

- Master's Thesis in Marine Ecology 2013 -

**Identification of preferential spawning areas
of the European squid, *Loligo vulgaris*,
Lamarck (1798)**

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Abstract

Identifying spawning areas is essential for ensuring sustainable exploitation of marine resources in general but may be critical for short-living organisms. Here, the spatio-temporal pattern of squid spawning is described and the preferred areas at a Western Mediterranean site have been identified for the European squid, *Loligo vulgaris*. Previous knowledge suggests that squid may undergo spawning migrations from deeper to shallower waters in order to maximize recruitment success. However, data on spawning are very scarce for the European squid because detecting and quantifying egg clutches at the wild is very challenging. Therefore, we designed an artificial device aimed to make possible spawning quantification. After deploying 30 of these devices covering a wide range of environmental conditions at a marine reserve, four main patterns have been identified: 1) squid spawns throughout the year but there is a peak set in May, 2) preferred depth for spawning is different between cold months (shallower) and warm months (deeper) 3) eggs clutches are more abundant at sandy bottoms, followed by rocky bottoms and no or very few clutches are found on phanerogams bottoms, and 4) certain sites has abnormally high abundance of eggs clutches. The first two findings are consistent with the existence of spawning migrations. The third finding may be interpreted as predator avoidance behavior of adults. The last finding may be interpreted as the results of selecting sites more exposed to marine currents, which may be related with either, increasing oxygen availability for embryonic development or facilitating dispersal of planktonic paralarvae. Overall, combining all the preferred environmental conditions, two hot-spots of spawning has been identified. We propose that temporal closure to fishing of such relatively small areas may be used as complementary management tool.

Keywords: Marine Protected Area (MPA), *Loligo vulgaris*, Western Mediterranean, Egg Clutches, Zero Inflated Poisson (ZIP), Spawning grounds, Spawning migrations.

Resumen

La identificación de las áreas para el desove es una herramienta esencial para asegurar la explotación de los recursos marinos en general, pero puede ser crítica para los organismos de ciclo de vida corto. En este sentido, se describe el patrón espacio-temporal de la reproducción del calamar europeo, *Loligo vulgaris* y se identifican sus áreas preferenciales de desove en el Mediterráneo occidental. Investigaciones anteriores sugieren que el calamar europeo realiza migraciones para el desove de aguas más a menos profundas, con el objetivo de maximizar su éxito de reclutamiento. Sin embargo, existen pocos datos sobre el desove del calamar ya que detectar y cuantificar las puestas de huevos en el medio natural es un gran desafío. Por consiguiente, hemos diseñado un dispositivo artificial para poder llevar a cabo la cuantificación del desove. Después de depositar 30 de estos dispositivos cubriendo un amplio rango de condiciones ambientales en una reserva marina, se han identificado cuatro patrones principales: 1) El calamar desova a lo largo de todo el año pero existe un pico de puesta en mayo, 2) la profundidad preferencial para el desove es diferente entre los meses fríos (menos profundidad) y cálidos (más profundidad), 3) las puestas de huevos son más abundantes en fondos de arena, seguidos de los fondos de roca y no se encontraron o se encontraron muy pocas puestas en fondos con fanerógamas marinas, y 4) ciertos lugares presentaron una elevada abundancia anormal de puestas. Los dos primeros resultados son consecuentes con la existencia de migraciones para el desove. El tercer resultado puede ser interpretado como un comportamiento de evasión frente a depredadores. El último hallazgo puede ser interpretado como el resultado de la selección de lugares más expuestos a corrientes marinas, que pueden estar relacionadas ya sea con la disponibilidad de oxígeno para el desarrollo embrionario o bien con la ayuda a la dispersión de las paralarvas planctónicas. En conjunto, combinando todas las condiciones ambientales preferenciales, se han identificado dos puntos calientes de desove. Proponemos que el cierre temporal de la pesca en estas áreas relativamente pequeñas, puede ser utilizado en un futuro, como una herramienta de manejo complementaria para la protección de dicha especie.

Prefacio

Este estudio ha sido realizado dentro del marco del Máster de Ecología Marina ofrecido por la Universidad de las Islas Baleares (UIB) del cual el director es el Dr. Gabriel Moyà Neil. Este estudio se enmarca dentro del proyecto CEFAPARQUES financiado por la Red de Parques Nacionales. El objetivo del proyecto, es identificar y caracterizar hábitats de preferencia para la freza y el alevinaje de cefalópodos con importancia comercial (calamar, sepia y pulpo) en los Parques Nacionales Marítimo Terrestres de las Islas Atlánticas de Galicia (PNIA) y el Parque Nacional de Cabrera (CNP). Se trata de un proyecto coordinado entre el grupo de Ecología y Biodiversidad Marina del Instituto de Investigaciones Marinas de Vigo (IIM) liderado por el Dr. Ángel Guerra, experto mundial en cefalópodos y el grupo de Ictiología del Instituto Mediterráneo de Estudios Avanzados (IMEDEA) de Mallorca, liderado por la Dra. Beatriz Morales.

Esta tesis ha sido supervisada por el Investigador titulado, Miquel Palmer, cuyas líneas de investigación son: la ecología cuantitativa de peces y, específicamente, los efectos de pesca (recreativas y comerciales) sobre las características de historia de vida tales como el movimiento de los peces, comportamiento, patrones de distribución, la morfología y el crecimiento.

Por último también me gustaría destacar el apoyo, disposición y ayuda que me ha ofrecido en todo momento el actual estudiante de doctorado Miguel Cabanellas Reboledo, un joven investigador que termina su tesis el presente año, titulada "Recreational squid jigging fishery". Sus líneas de estudio son: Ecología y biología de los cefalópodos, Aspectos de Fisiología y de comportamiento de los cefalópodos y Conservación y desarrollo sostenible de la pesca de cefalópodos.

Con esta tesis, pretendo clarificar parte del comportamiento reproductivo de una especie explotada por las pesquerías en nuestro Mar Mediterráneo, identificando sus zonas preferenciales para el desove. Con el objetivo en un futuro, tener en consideración estas zonas como herramienta para la protección de dicha especie. Por otra parte, este estudio, me ha aportado experiencia y visión en el campo de la investigación científica, tanto en el trabajo de campo a la hora de obtención de datos como en el desarrollo y expresión de los mismos.

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1. Introduction

1.1. Background

The increase of activity of the commercial fisheries in last decades implies an increase of the human impact on the living marine resources and coastal environments. In consequence, there is an urgent need for proper management and conservation of the marine resources. In the last decades, cephalopods have become an important human target group worldwide (Boyle and Rodhouse, 2005). In fact, the fish world trade in 2009 of cephalopods were 3398826 tones (<http://www.fao.org/fishery/statistics/>).

One of the most exploited species of cephalopods in the Mediterranean sea is the European squid, *Loligo vulgaris*, Lamarck (1798) (Guerra et al., 1994). This Loliginid generally inhabits a wide depth range (0-200 m) searching for temperate waters ranging between 12.5 and 20°C and in rather low (Mangold-Wirz, 1963).

This resource has high social-economical value and thus experiences large commercial fishing pressure (see the different fleets that exploit squid in Fig. 1A & B; (Guerra and Rocha, 1994; Ulaş, 2011 ; Cabanellas-Reboredo et al., 2011). For example, during a single year (2009) in the Balearic Islands, the declared annual catch by the professional (trawling and artisanal) fleet was 119.5 tons, representing 1.5×10^6 € (www.agriculturaipesca.caib.es). This squid is exploited by different professional fisheries: the trawling fleet and the small-scale artisanal fleet (Lefkadltou, 1998; Moreno, 2002; González and Sánchez, 2002; Cabanellas-Reboredo et al., 2012a). In addition, it suffers a high pressure by the recreational fishery (Guerra and Rocha, 1994; Cabanellas-Reboredo et al., 2012a). In fact, at Palma Bay (NW Mediterranean Sea), tens to hundreds of recreational boats (Fig. 1C) may concentrate at specific grounds (inshore waters at 20-35 m depth) and during the spawning season (winter-spring) of this species to capture this valued resource (Cabanellas-Reboredo et al., 2012a). It has been suggested that this vulnerability pattern may be related to the inshore-offshore spawning migrations performed by squid (Cabanellas-Reboredo et al., 2012b).

