

Biological parameters, ecology and population trends of the Mediterranean endemic skate, *Raja polystigma*, in the Balearic Islands

Francesca Ferragut-Perello ¹, Maria Valls ¹, Miquel A. Cortes-Pujol ², Sergio Ramirez-Amaro ¹, Beatriz Guijarro ¹, Francesc Ordines ¹.

¹IEO - Centre Oceanogràfic de les Balears, Moll de Ponent s/n, 07015 Palma, Spain.

(FF-P) (Corresponding author) E-mail: francesca.ferragut@ieo.es. ORCID-ID: <https://orcid.org/0000-0001-7688-6280>

(MV) E-mail: maria.valls@ieo.es. ORCID-ID: <https://orcid.org/0000-0001-9070-8181>

(SR-A) E-mail: sergio.ramirez@ieo.es. ORCID-ID: <https://orcid.org/0000-0002-0298-0749>

(BG) E-mail: beatriz.guijarro@ieo.es. ORCID-ID: <https://orcid.org/0000-0002-2083-4681>

(FO) E-mail: xisco.ordines@ieo.es. ORCID-ID: <https://orcid.org/0000-0002-2456-2214>

²Carrer Voltor 27, 07011, Palma, Spain.

(MAC-P) E-mail: miquelangelcortespujol@gmail.com. ORCID-ID: <https://orcid.org/0000-0002-6383-5609>

Summary: Elasmobranchs are a vulnerable group that has been overexploited for almost half a century in the Mediterranean. However, few elasmobranch species have been assessed because information on their basic biological parameters is lacking for most species. *Raja polystigma* is a poorly studied endemic skate in the Mediterranean. The aim of this work is to determine some basic life history parameters of this skate in the Balearic Islands. Data were collected from commercial catches and during MEDITS surveys. Total length varied from 17 to 59 cm for both sexes, with a higher proportion of females than males. Allometric and morphometric relationships differed between sexes, females being heavier for a given size. Females matured at a larger size than males (L_{50} 47.7 cm and 38.4 cm, respectively). Potential fecundity ranged between 11 and 45 yolked oocytes per female, and spawning was observed mainly during winter and spring. Persistent hotspots of adults and juveniles were detected on the shallow shelf off the northwest of Mallorca and east of Menorca, with adults being found mainly in the shallow and juveniles in deeper areas. Time series of abundance and frequency of appearance showed stable trends throughout the study period (2003-2018).

Keywords: speckled ray; biology; reproduction and condition; diet; fecundity; distribution; population indicators; western Mediterranean.

Parámetros biológicos, ecología y tendencias poblacionales de la raya endémica del Mediterráneo, *Raja polystigma*, en las Islas Baleares

Resumen: Los elasmobranquios son un grupo vulnerable que ha sido sobreexplotado durante casi medio siglo en el Mediterráneo. Sin embargo, pocas especies de elasmobranquios han sido evaluadas ya que falta la información sobre la biología básica de muchas especies. *Raja polystigma* es una raya endémica del Mediterráneo muy poco estudiada. El objetivo de este trabajo es conocer algunos parámetros biológicos básicos de esta raya en las Islas Baleares. La información fue obtenida de capturas comerciales y durante las campañas oceanográficas MEDITS. La longitud total varió entre 17 y 59 cm para ambos sexos, con una mayor proporción de hembras que de machos. Las relaciones alométricas y morfométricas fueron diferentes entre sexos, con hembras más pesadas para la misma talla. Las hembras maduraron a una mayor talla que los machos (L_{50} 47.7 cm and 38.4 cm, respectivamente). La fecundidad potencial osciló entre 11 y 45 ovocitos vitelados por hembra, y el desove se observó principalmente en invierno y primavera. Se detectaron *hotspots* persistentes de adultos y juveniles en la plataforma al noroeste de Mallorca y al este de Menorca, con adultos principalmente en áreas someras y juveniles en áreas más profundas. Las series temporales de abundancia y frecuencia de aparición mostraron tendencias estables a lo largo del período estudiado (2003-2018).

Palabras clave: raya manchada; biología; reproducción y condición; dieta; fecundidad; distribución; indicadores de población; Mediterráneo Occidental.

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INTRODUCTION

Batoidea infraclass (hereafter “batoids”), which include skates, rays, stingrays and their allies (Carrier et al. 2004), are considered vulnerable species (Dulvy et al. 2014). In general, the high vulnerability of batoids in comparison with most teleost fishes is related to vital traits such as their large body size, slow growth, late sexual maturation and low fecundity (Stevens et al. 2000, Dulvy and Reynolds 2002, Frisk et al. 2005). Although batoids are not usually target but by-catch species, these vital traits make them particularly vulnerable to the impacts of fishing exploitation, resulting in a global decline and a large proportion of species being assessed as overexploited (Stevens et al. 2000, Worm et al. 2013, Dulvy et al. 2014).

