Research paper

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

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## ABSTRACT

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

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

# 1. INTRODUCTION

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

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

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

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

The dusky grouper *Epinephelus marginatus* is an iconic and endangered Mediterranean grouper species that has received special conservation attention through time, being protected under the Barcelona and the Bern Conventions (Pollard et al., 2018). Effective conservation actions include restrictions or bans on fishing, mainly achieved through well-enforced Marine Protected Areas (MPAs), especially where fishing is prohibited (i.e., fully protected areas; (Giakoumi et al., 2017). Despite its endangered status, knowledge about its biology, particularly reproduction, is limited (Condini et al., 2017).

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

## 2. MATERIALS AND METHODS

### 2.1. Study species

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

### 2.2. Study sites

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

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

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

### 2.3. Acoustic data collection

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

### 2.4. Acoustic data processing

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

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

### 2.5. Behavioural observations

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

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

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

### 2.6. Analysis of sound production

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

Acoustic data were analysed using the following approaches:

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

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

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

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

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

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

The notation ':' indicates the interaction between categorical explanatory variables. Date was treated as a random effect ("1 | Date") to handle pseudo-replication (repeated measurements from the same sites).

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

### 2.7. Analysis of behavioural observations

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

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

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

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

### 2.8. Relationship between observed courtship activity and acoustic behaviour

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

## 3. RESULTS

### 3.1. Acoustic and visual behaviours

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

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

### 3.2. Temporal patterns of sound production

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

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

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

### 3.3. Temporal patterns of behavioural observations

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

### 3.4. Relationship between observed courtship activity and acoustic behaviour

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

## 4. DISCUSSION

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

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

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

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

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

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

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

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## CRediT authorship contribution statement

ED: Conceptualization, Investigation, Data curation, Formal analysis, Project administration, Validation, Visualization, Writing – original draft and review & editing; CM: Conceptualization, Supervision, Writing – review & editing; AN: Funding acquisition, Project administration, Writing – review & editing; RB: Data curation, Visualization, Writing – review & editing; AC: Formal analysis, Writing – review & editing CG: Resources, Software, Data curation, Writing – review & editing; PP: Investigation, Writing – review & editing; ET: Investigation, Resources, Writing – review & editing; PG: Conceptualization, Supervision, Writing – review & editing; LDI: Conceptualization, Resources, Supervision, Formal analysis, Writing – review & editing.

## Declaration of competing interest

The authors have no conflict of interest to declare.

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***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 *Epinephelus aeneus* and *Mycteroperca rubra*. |
| 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). |

