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1 Research paper

2 **Linking *Epinephelus marginatus*' courtship calls and observed behaviours in**
3 **the wild offers new insights into its reproduction and conservation**

4

5 Elena Desiderà^{1,2*}, Carlotta Mazzoldi^{2,3}, Augusto Navone⁴, Rémi Blandin⁵, Antonio Calò⁶, Cédric
6 Gervaise⁷, Pieraugusto Panzalis⁴, Egidio Trainito¹, Paolo Guidetti^{1,8,9}, and Lucia Di Iorio^{7,10}

7

8 ¹ Department of Integrative Marine Ecology (EMI), Genoa Marine Centre (GMC), Stazione Zoologica Anton
9 Dohrn–National Institute of Marine Biology, Ecology and Biotechnology, Villa del Principe, Piazza del
10 Principe 4, 16126 Genoa, Italy

11 ² Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy

12 ³ CoNISMa, Interuniversity National Consortium for Marine Sciences, Piazzale Flaminio 9, 00196 Rome,
13 Italy

14 ⁴ Marine Protected Area of Tavolara-Punta Coda Cavallo, Via Dante 1, 07026 Olbia, Italy

15 ⁵ Institute of Acoustics and Speech Communication, TU Dresden, 01062 Dresden, Germany

16 ⁶ Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Via Archirafi 20-22, 90123,
17 Palermo, Italy

18 ⁷ CHORUS Institute, Phelma Minatec, 3 Parvis Louis Néel, 38016 Grenoble, France

19 ⁸ National Research Council, Institute for the Study of Anthropic Impact and Sustainability in the Marine
20 Environment (CNR-IAS), Via de Marini 6, 16149 Genoa, Italy

21 ⁹ NBFC, National Biodiversity Future Center, Palermo 90133, Italy

22 ¹⁰ UMR 5110 CEFREM, CNRS, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan, France

23

24 * Corresponding author, at: Department of Integrative Marine Ecology (EMI), Genoa Marine Centre (GMC),
25 Stazione Zoologica Anton Dohrn–National Institute of Marine Biology, Ecology and Biotechnology, Villa del
26 Principe, Piazza del Principe 4, 16126 Genoa, Italy.

27 E-mail address: elena.desidera@szn.it

28 **ABSTRACT**

29 Groupers are marine fishes particularly vulnerable to overexploitation chiefly because
30 of their reproduction-related traits. The dusky grouper *Epinephelus marginatus* is
31 classified as 'Endangered' in the Mediterranean Sea, where protection measures have
32 proved critical for allowing population recovery and persistence of the species.
33 However, knowledge gaps remain regarding its reproductive behaviours and spawning
34 sites. As other grouper species, the dusky grouper is known to produce courtship-
35 associated sounds, which were validated in captivity, but no study has ever
36 established a link between sound production and visual behaviours recorded in the
37 wild. This study aims to characterise, both visually and acoustically, the behavioural
38 patterns of wild dusky grouper and to link visual courtship behaviours to the
39 associated sound production. The study was conducted over two consecutive
40 summers (2017 and 2018) using visual observations and passive acoustic monitoring
41 at two presumptive spawning sites within a Mediterranean Marine Protected Area in
42 the North-Western Mediterranean Sea. Results showed that courtship calls were
43 mostly recorded during evening hours at both study sites and were significantly
44 associated with visual reproductive behaviours. Results also indicated a temporal
45 partitioning in the species acoustic activity suggesting different behaviours occurring
46 at different times of the day. These findings have important implications for identifying
47 and monitoring dusky grouper spawning sites using passive acoustic methods,
48 therefore providing valuable information for the development and implementation of
49 effective conservation measures.

50

51 **Keywords:** Dusky grouper, sound production, passive acoustics, spawning, MPA,
52 Mediterranean Sea.

54 1. INTRODUCTION

55 The increasing global demand for seafood is driving the overexploitation of many
 56 marine fish stocks (FAO, 2020), with some species being more vulnerable than others
 57 due to their life history and behavioural traits (Jennings et al., 1998; Reynolds et al.,
 58 2005). In particular, reproductive behavioural traits are crucial determinants of species
 59 susceptibility to overfishing and their recovery capacity from exploitation (Biggs et al.,
 60 2021). Species with large size, long lifespan, late sexual maturity, sequential
 61 hermaphroditism and aggregating spawning behaviour, such as groupers, are at high
 62 risk (Erisman et al., 2013; Rowe and Hutchings, 2003). Groupers (Epinephelidae, Smith
 63 and Craig, 2007) are ecologically and economically valuable fishes known to mate in
 64 aggregations of several tens up to thousands of individuals occurring at predictable
 65 times and places (Prato et al., 2013; Sadovy de Mitcheson and Colin, 2012; Sala et al.,
 66 2001). Such aggregating behaviour makes them highly susceptible to overfishing,
 67 leading to a global population decline of over 80% (Sadovy de Mitcheson et al., 2020).
 68 Identifying and characterizing grouper spawning aggregations (i.e., in terms of fish
 69 densities, sizes, sex-ratio, and timing of spawning) is crucial for the proper
 70 conservation of these species (Sadovy de Mitcheson, 2016).

71 Directly identifying spawning sites in the wild is, however, challenging due to the
 72 significant effort required to monitor large areas over long periods and the elusive
 73 behaviour of grouper species toward divers. In this regard, passive acoustic
 74 monitoring (PAM) can be coupled with observational methods to non-intrusively assess
 75 temporal patterns of reproductive activity and help identify reproductive sites (Schärer
 76 et al., 2014, 2012a, 2012b).

77 Groupers are, in fact, known to produce sounds, some of which have been associated
 78 with visual courtship behaviours (Bertucci et al., 2015; Koenig et al., 2016; Rowell et
 79 al., 2019). Courtship calls are species-specific sounds emitted during courtship activity
 80 and can be used to spatially and temporally localise the occurrence of spawning
 81 aggregations (Locascio and Burton, 2015; Mann et al., 2010; Wall et al., 2014). PAM
 82 can be particularly valuable when monitoring reproductive behaviours that occur at
 83 multiple spawning sites simultaneously, in remote areas or where diving conditions
 84 are constrained (e.g., deep water, strong currents) or the visibility is limited (e.g.,
 85 turbid water, at dusk or nighttime).

