# Localizing individual fish using passive acoustic monitoring

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Running head: Acoustic localization of fishes

## 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 of arrival difference (TOAD) of sound recordings on a four-hydrophone array to pinpoint the location of male oyster toadfish, *Opsanus tau,* a sedentary fish that produces boatwhistles to attract females. Coupling the TOAD 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 acoustically sensitive 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.

## Highlights

- Passive acoustic localization pinpointed the location of toadfish, *Opsanus tau*
- The methodology was based on time of arrival differences and cross correlation

- Seven individual boatwhistles/toadfish were localized
- The method described is useful to investigate ecological trends
- Identifying acoustically sensitive areas can inform management decisions

**Keywords:** Passive acoustic monitoring, localization, fish ecology, environmental management

## 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 (Spiesberger and Fristrup, 1990; Mann, 2006; Locascio and Mann, 2011; Gebbie, 2015), 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 (Cato, 1998; Aubauer, 2000). Adding a second hydrophone, the bearing to a source can be calculated using the time of arrival difference (TOAD) (Spiesberger and Fristrup, 1990). However, at least three hydrophone are needed to pinpoint exact source location because multiple TOAD bearings can be calculated and intersected (Watkins and

Schevill, 1972; Spiesberger and Fristrup, 1990; Møhl *et al.*, 2001; Wahlberg *et al.*, 2001). 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 (Bass and McKibben, 2003; Amorim *et al.*, 2015). The oyster toadfish, *Opsanus tau*, is a benthic ambush predator that inhabits estuaries and coastal waters along the eastern 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 and 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 or 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). Individual male toadfish exhibit high site fidelity and repeatedly produce a similar boatwhistle for many weeks (Mensinger, 2014) making them an ideal study species for acoustic localization.

Acoustic recognition systems have evolved in many different animals to aid in situations where crowding or darkness reduce the roles of olfactory and visual cues. Batrachoidid fishes rely on their advertisement calls to attract females in turbid shallow waters or during night-time activity when visual cues are limited (Gray and Winn, 1961). 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, oyster toadfish were found to produce single, doublet and trains of grunts throughout May to September with vocalizations varying in pulse structure, duration and frequency components, suggesting that 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 produced “jamming” signals. For example, a male will produce a grunt during the tonal portion of the conspecific male boatwhistle and lowers the first harmonic to a rate that is unattractive to a female, preventing competing males from attracting females (Mensinger, 2014).

Acoustic recognition is beneficial when vocal animals defend long term territories, such as breeding toadfish, because animals may direct less aggression to familiar neighbors which are less likely to intrude their territories known as the "the dear enemy effect" (Temeles, 1994). 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 the spatial and temporal nature of spawning populations of fishes that actively produce advertisement calls to attract mates (Rice and Bass, 2009; Locascio and Mann, 2011). This type of non-invasive monitoring provides long term, continuous information on animal behavior and 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. Male toadfish are sedentary for extended periods as they guard eggs and cling young while calling to attract additional females (Gray and Winn, 1961). Toadfish also broadcast their boatwhistles in a physically variable and acoustically complex environment, making the species an excellent model for passive acoustic localization studies. 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), 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); however, this had minimal interference with acoustic analysis of toadfish vocalizations.

A four-channel digital acoustic recorder (ST4300, Oceans 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 – 30,000 Hz with sensitivities of 165.4, 165.0, 165.1, and 164.9 dB re 1V/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. 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. Additionally, the aperture of the array needed to be the same order of magnitude as the range to be covered (Møhl *et al.*, 2001). The hydrophones were deployed 1.0 meter from the water surface. The water depth was 2.4 - 3.4 meters 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 frequency 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 (Tindle *et al.*, 1978; Rogers and Cox, 1988). Temperature was recorded by a Hobo® Pendant model (± 0.1 ˚C), attached to the acoustic recorder and 1 m from the pond bottom, 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-hour 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.

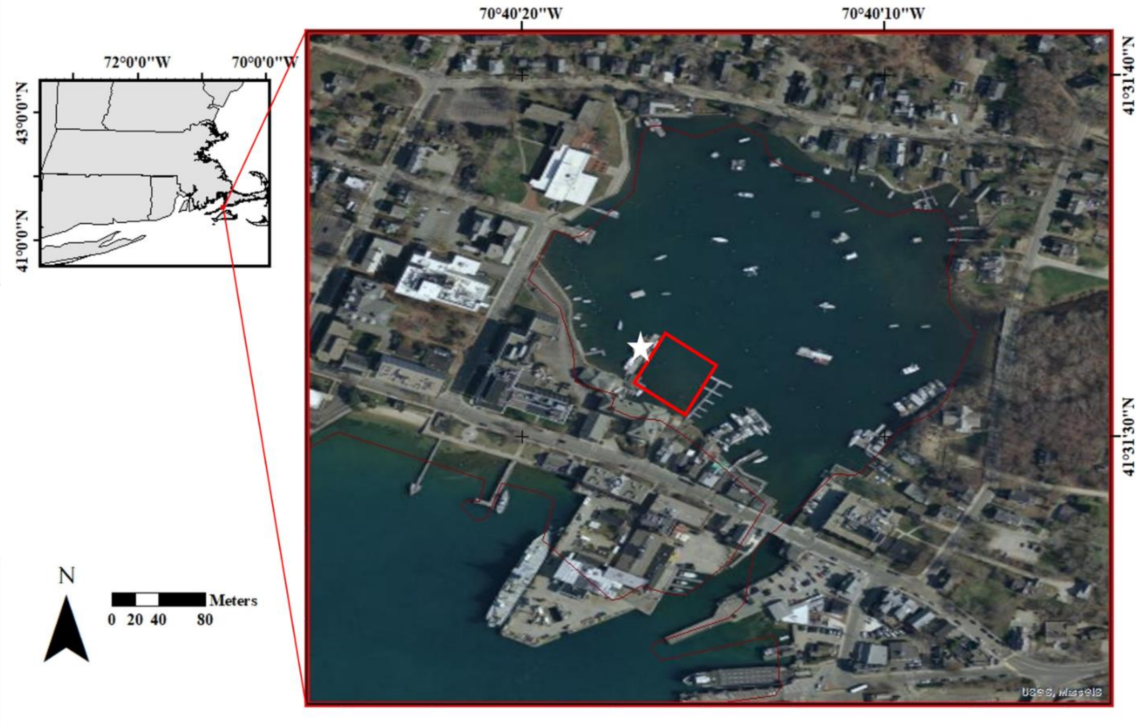


**Figure 1:** Map of Eel Pond, Woods Hole, MA, with inset showing position related to state. The red rectangle indicates the area where toadfish were localized. The four hydrophones were deployed along the dock indicated by the white star. Map produced using ArcGIS 10.3.1 (http://www.esri.com/software/arcgis/). Google ortho imagery 2014 was downloaded from the MassGIS website (https://www.mass.gov/service-details/massgis-data-layers/).

