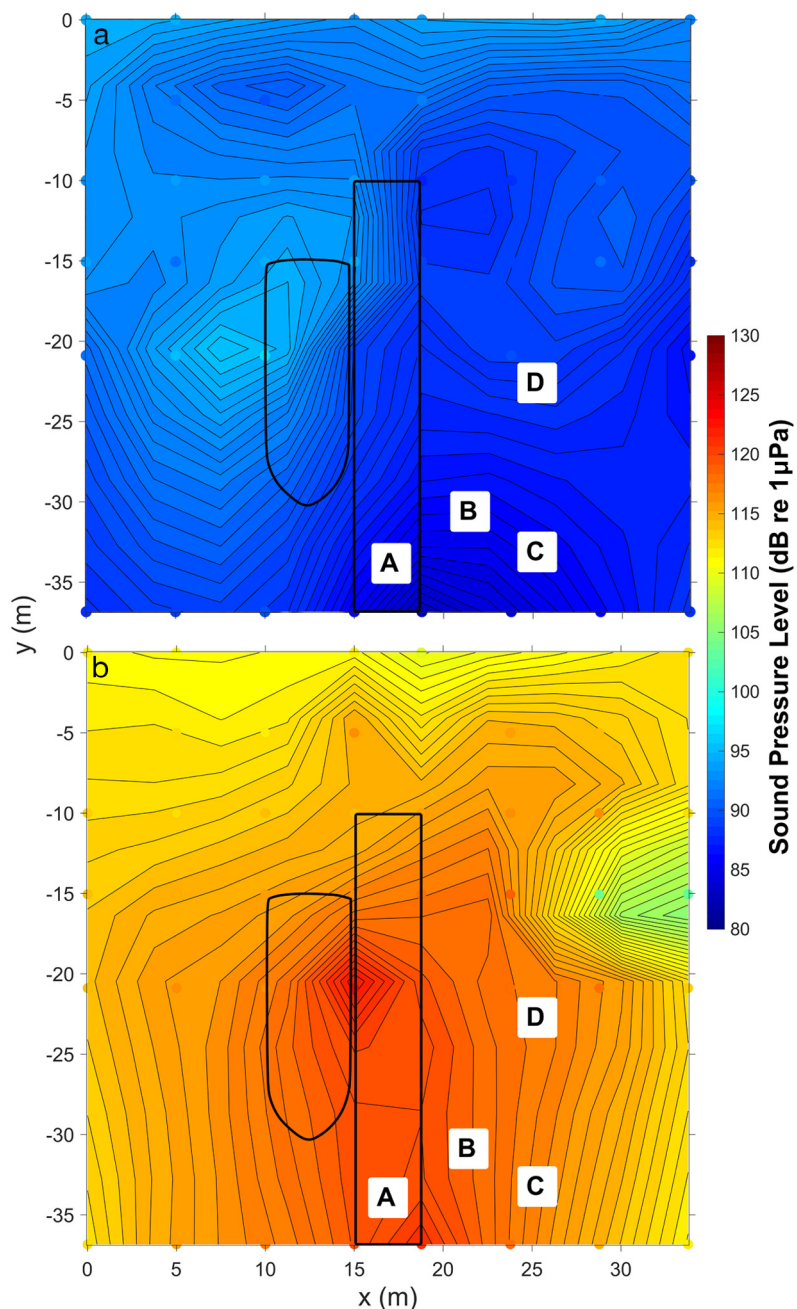


Fig. 5. (a) Ambient and (b) vessel sound playback sound maps showing the predicted sound field (root mean square sound pressure level at 100–500 Hz) at 1 m depth. The locations of 4 individual male toadfish are indicated with the white boxes (A–D). The dock and RV are outlined in black and the dots denote position of hydrophone while recording (cf. Fig. 1).

