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Fish sounds and boat noise are prominent soundscape contributors in an urban European estuary

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ABSTRACT

Passive acoustic monitoring is a valuable tool for non-intrusive monitoring of marine environments, also allowing the assessment of underwater noise that can negatively affect marine organisms. Here we provide for the first time, an assessment of noise levels and temporal soundscape patterns for a European estuary. We used several eco-acoustics methodologies to characterize the data collected over six weeks within May 2016 - July 2017 from Tagus estuary. Biophony was the major contributor dominated by fish vocalizations and the main driver for seasonal patterns. Maritime traffic was the major source of anthropogenic noise, with daily patterns monitored using 1584 Hz third-octave band level. This indicator avoided biophony and geophony, unlike other indicators proposed for the EU Marine Strategy Framework Directive. Furthermore, the frequency overlap between anthropophony and biophony demands precautionary actions and calls for further research. This study provides an assessment that will be useful for future monitoring and management strategies.

1. Introduction

Soundscape analysis has the potential to be a powerful tool for management and conservation efforts (Towsey et al., 2014; Sueur and Farina, 2015; Krause and Farina, 2016; Farina and Gage, 2017; Pavan, 2017; Farina, 2019). Supported by recent advances in passive acoustic monitoring (PAM), this non-invasive tool allows the evaluation of biodiversity, species density, habitat use, activity patterns and the impacts of human activities on marine organisms (Farina and Gage, 2017; Sueur et al., 2008; Obrist et al., 2010; Marques et al., 2013).

Anthropophony (man-made noise), Biophony (sounds from biological activity including vocalizations) and Geophony (sounds generated by geophysical and meteorological events) are the major components of a soundscape (Pijanowski et al., 2011). These components characterize each environment and can be particularly important for marine organisms (Montgomery and Radford, 2017; de Jong et al., 2020). Indeed, many marine organisms including cetaceans, fish and invertebrates use sounds to interpret their surroundings and/or to communicate (Kaatz, 2002; Cato et al., 2005; Remage-Healey et al., 2006; Vermeij et al., 2010; Chapuis and Bshary, 2010). For some, acoustic communication can be crucial for reproductive success (e.g. Amorim et al., 2016; Nabi et al., 2018). The major relevance of sound in marine environments is probably related to its velocity (travels five times faster in water than in air) and ability to propagate long distances (Mann and Lobel, 1997; Popper and Hawkins, 2009; Buscaino et al., 2011). However, man-made underwater noise has increased in the last decades and is now acknowledged as a chronic source of pollution that is changing underwater soundscapes and imposing new constraints on animals, including in their ability to communicate acoustically (Marine Strategy Framework Directive - MSFD, European Commission, 2008; Normandeau Associates, Inc, 2012). Anthrophonic noise can cause a wide range of effects, such as behavioural avoidance, temporary threshold shifts, or even death (Popper and Hastings, 2009). In this context, it is urgent to assess marine soundscapes, especially coastal and transitional areas (e.g. estuaries) in which high-density anthropogenic sound sources coexist with marine fauna.

Soundscapes change through space and time. Several articles have characterized aspects of the soundscape in underwater habitats. Most traditional approaches used short-term measurements at one or several locations or habitats (e.g. Radford et al., 2010; McWilliam and Hawkins,

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2013; Lillis et al., 2014). Recent technological advances and the greater affordability of acoustic equipment, however, currently allow the use of long-term recordings (e.g. Putland et al., 2017) encompassing days, months or even years. These enhanced recording capabilities have been accompanied by the development of powerful analytic methodologies (e.g. ecoacoustics indices, several long-term visualization techniques, and automatic detection and classification algorithms). Several studies focusing on ambient and anthropogenic noise (e.g. Merchant et al., 2014; Romagosa et al., 2017; Soares et al., 2020), mostly applied visual methods conjugated with the measurement of broadband and one-third octave band levels (SPL and TOL). In particular, the European MSFD suggested the use of 63 Hz and 125 Hz TOL to quantify anthropogenic noise, although several authors also recommended the assessment of sound frequencies up to 500 Hz in shallow waters (Merchant et al., 2014; Picciulin et al., 2016). On the other hand, studies focusing on the presence of vocal species and temporal patterns of acoustic activity typically used visual inspection of recordings to pinpoint animals vocalizations (e.g. Amorim et al., 2006; Luczkovich et al., 2008; Carriço et al., 2020a,b), or more recently used automatic recognition detection and classification approaches, such as simple energy thresholds and matched filter or unsupervised methods using machine learning like Gaussian mixture models (GMMs), artificial neural networks (ANN) and hidden Markov models (HMMs) (e.g. Monczak et al., 2019; Vieira et al., 2021). Lastly, some studies attempted to present an overview considering several aspects of a soundscape like biophony and anthropophony (e.g. Staaterman et al., 2014; Marley et al., 2016; Putland et al., 2017).

The Tagus estuary is one of the largest in Europe with an area of about 320 km^2, and its ecological and economic values are well recognized. The western area of the estuary is densely urbanized by the city of Lisbon and its metropolitan area, contrasting with its eastern upstream area that is legally protected as a natural reserve (Tavares et al., 2015) and classified as a Site of Community Importance (PTCON0009) and a Special Protection Area (PTZPE0010) under the European NATURA 2000 network (Directive 92/43/EEC; Directive 2009/147/EC; Fig. 1). This estuary is characterized by a high number of fish species and acts as a nursery area for several species (Costa and Bruxelas, 1989, Costa and Cabral, 1999; Table S1). More than fifty fish species have been reported in Tagus estuary (e.g. Cabral et al., 2001; Morais et al., 2017) including several soniferous species (Table S1). Moreover, according to Cabral et al. (2001) since the 1970s there have been some changes on the fish communities with an increase of more meridional highly vocal species such as the Lusitanian toadfish (*Halobatrachus didactylus;* Amorim et al., 2006) and the meagre (*Argyrosomus regius;* Lagardère and Mariani, 2006). These changes may be associated with global warming (Santos et al., 2002; Thiel et al., 2003), although the observed reduction in pollution levels could also be responsible for several changes (Cabral et al., 2001; Rodrigues et al., 2020). Concurrently, an increase in boat traffic has occurred (Ștefănescu et al., 2013). Nowadays, cargo and passenger ships dominate the traffic in the Tagus estuary as reported by the Automatic Identification System (AIS, i.e. a tracking system that uses transceivers on ships and large boats to report their geographical positions), with some routes being dominated by the ferryboats that connect Lisbon to Montijo, Cacilhas, Seixal and Barreiro (Rong et al., 2015). There are also several smaller boats (Vieira et al., 2020), however, the number and routes followed are not well known since they are not required to carry an AIS (Imo, 2001).

Here, we provide a first overview of the Tagus estuary soundscape. Using recordings from six weeks distributed over a year in a single location, the aims were to (1) describe the major contributors to the underwater soundscape; (2) provide a first assessment of noise levels; and (3) characterize major daily and seasonal patterns.

