'Posidonia meadows calling': a ubiquitous fish sound with monitoring potential

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Abstract
In the Mediterranean Sea, the seagrass Posidonia oceanica plays a key ecological role, and is protected by a range of legislation. Standard Posidonia monitoring programmes generally focus on the plant at different spatial and short temporal scales, without considering the organisms dependent on the ecosystem. Passive acoustic monitoring (PAM) has a high potential to non-intrusively monitor biological activities and biodiversity at high temporal resolution, and to assess ecosystem health. This is particularly relevant considering that Posidonia meadows host numerous sound-producing fish species. In this study, bottom-moored hydrophones were deployed in nine Western Mediterranean meadows covering a distance of more than 200 km to identify acoustic features potentially relevant to monitor this critical habitat. Among eight identified fish sound categories, we found a single type of sound (that we will refer to as /kwa/) dominating the soundscape of Posidonia meadows over a time span of 7 months. Compared to other low-frequency sounds, the /kwa/ presented unique characteristics that suggest it is produced by a fish via fast contracting muscles. The /kwa/ was the only sound detectable under anthropogenic noise conditions, and little affected by it. Cluster analyses performed on 13 acoustic features revealed a high degree of call diversity. /Kwa/ diversity, combined with its large-scale (all meadows), long-term (7 months) occurrence and low noise interference, make the /kwa/ a promising candidate for PAM of Posidonia meadows. Furthermore, variability in acoustic features suggests a central role of the /kwa/ in communication. Overall, this work sets the basis for establishing the relevance of the /kwa/ in monitoring P. oceanica meadows and developing PAM techniques for this critical habitat.

Introduction
Posidonia oceanica (L.) Delile, 1813 is an endemic flowering plant (Magnoliophyta) from the Mediterranean infralittoral. Although covering 1 or 2 % of the sea bottom, the multiple ecological roles of Posidonia make this habitat pivotal in the Mediterranean ecosystem. Posidonia is involved in the protection of the beaches from erosion, stabilization of the substratum, production of oxygen, reduction in bacterial pathogens and represents an important breeding and nursery habitat for many invertebrate and fish species (e.g. Boudouresque et al. 2012; Lamb et al. 2017). P. oceanica is protected by EU legislation and considered as a priority habitat (Council Directive 92/43/EEC & Council Directive 2000/60/CE).

Several long-term projects currently monitor Posidonia meadows and follow anthropogenic impacts (Lopez y Rojo et al. 2010; Holon et al. 2015a,b). The data collected essentially focus on the plant from a macroscale level, that is, the Posidonia leaf/shoot, to a local (meadow) or macroscale level (system) (Boudouresque et al. 2000; Descamp et al. 2011; Noël et al. 2012). Only recently, a
more ecosystem-based approach has been proposed (Pers-
sonic et al. 2014). Commonly, *P. oceanica* monitoring
methods are based on scuba diving transects (e.g. Bou-
douresque et al. 2007; Guillén et al. 2013) and more
recent techniques such as photogrammetry (Holm et al.
2015b), underwater photography and video footage
(Ardizzone et al. 2006) and aerial photography (Bonacorsi
et al. 2013) of *Posidonia* meadows. However, these meth-
ods do not consider the biological activities of organisms
that depend on *Posidonia*, and generally require consider-
able human and logistic efforts in return for data of rela-
tively low temporal resolution. There is therefore a need
to identify and establish new complementary and holistic
approaches to monitor at high temporal resolution this
total habitat, including organisms that live in *Posidonia*
meadows. Passive Acoustic Monitoring (PAM) has a great
potential to fill this gap and is receiving increasing atten-
tion as a means to acquire information on habitats, their
environmental status and changes in a large range of bio-
topes (Rountree et al. 2006; Kinda et al. 2013; Bertucci
et al. 2016). PAM offers a non-invasive and non-destruc-
tive approach that allows the study of invertebrate, fish
and marine mammal diversity and activities using their
sound emissions over long temporal scales (up to years)
(e.g. Rountree et al. 2006; Mellinger et al. 2007; Coquer-
eau et al. 2016). These animal sounds may act as biologi-
cal traits of the habitat highlighting species–environment
relationships and serve as environmental proxies (Picci-
ulini et al. 2013). Monitoring biogenic sounds can provide
unique information on both biological and/or ecological
processes and their spatio-temporal variability (Staat-
eman et al. 2014). For instance, acoustic diversity has been
associated with ecosystem health (Sueur et al. 2008; Ber-
tucci et al. 2016) and a recent study showed that biologi-
cal sounds can also be used to monitor key species on an
ocean basin level (Parmentier et al. 2017). In addition,
PAM allows the investigation of the presence of cryptic
species (Kéver et al. 2016), which is of particular interest
in habitats where the visibility is low and/or underwater
visual census are arduous to conduct such as in *Posidonia*
meadows. PAM is therefore highly promising for habitat
management, but in coastal habitats it also faces the chal-
lenge linked to the impact of noise from a variety of
human activities.

Fish sounds, which are typically produced in the low-
frequency bandwidth (<2000 Hz), are abundant in coastal
environments and vary as a function of time, space and
habitat (e.g. McCauley and Cato 2000; Ruppé et al.
2015). In the Mediterranean Sea, 38 fish species from 20
families have been identified to emit sounds (Table S1),
and some of them (e.g. Gobiidae, Sciaenidae, Ophidiidae)
are known to live in *Posidonia* meadows. However,
despite the ecological importance of this biotope, the high
number of fish species present in seagrass meadows
(Kalogirou et al. 2010) and the presence of many sound-
producing species, PAM of *P. oceanica* meadows has not
been reported to date. To be representative for habitat
monitoring, acoustic features associated with biogenic
sounds have to meet the following proposed criteria: (1)
Occur at large geographical scales in the same habitat and
(2) over long time periods (i.e. across seasons), (3) be
detectable also in the presence of anthropogenic noise,
and (4) show acoustic diversity, a parameter that has been
linked to habitat status (cf. Farina and Gage 2017).

