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Localizing individual soniferous fish using passive acoustic monitoring

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ABSTRACT

Identifying where fish inhabit is a fundamentally important topic in ecology and management allowing acoustically sensitive times and areas to be prioritized. Passive acoustic localization has the benefit of being a non-invasive and non-destructive observational tool, and provides unbiased data on the position and movement of aquatic animals. This study used the time difference of arrivals (TDOA) of sound recordings on a four-hydrophone array to pinpoint the location of male oyster toadfish, *Opsanus tau,* a cryptic fish that produces boatwhistles to attract females. Coupling the TDOA method with cross correlation of the different boatwhistles, individual toadfish were mapped during dawn (0523–0823), midday (1123–1423), dusk (1723–2023) and night (2323 – 0223) to examine the relationship between temporal and spatial trends. Seven individual males were identified within 0.5–24.2 m of the hydrophone array and 0.0–18.2 m of the other individuals. Uncertainty in passive acoustics localization was investigated using computer simulations as < 2.0 m within a bearing of 033 to 148° of the linear hydrophone array. Passive acoustic monitoring is presented as a viable tool for monitoring the positions of soniferous species, like the oyster toadfish. The method used in this study could be applied to a variety of soniferous fishes, without disturbing them or their environment. Understanding the location of fishes can be linked to temporal and environmental parameters to investigate ecological trends, as well as to vessel activity to discuss how individuals' respond to anthropogenic noise.

1. Introduction

Passive acoustic monitoring underwater has improved understanding of the repertoire and temporal distribution of soniferous aquatic animals. Many ecological applications would gain substantial benefits from knowing an animal's location (Spiesberger and Fristrup, 1990). The location of soniferous animals can also be linked to time of day, habitat type, salinity and temperature to investigate ecological trends, or used to monitor how individuals respond to anthropogenic sound, such as vessel traffic. As such, passive acoustic localization increasingly is used to locate soniferous animals, such as fish or marine mammals (Gebbie et al., 2015; Locascio and Mann, 2011; Mann, 2006; Spiesberger and Fristrup, 1990), which are difficult to observe using traditional visual methods. It also has the benefit of being a non-invasive and non-destructive observational tool, unlike underwater diver surveys (Barimo and Fine, 1998) or mark recapture studies (Marques et al., 2013), and provides unbiased data on the position and movement of the sound source in question.

Sound can propagate great distances in all directions underwater without the signal losing considerable energy (Urick, 1983). Acoustic localization uses the mathematics of acoustic propagation and parabolic geometry to determine source positions. Using one hydrophone, the distance to a sound source can be estimated from the amplitude and arrival times of the direct and surface reflected signals (Aubauer et al., 2000; Cato, 1998). Adding a second hydrophone, the bearing to a source can be calculated using the time difference of arrivals (TDOA) (Spiesberger and Fristrup, 1990). At least three hydrophones are needed to pinpoint exact source location because multiple TDOA bearings can be calculated and intersected (Møhl et al., 2001; Spiesberger and Fristrup, 1990; Wahlberg et al., 2001; Watkins and Schevill, 1972). Hydrophone arrays potentially can determine fish distributions that could not be obtained with single hydrophone recordings, but require a higher level of sophistication for setting up, operating and analyzing the data (Ricci et al., 2017).

Many fish sounds are species specific and repetitive, which enables passive acoustic recordings of sound production to be used to identify their distribution and behavior (Wall et al., 2013). Batrachoidid fishes (toadfish and midshipman) produce sounds through contractions of sexually dimorphic sonic muscles attached to the swimbladder, and are some of the best studied vocal fishes (Amorim et al., 2015; Bass and McKibben, 2003). The oyster toadfish, *Opsanus tau*, is a benthic ambush predator that inhabits estuaries and coastal waters along the

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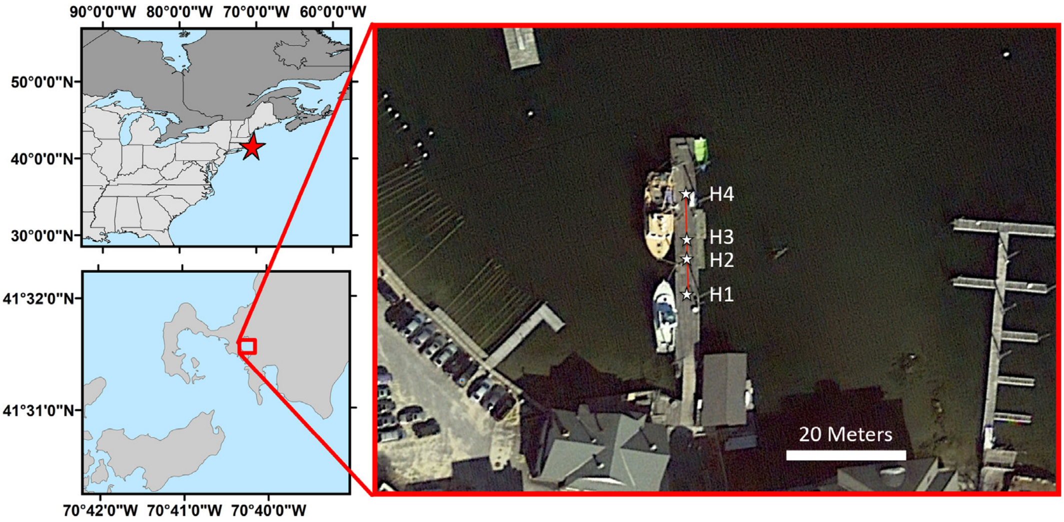


Fig. 1. Map of Eel Pond, Woods Hole, MA, with insets showing position related to state and country. The four hydrophones deployed along the dock are indicated by the white stars. Google ortho imagery 2014 was downloaded from the MassGIS website (https://www.mass.gov/service-details/massgis-data-layers/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

northeastern seaboard of the United States (Price and Mensinger, 1999). The toadfish has an unusually rich vocal repertoire for a teleost, produced by fast contracting sonic muscles along the swimbladder (Rome and Lindstedt, 1998). Both sexes of toadfish produce a variety of grunts associated with agnostic contexts while only males produce boatwhistles which have an initial broadband grunt-like segment, followed by a tonal portion (Maruska and Mensinger, 2009). At the beginning of the mating season, in late May to early June, male toadfish establish a nest and produce trains of boatwhistles to announce territorial ownership and position to other males as well as attract females into their nests (Fish, 1972; Winn, 1972).

