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Sound discrimination of two sympatric, threatened fish species allows for their *in situ* mapping

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Abstract

1. Human impacts on marine ecosystems are increasing and the number of fish species listed in the Red List is constantly growing. In the Mediterranean Sea, seven of the 10 bony fishes defined as Threatened by the IUCN are known to be vocal, including the target species of this study: the shi drum (*Umbrina cirrosa* Linnaeus, 1758) and the brown meagre (*Sciaena umbra* Linnaeus, 1758). As a result, non-invasive passive acoustic monitoring (PAM) can be used to pinpoint their distribution at sea. However, for PAM to be effective, reliable acoustic discrimination is required because the sounds they emit during reproduction are remarkably similar (i.e. short broadband pulsed sounds).

2. The shi drum and the brown meagre are closely related, elusive, vocal sciaenids, partially overlapping in their ecological niche. During summer 2019, three PAM surveys were conducted along the tidal inlets of the Venice lagoon (Italy). Here, the calls of both species have been recognized according to their temporal features: shi drum sounds were made up of a lower number of longer pulses with a different envelope, repeated at a lower rate than those of the brown meagre. Further, shi drum and brown meagre sounds of different origins (aquaculture and semi-natural conditions) were analysed and compared with those collected during the survey of the study area in order to validate the field collected data.

3. Call discrimination allowed for a fine-scale species mapping, showing a partially overlapping distribution of the two species along the inlets. This is the first case in which two sciaenids have been documented to share their reproductive habitat in the Mediterranean Sea.

4. This study demonstrates that it is feasible to acoustically monitor the target species even in those parts of the Mediterranean Sea where they co-exist. This, in its turn, could provide managers with the required data for effective conservation measures to be implemented.

KEYWORDS
biodiversity, coastal areas, endangered species, fish, fishing, monitoring, protected species, reproduction

1 | INTRODUCTION

Human impacts on natural ecosystems are increasing. Nowadays, over 100 million tons of fish are fished commercially each year, while another 900,000 tonnes are fished recreationally (Pauly & Zeller, 2016; Freire et al., 2020). The total number of marine fish species listed on the IUCN Red List is constantly growing (Davies & Baum, 2012). An effective management of fisheries resources is a fundamental requirement for maintaining fish stock abundances at target levels (Hilborn et al., 2020). In the Mediterranean Sea, 10 of the 442 native marine bony fish species are listed in threatened categories, which include the Critically Endangered, Endangered and Vulnerable species (Table 1; IUCN, 2020). Seven of these species have been proved to be vocal (Table 1). Vocal fish rely on sounds for defending territories, protecting the access to mates and for exhibiting courtship displays, conveying information on emitter identity, body size and fitness, which influence individual reproductive success (Lobel, Kaatz & Rice, 2010; Vasconcelos et al., 2012; Amorim, Vasconcelos & Fonseca, 2015; Casaretto et al., 2015). Several hundred fish species, belonging to different families and orders, are known to produce sounds using a large diversity of sound-producing mechanisms (Lobel, 1992; Amorim, 2006; Ladich & Fine, 2006; Fine & Parmentier, 2015; Lindseth & Lobel, 2018). The presence of vocal fish at sea can be detected by passive acoustic monitoring (PAM), a non-destructive, non-invasive, low-cost, replicable technique that uses hydrophones to record *in situ* the biological vocalizations and identifies the emitting species (Rountree et al., 2006). PAM represents a reliable alternative to traditional methods to survey fish spawning aggregations (FSAs) at sea. The assessment of FSAs is the basis of effective fisheries management and conservation strategies (Erisman

et al., 2015) and it is usually based on specimen net-harvest methods that, however, are not always efficient across diverse environments (Holt, Holt & Arnold, 1985; Lowerre-Barbieri, Chittenden & Barbieri, 1996; Colin, 2012). As a consequence, fish management would largely benefit from fisheries-independent methods that can sustainably monitor FSAs over different spatial and temporal scales.

At sea the FSAs of vocal species can generate intense choruses that greatly exceed local ambient noise levels (Parsons et al., 2016; Erisman & Rowell, 2017; Rice, Soldevilla & Quinlan, 2017). Fish choruses have been used as proxies to determine the timing and location of spawning aggregations, as well as for evaluating species distributions and essential habitats (Luczkovich et al., 2008; Tellechea et al., 2010b; Rice, Soldevilla & Quinlan, 2017; Monczak et al., 2019; Picciulin et al., 2020). Furthermore, the sound level of fish choruses provides insights into fish population abundances (Rowell et al., 2012; Rowell et al., 2017). PAM has already been proved as a valuable tool for locating FSAs of exploited species, such as cod, haddock and other gadidae (Rountree et al., 2006; Hawkins & Picciulin, 2019; Caiger et al., 2020). In the future, the use of fixed, autonomous recorders and of mobile acoustic sensors (e.g. wave and underwater gliders), together with automatic call detectors, will further expand our capability of monitoring vocal fish species over large spatial (i.e. on the order of tens of kilometres) and temporal (i.e. on the order of years) scales (Mann, Locascio & Wall, 2016; Lembke et al., 2018; Monczak et al., 2019; Pagniello, Cimino & Terill, 2019; Bolgan et al., 2020b).

The detection, characterization and discrimination of fish sound types is the key for developing effective PAM programmes in the Mediterranean Sea. To date, the sounds of ca. 40 vocal fish species have been characterized in this basin (Di Iorio et al., 2018). Despite this, most of the recorded sound types cannot still be attributed to a

TABLE 1 List of the 10 threatened bony fish species inhabiting the Mediterranean Sea according to the IUCN (2020), where vocal species are indicated

Threatened species	Common name	Family	Status of conservation	Vocal	Reference
Pomatoschistus microps	Common goby	Gobidae	Critically Endangered	Vocal	Blom et al., 2016
Pomatoschistus tortonesei	Tortonese's goby	Gobidae	Endangered	Not known to be vocal but it belongs to a vocal family	
Thunnus thynnus	Atlantic bluefin tuna	Scombridae	Endangered	Not known to be vocal	
Epinephelus marginatus	Dusky grouper	Epinephelidae	Endangered	Vocal	Bertucci et al., 2015
Merluccius merluccius	Common hake	Merluciidae	Vulnerable	Vocal	Groison, Kjesbu & Suquet, 2011
Labrus viridis	Green wrasse	Labridae	Vulnerable	Vocal	Steinberg et al., 1965
Sciaena umbra	Brown meagre	Sciaenidae	Vulnerable	Vocal	Picciulin et al., 2012
Umbrina cirrosa	Shi drum	Sciaenidae	Vulnerable	Vocal	Bolgan et al., 2020a
Dentex dentex	Common dentex	Sparidae	Vulnerable	Not known to be vocal	
Pomatoschistus minutus	Freckled goby	Gobidae	Vulnerable	Vocal	Blom et al., 2016

specific fish species and the contexts in which they are emitted remain unknown (Desiderà et al., 2019; Bolgan et al., 2020b). In the Mediterranean Sea, the broadest unidentified category of fish sounds consists of series of transient pulses (Desiderà et al., 2019). Within the pulsed sound category, the variability is usually mostly expressed in the time domain (i.e. duration, number of pulses, pulse repetition rate; Desiderà et al., 2019). In fish, this variability can promote reproductive isolation of closely related species, as well as enhancing mate choice or competitor recognition within the same species (Amorim, 2006). The temporal patterning of sounds was suggested to promote species recognition within assemblages of closely related sympatric species (Kihslinger & Klimley, 2002). In gobies, pulse rate and duration could be used to differentiate groups of closely related species (Malavasi, Collatuzzo & Torricelli, 2008). Temporal properties of the acoustic pulse trains produced by Pomacentrids also appear to be the most important feature in species recognition (Parmentier et al., 2009).