The aim of this study was to describe the sound pro-
duction associated with fish acoustic signalling within
Mediterranean *P. oceanica* meadows relevant for habitat
monitoring. We recorded nine meadows covering a dis-
tance of more than 200 km of the Western Mediterranean
coastline and over a temporal window of 7 months. We
focussed on one particular sound that appeared to
meet all criteria and evaluated its potential for monitoring
*Posidonia* meadows.

**Materials and Methods**

**Sampling**

Sound recordings were carried out as part of the CALME
acoustic monitoring programmes along the French Wes-
tern Mediterranean coast established by the RMC Water
Agency and the CHORUS Research Institute (www.med-
trix.fr). Recordings from nine different meadows were
used for this study. To allow for acoustic diversity com-
parisons, all recordings were made under low wind
regimes (<10 kN), and only meadows in good ecosystem
health were considered. Environmental status was based
on PREI (*Posidonia oceanica* Rapid Easy Index, Gobert
et al. 2009) index values (0.55–0.775) obtained from the
*P. oceanica* surveillance programmes TEMPO
(Andromède Océanologie 2015). All nine meadows were
recorded in 2015, with meadow *i* also sampled in 2014
(Table 1). Because of the considerable dis-
tances between the meadows’ locations (65 ± 45 km),
recordings could not be conducted simultaneously. One
recording was obtained at end of March, six in April, two
in June, one in July and one in August (Table 1). Data
were acquired using a HTI-92-WB hydrophone (High
Tech Inc., Long Beach, MS, USA) with a sensitivity of
−155 dB re 1 V/µPa and flat frequency response from
2 Hz to 50 kHz connected to an EA-SDA14 compact
autonomous recorder (RTSys®, France). The device was

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1RMC Water Agency/Andromède Océanologie, data from the cartography platform Medtrix (www.medtrix.fr)
bottom-moored with the hydrophone at 1 m from the seafloor (Fig. 1). It acquired sounds continuously at a 176 kHz sampling rate and 24 bit resolution. At each recording date, the recorder was submerged in the afternoon and recovered the next morning for a duration of at least 14 h. Recordings were made during the night because many temperate fishes usually vocalize and produce choruses predominantly at night (Cato 1978; McCauley and Cato 2000).

**Acoustic analyses**

**Data diagnostics for acoustic feature identification**

Long-term spectrograms of the entire night (12 h) and around dusk (5 h) were built with routines developed on Matlab® (R2012a) to visualize biogenic sound production. These long-term spectrograms, combined with a more detailed manual scrolling of the audio files, were used to explore *P. oceanica* soundscapes, assess the quality of the recordings and more particularly, the presence of low-frequency noise that acoustically masks fish sounds (Radford et al. 2014). Based on these diagnostics, one particular sound, aurally sounding like a */kwa/* (Audio S1), appeared to potentially meet the criteria proposed for acoustic monitoring features. We focussed on this particular sound to assess whether it represents an appropriate candidate for PAM of *Posidonia* meadows.

**Sound selection**

The long-term spectrograms were used to identify the time period of highest abundance of the */kwa/* that was used as subsampling unit for manual sound selection. Sound selection was carried out using RavenPro Sound Analysis Software 1.5 (Cornell Lab of Ornithology, USA) on audio files sub-sampled at 4 kHz. Each identifiable */kwa/* during 2 h of the peak sound production period (i.e. the chorus) was manually selected in order to carefully describe sounds and their variability in detail. To validate the temporal subsampling method, the acoustic features of the */kwa/* selected during the 2-h subsampling
period were compared to the ones selected during the rest of the night (files from the randomly chosen meadow e). Paired Student’s t-tests with a significance level (\( \alpha \)) of 0.05 were used to test for differences. Because of the important number of sound selections (21604 selections for the entire night, 7286 selections for the subsampling period of peak sound production) and consequently the size effect on the \( P \) value (Lin et al. 2013), we used Jackknife subsamples \( n_{\text{subsamples}} = 100 \) (group balance not enforced) & \( n_{\text{replicates}} = 1000 \) to carry out the statistical tests on data subsets using R 3.1.2. [function ‘sample’, R Core Team 2014].

**Sound description**

Acoustic features typically used for fish sound description were extracted from the sound selections (Fig. 2). These included call duration, peak frequency (i.e. dominant

**Table 1.** Locations, recording dates and depths of the studied Posidonia meadows.

<table>
<thead>
<tr>
<th>Meadow Name</th>
<th>GPS coordinates</th>
<th>Date</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Bastia</td>
<td>42.7052 N 9.4576 E</td>
<td>14/04/15</td>
<td>15 m</td>
</tr>
<tr>
<td>b Côte bleue</td>
<td>43.3255 N 5.1697 E</td>
<td>09/04/15</td>
<td>15 m</td>
</tr>
<tr>
<td>c Canaille</td>
<td>43.3246 N 5.1666 E</td>
<td>07/06/15</td>
<td>15 m</td>
</tr>
<tr>
<td>d Pierre plane</td>
<td>43.1919 N 5.5499 E</td>
<td>03/06/15</td>
<td>15 m</td>
</tr>
<tr>
<td>e Fautea</td>
<td>41.7214 N 9.4079 E</td>
<td>05/10/15</td>
<td>16 m</td>
</tr>
<tr>
<td>f Porto</td>
<td>41.6000 N 9.3649 E</td>
<td>15/04/15</td>
<td>17 m</td>
</tr>
<tr>
<td>g Sentosa</td>
<td>41.6331 N 8.8218 E</td>
<td>16/04/15</td>
<td>15 m</td>
</tr>
<tr>
<td>h Ajaccio</td>
<td>41.8847 N 8.6075 E</td>
<td>17/04/15</td>
<td>15 m</td>
</tr>
<tr>
<td>i Calvi</td>
<td>42.5802 N 8.7263 E</td>
<td>26/04/15</td>
<td>12 m</td>
</tr>
</tbody>
</table>