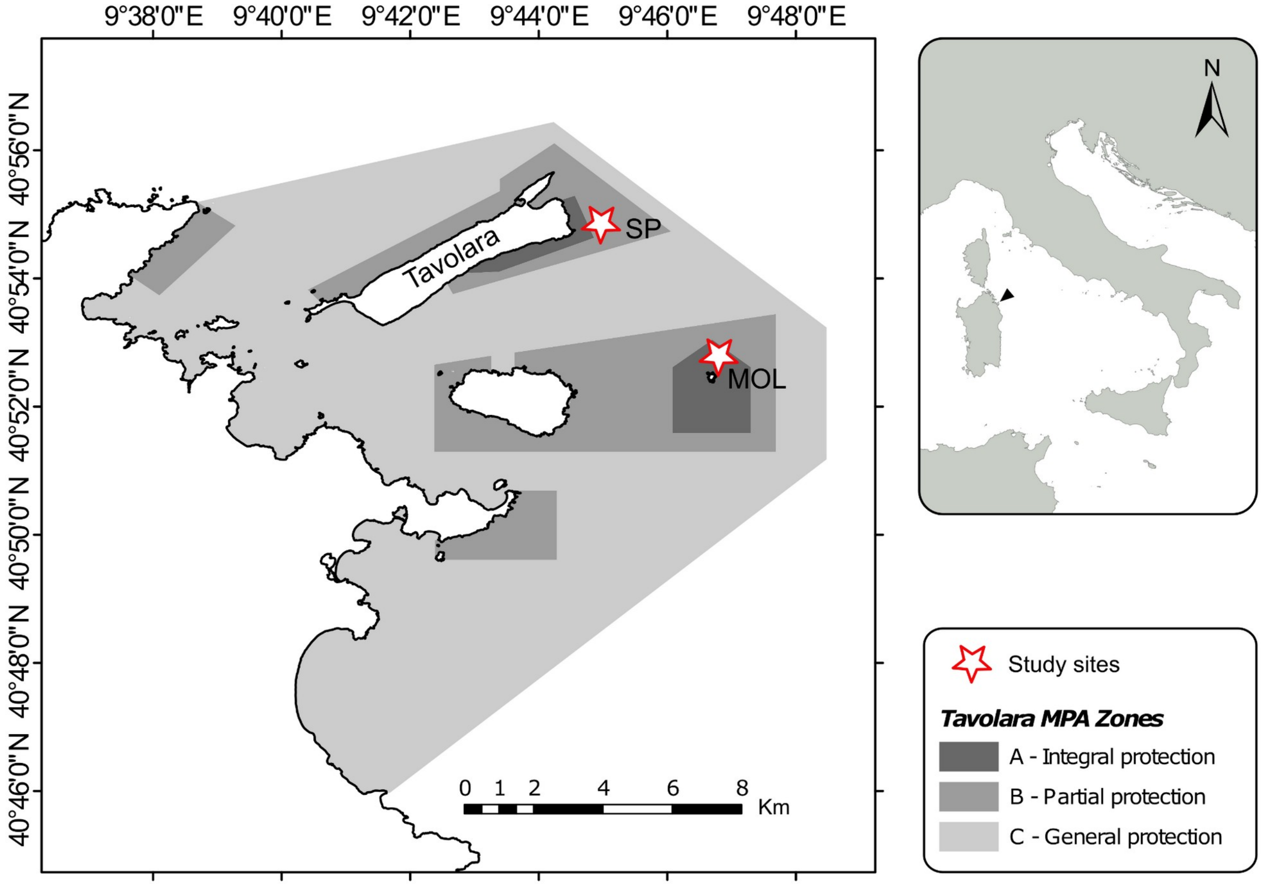


***Fig. 1 Map of the Tavolara-Punta Coda Cavallo Marine Protected Area (TPCC MPA) showing study sites and zonation.*** *A zones are fully protected (no-take, no-access). B and C zones are partially protected, with specific restrictions on human activities. In B zones, licensed local artisanal fishing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