The Mediterranean Sea has been reported as one of the regions where elasmobranchs are most threatened in the world (Dulvy et al. 2014). In this sea, up to 15 species of skates (Rajidae) are present (Meléndez et al. 2017), three of them considered endemic: *Leucoraja melitensis*, *Raja radula* and *Raja polystigma* (Dulvy et al. 2016). *Raja asterias*, formerly considered a Mediterranean endemism, was recently reported from the gulf of Cádiz, northeastern Atlantic (Ordines et al. 2017).

The speckled ray, *Raja polystigma* (Regan, 1923), is a small skate, with a maximum total length of about 60 cm (Stehmann and Bürkel 1984, Frodella et al. 2016). It is found in soft-bottom habitats ranging from 100 to 400 m depth and has been reported from the northwest Iberian Peninsula, the Gulf of Lion, the Ligurian and Tyrrhenian seas, the Tunisian and Algerian coasts, the Adriatic Sea and the Greek coast (Capapé and Quignard 1978, Capapé et al. 2006). It can be an abundant by-catch of the bottom trawl fisheries in some areas of the northwestern Mediterranean, such as the Balearic Islands (Ramírez-Amaro et al. 2015, Follesa et al. 2019) and Sardinia (Porcu et al. 2020), although it can be less abundant or rare in other areas, such as the Strait of Sicily (Geraci et al. 2021). According to the International Union of Conservation of Nature (IUCN), this species is listed as least concern, although its population has an unknown trend (Ungaro et al. 2015). The high intraspecific coloration variability, which makes it difficult to identify, and the confusion with *Raja montagui* (Cannas et al. 2008, Ramírez-Amaro et al. 2018) may have prevented the analysis of this trend (Frodella et al. 2016).

The reproduction of *R. polystigma* seems to show a large geographic variability in both seasonality and attainment of sexual maturity, even at medium spatial scales. Capapé and Quignard (1978) reported winter as the season with the highest spawning activity on the Tunisian coast, with spawning females (with presence of egg-cases in the oviducts) from 35 cm in total length (TL), with more than 80% of spawning females above 39 cm TL. Porcu et al. (2020) reported spring and summer as the spawning season and the smaller spawning females above 50 cm TL in Sardinia.

So far there is no complete biological and ecological description of the species in any of the areas where it has been studied. These data are needed for

many stock assessment models that allow the state of exploitation of the resources to be evaluated and are the basis of a suitable management of fisheries. Moreover, assessments are scarce for Mediterranean batoids in general, including *R. polystigma*, mainly because of their low economic value and the monitoring programmes aimed at collecting the data needed for stock assessment, which prioritize the populations of target species (Ungaro et al. 2015).

The aim of the present work is to study the main biological characteristics (population structure, distribution, condition, reproduction and diet) of *R. polystigma* in the Balearic Islands and to assess, for the first time, the trend of its population using the data collected during the time series of the MEDITS survey in this area.

MATERIALS AND METHODS

Data source

Information was obtained from two different sources: the monitoring of the commercial fleet in the European Data Collection Framework and the International Bottom Trawl Survey in the Mediterranean (MEDITS).

Commercial catches were collected by an observer on board the commercial bottom trawl fleet from Mallorca between May 2017 and February 2019. Samples were collected from four different harbours of Mallorca on the northwest (Sóller), northeast (Alcúdia and Cala Rajada) and southeast (Cala Figuera) coasts of Mallorca. This trawling fleet uses a square-mesh codend with a mesh size of 40 mm. During each commercial haul, duration, position, depth and vessel speed were recorded. These hauls were performed between 50 and 170 m depth. All the individuals of *R. polystigma* were transported to the laboratory of the Centre Oceanogràfic de Balears (COB), stored at -20°C and sampled biologically on a monthly basis. This biological sampling obtained in these individuals was as follows: individual TL and disc width (DW), both to the lowest mm, and total (TW), eviscerated (EW), liver (LW), digestive tract (SW) and gonad (GW) weights (± 0.1 g); GW includes the ovaries in females and the testes in males. In the case of females with vitellogenic oocytes, these were counted and measured to the lowest 0.1 mm. Egg capsules were also counted when present. Stomachs were used for diet analysis.

Sex and maturity stage were determined macroscopically following a maturity scale (Table 1) based on the description of maturity stages in Brown-Peterson et al. (2011) but adapted to the studied species. The commercial catch data were used for the following sections: population structure, reproduction, condition indices (evolution throughout the year is needed, as well as some of the biological information, e.g. EW, LW and SW, which is not available in the surveys' data), allometric growth (DW is needed but is not available in the surveys' data) and diet.

MEDITS surveys take place annually in late spring or early summer. In the present study, data and samples were collected between the years 2003 and 2018 in Geographical Subarea (GSA) 5 during these surveys.

Table 1. – Maturity scale for skates according to Brown-Peterson et al. (2011). F, females; M, males.