**Figure 2.** Acoustic representations of the /kwa/ and measured features (cf. main text for abbreviations). Centre: spectrographic view of a typical /kwa/ selection with the most energetic contour indicated as dotted white line; left: power spectrum; bottom: waveform of the sound. LFFT: 8192, sampling frequency = 15,6250 Hz, Kaiser 180 dB with 50% window overlap. Grey scale: between 50 and 95 dB re 1 \( \mu \text{Pa}^2/\text{Hz} \). \( 1/\text{HI} \) = pulse period.
frequency), pulse period and number of pulses (e.g. Amorim et al. 2008). However, these features do not allow to describe the complexity and diversity of the signal. Consequently, additional acoustic features were considered, including parameters used for characterizing harmonic or pseudo-harmonic² (Watkins 1968) sounds emitted by anurans and primates (e.g. Gerhardt 1981; Price et al. 2015) and for describing transient sounds emitted by anurans and primates (e.g. Gerhardt 1981; Price et al. 2015) and for describing transient sounds (Tucker and Brown 2005). Some features (e.g. duration, bandwidth, minimal and maximal frequency) were calculated within the selection box using custom-made Matlab codes. The considered features are listed in Table 2 and described in detail in the Data S1 (cf. Fig. 2).

Only the sounds with at least four pseudo-harmonics and a SNR ≥0 dB were included in the analyses. This allowed to only consider high-quality sounds (23,566 out of 44,257 selections). To facilitate comprehension and interpretation, the set of 23 features was subdivided into three subsets with increasing dependency on the sound level features:

1. Subset №1 {HI, ΔFsp, ΔFsp, ΔFcp, ΔFcp, Fmin, Fpeak, H1, H2, H3, H4, T, NP},
2. Subset №2 {BW, Entro, Fsp, Fcp, Fmax, NbH, Q3H},
3. Subset №3 {RL, SCL, SNR}

Subset №1 contains 13 features describing the spectral shape (contour) of the sound and the temporal pattern (NP & PP, i.e. 1/HI)), subset №2 comprises seven features, which describe the spectral shape (BW, Entro, Fsp, Fcp, Fmax, NbH) and the repartition of the relative acoustic power along the pseudo-harmonics (Q3H) and subset №3 comprises three features characterizing only the level of the sound without any information on the contour. Pearson correlations were carried out to highlight relationships between the 23 acoustic features.

**Occurrence**

The presence of the /kwaw/ was assessed for all recordings allowing to determine the extent of spatial and temporal occurrence. The selections from meadow e (14 consecutive hours, from 6 p.m. to 8 a.m.) used to test acoustic differences between the /kwaw/ of the period of highest

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Table 2. List of measured acoustic features (cf. Data S1 for detailed descriptions).

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>Number of pulses</td>
<td>Number of pulses within the temporal envelope</td>
</tr>
<tr>
<td>T</td>
<td>Duration (s)</td>
<td>Bandwidth based on the signal selection box</td>
</tr>
<tr>
<td>BW</td>
<td>Bandwidth (Hz)</td>
<td>Minimal &amp; maximal frequency based on the signal selection box</td>
</tr>
<tr>
<td>Fmin, Fmax</td>
<td>Minimal and maximal frequency (Hz)</td>
<td>The frequency at the maximum of the power spectrum</td>
</tr>
<tr>
<td>Fpeak</td>
<td>Peak or dominant frequency (Hz)</td>
<td>Start and end frequency of the most energetic contour C(t, f, fpeak).</td>
</tr>
<tr>
<td>ΔFes</td>
<td>End frequency minus start frequency (Hz)</td>
<td>Fes - Fs</td>
</tr>
<tr>
<td>ΔFsp</td>
<td>Start frequency minus peak frequency (Hz)</td>
<td>Fsp - Fpeak</td>
</tr>
<tr>
<td>ΔFcp</td>
<td>End frequency minus peak frequency (Hz)</td>
<td>Fcp - Fpeak</td>
</tr>
<tr>
<td>NbH</td>
<td>Number of pseudo-harmonics</td>
<td>Number of pseudo-harmonics between Fmin and Fmax.</td>
</tr>
<tr>
<td>HI</td>
<td>Pseudo-harmonic interval</td>
<td>Interval between the estimated using the complex auto-correlation function of $E(f)$ (Le Bot et al. 2015).</td>
</tr>
<tr>
<td>PP</td>
<td>Pulse period</td>
<td>The reciprocal of HI (i.e. 1/HI) It is therefore redundant to include both features in the analyses.</td>
</tr>
<tr>
<td>H1, H2, H3, H4</td>
<td>Pseudo-harmonic index</td>
<td>The index of the four most energetic pseudo-harmonics.</td>
</tr>
<tr>
<td>Q3H</td>
<td>Pseudo-harmonic ratio</td>
<td>Ratio of the energy contained in H1 + H2 + H3 to the energy contained in all pseudo-harmonics between $F_{\text{min}}$ and $F_{\text{max}}$.</td>
</tr>
<tr>
<td>Entro</td>
<td>Normalized linear entropy</td>
<td>$\frac{\int -\gamma(f) \log_{2} (\gamma(f)) df}{\log_{2}(\text{LEFT})}$</td>
</tr>
<tr>
<td>RL</td>
<td>Received Level in dB re 1 μPa</td>
<td>$RL = 10\log_{10}\left(\frac{1}{t}\int</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio in dB re 1μPa</td>
<td>Signal to noise ratio with the underlying Ambient Noise Level (ANL) in dB re 1 μPa. (Kinda et al. 2013; Mathias et al. 2016).</td>
</tr>
<tr>
<td>SCL</td>
<td>Sound cumulative level in dB re 1μPa²s</td>
<td>The SCL quantifies the energy contained in the signal. $\text{SCL} = 10\log_{10}\left(\int_{t} S_{t}(t)^{2} dt\right)$</td>
</tr>
</tbody>
</table>