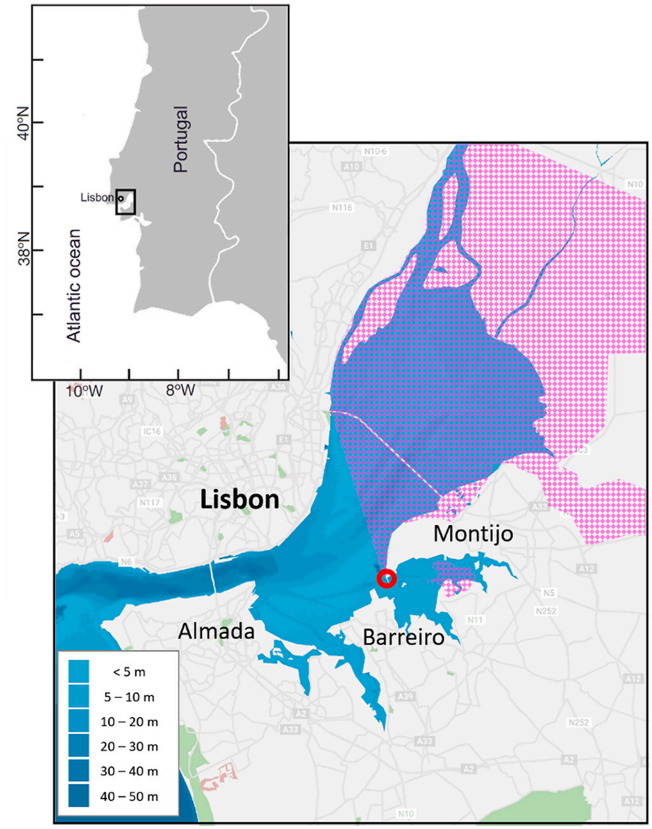

Fig. 1. Location of the underwater recording site (red dot) in the Tagus estuary. The areas classified as a Site of Community Importance (PTCON0009) and a Special Protection Area (PTZPE0010) under the NATURA 2000 network are represented in pink. Bathymetry data adapted from the Hydrographic Institute. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Acoustic recordings and meteorological data

Recording equipment was deployed in a pier with restricted access in the Portuguese Air Force base no. 6 (BA6, Montijo, Portugal; 38°42′N, 8°58′W; Fig. 1). This location is situated at the southern bank of the estuary and on the south-west boundary of the protected areas under the European NATURA 2000 network. It is also, near one of the major routes of passenger's ferryboats (Rong et al., 2015), and is a place where small open deck fishing boats are observed daily (Vieira et al., 2020). The presence of meagre (*A. regius*) and Lusitanian toadfish (*H. didactylus*) has previously been observed on this site (Vieira et al., 2020; Vieira et al., 2021).

Underwater recordings were obtained between May 2016 and July 2017, using 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) anchored at about 20 cm from the bottom. To minimise current-induced hydrodynamic noise the hydrophone was attached to a stainless-steel holder projecting from a concrete base. The signal from the hydrophone was recorded continuously by a 16 channel stand-alone data logger (Measurement Computing Corporation LGR-5325, Norton, Virginia, USA, 16 bits resolution; sampling rate of 22 kHz on 2016 and 4 kHz on 2017). Recordings from 2016 were downsampled from 22 kHz to 4 kHz. Depth at hydrophone location ranged from ca. 2 to 6 m depending on tide level.

Air temperature and wind speed were recorded by a weather station located at BA6. These data were sampled every 5 min. Tide level from

May 2016 to January 2017 was provided by Hydrographic Institute (Lisbon, Portugal; 38°42′N, 9°07′W).

2.2. Soundscape analysis

All acoustic data were analysed using R software (R Core Team, 2018).

We considered recordings made during six different weeks distributed within the period May 2016 - July 2017 (May, July and November 2016, and January, April and July 2017). This selection should capture an overview of the seasonal variation in the soundscape.

To investigate the sound level variation over the duration of recordings the following quantitative measures were computed: averaged broadband sound pressure level (SPL, 20-2000 Hz); and averaged sound level in third-octave bands (TOL). Spectral Probability Density plots (SPD), Long-Term Spectral Average plots (LTSA) and Cumulative Distribution Function of SPL (CDF) were also computed for each week to examine variations of the energy distribution along frequency. All these features were computed and calibrated adapting the code available by Merchant et al. (2015) (SPL, TOL, LTSA and CDF: FFT 1024, Hann window, 50% overlap, averaged for each minute; 1 s averaged PSDs were used to create the SPD). The correction factor for calibration was calculated using the hydrophone sensitivity, system gain and zero-to-peak voltage of the analog-to-digital converter. Recordings were also inspected both aurally and visually using Adobe Audition 3.0 software (Adobe Systems, San José, CA, USA) to identify the sound types corresponding to the geophony, biophony and anthropophony soundscape components. Note that this inspection was performed on the non-downsampled recordings.

TOLs pairwise correlations were performed considering 1-minute averages.

Sound level differences between the different selected weeks were evaluated using non-parametric one-way ANOVAs (Krustal-Wallis test), considering 1-hour arithmetic averages. This test was selected since homogeneity of variances and normality were not met. Post-hoc Dunn tests were used for pairwise comparisons.

To evaluate the relation between soundscape and abiotic factors, Generalized Additive Models (GAM) with a gamma distribution were used. GAM is a non-parametric, regression technique not restricted by linear relationships, and it is flexible regarding the statistical distribution of the data (Hastie and Tibshirani, 1990; Wood, 2017). The effect of the explanatory variables wind speed, tide level and tidal vertical velocity were assessed using data from May 2016 to January 2017, considering 1-hour arithmetic averages. Tidal vertical velocity is the time derivative of tidal water levels. Furthermore, the effect of time of day was also assessed. GAMs were chosen because preliminary analysis of the time-series indicated non-linear relationships (Hastie and Tibshirani, 1990; Wood, 2017). Thin plate regression splines were used, and all terms were subject to the second-order penalty. Parameters were estimated using restricted maximum likelihood (REML). The interactions of the covariates were taken into account with tensor product interactions (Wood, 2006). These interaction terms were only retained when they were significantly different from a zero (flat) function. This statistical analysis was conducted in R using the ́gam ́ function from ‘mgcv’ library. Model fit was evaluated through visual inspection of residual plots and diagnostic information produced using the ́gam.check ́ function (Wood, 2001).

3. Results

3.1. Soundscape sources

Fish sounds were responsible for major seasonal and daily changes in the soundscapes. Anthropogenic noise associated with boating repeatedly appeared throughout the day and overlapped the frequency band used by the fish. Daily tidal rhythms were also produced an abundancy of sounds. A set of one week long-term averaged spectrograms is shown in Fig. 2, representing Autumn (November 2016), Winter (January 2017), Spring (April 2017) and Summer (July 2017).

3.1.1. Biophony

Only fish calls from meagre and Lusitanian toadfish were recognized.

Lusitanian toadfish sounds include boatwhistles, double-croaks and grunt trains (sound type names as reported by Amorim et al., 2008). Boatwhistles were usually observed in bouts and in higher number (Fig. 2c). These calls occurred in all datasets (May, July and November 2016, and January, April and July 2017). Peak activity was observed in July 2017, with a noticeable pattern of the ca. 110 Hz and 220 Hz boatwhistles’ harmonics in the long-term averaged spectrogram (Fig. 2a,c, highlighted by the 2 yellow arrows). The week sampled in July 2016 also presented hours with noticeable presence of boatwhistles, but much less than July 2017. In November 2016 and January 2017, the presence of Lusitanian toadfish calls was sporadic.