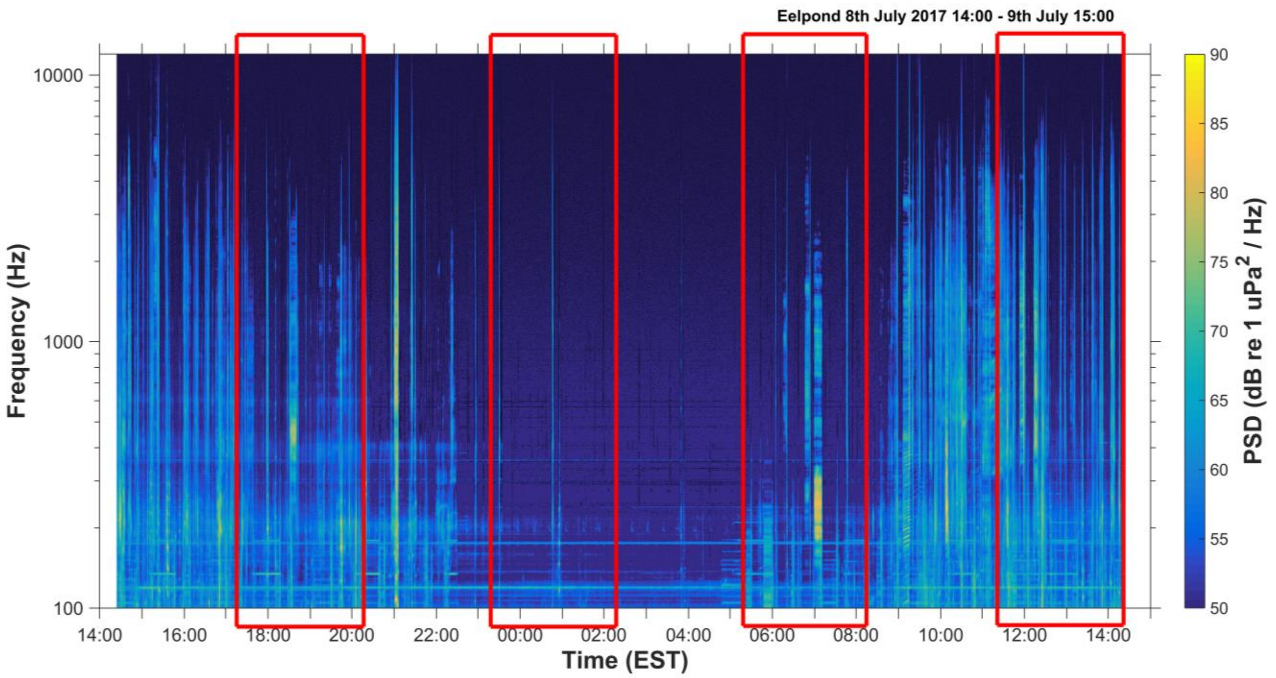


**Figure 2:** Spectrogram of one full day of recording (8th July 2017 14:00 to 9th July 2017 15:00) from Eel Pond, Woods Hole, MA, with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), produced using FFT length = 512 points, Hanning window and 50% overlap. The four red boxes indicate the four three-hour recordings used for boatwhistle analysis.

## 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 seconds (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 represent dusk (sunset - 2018), night (covering midnight), dawn (sunrise - 0517) and day (covering noon).

The number and timing of all boatwhistles [defined as a distinct initial grunt component preceding a tonal segment between 10 – 2000 Hz (Maruska and Mensinger, 2009)] were annotated (Fig. 3). To differentiate individual males, boatwhistle call characteristics including power spectrum and relative amplitude were used. To confirm boatwhistles were from the same toadfish, twenty random boatwhistles overall of each toadfish were cross correlated in MATLAB software (version 2014a), which outputted a matrix of the maximum correlation score for each pairing.

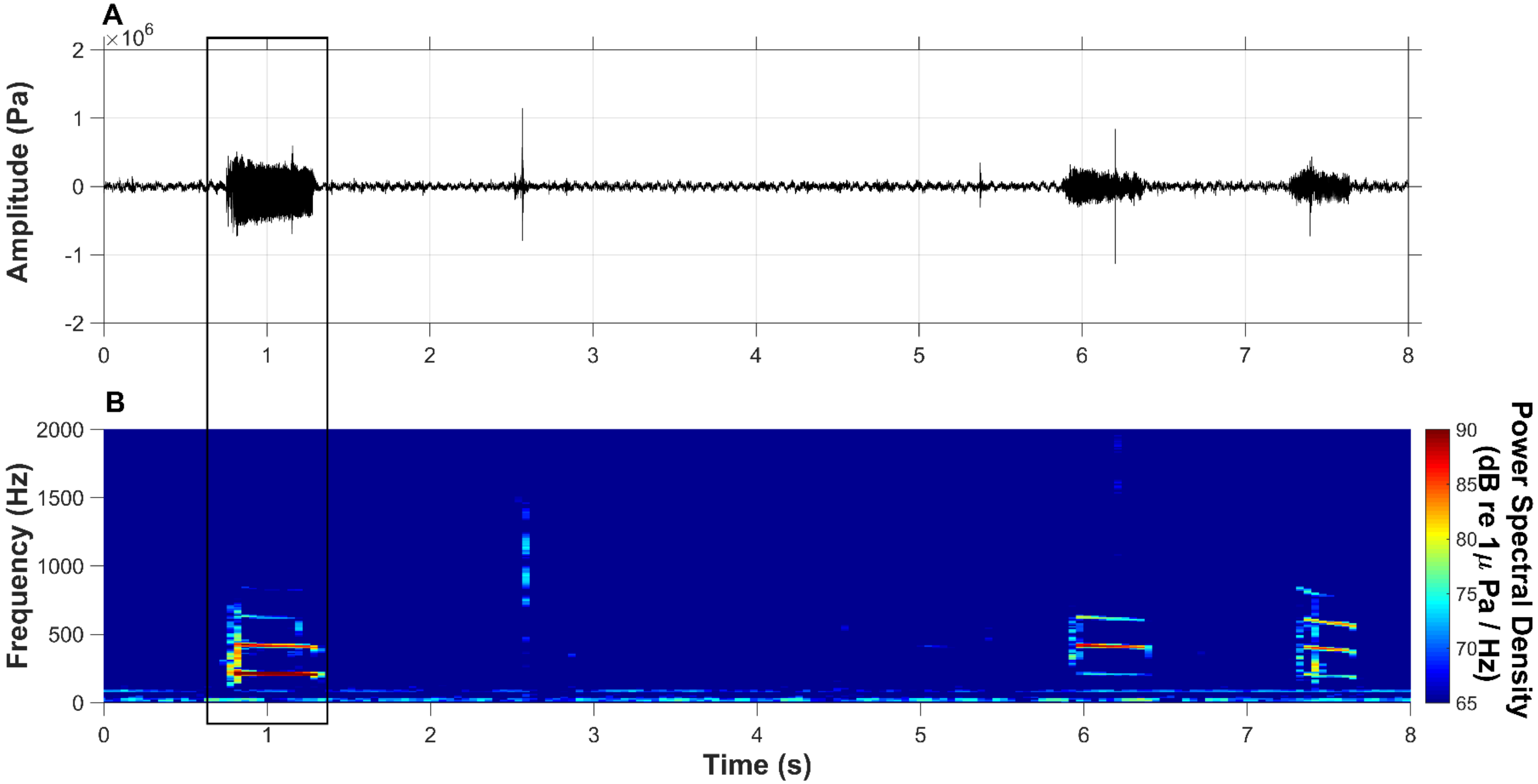


**Figure 3:** Three toadfish boatwhistles (from left to right toadfish 3, toadfish 6 and toadfish 5) taken from the 1723 - 2023 recording, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 2000 Hz with the colorbar showing power spectral density (dB re 1μPa$^2$/Hz). The black box indicates the boatwhistle (toadfish 3) used in Fig. 4.