²In the spectrographic view, pulse trains with fast repetition rates are indicated by the ‘pseudo-harmonic’ interval (harmonic of the amplitude-modulated function).
abundance at dusk and the rest of the night were also used to illustrate night-time patterns. Because selecting each /kwa/ over 14 h (from 6 P.M. to 8 A.M.) is extremely time-consuming considering the high number of /kwas/ present (7284 selections for meadow c), data from only one recording were used. Furthermore, the presence of the dusk chorus is also indicative of the night-time pattern in /kwa/ abundance. To evaluate the presence and relative abundance of the /kwa/ compared to other fish calls present in meadows, the /kwas/ and all other fish sound types of one entire night were also manually selected and their night-time patterns compared to the one of the /kwa/. Meadow c was randomly chosen between the three meadows in which sound detection was not masked by anthropogenic noise.

/Kwa/diversity

Visual inspection during the manual selection procedure suggested the existence of different types of /kwa/, particularly with respect to the spectral content and contour’s shape (Fig. 4). Cluster analysis was conducted to quantify this signal diversity. To reduce the dimensions for cluster analysis and eliminate redundancy due to high intercorrelation of the acoustic variables Principal Component Analysis (PCA) was performed. The PCA was conducted using all acoustic features of subset №1, because received-level dependent parameters may be linked to factors such as the distance of the vocalizing animals. The scores of the first three principal components of the PCA were used as input for the cluster analysis. Because the distribution of the PCA scores was multimodal, we considered the data to be distributed as a finite Gaussian mixture. For a given number of clusters, the maximum likelihood estimators of the mean and covariance matrix of each Gaussian component of the mixture were found using an expectation-maximization algorithm (McLachlan and Peel 2000). The minimum of the distribution of the Bayesian Inference Criteria (BIC) was used to estimate the optimal number of clusters (Penny et al. 2007). The clustering was realized using the gmdistribution.fit function of the Statistics Toolbox in Matlab® (R2012a).

Temporal variability

Acoustic features may change over time linked to adaptations to environmental factors (e.g. temperature, ambient noise) or morphological changes associated to specific behaviours such as reproduction (Connaughton and Taylor 1995; Ladich and Schleinzer 2014; Radford et al. 2014). Meadow recordings were ranked according to their recording date. The temporal variability in the /kwa/ was illustrated by plotting acoustic variables over time (given the rank). To test seasonal differences, comparisons were only conducted on meadows sampled in both spring and summer of the same year and at the same or almost the same position (meadows b and i). Welch’s t-test on Jackknife subsamples was used to test for significant differences. The significance level (α) was 0.05.

Results

Diagnostics

Six of the nine sites were subject to low-frequency anthropogenic noise also during night-time. This noise was mainly caused by distant shipping and potentially nearby harbour activity. Sound selection was therefore compromised or impaired due to acoustic masking (i.e. when the perception of one sound is affected by the presence of another sound). Only three of the nine site-recordings allowed the analysis of all types of fish sounds recorded. Consequently, fish sound diversity, which is recognized as an indicator of environmental status, was considered inappropriate for PAM of P. oceanica meadows. All nine sites were dominated by one particular sound, the /kwa/ with a frequency range above the noise responsible for the masking of all other fish sounds (Fig. 3). Together, these diagnostics suggested to focus on the /kwa/ to establish its potential as an acoustic monitoring feature.

Sound description

The /kwa/ is a pulse train of 0.27 ± 0.09 sec duration, characterized by 13 ± 6 pulses and a pulse period of 13 ± 4 msec (N = 23,566 sounds). Its waveform is characterized by a peak or dominant frequency of 747 ± 136 Hz modulated in amplitude by a periodic envelope with 1/HI oscillations. In the spectrographic view, this is visible as pseudo-harmonics around a 800 Hz contour with a mean pseudo-harmonic interval (HI) of 81.2 ± 30.6 Hz and a frequency bandwidth of 723 ± 280 Hz (Fig. 2). The average frequency contour is characterized by similar start and end frequencies around 750 Hz and a 70 Hz higher centre frequency indicative of a generally arch-shaped contour. The sound has an average received level of 95 ± 10 dB re 1 μPa (RMS) with three pseudo-harmonics comprising 85% of the signal’s power. All the detailed characteristics of the sounds are summarized in Table 3. Statistical comparison between the acoustic features of /kwas/ selected across one entire night and those selected during the 2 h of peak sound production at dusk revealed no differences (Table S2). This supports the selection procedure that was restricted to 2 h of the dusk chorus.
Occurrence