For PAM purposes it is crucial to identify a combination of acoustic features which allow the effective and consistent discrimination of pulsed vocalizations emitted by different fish species. Pulsed sounds are known to be produced by Sciaenids (Parmentier et al., 2014), which are present in the Mediterranean Sea with three genera comprising five species (Chao, 1986): the meagre *Argyrosomus regius* (Asso, 1801), the brown meagre *Sciaena umbra* (Linnaeus, 1758), the shi drum *Umbrina cirrosa* (Linnaeus, 1758), the Canary drum *Umbrina canariensis* (Valenciennes, 1843) and the fusca drum *Umbrina ronchus* (Valenciennes, 1843) (Chao, 1986). During reproduction, the shi drum and the brown meagre emit low-frequency pulsed sounds with main energy below 1 kHz (Picciulin et al., 2012; Lagardère & Parmentier, 2014; Picciulin et al., 2016). So far, shi drum sounds have been recorded only in captivity (Lagardère & Parmentier, 2014; Picciulin et al., 2016; Bolgan et al., 2020a). In aquaculture facilities, shi drum calls are emitted at significantly higher rates during spawning nights, when temporal features can provide information on the onset of reproduction (Bolgan et al., 2020a). In contrast, the presence of the brown meagre has been widely monitored by PAM in several coastal areas of the Mediterranean Sea (Bonacito et al., 2002; Picciulin et al., 2013; Colla et al., 2018; Parmentier et al., 2018; Desiderà et al., 2019; Di Iorio et al., 2020), where choruses provide a reliable natural indicator of this species' breeding sites (Picciulin et al., 2020).

Over-exploitation by fisheries is a major threat for the shi drum and the brown meagre, especially during spawning aggregations (Arneri, Coltella & Riannetti, 1998). As a result, these are two of the 10 Mediterranean species currently classified as Threatened in the IUCN Red List (Table 1; Bizsel et al., 2011; Yokes et al., 2011). A set of immediate measures are required to reduce catches, enhance their sustainable management and reinforce science-based management plans (Nieto et al., 2015). Shi drum and brown meagre distributions often overlap, as these two species inhabit similar coastal areas, characterized by sandy and rocky bottoms at depths ranging from −5 to −100 m (Tortonese, 1975). Both species are multiple-batch asynchronous spawners which share the same reproductive period, i.e. from May to August (Grau, Linde & Grau, 2009). In addition to similar

ecology, phylogeny and distribution, the target species share the same kind of sound-producing mechanism, i.e. the forced response model (Lo et al., 2015; Parmentier & Fine, 2016): sounds are emitted thanks to high-speed sonic muscles, which bilaterally surround the swimbladder (Connaughton, Fine & Taylor, 1997; Ramcharitar, Gannon & Popper, 2006). Consequently, the sounds emitted by these two species are similar in both the spectral and the temporal domains. The range of inter-specific temporal variability is particularly limited, with the mean number of pulses generally below five pulses per sound in both species (Picciulin et al., 2013; Lagardère & Parmentier, 2014; Picciulin et al., 2016). As intraspecific variability in temporal features is present in both species and is linked to behavioural motivation (Bolgan et al., 2020a; Picciulin et al., 2020), the discrimination of species-specific sounds at sea is further complicated. In order to successfully monitor the occurrence of these two sympatric species by using PAM, it is therefore imperative to identify a combination of acoustic features which can unequivocally discriminate inter-specific from intra-specific sound variability.

A preliminary study, which compared shi drum calls recorded in semi-natural conditions with brown meagre calls recorded in the wild, had shown that shi drum sounds are made up, on average, of a lower number of pulses repeated at a lower rate than brown meagre sounds (Picciulin et al., 2016). However, in this study, a single shi drum cohort of mature but young individuals was monitored in semi-natural conditions during a single-day recording session. As a consequence, the present paper aims to (i) validate the discrimination of shi drum and brown meagre calls at sea by using an enlarged dataset, and (ii) map the distribution of these two species at a short spatial scale by means of their sounds. To achieve these goals, shi drum and brown meagre sounds of different origins (aquaculture and semi-natural conditions) were analysed and compared with those collected during a recent PAM survey of the study area, i.e. the tidal inlets of the Venice lagoon (Venice). This allowed the discrimination and identification of the two species in the field and, consequently, the characterization of their distribution on a fine spatial scale.

2 | MATERIALS AND METHODS

2.1 | Study area

The Venice lagoon (North Adriatic Sea, 45° 24′ 47″ N, 12° 17′ 50″ E) is characterized by high levels of environmental heterogeneity which result in several habitats suitable for different fish species (Franco et al., 2006). During summer 2019, three acoustic surveys were conducted at 40 recording points distributed along the three tidal inlets connecting the Venice lagoon with the sea (Figure 1a). All three inlets, called Lido, Malamocco and Chioggia, are characterized by the presence of long rocky piers, which extend offshore. Since 2003, the inlets have been further modified by the construction of the Mo.S.E. (Modulo Sperimentale Elettromeccanico, i.e. Experimental Electromechanical Module), a system of mobile barriers which was built with the intention of limiting the flooding of the historical city of Venice. The
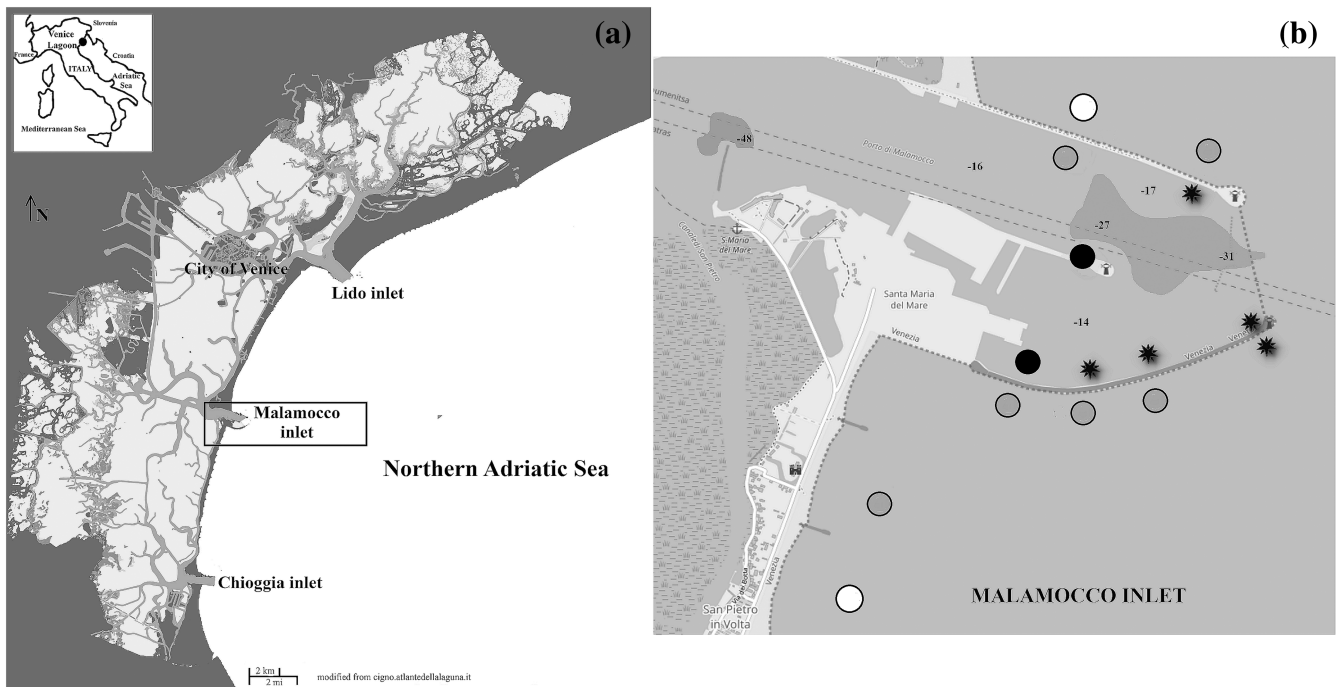
FIGURE 1 (a) Map of the study area. The three Venice inlets explored during the acoustic surveys. (b) Spatial distribution of the recording points (black stars) where *Sciaena umbra* and *Umbrina cirrosa* sounds have been recorded during summer 2019; the black and light grey dots indicate the stations where only *U. cirrosa* sounds and *S. umbra* sounds, respectively, have been recorded in the Malamocco inlet. White dots indicate the stations where no sciaenids sounds have been found. Bottom depth along the inlet is here indicated

