

1 Temperature mediates chorusing behaviour associated with  
2 spawning in the sciaenid *Argyrosomus regius*.

3 **Running page head:** Temperature mediates fish chorusing behaviour.

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17 patterns; rhythms; field study; automatic detectors

18

19 **ABSTRACT**

20 Climate change is leading to an increase in the frequency and intensity of daily temperature  
21 extremes in aquatic environments, posing a threat to ectothermic animals, such as fishes.  
22 Temperature shifts can impact their reproductive cycle and behaviour, including acoustic  
23 signalling associated with courtship and mating in vocal fishes. Here, we examined the effect of  
24 temperature and other environmental factors on spawning-related chorusing behaviour in the  
25 meagre. Using an automatic recognition system based on hidden Markov models coupled with  
26 third octave band sound level analysis we extracted meagre daily vocal activity over four years  
27 of passive acoustic monitoring (PAM) in the Tagus estuary (Portugal). Meagre's vocal activity  
28 showed a distinct diel pattern with choruses being most prevalent at dusk. Chorus activity  
29 started earlier in years with warmer springs, while the end of the season was concurrent with  
30 the rise of water temperature. Circa 70% of daily variations were explained by changes in  
31 water temperature. Tidal cycle, lunar phase and changes in daylength did not appear to affect  
32 vocal behaviour. Choruses were detected in days with mean water temperature ranging from  
33 15 °C to 25 °C, with stronger chorusing observed around 18 °C. Abrupt changes in temperature  
34 caused disruption in choruses, and likely in associated spawning. This study highlights the  
35 importance of temperature to fish reproduction and demonstrates that PAM can be a useful  
36 tool for long term conservation management plans. PAM may also allow us to predict how  
37 temperature shifts due to climate change may affect fish reproduction, using vocal fishes as  
38 model species.

39

## 40 **1. INTRODUCTION**

41 We are currently facing large-scale changes in climatic conditions that have far reaching  
42 consequences for biodiversity (Leadley 2010; Diffenbaugh & Field 2013). Warmer  
43 temperatures are likely to affect animals' behaviour, distribution and breeding patterns  
44 (Walther et al. 2002; Root et al. 2003; Dunn et al. 2010). Furthermore, changes in local  
45 weather and increasing frequency, intensity and duration of extreme climatic events, such as  
46 heatwaves, may negatively impact breeding success (Ummenhofer & Meehl, 2017; Marcelino  
47 et al. 2020). The effect of climate change is expected to be particularly severe for fishes since  
48 they can be highly vulnerable to temperature fluctuations (Brander 2007; Burraco et al. 2020).

49 Temperature and light influence fish behaviour and physiology (Cossins & Bowler 1987; Falcón,  
50 1999; Migaud et al. 2010), diel patterns (Binder et al. 2008; Idda et al. 2012; Feng & Bass,  
51 2016), and cause reproductive changes (Van Der Meer & Ivannikov, 2006; Meseguer et al.  
52 2008). Most temperate fish show reproductive seasonality (Pankhurst & Porter, 2003), with  
53 photoperiod being the 'proximate' driver (as defined by Bromage et al. 2001), while  
54 temperature is considered crucial for triggering gamete maturation at a short-term temporal  
55 scale (Van Der Kraak & Pankhurst, 1997). Furthermore, seasonal variation in temperature  
56 appears crucial to the reproductive cycle of temperate fish, influencing the quantity of eggs  
57 spawned (Donelson et al. 2010; de Mello et al. 2021). If temperature shifts cause multiple  
58 effects on the reproductive cycle of a fish species, then climate change associated with less  
59 pronounced temperature seasonal changes, and more frequent extreme events, will likely  
60 affect reproduction (in terms of timing, efficiency and success, for example).

61 Sciaenids, like most marine fish species of commercial importance, have multiple reproductive  
62 cycles over the course of their lifetime. Spawning generally occurs during a well-defined  
63 season, with external fertilisation and with no parental care of the eggs and young (Murua &  
64 Saborido-Rey, 2003). According to the rhythm of the maturation and release of oocytes in each

65 season, these species can also be classified as batch spawners, i.e., each female can release  
66 eggs in batches several times over a reproduction season (Brown-Peterson et al. 1988). In  
67 sciaenids, an increase in gametogenesis is usually observed during late winter or early spring,  
68 with a peak in the gonadosomatic index taking place a couple of months later (see scheme in  
69 Fig. 1; Nieland & Wilson 1993; Mar Gil et al. 2013; Prista et al. 2014). Sciaenids spawn mostly at  
70 dusk (Table 1) but the external triggering factors are not completely understood. Connaughton  
71 and Taylor (1996a), in laboratory conditions, observed an effect of temperature and daylength  
72 on sexual recrudescence and reported that later stages of spermatogenesis are inhibited by  
73 low temperatures. Further, gametogenesis was reported to stop in response to higher water  
74 temperatures in summer (Mylonas et al. 2013; Soares et al. 2015) and high temperatures may  
75 also diminish eggs' survival rate (Hubbs et al. 1969). Moreover, the timing of spawning can be  
76 dictated by light cycles (Meseguer et al. 2008). Moonlight luminosity and tide are also reported  
77 to affect spawning behaviour in some fish species (Ikegami et al. 2014).

78 Concurrent with spawning events, most species of the Sciaenidae family, known as croakers  
79 and drums, also engage in loud choruses at dusk (Fig. 1; Table 1). This vocal behaviour has  
80 been related to spawning (Mok & Gilmore, 1983; Montie et al. 2017; Vieira et al. 2019).  
81 Studies of the calling activity in sciaenids have observed a marked seasonal pattern, with a  
82 peak occurring during the spawning season (Connaughton & Taylor 1995; Tellechea & Bouvier,  
83 2011; Monzack et al. 2017). Furthermore, some field studies have revealed an association  
84 between collected eggs and fish calling activity (Mok & Gilmore, 1983, Luczkovich et al. 1999,  
85 Lowerre-Barbieri et al. 2008). Observations also report similar associations in captive fish  
86 (Connaughton & Taylor, 1996b; Montie et al. 2017; Vieira et al. 2019). For example,  
87 Connaughton and Taylor (1996b) reported a connection between calling, courtship and  
88 spawning. Since vocal activity of sciaenids can last several months in the wild, the use of  
89 passive acoustic monitoring (PAM) is a cost-effective and non-invasive tool for long-term

90 studies on the seasonal and daily reproductive behaviour of these species. Furthermore, it  
91 provides insights on geographical species density, distribution, or habitat use (e.g., Jaramillo-  
92 Legorreta et al. 2017; Monczak et al. 2017; Rowell et al. 2017).