Individuality in acoustic signaling arises when the within individual variation is smaller than the variation between individuals in one or more acoustic characteristics (Bee and Gerhardt, 2001). Differences in waveform, sound duration and distribution of energy in different harmonic bands can therefore identify different individuals. In southern Portugal, five individual lusitanian toadfish, *Halobatrachus didactylus,* were recorded, each with distinct boatwhistles (Dos Santos et al., 2000). Additionally, toadfish were found to produce vocalizations varying in pulse structure, duration and frequency components, suggesting toadfish have a complex acoustic communication system (Maruska and Mensinger, 2009). Acoustic signals may inform the receiver about species, sex identity, the sender's location, motivation and individual quality (Forlano et al., 2017). The calling rate and calling effort (percentage of time spent calling) of Batrachoididae has been found to indicate male condition (Vasconcelos et al., 2012) because these parameters reflect sonic muscle hypertrophy and larger gonads (Amorim et al., 2010). Sound dominant frequency, amplitude and fatigue resistance may also indicate body size (Bose et al., 2018), with larger fish tending to produce lower frequency, louder and longer sounds than smaller individuals (Conti et al., 2015). Additionally, boatwhistles are involved in male competition, as closely located individuals will produce "jamming" signals. For example, a male will produce a grunt during the tonal portion of the conspecific male boatwhistle that lowers the first harmonic to a rate that is unattractive to a female, preventing competing males from attracting females (Mensinger, 2014).

Despite the large number of experimental studies on toadfish vocalizations, surprisingly little is known about the occurrence and parameters of natural calls (Conti et al., 2015) and even less on the proximity of individual males. Previous studies have used invasive methods, such as locating and recording boatwhistles with SCUBA divers (Barimo and Fine, 1998) or restricting toadfish movements by placing individuals within artificial shelters (Zeddies et al., 2012). In comparison, fixed and towed hydrophones are now a popular tool for localizing fishes. This non-invasive monitoring provides long-term continuous information on animal behavior, abundance and calling measurements in settings that are otherwise difficult to sample (Ricci et al., 2017).

A naturally occurring population of toadfish is found in Eel Pond, MA with high site fidelity from May to August. The toadfish population in Cape Cod is at the northern extent of the population range. Toadfish were thought to be extirpated from Eel Pond since at least 1990 however, during hydrophone testing in 2014 boatwhistle calls were detected (Van Wert *per comms.*). Whether these toadfish migrated into Eel Pond or had escaped from the Marine Resources Center is not clear and the population number is unknown. Additionally, as a single hydrophone consistently picked up distinct boatwhistles from the dock area, it was hypothesized that the toadfish were confined to this physical structure (Van Wert *per comms.*). Individual male toadfish exhibit high site fidelity and only change vocalizations incrementally over the course of several days, allowing individual fish to be tracked for extended periods of time (Mensinger, 2014) and making toadfish an ideal study species for acoustic localization. The aim of the present study is to localize the position of individual nesting toadfish using recordings of their boatwhistles, and test the proximity of individual nesting males using a non-invasive method.

2. Materials and methods

2.1. Data collection

Oyster toadfish, *Opsanus tau,* vocalizations were recorded in situ from beneath the Marine Biological Laboratory (MBL) Marine Resources Center dock in Eel Pond, Woods Hole, MA (41° 31′32.28" N 70°40′16.74" W) (Fig. 1) northeastern USA, from Saturday July 8 14:23 to Sunday July 9, 2017 14:23. Recordings were taken during July, as this is within the peak calling period for the species in Eel Pond (Van Wert *per comms.*). The recordings were conducted over a weekend because dock access is restricted for the public and the large MBL research vessel moored at the dock does not operate. Small recreational vessel sounds were present in recordings (Fig. 2); but had minimal interference with acoustic analysis of toadfish vocalizations.

A four-channel digital acoustic recorder (ST4300, Oceans

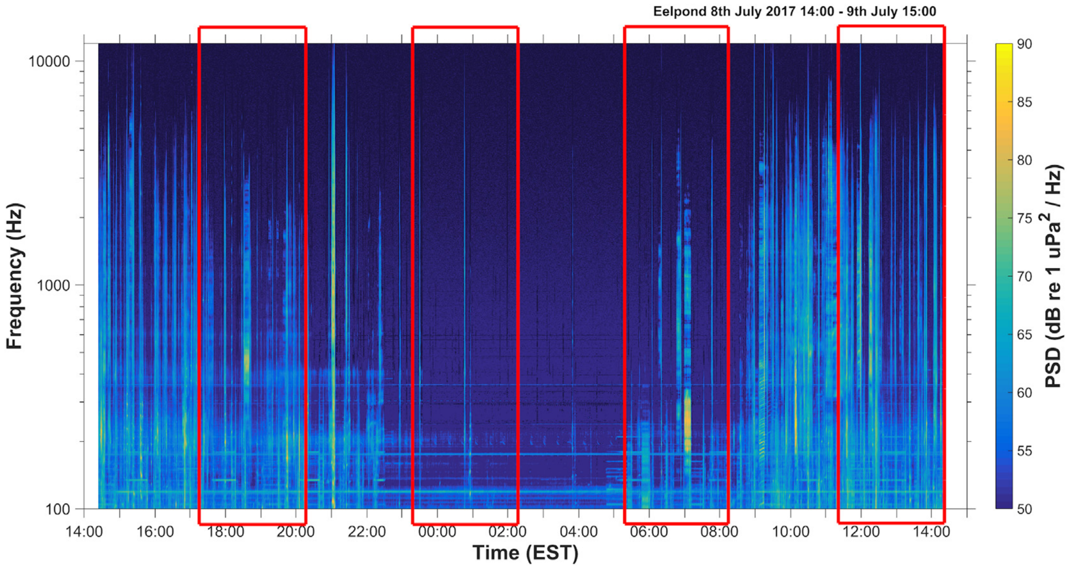


Fig. 2. Spectrogram of one full day of recording (8th July 2017 14:00 to 9th July 2017 15:00) from Eel Pond, Woods Hole, MA, with the colorbar representing power spectral density (dB re 1μPa^2/Hz), produced using FFT length = 512 points, Hanning window and 50% overlap. The four red boxes indicate the four three-hour recordings used for boatwhistle analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Instruments, NZ) was attached to four hydrophones (HTI 96 min, High Tech Inc., USA) programmed to sample at 24,000 Hz, 16 bits, continuously for the duration of the deployment. The four hydrophones all had a flat frequency response between 2 and 30,000 Hz with sensitivities of 165.4, 165.0, 165.1, and 164.9 dB re 1 V/μPa respectively.