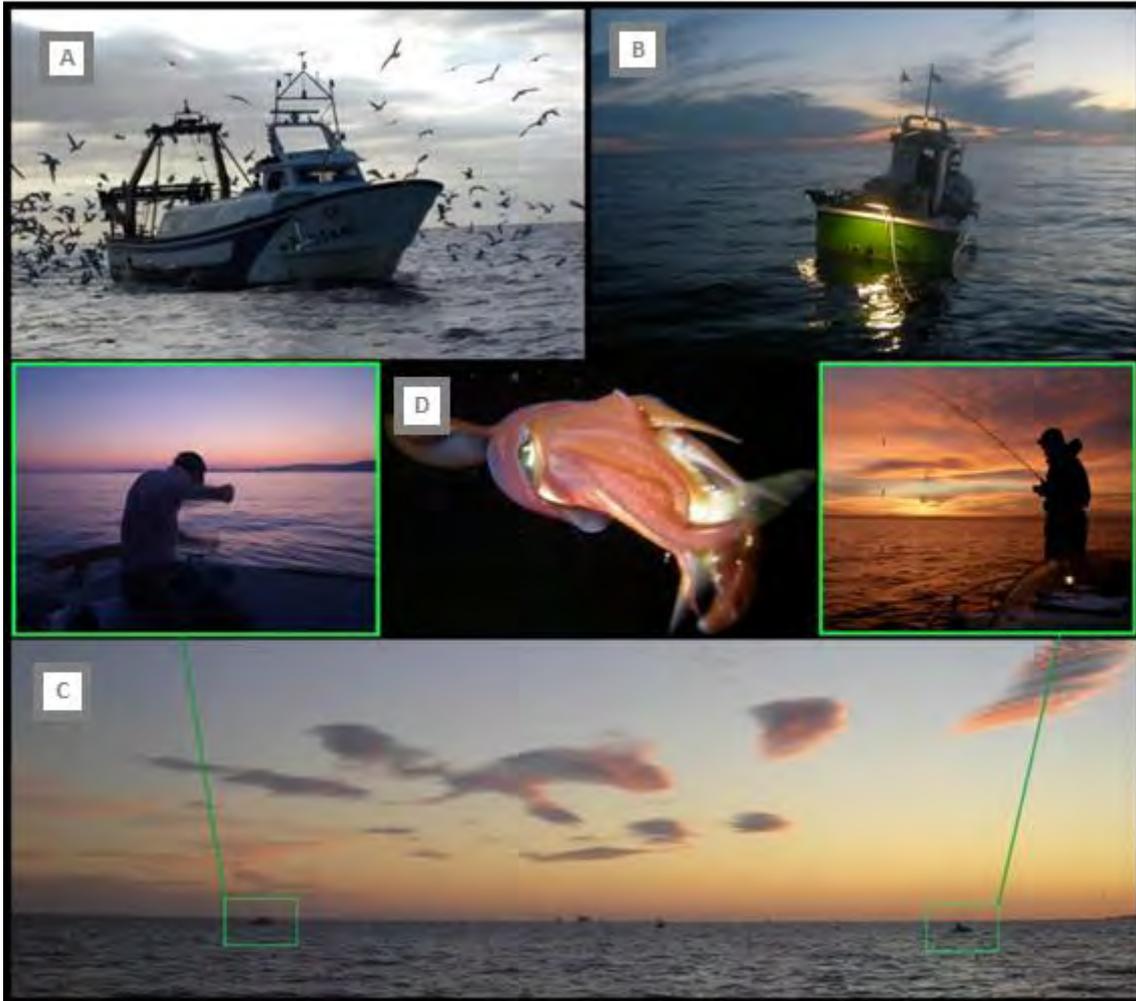


Figure 1. A) Trawling boat, B) Artisanal fleet, C) Recreational fleet with details of the jigging modalities performed by this fleet and D) The European squid *Loligo vulgaris*.

In these suggested migrations, the reproductive strategy is a partial ovulation and egg laying in separate batches during the spawning period (Rocha and Guerra, 1996; Rocha et al., 2001). Females lay eggs embedded in gelatinous strings at different hard bottom substrates, typically at the underside of rocky overhangs (Fig. 2) or on branched sessile organisms (Boletzky, 1998) and also fishing lines (Villanueva, 2000).

The structure of substrate is a factor that determines the clear adhesion of egg strings for a successful reproduction, but there are other environmental factors such as temperature and salinity (Villanueva, 2003; Sen, 2005a; Sen, 2005b) which can also affect the duration of embryonic development (Worms, 1983). Furthermore, the temperature can also influence growth rates of planktonic paralarvae (Villanueva, 2000).



Figure 2. *Loligo vulgaris* egg clutches visualized at PNIA waters (by Jorge Urcera).

Therefore, the spawning success and the optimal development of the paralarvae depend on specific environmental conditions. Accordingly the space-time distribution of this specie (i.e., inshore-offshore migration for spawning) may be related to reproduction, because squid should look for the environmental conditions for maximizing spawning success (Guerra et al., 1994; Villanueva et al., 2003; Cabanellas-Reboredo et al., 2012b).

This reproductive behavior searching for optimal conditions may be used as a management tools for protecting the preferential spawning grounds. In this sense, one of the management options used for protecting marine species is the implementation of Marine Protected Areas (MPA) (Roberts, 2005). The growing number of MPA worldwide, which increases at approximately 5% annually (Wood et al., 2008), reflects their usefulness for preserving marine biodiversity and fishery resources (Pauly et al., 2002). Despite the high mobility of the European squid could limit MPA usefulness (Cabanellas-Reboredo et al., 2012b), this species seems to aggregate at specific areas to spawn (Downey et al., 2010; Cabanellas-Reboredo et al., 2012b). The identification of where and when these spawning aggregations occur may be important for protecting the future recruits and for maximizing the spawning success.

1.2. Objectives

With this background, the aim of this master's thesis study is to identify the preferential spawning areas of *L. vulgaris* within an MPA. The specific objectives are: The identification of spawning areas in function of the prevailing conditions (benthic type, depth and temperature) and the description of the spatio-temporal variation in spawning intensity. This may allow elaborate a possible contingency plan in the future to protect and preserve the spawning and early juvenile areas of *L. vulgaris* at CNP.

2. Material and methods

2.1. Sampling area scenario

Experimental sampling took place at CNP, located at the south coast of Mallorca Island (North Western Mediterranean, Fig. 3A). This National Park comprises the archipelago of Cabrera, Conejera and 17 small islets which total just over 13 km² of land surface. Maximum depth within the boundaries of the park is 120 m. The local climate is semiarid Mediterranean, with an average annual temperature of 18°C and reduced rainfall of 350 mm on average.

CNP, having no river inputs, is girded by oligotrophic waters during most part of the year, but different seasonal oceanographic conditions allow having temporally favorable conditions for the survival of a large number of species. Important hydrographic channels (Fig. 3A) traverse the Balearic Islands. Cabrera currents also show significant changes associated with the seasonal wind regime (Fig. 3B). The combination of these environmental factors produce seasonal phytoplankton and zooplankton blooms, increasing the productivity and, consequently, the availability of food for larvae.

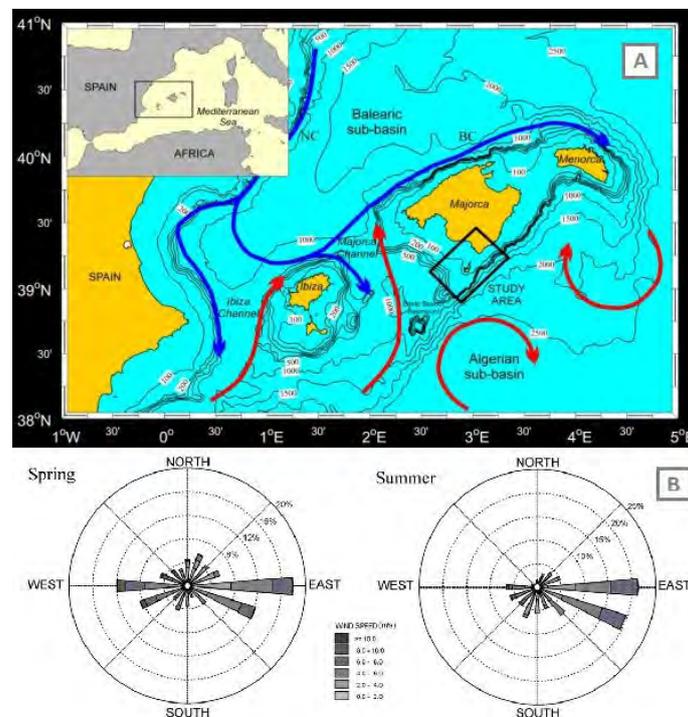


Figure 3. A) On upper, Balearic basin bathymetry including currents. B) Lower, wind roses which show seasonal differences in wind forcing. Data from Cabo Ses Salinas Station (by TALACA PROYECT web site).

Tidal regime, upwellings, surface water features and the impact of ocean currents on benthic formations are important parameters to consider in the characterization studies of preferential habitats for spawning of species of ecological significance (Basterretxea et al., 2012) like the European squid.

CNP was declared the first Spanish National Park in April 1991 (Law 14/1991), including 8703 hectares of sea with a great diversity of types of bottom, benthic communities and ichthyofauna (Garcia-Rubies, 1993; Ballesteros, 1994). Different management measures have been applied, from absolute prohibition of recreational and sport fishing to the establishment of a professional boat closed census under fishing gear (Fig. 4).