93 Here, we aimed to study what factors drive the chorusing behaviour in a sciaenid, the meagre  
94 (*Argyrosomus regius*). The meagre is a large marine teleost with a high value for recreational  
95 and small-scale commercial fisheries and aquaculture (Quéméner et al. 2002). Adults migrate  
96 to coastal reproductive areas, such as estuaries, to spawn (Haffray et al. 2012), where they  
97 make loud choruses (Lagardère & Mariani, 2006; Vieira et al. 2021a). In captivity, peak  
98 seasonal calling activity is concurrent with spawning events and the increase in water  
99 temperature (Vieira et al. 2019). We therefore expect that temperature variations drive calling  
100 activity and, ultimately, the spawning in this species in the wild. To investigate the importance  
101 of temperature and other environmental variables in the chorusing behaviour of wild meagre,  
102 we studied its temporal patterns using underwater sound recordings from the Tagus estuary  
103 (Portugal) over four breeding seasons (2016 to 2019). Temporal patterns of chorusing  
104 behaviour was extracted using an automatic recognition methodology based on hidden  
105 Markov models. Our study shows that long-term, multi-year PAM allows to investigate  
106 changes in fish chorusing behaviour in response to temperature, providing a tool to  
107 understand the impact of climate change and extreme events on fish reproduction.

## 108 **2. MATERIAL AND METHODS**

### 109 **2.1 Data acquisition**

110 Meagre calling activity was assessed from continuous sound recordings obtained in the Tagus  
111 estuary (Air Force Base 6, Montijo, Portugal; 38°42'N, 8°58'W) from April 2016 to September  
112 2019. A hydrophone (High Tech 94 SSQ; sensitivity - 165 dB re. 1 V/ $\mu$ Pa, flat frequency  
113 response up to 6 kHz  $\pm$  1dB) was deployed from a pier and anchored approximately 20 cm

114 from the bottom. A stainless-steel rod projecting from a concrete base was used to secure the  
115 hydrophone underwater. The hydrophone's cable was attached to the rod and concrete base  
116 to minimise extraneous hydrodynamic-induced noise. Sound was recorded to a 16 channel  
117 datalogger (LGR-5325, Measurement Computing Corp, Norton, MA, USA; 4 kHz sampling rate,  
118 16 bit, +/- 1V range). Depth at the deployment location ranged between ca. 2.5 and 6 m,  
119 depending on tide. At this place, two native vocal fish species are usually detected and can be  
120 easily distinguished: the meagre and the Lusitanian toadfish (*Halobatrachus didactylus*; Pereira  
121 et al. 2020; Vieira et al. 2021a). However, since 2018 we also have detected calls from an  
122 invasive sciaenid species: the weakfish (*Cynoscion regalis*; Morais et al. 2017; Wanjala, 2021).  
123 Furthermore, since 2018, water temperature was also measured (temperature datalogger  
124 USB-501-LT, Measurement Computing Corp). Air temperature recorded by the Air Force Base  
125 weather station over the four years was also analysed as a reference. Daily mean water  
126 temperature and air temperature showed similar patterns (Fig. S1). Solar (time of sunrise and  
127 sunset, and daylength) and lunar (lunar illumination or moon phase) variables were estimated  
128 for this location using 'suncalc' and 'lunar' libraries in R (Thieurmél & Elmarhraoui, 2019;  
129 Lazaridis, 2014). Tide range was calculated using tables available online for this location  
130 (tabuademaes.com).

## 131 **2.2 Automatic recognition of meagre chorus**

132 To detect and classify the meagre choruses, we used an automatic recognition system  
133 (adapted from Vieira et al. 2019a and described in Vieira et al. 2021b). In short, multiple  
134 hidden Markov models (HMMs) were trained using a dataset of sounds for each defined class  
135 (see below), and then each model was used to classify the recordings through maximum  
136 likelihood criteria (cf. Fig. 1 in Vieira et al. 2015).  
137 Firstly, the signal waveform was divided using a predefined window duration and then the  
138 following acoustic features were calculated: cepstrum and Mel-frequency cepstral (MFC) with

139 delta, and acceleration coefficients (as described in Young et al. 2006). Delta and acceleration  
140 coefficients are conventional features used to quantify short temporal variations. We used a  
141 32 ms window with a 50 % overlap, and frequency bandwidth of 20 – 2000 Hz. A Hamming  
142 window was applied to each frame. The MFC used a 26-filter bank and only the first 12 cepstral  
143 coefficients were selected.

144 As reported in Vieira et al. (2021b), we created a 50-state model to classify and discriminate  
145 each of the following classes: (1) choruses dominated by longer calls (mostly long grunts) with  
146 high rate of overlap, forming a mostly continuous roar, (2) choruses dominated mostly by long  
147 grunts with low rate of overlap, (3) choruses dominated by 1-3 pulse calls, and (4) choruses  
148 dominated by pulses and long grunts (usually with an intermediate rate of overlap; see Fig. 2).  
149 These calls and choruses are similar to what was previously reported for meagre in captivity  
150 (Vieira et al. 2019). Furthermore, we added a 14-state model to recognise long grunts with a  
151 high signal-to-noise ratio. This recognition system was trained to identify segments of choruses  
152 because most calls overlap other calls and, in many cases, it is not possible to discriminate the  
153 beginning and the end of each call. We also added models with 5 states for modelling  
154 background noise (silence), non-biological sound patterns with high energy and short duration  
155 (e.g., abiotic sound with high energy, such as mooring-related noise and breaking waves) and  
156 also a model for modelling boat noise (in the absence of meagre choruses). Models with 14  
157 states for modelling toadfish (*H. didactylus*) boatwhistles, double-croaks and grunt trains in the  
158 absence of meagre calls were also added as toadfish sounds are also common in the recording  
159 site (Vieira et al. 2021a).

160 For each model, a subset of samples was used to train the HMMs. The transition probabilities  
161 and the elementary segment probability densities of each state were estimated with the  
162 Baum–Welch algorithm (Baum et al. 1970). Initially we assigned at least 15 sounds to each  
163 model. After several preliminary tests (using a different dataset from the one used to evaluate

164 the system), we included additional sounds, especially in the most common and intra-variable  
165 sound classes. To obtain a good identification rate and accuracy we added several toadfish  
166 calls into the dataset used to train these models. The system used had a training set with 323  
167 files with different meagre choruses including several observed variations (multistyle training)  
168 and 700 sounds for the other models (165 silence, 19 boat noise, 25 other non-biological  
169 sounds, 19 boatwhistles, 141 double-croaks and 331 toadfish grunt trains).

170 In the recognition phase, using the Viterbi algorithm (Forney, 1973), each sound was matched  
171 against the estimated HMM for each sound class.

172 Vieira et al. (2021b) evaluated the output of the meagre sounds recognition system in the  
173 Tagus Estuary by measuring the identification rate and accuracy on a subsample of ca. 180  
174 min. Due to the continuous nature of the chorus, the identification rate and accuracy to  
175 recognise choruses was evaluated considering the percentage of seconds containing signals  
176 that were correctly classified, instead of the number of detection events. As reported by Vieira  
177 et al. (2021b), the overall identification rate reached 97.4%, and the accuracy was 96.7%.  
178 Furthermore, rapid visual inspection of daily spectrograms was used to qualitatively evaluate  
179 the reliability of the recognition system. We selected periods of 24h recordings of ca. 7 days  
180 per year and manually confirmed the accuracy of chorus type occurrence detected by the  
181 recognition system.

