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Detection of invasive fish species with passive acoustics: Discriminating between native and non-indigenous sciaenids

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ABSTRACT

Invasive alien species have been rising exponentially in the last decades impacting biodiversity and ecosystem functioning. The soniferous weakfish, *Cynoscion regalis,* is a recent invasive sciaenid species in the Iberian Peninsula and was first reported in the Tagus estuary in 2015. There is concern about its possible impacts on native species, namely the confamiliar meagre, *Argyrosomus regius,* as there is overlap in their feeding regime, habitat use, and breeding behaviour. Here, we characterised the sciaenid-like sounds recently recorded in the Tagus estuary and showed that they are made by weakfish as they have similar numbers of pulses and pulse periods to the sounds made by captive breeding weakfish. We further demonstrate that breeding grunts from weakfish and the native sciaenid, recorded either in captivity or Tagus estuary, differ markedly in sound duration, number of pulses and pulse period in the two species, but overlap in their spectral features. Importantly, these differences are easily detected through visual and aural inspections of the recordings, making acoustic recognition easy even for the non-trained person. We propose that passive acoustic monitoring can be a cost-effective tool for *in situ* mapping of weakfish outside its natural distribution and an invaluable tool for early detection and to monitor its expansion.

1. Introduction

Invasive alien species have been rising exponentially in the last decades representing a major driver of biodiversity loss and ecosystem functioning modification (Mormul et al., 2022). In the marine realm, the numbers of invasive alien species have been growing dramatically in the last decades. Generally, marine invaders are concentrated in coastal areas associated with transitional waters, such as estuaries, or close to harbours (Chainho et al., 2015; Moyle and Stompe, 2022; Rilov and Crooks, 2009). In a recent review for European marine waters, a total of 800 invasive alien species were identified and the Iberian coastal waters were among the mostly heavily invaded European sub-regions (Tsiamis et al., 2019).

During the early stages of invasion, most invasive species have low abundances, often staying undetected for several generations before reaching high species abundances (Lockwood et al., 2013). Early detection is challenging but critical for effective control actions as after establishment the eradication of invasive species is nearly impossible (Larson et al., 2020). Emerging techniques and approaches to detect early biological invasions have been successfully applied (reviewed by Larson et al., 2020), such as the usage of environmental DNA to detect Asian carp (*Hypophthalmichthys* spp.) (Jerde et al., 2013) or citizen science to detect European catfish (*Silurus glanis*) (Gago et al., 2016). Recently, passive acoustic monitoring (PAM) has been used for detecting and monitoring biological invasions (Ribeiro et al., 2022), though its application in aquatic environments is still in its infancy (Rountree and

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Juanes, 2017). PAM can be used to detect and monitor soniferous fish species and it is especially advantageous when they are cryptic or rare, as their sounds may still be easily detected (Picciulin et al., 2018), offering an enormous potential for the early detection of invasions (Juanes, 2018). Besides, PAM has low cost in comparison with other techniques such as fisheries monitoring programs (Juanes, 2018).

A recent alien fish in the SW coast of Iberia is the weakfish *Cynoscion regalis* (Bloch and Schneider, 1801), which has quickly invaded the coastal waters and several estuaries (Bañón et al., 2018; Gomes et al., 2017; Morais et al., 2017). This sciaenid is a cold-water tolerant fish that naturally occurs on the NE coast of North America, between the Florida coast and Cape Cod, inhabiting shallow coastal waters and estuaries; curiously, in its native area the weakfish biomass began to decline in the late 1990s and the stock is considered depleted since 2003 (ASMFC, 2022). In the Iberian Peninsula, it was first detected in the Gulf of Cadiz in 2011 (Spain, South coast of Iberia) and in the subsequent year in the Sado estuary (Portugal, West coast of Iberia) (Béarez et al., 2016). Currently, it is abundant in the Tagus estuary (Morais et al., 2017) where it could directly impact the native sciaenid, the meagre – *Argyrosomus regius* (Asso, 1801), through competition for habitat and food resources (Cerveira et al., 2021). The two species could also be competing for acoustic resources (*sensu* Bolgan et al., 2022) as both produce loud sounds associated with breeding (Connaughton and Taylor, 1996; Largardère and Mariani, 2006) and overlap in the seasonal and diel patterns of their calling. Meagre breeding choruses are one of the main biological contributors to the Tagus soundscape and can be detected from February to August depending on temperature (Vieira et al., 2021a, 2022). Weakfish calling in the Delaware was present from May–July (Connaughton and Taylor, 1995) and both species were recorded simultaneously in the Tagus estuary in June (this study). As in other sciaenids, both species call predominantly at dusk (Connaughton and Taylor, 1995; Vieira et al., 2022).

Presently, there is still limited knowledge about the distribution and dispersal patterns of this invasive fish in Iberian estuaries and its spatial occupation in relation to the native meagre, which is a highly valuable species for commercial and recreational fisheries (Prista, 2013). PAM could thus provide a useful tool to acquire this knowledge, namely to evaluate the habitat usage of weakfish in newly invaded areas, such as the Tagus estuary, and its habitat overlap with native species. However, to achieve this goal it is key to validate the detection of weakfish with PAM and to validate the ability to discriminate their sounds from the meagre, given that these sciaenids did not naturally co-occurred.