86 The widespread and systematic implementation of PAM for spawning site
 87 identification, however, relies on the accurate detection of courtship calls in acoustic
 88 recordings (Ibrahim et al., 2018), which requires a field-verified sound-behaviour
 89 pairing. In situ surveys combining direct behavioural observations with passive
 90 acoustic recordings are thus critical to link species-specific visual courtship behaviours
 91 to their associated calls. However, such surveys have been limited in number, greatly
 92 limiting the reliability of PAM for spawning site detection (Colin, 1990; Mann et al.,
 93 2010; Rowell et al., 2019). This is especially true in the Mediterranean Sea, where no
 94 studies have yet linked wild grouper behaviours with concurrent sound production,
 95 thereby preventing the use of PAM for identifying spawning aggregations and
 96 ultimately hindering the implementation or reinforcement of protection measures at
 97 critical spawning sites.

98 The dusky grouper *Epinephelus marginatus* is an iconic and endangered
 99 Mediterranean grouper species that has received special conservation attention
 100 through time, being protected under the Barcelona and the Bern Conventions (Pollard

101 et al., 2018). Effective conservation actions include restrictions or bans on fishing,
102 mainly achieved through well-enforced Marine Protected Areas (MPAs), especially
103 where fishing is prohibited (i.e., fully protected areas; (Giakoumi et al., 2017). Despite
104 its endangered status, knowledge about its biology, particularly reproduction, is
105 limited (Condini et al., 2017).

106 Our study aimed to characterise the behavioural patterns of wild dusky grouper, using
107 both visual and acoustic approaches. The specific aims included: 1) characterising the
108 species' sound production through PAM, 2) visually documenting behaviours and
109 associated colour patterns during the reproductive season, and 3) validating the link
110 between courtship calls and visual courtship behaviours at presumptive spawning
111 sites within a Mediterranean MPA. Ultimately, our study contributes to the
112 identification of spawning sites and supports the implementation of effective
113 protection measures therein.

114

115 **2. MATERIALS AND METHODS**

116 **2.1. Study species**

117 The dusky grouper forms spawning aggregations of tens of individuals in the
118 Mediterranean Sea during summer (June-September) (Hereu et al., 2006; Zabala et al.,
119 1997a). However, to our knowledge, evidence of these aggregations has only been
120 collected in six locations: the Medes Islands, Cerbère-Banyuls, Port-Cros, Lavezzi
121 Islands, La Revellata and Lampedusa Island (Culioli and Quignard, 1999; Hereu et al.,
122 2006; Louisy and Culioli, 1999; Marinaro et al., 2005; Marino et al., 2001; Pelaprat,
123 1999; Zabala et al., 1997a). During the spawning season, dusky grouper display
124 distinctive colour patterns indicating their reproductive conditions (Zabala et al.,
125 1997b). Males exhibit a silver streaked colour pattern during courtship and territorial
126 behaviours (Hereu et al., 2006; Zabala et al., 1997a, 1997b). Males are also known to
127 emit two main types of sounds: single and serial low-frequency pulses (< 200 Hz),
128 previously defined as “booms”, both validated in captivity with the latter associated
129 with courtship displays (Bertucci et al., 2015). Additionally, a low-frequency down-
130 sweeping sound (< 200 Hz), previously defined as “growl”, was recorded in the wild at
131 two known spawning sites and attributed to the dusky grouper (Bertucci et al., 2015).

132

133 **2.2. Study sites**

134 The study took place in the Tavolara-Punta Coda Cavallo Marine Protected Area
135 (hereafter TPCC MPA), located in the Western Mediterranean Sea (Sardinia, Italy). It
136 was established in 1997 but became effective around 2003-2004. The TPCC MPA is
137 divided into three zones with different protection levels (Fig. 1).

138 As part of a larger study, two unprotected and two protected sites were identified as
139 potential dusky grouper spawning sites based on local ecological knowledge and
140 scientific literature (Desiderà, 2020). Underwater Visual Census (UVC) data collected
141 at the four sites indicated that only the protected ones hosted significant grouper
142 densities, suggesting the occurrence of spawning aggregations (Desiderà, 2020).
143 Therefore, the present work was conducted at these two sites, namely “Secche Papa”
144 (SP) and “Molarotto” (MOL) (Fig. 1).

145 SP features two adjacent series of limestone pinnacles where diving is permitted while
146 fishing is forbidden. MOL is a granitic rocky outcrop located in the fully protected zone
147 of the TPCC MPA.

148

149 **2.3. Acoustic data collection**

150 The audio data were recorded from July 5th to July 31st, 2017, and July 18th to
151 September 23rd, 2018. Recorders were moored at depths of 25-40 m using sandbags,
152 with hydrophones situated 1.5 m above the bottom. One hydrophone was deployed at
153 MOL, while two to three hydrophones were used at SP to account for the influence of
154 bottom topography on sound propagation and signal detection. Despite using multiple
155 hydrophones, SP was treated as a single study site due to the close proximity of the
156 pinnacles. Recording cycles varied between sites depending on the recorders used. At
157 MOL in 2017, a recorder connected to a battery pack allowed for nearly continuous
158 recording. However, adverse weather conditions in the same year prevented
159 hydrophone recovery and deployment at SP, causing a gap in recordings. See
160 Supplementary Material for additional information on the recording equipment and
161 sampling schedule (Fig. S1).