182 For computations we used the HMM Toolkit (HTK, University of Cambridge, UK), modules  
183 written in C to create automatic recognition systems for human speech (Young et al. 2006).

### 184 **2.3 Data analysis**

185 All the computations except the HMM fitting and decoding were implemented in R (version  
186 3.5.1; R Code Team).



187 To characterise the diel patterns of the chorusing activity, the percentage of chorusing time  
188 per hour was computed from the automatic recognition system class labels. For this analysis,  
189 we discriminated choruses with long grunts (classes 1,2,4) from choruses presenting 1-3 pulse  
190 calls (classes 3,4). Additionally, to map the overall chorusing behaviour throughout the four  
191 breeding seasons, one-third octave band levels centred on 500 Hz (usually referred as third  
192 octave levels; hereinafter referred to as 500 Hz TOL) were measured and the labels of the four  
193 classes of chorusing obtained from the automatic recognition system were taken for each  
194 minute. TOL was computed and calibrated adapting the code available by Merchant et al.  
195 (2015) (FFT 1024, Hann window, 50 % overlap, averaged for each minute).

196 To quantify the changes in vocal activity throughout the reproduction season, we extracted the  
197 information on the chorus onset, chorus end and duration. To reduce the subjectivity of these  
198 chorus variables, we combined the labels of the automatic recognition system with a 20-  
199 minute moving average of one-third octave band levels centred on 500 Hz (500 Hz TOL). To  
200 calculate the onset, end and duration of choruses, we considered only chorus-labelled minutes  
201 where the 20-min averaged 500 Hz TOL was higher than 90 dB re. 1  $\mu$ Pa. This threshold was  
202 defined considering the Tagus estuary soundscape description by Vieira et al. (2021a).

203 According to that study, the 500 Hz TOL changes mostly due to the presence of meagre  
204 choruses and transient boat passages and is only consistently above 90 dB during a chorus.

205 Chorus duration was defined as the sum of the chorus labelled minutes per day. The onset/end  
206 was considered as the first/last minute where the condition holds. For this analysis we  
207 considered 24h periods starting at noon because chorusing activity usually starts at late  
208 afternoon and continues to the earlier hours of the next day.

209 To model chorusing behaviour as a response variable we considered the temperature, solar  
210 and lunar explanatory variables in a generalized additive models (GAM) framework, with an  
211 identity link for the gaussian distribution. Chorus duration, ranging from zero to 14h, was

212 selected as a good representation of vocal activity. Specifically, as explanatory variables we  
213 tested the effect of water daily mean temperature, temperature delta (minimum to maximum  
214 temperature range on a given day), moon cycle (tide range and lunar illumination or moon  
215 phase), seasonal variation in sunlight (daylength and time of sunrise and sunset) and  
216 progression of the season (Julian day). After preliminary analysis, water temperature was  
217 divided into two variables: the overall temperature seasonal trend (obtained by computing a  
218 linear trendline between March and August; see Fig. 3) and the daily temperature oscillations  
219 (obtained by subtracting the mean daily temperature from the trendline), from now on  
220 referred to as “temperature trend” and “temperature oscillation”, respectively. This analysis  
221 was only performed throughout 2018 and 2019 spawning seasons (since water temperature  
222 was only available for these years). GAM is a regression technique that can fit non-linear  
223 relationships, and it is flexible regarding the statistical distribution of the data (Hastie and  
224 Tibshirani, 1990; Wood, 2017). GAMs were chosen because preliminary analysis of the time-  
225 series indicated non-linear relationships (Hastie & Tibshirani, 1990; Wood, 2017). This additive  
226 model fits a smoothing curve through the data, in this case based on thin plate regression  
227 splines. Smooths were considered for all explanatory variables and removed only when the  
228 effective degrees of freedom (EDF) suggested a linear term. Parameters were estimated using  
229 restricted maximum likelihood (REML). When the main effect terms were significant, the  
230 interactions of the covariates were tested using tensor product interactions. These terms were  
231 only retained when they were significantly different from a zero (flat) function. This statistical  
232 analysis was conducted in R using the ‘gam’ function from ‘mgcv’ library (Wood, 2017). Model  
233 fit was evaluated through visual inspection of residual plots and diagnostic information  
234 produced using ‘gam.check’ function (Wood, 2001).

### 235 **3. RESULTS**

#### 236 **3.1 Diel pattern of the chorusing behaviour**

237 A clear diel pattern was observed in the four years (four breeding seasons) studied, with calling  
238 activity peaking near sunset (Fig. 3). We observed choruses dominated by longer calls (mostly  
239 long grunts), choruses dominated by 1-3 pulse calls, and choruses dominated by both pulses  
240 and long grunts. Choruses with the presence of short 1-3 pulse calls were observed before  
241 sunset and earlier in the day than choruses dominated by long grunts (Fig. 3). Figure 4 depicts  
242 how the diel pattern changes throughout the breeding season. Notice that the 500 Hz TOL, the  
243 labels from the automatic recognition system, and the inferred chorus onset and end, all show  
244 similar variations (cf. Fig. 4B, C and D). Choruses with long grunts were the most common.  
245 Figure 4C shows that choruses with short 1-3 pulse calls were not detected every day, mostly  
246 occurring in days with longer choruses (Fig. 4C).

247 Figure 5 shows how the time of onset and end of the chorus varied according to the duration  
248 of the chorus. Depending on the day, different chorusing timings were observed. However, the  
249 chorus always started after noon (from 12:00h to 21:00h) and usually ended within the period  
250 from 17:00h (afternoon) to 5:00h (end of the night). On average the chorus had its onset at  
251 17:38 h  $\pm$  1.6h (mean  $\pm$  SD) and ended at 23:16 h  $\pm$  2.1h. Significant correlations of chorus  
252 onset and end with chorus duration were observed ( $r^2$  of 0.6 and 0.5, respectively). Chorus  
253 duration was on average 3.1  $\pm$  3.1h but could last up to ca. 14h. As expected, longer choruses  
254 started earlier and ended later. Interestingly, chorusing activity was often centred around the  
255 sunset, suggesting that the onset was not directly triggered by the sunset.

### 256 **3.2 Phenology**

257 Acoustic activity presented variations across years. The first day with chorusing activity  
258 occurred on February 26, March 28, and March 3 in 2017, 2018, and 2019, respectively (Fig. 4).  
259 At these dates, the mean air temperature was 13.1, 13.1 and 12.7 °C, respectively. Consistent  
260 with later years, in 2016 the chorusing behaviour started before April (before the deployment  
261 of the acoustic recording device). Spring 2018 had lower temperatures than in 2017 and 2019.

262 It is worth noticing that in the study period of four years we observed the absence of chorusing  
263 activity after abrupt decreases in temperature. For example, in the beginning of the 2017  
264 breeding season choruses were not detected when air temperatures dropped below 12 °C.  
265 Even later in the breeding season a disruption is possible if a sudden drop in temperature  
266 occurs. For example, in May 2016, for nine consecutive days we did not detect chorusing  
267 activity after a decrease from 21 to 14 °C in air temperature (see Fig. 4).