Here, we aim to assess whether PAM can be used to detect an invasive fish and discriminate it from a native confamilar species under field conditions. Specifically, we aim to (i) ascertain that the recently recorded sciaenid-like sounds in Tagus estuary are from the invasive weakfish, (ii) distinguish between putative weakfish sounds and the sounds produced by the native meagre and (iii) demonstrate that the sounds from the two species can be mapped with PAM. To validate the source identity of weakfish sounds and the ability to monitor this species in nature, we used a comprehensive dataset including recordings made in captivity and in nature from weakfish and meagre. With this study, we demonstrate that monitoring fish mating sounds offers a scalable, effective method to assess the presence and distribution of vocal fish species and can be used as a new tool for early detection of invasive fishes.

2. Material and methods

2.1. General methods

The acoustic features of sounds from several datasets from previously published studies, as well as from new acoustic datasets were analysed (Table 1). We considered sounds made by captive fish during the breeding season, obtained from fish within their natural distribution, i.e., Northeast coast of North America, Delaware bay (weakfish) and Portugal (meagre), and sounds recorded in the wild, in the Tagus estuary, Portugal (Fig. 1). The source of captive weakfish sounds was Connaughton and Taylor (1996). Captive meagre voluntary sounds were sourced from Pereira (2019). Field records of meagre and weakfish

Table 1
Summary of the acoustic datasets used. TL – total length. BA6 – Air Force Base 6 at Montijo, Portugal.

	Dataset			
	1	2	3	4
Species	Weakfish	Meagre	Weakfish|Meagre	Weakfish|Meagre
Fish origin	Delaware bay, USA	F1 of wild fish from the South Portuguese coast	Tagus estuary, Portugal	Tagus estuary, Portugal
Location of recording	University of Delaware, College of Earth, Ocean and Environment	IPMA –EPPO, Olhão, Portugal	Logger 1 and 2, Tagus estuary	BA6 logger, Montijo
Context	Captive	Captive	*In situ*	*In situ*
Recording tank size	1500L	3600L	-	-
Group size	Mixed-sex groups (3–6 fish)	Mixed-sex group (6♂+2♀)	Unknown	Unknown
Date of recording	May/June 1993	May 2018	July 2020	June 2021
Fish TL (cm)	♂: 27-30 ♀: unknown (do not produce sound)	♂: 71–94 ♀: 88-90	Unknown	Unknown
Temperature	21–23 °C	21.6–22 °C	22 °C	17.1–21.8 °C
Sampling frequency/bit rate of analysed recordings	8 kHz/32 bit	8 kHz/16 bit	8 kHz/16 bit	4 and 8 kHz/16 bit
No. sounds analysed	15	28	30	–
Source	Connaughton and Taylor (1996)	Pereira (2019)	Present study	Present study

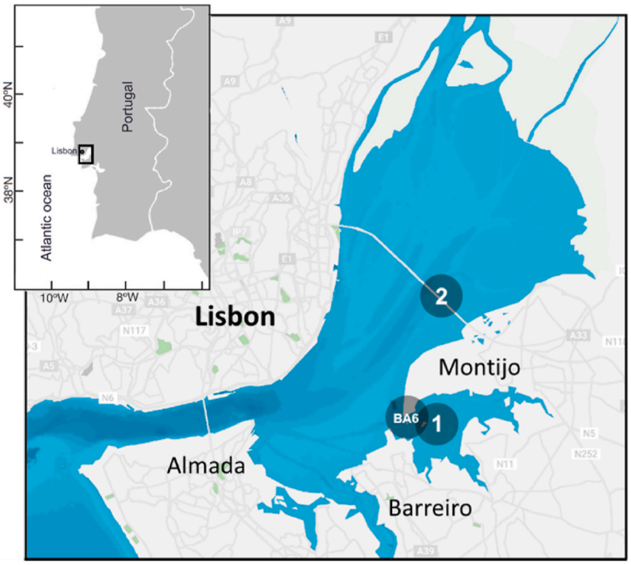

Fig. 1. Location of the two autonomous acoustic loggers (#1 and #2) and the land-based acoustic recorder placed at the pier of Air Force Base 6 (BA6); Tagus estuary, Portugal.

sounds were obtained from acoustic loggers deployed in the Tagus estuary (see details in 2.4).

2.2. Captive weakfish sounds

Weakfish were collected in May 1993 from the south-west portion of Delaware Bay (38°51’00.10″N, 75°10’00.74″W), near Lewes, Delaware. Captive fish were brought to the College of Earth, Ocean and Environment (CEOE), University of Delaware, Lewes (USA), where spawning was induced with human chorionic gonadotropin injections. Fish were recorded in May/June 1993 before and during successful captive spawning activity with a hydrophone (Edmund Scientific, Barrington, NJ; frequency response 10–6000 Hz), connected to a preamplifier (Hydrosonics, Ferndale, WA) and then to a grounded VHS recorder to reduce electrical noise (Connaughton and Taylor, 1996). This data corresponds to dataset 1 in Table 1.

2.3. Captive meagre sounds

The studied fish were offspring of wild individuals captured on the south Portuguese coast and maintained at the Instituto Português do Mar e da Atmosfera – Estação Piloto de Piscicultura de Olhão (IPMA–EPPO), Portugal (37°02′00.4″N, 7°49′12.0″W) (for more details, see Pereira, 2019; Pereira et al., 2020). Sounds made before and during spawning were recorded in May 2018 with a High Tech 94 SSQ hydrophone (High Tech Inc., Gulfport, MS, USA; sensitivity of −165 dB re. 1 V/μPa, frequency response up to 6 kHz within ±1 dB), connected to a stand-alone 16 channel datalogger (LGR -5325, Measurement Computing Corp, Norton MA USA; 12 kHz sampling rate 16 bit, ±1V range). Note that in this experiment, spawning was not induced. The sounds for analysis were chosen from a water temperature of 21.6–22 °C. This data corresponds to dataset 2 in Table 1.