The /kwa/ occurred within all 12 sampling nights of the 9 sites used in this study covering a time span from April until October. Overall, 44257 /kwas/ were selected. The average number of sounds per selection period of 2 h was 3248.25 ± 2830.05 (minimum: 40 sounds per 2 h, maximum: 9521 sounds per 2 h), that is, on average more than 27 sounds per minute. The /kwa/ production varied over the course of the night, with an important peak 2 h after sunset (33.72% of all the selections, calculated on meadow e). This period of highest abundance lasts around 2 h and corresponds to a mass phenomenon referred to as chorus (Cato 1978), which is clearly visible on the long-term spectrograms (Figs. 3 and 4). Compared to other identified fish sound types, the /kwa/ was the only call above 500 Hz. All other fish sounds were in the low-frequency (50–500 Hz) range. Overall, eight fish sound types were identified based on their acoustic characteristics (details available in Table S3). Quantitatively, more than 95% of all the recorded fish sounds were /kwas/. This percentage was not constant over the course of the night, as illustrated in Figure S1. After 1 A.M., the total number of fish sounds strongly decreased and the /kwa/ was almost the only one (almost 100%) recorded until sunrise.

Sound diversity

The correlation analysis between the 23 acoustic features revealed that 31 inter-correlations had absolute magnitudes greater than 0.4 comprehending 15 features. (Table S4). PCA was thus used to reduce the dimensionality of the features for cluster analysis. The first three axes of the PCA explained 43.8% of the variance (16.7% axis 1; 27.1% axes 1 and 2). All features of subset №1 (i.e. mainly describing the contour shape of the call) produced a sensitive variation (i.e. long projection of a feature’s unitary vector in the first three axes of the PCA) (Fig. S2). Their projections showed three groups of nearly collinear vectors (i.e. with a small angle between the feature’s unitary vectors): (1) \{ΔF_e, ΔF_sp\}, (2) \{T, HI (or PP)\} and (3) \{H1, H2, H3, H4\}. Features with collinear projections act in the same way in the PCA approximation and may not account to discriminate between different classes in a classification process. Cluster analysis performed on the three first components of the PCA revealed the existence of 11
Table 3. Summary statistics of the acoustic features (cf. Table 2 for abbreviation definitions) for sounds with more than four pseudo-harmonics during the 2-h sampling units.

<table>
<thead>
<tr>
<th></th>
<th>(\bar{x})</th>
<th>SD</th>
<th>SE</th>
<th>IQR</th>
<th>0%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>12.8</td>
<td>5.8</td>
<td>0.04</td>
<td>7</td>
<td>2</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>PP (s)</td>
<td>0.013</td>
<td>0.004</td>
<td>0.00003</td>
<td>0.005</td>
<td>0.005</td>
<td>0.011</td>
<td>0.014</td>
<td>0.017</td>
<td>0.041</td>
</tr>
<tr>
<td>HI (Hz)</td>
<td>81.23</td>
<td>30.61</td>
<td>0.16</td>
<td>27.5</td>
<td>10.5</td>
<td>62.5</td>
<td>72.5</td>
<td>90</td>
<td>200</td>
</tr>
<tr>
<td>(\Delta F_{SP}) (Hz)</td>
<td>7.74</td>
<td>104.07</td>
<td>0.54</td>
<td>33</td>
<td>-1130</td>
<td>-22</td>
<td>-4</td>
<td>11</td>
<td>1067</td>
</tr>
<tr>
<td>(\Delta F_{WS}) (Hz)</td>
<td>-6.43</td>
<td>105.09</td>
<td>0.55</td>
<td>51</td>
<td>-1068</td>
<td>-20</td>
<td>0</td>
<td>31</td>
<td>731</td>
</tr>
<tr>
<td>(\Delta F_{WSP}) (Hz)</td>
<td>1.31</td>
<td>30.87</td>
<td>0.16</td>
<td>12</td>
<td>-890</td>
<td>-9</td>
<td>-3</td>
<td>3</td>
<td>649</td>
</tr>
<tr>
<td>(\Delta F_{EP}) (Hz)</td>
<td>1.08</td>
<td>20.43</td>
<td>0.11</td>
<td>5</td>
<td>-878</td>
<td>-2</td>
<td>1</td>
<td>3</td>
<td>531</td>
</tr>
<tr>
<td>(F_{max}) (Hz)</td>
<td>540.71</td>
<td>131.83</td>
<td>0.69</td>
<td>175</td>
<td>85</td>
<td>447</td>
<td>518</td>
<td>622</td>
<td>1168</td>
</tr>
<tr>
<td>(F_{peak}) (Hz)</td>
<td>746.85</td>
<td>135.6</td>
<td>0.7</td>
<td>187.5</td>
<td>402.3</td>
<td>644.5</td>
<td>724.6</td>
<td>832</td>
<td>1884.8</td>
</tr>
<tr>
<td>H1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>H2</td>
<td>0</td>
<td>1</td>
<td>0.01</td>
<td>2</td>
<td>-7</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>H3</td>
<td>1</td>
<td>2</td>
<td>0.01</td>
<td>3</td>
<td>-9</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>H4</td>
<td>1</td>
<td>2</td>
<td>0.01</td>
<td>3</td>
<td>-10</td>
<td>-1</td>
<td>2</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>T (s)</td>
<td>0.27</td>
<td>0.09</td>
<td>0</td>
<td>0.11</td>
<td>0.07</td>
<td>0.2</td>
<td>0.25</td>
<td>0.32</td>
<td>1.09</td>
</tr>
<tr>
<td>BW (Hz)</td>
<td>722.82</td>
<td>280</td>
<td>1.99</td>
<td>386</td>
<td>168</td>
<td>506</td>
<td>694</td>
<td>892</td>
<td>1844</td>
</tr>
<tr>
<td>Entro</td>
<td>86.63</td>
<td>5.60</td>
<td>0.03</td>
<td>7.78</td>
<td>59.32</td>
<td>83.08</td>
<td>87.38</td>
<td>90.87</td>
<td>98.94</td>
</tr>
<tr>
<td>(F_s) (Hz)</td>
<td>754.59</td>
<td>161.82</td>
<td>0.84</td>
<td>210</td>
<td>294.8</td>
<td>637.8</td>
<td>734.3</td>
<td>847.8</td>
<td>1894.9</td>
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<tr>
<td>(F_o) (Hz)</td>
<td>748.17</td>
<td>134.87</td>
<td>0.7</td>
<td>185.5</td>
<td>386.3</td>
<td>647.3</td>
<td>727.7</td>
<td>832.8</td>
<td>1829</td>
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<tr>
<td>(F_{max}) (Hz)</td>
<td>1196.13</td>
<td>261.42</td>
<td>1.36</td>
<td>342</td>
<td>584</td>
<td>1009</td>
<td>1171</td>
<td>1351</td>
<td>2000</td>
</tr>
<tr>
<td>NbH</td>
<td>9.72</td>
<td>4.33</td>
<td>0.02</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>Q3H (%)</td>
<td>85.05</td>
<td>11.14</td>
<td>0.06</td>
<td>14.91</td>
<td>21.66</td>
<td>78.66</td>
<td>87.25</td>
<td>93.58</td>
<td>100.00</td>
</tr>
<tr>
<td>RL (dB re 1\mu Pa)</td>
<td>95.24</td>
<td>10.17</td>
<td>0.05</td>
<td>17</td>
<td>70</td>
<td>86</td>
<td>96</td>
<td>103</td>
<td>128</td>
</tr>
<tr>
<td>SCL (dB re 1\mu Pa^2/s)</td>
<td>89.31</td>
<td>10.09</td>
<td>0.05</td>
<td>16</td>
<td>65</td>
<td>81</td>
<td>90</td>
<td>97</td>
<td>123</td>
</tr>
<tr>
<td>SNR (dB)</td>
<td>11.19</td>
<td>3.76</td>
<td>0.02</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>34</td>
</tr>
</tbody>
</table>