Maturity stage	Sex	Description
Immature	F	Absence of visible follicles. Oviductal glands not detected.
	M	Non-solidified claspers, which do not reach the pelvic fin. Testes small or not distinct. Sperm not present in the sperm ducts.
Developing	F	Large vitellogenic oocytes not detected. Visible oviductal glands.
	M	Claspers reach or exceed the pelvic fin, but are not completely solidified. Testes are detectable but not forming sperm.
Spawning	F	Large vitellogenic oocytes detected.
	M	Claspers reach beyond the pelvic fin and are completely solidified. Presence of sperm in the sperm ducts.
Capsule present	F	Large vitellogenic oocytes detected along with the presence of capsules.
Regenerating	F	Large vitellogenic oocytes not detected. Flaccid and dilated ducts and uterus.

These data were obtained following the international MEDITS protocol. (For specific information on the sampling scheme see Bertrand et al. 2002, Spedicato et al. 2019). During the surveys, an experimental bottom trawl (GOC 73) with a codend mesh size of 18 mm (stretched mesh) is used to sample stations between 50 and 800 m depth. At each station, time, speed, position, depth and horizontal and vertical openings of the net during the haul (averaging 2.5 and 18 m, respectively) are recorded using a GPS and a SCANMAR system, respectively. Once the samples are on board, catches are sorted out by species, counted, sexed and weighed, and the individuals are measured (by TL in the case of *R. polystigma*). In the case of certain species, as *R. polystigma*, the stomach content analysis is also carried out. Density and biomass were standardized to 1 km² using the swept surface during each haul, calculated using the distance covered through the bottom and the horizontal opening of the net. These data were used for geographic and bathymetric distribution, population trend and diet. The only section using data from both sources was diet analysis.

Population structure

The size distribution for TL was obtained by season and for the total of individuals analysed. Differences in the TL distributions by season were assessed by applying the two-sample Kolmogorov-Smirnov test. Sex-ratio was calculated as the percentage of females regarding the total number of individuals in every 5 cm TL interval. These size intervals allowed us to have enough individuals to test for significant deviations from a 1:1 proportion of females and males using a chi-squared test. This analysis was used on the whole

population sex-ratio and also by season. When significant differences were found, standardized residuals of the chi-squared test were analysed in order to identify which sex predominated using the criterion established by MacDonald and Gardner (2000). This criterion sets an absolute value of ±1.96, from which the standardized residual is considered significant.

Allometric growth

The length-weight relationships (TL-TW, TL-EW and DW-EW) and morphometric relationship (TL-DW) were established by fitting a potential curve to the data from the biological samplings of the commercial samples. For all these relationships, we used the same individuals in order to avoid variability. Differences in these relationships between males and females were tested using analysis of covariance (ANCOVA). To do so, lengths and weights were log-transformed in order to linearize their relationship. For length-weight relationships, weight was set as the dependent variable, while length and sex were set as continuous and categorical predictors, respectively. For the morphometric relationship, DW was set as the dependent variable, while TL and sex were set as continuous and categorical predictors, respectively. In case of non-significant differences between sexes, these relationships were recalculated for the whole population.

Reproduction

The reproductive period was assessed from the seasonal evolution of the gonadosomatic index (GSI), calculated following

$$GSI = \frac{GW}{EW} * 100.$$

This index was only calculated with females in the stages “developing”, “spawning” and “capsule present” and with males in the stages “developing and “spawning”. Length at first maturity (at which 50% of the individuals are mature, L₅₀) was calculated for females and males by fitting the logistic curve

$$ML = \frac{e^{(a+b*L)}}{1+e^{(a+b*L)}}$$

where *ML* is the proportion of mature individuals for a given size class *L* and *a* and *b* are the parameters to be estimated. A log-likelihood function was used in SOLVER in EXCEL (Tokai 1997) to fit this model. Then, L₅₀ was calculated as

$$L_{50} = -\frac{a}{b}.$$

Potential fecundity (PF), defined as the maximum number of yolked follicles observed in spawning-capable females (Maia et al. 2012), was considered in this work as the maximum number of vitellogenic oocytes (plus capsules if they were present) observed in a female. Also, the range of PF in all the analysed females

was reported. Both PF and its range were determined for females in the “spawning” or “capsule present” maturity stages, both the maturity stages in which vitellogenic oocytes are present. The relationship between TL and PF was analysed by mean of linear regression of their log-transformed values. Mean PF values per season were also calculated.

Condition indices

Different condition indices were calculated to evaluate their variation throughout the year in relation to reproduction. To this end, we used the data collected from the monthly samplings of the commercial bottom trawl fleet. The Le Cren index (k), which measures the deviation of an individual from the average weight for a given length (Lloret et al. 2014), was calculated as

$$k = \frac{W}{W_t},$$

where W is the observed individual's weight (g) and W_t is the estimated weight of that fish obtained from the TL-W relationship. In the case of differences in the TL-W relationships between males and females, k was calculated for the males and females separately. The Le Cren index was calculated for both the TW and the EW. We also calculated the hepatosomatic (HSI) and digestivosomatic (DSI) indices as the percentage relative weight of the liver (LW) and digestive tract (SW) to EW, respectively. All these indices were calculated for all the individuals available.