Meagre produced massive choruses at dusk in May 2016 and April 2017 (see Fig. 2a,b, highlighted by the blue arrow). July 2016 also presented choruses on some days but with shorter duration. In these choruses the most common calls observed were the intermediate grunts (sounds with 7- 29 pulses) and long grunts (more than 30 pulses; sound type nomenclature as reported in Vieira et al., 2019). Fig. 2b represents the choruses dominated by long grunts with high rate of overlap, forming a mostly continuous roar. Note that 1-3 pulsed calls were also observed, usually produced in a bout. In November 2016 and January 2017 meagre calls were also detected (Figs. S3 and S4). In the colder months, only short grunts were detected.

3.1.2. Anthropophony

The most abundant anthropogenic noise was produced by boats. In this location it is common to observe small boats (mostly open deck fishing boats with outboard engine) and ferryboats that transport passengers between Lisbon and Montijo (examples in Fig. 3a and b). Most boat passages occurred from ca. 5 am to 23 pm. Noise from small boats and ferryboats passing-by usually presented Lloyd's mirror effect (Fig. 3a) and occupied a wide frequency range from ca. 20 Hz up to the Nyquist limit of our recordings. The Lloyd's mirror effect is the result of the interactions of out-of-phase reflections of the sound in the recording position and shows a shift on the frequencies observed according to the distance of a moving source, usually presenting a symmetrical spectrogram between approach and departure from the recording device (Carey, 2009). Some small boats also produced noise restricted to low frequencies ($<$ 200 Hz; example in Fig. 3b). At this site, it was also possible to detect some airplanes landing or taking off from the air force base (Fig. 3c; sound type confirmed with simultaneous visual observation in the recording site) as well as several other anthropogenic sounds from unknown sources.

3.1.3. Geophony

Tidal currents and wind generated noise on the several datasets. Ebb and flood tide produced low frequency sounds (e.g. Fig. 3d). Tidal flow also increased hydrophone self-mooring sounds and or knock sounds produced by the impact of objects flowing within the water current (Fig. 3e). The latter can occur across the recorded spectrum, but most energy was concentrated at low frequencies. Low frequency short span waves breaking sounds occurred throughout the dataset (e.g. Fig. 3f). We could identify two sources of wave breaking sounds: increased wind speed and boat passages (see end of boat noise in Fig. 3a).

3.2. Sound level patterns

Averaged broadband SPL (20-2000 Hz) was highly correlated with averaged sound level in 39 Hz to 630 Hz third-octave bands (TOL) (Fig. S7; $r > 0.6$). Furthermore, pairwise correlation between TOLs presented high correlation ($r > 0.4$) in 4 groups: (1) very low frequencies

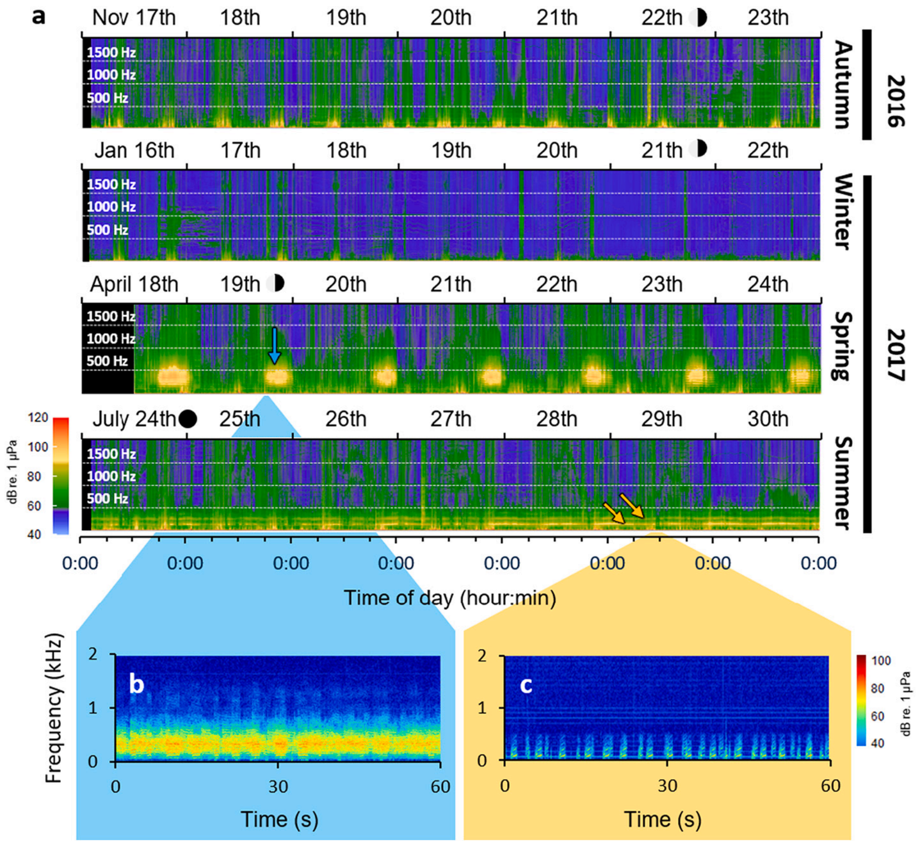

Fig. 2. Long-term spectrograms from Tagus estuary representing the several seasons from November 2016 to July 2017. (b,c) depicts in detail the detected fish sounds: (b) depicts a 1-min spectrogram from April 2017 with a meagre chorus dominated by long grunts with high rate of overlap, forming a mostly continuous roar; and (c) depicts a 1-min spectrogram with a bout of Lusitanian toadfish boatwhistles detected in July 2017. Arrows highlight examples of the presence of meagre (blue) and the presence of 110 Hz and 220 Hz boatwhistles' harmonics visible during Lusitanian toadfish chorus (yellow). Long term averaged spectrogram settings: sampling frequency: 4 kHz, FFT size: 1024, window type: Hann, 50% overlap, averaged for 1-minute segments. Spectrogram settings used a FFT size of 512. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

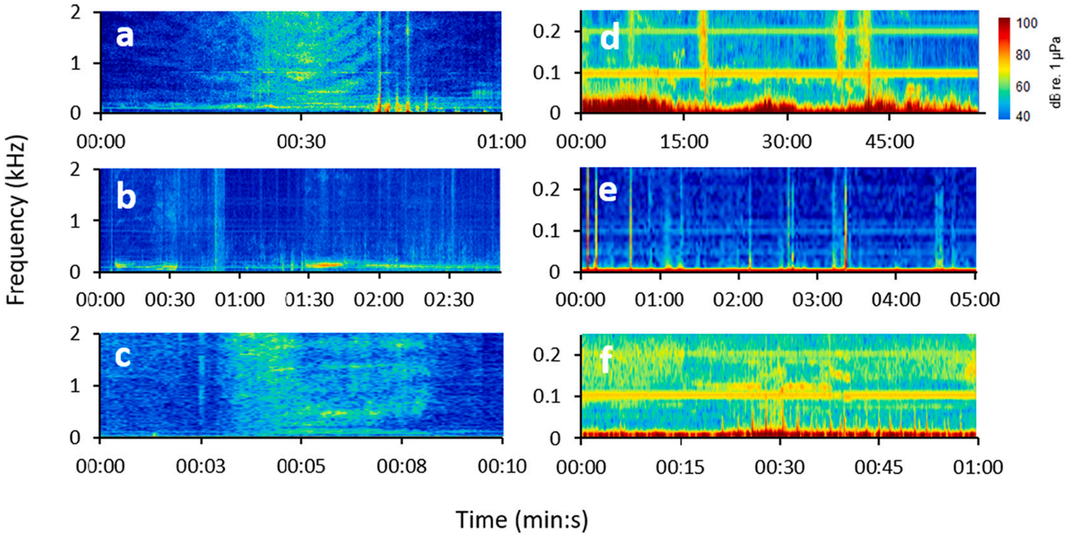

Fig. 3. Spectrograms of anthropogenic and geophonic sounds: (a) ferryboat in April 2017; (b) small boat manoeuvring in April 2017; (c) airplane of the air force detected in April 2017; (d) 1 h-spectrogram with low frequency tidal influence noise in May 2016 (see red area); (e) flow noise in January 2017 observed as broadband short noise (green and red vertical lines); and (f) consecutive breaking surf wave sounds in May 2016. Note a 50 Hz electrical noise (and respective harmonics) from the recording setup is also noticeable in panels (d-f). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