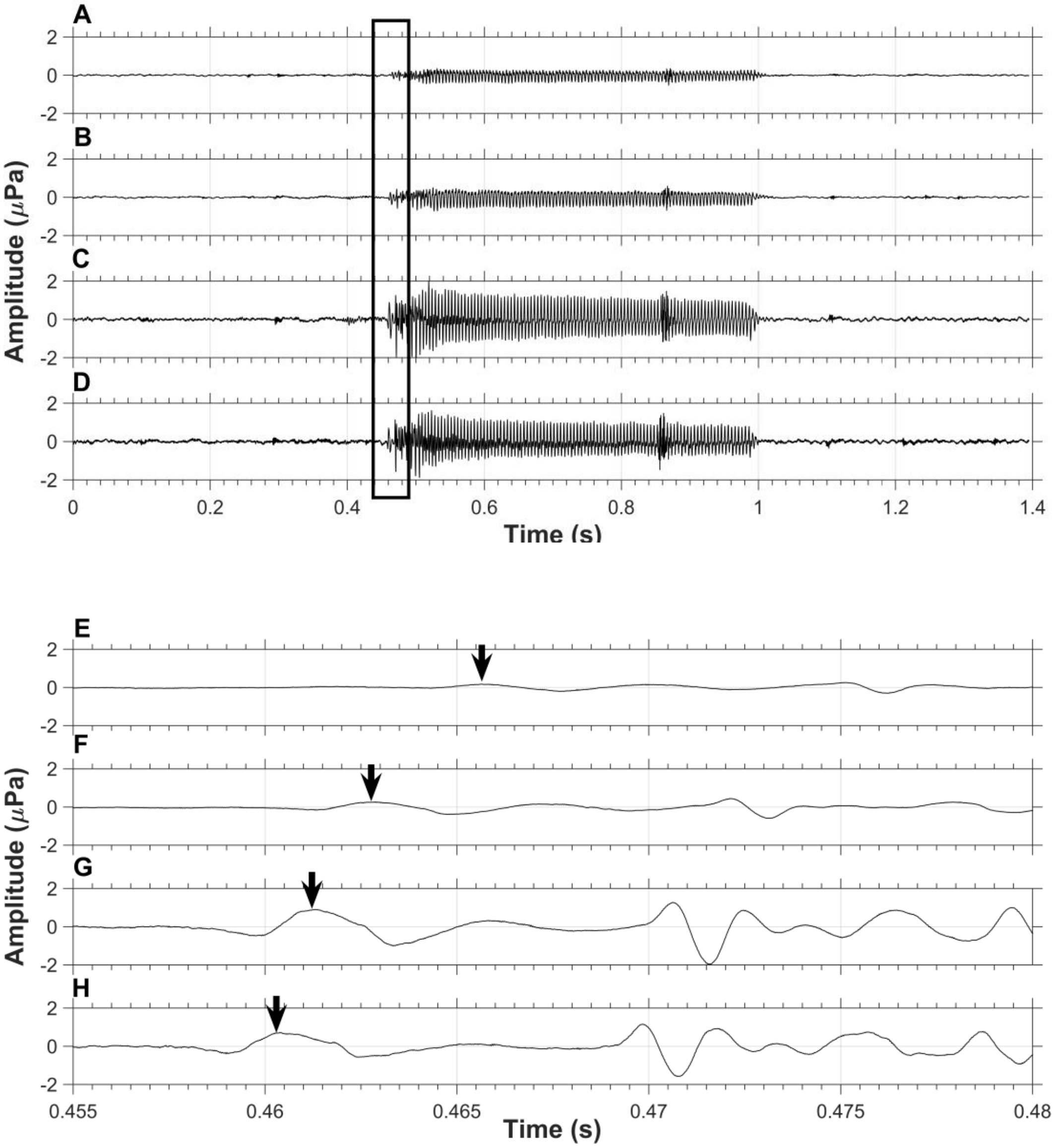


**Figure 4:** A – D) Waveform of toadfish 3 as recorded by each of the four hydrophones (h1, h2, h3. h4). The black box represents the section of the waveform used in E – H, which shows a zoomed in view of the waveform as recorded by each of the four hydrophones. The arrows on E – H indicate the time of arrival of the toadfish boatwhistle at each hydrophone.

### 2.3 Localization of boatwhistles

The waveform of each boatwhistle was analyzed in LabChart (version 8) using a scrolling display of 1 second (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 of arrival differences (TOADs) 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 TOAD method established by Watkins and Schevill (1971) and developed by Spiesberger and Fristrup (1990). For each pair of hydrophones (h1 and h2, h1 and h3, h1 and h4, h2 and h3, h2 and h4, h3 and h4) there are two possible solutions. To determine which of the two solutions was correct; a branch choosing function (BCF) was developed to select the correct solution based on the angle between receivers and the order of reception. For example, if the boatwhistle is received at h2 then h3 and h4, BCF = 1, but if they are received in another order, the source is in a region with BCF = -1. If BCF = +1 then S+ is the source position, whereas when BCF = -1 then S- is the source position.

To investigate the uncertainty associated with the estimated X Y location of the source, a computer simulation placed the four hydrophones in a linear (180˚) array with a separation replicating the distances between hydrophones used in the field experiment and the source was moved from 0 to 360˚ (in 1˚ steps) around the hydrophone placed at the origin. The accuracy was calculated as the mean Euclidean distance between the true (actual) and estimated source position. To investigate proximity of toadfish the distance between epicenters of each identified individual was calculated.

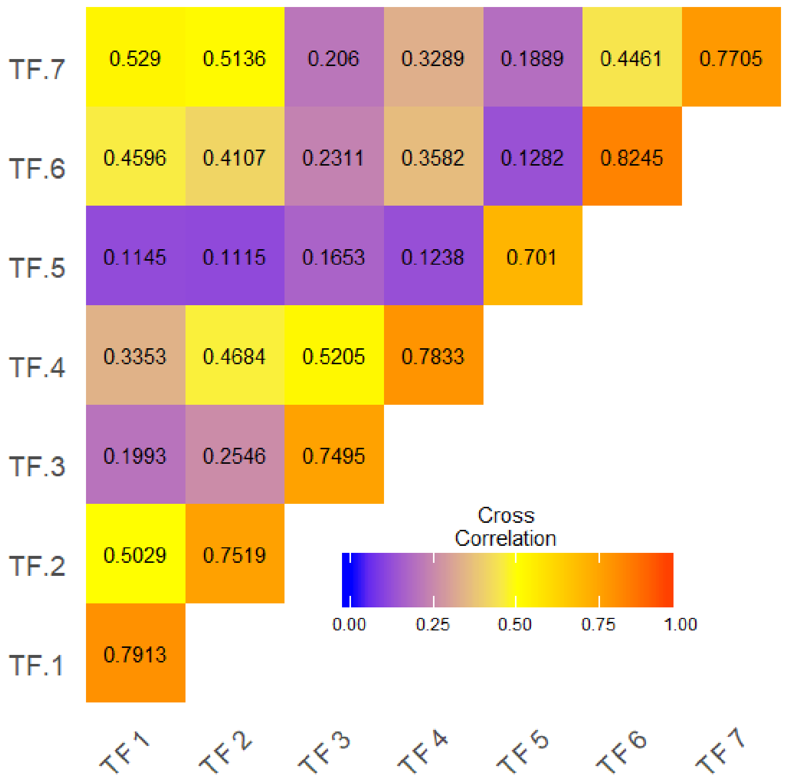
## 3. Results

### 3.1 Identification of boatwhistles

A total of 1839 boatwhistles were identified in the four recording periods analyzed (Table 1). Seven unique boatwhistles were identified through spectrographic analysis (Supplementary Info. 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%). Additionally, the majority of boatwhistles were identified during the dusk (41.3%) and night (52.3%) recordings. The boatwhistles were confirmed to come from a unique source when median cross correlation score was between 0.70 – 0.83 when each boatwhistle was correlated (e.g. TF 1 against other TF 1), whereas median scores ranged between 0.11 – 0.52 when different boatwhistles were correlated (e.g. TF 1 against TF 4) (Figure 5). Anecdotal observations of the boatwhistles showed no clear pattern in calling individuals (Supplementary Info Fig. S8). Although, 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).

**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<br>8th July 2017 | 760 | 10 | 186 | 90 | 214 | 69 | 123 | 22 | 11 | 35 |
| 2323-0223<br>8th July 2017 | 963 | 1 | 301 | 93 | 271 | 118 | 113 | 24 | 11 | 31 |
| 0523-0823<br>9th July 2017 | 32 | 0 | 6 | 5 | 7 | 4 | 6 | 4 | 0 | 0 |
| 1123-1423<br>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 | | | | | | |

**Figure 5:** Cross correlation matrix of the seven-different toadfish boatwhistles. The colorbar and number on each panel represents the median cross correlation index.

### 3.2 Localization of boatwhistles

1826 boatwhistles were localized using the TOAD method (Table 1), 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 and night time recordings (Fig. 6, Table 1), with an increase in the number of boatwhistles occurring around 1830 (Fig. 7), coinciding with dusk and toadfish 1 through 4 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). The 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).