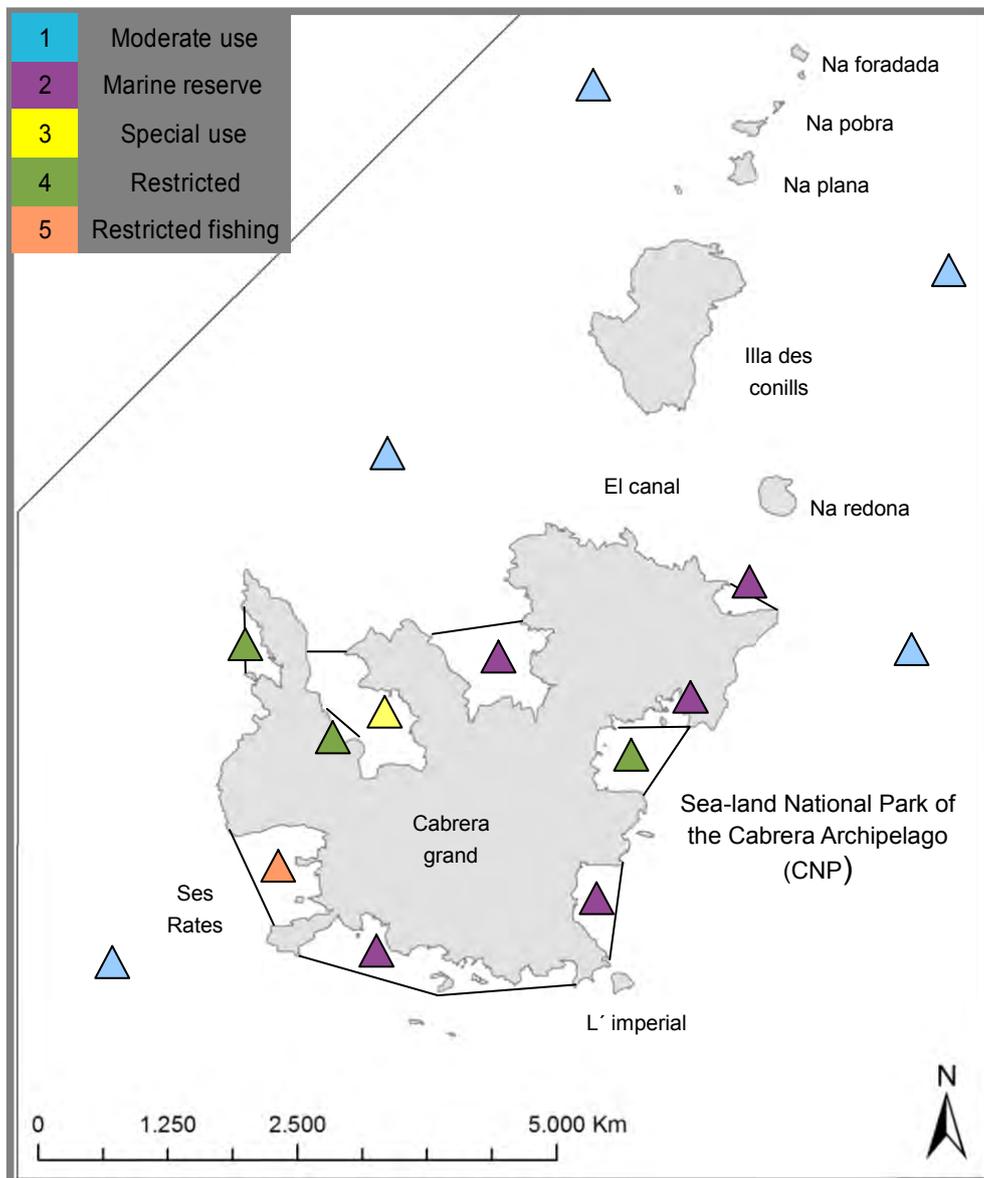


Figure 4. Zoning restrictions of CNP.

These management tools aim to enhancing fisheries. MPA have been shown to have many benefits, such as enhancing marine biodiversity and biomass (Halpern, 2003) and increasing ecosystem resiliency (Gell and Roberts, 2003).

2.2. Period of study

Fieldwork covered the annual cycle of the European squid, from June 2012 to June 2013. Therefore, 11 samplings have been performed with a monthly periodicity (note that the sampling of February could not be developed due to bad sea condition). This periodicity was based on previous studies that determined an average embryonic development of 1 month for *Loligo vulagris* (Şen, 2005b). Sampling dates were set in accordance on weather conditions and availability of the IMEDEA's boat (ROADMAN 11.2).

2.3. Design and sampling methodology

30 artificial devices (AD; Fig. 5A) were deployed over three different main benthic habitats (Sandy Bottom, Rocky Bottom and Phanerogams; see below predictive variables in Data analysis) and covering a 0 to 50 m depth range around the CNP (Fig. 6). The utilization of artificial structures was inspired on previous experiments, in which squid attached egg clutches at the ropes of acoustic tracking structures (Cabanellas-Reboredo et al., 2012b).

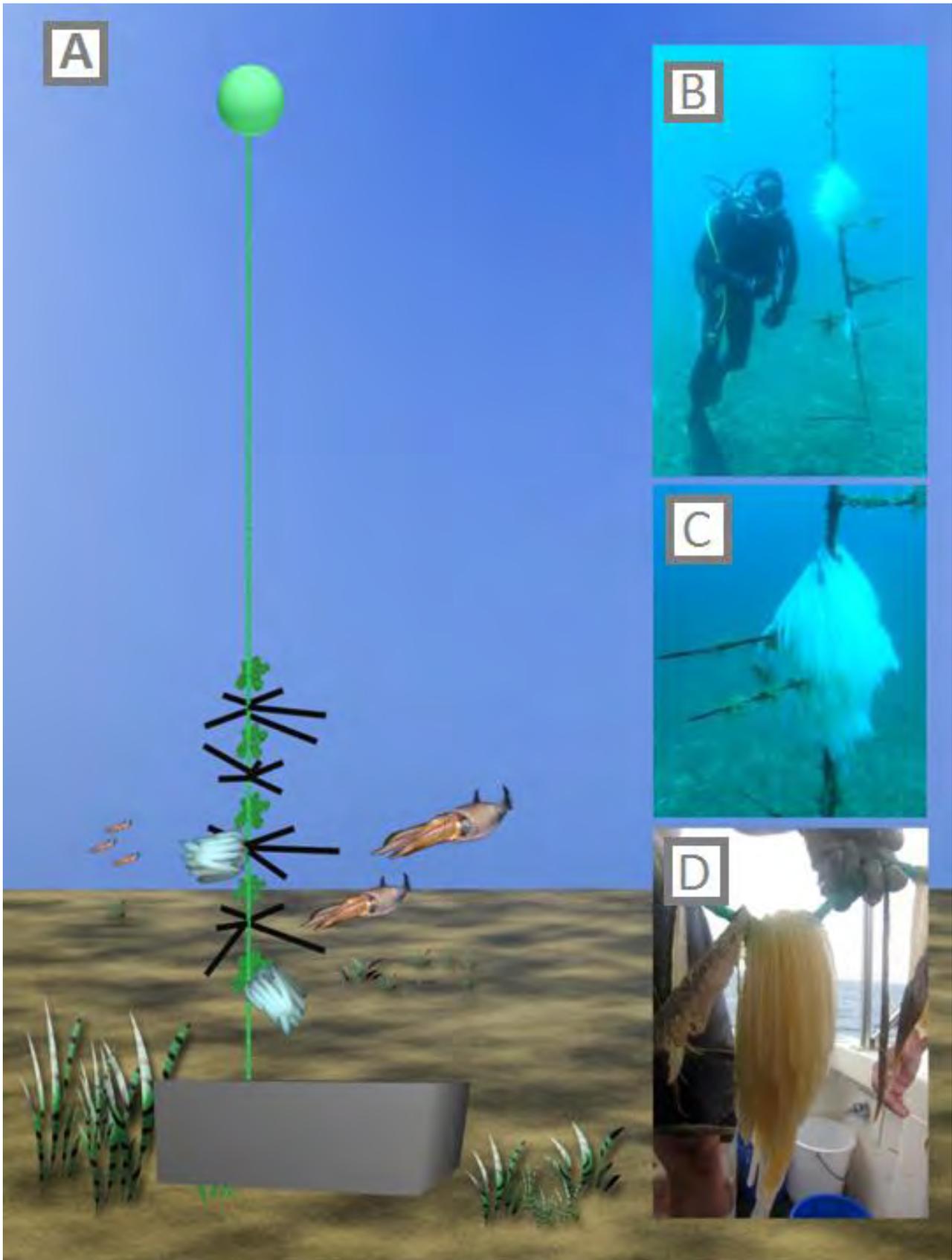
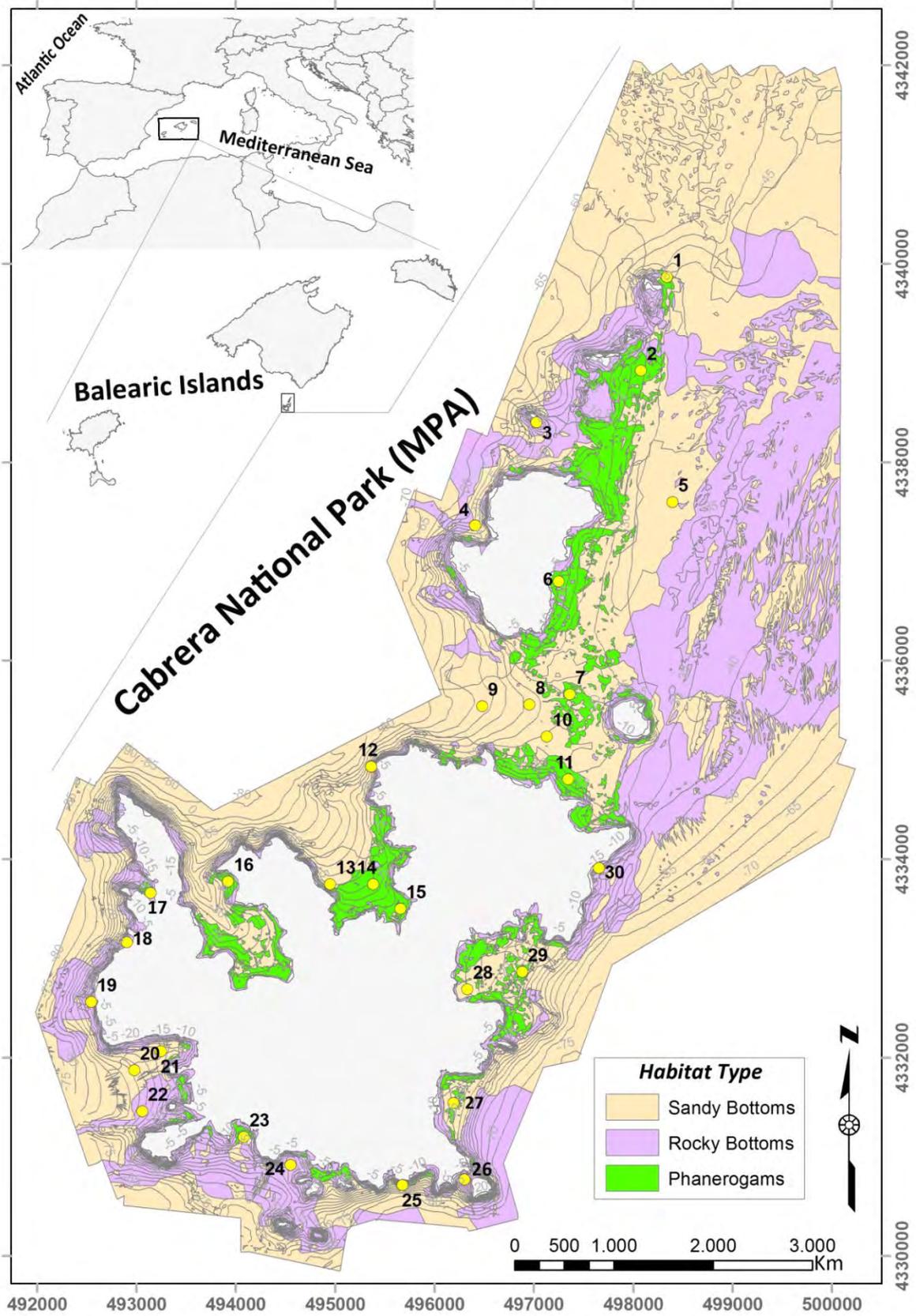


Figure 5. Artificial Device (AD) where squid attached egg clutches: A) Structure of the AD formed by a rope (\varnothing 1.2 cm), a buoy and a weight on the bottom for fixing the structure. The first two meters of rope from bottom contain 5 knots and 16 plastic flanges among these knots aimed to increase the attachment surfaces. B) & C) Egg clutches located on rope or flanges.