Mo.S.E. construction has contributed to a substantial modification of the inlet morphology (Toso et al., 2019). The inlets now present artificial hard substrates in an otherwise soft and mobile sea floor, which increase habitat heterogeneity. It is known that these artificial structures can act as an attraction site for the brown meagre (Fabi, Panfili & Spagnolo, 1998; Bonacito et al., 2002; Colla et al., 2018). Along the Venice inlets, reproductive individuals have been previously acoustically identified (Picciulin et al., 2013), whereas the presence of the shi drum has been reported only anecdotally by local recreational fisherman.

2.2 | Sampling design

The recording locations were located along the Lido inlet (north-eastern inlet; 13 recording points), the Malamocco inlet (central inlet; 15 recording points) and the Chioggia inlet (south-western inlet; 12 recording points) on both the lagoon and the marine sides. Within each inlet, these recording points were located ca. 300 m apart; this distance was decided on the basis of the mean propagation range of brown meagre calls, which was calculated from the sound source levels and assuming cylindrical spreading loss; for details see Codarin et al. (2009).

All recording points within one inlet were acoustically monitored on the same day, in three repetitions, i.e. three days per inlet (28 June, 5 August and 29 August for the Lido inlet; 1 July, 29 July and 28 August for the Malamocco inlet; 5 July, 1 August and 27 August 2019 for the Chioggia inlet). All acoustic recordings were achieved

during summer from 19:00 to 24:00, in correspondence to the seasonal and diel peak of the brown meagre vocal activity (Picciulin et al., 2012; Parmentier et al., 2018). Explorative recordings conducted during the afternoon confirmed the absence of fish sounds at this time.

2.3 | Acoustic recordings

Recordings were obtained using a pre-amplified Colmar GP1280 hydrophone (sensitivity −170 dB re 1 V/µPa, frequency range 5–90 kHz) connected to a Tascam DR40 Handy Recorder (Tascam Corporation, California; sampling rate 44.1 kHz, 16 bit) generating .wav files. Prior to each survey, the signal was calibrated using a generator of sine waves of known voltage. The hydrophone was lowered from an open boat to an average depth of 4 m (depth range 2–6 m). Each recording lasted 5 min. Recordings were carried out with sea state <2 on the Douglas scale, and with wind speed <10 km/h. Water temperature was measured prior to each recording by using a digital thermometer (HANNA Checktemp® 1 HI98509 ± 0.1°C), and ranged from 26.6 to 28.5°C for the acoustic recordings containing fish sounds.

2.4 | Acoustic and statistical analyses

A total of 120 acoustic samples (sampling rate 44.1 kHz, 16 bit) were collected and analysed minute by minute using Adobe Audition

(Adobe Systems, San Jose, CA). Files were downsampled at 4 kHz and spectrograms were visualized using a 256 point FFT (Hanning window, frequency resolution 15.6 Hz). Aural and visual assessment of the spectrograms allowed for the identification of two kinds of pulsed sounds in the acoustic samples. In order to characterize their acoustic features, sound analysis was conducted on a subsample of 50 sounds (i.e. a individually identifiable sequence of pulses) per sound that may correspond to the two species with an optimum signal-to-noise ratio. The following sound parameters were measured: (i) number of pulses per sound unit; (ii) pulse period (peak to peak time interval between consecutive pulses in a sound, ms); (iii) pulse duration (ms); (iv) pulse peak frequency (the frequency with the highest energy, Hz); and (v) pulse bandwidth (the difference between max and min frequency, Hz). Temporal features were measured from the waveform, whereas spectral analysis was run automatically using Avisoft SASLab Pro software (FFT 256 points, Hanning window, frequency resolution 15.6 Hz).

The same analysis was run also on a subsample of: (i) *U. cirrosa* sounds recorded in semi-natural conditions by Picciulin et al. (2016) (N = 52); (ii) *U. cirrosa* sounds recorded during spawning nights in aquaculture facilities by Bolgan et al. (2020a) (N = 33); and (iii) *Sciaena umbra* sounds recorded in the Gulf of Trieste (Northern Adriatic Sea, Italy) by Picciulin et al. (2012) (N = 50); for full details on sound recordings and animal conditions, refer to the above-mentioned studies. Comparisons between sound types were made with non-parametric tests (Kruskal–Wallis test, Mann–Whitney U-test; Sokal & Rohlf, 1995), with an alpha level of 0.05.