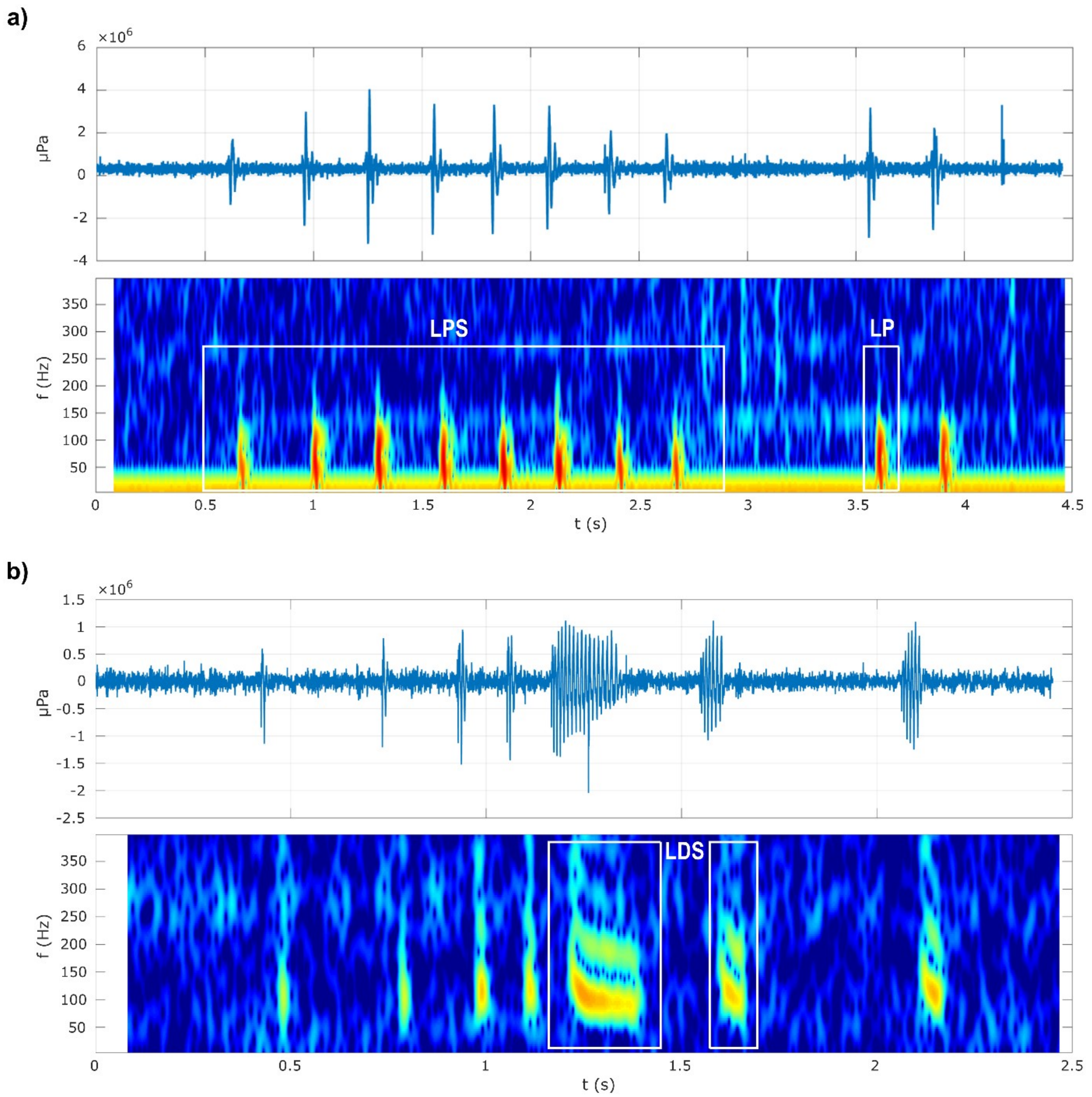


***Fig. 2 Spectrograms and oscillograms of dusky grouper sound types. a)*** *LP: Low-frequency Pulse; LPS: Low-frequency Pulse Series.* ***b)*** *LDS: Low-frequency Downsweeping Sound. LP and LPS are confirmed dusky grouper sounds recorded in captivity and in the wild, with LPS sounds linked to courtship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