D) Detail of the egg clutches while sampling on board.
Figure 6. Location of the study area and distribution of artificial devices (yellow circles) on three different main benthic habitats (note: each isobaths represent 5 m).

Each sampling consisted in set sail with IMEDEA's boat (Fig. 7A) from Cala Nova Port to CNP (journey time: 2 hours and 30 minutes). At CNP, we located the AD previously marked by GPS (Fig. 7B) and recovered the artificial structure with an electric winch (Fig. 7C and 7D). On board, we observed and counted the number of egg clutches and egg strings (Fig. 7E). A subsample was fixed with ethanol 70% for possible future embryological analysis (Fig. 7F). Then, we removed the egg clutches (to avoid over counting them in the next sampling date) and relocated the artificial devices at the same position. We deployed a CTD (YSI Cast Away) to record the hydrographic parameters prevailing in the area. Finally, we collected all the information in a summary report for posterior data analysis (Fig. 7G). In addition, during sampling, *Lepas anatifera* (Linnaeus, 1758) was found on a relatively large number of AD buoys (see results; Fig. 14). The presence/absence of this barnacle was recorded.

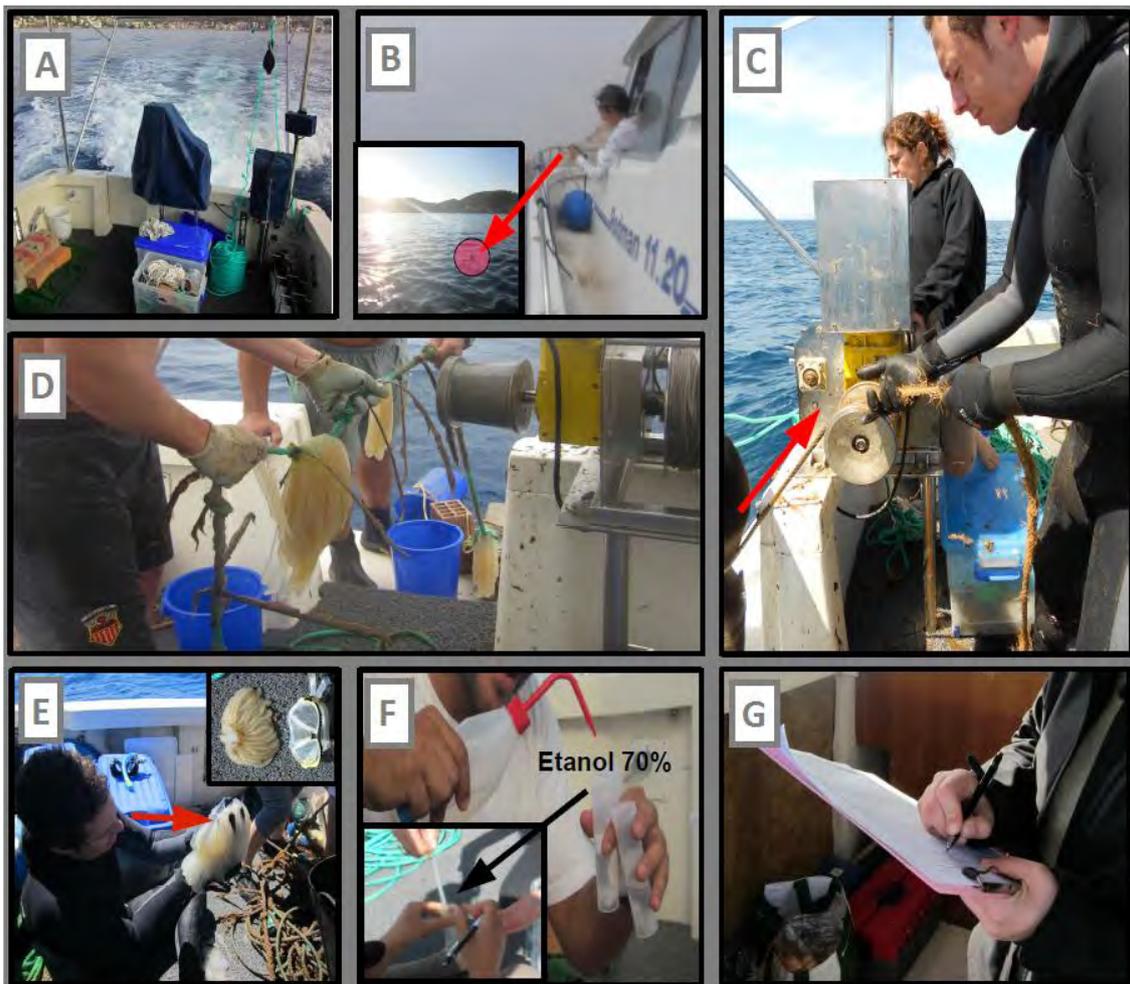


Figure 7. Monthly methodology and fieldwork with AD.

After each sampling and in order to test if the number of squid egg clutches in the natural environment are correlated to the number of clutches in artificial devices, a visual surveys by scuba diving was completed (Fig. 8). Each visual survey consisted in a transect of 50 m². All transects were recorded with video cameras (Canon Ixus 105 and Go-Pro HERO 3) which allowed to identify different habitats characteristics. Twelve's diurnal and one nocturnal visual census were undertaken in the CNP waters. These surveys comprised a total of 18 diving hours.



Figure 8. Visual surveys by scuba diving searching for egg clutches around CNP waters.

2.4. Data analysis

2.4.1. Predictive variables

Raw data for all variables were obtained from diverse sources and are at different spatial scales. Therefore, the input data for the analyses were first prepared with the R package (*raster* library; (Van Etten, 2012) and ArcGIS 9.2 (ESRI) to fit the raw data to a common statistical unit (AD-Month). Habitat type (HT) and Depth (D) were obtained from PROYECT LIFE Posidonia web (http://lifeposidonia.caib.es/user/index_cs.htm) at a fine scale (5 m²). Around 24 benthic habitats, characterized by LIFE Project, were grouped in three main Habitat types: 1) Sandy bottoms (HTS), 2) Rocky bottoms (HTR) and 3) bottoms covered by phanerogams (HTP) (Table 1). The daily Sea Surface Temperature (SST; in °C) was obtained from MyOcean web (<http://www.myocean.eu/>) and spatial resolution of 1 Km².

Habitat types	LIFE Project habitat classification
1. Sandy bottoms (HTS)	Fine sand, Coarse sand, Poorly calibrated sand, Coralligenous, Dispersed coralligenous, Coastal dendritic, Precoralligenous, Dispersed precoralligenous,
2. Rocky bottoms (HTR)	Dispersed sciaphilous community, Litoral rock sciaphilous community, Infralitoral rock photophilic community, Dispersed photophilic community, <i>Peyssonnelia</i> coastal detrital, <i>Vidalia</i> coastal detrital, Pebbles coastal detrital, Precoralligenous on hard bottom.
3. Phanerogams (HTP)	Dense <i>Cymodocea</i> , Dispersed <i>Cymodocea</i> , Isolated seagrass, Seagrass with batches, Continuous seagrass, Degraded seagrass, Rocky seagrass, <i>Cymodocea-Caulerpa</i> grassland.

Table 1. Reclassification of the Habitats types from LIFE PROYECT characterization.

2.4.2. Zero-Inflated Poisson Model

The conventional approach for relating environmental variables with species abundance is the use of linear models as, for example, analysis of variance (ANOVA) or regression. The three basic implicit assumptions of these methods are: 1) that the residuals must be normally distributed, 2) variance

homoscedasticity and 3) independence of observations (Zuur et al., 2009). However, preliminary inspection of the variable response (*Egg Clutches_{ij}* ; number of egg clutches at the AD i^{th} and per sampling period j^{th}), suggested non normal distribution of our data and, therefore, more sophisticated methods are needed. The most usually distribution assumed in the case of count is the Poisson distribution because it is discrete and positive-defined (Zuur et al., 2009). However, the Poisson distribution implies an astringent condition on the relationship between mean and variance (Millar, 2011). Most of the real data sets do not fit such a constraint because the problem of zeroes excess (it is said that the distribution is over dispersed; Zuur et al., 2009). Frequently, the observed counts where the results of two processes. The count process itself may give any value including zero (“true” zeroes: egg clutches are not here because the habitat is unsuitable). However, for a variety of reasons, egg clutches may be laid at these environmental conditions but they are not currently laid detected (“false” zeros). The resulting distribution is typically a mixing of a Poisson distribution and a binomial distribution (the later distribution determines the probability of obtaining a false zero). This family of models is known as Zero-Inflated Poisson (ZIP) models (Zuur et al., 2012). A Zero Inflated Poisson (ZIP) model was applied to model the response variable (number of egg clutches by AD and per month) as a linear combination of the potential explanatory variables: Habitat Type (Sandy: *HBT*, Rocky: *HTR* and Phanerogams: *HTP*), Depth (*D*) and Sea Surface Temperature (*SST*). In turn, a random effect term (*Month*) was included in order to take into account that the samples taken at the same sampling event are no independent but structured by months. The ZIP model considered was

$$\begin{aligned}
 EggClutches_{ij} &\sim ZIP(\mu_{ij}, \pi) \\
 ExpEggClutches_{ij} &= \mu_{ij}(1 - \pi) \\
 Log(\mu_{ij}) &= \beta_0 + \beta_1 HTS + \beta_2 HTR + \beta_3 D + \beta_4 SST + \beta_5 D * SST + MonthEffect_j \\
 MonthEffect_j &\sim Normal(0, \sigma) \\
 Logit(\pi) &\sim Normal(0, \sigma_\gamma)
 \end{aligned}$$

where i denotes the 30 AD examined per sampling and j the number of samplings (11).