268 The end of the reproductive season was characterized by intermittent chorusing, i.e. days with  
269 chorus alternated with days where chorusing activity was absent. The last day with chorus  
270 occurred on August 14, July 4, August 20 and July 28 in 2016, 2017, 2018, and 2019,  
271 respectively. At these days, the mean air temperature was 24.6, 21.9, 21.4 and 22.1 °C,  
272 respectively. The earlier halt of chorusing activity in 2017 is in line with a warmer June (Fig. 4).

### 273 **3.3 Seasonal variations**

274 The GAM revealed that chorus duration (representing the overall calling activity) was only  
275 significantly affected by water temperature-related variables (Fig. 6; Table 2; see graphical  
276 representation of the parameters tested in Fig. S2). A non-linear relation of chorus duration  
277 with temperature trend was present ( $p < 0.001$ ). Chorus duration was longer when the water  
278 temperature was close to 18 °C. In days with lower or higher water temperatures chorusing  
279 started later, suggesting the existence of an optimal temperature range for the occurrence of  
280 this behaviour. During 2018 and 2019, choruses were only present in days with mean water  
281 temperature within 15 to 25 °C and air temperature from 12 to 32 °C. Calling activity was also  
282 affected by temperature oscillations on a shorter timescale (Fig. 6; Table 2). An increase in  
283 temperature oscillation was usually followed by an increase in chorus duration (Fig. 6).  
284 However, the interaction between these two variables shows that, when the temperature  
285 trend is over ca. 20 °C, the inverse can happen. A model considering only these two  
286 temperature variables can explain 70% of the chorus duration variance. Tide range and

287 moonlight were removed from the model, since no significant effects were attributed to these  
288 variables. It is worth noticing, though, that water temperature is usually affected by tide.  
289 Similarly, Julian day was excluded from the model. Notice, however, that temperature trend  
290 increases throughout the summer. Variations on daylength and the times of sunrise and  
291 sunset, were not significant either.

## 292 **4. DISCUSSION**

293 The present study shows how wild meagre chorusing behaviour recorded in the Tagus estuary  
294 (Portugal) changes throughout the day and throughout the reproductive season. Temperature  
295 was the major factor affecting the chorusing activity. The first choruses were detected earlier  
296 in the year when air temperature increased above 13 °C and underwater temperature was  
297 above ca. 15 °C. Choruses reached a peak in duration around 18 °C, which appeared to be the  
298 species optimal water temperature for chorusing and possibly spawning. The high temperature  
299 during the summer months appeared to cue the end of the reproductive season. Temperature  
300 oscillations were also responsible for changes in the daily calling activity, in some cases causing  
301 the inhibition of chorusing behaviour.

### 302 **4.1 Identifying meagre sounds in nature**

303 It is always a challenge to confirm the identity of vocal species using PAM in the wild. In  
304 captivity, we have associated meagre's acoustic activity with spawning and observed that the  
305 production of 1-3 pulses calls and long grunts also peaked around sunset time (Vieira et al.  
306 2019). The similarity of field and captivity recorded sounds support the correct identification of  
307 sounds in nature. As aforementioned, in the area where the present work was conducted, only  
308 the calls of the Lusitanian toadfish were previously reported (Vieira et al. 2021a). Toadfish calls  
309 are very different from the meagre's precluding misidentification of the calls (for more details  
310 see Material and Methods). Recently, another sciaenid was found in the Tagus estuary, the

311 invasive weakfish (Morais et al. 2017). In 2020, choruses of the weakfish were detected in  
312 other sites within the Tagus estuary. This species is known to produce grunts (Connaughton  
313 and Taylor 1995) and can be distinguished from the ones produced by the meagre for having  
314 longer pulse periods (weakfish:  $47 \pm 7$  ms, meagre:  $23 \pm 9$  ms; Wanjala 2021). During this  
315 study, some vocalisations of this invasive species were detected since 2018. The temporal and  
316 geographical distribution of this invasive species in Tagus estuary remains to be investigated.

#### 317 **4.2 Diel pattern of the chorusing behaviour**

318 In sciaenids, spawning exhibits diel periodicity (Table 1). Indeed, in several species of this and  
319 other fish families, the final oocyte maturation (FOM) appears to follow a diel pattern. For  
320 example, in the spotted seatrout the plasma levels of the primary maturation-inducing steroid  
321 peak ca. 14h before ovulation, the FOM is mostly completed within 8 to 10h and by dusk  
322 ovulation and spawning occurs (Brown-Peterson et al. 1988; Crabtree & Adams, 1998). In  
323 *Pagrus major*, the plasma levels of estradiol-17 b in females peak at 07:00h and spawning  
324 takes place between 18:00h and 19:00h (Matsuyama et al. 1988). Furthermore, Meseguer et  
325 al. (2008) showed how spawning onset in *Sparus aurata* shifted according to the artificially set  
326 light-dark cycles. This highlights the relevance of sunlight in regulating daily reproductive  
327 rhythms in several species. In the meagre and in other sciaenids (Table 1), the chorusing  
328 behaviour mirrors these diel patterns. The chorusing and mass spawning behaviours occurring  
329 at dusk or dawn are considered a possible adaptation to limit the predation on pelagic eggs  
330 (Hobson & Chess, 1978; Lobel 1978; Ferraro, 1980; Holt et al.1985). In our study, although the  
331 timing for the onset and ending of the chorus could vary, chorusing activity was usually  
332 centred at sunset, suggesting that it could coincide with the spawning peak on that day.  
333 Further studies should measure the daily density of eggs released in the wild to quantify its  
334 relationship with chorus duration and the centre of the chorusing activity.

335 On some days, a series of calls made of 1-3 pulses could last for more than one hour and  
336 preceded the choruses of long grunts. This vocalization pattern was also observed in captivity  
337 during breeding season (Vieira et al. 2019), suggesting it could have an important function in  
338 spawning. No similar acoustic pattern was observed in other sciaenids to support this  
339 hypothesis, but other fish species can vary the pulse emission pattern with proximity to  
340 spawning. For example, the haddock (Gadidae) emits a series of knocks at the start of  
341 courtship but, as courtship progresses, pulse repetition rate increases until the pulses are  
342 emitted so close together that they are heard as hums during the spawning embrace (Hawkins  
343 & Amorim 2000). To understand more about the function of the short 1-3 pulse calls a  
344 behavioural study measuring visual and acoustic cues during spawning events must be carried  
345 out.