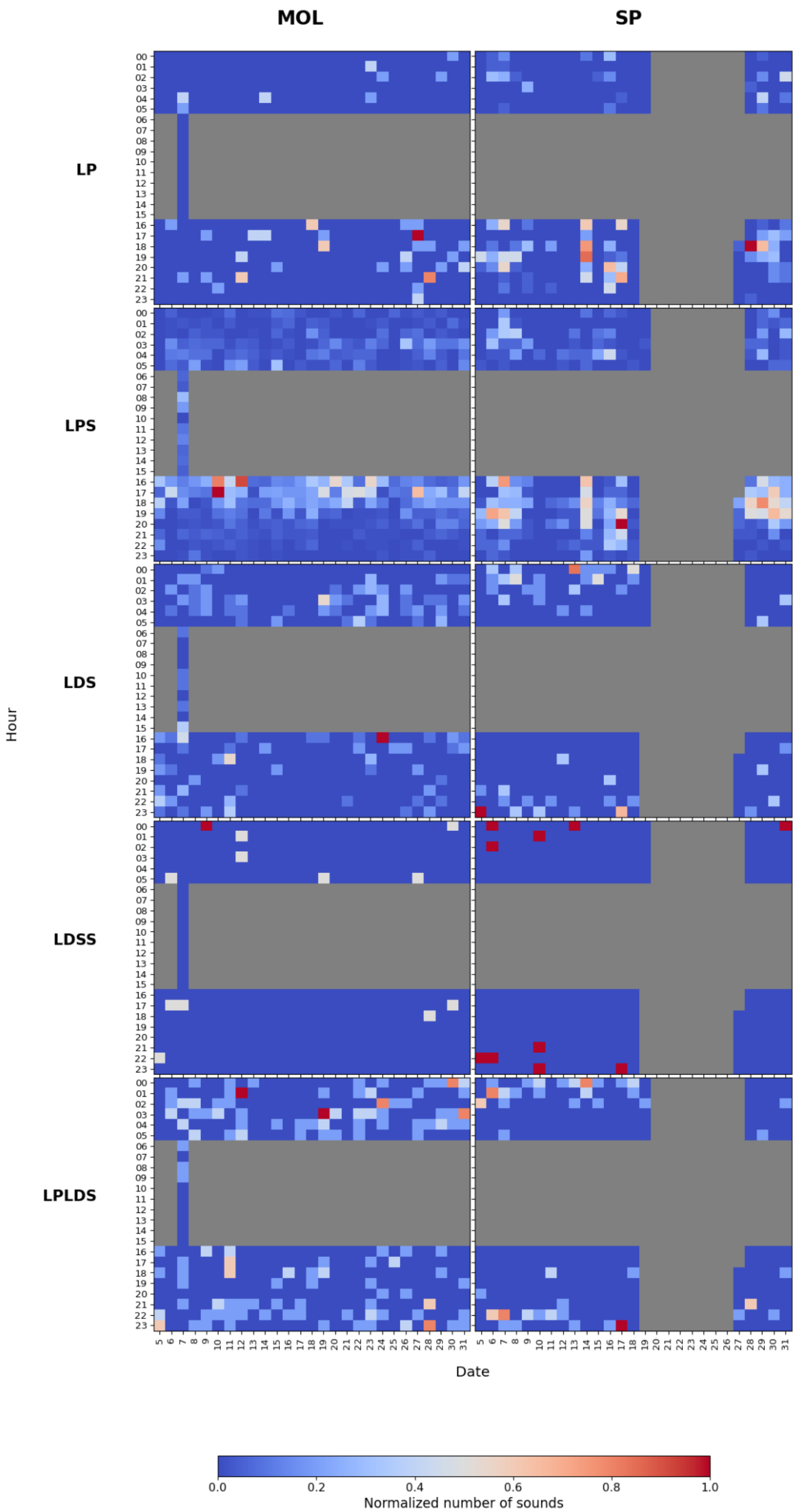


***Fig. 3 Normalized (0-1) values of dusky grouper sounds detected by sound type and study site per hour interval from July 5th to 31st, 2017.*** *On July 7th, the entire day's audio recordings were analysed at MOL. Grey areas indicate gaps in acoustic data (unrecorded or unanalysed data).*