However, currently there is no closed statistical package allowing the fit

of such a ZIP models including random effects. Therefore, this model was fitted using the Bayesian machinery as implemented in JAGS (<http://mcmcjags.sourceforge.net/>) and using the R2jags library (<http://cran.rproject.org/web/packages/R2jags/index.html>) from the R package (<http://www.r-project.org/v2.15-2>).

Conventional tools were used for assessing proper mixing of the Monte Carlo Markov chains (MCMC), its convergence and lack of autocorrelation (thinning interval = 500; Number of chains = 3; Sample size per chain = 1000). After model fitting, model residuals were inspected for over dispersion (Zuur et al., 2012). The occurrence of an identifiable effect of any putative explanatory variable was evaluated based on 95% Bayesian credibility intervals (CI) for β_s (and whether these intervals included zero).

2.4.3. Complementary variables

To improve the interpretation of the results, some complementary variables (not included in the ZIP model) has been examined. *Lepas anatifera* has been recruited in certain AD buoys (Fig. 14). This unexpected finding allowed us to use the presence/absence of this filter-feeder species as a proxy (bioindicator) of zones with optimum currents and food availability (Inatsuchi et al., 2010). The presence/absence of this barnacle (for all study period and on each AD) was related to explanatory variables (Habitat types and Depth) using a Generalized Linear Model (GLM), as implemented at the library *lme4* of the R package: (<http://cran.r-project.org/web/packages/lme4/index.html>).

Another complementary variable was Sea Surface Chlorophyll (SSC; in mg m^{-3}) was used to interpret the results. This variable was downloaded from MyOcean web with spatial resolution of 1 Km^2 . In order to avoid a high number of missing values, monthly average values of SSC could only be obtained.

3. Results

Spawning activity of *L. vulgaris* occurs during all-year-round, reaching a maximum peak at spring season (maximum number of egg clutches recorded in March; Fig. 9). This squid seems to prefer spawning grounds between 25 m to 50 m avoiding shallow waters (from 5 m to 15 m depth; Fig. 10). AD_s accumulated a total of 242 egg clutches of which 72.3 % were located on HTS (Fig. 11). Two zones seem to be especially suitable for spawning: Ses Rates and El Canal (Fig. 10). Egg clutches at AD were witnessed during all months of sampling in the Canal. On contrary that at Ses Rates, were in January none egg clutches were found (Fig. 9).

The results of the ZIP model are summarized at Table 2. These results demonstrated an effect of Habitat Type on the spawning preferences of *L. vulgaris*. Specifically, HTS and with a lesser extent HTR showed larger number of egg clutches than the reference habitat, HTP (Table 2; CI including zero). Concerning Depth, the ZIP model showed a negative effect of this variable (the deeper the AD the larger the number of egg clutches) on the squid spawning preference. SST by itself does not affect the spawning preferences of *L. vulgaris*. However, the interaction between depth and SST showed a relevant effect on the spatio-temporal spawning preferences of squid due to searching optimal temperature ranges to ensure normal development and hatching of paralarvae. (Table 2).

We also mapped (Fig. 12 & 13) the expected mean number of egg clutches at the sampling area. At Figure 12 we can observe a comparison between two different temporally seasons, winter and summer. At September, egg clutches appear from 30 m to 50 m, but at February egg clutches, are predicted to increase the depth range reaching until 15 m. The expected spawning activity during each sampled month around CNP waters are detailed at Figure 13.

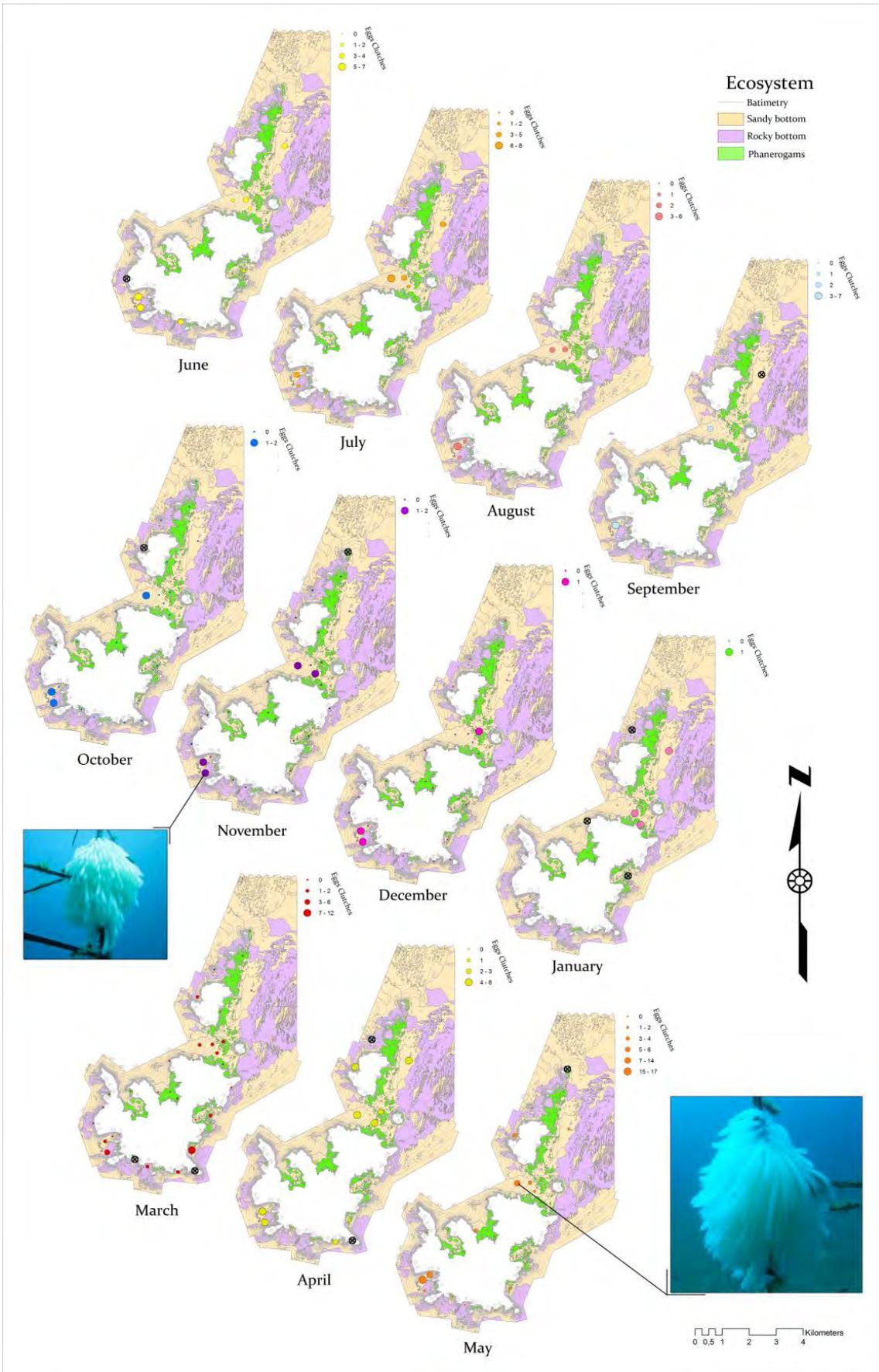


Figure 9. Egg clutches accumulated in AD at each sampling month. Spawning was observed during all year of sampling at different depths. Black symbol (⊗) indicates AD lost.