The four hydrophones (h1, h2, h3, h4) were deployed in a linear array 5.6 (h1 to h2), 3.0 (h2 to h3) and 8.1 (h3 to h4) meters apart respectively along the dock, with h1 23.2 m from shore and h4 39.9 m from shore. These distances were chosen in anticipation that individual toadfish boatwhistles produced near the dock would likely be recorded on a minimum number of three hydrophones required for localization. The aperture of the array also needed to be the same order of magnitude as the range to be covered (Møhl et al., 2001). Owing to the fact the hydrophones were placed in a linear array underneath the dock (Fig. 1), there would be a right-left ambiguity (Spiesberger and Fristrup, 1990) in calculated toadfish locations. The area to the northwest (or left) of the MRC dock is shallower than the southeast side (right) and is delineated by a seawall and numerous small recreational watercraft. A greater proportion of the northwest section is also exposed at low tide and no toadfish have been observed under the very limited hard substrate when exposed by tidal conditions. Therefore, it was hypothesized that all toadfish locations would be under or southeast of the dock. Calculated toadfish locations were therefore limited to $x > 0$.

All four hydrophones were mounted 1.0 m from the water surface, the dock moved with the tide so depth from the water surface remained consistent throughout recording. The water depth under the dock was 2.4–3.4 m depending on tidal conditions and the pond bottom consisted primarily of soft sediments interspersed with rocky substrate. A theoretical cut-off frequency (~185 Hz) (below this sounds cannot be accurately recorded) for the study area was calculated using the absolute cut-off frequency equation, with the velocity for sound propagation in a soft sediment substrate (1600 ms^{-1}) (Hamilton and Bachman, 1982) and 3.4 m water column (Rogers and Cox, 1988; Tindle et al., 1978). Temperature was recorded by a Hobo® Pendant model (± 0.1 °C), attached to the acoustic recorder and used to calculate the sound speed of the water during each three-hour recording period (Del Grosso, 1974). Temperature varied by 5.6 °C over the course of the 24-h period at the water surface and by 2.0 °C at the pond bottom. The water column was well mixed (not stratified) and atmospheric conditions were clear and calm during recordings.

2.2. *Identification of boatwhistles*

Four three-hour sound recordings (0523–0823, 1123–1423, 1723–2023 and 2323–0223) were reviewed aurally and visually using a scrolling spectrographic display of 10 s (Hanning window, FFT length = 512 with 50% overlap, providing a frequency resolution of 46.8 Hz, and a time resolution of 0.4 ms) in Raven Pro 1.5.0 software. These times were chosen to include dusk (sunset – 2018), night (covering 0000), dawn (sunrise – 0517) and day (1200).

The number and timing of all boatwhistles [defined as a distinct initial grunt component preceding a tonal segment between 10 and 2000 Hz (Maruska and Mensinger, 2009)] were annotated. Primarily, to differentiate individual males, a combination of waveform shape, spectrogram composition and relative amplitude was used (Fig. 3) Spectrogram shape was most useful with visible differences in the waveform (for example TF 3 and TF 7) (Supplementary Figs. S3 and S7). Amplitude differences was used because individual toadfish only produce boatwhistles of very consistent sound pressure level (SPL) throughout 24 h periods and rarely move from their habitat during vocalizations (Mensinger, 2014). Therefore, the hydrophone would consistently record the same SPL. The number of harmonics was found to not be a reliable indicator of different individuals because higher frequencies attenuate quickly over short distances and so were not detected by the hydrophone (Urick, 1983). After visual analysis, to confirm boatwhistles were from the same toadfish, the call duration was measured and compared between calls. It was shown from saltwater pond recordings of toadfish, at known locations, that the call duration of boatwhistles did not change over the course of an evening and only incrementally over the course of consecutive days (Mensinger, 2014). Twenty random boatwhistles of each toadfish (taken from hydrophone 1) were cross correlated in MATLAB software (version 2014a), which outputted a matrix of the maximum correlation score for each pairing. All boatwhistles for cross correlation were taken from the same three-hour period to minimize the effect of water temperature on pulse repetitive rate. Additionally, each boatwhistle was extracted within a 5 s clip to run cross correlation to ensure both the leading grunt and tonal portion (Maruska and Mensinger, 2009) were included in analysis.

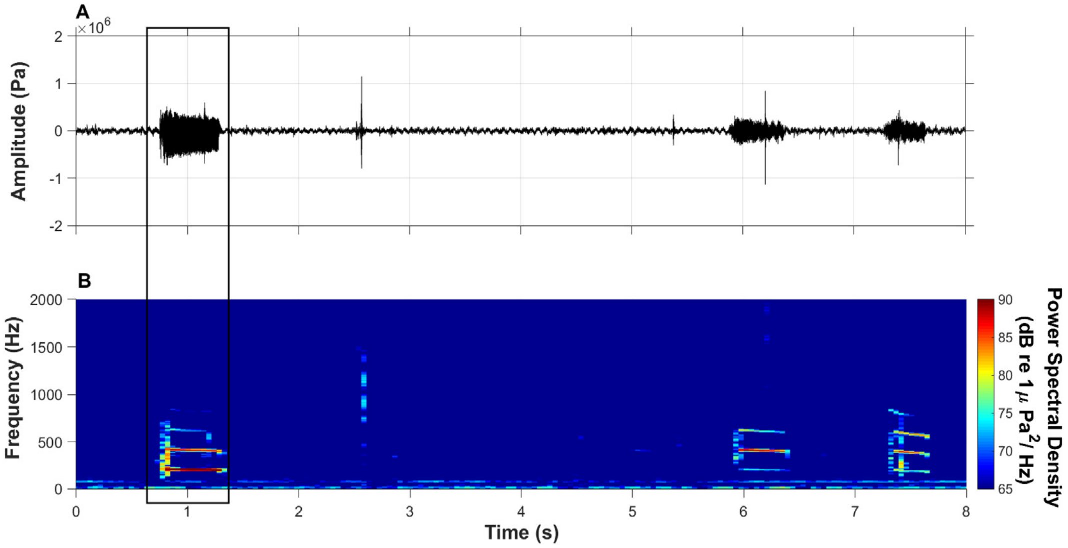


Fig. 3. Three toadfish boatwhistles (from left to right toadfish 3, toadfish 6 and toadfish 5) taken from the 1723–2023 recording, A) Waveform of the signal, B) Spectrogram of the signal between 10 and 2000 Hz with the colorbar showing power spectral density (dB re 1μPa^2/Hz). The black box indicates the boatwhistle (toadfish 3) used in Fig. 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Localization of boatwhistles