In order to evaluate if independent and mutually exclusive sound classes could be identified, a principal component analysis (PCA) was performed in Minitab 18 on the sounds recorded *in situ* during the 2019 summer campaign, as well as on the sounds recorded in captivity, in semi-natural conditions and in the Gulf of Trieste. All acoustic features were inspected for cross-correlation; many acoustic features were found to be highly correlated (e.g. duration and number of pulses). PCA was therefore performed using bandwidth, peak frequency, number of pulses, pulse period and pulse duration as variables. The correlation matrix was used to calculate the principal components, as variables had different scales and needed to be
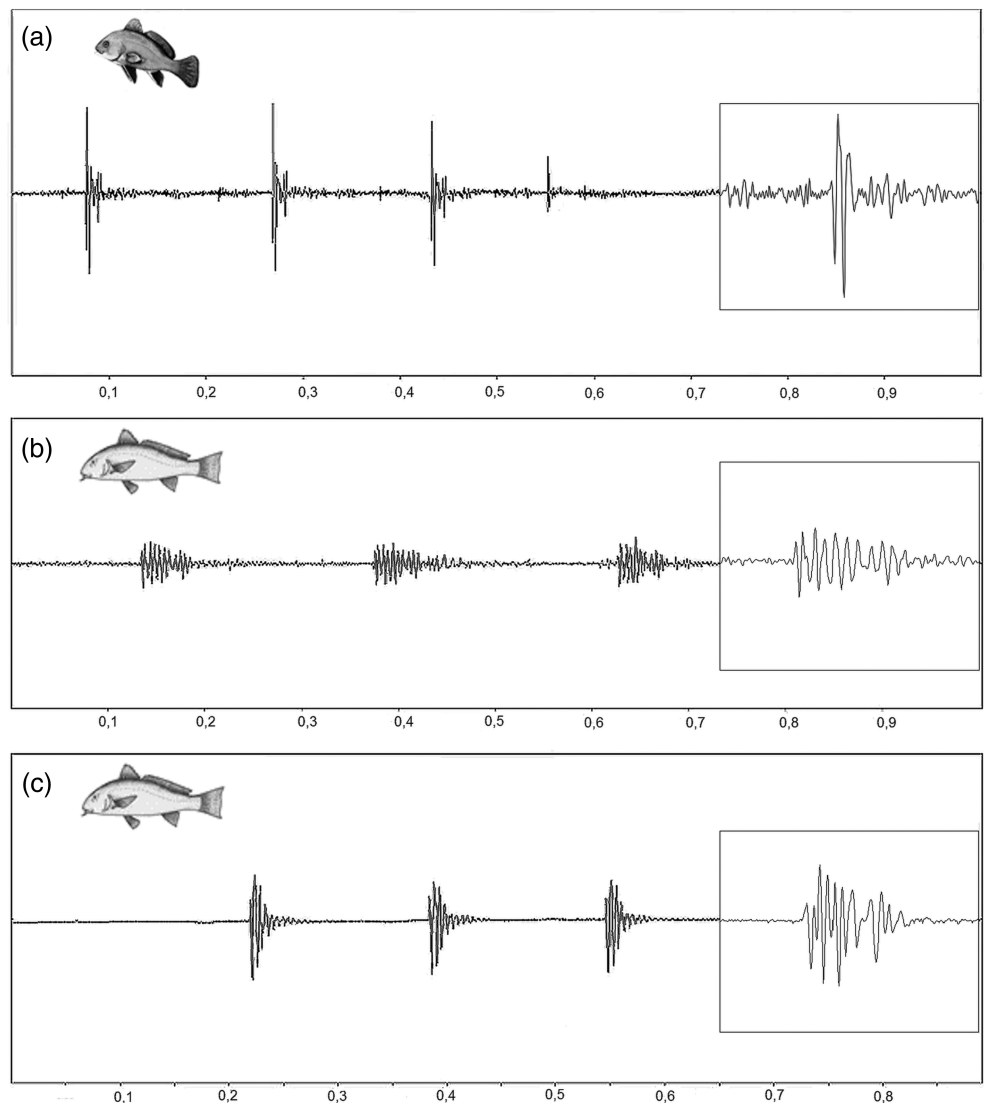

FIGURE 2 Pulse envelope of *S. umbra* (a); and *U. cirrosa* (b) sounds recorded at sea along the Venice inlets, compared with the pulse envelope of *U. cirrosa* sounds recorded in semi-natural conditions (c). Insets show a detail of a single pulse for each panel

standardized. A scree plot for the first five components and a score plot for the first two components (which cumulatively explained 78% of the variance) were generated.

Discriminant analysis was performed in Minitab 18 for (i) calculating the species discriminant functions that best discriminated the brown meagre sounds ($N = 50$) recorded in the Trieste Gulf and the shi drum sounds collected in captivity ($N = 85$) and (ii) predicting to which species the sounds recorded in the Venice inlets belong, according to the measured values in the discriminant functions (predictors comprise number of pulses, duration pulse, pulse-period, peak frequency and bandwidth). Finally, the stations where *S. umbra* and *U. cirrosa* sounds have been recorded were identified.

3 | RESULTS

3.1 | Sound characterizations

Two types of pulsed sounds, which mainly differed in their pulse envelope, were visually and aurally identified in the recordings collected in the Venice inlets. In particular, the first type was characterized by the typical brown meagre pulse envelope, i.e. a sharp increase in acoustic energy the amplitude of which decays rapidly, followed by a series of damped amplitude-modulated oscillations (Figure 2a; Parmentier et al., 2018). As a result it was considered as potentially associated with *S. umbra*. In the second type, the pulse envelope lacks the first, sharp peak of acoustic energy (Figure 2b), thus resembling the pulse envelope of shi drum sounds recorded in semi-natural conditions (Figure 2c). Based on this visual observation, this type was potentially associated with *U. cirrosa*.

In comparison with the first type, the second type of pulse appears longer. Furthermore, the second type of pulsed sound seems to be characterized by a lower number of pulses and by a longer pulse period; this is clearly visible when the two types are recorded simultaneously at sea, and thus with the same water temperature (Figure 3).

The PCA run on these two types of pulsed sounds, as well as on shi drum sounds recorded in both captivity settings and on brown meagre sounds recorded in Trieste, showed that 57% of the variance is explained by temporal features (i.e. number of pulses, pulse duration and pulse period) as well as bandwidth (PCA; Table 2, Figure 4). The first component was fairly efficient in separating brown meagre (positive PCA values) from shi drum sounds (overall negative PCA values);
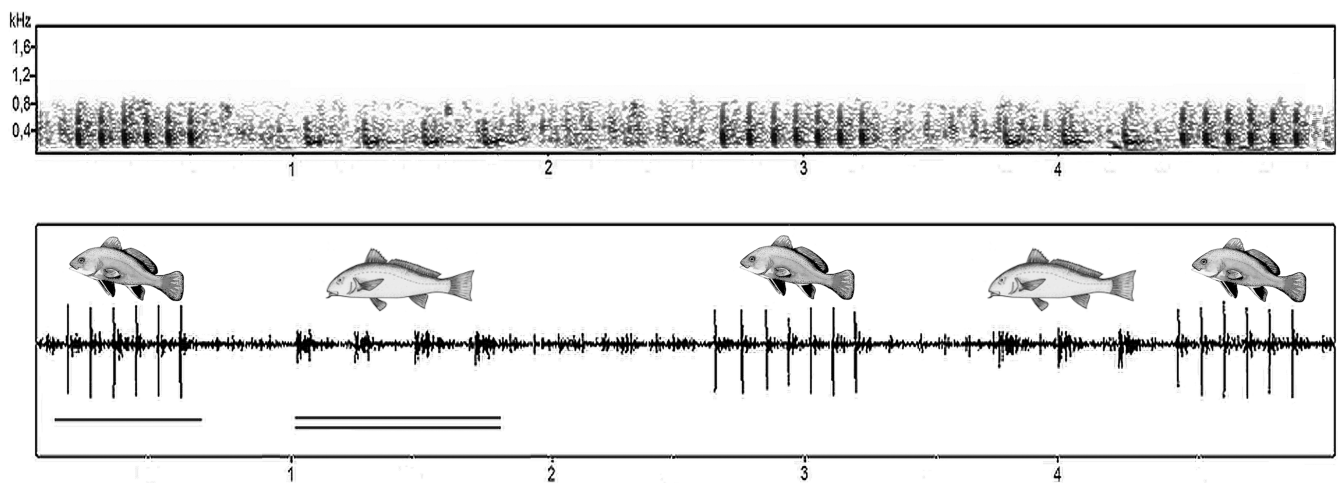