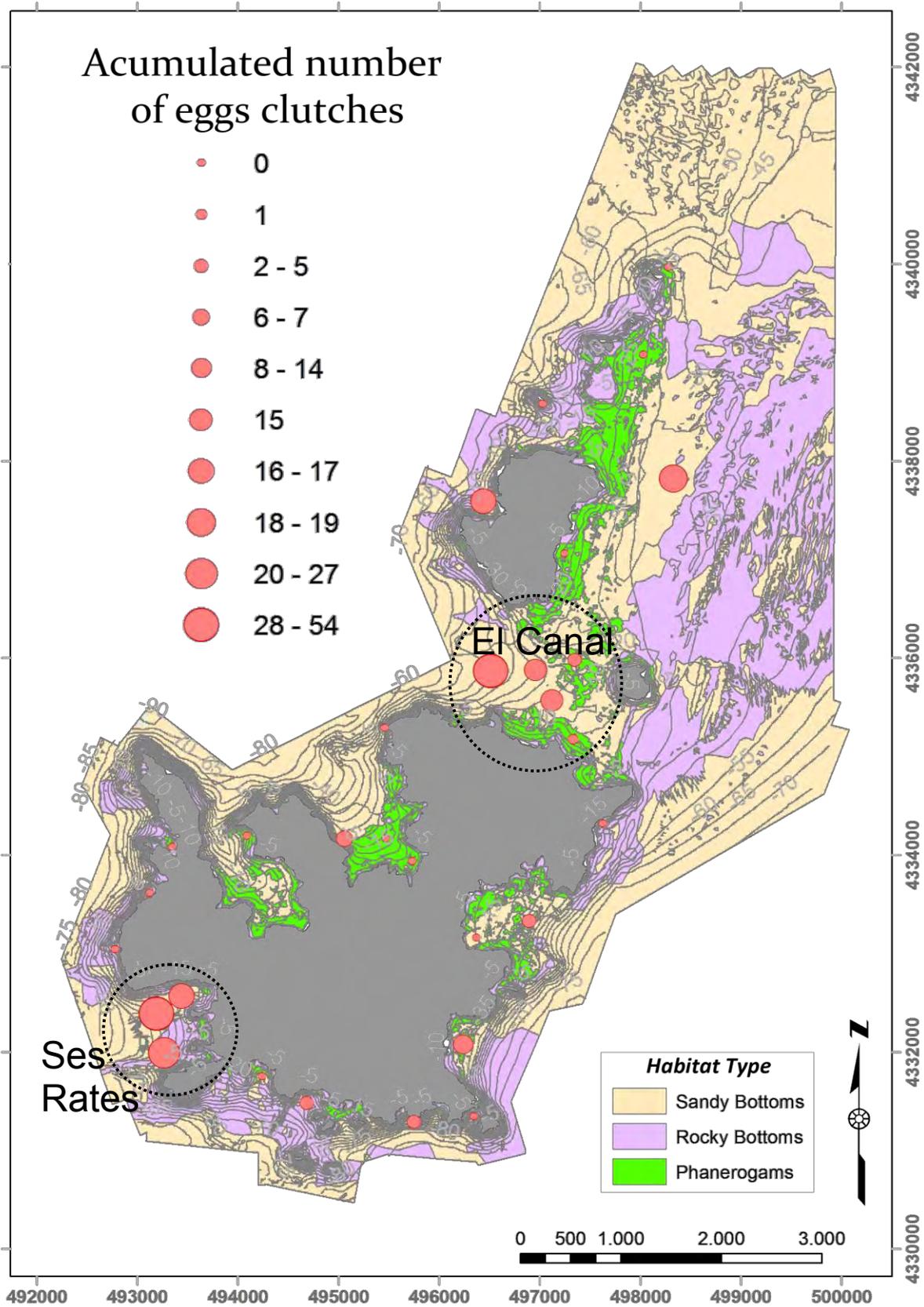


Figure 10. Spatial distribution of the accumulated number of egg clutches by each AD during one year of sampling. Two preferential zones for spawning were observed during almost all year: Ses Rates and El Canal.

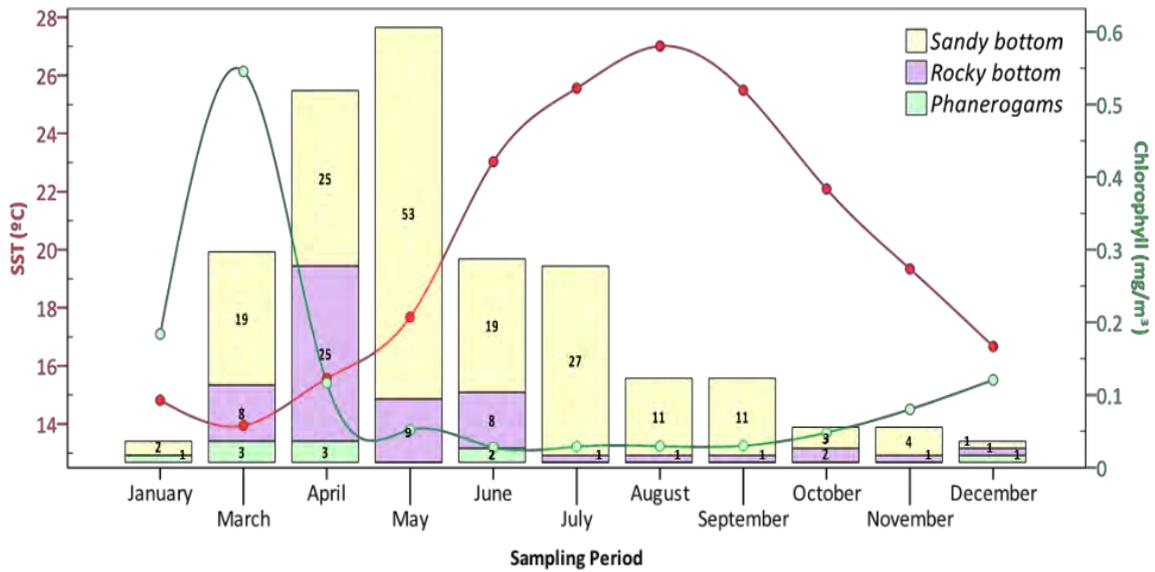


Figure 11. *Loligo vulgaris* spawning (cumulated number of egg clutches per month) related with SST (red line) and Chlorophyll (green line).

Parameters	Description	Mean	SD	Bayesian Credibility Intervals			
				2.5%	Median	97.5%	
π	False zeroes parameters	0.281	0.078	0.135	0.278	0.434	
Fixed factors	β_0	Grand mean	-2.445	0.609	-3.800	-2.390	-1.355
	<i>HTS</i>	Habitat type sandy	1.772	0.388	1.056	1.747	2.605
	<i>HTR</i>	Habitat type rocky	1.016	0.403	0.271	0.989	1.883
	<i>D</i>	Depth	-0.076	0.008	-0.093	-0.076	-0.060
	<i>SST</i>	Sea Surface Temperature	-0.126	0.099	-0.321	-0.126	0.056
	<i>D*SST</i>	Depth*Sea Surface Temperature Interaction	-0.007	0.002	-0.011	-0.007	-0.004
Random	σ_v	Month effect	1.331	0.475	0.739	1.242	2.445

Table 2. Summary statistics for the posterior distributions of fixed and random effects. Relevant fixed effects are highlighted in grey.

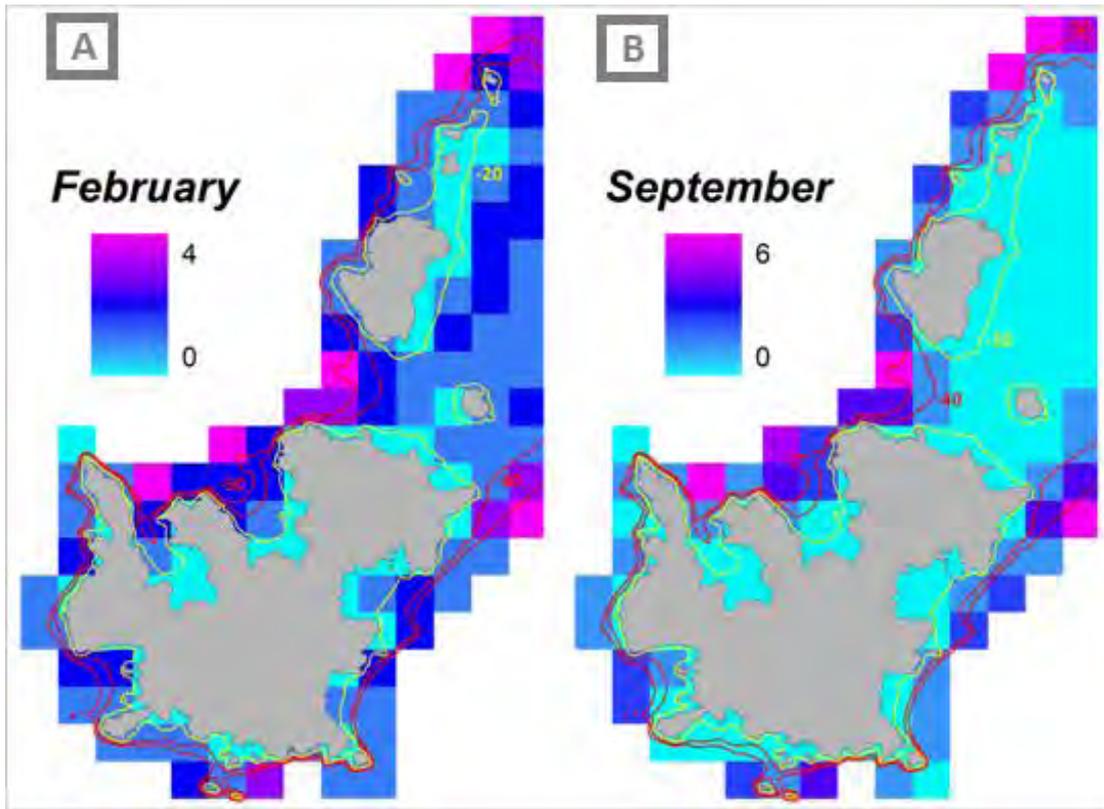


Figure 12. Predicted maps of expected mean number of egg clutches in: A) a cold month and B) a warm month. Isobaths of 50 m and 40 m are represented with red lines. Isobaths of 20 m depth is represented with a yellow line.