2.4. Wild weakfish and meagre sounds from the Tagus estuary

Round-the-clock recordings (recording 30 min every hour) of breeding sounds were collected in 2020 at the end of the breeding season from two custom-made autonomous acoustic loggers that were deployed under navigational buoys, anchored at the chain, in the Tagus estuary (logger 1, 38°41′30.55″N, 9°01′59.23″W and logger 2, 38°45′08.14″N, 9°02′13.25″W, Fig. 1). The custom-made loggers consisted of an Audiomoth recorder (Hill et al., 2018) coupled to a piezoelectric ceramic disc placed inside a PVC waterproof case. Sounds were analysed from two days (29–30 July 2020) when water temperature was at 22 °C (dataset 3, Table 1), a temperature comparable with datasets 1 and 2 for captive weakfish and captive meagre. Recordings were inspected visually and aurally around sunset for the presence of putative weakfish sounds that resembled the ones described by Connaughton and Taylor (1996) and other authors (e.g. Luczkovich et al., 2008), and of typical meagre sounds (Vieira et al., 2019, 2022). Note that we have been recording sounds from meagre since 2013 from a land-based acoustic recorder (see below) placed at the pier of Air Force Base 6 (BA6), Montijo, Portugal (38°41′25.7″N, 9°02′55.6″W) and since 2000 in nearby locations (well before the first record of the presence of weakfish; Morais et al., 2017). A previous study has characterised the meagre vocal repertoire and validated the occurrence of meagre sounds at this site (Pereira et al., 2020).

Sounds with a good signal to noise ratio, non-overlapping with other fish sounds or boat noise were chosen for analysis. Note that the spectral component of the recordings made by the custom-made loggers were nonlinear with the lower frequencies underrepresented due to the PVC case (Fig. S1), though sounds from different species could be easily detected and recognised (see results).

In addition, to evaluate the potential for *in situ* mapping of weakfish and meagre with PAM, we considered one full day of round-the-clock recordings collected in 2021 (12 June) from the above-mentioned land-based acoustic recorder placed at the pier of BA6. Here, sounds were collected by a High Tech 94 SSQ hydrophone anchored, at about 20 cm from the bottom to a stainless-steel holder projecting from a concrete base where the cable was attached to minimise current-induced hydrodynamic noise. The hydrophone signal was recorded by a 16 channel stand-alone data logger (LGR–5325, Measurement Computing Corp, Norton MA USA; 4 kHz sampling rate 16 bit, ±1V range). Water depth varied between 3 and 6 m, depending on the tides. Recordings from the BA6 logger were used as weakfish sounds were regularly detected in this logger in 2021, rendering an excellent opportunity to test the feasibility of mapping these two species with PAM, as in these recordings the spectral properties of the calls were preserved (dataset 4, Table 1).

2.5. Sound analysis

Acoustic features of sounds from datasets 1–3 (Table 1) were measured. Captive weakfish sound recordings from North America from Connaughton and Taylor (1996) were converted from Canary bioacoustic software (The Cornell Lab of Ornithology, Ithaca NY, USA) format into wav files with Cool Edit Pro with an 8 kHz sample rate and 32-bit (32-bit iEEE float 24.0) resolution. The sounds were normalized to 90%. Captive meagre recordings were also down-sampled to 8 kHz with a 16-bit resolution. Eight kHz was chosen to match the autonomous acoustic loggers that recorded weakfish and meagre sounds in the Tagus estuary. Sound analysis was carried out with Raven Pro 1.6 (The Cornell Lab of Ornithology, Ithaca NY, USA). Temporal parameters were measured from oscillograms and included: sound duration (ms), the time from the onset of the first pulse to the offset of the last pulse; number of pulses in a sound; mean pulse period (ms), the average time between the peaks of consecutive pulses in a sound. The peak (or dominant) frequency, the frequency with the highest energy in the sound (Hz), was measured from power spectra (8 kHz, FFT size 1024 points, Hamming window, 50% time overlap) with Raven custom tools.

To explore the possibility of mapping the two species in the Tagus estuary we aurally and visually inspected a 24 h field recording (dataset 4, Table 1) to annotate sciaenid sounds. Following Vieira et al. (2021a), we made a long-term averaged spectrogram (LTAS) to represent these recordings. Power spectral density (PSD) (used to create LTAS) was computed adapting the code available by Merchant et al. (2015) (FFT 1024, Hann window, 50% overlap, averaged for each minute for LTAS).

2.6. Statistical analysis

Statistical analysis was conducted with STATISTICA (version 13, TIBCO Software inc., Palo Alto, CA, USA) and SPSS (version 27, IBM SPSS Statistics for Windows). T-tests were performed to assess differences in sound features between populations (weakfish) and species. As sound duration is the result of the number of pulses in a sound and pulse period, this parameter was not included in these tests. When there was no homogeneity of variances, unequal variance t-tests were used. Data met the assumptions of normality. Comparisons were made between the acoustic features of the different populations/species to answer different questions as follows:

i) Are putative weakfish sounds from the Tagus estuary from the weakfish?