162

163 **2.4. Acoustic data processing**

164 Audio recordings were down-sampled to 4 kHz since dusky grouper calls are low in
165 frequency (<450 Hz, Bertucci et al., 2015). Low-frequency pulses and downsweeping
166 sounds were a priori identified as known dusky grouper sound types (Bertucci et al.,
167 2015). After inspecting a full day of audio recordings from MOL (July 7th, 2017) using
168 Raven PRO 1.5 software (The Cornell Lab of Ornithology), we found that these sound
169 types could occur individually or in combinations. To classify these sounds, we defined
170 five main sound types (Fig. 2, Audio Files S1, S2): (1) Low-frequency (always referred
171 to as with a peak frequency <200 Hz) single Pulses (LP), (2) Low-frequency Pulse
172 Series (LPS) made up of at least three LP sounds, (3) Low-frequency Down-sweeping
173 Sounds (LDS), (4) Low-frequency Series of Down-sweeping Sounds (LDSS) made up of
174 at least three LDS sounds, and (5) a combination of Low-frequency Pulses and Down-
175 sweeping sounds (LPLDS).

176 Using the same custom-built MATLAB interface and procedure described in Desiderà et
177 al. (2022), audio recordings were audio-visually inspected to quickly identify and
178 attribute detected sounds to one of the aforementioned five sound types. Sound
179 selections were summarized in .csv output files and used for further analysis as
180 detailed in the following sections.

181

182

2.5. Behavioural observations

183 Dusky grouper spawning was reported to occur around sunset (Hereu et al., 2006;
184 Zabala et al., 1997a), but no systematic monitoring had been conducted at other
185 times of the day. To investigate the timing of dusky grouper spawning and collect data
186 on reproductive behaviours, direct observations were conducted at the study sites
187 during the summers of 2017 and 2018 (from June to late September-early October).
188 Observations were made during three time slots: after sunrise (04:00-07:00 hours
189 Universal Time Coordinated, UTC), during daytime (07:01-11:00 hours UTC), and
190 before sunset (15:00-19:00 hours UTC). Three SCUBA divers, one observer per dive,
191 recorded in situ observations of dusky grouper behaviours onto an underwater board,
192 noting the start and end times of each survey and the time of observations.
193 Additionally, a high-definition camera (Sony Cyber-shot DSC-RX100) was used to
194 record dusky grouper behaviours.

195 The sex of individuals was inferred from their behaviours and colour patterns. Males
196 were identified by their silver streaked colour pattern and females were identified
197 when being courted by males (Zabala et al., 1997b). If there was insufficient evidence,
198 sex was left unspecified.

199 Observed behaviours were classified as territorial or reproductive, following the
200 terminology described in the literature for the dusky grouper (Table 1).

201

202

2.6. Analysis of sound production

203 To describe the temporal patterns of dusky grouper sound production at the study
204 sites, the audio recordings collected in July 2017 were analysed. This period was
205 selected based on the intense patrolling and courtship activity observed, along with
206 previous studies reporting a peak in spawning during July in the Western
207 Mediterranean (Balearic Islands, Reñones et al., 2010). Because fish emit sounds
208 mainly at night (Bertucci et al., 2015; Parsons et al., 2016), and nighttime sound
209 production is less masked by anthropogenic noise, only data recorded from 16:00 to
210 6:00 hours UTC were processed as per section 2.4. Furthermore, the chosen period
211 encompasses dusky grouper reproductive activity (Hereu et al., 2006; Zabala et al.,
212 1997a). Diel 14-hour intervals (from 16:00 to 6:00 hours UTC) were divided into three
213 subgroups encompassing sunset (16-20), midnight (20-24), and sunrise (00-06) to test
214 for differences in sound abundance across these time intervals.

215 Acoustic data were analysed using the following approaches:

216 1) Visualising sound production patterns: The hourly number of detected sounds was
217 rescaled to the range of 0-1 by sound type and study site (min-max normalization), to
218 ensure data comparability. Normalized data were used to create bar plots and heat
219 maps, which visualized sound production patterns over the analysed 14-hour interval
220 and across different days, respectively.

221 2) Testing the relationship between hourly sound abundance and sound type across
222 time periods: Hourly sound abundance (response variable) was modelled as a function

223 of sound type (categorical with five levels: LP, LPS, LDS, LDSS, LPLDS), study site
224 (categorical with two levels: SP and MOL), and time period (categorical with three
225 levels: 16-20, 20-24, 00-06).

226 Data exploration followed the protocol by Zuur et al. (2010). A zero-inflated negative
227 binomial (ZINB) model was used to account for the high percentage of zeros and
228 overdispersion. To account for differences in recording cycles, an offset (natural
229 logarithm of the amount of minutes effectively recorded per hour) was included in the
230 model. Model selection was based on the Akaike Information Criteria (AIC) after model
231 testing using the package “glmmTMB” (Brooks et al., 2017). The model used for this
232 study was the following ZINB GLM model (in words):

233 *count part of the model:* Sound.abundance ~ Site + Sound.type + Time.period +
234 Site:Time.period + Sound.type:Time.period + Site:Sound.type +
235 offset(LogMinutes) + (1 | Date)

236 *binary part of the model:* ~ Site + Sound.type + Time.period

237 The notation ‘:’ indicates the interaction between categorical explanatory variables.
238 Date was treated as a random effect (“1 | Date”) to handle pseudo-replication
239 (repeated measurements from the same sites).

240 Model validation was conducted using the package “DHARMA” (Hartig, 2022) by
241 plotting residuals versus fitted values and versus each covariate in the model, and by
242 verifying that the model could cope with the number of zeros in the dataset (Figs S2,
243 S3, S4). Residuals were also assessed for temporal correlation. Post-hoc tests were
244 performed using the package “emmeans” (Lenth, 2022).