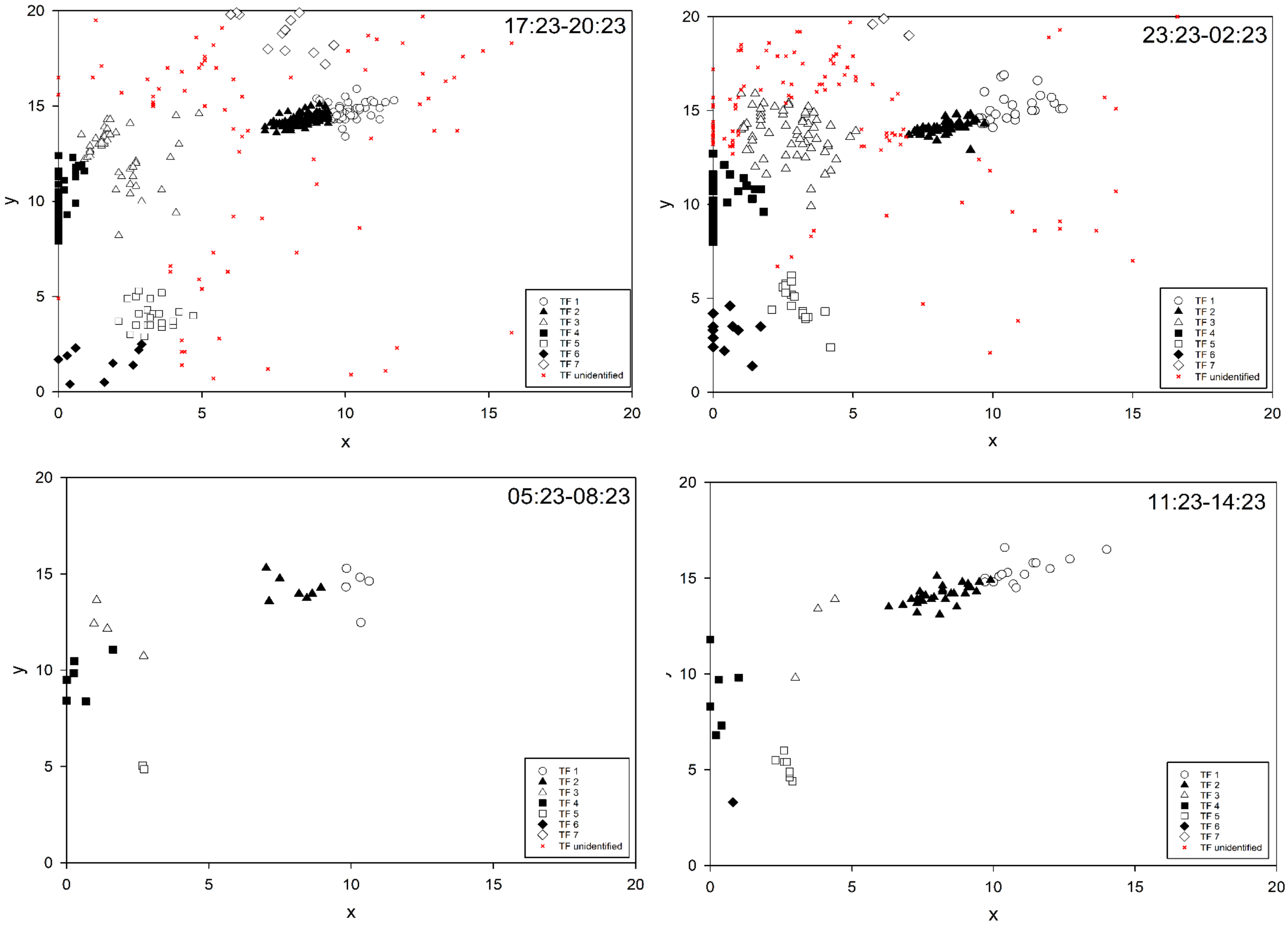


**Figure 6:** Maps of the seven-different toadfish (TF) during A) 1723 – 2023, B) 2323 – 0223, C) 0523 – 0823 and D) 1123 – 1423. TF 1 represented by open circles, TF 2 by closed triangles, TF 3 by open triangles, TF 4 by closed squares, TF 5 by open squares, TF 6 by closed diamonds, TF 7 by open diamonds and unidentified toadfish boatwhistles are shown by red crosses.

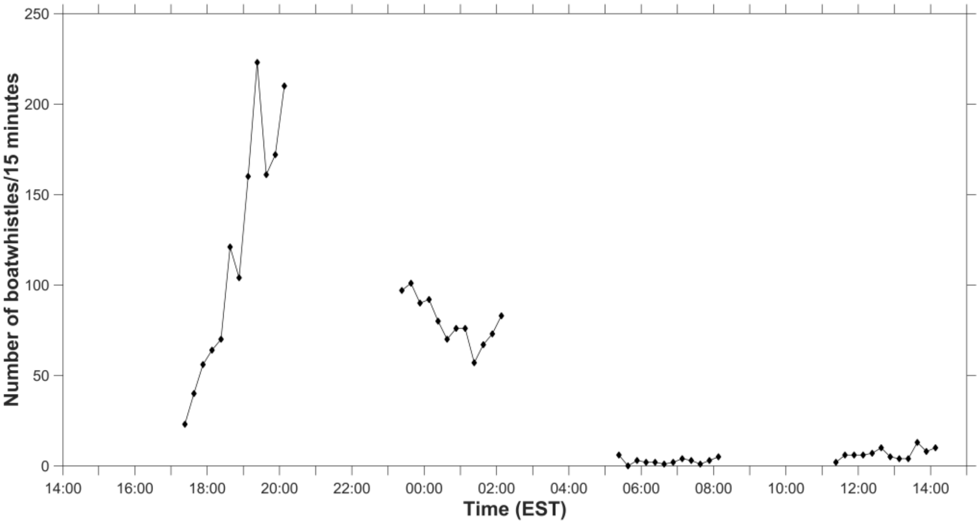


**Figure 7:** Total number of boatwhistles identified every 15 minutes over the four three-hour recordings analyzed.

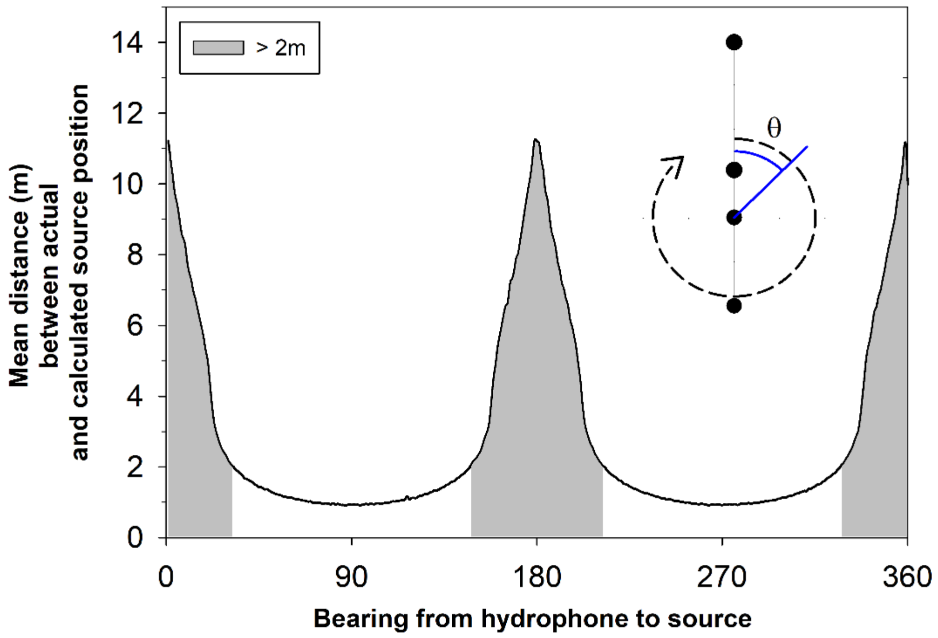


**Figure 8:** Mean Euclidean distance between actual and calculated source position (m) when a computer simulation changed the bearing from hydrophone to source between 0 – 360˚. The four hydrophones were placed in a 180˚ geometry with a separation of 5.6, 3.0 and 8.1 meters to replicate the field investigation, 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.

## 4. Discussion

The hydrophone array successfully located the positions of seven vocalizing male 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 for long periods to determine temporal and seasonal variation in boatwhistle production. 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 TOAD calculation varies depending on the sound velocity of the medium, whether ambient noise or other interference masks the signal (Aubauer, 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, 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 TOAD 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 and receiver position within field recordings (Cato, 1998; Wahlberg *et al.*, 2001; Thode *et al.*, 2004; Barlow and Griffiths, 2017). 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 TOAD localization method used in this study was calculated as < 2 m within 033 to 148˚ of the confines of the linear array. Uncertainty increases rapidly when the sound source approaches the plane bisecting at right angles between two hydrophones (the area close to the array) because the position becomes equidistant from each of the hydrophones and the TOAD approaches zero making the choice between two solutions indeterminate (Cato, 1998). Uncertainty in acoustic localization calculations improves as the hydrophone spacing increases, because uncertainty in TOADs are proportionally smaller (Cato, 1998; Macaulay *et al.*, 2017). However, array spacing is ultimately limited by the requirement that the hydrophones must be close enough together to ensure that there is adequate signal to noise ratio for the signal received on the hydrophone farthest from the source (Samaran *et al.*, 2010). 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.

The toadfish population in Cape Cod is at the northern extent of the population range and declining local toadfish populations resulted in the MBL Marine Resources Center supplementing locally caught fish with fish from out of state.  Toadfish were also thought to be extirpated from Eel Pond since at least 1990 however, during hydrophone testing in 2014 boatwhistles 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 however the population number was unknown. Additionally, as a single hydrophone consistently picked up distinct calls from the dock area, it was hypothesized that the toadfish were confined to this physical structure (Van Wert *per comms.*). 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. As vocalizing toadfish seek hard substrate, it was hypothesized that toadfish were restricted to the dock area. However, Eel Pond is an active marina and rocks or other detritus may be available near the deeper water near the end of the dock and not visible from the surface.

Passive acoustics offered an opportunity to estimate and localize the previously uncharacterized toadfish population in Eel Pond. It provides an alternative survey mode for species, like oyster toadfish, that are cryptic and hidden in nesting sites. 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 TOAD method which revealed three different clusters of vocalizing toadfish. The ability to discriminate between individuals or groups of individuals is important for the establishment of social relations and implies individual distinctiveness (Bradbury and Vehrencamp, 1998). Batrachoidid fishes form dense breeding aggregations and live in turbid environments where vision is impaired. It is unknown whether toadfish attack other nests within close proximity or direct less aggression towards familiar neighbors. In this study, 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. These three clusters may recognize their neighbors and therefore be less territorial because of proximity.