FIGURE 3 Spectrograms (above; FFT 256 points, Hanning window, frequency resolution = 15.6 Hz) and oscillograms (below) of three sounds produced by *S. umbra* (underscored by a single line) and two sounds almost simultaneously produced by *U. cirrosa* (underscored by a double line) recorded at sea

Variable	PC1	PC2	PC3	PC4	PC5
Number of pulses	**0.422**	−0.529	0.266	−0.473	−0.480
Pulse duration (ms)	**−0.498**	−0.207	−0.293	−0.722	0.319
Pulse period (ms)	**−0.538**	0.241	−0.096	−0.014	−0.802
Peak frequency (Hz)	0.308	**0.785**	0.184	−0.504	0.017
Bandwidth (Hz)	**0.416**	0.046	−0.895	−0.007	−0.157
Eigenvalue	2.87	1.01	0.62	0.33	0.16
Percentage	57.4	20.2	12.4	6.7	3.3
Cumulative %	57.4	77.6	90.0	96.7	100

TABLE 2 Principal component analysis; relevant coefficients, eigenvalues, percentage of the variance and cumulative percentage of the variance explained by the first five principal components (PCs)

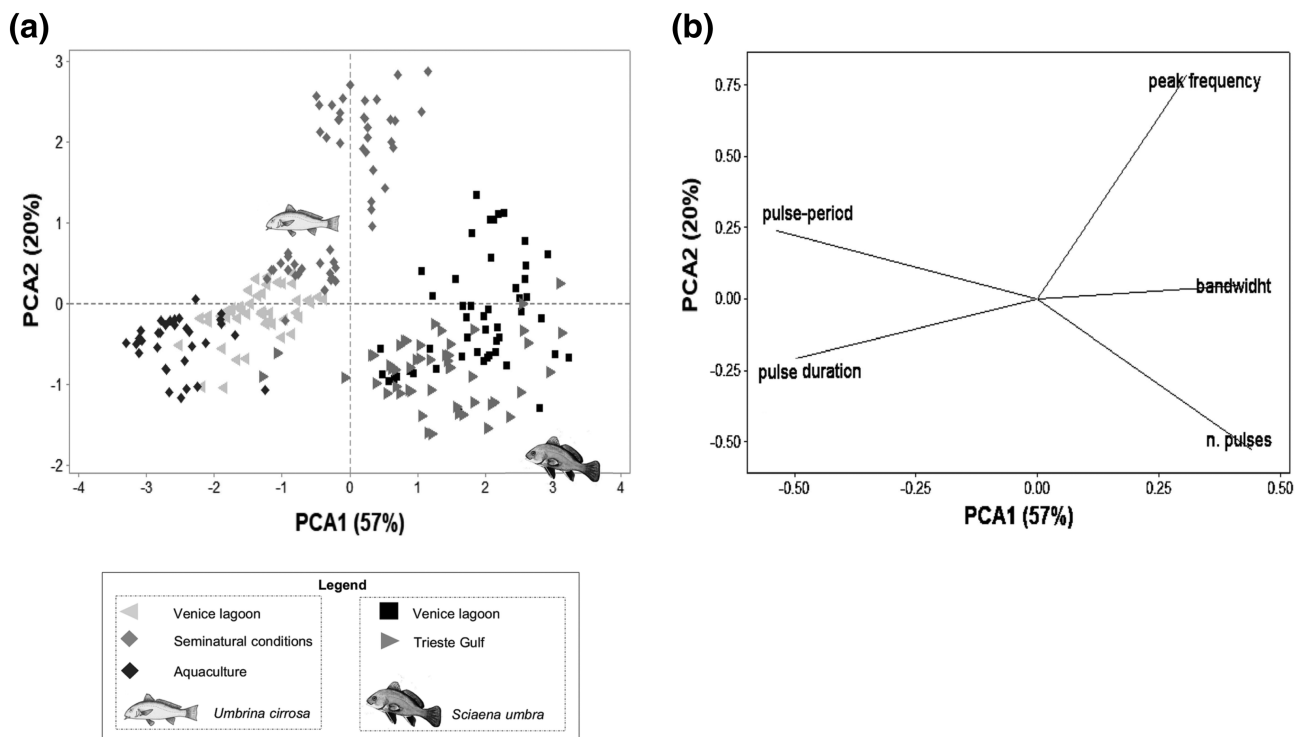*Note:* The main contributors to the first two components are highlighted in bold.

FIGURE 4 (a) Score plot and (b) loading plot of the principal component analysis (PC1 and PC2) carried out on sounds recorded along the Venice inlets as part of this study (N = 100), on the spawning sounds of *U. cirrosa* recorded in aquaculture (Bolgan et al., 2020a; N = 33) and in semi-natural conditions (Picciulin et al., 2016; N = 52), as well as *S. umbra* sounds recorded in the Trieste Gulf (Picciulin et al., 2012; N = 50). Variables: number of pulses, pulse duration, pulse period, peak frequency and bandwidth

in particular, the sounds recorded in the Venice inlets and classified as sounds of the first type clustered together with brown meagre sounds recorded in Trieste (Figure 4). On the other hand, the second type of pulsed sounds recorded in the Venice inlets clustered together with shi drum sounds recorded in captivity (Figure 4). The scree plot, therefore, clearly shows two clusters which correspond to the two species; the only exceptions were some shi drum sounds recorded in semi-natural conditions.

Overall, 100% of sounds were assigned to the correct species by the discriminant function analysis run on the sounds collected in captivity (DFA, d.f. = 5, P < 0.001, Table 3). Furthermore, 50 out of 50 sounds manually assigned to *Umbrina cirrosa* and 49 out of 50 sounds manually assigned to *Sciaena umbra* were attributed to the correct species by the discriminant functions. This analysis fully

confirms that it is possible to discriminate the sounds emitted by these two species at sea.

Table 4 reports the acoustic features of all the sounds used for the PCA; statistical differences between the considered data sets are highlighted here.

3.2 | Sound distribution in the inlets

In summer 2019, brown meagre vocalizations were more abundant than shi drum ones. In particular, brown meagre sounds were identified in 53 recordings, while shi drum sounds were recorded in only 16 (total N recordings = 120, summary in Table 4). Brown meagre sounds were not homogenously distributed along all of the three inlets, with the largest number of sound samples containing this species' sounds (42% of the samples) recorded at the internal stations of the Lido and Malamocco inlets. The shi drum sounds were even more spatially confined, as they were present only within the Malamocco inlet.