245

246 **2.7. Analysis of behavioural observations**

247 To examine the relationship between the behaviours observed during the summers of
248 2017 and 2018 and their dependence on time, the number of behaviours observed per
249 dive (response variable) was modelled as a function of behavioural category
250 (categorical with five levels: stimulation, courtship, patrol, chase, bumping) and time
251 of the day (categorical with three levels: after sunrise, daytime, before sunset). The
252 same protocol explained in section 2.6 was used for data exploration, model selection
253 and model validation (Figs S6, S7, S8). To account for differences in observation
254 duration, an offset (natural logarithm of the actual observation time per dive) was
255 included in the model, while site was excluded due to the small number of
256 observations per site by time period. The model used was the following GLM model
257 with negative binomial distribution (in words):

258 Behaviour.abundance ~ Category + Time.period + offset(LogMinutes) + (1 |
259 Date)

260 Date was treated as a random effect (“1 | Date”) to handle pseudo-replication.

261 In both this section and the previous one (2.6), all calculations were performed using R
262 version 4.2.1 (R Core Team, 2022), along with the support file by Zuur et al. (2009)
263 and the specific R packages listed in the Supplementary Material.

264

265 **2.8. Relationship between observed courtship activity and acoustic**
266 **behaviour**

267 To characterize and validate sound production activity associated with reproductive
268 behaviours, only dives with concomitant acoustic recordings were analysed. Dives
269 were categorised as either C (“Courtship”) or NC (“No-Courtship”) based on the
270 presence or absence of courtship behaviours, respectively. Only audio files recorded
271 within a three-hour interval overlapping each dive were considered. The audio
272 recordings were labelled as C or NC corresponding to their respective dives and
273 processed using the MATLAB interface previously mentioned. The number of sounds
274 by sound type was assessed for each three-hour interval and differences in the
275 weighted number of sounds (i.e., number of sounds per effective hour of recording)
276 were tested between C and NC recordings. Considering the non-normal distribution of
277 the data, Kruskal-Wallis tests were used to investigate if specific sound types were
278 associated with courtship behaviour in R version 4.2.1 (R Core Team, 2022).

279

280

281 **3. RESULTS**

282 **3.1. Acoustic and visual behaviours**

283 The audio recordings collected at the two study sites during July 2017 yielded a total
284 of 4353 dusky grouper sounds (Table S1). The courtship call (LPS) was the most
285 abundant sound type at both study sites.

286 Regarding observations, a total of 156 behaviours were documented across 117 dives
287 (n=35 in 2017, n=82 in 2018) (Table S2), categorised as follows: after sunrise (n=32
288 dives), during daytime (n=23 dives), and before sunset (n=62 dives). In 53% of these
289 dives, at least one reproductive behaviour was observed (n=17 in 2017, n=45 in
290 2018). Courtship was the most commonly observed behavioural category at both
291 study sites (Table S2).

292

293 **3.2. Temporal patterns of sound production**

294 Temporal patterns in sound production were comparable between the two study sites
295 although a clear site-specific trend was observed (Figs 3-4).

296

297 While all sound types were emitted throughout the investigated period, production
298 rates of each sound type varied over time and between study sites (Fig. 3). Peaks in
299 sound production were not synchronized between study sites.

300 Dusky grouper were acoustically active from evening to morning hours, with different
301 sound types detected at different times of the day (Fig. 4). LPS (courtship call) was
302 significantly more prevalent during the evening (16-20 UTC) compared to other time
303 periods and sound types (Fig. S5). LP showed a similar temporal pattern to LPS sounds
304 (Figs 3-4). In contrast, LDS, LDSS, and LPLDS were more abundant later at night and in
305 the early morning at both study sites.

306

307 **3.3. Temporal patterns of behavioural observations**

308 Among the observed behaviours, courtship was the most frequently documented,
309 particularly before sunset compared to other times of the day (Fig. S9). Statistically
310 significant differences were found between courtship before sunset and all other
311 behavioural categories and times, except for courtship after sunrise and chase before
312 sunset (Table S4).

313

314 **3.4. Relationship between observed courtship activity and acoustic** 315 **behaviour**

316 Courtship was observed in 19 out of the 37 dives with simultaneous acoustic
317 recordings. Analyses showed that LPS sounds were significantly more common during
318 dives with courtship (C) compared to dives without courtship (NC) ($N=37$, $\chi^2 = 6.6$, $p =$
319 0.01), confirming their classification as courtship calls (Fig. 5). The other sound types,
320 except for LP, did not significantly differ between C and NC dives (all $p > 0.05$),
321 suggesting that they are not directly related to courtship but possibly associated with
322 other behaviours, such as resource defence (Fig. 5).

323

324 **4. DISCUSSION**

325 In this study, we showed that the dusky grouper courtship call (LPS) was the most
326 frequently recorded sound type at both sites and was associated with visual courtship
327 behaviour in the wild.

328 Dusky grouper courtship calls (LPS) were more frequently recorded at dusk (16-20
329 hours UTC) at both study sites. However, peaks in acoustic activity were not
330 synchronized between sites, possibly due to the asynchronous reproductive cycle of
331 the species at the population level (i.e., different stages of gonadal development
332 across individuals) (Reñones et al., 2010). This spawning asynchronicity was also
333 documented by direct observations in the Medes Islands, where different monitored
334 males did not spawn on the same days (Hereu et al., 2006).

335 In our study, dusky grouper courtship behaviours were predominantly observed before
336 sunset (15-19 hours UTC), coinciding with the peak detection of courtship calls. This
337 result provides strong indications on the timing of reproductive behaviours.
338 Additionally, both LPS and LP sound types were significantly associated with the
339 observation of courtship behaviours, suggesting that the study sites are dusky grouper
340 spawning sites, although no direct observation of spawning was made. Further
341 investigations are needed to verify if peaks in LPS production indicate actual spawning
342 events. If so, passive acoustic recordings could be used to monitor the spatio-temporal
343 occurrence of dusky grouper spawning events, as demonstrated in other grouper
344 species (Rowell et al., 2019).