\(N = 23566\). IQR, Inter Quartile Range; SD, Standard Deviation; SE, Standard Error.

\(^{1}\)Most probable H.

**Figure 4.** Spectrographic views of /kwas/ over different temporal scales. (A) Long-term spectrogram of an entire night on a P. oceanica meadow (meadow i, July 2015); (B) 3-h spectrogram showing massif /kwa/ chorus after sunset (dark horizontal band around 800 Hz); (C) spectrogram showing rhythmic repetitions of single /kwa/; (D) spectrogram showing different types of /kwa/. LFFT: 8192, sampling frequency = 156250 Hz, Kaiser 180 dB with 50% window overlap. Grey scale: between 50 and 95 dB re 1\mu Pa^2 Hz^{-1}. 

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classes (Fig. 5), which correspond to the minimum BIC value. These results suggest that /kwa/ have a high intrinsic variability.

**Temporal variability**

RL, Entro, PP, NP, BW and the frequency features ($F_{\text{min}}$, $F_{\text{peak}}$, $F_s$ and $F_e$) varied over time. RL showed a maximum in July, while the entropy showed an opposite trend with a minimum in June. BW and NP also peaked in June but the number of pulses decreased more rapidly than the bandwidth, which was almost constant until October. Peak frequency and pulse period showed opposite trends, with $F_{\text{peak}}$ increasing during the summer months and PP decreasing (minimum in August) (Fig. 6). To avoid effects linked to spatial variability, the seasonal variability in two specific meadows, for which both spring and summer data were available (meadows $b$ and $i$), was analysed separately. The same trends were confirmed: the RL was significantly higher in summer than in spring, while the linear entropy followed an opposite trend (Table 4, Table S5, Fig. 7). PP significantly decreased in summer compared to spring, with August showing the smallest values (in meadow $i$, Table 4 & Table S5). Seasonal peak frequency comparison in meadows $b$ and $i$ resulted in overall greater values in summer than in spring. $F_{\text{min}}$, $F_s$ and $F_e$ showed the same behaviour as $F_{\text{peak}}$. Although preliminary, these results indicate a potential seasonal effect on the acoustic structure of the /kwa/. During the summer, fish appear to produce more powerful and spectrally structured sounds with a greater bandwidth (and thus more pseudo-harmonics), higher dominant frequencies and smaller pulse periods. Because the sampling campaign was not designed to study seasonal acoustic variation, these results need to be confirmed with appropriate long-term data.
Discussion

Acoustic features for *P. oceanica* monitoring

Our study identified and characterized an acoustic feature potentially relevant for monitoring *P. oceanica* meadows, a key habitat protected by numerous legislations. According to the proposed criteria such an acoustic feature has to: (1) be measurable over large geographical scales in the same habitat; (2) occur across seasons, so independent of time-specific behaviours such as reproduction of the sound emitter (e.g. Amorim et al. 2006); (3) be poorly affected by noise interference, (4) show acoustic diversity that can be influenced by the ecological status of the habitat.