#### 346 **4.3 Phenology**

347 Many sciaenids have been reported to produce choruses during the spawning season (Table 1),  
348 suggesting that passive acoustic monitoring may be used to monitor the reproductive  
349 phenology of these species (Montie et al. 2015; Monzak et al. 2020). In the Tagus estuary we  
350 detected the presence of meagre choruses from February to August, with some variations  
351 possibly related to differences in temperature. Indeed, higher temperatures in spring triggered  
352 earlier chorusing activity. Similar changes in the beginning of the overall chorusing activity  
353 were observed in a North-American estuary where several sciaenid species occur (Monczak et  
354 al. 2020). On the other hand, when summer temperatures rise above some point, it signals the  
355 end of the reproductive season. A major role of temperature in determining the end of the  
356 spawning season has been previously hypothesised (e.g. Marsh 1980; Hubbs 1985). This  
357 hypothesis is further supported by observations where higher temperatures stopped  
358 gametogenesis in captive meagre (Mylonas et al. 2013; Soares et al. 2015).

359 The overall acoustic seasonal pattern of the meagre is also consistent with other studies. For  
360 example, the gonadosomatic index of this species was found to increase in February/March  
361 and to decrease in July (Fig. 1; wild fish: Abou Shabana et al. 2012; captivity: Mar Gil et al.  
362 2013). In accordance with our observations in the wild, the monthly acoustic activity in captive  
363 meagre also started to increase during February/March, and this increase was concurrent with  
364 changes in water temperature and accompanied by a rise in the number of spawning events  
365 (Vieira et al. 2019). In fact, temperature has been suggested as an important seasonal factor  
366 triggering the spawning season (Lange & Greve 1997) and an increase in vocal activity of fishes  
367 (Montie et al. 2015). Globally, the seasonal temperature variations may determine the  
368 regeneration and development of the gonads (Mello et al. 2021) and can also trigger the  
369 development of the sonic muscles (Connaughton & Taylor 1994). Together, water temperature  
370 seems to play a fundamental role in different aspects of reproduction which makes fish highly  
371 susceptible to gradual temperature rise or to sudden temperature oscillations.

372 Why is the end of the reproductive season characterised by intermittent calling activity? At the  
373 end of the spawning season there were several days without choruses, even at high  
374 temperatures, followed by a few days with high calling activity. This behaviour might be the  
375 consequence of the “desperado effect” (Grafen 1987). High-quality males are expected to  
376 reproduce when the conditions are optimal, since this would increase the number of eggs  
377 released by females and the survival rate of embryos. However, low-quality individuals may  
378 have less opportunities to reproduce during this period due to high mating competition (Uusi-  
379 Heikkilä 2020). Consequently, they may try to reproduce even when environmental conditions  
380 are not the best, as it may be better to reproduce at suboptimal conditions than not to  
381 reproduce at all. Furthermore, meagre is a batch spawner species, and some females may still  
382 be able to release a last batch (cf. Fig. 1). Note that according to the detailed description of the  
383 spawning behaviour in another sciaenid by Connaughton & Taylor (1996b), a pair usually



384 swims away from the group to the surface for gamete release. Although meagre's spawning  
385 behaviour is not known yet, if similar to the weakfish, the existence of paired spawning would  
386 further support our hypothesis for the intermittent calling behaviour observed at the end of  
387 the breeding season.

#### 388 **4.4 Seasonal variations**

389 The observed variation in chorus duration throughout the spawning season appeared to be  
390 related to changes in water temperature and not photoperiod. Our data suggest that  
391 chorusing, and consequently spawning, have an optimal water temperature at about 18 °C  
392 since at this temperature longer chorus durations were observed. However, the relationship  
393 between chorusing or spawning and temperature in the wild is still not well documented. It is  
394 known that temperature affects fish metabolic rate (Clarke & Johnston 1999) and the latency  
395 of the hormonal induction of spawning (Drori et al. 1994). It is possible that temperature cues  
396 activate the FOM and, consequently, an increase of the associated behaviours such as  
397 chorusing. Male reproductive cycles are less well known (Prista et al. 2014), although male fish  
398 usually only have a short period to fertilise the eggs. Note that in the meagre both males and  
399 females can produce sounds (Pereira et al. 2020), but detailed information on the "spawning  
400 ritual" is still lacking. On the other hand, it is known that fish might reduce vocal activity in  
401 response to the lower and higher temperatures that would limit spawning activity (Amorim et  
402 al. 2006; Parsons et al. 2016). Furthermore, several sciaenids sharing the same estuary can  
403 exhibit chorusing behaviour at different periods characterized by different temperatures  
404 (Montie et al. 2015), possibly according to their optimal spawning requirements.

405 Neither tidal range nor moon phase appeared to significantly explain the variations observed  
406 in the meagre chorusing behaviour. Previous studies have reported the influence of lunar light  
407 in fish spawning. Aalbers (2008), for example, indicates that in the white seabass, spawning  
408 occurred mostly for four days from the beginning of the new moon. Also, in the *Siganus*

409 *doliatus* (Siganidae), a pattern of gonadal development and spawning related to the lunar light  
410 was observed (Takemura et al. 2004; Park et al. 2006). Furthermore, several variations in  
411 acoustic activity throughout the spawning season have also been linked to the lunar cycle in  
412 different species. For example, Gilmore (1994) and Lowerre-Barbieri et al. (2011b) observed  
413 that calling in the spotted seatrout occurred more often around full moon. However, Monczak  
414 et al. (2017) observed longer choruses of the same species on the first and third quarter  
415 phases, suggesting that fish sing more in neap tides, when the tidal range is smaller, and the  
416 currents are slower. In the Tagus estuary, Lusitanian toadfish nesting in the intertidal zone also  
417 showed an effect of low tides on the vocal activity, but no effects were observed in deeper  
418 areas (Vieira et al. 2021c). We believe that in most studies on estuarine areas the effect  
419 reported might be coincidental or just due to restrictions of the water depth in intertidal areas  
420 (as observed in Vieira et al. 2021c). To discriminate effects due to lunar light, tides and  
421 currents from changes related to other environmental variables may require studying locations  
422 with different depths and similarly long or even longer data sets.

423 Our data suggests that intra-annual temperature oscillations may affect fish vocal mating  
424 behaviour, and ultimately their reproductive success. Our results showed that negative  
425 temperature oscillations in spring, and positive in summer, can strongly affect the chorusing  
426 behaviour, and even halt chorusing behaviour for several days. It is not clear what can be the  
427 possible consequences of the observed temperature oscillations for fish populations, but an  
428 increase in local weather instability may likely negatively affect reproductive outcome. This is  
429 especially relevant in the current climate change context.