345 Unlike LPS calls, the presumptive dusky grouper sound types (LDS, LDSS and LPLDS)
346 were mostly recorded later at night, corroborating previous findings on the “growl-like
347 sounds” (Bertucci et al., 2015). This nocturnal acoustic activity challenges the
348 prevailing notion that the dusky grouper is primarily a diurnal species (Azzurro et al.,
349 2013; Koeck et al., 2014; Pastor et al., 2009). Grouper species have a diverse vocal
350 repertoire as they can emit pulse and tonal sounds individually, in series or in
351 combination (Mann et al., 2010; Rowell et al., 2019; Schärer et al., 2012a, 2012b).
352 However, most studies have mainly focused on identifying courtship-associated
353 sounds and their temporal patterns, rather than the entire repertoire of grouper
354 sounds (but see Santiago et al., 2020). Here we found a clear temporal partitioning in
355 dusky grouper acoustic activity, with distinct sound types produced at different times
356 of the day, suggesting the occurrence of different behaviours at different times.
357 Validating this temporal partitioning in the wild requires identifying the species’ visual
358 nocturnal behaviours and associated sound types, which can be challenging due to
359 their timing. Possible methods include nocturnal diving, although it can impair fish
360 behaviour (“light shock effect”, (Reeb, 2002)), or using fixed cameras with infrared
361 illumination or acoustic cameras (Mallet and Pelletier, 2014; Martignac et al., 2015) for
362 a less invasive behavioural monitoring.

363 Despite differences in sampling effort, potentially influencing the number and
364 temporal distribution of detected sounds, our findings suggest that the recording
365 cycles used in this study effectively capture both short-term (diel 14-h periods) and
366 long-term trends (about one month) in dusky grouper acoustic activity. The most
367 comprehensive acoustic activity pattern was recorded at MOL, despite recent
368 evidence showing a lower dusky grouper abundance at MOL compared to SP, as
369 determined through underwater visual census and photo-identification during the
370 same investigated period (Desiderà et al., 2021). This suggests that the findings at
371 MOL are likely attributed to continuous recordings and minimal human-made noise. In
372 fact, anthropogenic noise from SCUBA diving and boat traffic is higher at SP than at
373 MOL, potentially affecting sound detection and dusky grouper behaviour at that site.
374 Tourism in the study area peaks in July-August (Hogg et al., 2017), exposing the
375 species to increased motorboat noise during the spawning season and potentially
376 impacting its behaviours and reproductive success at SP (Amorim et al., 2022; Popper
377 and Hawkins, 2019). In fact, our findings highlight the importance of acoustic

378 communication for the dusky grouper and potential vulnerability to anthropogenic
379 noise, which should be further investigated.

380 Knowledge on the timing of reproductive activity can aid environmental managers in
381 mitigating anthropogenic disturbances at spawning sites. Implementing noise
382 reduction measures, such as reducing vessel speed and restricting SCUBA diving
383 during critical hours in summer, may enhance the species reproductive success at
384 critical habitats (Nedelec et al., 2022). Such conservation measures would not only
385 benefit the dusky grouper but also other species aggregating in the same areas
386 (Erisman et al., 2017; Sadovy de Mitcheson, 2016). Indeed, multiple grouper species
387 are known to spawn either simultaneously or at different times at the same sites, as
388 observed in the Mediterranean Sea (Aronov and Goren, 2008) and specifically within
389 the TPCC MPA (Desiderà et al., 2022).

390 In conclusion, our study provides valuable insights into the reproductive biology of the
391 dusky grouper and highlights the effectiveness of passive acoustic monitoring (PAM) in
392 studying its reproduction globally, including the Mediterranean Sea. By reliably
393 identifying reproductive activity through dusky grouper courtship calls (LPS), we can
394 determine the timing and location of spawning, which is crucial for conserving critical
395 habitats often shared by multiple ecologically valuable species. Protecting such
396 habitats is paramount, as it ensures grouper long-term survival, preserves
397 biodiversity, and maintains the ecological functioning of the marine ecosystem.
398 Therefore, prioritizing the protection of these high-quality reproductive habitats
399 becomes more important than focusing solely on expanding the protected ocean
400 surface area.

401

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411

412 **CRedit authorship contribution statement**

413 ED: Conceptualization, Investigation, Data curation, Formal analysis, Project
414 administration, Validation, Visualization, Writing – original draft and review & editing;
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422

423 **Declaration of competing interest**

424 The authors have no conflict of interest to declare.

425

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428

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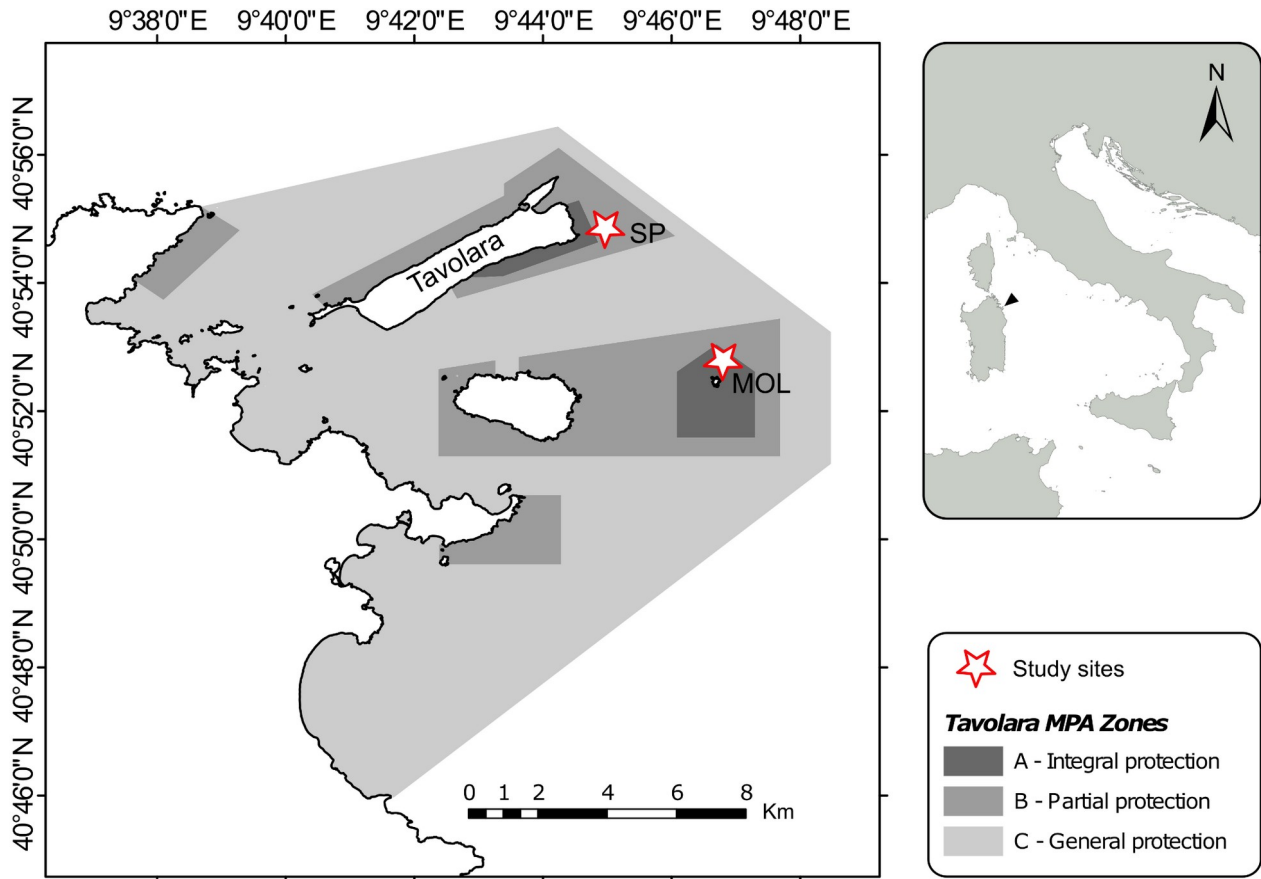
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634 **Table 1 Descriptions of dusky grouper observed behaviours.**