Captive North America weakfish sounds at 21–23 °C (dataset 1, Table 1) were compared with putative weakfish sounds recorded in the Tagus estuary at 22 °C (dataset 3). Frequency parameters were not used to compare the two populations as recordings from dataset 3 had the lower frequencies under-represented, as previously mentioned.

ii) Can we discriminate between meagre and weakfish sounds?

To answer this, we compared sound features between species from recordings obtained both in captivity and *in situ*: a) captive weakfish sounds (dataset 1, 21–23 °C) were compared with captive meagre sounds (dataset 2, 21.6-22 °C); b) sounds recorded in the Tagus estuary of weakfish and meagre were compared (dataset 3, 22 °C).

To further ascertain the ability to discriminate the two species in nature (Tagus estuary) based on sound features, we performed a principal component analysis (PCA) to obtain composite scores for the considered sound features and thus eliminate redundancy caused by intercorrelation among these variables. For this analysis we considered the variables sound duration, number of pulses and mean pulse period. Only PC1 presented an eigenvalue larger than 1 and explained 95.3% of data variance. Sound duration and number of pulses presented factor loading scores on PC1 of 0.97 and 0.99 respectively, while mean pulse period loaded negatively (−0.97) on PC1. We then compared PC1 scores between species with a *t*-test.

3. Results

3.1. Validation of the presence of weakfish in the Tagus estuary

We compared captive weakfish sounds with putative weakfish recorded in the Tagus estuary. Weakfish sounds recorded from captive adult fish collected from their native range did not differ in the number of pulses ($N_{\mathrm{N.\ America}} = 15$, $N_{\mathrm{Portugal}} = 30$, *t*-test, $t = 0.73$, $P > 0.05$) or pulse period (*t*-test = 0.16, $P > 0.05$) from the sounds recorded in the Tagus estuary (Fig. 2; Table 2). Sounds recorded in both locations present a remarkable similarity (Fig. 3).

3.2. Discrimination between weakfish and meagre sounds

3.2.1. Sounds recorded in captivity

Sounds made by captive fish during the breeding season differed significantly between species in the number of pulses ($N_{\mathrm{weakfish}} = 15$, $N_{\mathrm{meagre}} = 28$, *t*-test, $t = -4.54$, $P < 0.001$) and in the mean pulse period ($t = 10.88$, $P < 0.001$) but not in the peak frequency ($t = 0.25$, $P > 0.05$). Although there was considerable variability in the number of pulses in the analysed sounds, meagre grunts were characterised by a higher number of pulses reaching 130 pulses in contrast with the maximum of 14 pulses found for the weakfish (Fig. 4; Table 2). The pulse period was shorter in the meagre and its range did not overlap the pulse period range of the weakfish (Fig. 4; Table 2), suggesting that this is a reliable parameter to easily distinguish the two species.

3.2.2. Sounds recorded in the Tagus estuary

The sounds of the wild meagre and weakfish in the Tagus estuary were compared. As observed for captive fish, weakfish sounds presented a significantly lower number of pulses ($N_{\mathrm{weakfish}} = 30$, $N_{\mathrm{meagre}} = 30$, *t*-test, $t = -37.39$, $P < 0.001$) and a significantly longer mean pulse period and without overlap ($t = 30.14$, $P < 0.001$) than meagre (Fig. 5; Table 2). A Principal Component Analysis retained a single compositive score explaining over 95% of data variance which integrated sound duration, number of pulses and mean pulse period, all with factor loading scores of 0.97 or higher (note that only pulse period loaded negatively on PC1). PC1 values differed significantly between species with meagre presenting higher and non-overlapping values ($t = -31.03$, $P < 0.001$; Fig. 5C).

Table 2
Descriptive statistics for weakfish and meagre sounds made during the breeding season. Captive weakfish sounds (North America, University of Delaware) refer to dataset 1 in Table 1 (sourced from Connaughton and Taylor, 1996). Captive meagre sounds (Portugal) refer to dataset 2 (sourced from Pereira, 2019). The sounds of wild weakfish and meagre were recorded in Portugal (Tagus estuary, TE) and refer to dataset 3 (present study). Note that frequency parameters were not analysed for wild fish due to frequency-domain nonlinearity of the custom-made autonomous acoustic loggers.

		Weakfish		Meagre	
Acoustic parameters		Captive - N America	TE - Portugal	Captive - Portugal	TE - Portugal
	N	15	30	28	30
Sound duration (ms)	mean	290.9	320.4	516.8	597.9
	SD	94.2	77.4	499.3	63.6
	Min	133.7	226.8	37.0	475.5
	Max	528.3	504.4	2176.0	728.7
Number of pulses	mean	6.1	5.6	31.8	32.6
	SD	2.8	1.5	29.8	3.6
	Min	3	4	3	26
	Max	14	9	130	41
Pulse period (ms)	mean	58.3	57.7	16.4	17.4
	SD	14.9	7.3	1.8	0.7
	Min	30.8	45.4	12.3	16.4
	Max	96.1	90.4	22.3	18.9
Peak frequency (Hz)	Mean	269.9		261.6	
	SD	38.8		171.1	
	Min	217.3		117.0	
	Max	351.6		586.0	

3.3. Potential for in situ mapping of meagre and weakfish with PAM

Aural and visual inspection of the field recordings from the Tagus estuary in Portugal (dataset 3) depicted obvious differences (Audio S1; Video S1) in the sounds produced by the two species at a given temperature (same file), with weakfish pulse emission rate being obviously slower to the human ear. A visual assessment of the oscillograms and spectrograms showed that the differences ascertained by statistical analyses were easily picked up by sight, with weakfish sounds being