The waveform of each boatwhistle was analyzed in LabChart (version 8) using a scrolling display of 1 s (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) on the four hydrophones. TOAs were standardized as the instance of the tallest point in the first oscillation of the waveform during the boatwhistle (Fig. 4). Time difference of arrivals (TDOAs) for boatwhistles at hydrophones 2, 3 and 4 (d2, d3, d4) were determined by expressing times relative to the TOA at hydrophone 1 (d1). The four hydrophone positions were converted into vector coordinates (h2x, h2y, h3x, h3y, h4x, h4y) using hydrophone 1 (h1x, h1y) as the origin reference (0,0).

Individual toadfish were then localized using the TDOA method established by Watkins and Schevill (1972) and developed by Spiesberger and Fristrup (1990) (more information in appendix). To investigate the uncertainty associated with the estimated X Y location of the source, a Monte Carlo computer simulation placed the four hydrophones in a linear (180°) array with a separation replicating the distances between hydrophones used in the field experiment. The source was placed 5 m away and moved from 0 to 360° (in 1° steps) around the hydrophone placed at the origin. In the simulation the arrival time uncertainty was taken as +/− 0.001 s to account for human uncertainty in manually selecting the TOA at each hydrophone. The model was run 1000 times, each time using different randomly selected values for the arrival time uncertainty at each of the four hydrophones. The output accuracy was calculated as the mean Euclidean distance between the artificially placed sound source and calculated source position, as well as standard deviation. To investigate proximity of toadfish the distance between epicenters of each identified individual was calculated.

3. Results

3.1. Identification of boatwhistles

Seven unique boatwhistles were identified through spectrographic analysis (Supplementary Figs. S1–S7 and Table 1). The highest number of boatwhistles from a single source was identified as coming from toadfish (TF) 2 (28.7%). When each boatwhistle was correlated (e.g. TF 1 against other TF 1) median cross correlation scores were between 0.70 and 0.83 (95% confidence interval), whereas when different boatwhistles were correlated (e.g. TF 1 against TF 4) median cross correlation scores ranged between 0.11 and 0.52 (95% confidence interval) (Fig. 5). Very few boatwhistles were observed to overlap however no clear pattern of call sequence was noted in calling individuals (Supplementary Fig. S8). However, it was noted that TF 1, TF 2, and TF 7 would often follow each other, as would TF 3 and TF 4. Interestingly TF 1, TF 2, and TF 7 had the closest similarity according to cross correlation (0.50–0.69) as did TF 3 and TF 4 (0.52–0.68) (Fig. 5).

3.2. Localization of boatwhistles

Using the TDOA method 1826 boatwhistles were localized (Table 1) and only 25 (1.4%) boatwhistles could not be localized as the signal was obscured by noise on one or more of the four hydrophones. In Eel Pond, the highest number of boatwhistles were detected during the dusk (41.3%) and night (52.3%) recordings (Fig. 6, Table 1), with an increase in the number of boatwhistles occurring around 1830 (Fig. 7). At dusk, toadfish 1 through 4 were also detected most often during the study period (Table 1). Individual toadfish were consistently found in the same areas during the four different recording periods, with all occurrences within a 1.4, 1.1, 2.4, 2.1, 0.8, 1.0 and 0.8 m radii of the epicenter (Fig. 6). Additionally, using the epicenter of each individual toadfish, TF 1, TF 2, and TF 7 were within 4.8 m of each other, TF 3 and TF 4 were within 3.4 m of each other and TF 5 and TF 6 were within 3.2 m of each other (Fig. 6). TF 4 and TF 6 were found to be positioned frequently at x = 0, indicating they were found directly beneath the dock where the hydrophones were deployed. Unidentified boatwhistles (Fig. 6A, B) were localized at scattered positions throughout the 400 m^2 area. Uncertainty associated with the estimated X Y location of the source was calculated as < 2.0 m within 033 to 148° (Fig. 8).

4. Discussion

The hydrophone array successfully located the positions of seven vocalizing male oyster toadfish, *Opsanus tau,* in Eel Pond. Call number varied throughout the day with higher numbers of boatwhistles during dusk and night recordings. The methodology used in this study has the potential to track individual toadfish or other cryptic soniferous fish to determine spatial temporal variation in boatwhistle production.