under 35 Hz; (2) frequencies between 35 and 140 Hz; (3) frequencies between 140 and 891 Hz; and frequencies higher than 891 Hz. Only TOLs with centre frequency of 63, 125, 500 and 1584 Hz were selected for further analysis. Very low frequencies were excluded. The 63 and 125 Hz centred TOLs were selected because they are recommended by the MSFD of the European Union to monitor low-frequency anthropogenic noise. The 500 Hz TOL was selected taking also into account that several authors recommended the use of such frequencies in shallow waters (Merchant et al., 2014; Picciulin et al., 2016). Lastly, the 1584 Hz TOL was selected to represent the group of highest recorded frequencies (Fig. S7). Descriptive statistics for SPL, 1548, 500. 125 and 63 Hz TOL, atmospheric temperature, wind speed and tide level are presented in Table S2.

3.2.1. Wind and tidal influence

Sound level was affected by the tide and wind speed. Tide level and tidal vertical velocity affected non-linearly the sound levels at every band except TOL 1584 Hz (see Fig. 4; Table 1). GAM's also indicate an increase of sound level at lower frequencies during high tide ($p < 0.001$). On the TOL 63 Hz and SPL the increase was especially high at the ebb tide ($p < 0.001$; cf. tidal velocity in Fig. 4). SPLs increase at ebb-current was ca. 20 dB greater than in the stand of the tide (when tidal velocity is zero). Wind speed proportionally increased sound level at all analysed bandwidths ($p < 0.001$), although the increase in sound level was higher at the 63 Hz TOL.

3.2.2. Daily temporal patterns

Daily patterns of noise levels were driven by meagre choruses and boat passages. Meagre chorus raised SPL and 500 Hz TOL at dusk (Fig. 2a,b; cf. Figs. S1, S2 and S5). This can be observed in the GAM as a non-linear relation of those sound level metrics with time of day increasing since 15 h and peaking at ca. 20-23 h (Fig. 4 and Table 1; $p < 0.001$). Lower frequency TOLs (63 and 125 Hz) showed a non-linear correlation, with lower levels during the day (Fig. 4; $p < 0.001$). This can be also be partially observed in the SPL. Using SPL and 500, 125 and 63 Hz TOLs, the marked daily pattern of marine traffic at this site could not be observed. However, boat noise incidence could be monitored using 1-min averaged 1584 Hz TOL (cf. Figs. S1-6). Anthropogenic noise produced by boats passing-by increased the 1584 Hz TOL from ca. 5 am to 11 pm (Figs. S1-6). This can be observed on the GAM also presenting a non-linear correlation with time of day (Fig. 4; $p < 0.001$). A peak at ca. 8 am and a smaller peak at ca. 6 pm can also be observed and are concurrent with the rush hour periods when more ferryboat passages occur. Furthermore, Sundays presented lower incidence of boat passages (see May 8th, Nov 20th and July 30th in Figs. S1, S3 and S6).

3.2.3. Seasonal temporal patterns

Some clear differences were observed throughout the sampled days representing spring, summer and autumn/winter. There was a significant difference in broadband SPL (20-2000 Hz) according to month (Krustal-Wallis test, $\chi^2_{(5)} = 387.2$, $p < 0.001$; Fig. 5). During January, the broadband SPL was lower (90.5 ± 5.4 dB re. 1μPa; mean ± SD; Dunn test, $p < 0.001$) than the other months. November was the second-to-last (95.2 ± 6.6; $p < 0.001$) in broadband SPL. The highest SPL were