The /kwa/ occurred in all analysed *P. oceanica* meadows along 200 km of the Western Mediterranean littoral, supporting a strong relationship with this habitat. /Kwas/

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**Figure 6.** Normalized mean (between 0 and 1) and standard deviation of acoustic features as a function of time: the entropy (grey) and the received level (light blue) at the bottom, the bandwidth (yellow) and the number of pulses (green) in the centre, and peak frequency (orange) and pulse period (dark blue) at the top. Each dot represents a single night-time recording. The dotted black curve in the top panel represents the Mediterranean surface temperature trend during the same time period measured at three stations (data provided by ‘Service d’Observation en Milieu Littoral, INSU-CNRS’).
were present across seasons, over a time span of 7 months (April until October) and dominated the fish sound production in meadows with water depths around 15 m. Overall, there were over 20 times more /kwas/ than all other fish calls combined in the analysed recordings. /Kwas/ were present during the whole night, with a peak production 2 h after sunset (33.72% of all night-time selections) corresponding to a chorus of at least 2 h duration. Furthermore, the /kwa/ occupies a frequency window (747 ± 136 Hz) that is at least three times higher than the one of the majority of Mediterranean fish sounds recorded to date (cf. Table S2). Consequently, compared to all the fish sounds identified in this study, the /kwa/ was the only low-frequency sound that showed poor anthropogenic noise interference. This is particularly relevant considering that P. oceanica monitoring programmes include meadows affected by human activities (e.g. fishing, recreational navigation) that can acoustically mask low-frequency animal sounds (e.g. most fish sounds) (e.g. Radford et al. 2014). In fact, 67% of the recorded Posidonia meadows presented low-frequency noise also at night, mainly related to shipping that impaired the selection of all fish sounds, except for the /kwa/. Finally, /kwas/ also present a high diversity, as revealed by the cluster

Table 4. Mean values ± SD of some acoustic features for separated sites and months, SNR ≥ 10 and NbH ≥ 4.

<table>
<thead>
<tr>
<th>Meadow</th>
<th>Month</th>
<th>N</th>
<th>RL (dB re 1 µPa)</th>
<th>Entro</th>
<th>Fpeak (Hz)</th>
<th>BW (Hz)</th>
<th>HI (Hz)</th>
<th>PP (s)</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>April</td>
<td>1843</td>
<td>91 ± 5</td>
<td>85.9 ± 3.6</td>
<td>621 ± 61</td>
<td>691 ± 199</td>
<td>70.8 ± 38.9</td>
<td>0.017 ± 0.005</td>
<td>11.4 ± 5.6</td>
</tr>
<tr>
<td>June</td>
<td>4876</td>
<td>105 ± 5</td>
<td>81.2 ± 4.7</td>
<td>677 ± 66</td>
<td>824 ± 231</td>
<td>78.8 ± 27.1</td>
<td>0.013 ± 0.002</td>
<td>12.3 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>April</td>
<td>842</td>
<td>96 ± 4</td>
<td>89.9 ± 3.6</td>
<td>664 ± 68</td>
<td>520 ± 189</td>
<td>65.9 ± 23.2</td>
<td>0.016 ± 0.003</td>
<td>10.8 ± 4.7</td>
</tr>
<tr>
<td>July</td>
<td>827</td>
<td>113 ± 4</td>
<td>83.9 ± 5.6</td>
<td>818 ± 146</td>
<td>755 ± 332</td>
<td>81.9 ± 19.9</td>
<td>0.013 ± 0.002</td>
<td>12.6 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>2899</td>
<td>103 ± 7</td>
<td>83.6 ± 5.5</td>
<td>856 ± 137</td>
<td>786 ± 316</td>
<td>95.7 ± 20.4</td>
<td>0.01 ± 0.002</td>
<td>13 ± 5.4</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. Feature comparisons of /kwas/ between spring (left) and summer (right) from meadow i and b, for which data across seasons were available from the same recording positions. Two night-time recordings were used for each season (09/04/15 and 26/04/15; 07/06/15 and 07/06/15). *P < 0.01, **P < 0.001.

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analysis. Although it remains to be tested, differences in /kwa/ diversity could be relevant to depict habitat differences and underlie environmental status. In terrestrial animals, such as birds or anurans it has been shown that acoustic diversity decreases with poor habitat quality (i.e. urbanization, habitat loss, degradation, fragmentation) (Laiolo and Tella 2007; Laiolo et al. 2008; Pillsbury and Miller 2008) and that condition-dependent traits in male songs, relevant for reproduction, are reduced at the level of the population (McGregor 2005; Grava 2006). Although temporal signal variation remains to be fully validated, it has to be taken under consideration when performing diversity comparisons across seasons, as it may affect /kwa/ diversity at the population level (e.g. Connaughton et al. 2002). Together, these results indicate that the /kwa/ meets all criteria relevant for PAM of P. oceanica meadows. Whether it actually reflects differences associated to habitat quality for application in monitoring programmes, remains to be tested.

The /kwa/, its origin and potential role

/Kwas/ are composed of 13 ± 6 pulses separated by 13 ± 4 msec intervals and have a mean dominant frequency of 747 ± 136 Hz. The acoustic structure of the /kwa/ shares similarities with other fish sounds. Pulses are emitted at a regular frequency of 81 Hz, implying that each single pulse corresponds to a unit of muscle activity that takes place every 12 msec. This information clearly supports the use of fast contracting muscles for sound production. In species with similar contraction periods, sounds produced by drumming muscles are characterized by a relatively low fundamental frequency (<300 Hz) (Parmentier et al. 2013; Boyle et al. 2015) that generally corresponds to the contraction rate of the sonic muscle (Zelick et al. 1999). In the /kwa/, the pulse period of 81 Hz is not responsible for the dominant frequency of the call, which ranges between 700 and 1000 Hz. This dominant frequency appears to correspond to one of the harmonics of the pulse period as reported in various fish families (Amorim et al. 2004; Rice and Bass 2009; Parmentier et al. 2016). Within the call, each pulse consists of different peaks with the peak period corresponding to the dominant frequency of the call. Each muscle twitch produces multiple vibrations causing the radiated sound to have a different dominant frequency than the muscle contraction rate. The tension that increases in the contracting muscles makes it oscillate like a guitar string (Sprague 2000). This muscle vibration provides the dominant frequency of the sound. Scorpaeniformes comprise species capable of producing harmonic sounds with fast contracting muscles (Amorim et al. 2004; Connaughton 2004) thus representing good candidates as the source of the /kwa/. In Mediterranean Posidonia meadows, the most abundant and nocturnal species of this order are from the Scorpaenidae family (Kalogirou et al. 2010).