Diet analysis

All preys were identified to the lowest possible taxonomic level and counted, and their volume was measured using a trophometer, a calibrated device used in previous studies for this purpose (Olaso et al. 1998, Valls et al. 2011). The diet was described using the following indices: 1) frequency of occurrence (%F), calculated as the percentage of stomachs with a specific type of prey in relation to the total number of stomachs containing food; 2) numerical (%N) and volumetric (%V) composition, calculated as the percentage contribution of each prey to the whole content in number or volume, respectively; 3) index of relative importance, calculated using the formula, which was standardized as

$$\%IRI = \left(\frac{IRI}{\sum IRI} \right) * 100,$$

according to Cortés (1997); and 4) the vacuity index, calculated as the percentage of empty stomachs.

Geographic and bathymetric distribution

To assess the geographic and bathymetric distributions of *R. polystigma*, we used data collected during 832 hauls of the MEDITS surveys between 2003 and 2018. Individuals were classified into adults (above

the L_{50}) and juveniles (below the L_{50}), and this sorting was used for both geographic and bathymetric distributions.

Maps of predicted abundance and persistence of adults and juveniles were created using the standardized abundance (n/km^2) at each MEDITS sampling station. To this end, generalized additive models (GAMs) were used to predict the mean values in each of the $0.01 \times 0.01^\circ$ cell grids of the whole study area by year for juveniles and adults. The standardized abundances of the two population fractions were modelled using a two-stage GAM approach (Barry and Welsh 2002), owing to the presence of a larger proportion of zeros than expected from a Gaussian distribution (zero inflation). First, presence-absence was modelled with a logit link function and, second, the log-transformed abundance conditional on presence was modelled using an identity link function. The explanatory variables were year, included as a factor, and the smoothed effects of depth and the geographic position of the haul (latitude and longitude). Final model election was based on significance of the explanatory variables and minimization of the value of the Akaike information criterion. Finally, assuming independence of the two model stages, abundance predictions were obtained by multiplying the prediction of both stages. Although no specific correlation term was included in the modelling, the introduction of latitude and longitude as predictor variables implicitly incorporated the spatial structure. The mgcv package (Wood 2011) of the R statistical software (R Core Team 2018) was used for the whole modelling process.

Once the abundance maps were created, we identified the hotspot areas of each population fraction on a yearly basis following a similar approach to that of Colloca et al. (2015). Initially, hotspot areas were identified as every grid cell of every particular year in which the predicted value was above the upper 95% confidence interval of the mean abundance for the whole time series. Second, hotspot persistence was defined as the percentage of years for which a particular cell was considered a hotspot. Third, we defined areas considered as persistent hotspots as those areas with persistence above 70%. Finally, spatial overlap of persistent hotspots of adults and juveniles was defined as cell grids in which both population fractions show persistent hotspots. Maps with the mean annual abundance and persistence of both population fractions and of the overlap of persistent hotspots were created using the Surfer 8 software.

To assess the bathymetric distribution, Generalized linear models (GLMs) and linear regression were used to evaluate the effect of depth (set as the continuous predictor) on the presence of the species at each sampling station and to obtain a bathymetric distribution model for each species. These models were applied for the whole population, the adult population and the juvenile population. The response type (unimodal or linear) of each fraction of the population was chosen first on the basis of resulting significant and second on the basis of the lowest value of the Akaike information criterion.

Population trend

Frequency of appearance and annual abundance were used as indicators based on the surveys' data to assess the population state of *R. polystigma*. We calculated the frequency of appearance and the annual abundance for each year (2003-2018) during the time series of the MEDITS surveys by dividing the number of sampling stations in which the species appeared by the total number of hauls carried out each year within the species' bathymetric range and by calculating the mean annual individuals per km², respectively. Baselines were calculated as the mean frequency of appearance and the mean annual abundance of the first three years (2003-2005).

We also assessed the evolution of the mean PF per km² (population PF). To do so, we first applied the PF equation (which relates the TL and the number of oocytes produced) to estimate the fecundity (in number of produced oocytes) of each adult female (above the L₅₀) in the MEDITS data. Then, using the swept area in the haul, we calculated the estimated number of oocytes produced per km² in each haul. The average number of oocytes per km² was calculated from all hauls carried out within the bathymetric range of distribution of *R. polystigma*, including those in which the species was not present (i.e. counting as zero). A linear regression model was used to detect significant temporal trends in frequency of appearance, abundance and population PF.

RESULTS

Population structure

Of the 236 individuals of *R. polystigma*, 98 were males and 138 females. The sizes ranged between 17 and 59 cm TL and between 11 and 39 cm DW. The species showed a size distribution with a mode between 37 and 39 cm (Fig. 1).

The size distribution changed throughout the year (Fig. 2). In the winter and summer seasons TL showed the widest range (between 16 and 60 cm TL in both seasons). In spring, the smallest and largest sizes did not appear and there was a mode in the sizes between 40 and 42 cm of TL. In summer, the mode was observed in sizes between 37 and 39 cm TL. In autumn, the mode was detected in 28 to 30 cm TL. Significant differences between the TL distributions by season resulting from the Kolmogorov-Smirnov test were found between summer and winter (p<0.05) and between autumn and spring (p<0.01).