The communication space of oyster toadfish has previously been speculated as ~ 10 meters during recordings taken in Florida (Fish, 1964), but water depth and sediment type was unspecified. A communication space of ~ 5 meters was given for toadfish in 1 meter 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.*, 2017). 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 λ/2π 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)

Long term monitoring provides information on daily and seasonal activity, as well as movement patterns. Localization of consecutive vocalizations by individual fish is also useful for validating results and improving precision. Although for the purposes of this study, the toadfish were only monitored for a 24 hour period, individual and temporal variations were evident.  In Eel Pond, the highest number of boatwhistles were detected during the dusk and night time recordings, with an increase in the number of boatwhistles occurring around 1830. Males in chorus may benefit from increased mate attraction, reduced assessment costs or reduced predation risks (Gerhardt, 1978). Whether “silent” males benefit from neighbor’s vocalizations remains to be determined.

Calling rate may also be condition dependent in Batrachoididae, with higher calling rates (up to 20 boatwhistles per minute), indicative of high reproductive success in this species (Vasconcelos *et al.*, 2012). It could therefore be suggested that toadfish 1 - 4 could be the most reproductively successful. Alternatively, other identified fish may be egg guarding already and long term evaluation would be needed.  The advantage of passive acoustic monitoring is that the nest sites are localized and could allow divers or cameras to locate nest and check for egg number which would be difficult in murky conditions without approximate nest locations. A limitation of this study is that the results are based on 24-hour period, future research efforts would therefore also utilize the long-term capabilities of passive acoustic monitoring to assess toadfish populations over a longer time scale.

In this study, it was assumed that boatwhistles would change minimally in frequency range or duration over the 24-hour period, which is consistent with daily call variation in the genus *Opsanus* (Mensinger, 2014). However, over seasonal time frames, boatwhistle 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 pulse repetitive rate (PRR) of oyster toadfish boatwhistles increasing by 11 Hz for every 1˚C increase in temperature (Ricci *et al.*, 2017). Over the 24-hour period, temperature varied by 2.0 ˚C at the pond bottom, therefore the PRR of the boatwhistles may have varied. However, differences in frequency would not impact the TOAD method used in this study as it took the peak in the first waveform to be the time of arrival. Future passive acoustic monitoring could allow individual monitoring and more precise correlation of temperature and PRR to be researched.

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. Finally, 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.

## Acknowledgements

We would like to thank John Atkins, Mark Johnson and Ben Schlaer for their guidance in the acoustic localization method used and Emily Fleissner for helping to identify toadfish boatwhistles. We also thank Jenni Stanley for help with initial hydrophone set-up and the Marine Resources Center at MBL for dock space and resources. Funding was provided by National Science Foundation grants IOS 1354745, DOB 1359230 and REU Program Award 1659604 awarded to AFM.

## Competing financial interests

None

## Data Accessibility

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

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# Supplementary Information

# Localizing individual soniferous fish using passive acoustic monitoring

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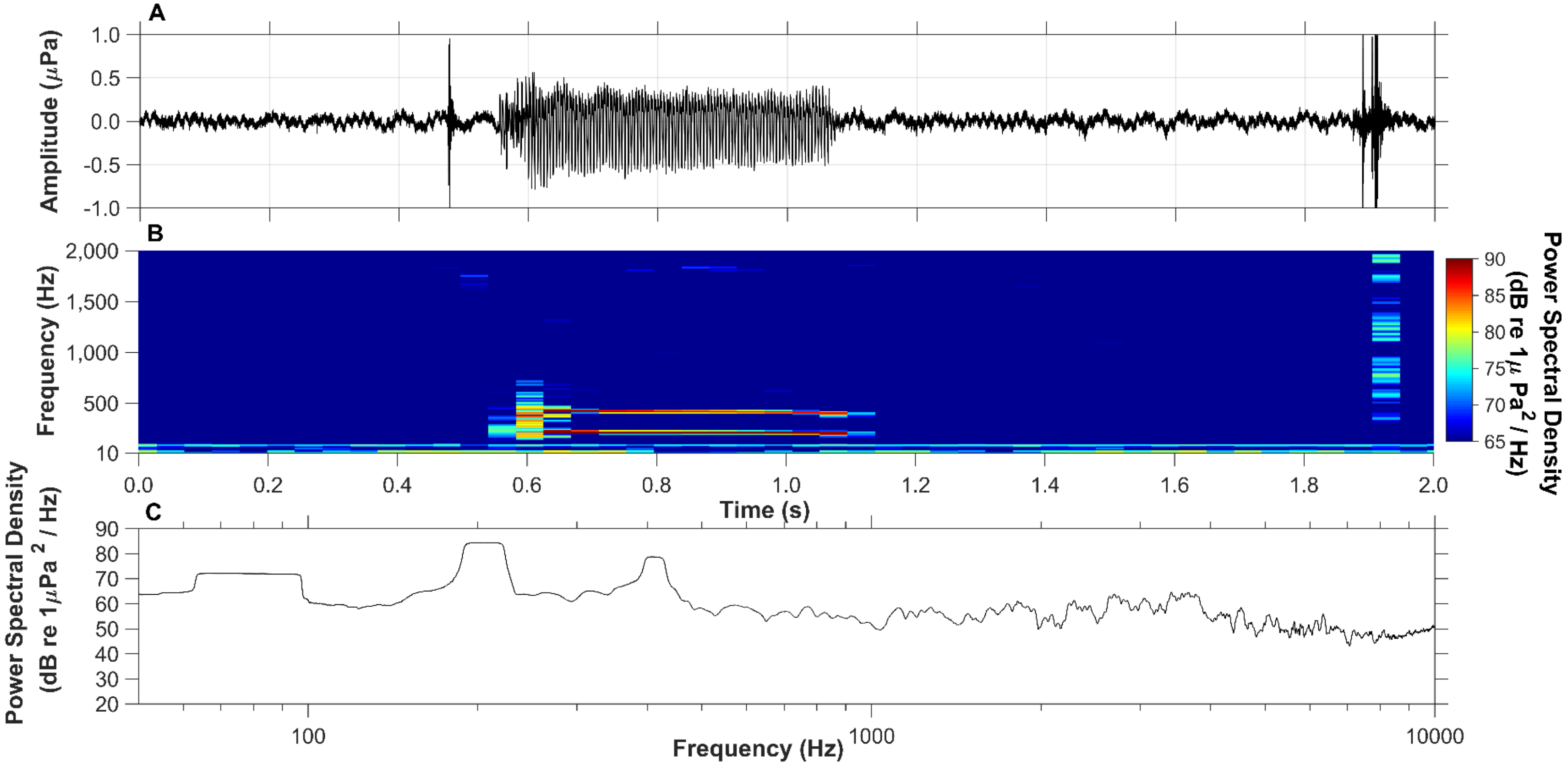