Both species showed a decline in their vocal activity throughout the summer: *S. umbra* vocalizations were present in 23 different stations at the end of June, 17 at the end of July and 13 at the end of August (out of 40 recording stations monitored per month). Similarly, shi drum calls were mostly recorded in June (at seven stations) and in July (at seven stations), whereas they were present in only two acoustic samples recorded in August. Although all acoustic monitoring

TABLE 3 Classification summary of the discriminant function analysis (linear)

True group		
Put into groups	*Umbrina cirrosa*	*Sciaena umbra*
Umbrina cirrosa	85	0
Sciaena umbra	0	52
Total number	85	52
Number correct	85	52
Proportion	100%	100%

TABLE 4 Average (± SD) and range of the acoustic features (in brackets) of the brown meagre *Sciaena umbra* and the shi drum *Umbrina cirrosa* sounds recorded along the Venice inlets compared with *U. cirrosa* sounds recorded in semi-natural conditions and in captivity and *S. umbra* sounds recorded at sea along the Gulf of Trieste (Italy)

	Sample size (N)	Number of pulses	Pulse duration (s)	Pulse period (s)	Peak frequency (Hz)	Bandwidth (Hz)
Umbrina cirrosa (Venice inlets)	50	3.2 ± 0.7 (2–5)	0.04 ± 0.01 (0.02–0.08)	0.23 ± 0.01 (0.18–0.26)	202 ± 13 (180–230)	212 ± 65 (120–350)
Umbrina cirrosa (semi-natural conditions; Picciulin et al., 2016)	52	2.1 ± 0.3 (2–3)**	0.03 ± 0.006 (0.02–0.04)**	0.19 ± 0.01 (0.14–0.22)**	391 ± 150 (180–630)**	388 ± 159 (160–670)**
Umbrina cirrosa (captivity; Bolgan et al., 2020a)	33	2.4 ± 0.7 (2–5) **	0.06 ± 0.009 (0.04–0.08)**	0.27 ± 0.02 (0.2–0.31)**	115 ± 8.3 (100–120)**	253 ± 96 (120–530), n.s.
Sciaena umbra (Venice inlets)	50	5.6 ± 1.3 (3–9)**	0.02 ± 0.007 (0.01–0.04)**	0.07 ± 0.009 (0.01–0.04)**	324 ± 103 (170–500)**	434 ± 88 (210–580)**
Sciaena umbra (Gulf of Trieste, Picciulin et al., 2012)	50	5.6 ± 1.3 (3–9)**	0.03 ± 0.009 (0.01–0.05)**	0.03 ± 0.009 (0.07–0.1)**	232 ± 77 (100–420), n.s.	462 ± 131 (230–760)**

**Statistically significant difference (P < 0.001) between the *Umbrina cirrosa* sounds recorded along the Venice inlets compared with the other sounds features (*U*-Mann non-parametric test; n.s., not statistically significant different).

sessions were undertaken from 19:00 to 24:00, the shi drum sounds were recorded at earliest at 19:00 and at latest at 21:45, whereas brown meagre calls were collected throughout the whole recording time-period.

The case of the Malamocco inlet is of particular interest (Table 5): in June, most of the internal stations (five out of the seven) presented shi drum sounds. Furthermore, a simultaneous co-occurrence of both species calls was found at one station, while the last station was characterized by only brown meagre sounds. In July, brown meagre vocal activity expanded along the inlets; the co-occurrence of these two scieanids calls was detected in four stations, whereas brown meagre and the shi drum sounds were found separately in only one station. No vocal co-occurrence between species was observed in August, when the brown meagre vocalizations were the most common in the area. Interestingly, the acoustic samples with vocal co-occurrence of these two species were mostly characterized by a mixed chorus and/or the concomitant presence of many vocal individuals of both *S. umbra* and *U. cirrosa*.

In June and July 2019, the shi drum vocal activity was recorded in only one out of nine external Malamocco stations (station 1; Figure 1b), but in August these locations presented exclusively brown meagre vocalizations.

All together, through the summer period, four out of 40 recording stations were simultaneously or alternately occupied by both scieanid species.

4 | DISCUSSION

This is the first case in which very similar pulsed sounds, simultaneously produced by closely related sympatric fish species, have been effectively discriminated in the Mediterranean Sea. Similarly, along the Atlantic coast, sympatric sciaenids have been discriminated and located at sea thanks to their vocalizations; however, their degree of inter-specific difference appears greater than that characterizing shi drum and brown meagre sounds (Sprague et al., 2000; Luczkovich et al., 2008; Monczak et al., 2017; Monczak et al., 2019). The proposed characterization of shi drum sounds allows for its acoustic monitoring at sea, as is already the case for the brown meagre (Picciulin et al., 2016; Parmentier et al., 2018; Di Iorio et al., 2020).

4.1 | Sounds characterization, detection and discrimination

In this study, two different types of pulsed sounds were identified in the monitored area. Both of them consisted of short, low-frequency trains of pulses that share similarities with sounds described for Mediterranean sciaenids (Picciulin et al., 2012; Parmentier et al., 2018; Bolgan et al., 2020a). Generally, sciaenids sounds share common features, such as an acoustic waveform of the pulse which decays rapidly, short pulses separated by intervals of no sound, a pulse sound spectrum containing energy within a wide bandwidth and finally a

TABLE 5 Presence of (i) *Sciaena umbra* sounds (horizontal lines, light grey), (ii) *Umbrina cirrosa* sounds (light grey) and (iii) both species sounds simultaneously (black) per monitored station in the Venice inlet along the summer period 2019

External stations	June	July	August	Internal stations	June	July	August
Lido inlet							
1	Su	Su	Su	1	Su	Su	Su
2	Su	Su	Su	2	Su	Su	Su
3	Su		Su	3	Su		Su
4	Su			4	Su	Su	
5				6	Su	Su	Su
6	Su			7	Su	Su	Su
Malamocco inlet							
1	Uc	Uc	Su	1	Uc	both	Uc
2	Su	Su		2	Uc	both	Su
3	Su			3	Uc	both	Uc
4				4	Uc	Uc	
6	Su			5	Uc	Uc	
7	Su			6	Su	Su	Su
8				7	both	both	
9							
Chioggia inlet							
1	Su	Su	Su	1	Su		
2				2	Su		
3				3	Su	Su	Su
4				4	Su	Su	
5	Su			5			
6				6		Su	

(Cell shading key: Su = *Sciaena umbra* sounds, horizontal lines light grey; Uc = *Umbrina cirrosa* sounds, light grey; both = both species, black)

relatively limited variability in the call peak frequency (Sprague, 2000; Connaughton, Fine & Taylor, 2002; Parmentier et al., 2014). One of the two types of pulsed sounds recorded within the Venice inlets is attributable to the brown meagre because of its typical pulse envelope and temporal features, i.e. sounds made up of four to seven pulses, with a pulse period of ca. 70–145 ms and a pulse duration of ca. 16–27 ms (Picciulin et al., 2012; Parmentier et al., 2018). All of these features have been proved to be consistent over space and time, despite the fact that intra-specific variation depends on context, size and motivation or the influence of recording settings and conditions (Parmentier et al., 2018; Picciulin et al., 2020).