Behaviour	Category	Description
Patrol	Territorial	Males swim rapidly within 1-2 m off the bottom throughout their territory, typically below the thermocline. Patrols are particularly intense during evening hours.
Chase	Territorial	When encountering another male within its territory, the male rapidly chases the intruder, even covering relatively long distances (several meters), before returning to patrolling. Chases have been observed between suspected females and dusky groupers targeting individuals of other grouper species, such as <i>Epinephelus aeneus</i> and <i>Mycteroperca rubra</i> .
Bumping	Territorial	Male-male as well as female-female aggressive interactions involve the raising of the dorsal fin and striking each other's sides by tilting the body, typically near the bottom. Females have been observed displaying an "aggressive zebra colour pattern" as mentioned by Zabala et al. (1997b). Subsequent to these interactions, one individual moves away (Video S1).
Stimulation	Reproductive	A single individual, presumed to be male, was observed nudging the abdominal area of a presumed female, possibly to sense her readiness to spawn through pheromone release occurring at the vent (personal communication, Y. Sadovy). This behaviour was displayed by a presumptive male exhibiting a light colour pattern and a straight dorsal fin, while the presumed female showed a bulging belly and a uniformly dark livery. This behaviour suggests that spawning is imminent, likely within 24 hours. A video capturing this behaviour is available (Video S2)
Courtship (Approach and Display)	Reproductive	A single male approaches a single female from behind, tilts to a horizontal position, and shakes its posterior part over the female (referred to as "ritualized caudal flapping" by Zabala et al., 1997a). Concurrently with the caudal flapping, males emit low-frequency pulse-series (Bertucci et al., 2015). These sounds were once heard in situ by one of the authors (ED) (Video S3).