## 430 **5. CONCLUSIONS**

431 Here we characterised wild meagre chorusing behaviour over four spawning seasons using  
432 PAM. Meagre, as other sciaenids, presented a well-defined diel pattern of vocal activity with  
433 choruses often centred at sunset. A chorus dominated by long grunts was the most common,

434 but a chorus of short calls with 1-3 pulses can also occur usually earlier in the day. Importantly,  
435 we found that water temperature plays an important role in initiating and regulating both daily  
436 and seasonal patterns of chorusing activity, suggesting that even small temperature changes  
437 might affect fish reproduction. Vocal activity peaked around 18 °C, which appeared to be the  
438 species optimal water temperature for chorusing and possibly spawning. Moreover, changes in  
439 vocalisation (and likely reproductive) phenology and disruptions due to temperature  
440 oscillations were also observed. In conclusion, we found that temperature plays an important  
441 role in triggering the onset of the seasonal activity and, in general, regulating chorusing  
442 behaviour. Together, our data suggests that even small temperature changes might affect  
443 reproduction in meagre and likely other fish species, making fishes susceptible to global  
444 warming and extreme temperature events. Using vocal fishes as models, we suggest that long  
445 term conservation and management plans can take advantage of PAM associated with water  
446 temperature recordings to make predictions about how shifts in temperature due to climate  
447 change may affect fish reproduction, namely the distribution of breeding fish and the timing  
448 and incidence of spawning.

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**Table 1.** Sciaenid spawning and chorus daily patterns during the mating season.

Species	Spawning timing*	Chorus timing*	Location	Reference
Atlantic croaker ( <i>Micropogonias undulatus</i> )	1 to 2h	After sunset	USA	Holt et al. (1985); Gannon (2007)
Black drum ( <i>Pogonias cromis</i> )	1 to 2h	-1 to 1h	USA	Holt et al. (1985); Locascio and Mann (2011) Monczak et al. (2017)
Brown meagre ( <i>Sciaena umbra</i> )		0 to 2h	France	Parmentier et al. (2017)
Meagre ( <i>Argyrosomus regius</i> )		-5 to 7h	Portugal	Vieira et al. (2019) Current study
Red drum ( <i>Sciaenops ocellatus</i> )	0 to 3h	0 to 5h	USA	Holt et al. (1985); Luczkovich et al. (2008) Monczak et al. (2017)
Sand seatrout ( <i>Cynoscion arenarius</i> )	1 to 2h	-2 to 6h	USA	Holt et al. (1985); Locascio and Mann (2008)
Silver perch ( <i>Bairdiella chrysoura</i> )	-1.5 to 2h	0.5 to 9h	USA	Holt et al. (1985); Luczkovich et al. (2008) Monczak et al. (2017)
Silver croaker ( <i>Pennahia argentata</i> )	-0.5 to 1.5h		Japan	Yamaguchi et al (2006)
Southern kingfish ( <i>Menticirrhus americanus</i> )	-3 to 0h		USA	Holt et al. (1985)
Spotted seatrout ( <i>Cynoscion nebulosus</i> )	-1.5 to 2h	-1 to 4h	USA	Holt et al. (1985); Montie et al. (2017) Luczkovich et al. (2008) Monczak et al. (2017)
Weakfish ( <i>Cynoscion regalis</i> )		0 to 3h	USA	Connaughton and Taylor (1995) Luczkovich et al. (2008)
White seabass ( <i>Atractoscion nobilis</i> )	-2 to 3h	-2 to 3h	USA	Aalbers (2008)
Whitemouth Croaker ( <i>Micropogonias furnieri</i> )		-13 to -11h and -4 to 3h	Uruguay	Tellechea and Bouvier (2011)

\*approximate range relative to sunset

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Table 2. Results of generalized additive model (GAM) selected for interpretation assessing the parameters that explain the daily variation of meagre chorus duration throughout 2018 and 2019 spawning seasons.

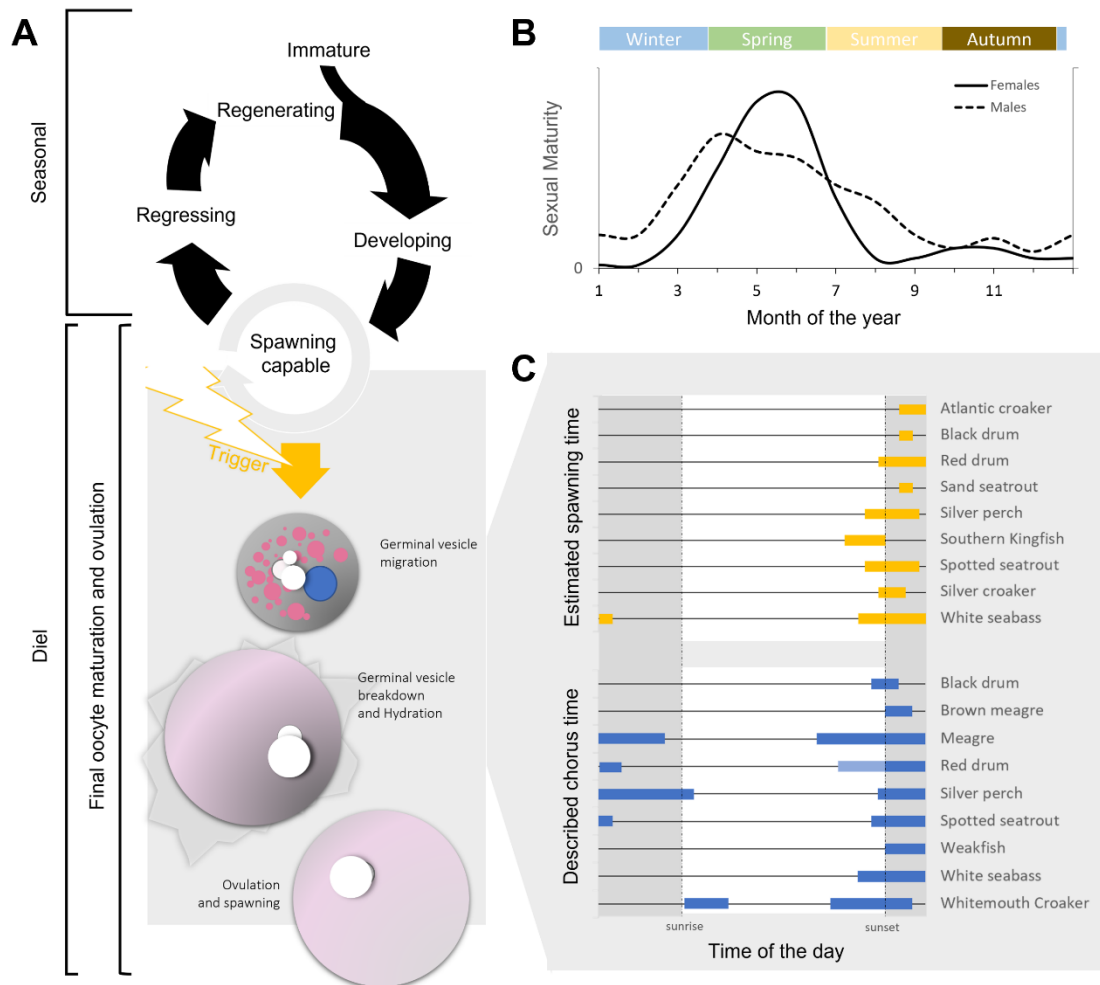
n		293		
r <sup>2</sup>		0.68		
Deviance explained		70%		
<b>Parametric coefficients (linear terms)</b>				
	<b>Estimate</b>	<b>Std. error</b>	<b>t-value</b>	<b>p-value</b>
Temperature oscillation (°C)	1.79	0.33	5.49	< <b>0.001</b>
<b>Predictor spline (smooth terms)</b>				
	<b>EDF</b>	<b>Ref-df</b>	<b>F</b>	<b>p-value</b>
Temperature trend (°C)	9.38	15	23.36	< <b>0.001</b>
<b>Random effects</b>				
Year	0.99	1	72.48	< <b>0.001</b>
<b>Tensor product interactions</b>				
Temperature oscillation × trend	7.30	16	4.34	< <b>0.001</b>

Number of days with chorus (n), fit of the model (r<sup>2</sup>), percentage of deviance explained, estimated degrees of freedom (EDF); and p-values are represented. In bold p-value lower than 0.001. Tide range (m), Lunar illumination, Daylength (h), Sunset (h), Sunrise (h), and Julian day were main effects tested and removed from the model.