The second type of pulsed sounds shows a completely different pulse envelope. This envelope was found in shi drum sounds recorded in captivity and in semi-natural conditions, but also in sounds recorded at sea, which excludes tank-induced distortion of the pulse waveform (Parmentier et al., 2014). Furthermore, the observed pulse envelope resembles that described for the congeneric species, the Argentine croaker *Umbrina canosai* (Tellechea, Fine & Norbis, 2017). In terms of sound temporal features, this sound type presents a lower number of longer pulses than the brown meagre, in accordance with previous descriptions of shi drum sounds recorded in captivity (Picciulin et al., 2016; Bolgan et al., 2020a). The sound temporal features and the pulse envelope provide, therefore, a set of variables that can be reliably assigned to the shi drum, as confirmed by both principal component analysis and discriminant function analysis. This observation is in accordance with other studies that suggest that the time interval between pulses, as well as pulse envelope, can be considered as cues for species-specific recognition between sympatric sciaenids (Locascio & Mann, 2008; Tellechea, Fine & Norbis, 2017). In terms of spectral features, the bandwidth contributed to the species discrimination, being narrower for the shi drum, whereas sound peak frequency appeared less informative. An intra-specific variability of shi drum vocalizations is evident in the present results. Intra-specific sound variability in sciaenids owing to differences in fish size, ontogenetic stage and reproductive motivation has been reported by other studies (Connaughton, Taylor & Fine, 2000; Tellechea et al., 2010a; Tellechea, Fine & Norbis, 2017; Bolgan et al., 2020a; Pereira et al., 2020; Picciulin et al., 2020).

4.2 | PAM-based distribution of the target species in the Venice inlets and behavioural context of their sound production

So far, *U. cirrosa* has been found within the Venice lagoon particularly, but not exclusively, at the juvenile or subadult stage (Fabi et al., 2002; Franco et al., 2009; Franzoi, Franco & Torricelli, 2010); this paper highlights for the first time the presence of mature individuals engaged in reproductive activity along the Venice inlets. Here, shi drum vocalizations peaked in June and July, while fewer sounds were recorded in August; this pattern mirrors the reproductive season of this species (Barbaro et al., 2009). In addition, the shi drum vocalized during night-time hours, which is in accordance with the diel pattern

recorded in captivity close to spawning events (Bolgan et al., 2020a). Sciaenids are characterized by a lekking reproductive system (Ramcharitar, Gannon & Popper, 2006). A lek is an arena in which several males aggregate to exhibit courtship displays and where mating occurs; in leks, males are chosen on the basis of individual qualities, which are often expressed by visual or acoustic displays, since no significant resources, such as food, nests or parental care are presented to the females (Höglund & Alatalo, 1995). As a result, vocalizations play an important role in the sciaenid reproduction, leading to the formation of large choruses at the spawning coastal areas (Ramcharitar, Gannon & Popper, 2006). Generally, sciaenid reproduction involves multiple courting males closely pursuing the female, while producing sounds and repeatedly nudging her urogenital region until spawning is acheived (Picciulin et al., 2020). In the shi drum, male competition has been reported, where the stronger male in the group gains higher fertilization success (Basaran et al., 2009).

The presence of the brown meagre has also been previously reported in the Venice lagoon (Franco et al., 2006), but reproductive individuals were acoustically identified only along the tidal inlets (Picciulin et al., 2013), which is confirmed by the present paper. This study further demonstrated a temporal and spatial overlap between these two sciaenids during their reproductive period: the two species were vocally active at the same time but in separate locations along the Venice inlets but they were also simultaneously chorusing in an area with a radius smaller than 300 m (the brown meagre calls were estimated to be detectable at approximately 300 m by Codarin et al. (2009); no data are available for the shi drum).

4.3 | Spatial distribution and sympatry of the target species along the Mediterranean Sea

Reproductive activities within bays and lagoons and near sea inlets have been reported for many sciaenids owing to their high adaptability to the fluctuating physical conditions typical of these habitats (Vieira & Musick, 1994). In the context of estuarial areas, both *S. umbra* and *U. cirrosa* have been defined as 'marine stragglers' (Koutrakis, Tsikliras & Sinis, 2005), i.e. species that spawn at sea and typically enter estuaries only in reduced numbers and are most frequently found in the lower reaches, where the salinity is similar to that of the marine environments (Potter et al., 2014). *Umbrina cirrosa* has also been described as a 'marine seasonal migrant', which enters lagoons mainly during the spring and summer months in order to take advantage of the high abundance of prey available in these coastal systems (Franzoi, Franco & Torricelli, 2010).

The shi drum can be found inside lagoons and along the estuaries of the Mediterranean Sea (Sobrino et al., 2005; Dulčić & Glamuzina, 2006), where the catch of young individuals confirmed their role as nursery and feeding areas for the species (Koutrakis, Tsikliras & Sinis, 2005). *Umbrina cirrosa* has been reported in Aegean, Spanish, French, and Turkish estuarine systems (Koutrakis & Tsikliras, 2003; Lobry et al., 2003; Sobrino et al., 2005; Innal, 2020), whereas *S. umbra* – but not *U. cirrosa* – was present in the fish

checklists of the coastal lagoons along the central-western shore of Italy (Tyrrhenian basin; Mariani, 2001). The co-occurrence of both brown meagre and shi drum along three estuarine systems in the Croatian waters can be inferred from Dulčić & Glamuzina (2006) and D'Adamo et al. (2009) for the Lesina, Varano and Alimini Adriatic coastal lagoons.

Non-overlapping spawning areas of sympatric marine species which avoid offspring competition for food, shelter and/or space has been demonstrated for Clupeiformes (families Clupeidae and Engraulidae), Sparidae and Mugilida (Tsikliras et al., 2010). This is clearly not the case of the target species, both being summer spawners (*sensu* Tsikliras et al., 2010), with a co-occurring reproductive season. Although not specifically related to their spawning period, other *in situ* monitoring supports the observed sciaenid sympatry even outside the estuarine systems: both *S. umbra* and *U. cirrosa* have been reported to colonize the artificial reefs located on sandy–muddy bottoms along the Italian coast (Bombace et al., 1994; Fabi et al., 2004; Relini et al., 2007). These species also co-occur in a few Mediterranean marine reserves, such as the marine protected area of Sazani Island, Karaburuni Peninsula (Albania; Kashta et al., 2011), and the Columbretes Islands reserve (Spain), the latter being characterized by volcanic rock and coralligenous habitats (maërl beds), with patches of sand and mud that extend down to depths of 80 m (Stobart et al., 2009). The co-occurrence of these species has also been reported in field monitoring over vast coastal areas (i.e. 400 linear km; Gordoa, 2009).

It has to be considered that all of the above-mentioned studies do not specify whether the target species were simultaneously present at the same site, or if they were present in different periods of the year; furthermore, these studies did not focus specifically on the fish spawning season. Based on the current state of knowledge, therefore, the real spatial extension of brown meagre and shi drum sympatry in the Mediterranean Sea is unknown, both during the spawning season and outside of it; further studies in this sense are therefore warranted.