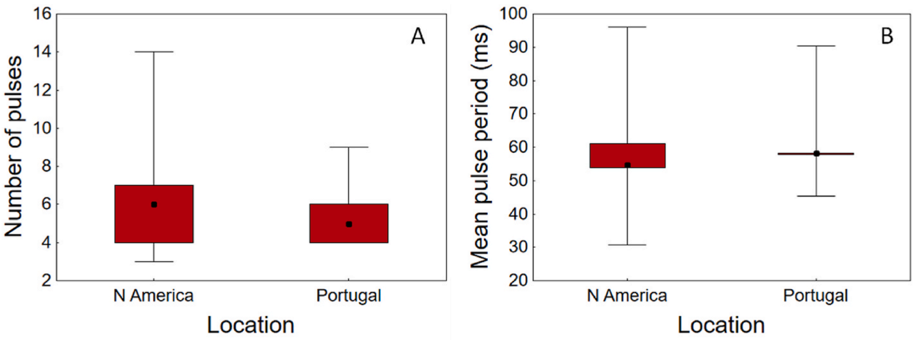

Fig. 2. Comparison of the (A) number of pulses and (B) mean pulse period (ms) of weakfish grunts produced during the breeding season from fish recorded in captivity – North America (University of Delaware; Connaughton and Taylor, 1996) and in nature – Portugal (Tagus estuary; present study). The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers).

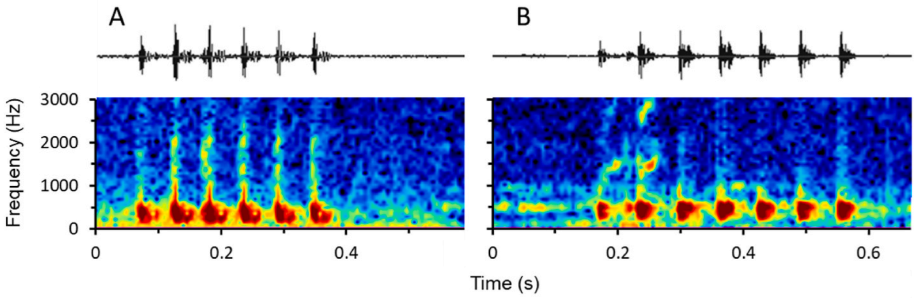

Fig. 3. Oscillograms and spectrograms of weakfish breeding sounds recorded in (A) captivity – North America (University of Delaware; Connaughton and Taylor, 1996) and (B) in nature – Portugal (Tagus estuary; present study). Spectrogram configuration: Sampling frequency, 8 kHz; FFT size, 1,024; window points: 96; window type, Hanning; overlap samples per frame, 50%.

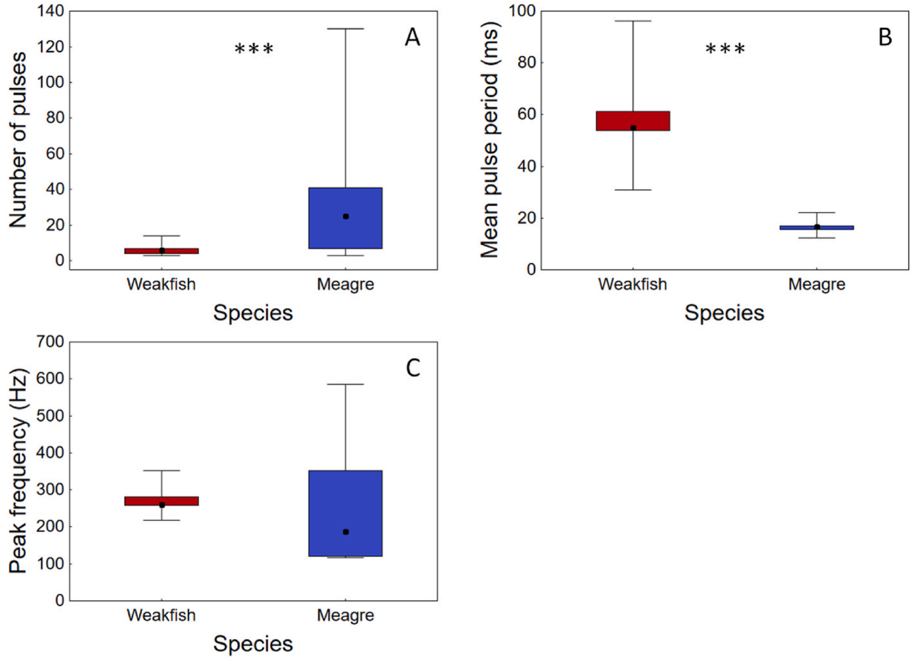

Fig. 4. Comparison of the (A) number of pulses, (B) mean pulse period (ms), and (C) peak frequency (Hz) of grunts produced by captive weakfish (North America, University of Delaware; Connaughton and Taylor, 1996) and captive meagre (IPMA-EPPO; Portugal, Pereira, 2019) during the breeding season. The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers). *** = $P < 0.001$.

shorter, with fewer pulses and longer pulse periods than meagre (Fig. 6A). The sounds obtained from the land-based recorder (dataset 4) were also inspected. Sounds from weakfish were more distant from this logger and were not analysed but illustrate that as in sounds recorded from captive fish, the frequency ranges from wild meagre and weakfish overlap in the Tagus estuary (Fig. 6B). Note that the spectral properties are well preserved by the HTI hydrophone used in the recordings (Fig. 6B).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.marenvres.2023.106017

In addition, we mapped sound occurrence of these two sciaenids for 24 h in the Tagus estuary. Most sounds were detected at the end of the day, with the presence of high intensity choruses (very clear in the LTAS) (Fig. 7A). However, in the LTAS it is difficult to distinguish between species because they occur in overlapped frequency bands. Through aural and visual inspection of the short-term spectrograms (Fig. 7B and C), it was clear that the choruses were mostly dominated by the meagre, and that the weakfish sounds had less energy, suggesting that they were further away. For this visual inspection, the spectrograms with a FFT size of 256 points (temporal resolution of 64 ms and frequency resolution of 15.6 Hz) were used to maximize the visual differences due to the differences in pulse period (cf. Fig. 7C).