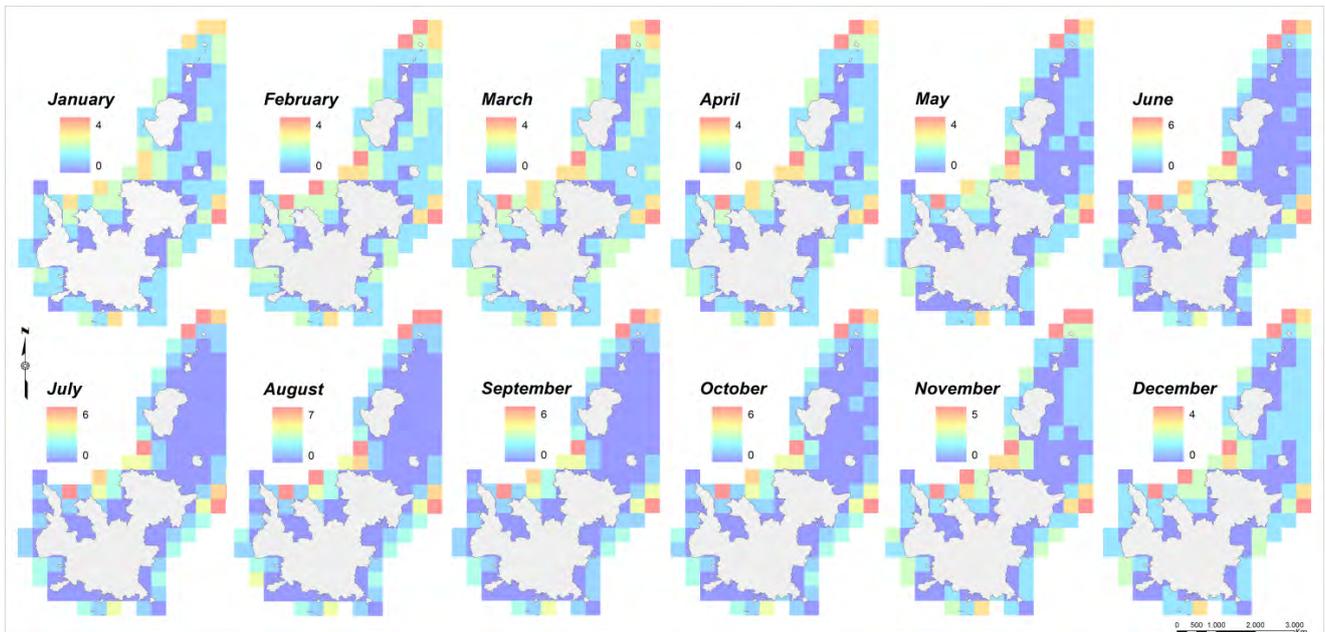


Figure 13. Monthly prediction maps of the expected mean number of egg clutches for one year around at CNP waters by ZIP model.

The unexpected recruitment of *L. anatifera* (Linnaeus, 1758) was found on a relatively large number of AD buoys when the structures were recovered (Fig. 14). The presence/absence of *L. anatifera* versus egg clutches has been analyzed. We found a strongly relation between presence/absence of *L. anatifera* and the variables Depth and Habitat types. That is, the presence of this organism was related to deeper HTS (GLM results $p < 0.05$). Moreover, the presence of this barnacle was linked with squid spawning (see discussion; habitat type).

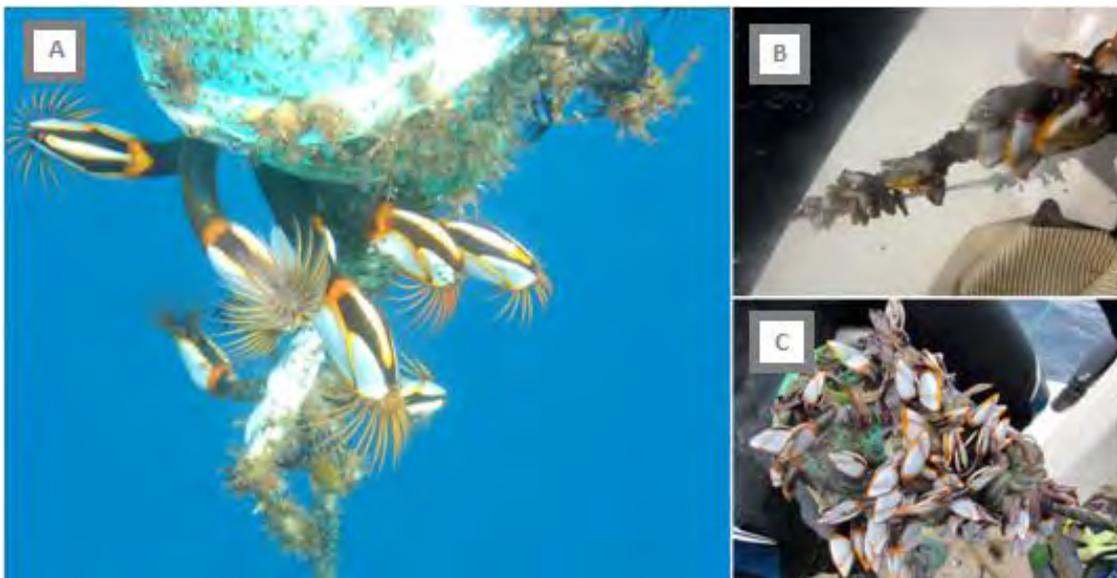


Figure 14. A), B) and C) Example of buoy and rope of and AD covered with *L. anatifera*. AD n° 24, date: 14 of february of 2013 (by Miquel Gomila).

Finally, no egg clutches were founded into the wild during the underwater transects. Figure 15, describes the distribution of visual surveys by geographical location, HT, AD, depth and presence of most relevant organisms in the area.

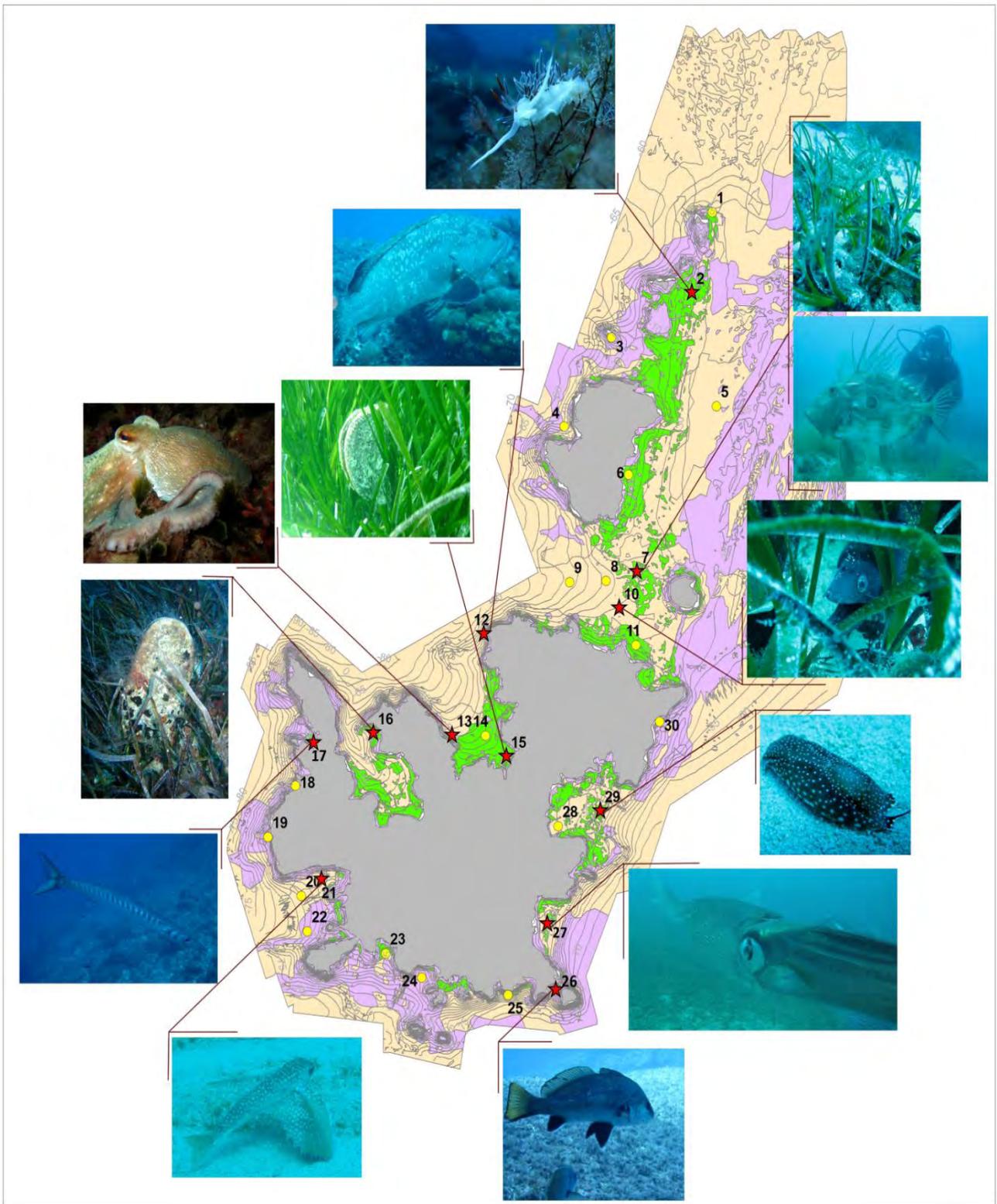


Figure 15. Map of the visual surveys sampled around CNP waters. Each survey has a photo with the most characteristic organism of transect.

4. Discussion

This study describes the preferred spawning area of the European squid, *Loligo vulgaris*. It was well known that the distribution and abundance of species in different marine environments are affected by the characteristics of the bottom sediments together with the oceanographic properties (Valavanis et al., 2004). Most of the cephalopods have the ability to adjust their bathymetric range and their life cycle according to the prevailing environmental conditions (Boyle and Rodhouse, 2005). Our results suggest that the preferential spawning areas are sandy bottoms. This preference may be related to the marine currents prevailing in this zones and the absence of potential predators. On the other hand, all-year-round *L. vulgaris* spawn was performed at 30 m to 50 m, although during colder months, squid expand their spawning activity to shallower waters (suggesting inshore migrations). Finally, the spawning peak in spring may be related with the higher values achieved by the cascade of the phytoplankton-zooplankton. This fact could ensure food availability to paralarvae.