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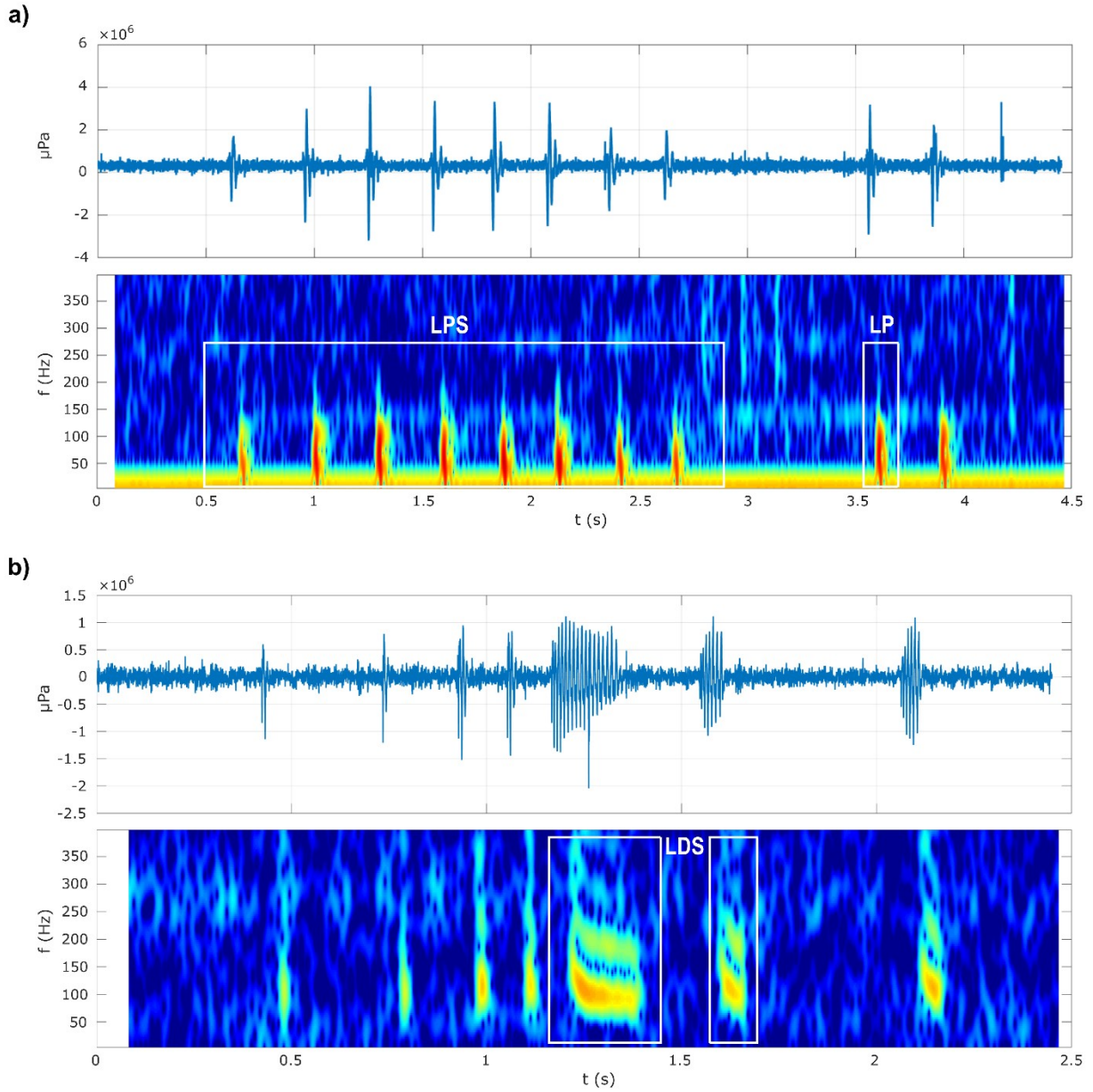
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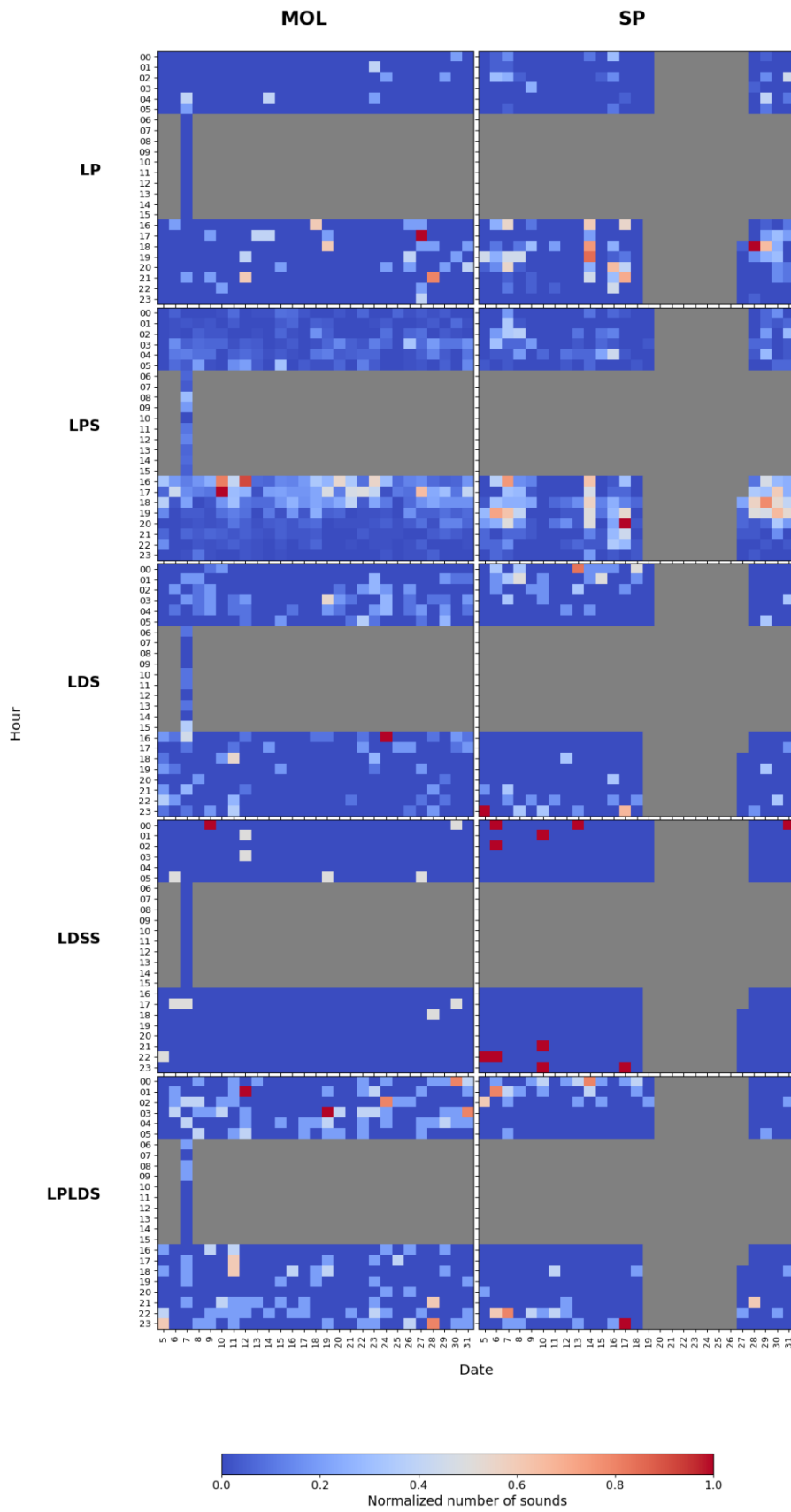
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Fig. 1 Map of the Tavolara-Punta Coda Cavallo Marine Protected Area (TPCC MPA) showing study sites and zonation. A zones are fully protected (no-take, no-access). B and C zones are partially protected, with specific restrictions on human activities. In B zones, licensed local artisanal fishing and diving are allowed, while in C zones, recreational fishing is also allowed. Spearfishing and grouper fishing are prohibited throughout the MPA.