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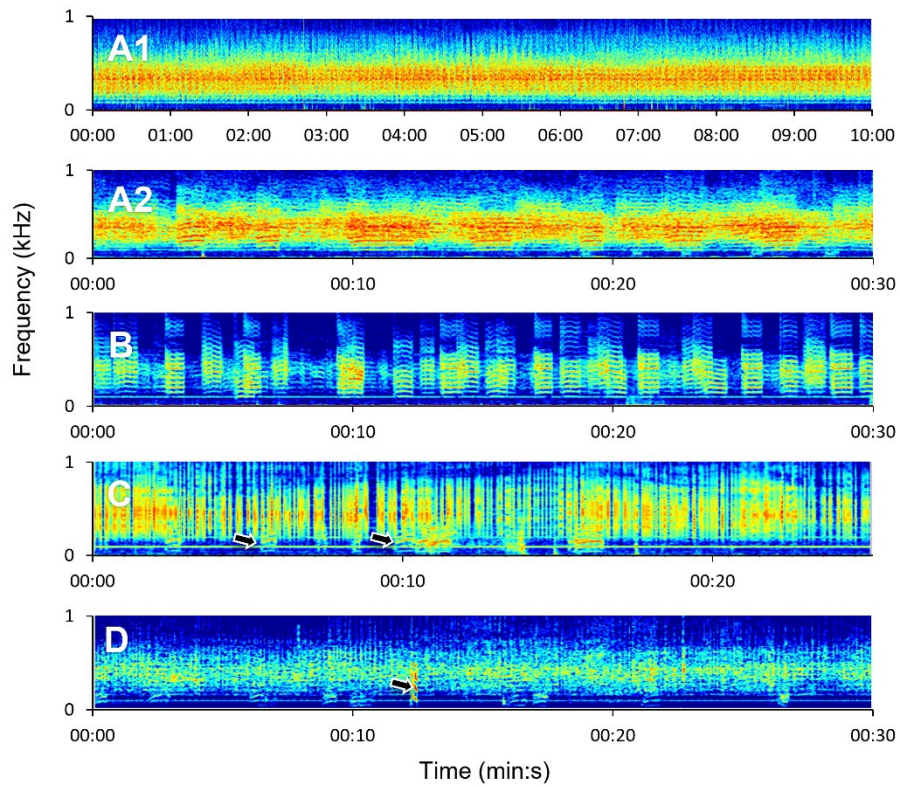




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711 Fig. 1. Sciaenids reproductive cycle: seasonal gonad development and diel final oocyte  
 712 maturation. A) Sciaenids produce pelagic eggs that are released in batches over a spawning  
 713 season with only a portion of the oocytes being released in each spawning event. The final  
 714 oocyte maturation usually occurs in less than 24h. Several environmental and social  
 715 parameters might be responsible for triggering spawning and the quantity of eggs released  
 716 (adapted from Lowerre-Barbieri et al. 2011a and Costa, 2015). B) In the north hemisphere,  
 717 males and females are capable of spawning mostly during spring (adapted from Mar Gil et al.  
 718 2013). C) Studied sciaenids usually spawn at dusk, concurrent with their typical chorusing  
 719 behaviour (see Table 1).

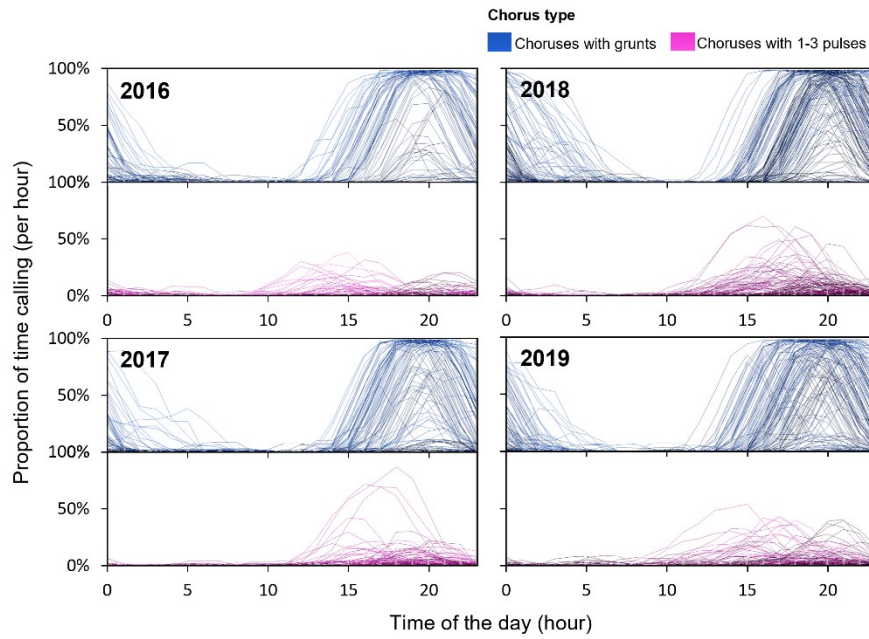
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722 Fig. 2. Spectrograms of choruses produced by meagre. (A1, A2) choruses dominated by long  
 723 grunts with high rate of overlap, forming a mostly continuous roar (class 1), (B) choruses  
 724 dominated by long calls (mostly intermediate and long grunts) with lower rate of overlap (class  
 725 2), (C) choruses dominated by pulses (calls with 1 to 3 pulses; class 3) and (D) choruses  
 726 dominated by pulses and long grunts (class 4). In (C) and (D) lower frequency calls produced by  
 727 Lusitanian toadfish males are also visible (black arrows).