4. Discussion

The weakfish is a recent invasive species in the Iberian Peninsula and was first reported in the Tagus estuary in 2015 (Morais et al., 2017). There is concern about its possible impacts on native species, including the confamiliar meagre, as they consume the same prey and share the same habitat (Cerveira et al., 2021). Moreover, they could be competing for acoustic resources as both engage in dense choruses during breeding season and the spectral features of their sounds overlap (Connaughton and Taylor, 1995; Largardère and Mariani, 2006). Here, we provide evidence that PAM can be a cost-effective tool to monitor the spatial usage of weakfish during the breeding season outside its natural distribution. Being able to effectively detect and monitor the spatial use and activity of the weakfish provides a critical tool for its management (Juanes, 2018).

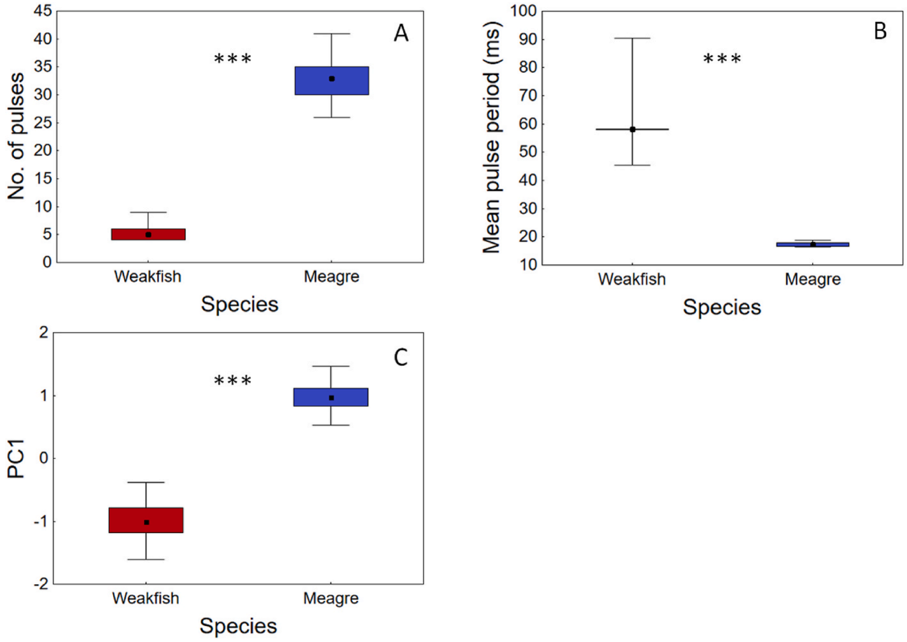

Fig. 5. Comparison of the (A) number of pulses, (B) mean pulse period (ms) and of the (C) compositive variable representing sound duration, number of pulses and mean pulse period (first principal component of a PCA), of grunts produced by wild weakfish and meagre in the Tagus estuary during the breeding season. The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers). *** = P < 0.001.