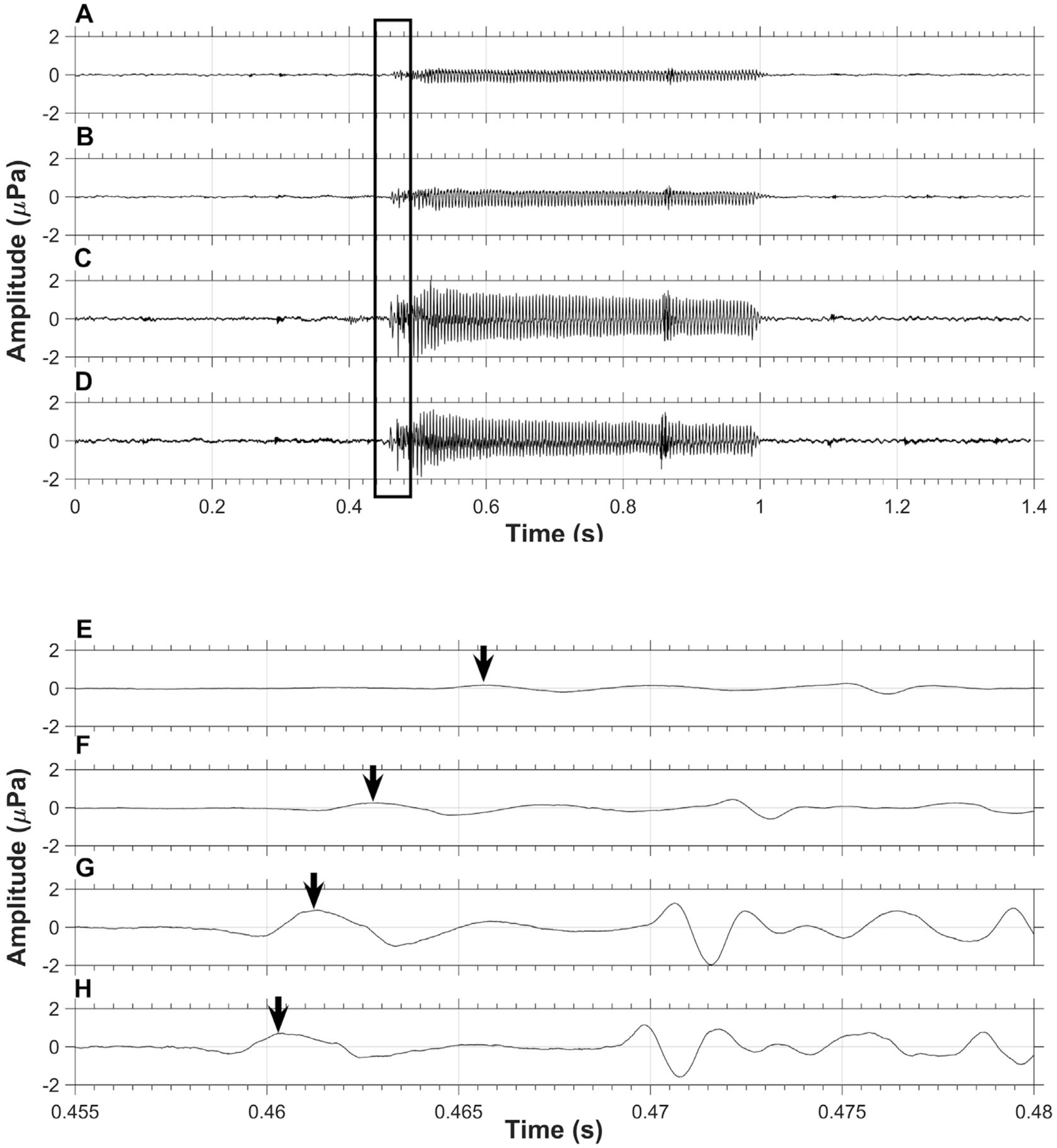


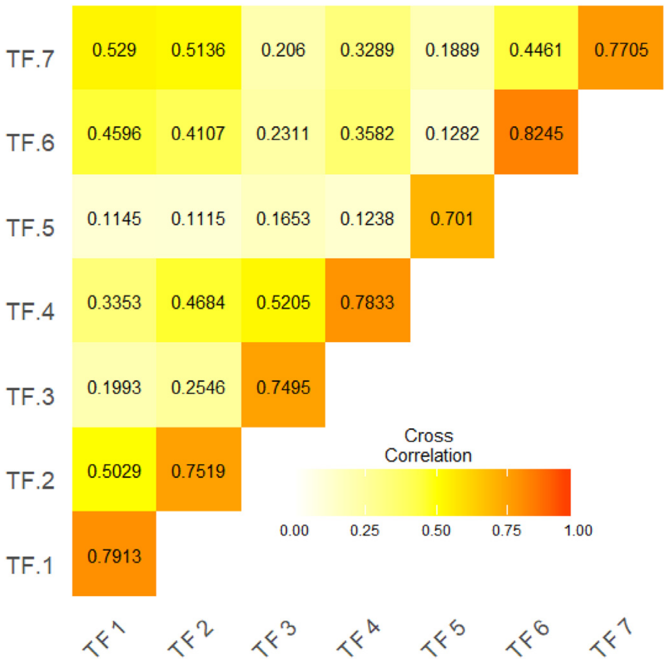
Fig. 4. A–D) Waveform of toadfish 3 as recorded by each of the four hydrophones (h1, h2, h3, h4). The black box represents the section of the waveform used in E–H, which shows a zoomed in view of the waveform as recorded by each of the four hydrophones. The arrows on E–H indicate the time of arrival of the toadfish boatwhistle at each hydrophone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Additionally, by localizing nesting males and monitoring the soundscape of the area, the effect of exposure to anthropogenic sound could be investigated.

Localization of individual toadfish was based on solving a set of hyperbolic equation each described by a pairwise difference in the time of arrival at three of more hydrophones. Uncertainty in the TDOA calculation varies depending on the sound velocity of the medium, whether ambient noise or other interference masks the signal (Aubauer et al., 2000) and hydrophone position (Spiesberger, 1999). For shallow water environments the sound velocity is relatively homogenous, so the associated uncertainty caused by signal distortion is negligible (Clay and Medwin, 1977). However, erroneous registration on just one hydrophone can potentially offset the estimated position by many meters, even if the signal was detected correctly at the other hydrophones in the array (Baktoft et al., 2017). In terms of hydrophone positions, the TDOA model performs well inside of a hydrophone array, but estimation deteriorates outside (Spiesberger, 1999). Some sophisticated studies of the uncertainty in calculation have investigated the effect of sound velocity

Table 1
Number of boatwhistles detected in each recording and number identified as each different toadfish (TF) boatwhistle.