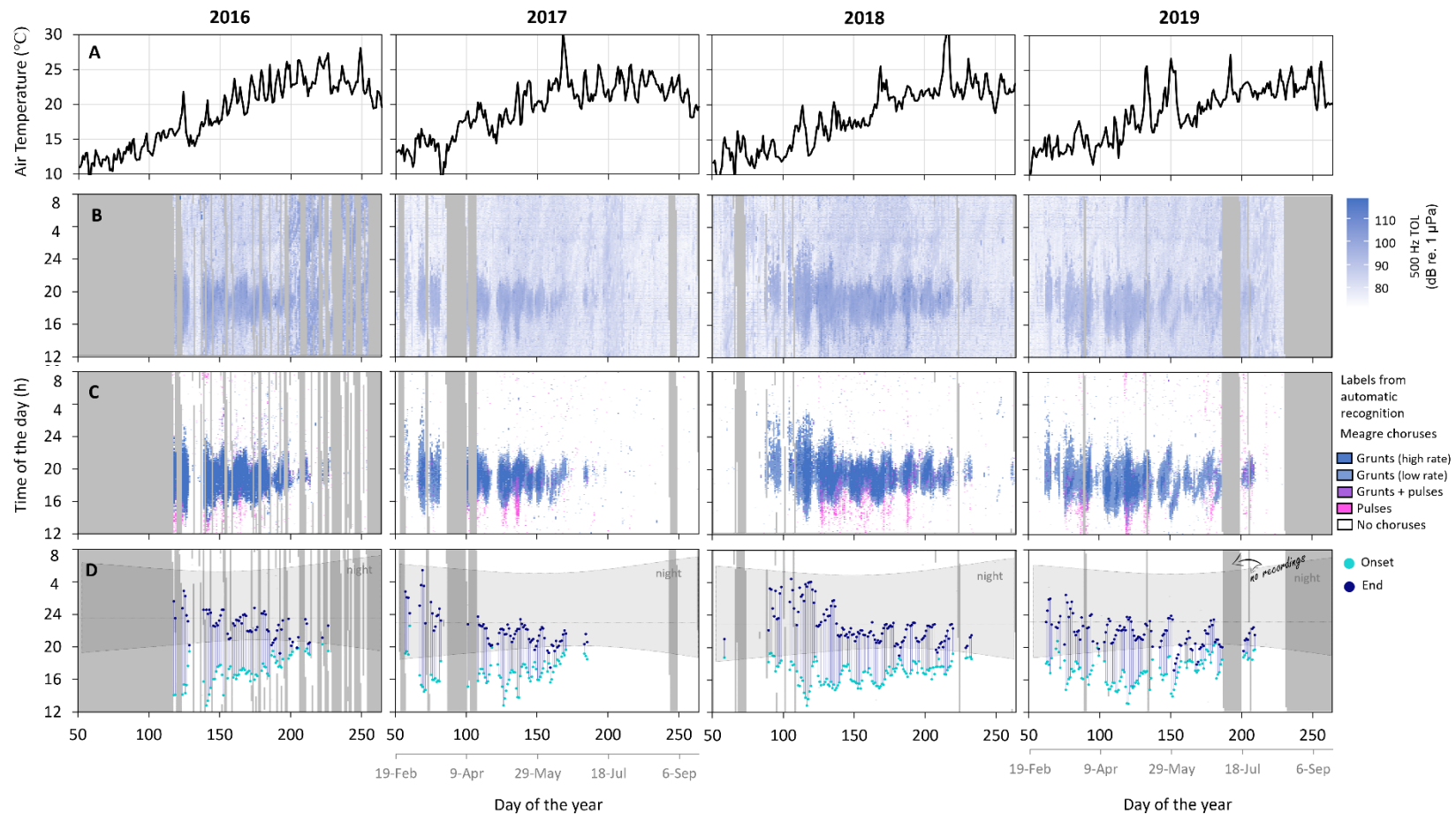
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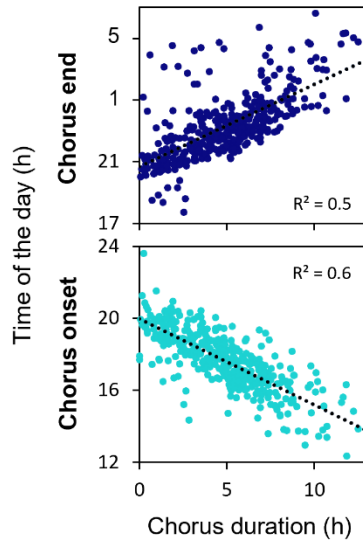
730 Fig. 3. Diel variations of calling activity in 2016, 2017, 2018 and 2019. The percentages of time  
 731 with choruses were obtained from the automatic recognition system. For each year the  
 732 proportion of time with choruses that includes long grunts (blue) and/or 1-3 pulses calls (pink)  
 733 are represented.

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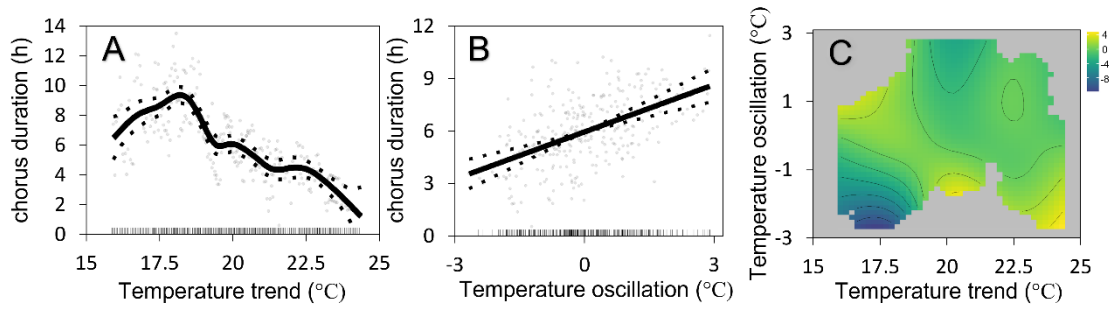
736 Fig. 4. Chorusing behaviour throughout the spawning seasons of 2016, 2017, 2018 and 2019. (A) Daily air mean temperature at the recording site; (B) Daily  
 737 patterns of third octave band levels (TOL) centred at 500 Hz driven by meagre choruses; (C) Output of the automatic recognition system showing the  
 738 following meagre choruses: Grunts (high rate) – class 1, Grunts (low rate) – class 2, Grunts + pulses – class 4 and Pulses – class 3; (D) Onset and end of each  
 739 daily chorus combining the output of recognition system and the 500 Hz TOL. Note that the vertical axis goes from 12 pm (noon) to 8 am to encompass the  
 740 choruses that usually begins during the afternoon and ends at night. Vertical gray regions in panels B - D represent days with no records.



741

742 Fig. 5. Correlation between chorus onset and end with chorus duration. Choruses usually begin  
 743 during the afternoon and end at night. Notice that usually longer choruses start earlier and  
 744 end later at night, remaining mostly centred at sunset.

745



746

747 Fig. 6. The effect of predicted drivers on daily chorus duration. Temperature trend plot (A)  
 748 representing the GAM partial effects splines, while temperature oscillation (B) was assumed as  
 749 linear (see methods). Functions are presented as solid lines, dashed lines denote confidence  
 750 interval, and dots indicate the partial residuals. Marks along the x-axis represent the  
 751 distribution of the data. The effect of the interaction between the two variables is also  
 752 represented as a heatmap plot (C).

753