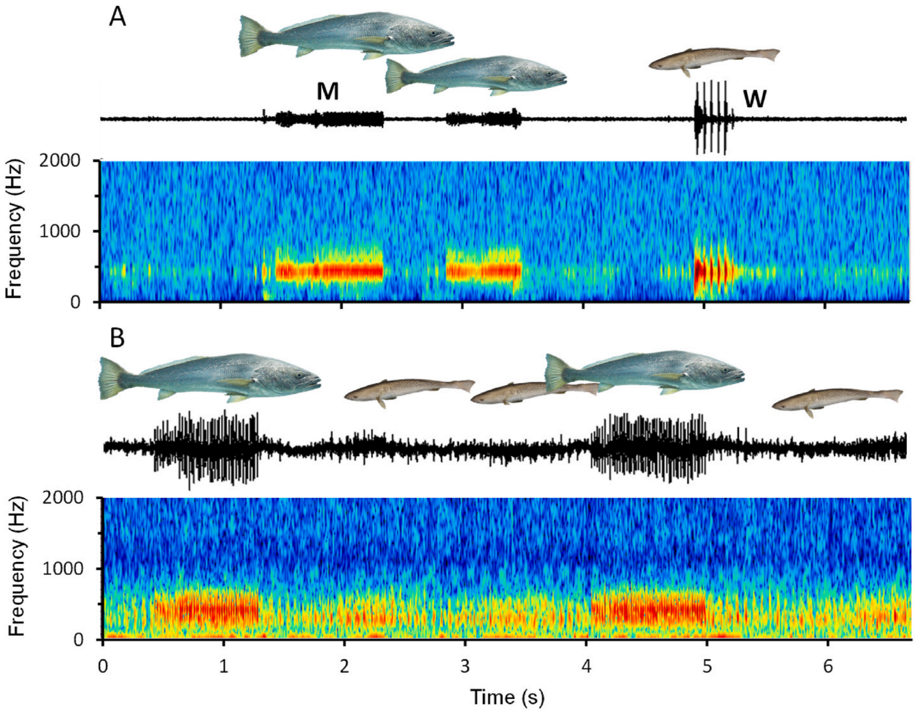

Fig. 6. Oscillogram and spectrogram showing the co-occurrence of weakfish (W) and meagre (M) in the Tagus estuary in (A) July 2020 in logger 1 (an example from dataset 3) and (B) July 2021 in Air Force Base 6 logger. Note that in (B) it is possible to observe an almost continuous occurrence of weakfish grunts, creating a chorus. Spectrogram configuration: Sampling frequency, 8 kHz; FFT size, 1024; window points: 96; window type, Hamming; overlap samples per frame, 50%.

4.1. Validation of the presence of weakfish in the Tagus estuary

We show that the sciaenid-like sounds recently recorded in the Tagus estuary have similar number of pulses and pulse period to the sounds recorded from captive breeding weakfish, indicating that they are from the weakfish. Using recorded sounds from captive fish to validate field recordings is important to avoid misidentification of sound producers (Luczkovich and Sprague, 2002). For example, the sounds of the striped cusk-eels have been incorrectly identified as weakfish ‘chatters’ due to lack of such validations (Sprague and Luczkovich, 2001). Meagre sounds have been recorded in the Tagus estuary since 2000 (unpublished data) and have been previously validated with recordings of captive fish of different sizes and breeding conditions (Pereira et al., 2020). Fig. S2 depicts meagre grunts recorded during the breeding season in captivity

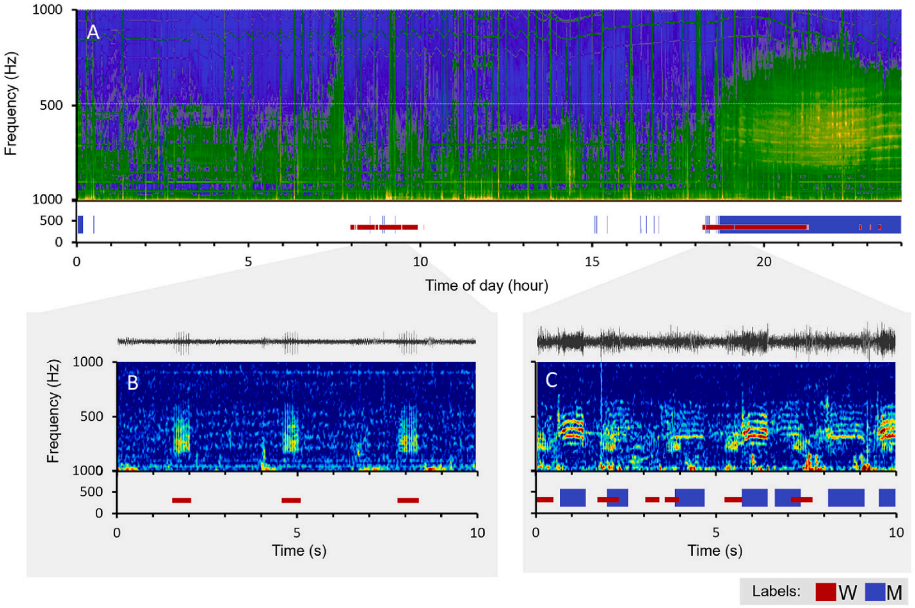

Fig. 7. Long-term averaged spectrogram (A, top panel) showing the co-occurrence of weakfish (W) and meagre (M) through 24 h in July 2021 (BA6 logger), with segments expanded to 10 s spectrograms (bottom panels). The 10 s spectrograms depict (B) weakfish grunts and (C) chorus with meagre and weakfish grunts. Under each spectrogram, sound labels restricted to the usual species-specific frequency range are used to indicate the presence of each species. Spectrogram settings: Sampling frequency: 4 kHz; FFT size: 1024; window type: Hamming, 50% overlap; for the insets to observe individual sounds, a FFT size of 256 was used. Note a 50 Hz electromagnetic induced noise (and respective harmonics) from the recording setup is also noticeable in the LTAS. W – weakfish; M – meagre.

and in nature.

4.2. Discrimination between weakfish and meagre sounds

We further demonstrate that grunts recorded either in captivity or in nature (Tagus estuary) during the breeding season differ markedly in sound duration, number of pulses, and pulse period between the weakfish and the meagre. Importantly, these differences are easily detected through visual and aural inspections of the recordings made in the Tagus estuary, making acoustic recognition feasible even for the non-experts.

The Sciaenidae family has numerous soniferous species that produce sounds (Borie et al., 2014; Ramcharitar et al., 2006). Although sciaenid sounds are species-specific (Luczkovich et al., 2008; Picciulin et al., 2021) they are characterised by a similar acoustic structure: they are made of pulse trains with most acoustic energy below 1 kHz (for an exception see Mok et al., 2020), lacking frequency modulation. Sciaenid fishes produce these sounds thanks to high-speed sonic muscles originating from the hypaxial musculature, bilaterally surrounding the swimbladder and inserting on a central tendon dorsal to the swimbladder (Connaughton et al., 1997; Hill et al., 1987; Ramcharitar et al., 2006). Fast contractions of these sonic muscles drive the swimbladder in a transient response, where each muscle-twitch corresponds to one pulse within the call (Connaughton et al., 2000; Parmentier and Fine, 2016; Sprague, 2000). In Sciaenid fishes, therefore, the number of pulses corresponds to the number of sonic muscle twitches and pulse period corresponds to muscle contraction rate (Parmentier and Fine, 2016; Sprague, 2000). This implies that the observed differences in acoustic features characterising meagre and weakfish sounds are related to faster and more numerous sonic muscle contractions (per sound unit) occurring in the meagre. That is, species-specific acoustic differences are likely related to species-specific morphological, histological, and/or physiological differences at the level of the sonic apparatus and of the underlining neuromotor system including the central pattern generator, which deserve further investigation.