The chi-squared test indicated that there were significant differences from the 1:1 sex proportion for the whole population (p=0.03), with a higher proportion of females. By size class, there were no significant differences except for the larger classes, in which females predominated among individuals larger than 40 cm (Fig. 3). Standardized residuals of the size classes 45 to 49 cm and 50 to 54 cm showed significant differences ($z_{45-49} = \pm 2.16$, $z_{50-54} = \pm 2.07$), indicating a higher proportion of females, whereas in the 55 to 60 cm class all the individuals were females. Analysed by season,

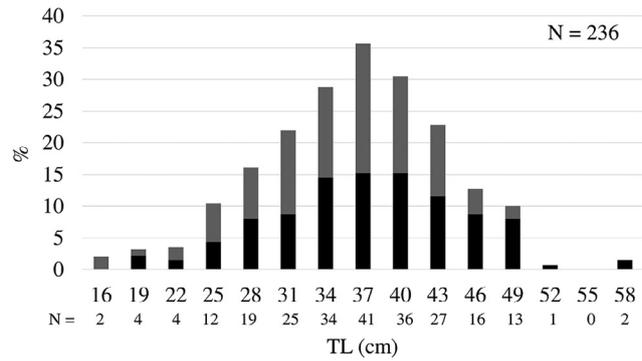


Fig. 1. – Size distribution of the population of *Raja polystigma* as a percentage of individuals grouped by 3 cm intervals. Females in black and males in grey.

no significant differences from the 1:1 sex proportion were found.

Allometric growth

The ANCOVA analyses showed significant differences (p<0.05) between the two sexes for the relationships TL-TW, TL-EW and TL-DW. On the other hand, the DW-EW relationship showed no significant differences between males and females (p=0.17). Except for the TL-DW relationship, the remaining allometric coefficients (*b*) of the tested regressions were greater than 3 (Table 2), indicating a positive allometry growth.

Table 2. – Parameters of the length-weight and morphometric relationships (potential regression) calculated for *Raja polystigma* and results of the ANCOVA comparisons between the two sexes. F, females (n=133); M, males (n=84). Between brackets the 95% confidence interval for the *b* parameter. TL, total length; TW, total weight; EW, eviscerated weight; DW, disc width.

Relationship tested	Sex	<i>a</i>	<i>b</i>	R ²
TL-TW	F	0.0006	3.62 (3.52-3.72)	0.97
	M	0.0012	3.38 (3.26-3.49)	0.98
TL-EW	F	0.0005	3.62 (3.51-3.71)	0.97
	M	0.001	3.4 (3.28-3.52)	0.98
DW-EW	F	2·10 ⁻⁶	3.37 (3.23-3.45)	0.97
	M	3·10 ⁻⁶	3.34 (3.26-3.41)	0.99
	whole population	3·10 ⁻⁶	3.33 (3.24-3.38)	0.97
TL-DW	F	0.4694	1.05 (1.03-1.08)	0.98
	M	0.5921	1.01 (0.98-1.04)	0.97