Sound recording	Total number of boatwhistles	Unable to localize	Unable to identify	TF 1	TF 2	TF 3	TF 4	TF 5	TF 6	TF 7
1723–2023 8th July 2017	760	10	186	90	214	69	123	22	11	35
2323–0223 8th July 2017	963	1	301	93	271	118	113	24	11	31
0523–0823 9th July 2017	32	0	6	5	7	4	6	4	0	0
1123–1423 9th July 2017	84	2	6	16	35	4	6	10	4	1
Total	**1839**	**25**	**487**	**204**	**527**	**195**	**248**	**60**	**26**	**67**



	TF 1	TF 2	TF 3	TF 4	TF 5	TF 6	TF 7
TF.7	0.529	0.5136	0.206	0.3289	0.1889	0.4461	0.7705
TF.6	0.4596	0.4107	0.2311	0.3582	0.1282	0.8245	
TF.5	0.1145	0.1115	0.1653	0.1238	0.701		
TF.4	0.3353	0.4684	0.5205	0.7833			
TF.3	0.1993	0.2546	0.7495				
TF.2	0.5029	0.7519					
TF.1	0.7913						

Fig. 5. Cross correlation matrix of the seven-different toadfish boatwhistles. Twenty random boatwhistles chosen for each. The colorbar and number on each panel represents the median cross correlation index. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and receiver position within field recordings (Barlow and Griffiths, 2017; Cato, 1998; Thode et al., 2004; Wahlberg et al., 2001). For example, sperm whale clicks were localized with a precision of 2–138 m using an array of three hydrophones (Møhl et al., 2001). Accuracy of the TDOA localization method used in this study was calculated as < 2 m within 033 to 148° of the confines of the linear array. It is important to note that field verification of the methodology would be the next step in this research: placing a known sound source at a fixed location to understand how the theoretical data quality translates to what is possible to achieve in the field.

Using a linear array there was also right-left ambiguity in the calculated source positions. An assumption in this study was that all toadfish were located to the right or southeast of the dock with a restriction that $x > 0$. Two toadfish (TF 4 and TF 6) were found to be located at $x = 0$, suggesting they were positioned directly underneath the dock and hydrophones (Fig. 6). The substrate of Eel Pond is characterized by fine silt and overhead views during periods of peak water clarity reveal only a few large rocks visible in the shallow water (< 2 m) which extends to approximately half the dock length. Vocalizing toadfish seek hard substrate therefore the dock provides suitable habitat. However, Eel Pond is also an active marina and rocks or other detritus may be available in the open water and the localization showed that several of the toadfish were away from the dock. Testing detection methods and validating localization techniques are both necessary for understanding the accuracy of individuals positions before it is passed on as evidence for management. Future experiments would position four hydrophones at the same depth in a “T shaped” array with the reference hydrophone at the origin, because combining a right angled and linear array would reduce uncertainty in calculations with full 360° coverage (Putland et al., 2016; Spiesberger and Fristrup, 1990).

Another method researchers have used to collect information on fish movements is active acoustic telemetry. An ultrasonic transmitter is surgically implanted or attached externally to the fish and an acoustic receiver listens for the transmitted signal, recording the tagged fish's unique code along with the date and time. In the same way as the passive telemetry described in this study, an array of closely located receivers uses the TDOA method to localize individuals with a potential precision of 1–2 m (O'Dor et al., 2011). The advantages of active telemetry are that multiple fish can be tracked simultaneously and tags can be fitted with sensors capable of recording swimming speed, swimming direction and heart rate (Thorsteinsson, 2002) which could improve knowledge on the energetic costs associated with migration or reproductive seasons (Cooke et al., 2008). However, acoustic transmitters typically operate at a frequency between 30,000–300,000 Hz (Thorsteinsson, 2002) and the distance at which a transmitter can be detected depends upon the power and frequency of the tag, array geometry and array spacing. Transmitters and receivers are also relatively expensive, and because of proprietary interests, transmitters made by one company cannot be detected by receivers made by another company, which may lead to foregone opportunities to collect data on the movements of a tagged fish (Grothues, 2009). Using passive acoustic telemetry there is also no human interaction with the study fish. External attachment of tags can cause scale abrasion and muscle damage (Bridger and Booth, 2003) and the tag placement may affect the swimming ability of some fish. Additionally, internal tags are not suitable for many species because anesthetics are required (Bridger and Booth, 2003), and signal attenuation can occur when transmitters are implanted into a very large fish (Thorsteinsson, 2002).

The main advantage of using passive acoustics to monitor toadfish boatwhistles (or another reproductively active fish), is that the nest sites were localized and subsequent divers or cameras could be used to locate each nest and check for egg number which would be difficult in murky conditions without approximate nest locations. Furthermore, in Eel Pond, passive acoustics offered an opportunity to estimate a previously uncharacterized toadfish population. Seven different boatwhistles were identified in this study, distinct in terms of waveform (amplitude modulation) and spectral characteristics, suggesting at least seven individual male toadfish were resident in the 300 m^2 study area within Eel Pond. The seven different boatwhistles were also successfully localized using the TDOA method which revealed three different clusters of vocalizing toadfish. TF 1, TF 2, and TF 7 were within 4.8 m of each other, TF 3 and TF 4 were within 3.4 m of each other and TF 5 and TF 6 were within 3.2 m of each other (Fig. 6). However, these three clusters showed the closest similarity in terms of cross correlation (Fig. 5), so there is the possibility that the identified locations for each cluster could be of one individual.

In this study, data was processed manually to calculate TDOAs and separate different toadfish, as such it was suited to researching the four-time slots chosen within a one-day period. Long term passive acoustic monitoring efforts have recently become more prevalent to monitor fish and other aquatic organisms. Yet, large datasets associated with long term passive acoustic monitoring can be impractical to manually process (Hartog et al., 2009), necessitating the development of automated detection methods to screen for different toadfish boatwhistles and select the TDOA. Existing methods for automated detection of bioacoustics signals includes energy summation, matched filtering, spectrogram correlation and hidden Markov models (HMMs) (Mellinger, 2004; Munger et al., 2005; Putland et al., 2018b; Ren et al., 2009). For example, cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) both have stereotyped calls that have been used to train a detection algorithm based on fundamental frequency, sound duration and number of pulses (cod) or knocks (haddock) (Urazghildiiev and Parijs, 2016). This detector was subsequently used to estimate how fish communication would be affected by overlapping man-made sound (Stanley et al., 2017). Further work would use the dataset collected in this study to train an automated detector before investigating spatial variation of toadfish nesting sites over a longer time period at Eel Pond. A HMM would be recommended because it can account for changes in acoustic characteristics over time (Ren et al., 2009). Over seasonal time frames, boatwhistle pulse repetitive rate (PRR) can change with temperature because the central pattern generator that drives sonic muscle contraction is influenced by temperature (Bass and Baker, 2004), with the