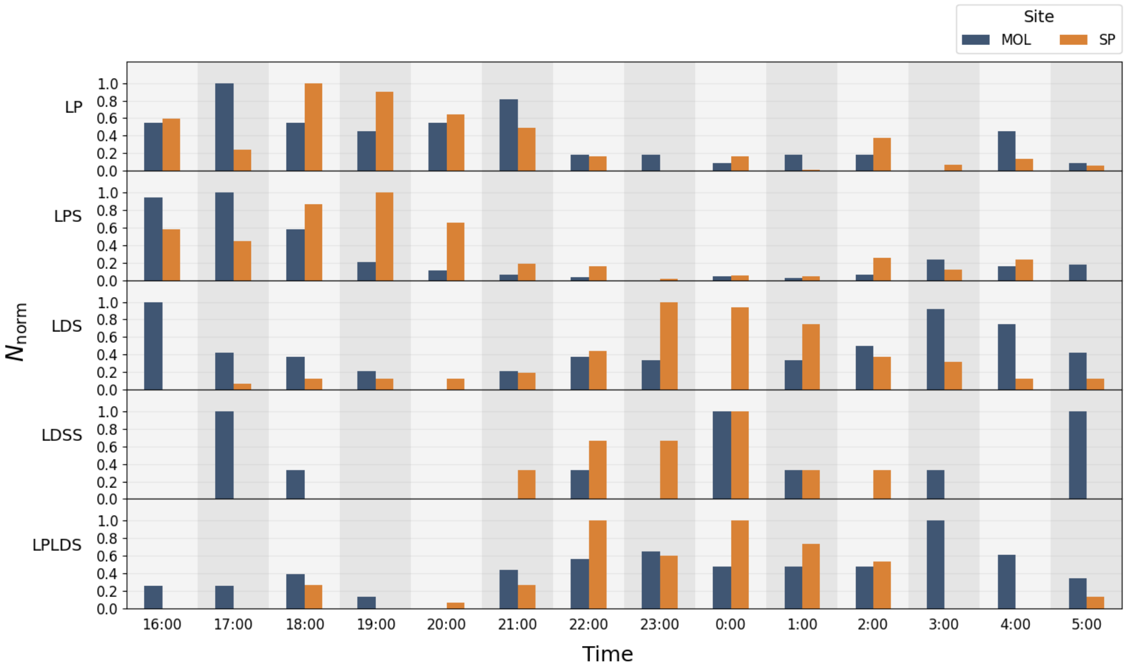


***Fig. 4 Cumulative diel variation of normalized sound abundances (0-1) of the five sound types recorded at the two sites over the month of July from 16:00 to 6:00 hours UTC.*** *MOL: Molarotto, SP: Secche del Papa.*

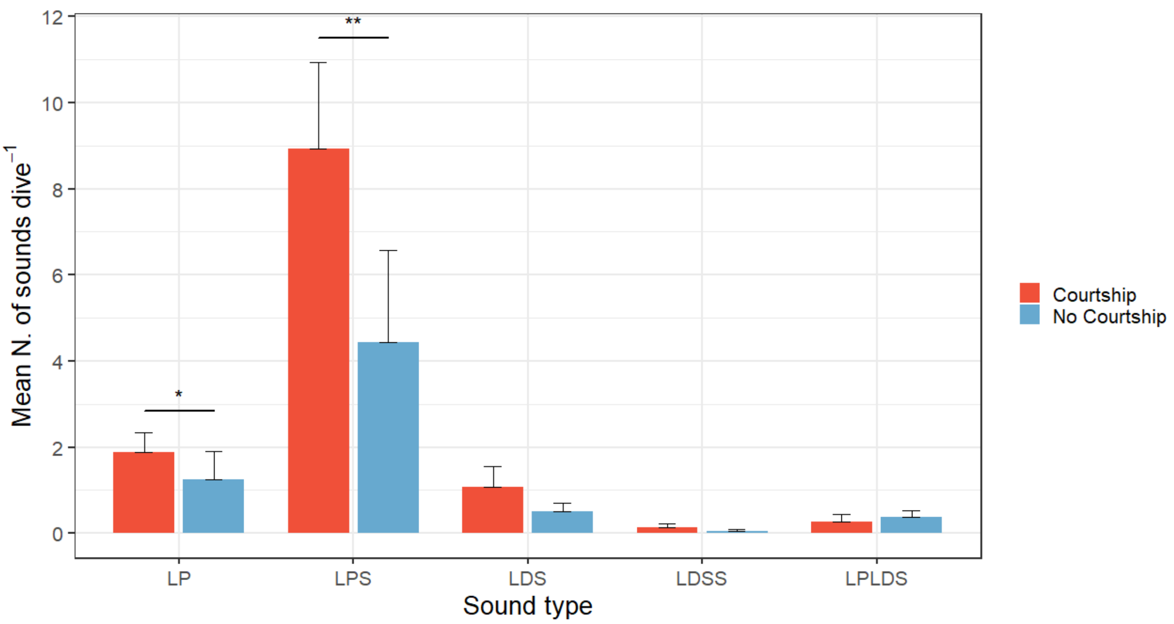


***Fig. 5 Mean (± SE) number of sounds per sound type during dives with dusky grouper courtship behaviour (C) and without (NC) (N=37, C=19 and NC=18 dives), across both study sites****. * p < 0.05, ** p < 0.01.*