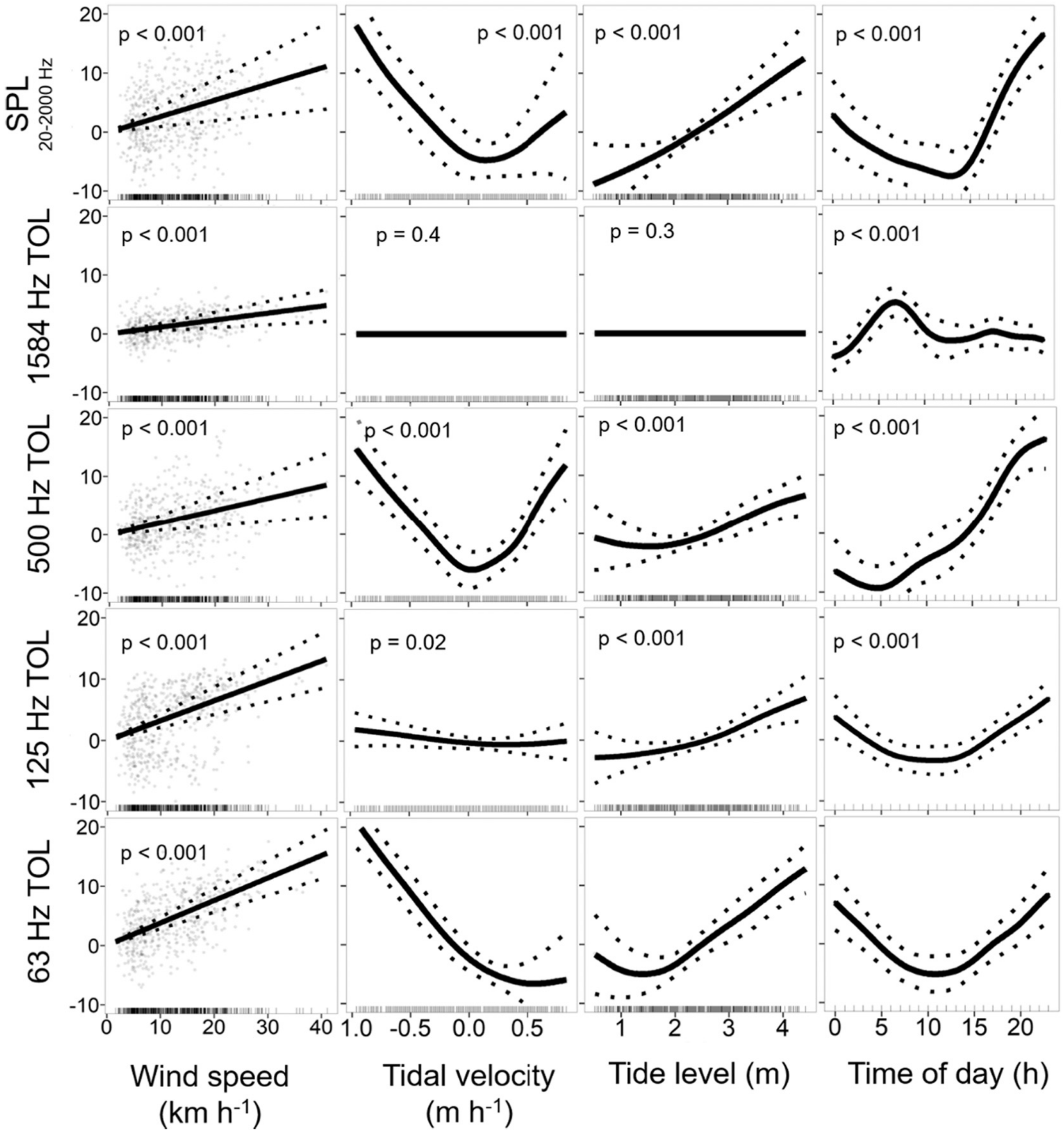

Fig. 4. The effect of variation in predicted drives on the SPL (20-2000 Hz), 1584 Hz TOL, 500 Hz TOL, 125 Hz TOL and 63 Hz TOL from May, July, November 2016 and January 2017. In the model we used the hourly averages ($n = 577$). Plots represent the GAM partial effects splines for each model (see Table 1), except for wind that was assumed as linear. Functions are presented as solid lines, dashed lines denote confidence interval, and dots indicate the partial residuals.

Table 1
Results of generalized additive models (GAM) assessing the parameters that explain the daily patterns of SPL and TOLs.

	SPL		1584 Hz TOL		500 Hz TOL		125 Hz TOL		63 Hz TOL	
n	577		577		577		577		577	
r^2	0.70		0.48		0.68		0.50		0.70	
Deviance explained	72.6%		51.7%		71.4%		53.4%		72.6%	
Linear predictor	EDF	*p*-value	EDF	p-value	EDF	p-value	EDF	p-value	EDF	p-value
Wind speed (km h^{-1})		0.002		< 0.001		0.002		< 0.001		< 0.001
Predictor spline		p-value		p-value		p-value		p-value		p-value
Tide level (m)	1.5	**< 0.001**	0.0	0.4	2.2	**< 0.001**	2.0	**< 0.001**	3.4	**< 0.001**
Tidal velocity (m h^{-1})	3.5	**< 0.001**	0.0	0.3	4.8	**< 0.001**	0.9	**0.02**	3.6	**< 0.001**
Time of day (h)	5.6	**< 0.001**	7.9	**< 0.001**	5.4	**< 0.001**	3.3	**< 0.001**	4.2	**< 0.001**
Tensor product interactions	EDF	p-value	EDF	p-value	EDF	p-value	EDF	p-value	EDF	p-value
Tide × Tidal velocity	10.1	**< 0.001**	5.9	**< 0.001**	4.1	**0.002**	7.7	**< 0.001**	10.0	**< 0.001**
Tide × Wind	2.4	**0.006**	6.7	**< 0.001**	3.2	**< 0.001**	4.4	**< 0.001**	1.2	**0.01**
Tide × Time of day	13.1	**< 0.001**	7.8	**< 0.001**	14.2	**< 0.001**	9.5	**< 0.001**	14.1	**< 0.001**
Tidal velocity × Wind	2.9	**< 0.001**	7.1	**< 0.001**	3.1	**0.003**	2.6	**0.01**	1.7	**0.04**
Tidal velocity × Time of day	11.1	**< 0.001**	5.4	**< 0.001**	13.4	**< 0.001**	9.4	**< 0.001**	11.5	**< 0.001**
Wind × Time of day	5.7	**< 0.001**	3.6	**0.006**	7.4	**< 0.001**	1.9	**0.04**	2.8	**0.02**

Number of hours with calling rate at each location (n), fit of each model; (r^2), percentage of deviance explained; EDF, estimated degrees of freedom; and *p*-values are represented. In bold p-value lower than 0.05. In the formulation of GAM models, wind speed was inserted as a linear (parametric coefficients are omitted).

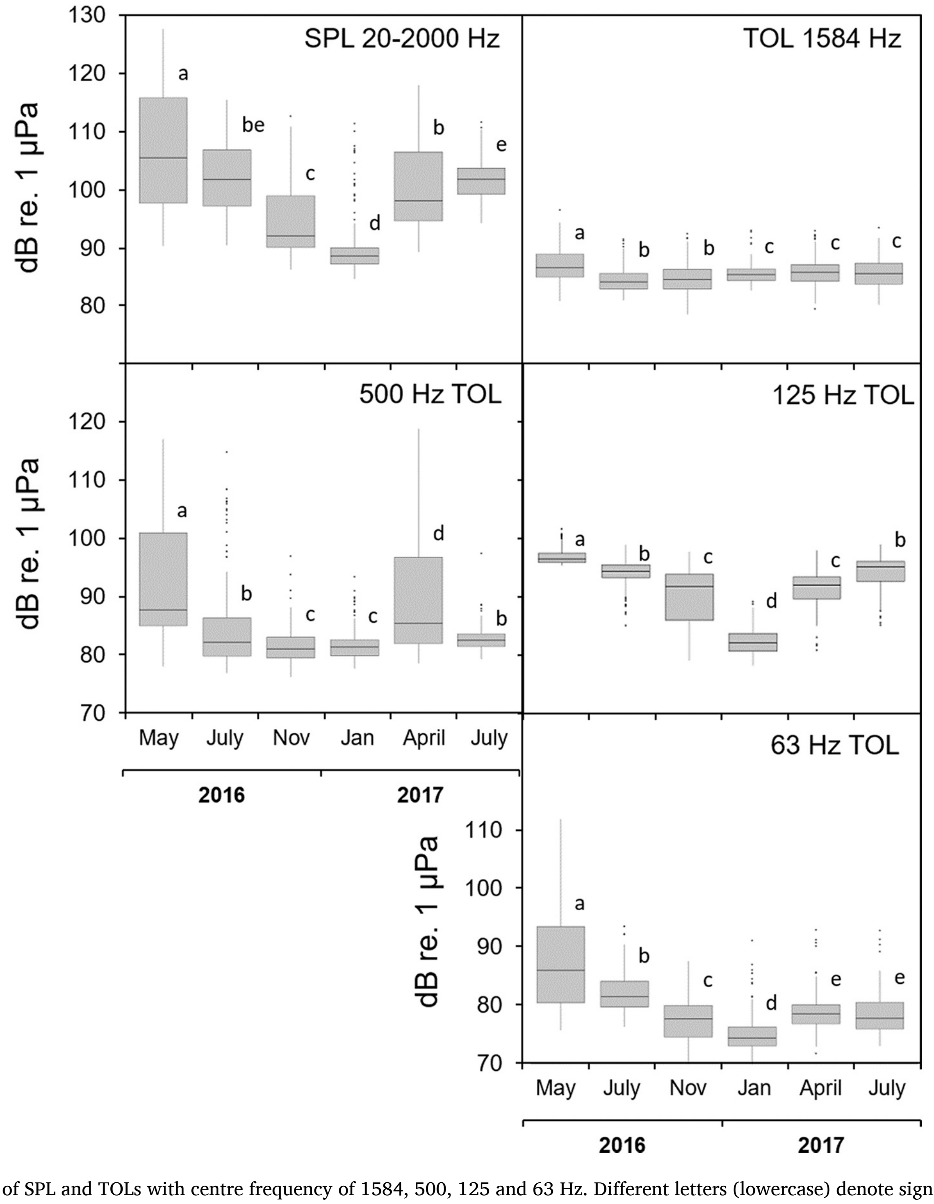

Fig. 5. Seasonal variation of SPL and TOLs with centre frequency of 1584, 500, 125 and 63 Hz. Different letters (lowercase) denote significant pairwise differences (Dunn test, $n = 928$; $p < 0.05$).