4.1. Habitat type

The preferred habitat type of *L. vulgaris* for spawning at CNP was HTS. This habitat preference has been also observed in other related loliginid species *Loligo reynaudii* (Sauer and Smale, 1993) and *Loligo plei* (Vecchione, 1988), whose spawning aggregations are mainly observed at sandy bottoms and seems in dependent of solid the availability of surfaces for egg-laying. At a similar geographic region (Central and NW, Mediterranean Sea), coasts with dominant sand and sandy-muddy bottoms have been proposed as potential spawning areas for *L. vulgaris* (Valavanis et al., 2002; Sanchez et al., 2008). However, some loliginids like *Loligo forbesi* choose rocky bottoms to form spawning aggregations (Smith et al., 2013). Attending only to the larger prevalence of natural structures to attach egg clutches of HTR (Hanlon and Messenger, 1996), this habitat type seems to be more a priori appropriated for spawning than HTP. Our results support this statement. Nonetheless, we demonstrate that sandy bottoms are preferred over any other habitat type and we suggest that it may be related to the great abundance of the potential

predator *Ephinephelus marginatus* associated to CNP rocky bottoms (Reñones et al., 1997; Reñones et al., 1999). Presence of egg clutches attached at AD located over rocky bottoms was mainly observed during the cold season (Fig. 9 & 11). This spatio-temporal pattern may be linked to *E. marginatus* migrations to deeper water during winter, decreasing their abundance at shallower waters (Harmelin and Harmelin-Vivien, 1999) and consequently decreasing the predation pressure on rocky spawning grounds. In fact, in past studies it has been observed that predator induce disruptions in egg deposition and cause the absence of chokka squid (*L. reynaudii*) from certain habitat types (Smale et al., 2001).

But not only presence of predators triggered a positive selection for HTS as preferential spawning ecosystem. Our results also suggested a relationship with the oceanographic conditions that prevails on this type of habitat (e.g., optimal currents). Filtering organisms (*Lepas anatifera*) which were found in AD buoys (Fig.14), served as indicators of zones more exposed to currents. The presence of filter-feeders was higher at HTS sites. This barnacle usually recruits at zones where currents and food availability are adequate for their survival (Inatsuchi et al. 2010). Therefore, it is not unreasonable that squids prefer habitats influenced by currents, which may be related to several aspects of the paralarvae survival, such as oxygenation of the egg masses or paralarvae dispersion. Some studies demonstrated that large aggregation egg can reduce ambient oxygen availability causing severe problems on the development and survival of embryos (Cohen and Strathmann, 1996; Steer and Moltschanivskyj, 2007). Other loliginid species, for example, *Doryteuthis* (formerly *Loligo*) *gahi*, attach their egg clutches at the margins of the Kelp forest, exposing them to optimal currents (Arkhipkin et al., 2000). In the same way, the chokka squid (*Loligo reynaudii*) mainly spawns on areas where the bottom dissolved oxygen is increased by wave action and prevailing currents (Roberts, 2005). After hatching, the paralarvae are dispersed by currents and eventually reach nursery areas that should ensure feeding and growth during the early life cycle of squid (Roberts and Van Den Berg, 2002; Roberts and Van den Berg, 2005; Martins et al., 2013). Further investigation in this field is needed in order to disentangle the potential role of currents in squid paralarvae distribution (i.e., presenting adequate oxygen level versus paralarvae dispersal).

4.2. Seasonal patterns

Our study suggests that *L. vulgaris* not only has preference for some specific habitats types to spawn. In addition, this squid seems to search the opportune season to maximize the survival of their offspring. Results shown a spawning peak synchronized with the bloom of nutrients (cascade phytoplankton-zooplankton, see Fig. 11). Some studies (Villa et al., 1997) observed an increasing number of *L. vulgaris* egg clutches during periods of higher zooplankton abundance. Despite the European squid spawns all-year-round, it focuses their reproductive effort on specific seasons (Guerra and Rocha, 1994; Moreno et al., 1994; Arkhipkin, 1995) and this pattern seems to be related to environmental conditions, which may be specific to each geographical area (Moreno et al., 2002; Boavida-Portugal et al., 2010). In agreement with the results obtained at the Central Mediterranean Sea (Šifner and Vrgoč, 2004), the spawning peak in CNP was centered on winter-spring season (Fig. 11). Chlorophyll peaks during March and also after this primary production peak at CNP, the mesozooplankton biomass reached the maximum concentrations (Álvarez et al., 2012) coinciding with the maximum number of egg clutches recorded (Fig. 11). Consequently, this finding reinforces the idea that the European squid matches their spawning with the peak of nutrients, probably for maximizing food availability for the paralarvae, one of the crucial phases for the survival of early life stages (Vidal et al., 2002; Vidal et al., 2005).

4.3. Spatio-temporal migrations

SST by itself has no relevant effect on spawning (Table 2). However the interaction between SST and Depth significantly affects spawning (see model prediction, Fig. 12 & 13). *L. vulgaris* spawned all-year-round at deep waters (50-40 m depth), and during cold months (when inshore waters reached lower temperature values), squid increased their spawning area reaching inshore waters (40-17 m depth). This result is compatible with the hypothesis of seasonal inshore-offshore migrations of this species suggested by others studies in the Mediterranean Sea (Guerra et al., 1994; Valavanis et al., 2002; Cabanellas-Reboredo et al., 2012b). Other loliginids, like *L. reynaudii*, perform this type of movement patterns, being temperature one of the main environmental drivers involved on the inshore spawning migrations experienced

by this species (Sauer et al., 1991). We suggest that *L. vulgaris* searches for a suitable range of temperature for maximizing hatching success. Reproductive success seems to be maximized within a relatively narrow range of sea temperatures, which is 12°C to 17°C for *L. vulgaris* (Villanueva et al., 2003), and such a range maximizes hatching success too (Şen, 2005b).

In spite that our study was focused at the shallowest part of the neritic zone of CNP, there are evidences of spawning activity at deeper areas (90-100 m; personal observations from artisanal fishery). Therefore, further studies should be developed in order to identify deeper spawning grounds in the Mediterranean. This can help to disentangle future probable implications on the stock management of *L. vulgaris*, due to it is probably that these deeper spawning grounds could be disturbed by human activity (e.g., by trawl fishery).

4.4. Visual surveys

No egg clutches were founded in visual transects of natural environment. This result may be due to logistic problems. The design of the transects covered a relativity small sampling area and depth range of Scuba diving only covered until 25 m depth. We suggest to realize transects with a higher number scuba divers in the future to cover more sampling area and/or using a sea scooter propulsor device to reach more depth and distance.

4.5. Management proposals

Management planning may benefit from knowing the preferential spawning areas of this species. Resource management of cephalopods has been performed with different techniques (Pierce and Guerra, 1994; Otero et al., 2005), which have led from the imposition of minimum legal size, establishment of closed seasons, catch quotas and fishing power limitation. However, due to their specific biological characteristics (short life cycle, breeding once in its life, and high turnover rate of annual biomass) most management techniques have not worked properly with cephalopods, and have not been effective in preventing the acute decrease of some stocks (Guerra et al., 2010). Limiting the access to the resource by MPA may ensure limited success in protecting mobile species. For such species, several authors have acknowledge that MPAs may not offer effective protection (e.g. Afonso et al.,

2009; Gerber et al., 2003; Kramer and Chapman, 1999; Nowlis and Roberts, 1999), particularly because of the small size of most coastal MPAs. This seems to be the case for *L. vulgaris*, may perform seasonal migrations between shallow and deeper waters (Cabanellas-Reboredo et al., 2012b).

However, MPAs may still play a role in protecting *L. vulgaris* populations reproductive output, provided that squid encompass most of the spawning effort where this species attaches eggs to specific spawning. Moreover, protecting spawning grounds may be relevant for the management of cephalopods because landings are strongly dependent on the annual recruitment of paralarvae and more specifically on a combination of environmental factors that control paralarvae distribution and abundance within Essential Fish Habitat (EFH) areas where growth and reproduction occurs (Sánchez et al., 2008). In fact, recently, the protection of preferential reproductive EFH for other cephalopod, *Octopus vulgaris*, has been proposed in another Marine National Park (Guerra et al., submitted).

We conclude that to protect reproductive EFHs like the area of Ses Rates and the zone of El Canal could be favourable to *L. vulgaris* fishery. However, large fishing effort concentrates at these areas (Frontera et al., 1993) suggesting that the fishermen are taking advantage of these EFH. As the species is highly mobile and could export biomass outside the Park, a compromise solution maybe protect them only during the peak spawning and hatching period in spring.

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7. Publications and divulgation activities

1. Cabanellas-Reboredo, M., **Calvo-Manazza, M.**, Miquel Palmer, Jorge Hernández Urcera, Manuel E. Garci, Ángel F. González, Ángel Guerra, Beatriz Morales-Nin. Submitted in 2013. "New insights for cephalopods management: Identification of preferential spawning areas for the European squid." *Marine Biology*.
2. Ángel Guerra, Jorge Hernández-Urcera, Manuel E. Garci, Marta Sestelo, Marcos Regueira, Ángel F. González, Cabanellas-Reboredo, M., **Calvo-Manazza, M.** and Beatriz Morales-Nin. Submitted in 2013. "Spawning habitat selection by *Octopus vulgaris*: New information for management of this resource within a National Park." *Estuarine, Coastal and Shelf Science*.
3. **Calvo-Manazza, M.**, Cabanellas-Reboredo, M., Miquel Palmer, Jorge Hernández Urcera, Manuel E. Garci, Ángel F. González, Ángel Guerra, Beatriz Morales-Nin. (2013). Identification of preferential spawning areas for the European squid. 40th CIESM Congress (Marseille, France, 28 October - 1 November 2013).
4. Cabanellas-Reboredo, M., **Calvo-Manazza, M.**, Morató, M., Palmer, M., Hernández Urcera, J., Garci, M.E, González, Á.F., Guerra, A. and Morales-Nin, B. (2013). Preferential spawning areas for the European squid at Cabrera National Park. VI Jornades de Medi Ambient de les Illes Balears (Octubre 2013).
5. Morató, M., Deudero,S., Vázquez-Luis, M., Álvarez, E., Cabanellas-Reboredo, M. and **Calvo-Manazza, M.** (2013). Experiència de sembra de juvenils de *Pinna Nobilis* com a proposta de gestió activa. VI Jornades de Medi Ambient de les Illes Balears (Octubre 2013).
6. **Calvo-Manazza, M.** and Pep Pomares. (2013). La pesca del calamar de potera (*Loligo vulgaris*). Jornadas del Calamar de Formentera, organizadas por Nautica Pins-Awa Shima ,Consell Insular y Carbonicas Tur (Febrero 2013).