4.4 | The ecological requirements of the target species and their relation to the observed fish distribution

Adult aggregations of shi drum generally occur along sandy beaches, mixed rock and sand surf-zones and subtidal reefs (Hutchings & Griffiths, 2005), features which characterize the study site. On the other hand, the local distribution of *S. umbra* is probably due to its preference for the artificial, hard structures that resemble its typical reproductive habitat, consisting of rocky reefs with holes and shelters close to soft substrates acting as feeding grounds (Fabi, Panfili & Spagnolo, 1998; Bonacito et al., 2002; Colla et al., 2018). To date, no research has focused on the species-specific abiotic habitat preferences (temperature, salinity, depth, water current) of the target species. It is known, however, that meteorological factors, such as wind speed and direction as well as the amount of rainfall, affect *U. cirrosa*

movements between the lagoon and the open sea (Milardi et al., 2019). Being an important species for aquaculture (Chaves-Pozo et al., 2019), the shi drum is also known to cope with full-strength seawater to iso-osmotic salinity, while hypo-osmotic water induced low growth performance (Mylonas et al., 2009).

Any (or many) of the above mentioned abiotic factors – as well other biotic features such as the prey availability – could potentially explain why shi drum vocalizations were recorded only in the Malamocco inlet, in contrast to the brown meagre, which was distributed along all three inlets. It is worth underlining that the deepest point of the Venice lagoon (about −48 m; Sarretta et al., 2010) is located very close to the Malamocco inlet. Moreover, in the internal channel of the Malamocco inlet, the bottom reaches −27 m, while the depth in the rest of study areas varies between −4 m and −17 m at Lido, and between −8 m and −13 m at Chioggia. All together, these findings suggest that stratigraphic variability – and/or the depth-linked variability of other parameters such as the water temperature, shelter availability, prey composition and so on – is a possible attracting factor for the shi drum. Interestingly, stratigraphic habitat partition has been already suggested for other sympatric sciaenids (Luczkovich et al., 2008). Water currents could also play a role in the observed fish distribution, since the internal channel of the inlets presents high water velocities (over 1 m s^{-1}). The main factors affecting such water flow are wind and tide (±50 cm during spring tide): the exchange of water through the inlets is about a third of the total volume of the lagoon in each tidal cycle (Gacic & Solidoro, 2004). The MoSE project (the construction of a mobile barrier to safeguard the Lagoon of Venice) changed the structure of the Venice inlets and the decreased depth of the Malamocco channel further increased the local current intensity (Ghezzo et al., 2010). Despite the emerging patterns, more extended studies are needed in order to fully explore which habitat features could relate to the shi-drum and the brown meagre distribution.

4.5 | Potential of PAM for endangered and exploited fish species

Available evidence indicates that, in general, in the Mediterranean Sea, fish populations are rapidly declining as a consequence of a number of threats, worsened by particularly intense fishing activity in coastal and pelagic waters. The brown meagre and the shi drum populations have declined by up to 67% on average over the last 25 years, mainly because of commercial fisheries and recreational spearfishing (Malak et al., 2011). At present, both species are listed in Appendix III of the Bern Convention and in Annex III of the Barcelona Convention. Despite this, the brown meagre and the shi drum do not benefit from any specific form of protection outside of marine protected areas. Even in marine protected areas, the recovery of fish populations is a long-term process. Brown meagre abundance and biomass have been proved to increase according to the reserve protection level and the duration of the protection; however, the time required to reach the population carrying capacity might exceed

50 years of full protection (Garcia-Rubies, Hereu & Zabalà, 2013; Harmelin-Vivien et al., 2015). In unprotected areas data are lacking for both species, despite the need for fine-scale monitoring and for a better understanding of their biology, threats and conservation requirements (Malak et al., 2011). Generally, populations that spawn in high density at relatively few spawning sites, as is the case for the target species, are expected to have lower reproductive resilience to disturbance (Lowerre-Barbieri et al., 2017). The identification, mapping and monitoring of their spawning grounds is therefore a priority for environmental managers. Given that these species are vocally active during reproduction, PAM represents a non-invasive technique to achieve these goals over yearly temporal and vast basin spatial scales (ie. the north-western Mediterranean; Di Iorio et al., 2020). This is the first case in which two sciaenid species have been documented to share their reproductive habitat in the Mediterranean Sea, as is the case for many other sciaenids inhabiting the Atlantic coast (Holt & Holt, 2000; Soares & Vazzoler, 2001; Monczak et al., 2017; Monczak et al., 2019). By finely localizing the brown meagre and the shi drum distributions in the Venice inlets, the present paper confirms that PAM is an efficient technique for *in situ* mapping of these vulnerable species when they co-occur. Results could be replicated in other areas, in order to expand the knowledge of their reproductive behaviour and locations. In turn, this will also help to define habitats or environmental factors which support the reproductive process, in order to define protection measures.

Generally speaking, studies on vocal activities can contribute to conservation outcomes if those vocalizations are informative about species that present a conservation problem. In this context, fish bioacoustic monitoring is a useful tool for measuring the presence/absence of vulnerable species, as in the present study; similarly, the PAM-based approach could potentially be applied to other IUCN threatened Mediterranean bony fish known to be vocal, such as the dusky grouper *Epinephelus marginatus* (Bertucci et al., 2015) and the green wrasse *Labrus viridis* (Steinberg et al., 1965). An information gap in IUCN Data Deficient species could also be filled when rare or cryptic species are vocal, as has been demonstrated for the cruskel-eel acoustically detected in an Italian coastal marine reserve (Picciulin et al., 2019). PAM could help to achieve a more comprehensive picture of fish diversity in different habitats: Desiderà et al. (2019) used PAM to reveal a strong relationship between taxonomic diversity and acoustic diversity in a rocky reef habitat. Evaluating species diversity in target areas can have major implications for conservation, considering the biodiversity changes and losses that many marine habitats are facing. Bioacoustics also offer a means to monitor and evaluate the effectiveness of already existing conservation actions, as highlighted by Di Iorio et al. (2020) with the probability of detecting *S. umbra* calls in different protected areas being shown to change in relation to their level of protection and enforcement and the onset of the protection measures.

Conservation-related studies focus on behaviours that affect population persistence (Anthony & Blumstein, 2000), such as the reproductive behaviour. Most vocal fish produce sounds associated with courtship and spawning (Amorim, Vasconcelos & Fonseca, 2015), so that the timing and location of spawning in exploited species, such as

the Atlantic cod (Fudge & Rose, 2009) and the haddock (Casaretto et al., 2015), have been successfully determined using PAM. Monitoring the reproductive performance of wild, threatened populations is an essential requirement for their effective management as fishery resources. More recently, haddock behaviour and population characteristics have been inferred from their sounds recorded at 75 m depth in cold Arctic waters (Buscaino et al., 2020), giving gender and maturity information on an exploited fish species located in a habitat that is otherwise difficult to access and monitor. Such qualitative assessments could form the basis of local fisheries management and conservation strategies. Quantitative data on fish abundance could be obtained by PAM by comparing the sound levels produced by a spawning fish with simultaneous measurements of density from active acoustic surveys, as demonstrated for a scieanid species (Rowell et al., 2017).

Summing up, PAM is a non-invasive, low-cost, replicable and efficient technique that can aid conservation and monitoring programmes by capturing individual- and group-level data, by defining and monitor the distribution of a species, by revealing the behaviours that enable that species' persistence and recovery and by identifying the environments in which these are supported (Teixeira, Maron & van Rensburg, 2019). As a result, the PAM-related potential for conservation achievement is substantial.

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