recorded in May 2016 (106.8 ± 9.7 re 1μPa; $p < 0.001$), concurrent with the peak of meagre calling activity. The presence of meagre choruses could be assessed with 500 Hz TOL, presenting also significant differences among months (Krustal-Wallis test, $\chi^2_{(5)} = 240.6$, $p < 0.001$; Fig. 5). In accordance, the long-term spectrograms of May 2016 (Fig. S1) and April 2017 (Fig. 2) presented significantly higher 500 Hz TOL ($p < 0.001$) than the other months although significant differences were also observed within these two months (92.7 ± 11.1 and 90.9 ± 12.2 dB re. 1μPa, respectively; $p = 0.003$). November 2016 and January 2017 exhibited the lowest values of 500 Hz TOL (81.7 ± 3.1 and 81.6 ± 2.7 dB re. 1μPa; p < 0.001), and no significant differences were found between these colder months ($p = 1$). July 2016 showed no significant differences from July 2017 ($p = 0.4$). Seasonal significant differences were also found at 125 Hz TOL (Krustal-Wallis test, $\chi^2_{(5)} = 597.2$, $p < 0.001$) and 63 Hz TOL (Krustal-Wallis test, $\chi^2_{(5)} = 381.0$, p < 0.001; Fig. 5). May 2016 presented the highest values (125 Hz TOL: 97.0 ± 2.0 dB re. 1μPa, $p < 0.001$; 63 Hz TOL: 88.5 ± 9.1 dB re. 1μPa; $p < 0.001$), while January 2017 presented the lowest values (125 Hz TOL: 82.5 ± 3.9 dB re. 1μPa, $p < 0.001$; 63 Hz TOL: 75.3 ± 3.6 dB re. 1μPa; p < 0.001). Furthermore, 125 Hz TOL presented non-significant differences between July 2016 and July 2017 ($p = 0.66$) or between November 2016 and April 2017 ($p = 0.37$). 63 Hz TOL presented marginally non-significant differences between November 2016 and July 2017 ($p = 0.07$). The 63 and 125 Hz centred TOLs appear to be at least partially concurrent with both fishes calling activity (see Fig. 2). 1584 Hz TOL presented smaller variations throughout the sampled months, but still significant differences were observed (Krustal-Wallis test, $\chi^2_{(5)} = 87.0$, $p < 0.001$; Fig. 5). July and November 2016 showed significantly lower 1584 Hz TOL levels (84.5 ± 2.3 and 84.7 ± 2.5 dB re. 1μPa), while May 2016 presented the highest values (87.2 ± 3.2 dB re. 1μPa; $p < 0.001$).

Spectral Probability Density plots in Fig. 6, depicting the different percentiles (1%, 5%, median, 95%, 99%) and the Root Mean Square level (RMS), illustrate the contribution of the different sound sources to the soundscape. The SPD plots of May 2016 and April 2017 exhibit a major peak observed in the higher percentiles (95%, 99%) and shadowed in a darker blue, which represents the presence of meagre choruses between ca. 200 and 700 Hz. July 2016 shows also the presence of meagre and Lusitanian toadfish, but less pronounced (i.e. lower incidence of these sounds). The SPD computed from July 2017 recordings presents two pronounced peaks at about 110 and 220 Hz, especially at the higher percentiles (95%, 99%), which represents the second and third harmonics of the boatwhistles produced by the Lusitanian toadfish. This SPD denotes the absence of meagre calling activity as the peak between ca. 200 and 700 Hz is absent. In all SPD plots we can observe the presence of higher intensity sounds with lower incidence (lighter blue). Those represent mostly anthropogenic noise from passing boats. Peaks at ca. 1800 Hz up to 2000 Hz, observable in almost all months, might be associated with tonal acoustic signatures observed in some ferryboats. Several tonal noises with low intensity (possibly anthropogenic noise from other sources) can also be observed in all the SPD plots (e.g., peak at ca. 1000 Hz and peaks at ca. 1600 Hz). On all plots a 50 Hz electrical noise (and respective harmonics) from the recording setup is also noticeable.

The cumulative distribution function of SPL for each week is represented in Fig. 7. Each curve presents the proportion of time where a given sound level is reached. Lower sound levels are observed in the winter/autumn period, with sound levels under 100 dB re. 1μPa ca. 80% of time. Nevertheless, November has consistently higher SPL than January (see also Fig. 5). The level exceedance for 5% of the time are 115 dB re. 1μPa for November, and 107 dB re. 1μPa for January. May 2016 and April 2017, show a characteristic curve with a light dome between ca. 20 and 30%, possibly related with meagre choruses that dominate the soundscape at dusk. The level exceedance for 5% of the time are higher in these months with 124 dB re. 1μPa in May 2016, and 117 dB re. 1μPa in April 2017. The July 2017 shows a straighter curve, probably by the presence of Lusitanian toadfish chorus through day and night. The level exceedance for 5% of the time are 115 dB re. 1μPa in

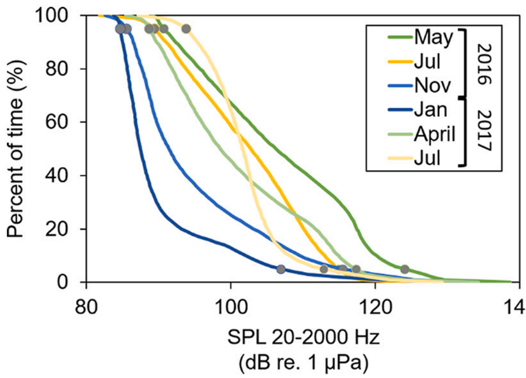

Fig. 7. Cumulative distribution function for the seasonal variation of sound pressure level (SPL). Grey circle markers indicate level exceedance for 95% and 5% of the time. SPL averaged over 60 s windows.

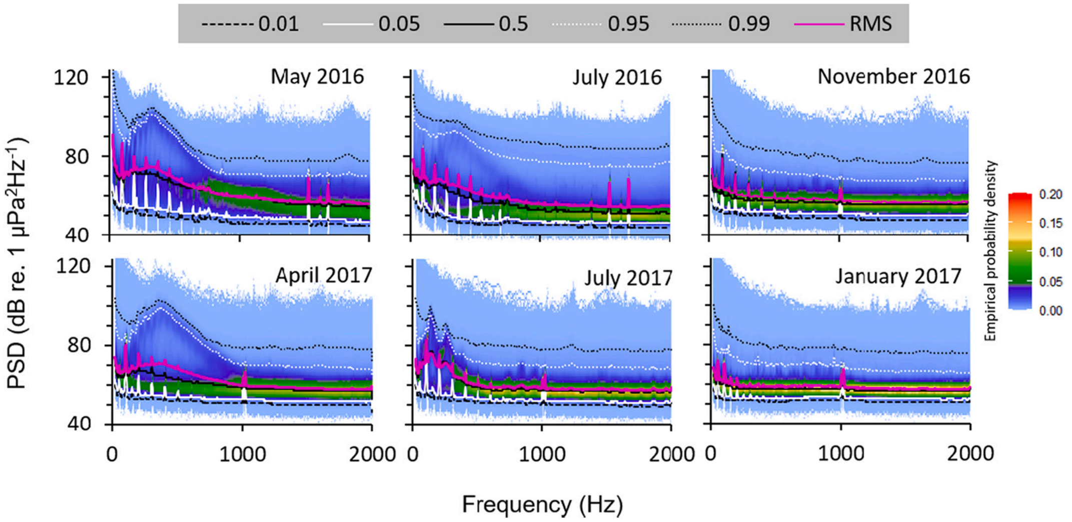

Fig. 6. Spectral probability densities level (SPDs), percentiles and root-mean-square (RMS) level for each week selected in May, July and November of 2016, and January, April and July of 2017. Note that plots are not in chronological order.