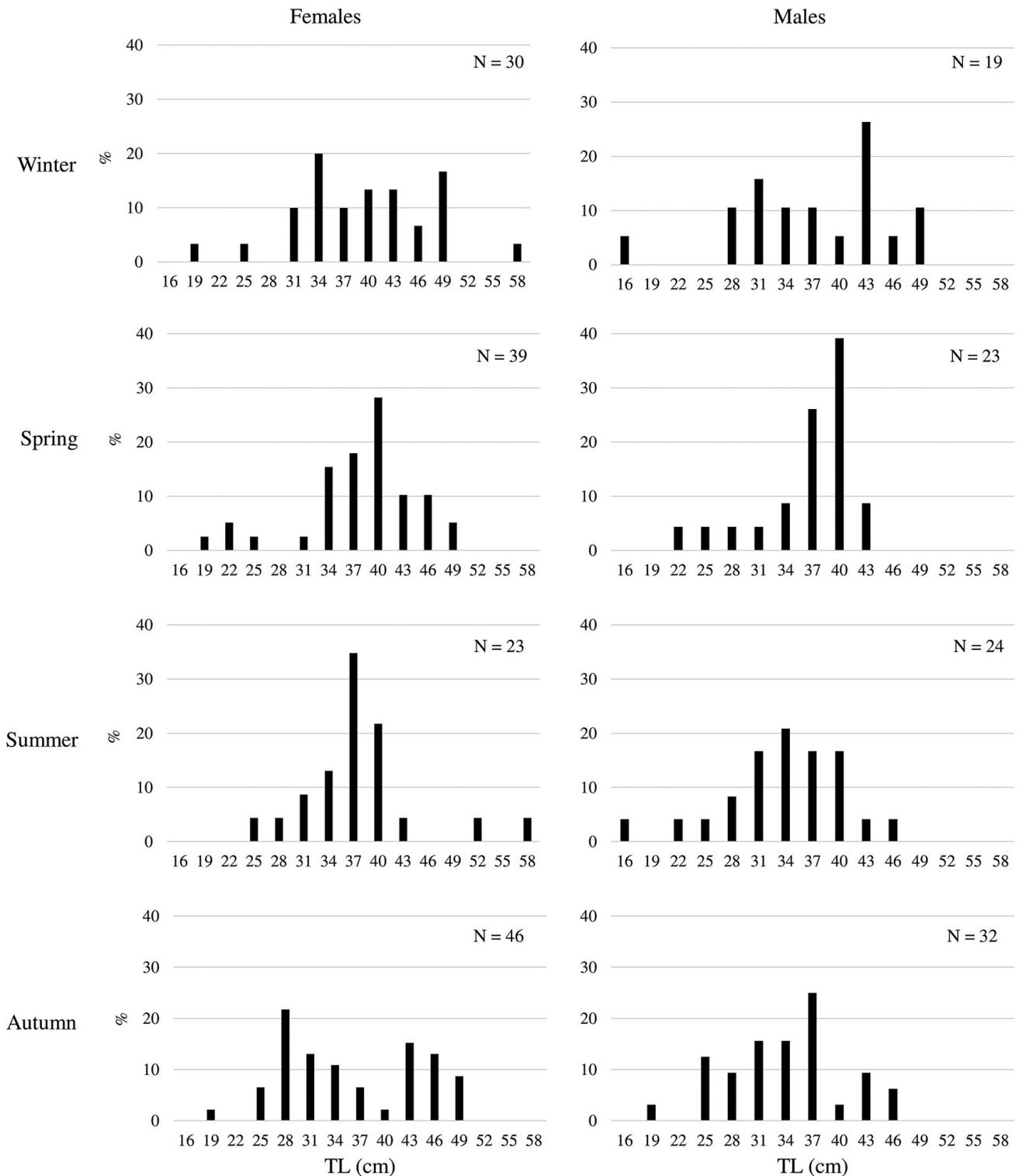


Fig. 2. – Size distribution of the population of *Raja polystigma* by sex as a percentage of individuals, grouped by 3 cm intervals, by seasons, regarding total length (TL).

Reproduction

The size ranges (TL) observed for every maturity stage in females were 20 to 41 cm for immature individuals, 27 to 45 cm for developing individuals, 40 to 51 cm for spawning individuals, 44 to 58 cm for capsule

present individuals and 40 to 59 cm for regenerating individuals. In males, the size ranges were 17 to 39 cm for immature individuals, 33 to 44 cm for developing individuals and 37 to 52 cm for spawning individuals.

Females showed a maximum GSI in summer (Fig. 4), whereas in winter they showed the maximum pro-

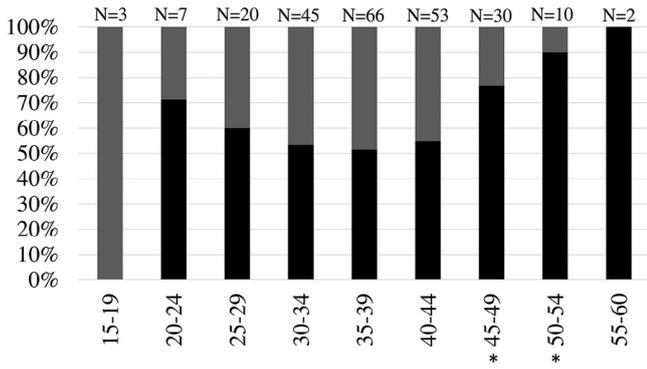


Fig. 3. – Sex-ratio in *Raja polystigma*, showing the percentage of males (in grey) and females (in black) for every 5 cm size interval (cm) of total length. Significant differences in the chi-squared test ($p < 0.05$) are indicated with asterisks.

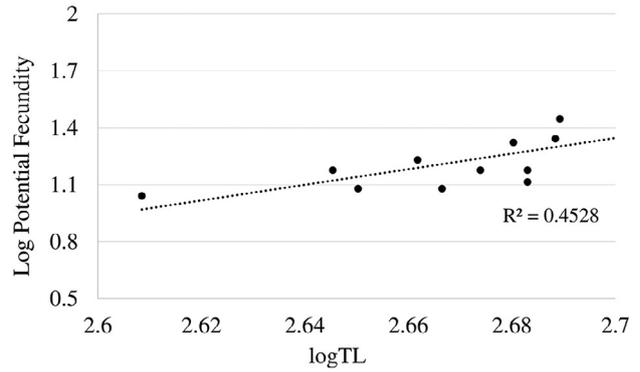


Fig. 5. – Potential fecundity (number of vitellogenic oocytes and capsules per individual) relative to total length (TL). Dotted line indicates significant regression.