In fish, which are poikilotherms, the activity of the neuromotor system controlling sonic muscle contraction depends on temperature (Ladich, 2018). In the weakfish, higher temperatures have been shown to increase pulse repetition rate within the same call (i.e., to decrease pulse period; Connaughton et al., 2000). A similar effect of temperature was observed in the meagre (Vieira et al., 2019). In this study, we compared sounds from captive specimens made at similar temperatures. Wild weakfish and meagre vocalizations were recorded in the same location and at the same time, thus in the same temperature conditions; therefore, the influence of environmental factors, such as water temperature on pulse repetition rate can be excluded.

4.3. Potential for in situ mapping of meagre and weakfish with PAM

Several studies have made use of PAM to distinguish and monitor different fish species, namely sciaenids. For example, Luczkovich et al. (2008) recorded sounds produced by spawning fish in Pamlico Sound, North Carolina (USA), including the weakfish and three other sciaenids (red drum *Sciaenops ocellatus*, spotted seatrout *Cynoscion nebulosus* and silver perch *Bairdiella chrysoura*) and mapped their specific spawning areas and spawning times. Picciulin et al. (2021) recorded sounds produced by the elusive *Umbrina cirrosa* (shi drum) and *Sciaena umbra* (brown meagre) in tidal inlets of the Venice lagoon (Italy) and showed through manual acoustic analysis that their sounds were distinguishable by their temporal features: shi drum sounds were made up of a lower number of longer pulses, repeated at a lower rate than those of the brown meagre. Picciulin and colleagues thus showed that PAM allows for fine-scale mapping of these two species. Monczak et al. (2019) used long-term recordings (several months) from the May River estuary (South Carolina, USA) to create an acoustic library for four sciaenid species (black drum *Pogonias cromis*, silver perch *Bairdiella chrysoura*, spotted seatrout *Cynoscion nebulosus* and red drum *Sciaenops ocellatus*) and to subsequently create a signal detector to automatically detect, classify and quantify the number of calls from these species in the recordings. These three examples illustrate that PAM, especially when combined with automatic detection procedures, can be an invaluable and powerful tool to detect and monitor both alien and indigenous soniferous fish species. In addition, it could provide insight of the spawning potential (e.g. by evaluating the overlap between sounds and the loudness of the choruses, Vieira et al., 2019, 2022) and degree of competition (temporal and spatial overlap of breeding choruses, Luczkovich et al., 2008) of fish species that make conspicuous breeding sounds. Our study shows that PAM can be used to detect and monitor weakfish in a busy and noisy estuary such as the Tagus (Vieira et al., 2021a), even when co-occurring with other soniferous fish species from the same family. It further confirms that it is possible to use fish sounds for *in situ* mapping.

Although advancements in recording and analytical technology have rendered PAM a promising tool for monitoring soniferous fishes (Gannon, 2008; Lindseth and Lobel, 2018; Rountree et al., 2020) there are still limitations that need to be overcome. For example, many fishes are yet to be auditioned and their sounds described (Gannon, 2008; Lindseth and Lobel, 2018; Rountree et al., 2020). In addition, anthropogenic noise, which can mask fish acoustic communication, may also hinder the detection of fish sounds (Vieira et al., 2021b, 2022). However, once the acoustic behaviour of a target species has been well described, PAM, in association with automated detection methods (e.g. Vieira et al., 2019), could be effective for early detection of invasive species and for monitoring their expansion. PAM could complement other useful surveillance techniques such as environmental DNA metabarcoding and citizen science (Larson et al., 2020). Indeed, the rapid spread of weakfish along Iberian coastal waters was described through citizen science (angler) records or incidental collections done by commercial fishermen (Morais et al., 2017). But although anglers are often the first to provide new records about invasive fishes in new areas (Gago et al., 2016; Martelo et al., 2021), most of this information requires data curation and validation that could be time consuming, being recurrently biased for specific localities, depending for example on species' popularity among recreational fishermen (Martelo et al., 2021). PAM could thus compensate for some of these shortcomings.

4.4. *Final remarks*

We show that the non-indigenous weakfish can be monitored in the Tagus estuary with PAM and distinguished from a native sciaenid, the meagre, by the temporal patterns of their grunts. Most importantly, we demonstrate that PAM allows the discrimination of the two species while in the same area and when calling simultaneously through visual and aural inspections of the recordings. Complementing PAM with automatic detection and recognition methods should be feasible (Vieira et al., 2019) allowing for scalable monitoring of this invasive fish, which is critical for conservation efforts. We suggest that the present study provides baseline information for a passive acoustics monitoring program that could be implemented along the Iberian coast to monitor the spread of the invasive weakfish northward. For example, PAM stations could be implemented in existing facilities such as CoastNet (coastnet.pt), a research infrastructure aiming at monitoring important ecosystems of the Portuguese coast.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106017.

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