July 2016, and 113 dB re. 1μPa in July 2017. Note that all the curves show a tail past the 5% mark, which includes observations exceeding this mark by up to 23 dB.

4. Discussion

The acoustic characteristics of marine habitats are crucial to many animals that communicate through sound or use acoustic cues to exploit their environment. This study accomplishes an important first step in characterising the soundscape of one of the largest European estuaries surrounded by a densely populated urban area. The soundscape in the shallow waters of the Tagus estuary was dominated by fish sounds with additional significant energy from anthropogenic noise and sounds produced by wind and tidal currents. Wind influenced the overall frequency range while tidal flow affected the lower frequencies of the soundscape. Boat noise was the most common anthropogenic noise and influenced all analysed bandwidths. To the best of our knowledge this is the first study characterising the soundscape in a European estuary and measuring noise levels in different times of the year.

4.1. Soundscape sources

4.1.1. Biophony

Fishes were the major contributors to the underwater soundscape. In the recorded location it is clear the presence of two of the most abundant vocal species present in the Tagus estuary (Table S1): Lusitanian toadfish and meagre. Both species are highly vocal and produce sounds especially during the spawning season (Amorim et al., 2006; Vieira et al., 2019). In the long-term spectrogram and in the SPD plots the presence of meagre was easily identified by their choruses (May and July 2016, and April 2017). Furthermore, sounds from both species were detected even in winter, indicating that several individuals of both species are residents in the estuary. Interestingly, there are other Atlantic temperate estuaries also dominated by *sciaenids* and toadfishes sounds (Rice et al., 2016; Monczak et al., 2017; Mueller et al., 2020).

Toadfish was only easily identified through long-term graphical methods in July 2017, when the calling rate was exceptionally high. Furthermore, fish sounds in winter were only detected by visual inspection using short-term spectrograms. Therefore, the best approach to be used when analysing biophony is to combine long-term graphical representations (e.g. long-term spectrograms and SPD) with other techniques like manual annotation or automatic recognition of sounds (e.g. Vieira et al., 2015). Note however, that automatic recognition methods enable fast detection, but usually require previous knowledge of the sound types present in a soundscape.

Sounds from other fish species were not detected. In Tagus estuary several other species are vocal or potentially vocal (Table S1), but several reasons might explain their apparent absence. Several species from the Gobiidae family can produce sounds, but they produce sounds with low intensity. In this family the communication range is usually of few centimetres (Lugli et al., 2003, Amorim et al., 2018; cf. Fig. 6 of Carriço et al. (2020b) for the estimation of the attenuation of several fish species' vocalizations), therefore it is highly improbable to record these species even if present. On the other hand, other species like the invasive *Cynoscion regalis* (Morais et al., 2017) might prefer other locations within the estuary. This species is highly vocal and can produce choruses like the meagre (Connaughton and Taylor, 1995). Hence, is possible that this species does not occur near our recording site but might be detected in other locations in the Tagus estuary. The same may happen in relation to other vocal species that may prefer other habitats in the estuary. This remains to be investigated.

Several potentially vocal fish species might produce sounds that could have been disregarded. Several species can, for example, produce more isolated sounds (not all vocal fish species make choruses, Amorim et al., 2015) that might thus be easily ignored if sounds are only sporadically produced. In addition, the absence of previous characterization of the sounds produced by vocal species limits the ability to detect and recognize them. There is still a major number of species that are not even confirmed as being vocal. Several studies attempted to describe unknown sounds potentially produced by fishes (Straight et al., 2014; Parsons et al., 2016; Carriço et al., 2019; Rountree et al., 2020), but were still constrained by similarities to known fish sounds. It is also important to notice the lack of a central database of sounds that could be used as reference for each species or group of species. Efforts to characterize the repertoire of vocal fish species are urgent to support soundscape analysis and ecological assessment.

4.1.2. Anthropophony

Anthropophony was dominated by noise from boats, both ferryboats and small boats with outboard engines usually used by fishermen. In the Tagus estuary an increase in boat traffic has occurred since the seventies (Ştefănescu et al., 2013). Cargo and passenger ships dominate the traffic reported by the Automatic Identification Systems (AIS), including a ferryboat route near our recording site (Rong et al., 2015). As reported by Vieira et al. (2020) for this location, ferryboats produce the most audible noise with higher traffic during weekdays. Boat noise has been mostly described as variable broadband sound (Hildebrand, 2009; Lester et al., 2013; Li et al., 2015), and in our case regularly presents the Lloyd effect typical from a passing-by sound source. Boat noises affect aquatic organisms' acoustic communication, behaviour and fitness (e.g. Bruintjes and Radford, 2013; Sebastianutto et al., 2011; de Jong et al., 2020). Furthermore, the observed frequency overlap with the biophony maximize its detrimental effects on the communication space of vocal species (Erbe et al., 2016; Putland et al., 2018; Alves et al., 2021). Thus, the possible higher presence of anthropogenic noise in the northern area of the estuary demands for further research using recordings on multiple sites.

Other noises were also detected, several of unknown origin. Several factories operate in the opposite margins of the recording site, including a small port for cargo ships. Furthermore, at the northern area of the estuary large cargo ships, cruisers and dredgers are common. These vessels likely have much higher source levels and can be heard at longer distances (Veirs et al., 2016). Small boats with outboard engines usually produce sound levels with 120 - 160 dB re. 1 μPa at 1 m depending, for example, on engine and speed (Barlett and Wilson, 2002; Hildebrand, 2009; Erbe, 2013; Lester et al., 2013). Other boats and large vessels can produce louder sounds from 130 to 190 dB re. 1 μPa at 1 m (Merchant et al., 2012; Hildebrand, 2009; McKenna et al., 2012; Wittekind, 2014; Veirs et al., 2016; Putland et al., 2018).

Our recording site is located within an Air Force base and, as such, several airplanes and helicopters pass by at low altitudes. The low frequency noise produced by aircraft can be heard underwater, but it is currently sporadic and exhibit lower intensity than the recorded boat noise. Note, however, that this area is the proposed location for a new large commercial airport (Alves and Dias, 2020) that would substantially increase air traffic noise and likely underwater noise.