The RV investigated in this study operated soon after sunrise, corresponding with the natural daily decline in toadfish calling. Therefore, it was imperative to compare experimental and control days to ascertain that decreased calling was due to vessel sound and not to the normal daily fluctuations in toadfish calling. Results of this study indicated a sig-

nificant decline in post-exposure calling compared to pre-exposure on experimental days, but not on control days. On control days, there was a slight decrease (–4.5%) in call change, which is consistent with the typical decrease in calling following sunrise, but there was a greater decrease on experimental days after vessel sound exposure (–31.6%). Interestingly, the median pre-exposure call rate was lower on control days than on experimental days. Controls had to be adjacent with the experimental days and have no vessel activity, thus limiting the number of days available to analyze. The lower median call numbers on control days may be attributed to the prolonged effects of anthropogenic sound, but further studies are warranted. Additionally, it would be important to monitor the time it takes following the presence of vessel sound for calling activity to return to pre-exposure levels.

Recent reviews have suggested that long-term field studies are needed to assess the effects of anthropogenic sound on the behavior of fishes with a focus on both the individual and the population (Popper & Hawkins 2019). In our study, the use of PAM allowed for a greater understanding of the soundscape in Eel Pond and allowed individual toadfish and their response to vessel sound to be continuously monitored. Accurate localization of individual male toadfish required high-fidelity signals, and the low density of males and shallow environment combined to limit the number of signals. Of the 8 identified male toadfish, 4 produced boatwhistles of sufficient amplitude to allow individual localization. The male toadfish were 10.1–16.3 m away from the RV motor and showed individual

variation in calling behavior. Even though TF D was closest to the RV motor, it was not exposed to the highest sound levels, which could be due to non-linear shallow-water sound propagation. Although TF A was subjected to the highest sound exposure levels and had the largest decrease in call change, the low sample size of localized fish and relatively small vari-

ation in sound exposure precluded our ability to correlate exposure levels to call reduction at the level of the individual. Further studies are needed to understand how individual calling behavior is affected by vessel sound and how individuals may differ compared to population response. Additionally, it would be important to determine the minimum sound level that elicits a behavioral response, how different sound levels affect changes in calling, and if these differences in sound exposure induce long-term effects.

A number of studies have elucidated the impact of anthropogenic sound on the behavior and physiology of both marine and freshwater fishes (e.g. Popper 2003, Slabbekoorn et al. 2010, Popper & Hawkins 2019). Many of these past studies were conducted in tanks, and while laboratory studies can determine changes in hearing sensitivity following sound exposure, sound propagation can be dramatically different than the *in situ* soundscape (Duncan et al. 2016). Additionally, tank and laboratory studies usually confine fish to a limited area, in contrast to natural conditions where fish can swim away from aversive stimuli. Field-based studies that have assessed the effect of anthropogenic sound on fish behavior have typically used speaker playbacks (Picciulin et al. 2010, Brintjes & Radford 2013, Simpson et al. 2015, 2016, Krahforst et al. 2016, Nedelec et al. 2017). While these studies are important, sound playbacks do not represent the full range of sound produced by motorboats. This is one of the first studies to look at exposure to vessel sound from an RV rather than sound playback via an underwater speaker and provides a new perspective on the effects of *in situ* vessel sound on fish behavior.

Sound has both pressure and particle motion components, and in this study, we specifically assessed how toadfish calling behavior may be affected by the SPL component of the vessel sound. We were unable to assess particle motion due to equipment limitations, but future studies could assess the effect of particle motion by placing accelerometers at the same location as hydrophones. Additionally, a non-linear hydrophone array or a linear array with hydrophones at different depths with vertical separation could be deployed, which would measure shallow-water sound propagation more precisely and allow for more accurate toadfish localization. We acknowledge that sound recorded on hydrophones 1 m above the substrate may differ from sound perceived by toadfish on the bottom due to depth-dependent propagation. However, due to methodological restraints, we were unable to place our hydrophones on the bottom substrate. It would be important for future stud-

ies to conduct advanced sound propagation analysis at Eel Pond to understand how vessel sound propagates in the 3D space.

In summary, our study characterized and monitored a population of oyster toadfish and assessed the impact of *in situ* vessel sound on male calling behavior. Eel Pond provided a unique study location, as the drawbridge restricted RV activity to set times during the week at the same location. We found that vessel sound has the ability to decrease calling behavior of toadfish and potentially mask boatwhistles. This study adds to the growing literature on the negative impacts of anthropogenic sound and a new area of research on the effects of *in situ* vessel sound on behavior.

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