/Kwas/ form a specific sound category that can easily be distinguished from other fish calls. The /kwa/ appears to occupy an exclusive frequency-niche that reduces masking interference by other fish sounds and promotes intraspecific communication (McCauley and Cato 2000; Hastings and Širović 2015). /Kwas/ also present a high diversity in spectral shape and temporal pattern (subset №1 features) as revealed by cluster analysis. The factors responsible for this high signal variability remain to be elucidated, but these findings suggest the transmission of multiple messages, and/or a link to different species, as observed in Sciaenidae (Picciulin et al. 2016) and Gobiidae (Pedroso et al. 2012; Blom et al. 2016), and/or inter-individual differences. For instance, across taxa, differences in the number of harmonics as well as in the energetic distribution across harmonics have been attributed to individual differences (e.g. penguins: Searby et al. 2004; toadfish: Amorim and Vasconcelos 2008; monkeys: Price et al. 2015), and/or allow individuals to occupy a greater frequency window and thus enhance signal transmission (Brumm and Fishman 2006; Radford et al. 2014). Combined with the presence of stereotyped, rhythmic sequences, sometimes involving different types of /kwas/, our findings support a communicative function of this fish call.

/Kwas/ showed temporal differences in frequency features (BW, F̄min, F̄peak, F̄s and F̄e), temporal features (NP, PP) and amplitude-related features (RL, Entro). Hydrophone position, distance and number of calling fishes, environmental factors such as lunar cycle, or ambient noise (Connaughton and Taylor 1995; Radford et al. 2014) may contribute to the observed temporal variations. However, the interplay between the changes in acoustic features shows similarities to the one observed in other temperate fish species that have been attributed to physiological or morphological changes during the reproductive season. For instance, similar to many different fish species, peak frequency and water temperature increased over the course of the months and call amplitude was 14 to 17 dB higher in summer than in spring. A rise in temperature is known to increase the activation rate and the velocity of the sonic muscle (e.g. Connaughton et al. 2002; Ladich and Schleiner 2014; Kéver et al. 2015), while an increase in call amplitude has been reported as a result of sonic muscle hypertrophy during the spawning period (Connaughton et al. 2002; Rowe and Hutchings 2004; Nguyen et al. 2008). The identification of the /kwa/-producing species combined with long-term data acquired within the same Posidonia meadow will allow to verify the here observed seasonal trends in acoustic features and evaluate their potential role.
Conclusions
This work is foundational in describing a ubiquitous Mediterranean coastal fish sound that meets the proposed criteria relevant for PAM of P. oceanica seagrass meadows. It sets the bases for future studies aiming at revealing if the /kwal/ can be used as an environmental proxy for habitat monitoring. Fish sounds are used in communication; they reflect an organism’s activity and play a role in the species survival (Ladich 2015). Across taxa, there is evidence that environmental disturbance and habitat quality are reflected in the acoustic behaviour and variation in animal communities and populations (Riede 1998; van Oort et al. 2006; Pillsbury and Miller 2008; Laiolo 2010; Rosenthal and Stuart-Fox 2012). The next step to further evaluate if the /kwal/ is valuable for PAM of P. oceanica meadows would be to test /kwal/ diversity, chorus output and calling activity in relation to environmental variables and the status of P. oceanica seagrass meadows, characterized by different quality index values (e.g. BiPo, PREI, EBQI). Besides identifying a possible environmental proxy of a key habitat, this work also describes a sound with unique features compared to other known Mediterranean fish vocalizations that is potentially significant in the study of fish communication. Future studies need to be designed to further elucidate the source of the sound, the communicative role of the /kwal/ and its diversity.

Acknowledgments
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Conflict of Interest
All authors declare that they have no conflict of interest.

References


Acoustic Monitoring of Posidonia Meadows


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### Supporting Information

Additional supporting information may be found online in the supporting information tab for this article.

**Figure S1.** Number of fish sounds selected over the course of one night (meadow c).

**Figure S2.** Two views of the projections of the unitary vectors of the subset №1 features on the first three axes of the PCA, thick lines: black: single features whose projections are not collinear, blue, green and red: three sets of features whose projections are nearly collinear.

**Table S1.** Alphabetic list of known sound-producing fish families of the Mediterranean Sea, for which at least one reference was found in literature. The reference list is likely incomplete. Gobiidae of brackish Adriatic waters excluded.

**Table S2.** Comparison of the /kwa/ features during the subsampling period (i.e. the 2 h of peak production) versus the rest of the night.

**Table S3.** Fish sound categories present in the entirely analysed meadow recording.

**Table S4.** Pearson correlations for each studied feature for the sounds with ≥4 pseudo-harmonics during the 2 h of peak /kwa/ emission. \( N = 23,566 \).

**Table S5.** Summary table of seasonal feature differences represented as means (top row) and medians (bottom row) for 1000 \( P \)-values.

**Audio S1.** Example of typical /kwas/ recorded in Corsica, France.

**Data S1.** Description of the acoustic features used for sound description.