4.1.3. Geophony

Geophony had a significant influence on the recorded Tagus estuary soundscape. Wind speed caused an increase in sound levels. This relation of wind speed with sound level is mostly a consequence of the relation between wind speed and water wave height and energy. This relation is well known (Wenz, 1962) and has been reported in other shallow waters (Haxel et al., 2013; Halliday et al., 2020). Changes in tide level and tidal current, which are related to tide ebbing and flowing, also influenced non-linearly the sound level. Tide ebbing was responsible for the highest increase in noise levels in all the frequency bandwidths. This is probably related to a combination of hydrophone self-mooring sounds, and other sounds related to the increased water currents and waves. van Geel et al. (2020) also observed a substantial tidal influence on ambient sound measurements in several locations and identified methods to exclude such sounds and highlight other sound sources. Such methods might be considered in measuring ambient noise on estuaries and other coastal areas.

4.2. Sound level patterns

4.2.1. Daily temporal patterns

We found time-specific differences in sound level, mostly due to rhythms in fish sound production and traffic of marine vessels. The analysis of SPL and 500 Hz TOL daily patterns reflected the clear diel cycles of meagre choruses (e.g. Fig. 4). Meagre peak activity occurred at dusk/beginning of night, consistent with observations in captivity (Vieira et al., 2019). Similar acoustic behaviour, i.e. restricting the calling activity to a well-defined time of day, can be found in several other species (e.g. Connaughton and Taylor, 1995; Luczkovich et al., 2008; Mann et al., 2010; Montie et al., 2017; cf. Table 1 in Vieira et al., 2021). On the other hand, for example, the splendid toadfish species in Cozumel (Caribbean Sea), just avoid singing during the day, likely due to the high presence of anthropogenic noise (Pyć et al., 2020). In our dataset, Lusitanian toadfish did not present a well-defined diel rhythm as reported by Vieira et al. (2021) but further studies should investigate the possible constrictions of the vocal activity due to the presence of boat noise. The lower 63 and 125 Hz TOLs during the day are consistent with the Lusitanian toadfish calling activity at this location (cf. set-up 3C in Vieira et al., 2021).

Marine traffic at this site has a marked daily pattern that can be observed in the analysis of 1584 Hz TOL. This higher frequency is ideal to observe these patterns because it includes boat noise while avoiding the frequency bands dominated by fish sounds. The 1584 Hz TOL daily pattern showed a significant decrease from ca. 11 pm and 5 am, and a peak boating activity at ca. 8 am. This pattern is similar to the one reported by Vieira et al. (2020) using automatic recognition on recordings from the same location. The presence of daily patterns in anthropogenic noise seems common in coastal waters. Haviland-Howell et al. (2007) in coastal waters (North Carolina) and Smott et al. (2018) in May River estuary (South Carolina) also observed clear daily patterns, although more associated to recreational boaters with higher prevalence during late morning and afternoon, and higher traffic in weekends. Marley et al. (2016) in an Australian urban estuary also reported boats as the most prevalent source of anthropogenic noise with peaks around 7 am, 10 am–2 pm, and 4–7 pm.

4.2.2. Seasonal temporal patterns

Major seasonal differences were caused by fish calling activity. Spring (May 2016 and April 2017) was characterized by large and intense meagre choruses, causing a significant increase in the 500 Hz TOL. This is concurrent with the meagre vocal activity studied in captivity (Vieira et al., 2019) and is similar to what is observed is other sciaenids (e.g. Connaughton and Taylor, 1995). Lusitanian toadfish had the highest activity in July 2017. Amorim et al. (2008) reported seasonal changes of the calls produced by the Lusitanian toadfish. According to this study the calls were mostly produced in spring and summer with a peak in July. This was also concurrent with nest occupancy (Amorim et al., 2010).

No noticeable seasonal changes in anthropogenic noise were observed, although the SPL level exceedance for 5% of the time were higher in spring with 124 dB re. 1 μPa in May 2016, and 117 dB re. 1 μPa in April 2017. Only a perceptible decrease in the weekends was observed. This can be explained by the type of boats that produce the anthropogenic noise in the studied location. Most small boats are fishing boats, often also used in the catch of clams, and passenger ferryboats connecting Lisbon to Montijo, i.e. boat types with no clear seasonal activity, and that are more common on business days. In contrast, seasonal changes in anthropogenic noise are found, for example, in the touristic area of Ria Formosa, where significant changes occur due to increased vessel traffic intensity in the summer months (Soares et al., 2020).

4.3. Soundscape and ambient noise assessment

Here we described an estuarine soundscape and highlight the need to use several methods to visualize and quantify different sound sources. As reported by Carriço et al. (2020a) long-term spectrograms and SPD plots are important visual tools to describe marine soundscapes. Furthermore, SPL and TOLs provide a way to quantify acoustic levels and assess temporal differences.

Indicator 11.2.1 of the European MSFD highlights the importance of describing and measuring ambient noise. However, as reported by the Technical Subgroup on Underwater Noise (TSGN), this poses the obvious problem of how to deal with identifiable sources that contribute to the local soundscape and that add to the measured pressure levels. Consequently, they suggested a more operational definition of the European MSFD more in line with the term ‘soundscape’.

This study highlights the necessity to use a higher frequency band than suggested by European MSFD to monitor anthropogenic noise. Specifically, the commission prescribed the use of 63 and 125 Hz TOLs, due to previous reports of Van der Graaf et al. (2012) on the noise produced mostly by ships. However, as our study demonstrates, these frequency bands are also occupied by fish sounds, that at least during some seasons are the major source contributing to elevated sound pressure levels. Furthermore, as reported by Merchant et al. (2014), low frequency noise from tidal flow and waves might also contaminate these low frequency bands. On the other hand, higher frequency bands might be more appropriate, at least in temperate estuaries, to indicate the presence of boat noise that also presents energy in higher frequencies up to 10 kHz or even more. In the case of the Tagus estuary, 1548 Hz TOL presented good results, maybe even better that suggested before (Merchant et al., 2014; Picciulin et al., 2016). As such, the suggestion of TSGN to also measure higher frequencies appears appropriate, since these data can be obtained without considerable extra costs and may prove relevant in different shallow water habitats. Furthermore, note that in addition to underwater anthropogenic noise evaluation and management, soundscape analysis can provide data to monitor biodiversity.

5. Conclusions

In conclusion, in the Tagus estuary, biophony is responsible for significant seasonal variations mostly due to the presence of two vocal fish species: Lusitanian toadfish and meagre. Geophony and anthropophony substantially contributed to an increase of the soundscape sound level. However, only the combination of several methodologies allowed to interpret the shifts in recorded sound levels. Additionally, the inclusion of TOLs at frequencies higher than 1000 Hz may be important for MSFD noise monitoring in shallow waters. This study highlights the potential impact of boat noise. The continuous increase in anthropophony and its frequency overlap with biophony demands precautionary actions and calls for further research. Furthermore, biodiversity changes due to direct human actions and/or climate change might be observed through changes in soundscapes. This study serves as a steppingstone for soundscape and ambient noise assessment in European estuaries and may represent a baseline to monitor the Tagus estuary ecosystem.

CRediT authorship contribution statement

Conceptualization, P.J.F., M.V. and M.C.P.A; Formal analysis, M.V.; Funding acquisition, P.J.F., M.C.P.A.; Investigation, M.V.; Methodology, M.V.; Project administration, P.J.F. and M.C.P.A.; Software, M.V.; Supervision, P.J.F. and M.C.P.A.; Visualization, M.V., M.C.P.A and P.J.F.; Writing—original draft, M.V.; Writing—review & editing, P.J.F. and M. C.P.A.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112845.

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