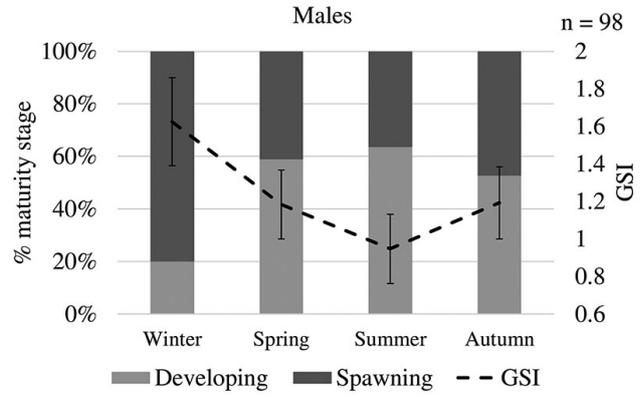
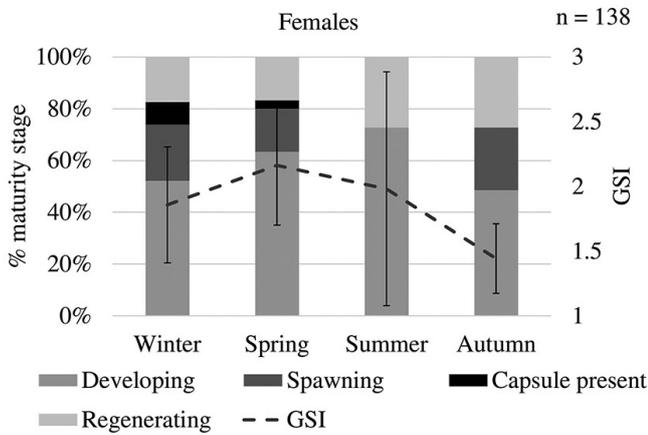


Fig. 4. – Frequencies of maturity stages and values of GSI throughout the year in females and males of *Raja polystigma*.

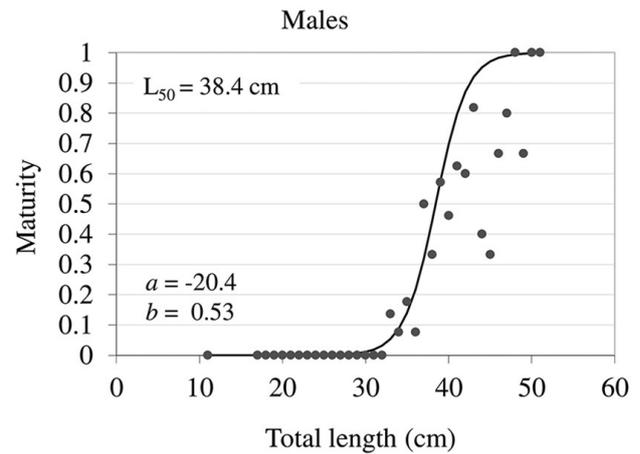
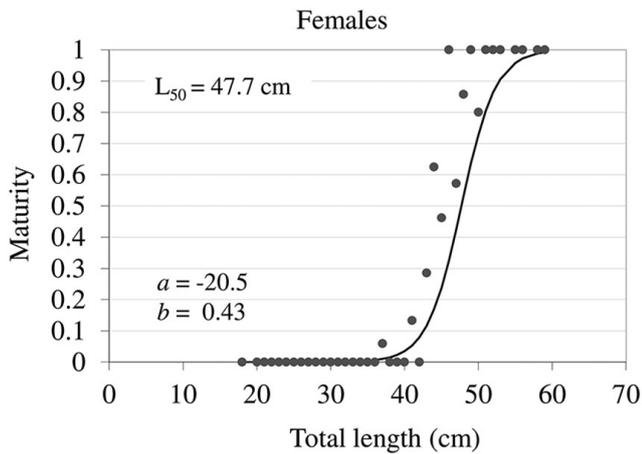


Fig. 6. – Logistic curves for length-at-maturity with their parameters (a and b) and L50 (cm) for females (F) and males (M) of *Raja polystigma*.

portion of spawning individuals and the maximum proportion of individuals with egg capsules present. Egg capsules were also present in spring, but they did not appear in summer and autumn. In summer, all females showed a developing or regenerating stage. The proportion of regenerating stage was highest in the sum-

mer and autumn months. The GSI values for males were the highest and very similar in autumn, winter and spring, whereas a the lowest GSI was observed in summer. The highest proportion of spawning males was found in winter. In the rest of the seasons, proportions of spawning and developing individuals were similar