**Figure S1:** Example of toadfish boatwhistle ID 1, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

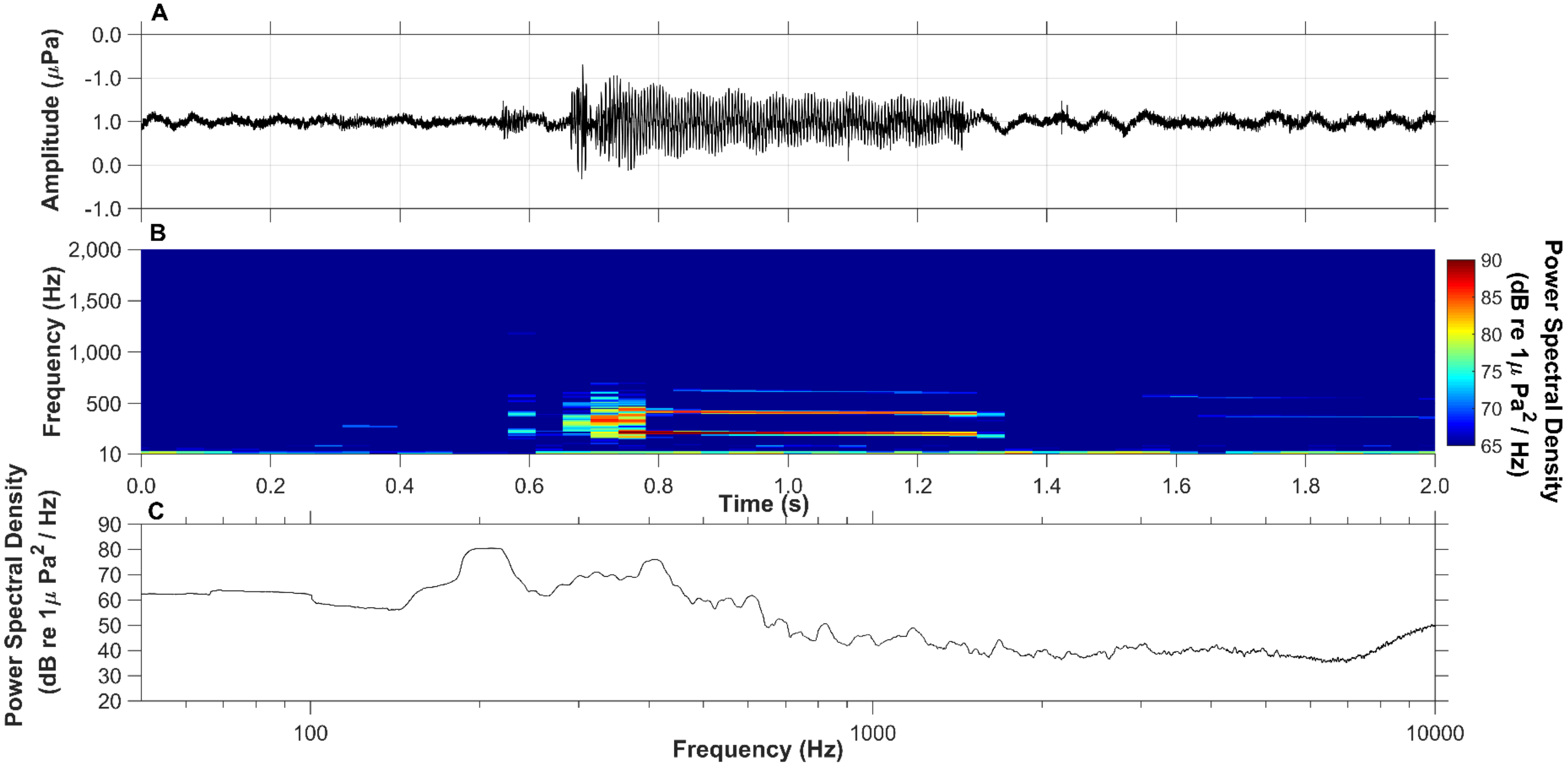

**Figure S2:** Example of toadfish boatwhistle ID 2, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

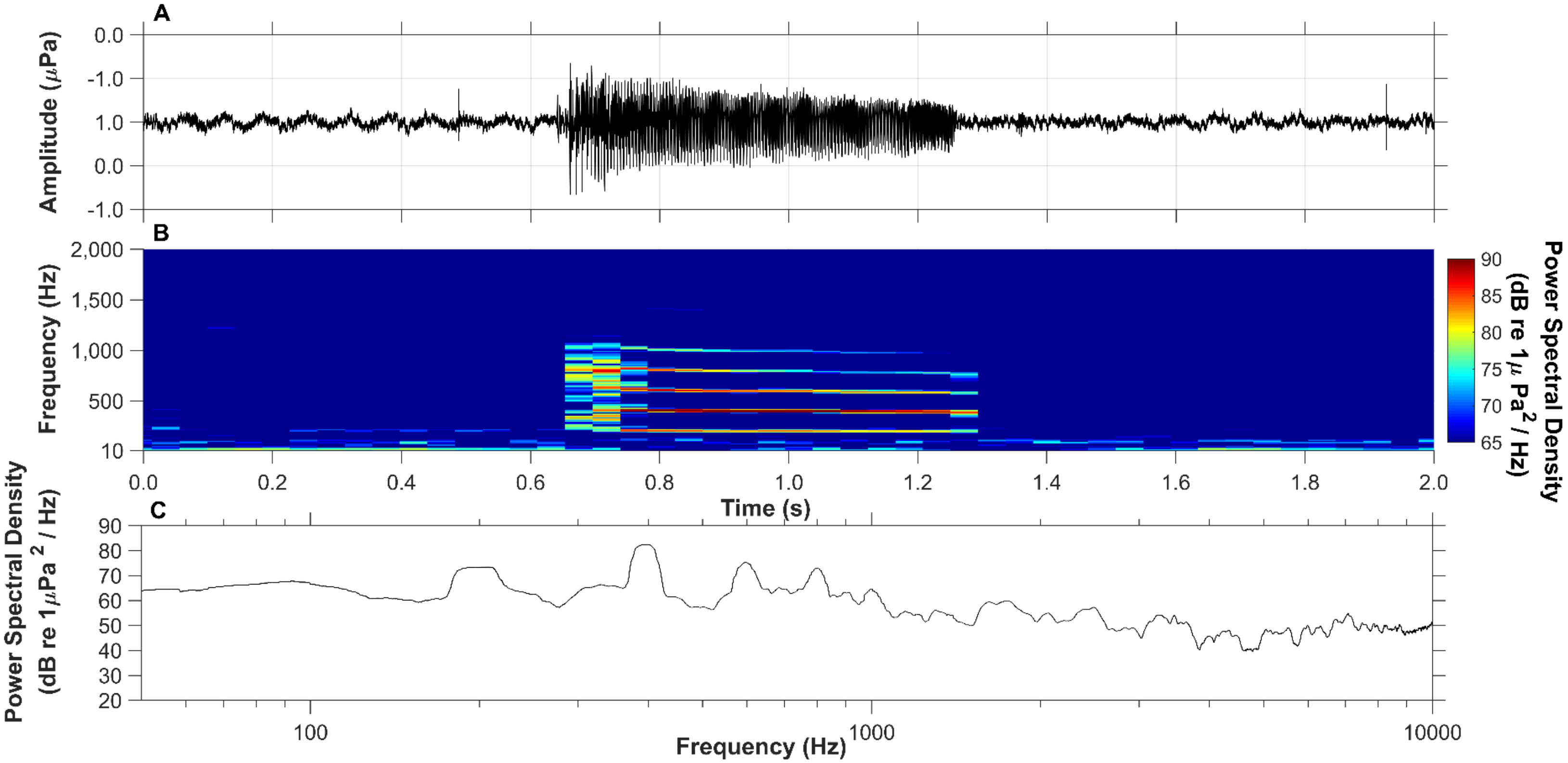

**Figure S3:** Example of toadfish boatwhistle ID 3, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

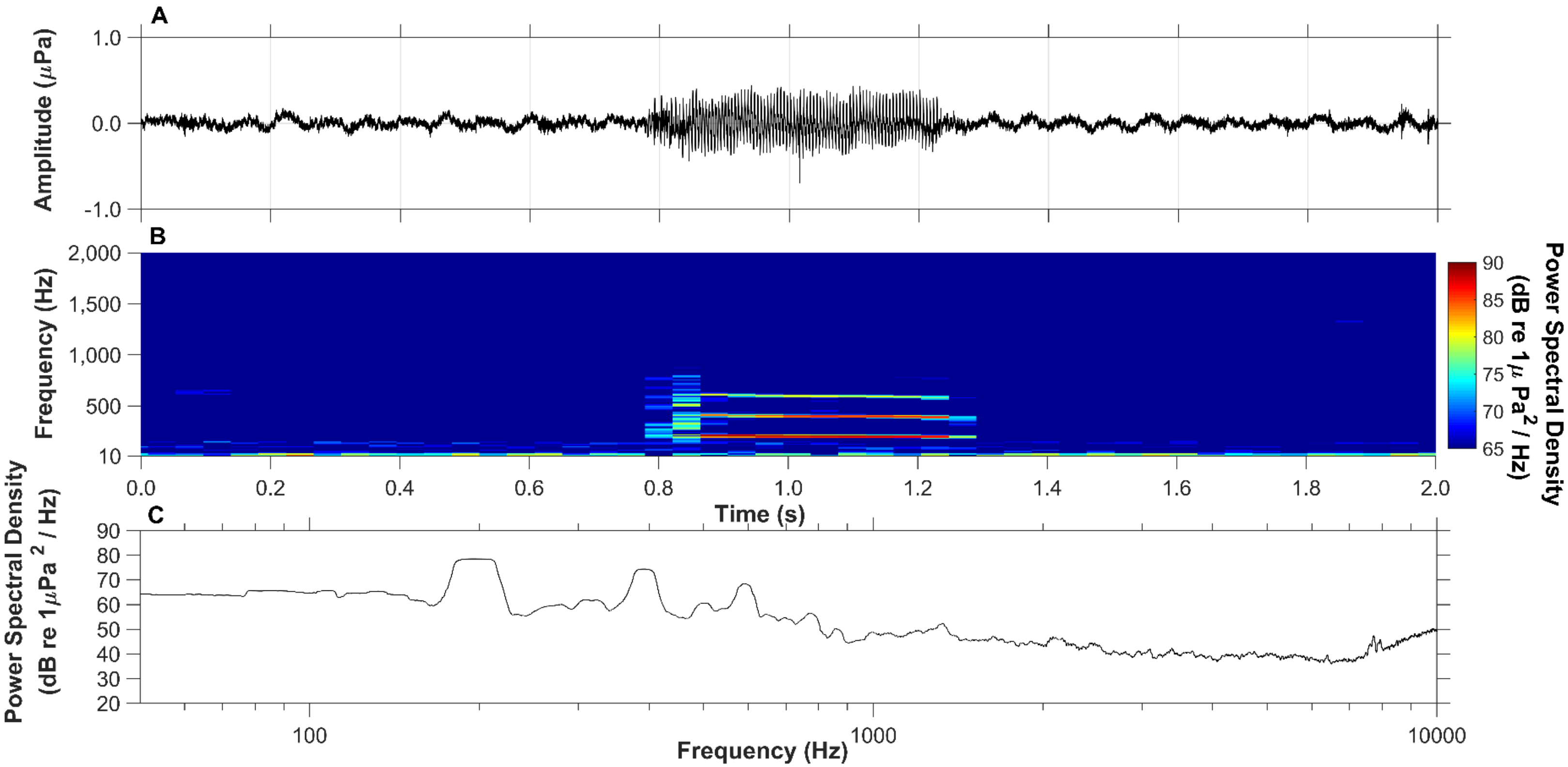