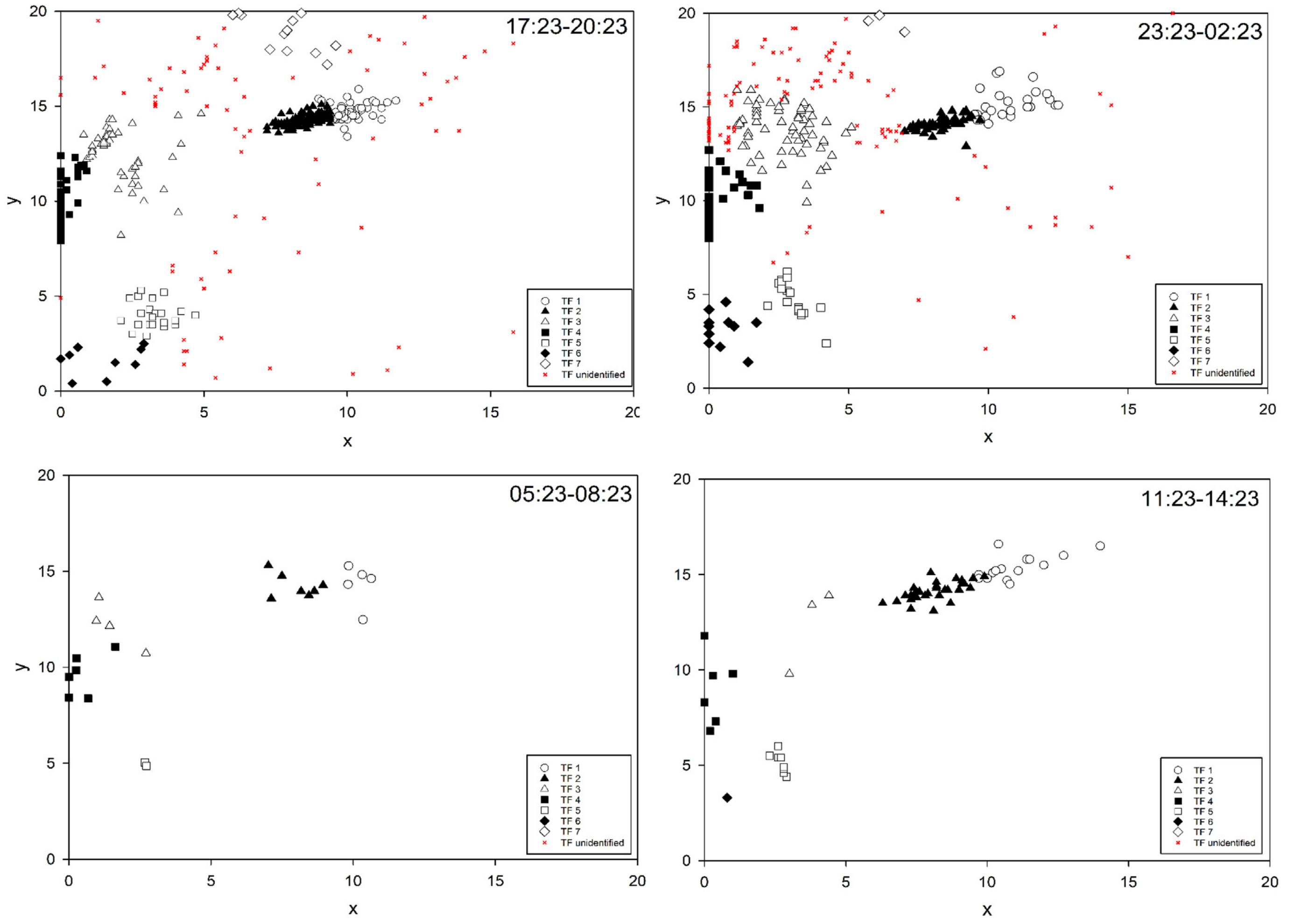


Fig. 6. Maps of the seven-different toadfish (TF) during A) 1723–2023, B) 2323–0223, C) 0523–0823 and D) 1123–1423. TF 1 represented by open circles, TF 2 by closed triangles, TF 3 by open triangles, TF 4 by closed squares, TF 5 by open squares, TF 6 by closed diamonds, TF 7 by open diamonds and unidentified toadfish boatwhistles are shown by red crosses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PRR of oyster toadfish boatwhistles increasing by 11 Hz for every 1 °C increase in temperature (Ricci et al., 2017). As such, seasonally specific male toadfish locations would need to be validated using an additional hydrophone close to the expected nesting sites. Over the 24-h period, temperature varied by 2.0 °C at the pond bottom, suggesting PRR of the boatwhistles may have varied and subsequently measures of PRR were not taken into consideration when assessing individuals. Differences in frequency would not impact the TDOA method used in this study as it took the peak in the first waveform to be the time of arrival. Moreover, the cross-correlation technique considered the duration of the boatwhistle in addition to the shape of the waveform to make objective rather than subjective assessments. However, future passive acoustic

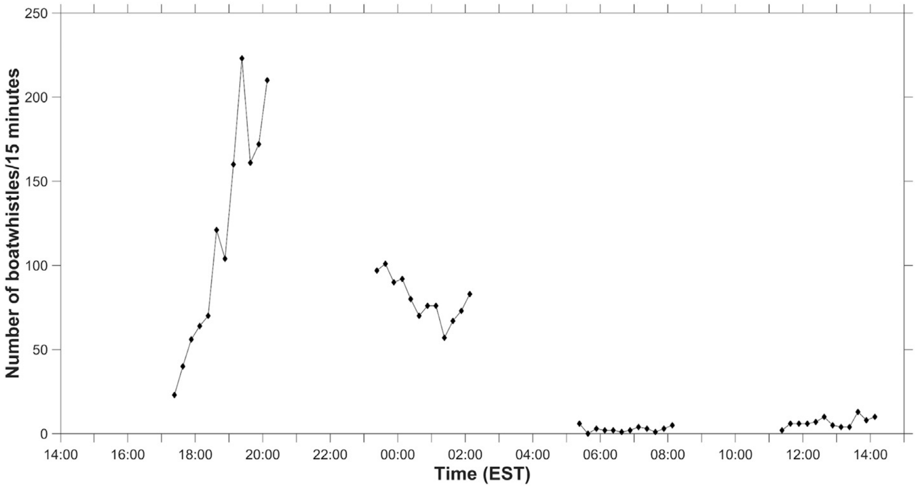


Fig. 7. Total number of boatwhistles identified every 15 min over the four three-hour recordings analyzed.