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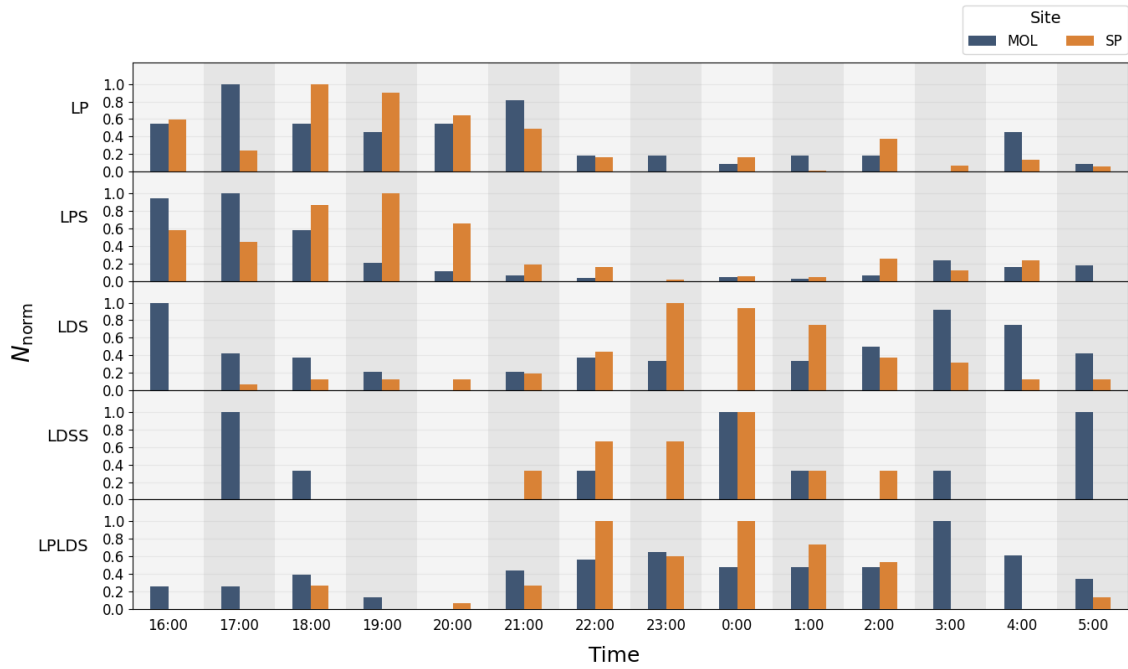
644 **Fig. 2 Spectrograms and oscillograms of dusky grouper sound types. a) LP:** Low-frequency Pulse;
 645 **LPS:** Low-frequency Pulse Series. **b) LDS:** Low-frequency DownswEEPing Sound. LP and LPS are
 646 confirmed dusky grouper sounds recorded in captivity and in the wild, with LPS sounds linked to courtship
 647 activity in captivity; LDS sounds were only recorded in the wild and attributed to the species (Bertucci et al.,
 648 2015).



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650 **Fig. 3 Normalized (0-1) values of dusky grouper sounds detected by sound type and study site per**
 651 **hour interval from July 5th to 31st, 2017. On July 7th, the entire day's audio recordings were analysed at**
 652 **MOL. Grey areas indicate gaps in acoustic data (unrecorded or unanalysed data).**

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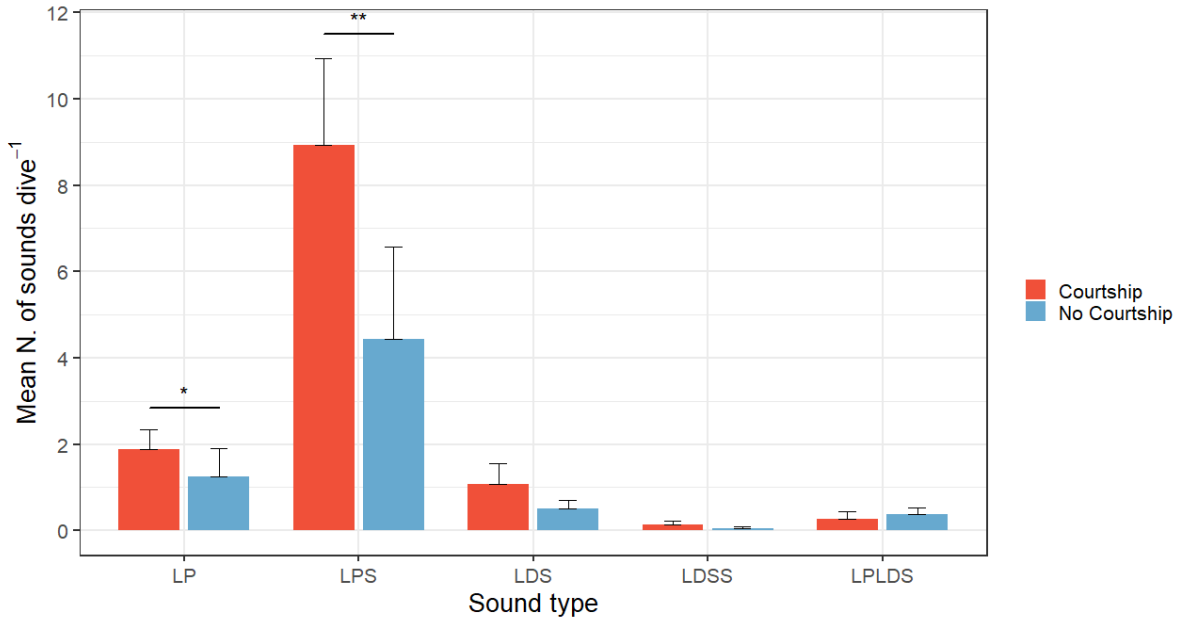


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655 **Fig. 4** Cumulative diel variation of normalized sound abundances (0-1) of the five sound types
 656 recorded at the two sites over the month of July from 16:00 to 6:00 hours UTC. MOL: Molarotto, SP:
 657 Secche del Papa.

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661 **Fig. 5** Mean (\pm SE) number of sounds per sound type during dives with dusky grouper courtship
 662 behaviour (C) and without (NC) ($N=37$, $C=19$ and $NC=18$ dives), across both study sites. * $p < 0.05$, ** p
 663 < 0.01 .

664