**Figure S4:** Example of toadfish boatwhistle ID 4, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

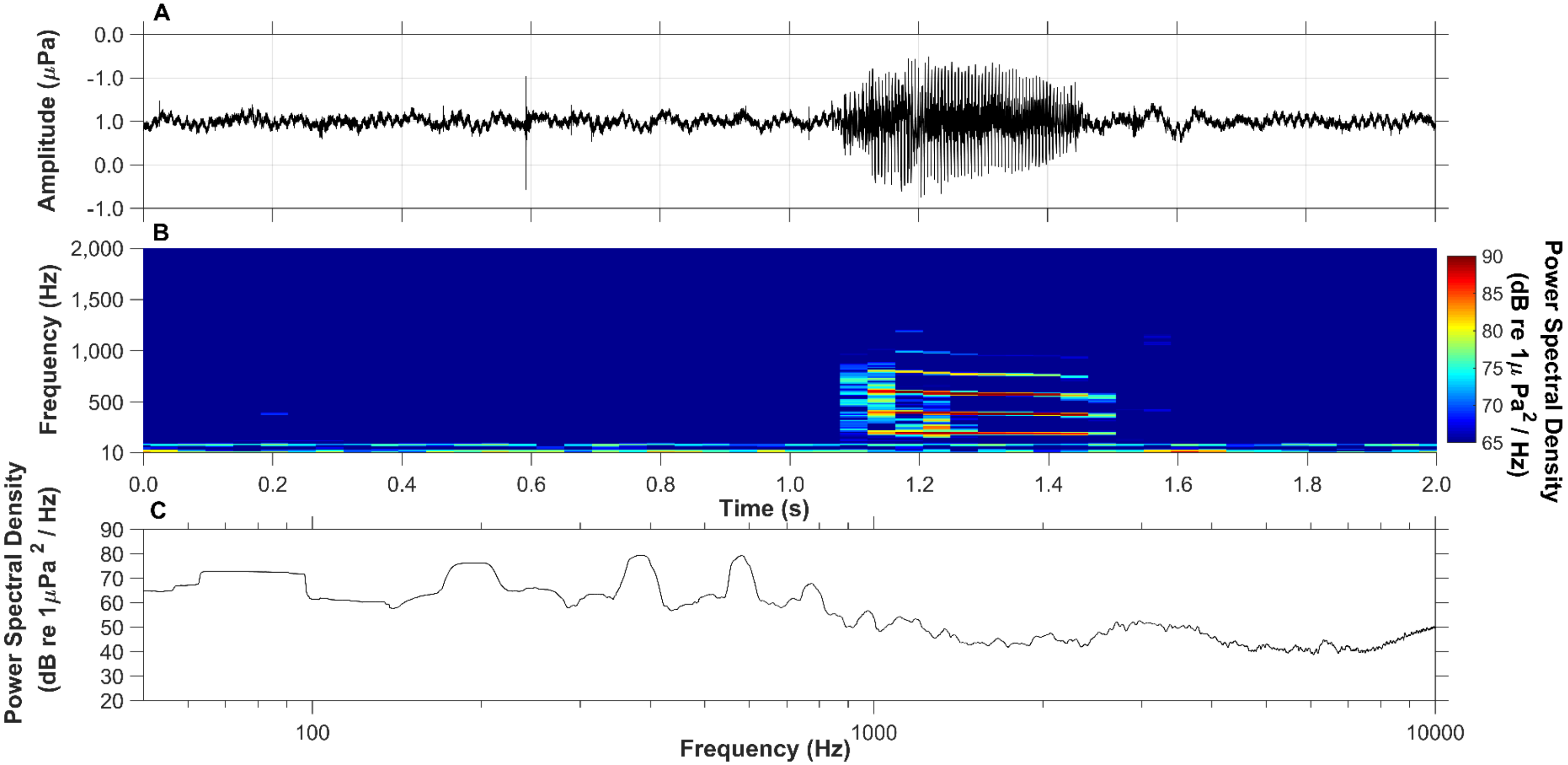

**Figure S5:** Example of toadfish boatwhistle ID 5, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

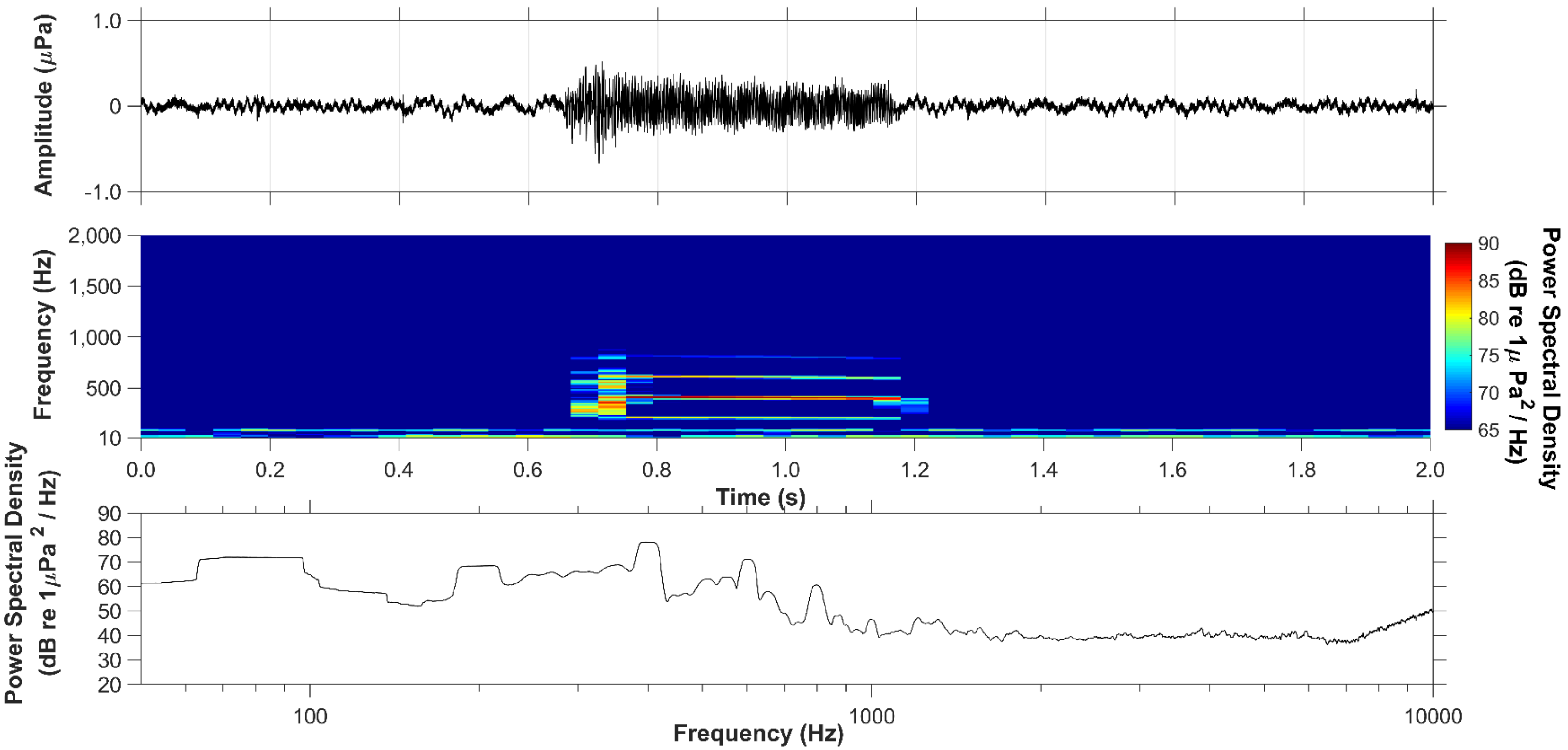

**Figure S6:** Example of toadfish boatwhistle ID 6, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal

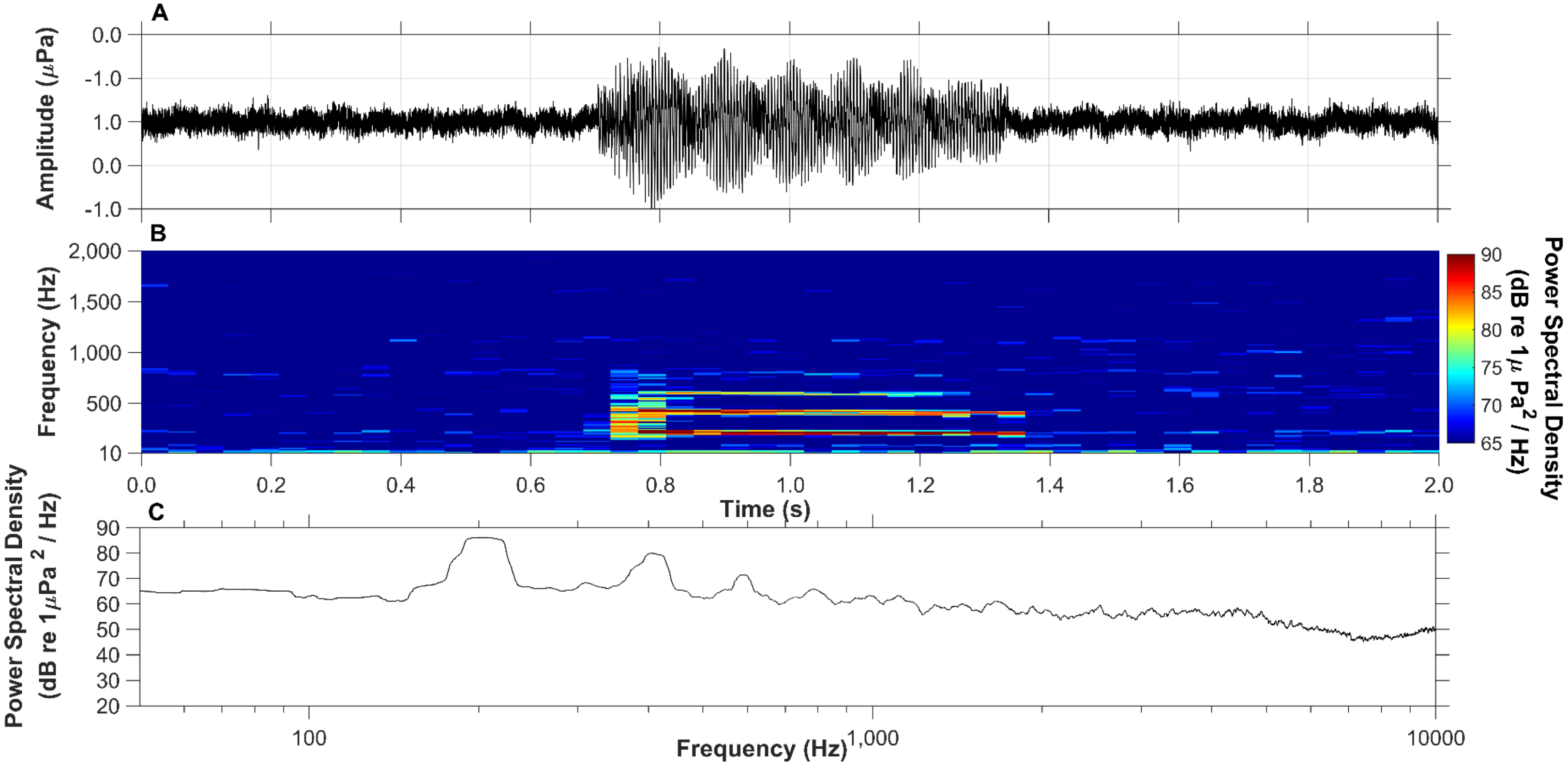