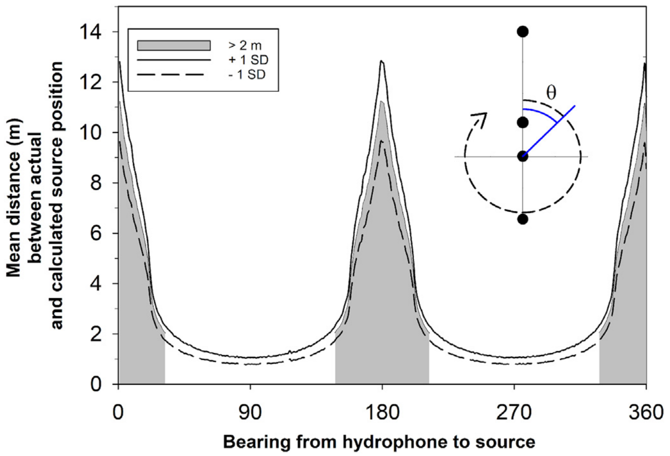


Fig. 8. Mean Euclidean distance between actual and calculated source position (m) +/−1 standard deviation (SD) when a computer simulation changed the bearing from hydrophone to source between 0 and 360°. The four hydrophones were placed in a 180° geometry with a separation of 5.6, 3.0 and 8.1 m to replicate the field investigation, and the source at 5 m away. The shaded region below the line indicates when the mean distance between actual and calculated source position was > 2 m.

monitoring is warranted to monitor caged individuals and correlate temperature and PRR of boatwhistles produced.

The communication space of oyster toadfish has previously been speculated as ~10 m during recordings taken in Florida (Fish, 1964), but water depth and sediment type was unspecified. A communication space of ~5 m was given for toadfish in 1-m water depth with sandy-silt substrate (Fine and Lenhardt, 1983). However, it was stated by the authors of this study that some of the boatwhistle frequencies would be below the absolute cut-off frequency (~1000 Hz) (Fine and Lenhardt, 1983) meaning theoretically acoustic propagation cannot be measured and accurate recordings of sound cannot be taken (Officer, 1958). To accurately investigate an animal's communication space, the acoustic behavior (source level, frequency range and/or hearing threshold) of the species in question the local sound propagation conditions must be understood (Putland et al., 2018a). Propagation of low frequencies in shallow waters is a very complex phenomenon where refraction and reflection will play an important role (Bass and Clark, 2003; Mann, 2006). Water depth in Eel Pond was < 3.4 m and toadfish boatwhistles have a pulse repetitive rate of ~200 Hz (λ ~7.5 m), meaning that sound propagation will directly be impacted by the surface and bottom reflections because of the frequency cut-off phenomenon (Rogers and Cox, 1988). Vertically separated hydrophones should be used in future research to account for modal structures and dispersion associated with the complex boundary conditions and the properties of the substrate must be considered (Locascio and Mann, 2011).

The radiation pattern of the sound source, in this case the toadfish will also influence sound attenuation. The sound producing swimbladder in toadfish was described as a complex mixed sound radiator with monopole, dipole and quadrupole components (Fine et al., 2001). The acoustic near field of such a source (usually up to $\lambda/2\pi$ meters) can be quite complex with acceleration, velocity, net fluid displacement and sound pressure decreasing faster than expected for a geometric spreading model [6 dB per doubling distance (Bass and Clark, 2003)]. This may explain why in previous studies, the amplitude of toadfish boatwhistles decreased rapidly very close to the sound producing fish, with a steep slope in the first few meters, while further afield the attenuation in lower and becomes more uniform (Alves et al., 2016). For example, the amplitude of gulf toadfish, *Opsanus beta,* boatwhistles reduced by 22 dB within 2.5 m of the source (in 1 m water depth) (Remage-Healey and Bass, 2006). The acoustic adaptation hypothesis states that individuals have structural adaptations to permit the continued use of acoustics in the habitat (Hopkins, 1988). For example, high source levels of Batrachoididae (such as 135 dB re 1μPa) vocalizations may allow longer sound propagation (Jordão et al., 2012).

Scientists and managers are concerned about the effect of anthropogenic sound on aquatic life as it may affect communication, behavior, fitness and reproductive success. Eel Pond is connected to the Woods Hole channel by a narrow canal with a drawbridge which allows both small recreational motorboats and larger commercial fishing vessels to enter the area. Toadfish may subsequently change their behavior in response to increasing amounts of sound. For example, repeated exposure to vessel sound was found to affect parental behavior, including feeding, nest maintenance and defense in the spiny chromis, *Acanthochromis polycanthus,* and thereby reduced the likelihood of offspring survival (Nedelec et al., 2017). It would therefore be interesting to investigate if toadfish choose nesting sites based on the ambient soundscape of the area (geological, biological and anthropogenic sounds), by mapping the area over multiple years to distinguish if the breeding area is changing over time in response to harbor development. Additionally, in a disrupted soundscape, when individuals remain in proximity to sound, there is evidence that some fish species attempt to compensate for exposure by altering the amplitude, frequency or duration of the sounds they produce to maintain a constant signal to noise ratio (Radford et al., 2014). It was found that toadfish increased the power spectral density of boatwhistles by 6.8 dB during and 8.7 dB re 1μPa after playback of inboard and outboard motor noise in estuarine areas of North Carolina (Luczkovich et al., 2016). Preliminary studies in Eel Pond suggest that toadfish produce fewer boatwhistles post exposure to boat noise. However, the effect of anthropogenic sound on fish acoustics is difficult to determine without knowing the exact position of the individual. By localizing the nest location and monitoring the soundscape of the area, changes in acoustic behavior could be correlated to exact sound exposure levels of anthropogenic sound.

5. Conclusion

Passive acoustic localization successfully allowed individual differences in call amplitudes, waveforms and spectra to be identified, and provided the location of individual toadfish within Eel Pond, MA. The method used in this study could be used to identify soniferous fish in other shallow water environments. Knowing when, where, and how often animals are producing sounds would also allow acoustically sensitive times and area to be prioritized during management strategy.

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Competing financial interests

None.

Data accessibility

Following publication, the data will be made available on the Dyrad Digital Repository.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoinf.2018.08.004.

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