**Figure S7:** Example of toadfish boatwhistle ID 7, A) Waveform of the signal, B) Spectrogram of the signal between 10 – 5000 Hz with the colorbar representing power spectral density (dB re 1μPa$^2$/Hz), C) Power spectral density (dB re 1μPa$^2$/Hz) of the signal.

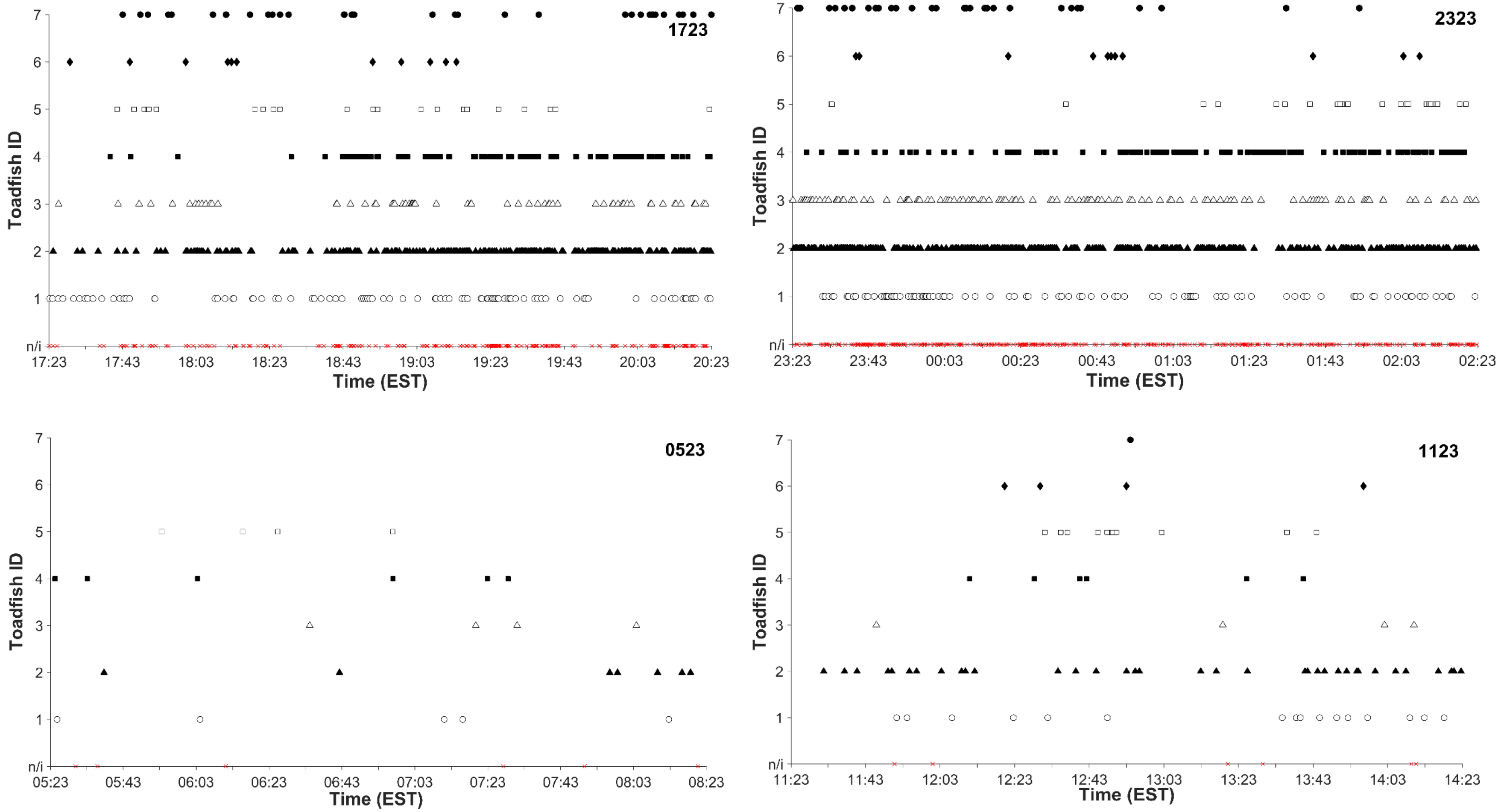

**Figure S8:** Timing of the seven different boatwhistle types during A) 1723 – 2023, B) 2323 – 0223, C) 0523 – 0823 and D) 1123 – 1423.