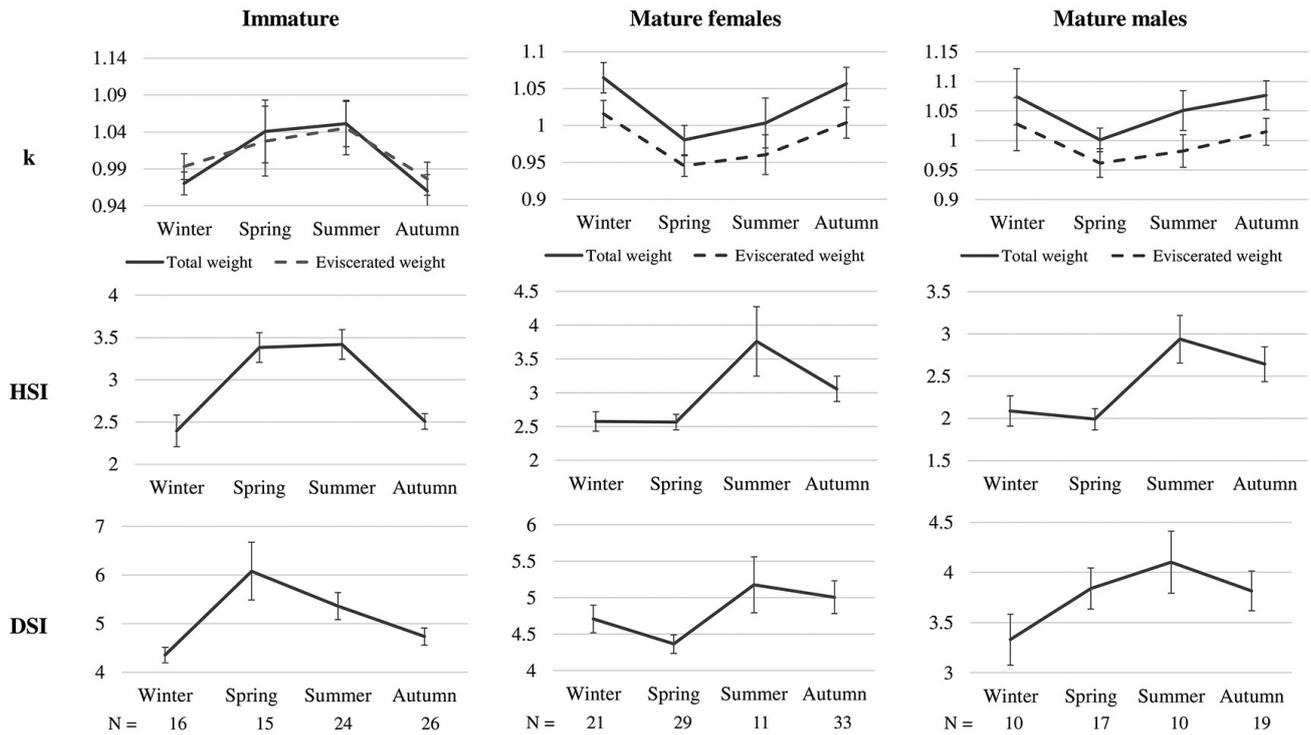


Fig. 7. – Le Cren index (k) for evolution of total and eviscerated weight, hepatosomatic index (HSI) and digestivosomatic index (DSI) by seasons for immature individuals, mature females and mature males of *Raja polystigma*.

(Fig. 4). The proportion of immatures by season was 23% in both winter and spring, 52% in summer and 28% in autumn for females and 47% in winter, 26% in spring, 54% in summer and 40% in autumn for males.

Females showed between 11 and 45 yolked oocytes per individual (PF = 45), with a significant and positive relationship between PF and TL ($p=0.008$) (Fig. 5). Mean values of $PF \pm SE$ per season were 18.83 ± 26.64 , 12.72 ± 55.06 and 23.57 ± 67.5 , for autumn, spring and winter, respectively.

The smallest sexually active individuals found measured 41 and 33 cm for females and males, respectively. The L_{50} was also larger for females (47.7 cm) than for males (38.4 cm) (Fig. 6).

Condition indices

Immature individuals showed an annual cycle based on condition indices (Fig. 7). Their HSI and k followed the same pattern, showing minimum values in winter and continuous recovery until their maximum values in summer. Their DSI pattern showed its maximum in spring and then it started to decrease to a minimum in winter.

Mature males and females showed a similar seasonal pattern of variation of condition indices. For both sexes, the Le Cren index (k) showed maximum values in autumn and winter for both sexes, an abrupt decrease in spring and a recovery from then on (Fig. 7). The pattern for HSI and DSI was different from that for k , but again similar between males and females (Fig. 7), with

a maximum during the summer months. After summer, values started decreasing until winter (in males) and spring (in females), and then they started to increase again until a peak in summer.

Diet

A total of 152 stomachs of individuals ranging from 18 to 58 cm TL were analysed. Of these, 12 were empty (vacuity index = 7.9%). The main taxonomic groups of prey were decapod crustaceans, which were present in 57% of the stomachs representing 56% of the index of relative importance (IRI), fish, which were present in 19% of stomachs representing 19% IRI and amphipods, which were present in 31% of stomachs representing 12% IRI. Isopods, lophogastrids, mysidaceans and annelids were also frequently found in the stomach contents (17-26% of the stomachs), but with lower %IRI (around 3% IRI). Molluscs and cnidarians completed the diet with low frequencies and %IRI (Table 3).

Within decapods, 16 prey items were identified, unidentified natantians being the main prey, representing 11.7 of %IRI. *Processa* spp and *Solenocera membranacea* were among the identified species showing the highest frequencies (15% and 12%, respectively) and %IRI (4.9 and 4.6, respectively). Among fish, *Gymnammodytes cicerelus*, despite not being as frequent as the previous species, was the most important prey in terms of %IRI (26%) owing to its larger percentage of volume (39%).

Table 3. – Frequency of appearance (F), percentage number (%N), percentage volume (%V), index of relative importance (IRI) and percentage IRI (%IRI) for each prey of *Raja polystigma*.

Prey	F	%N	%V	IRI	%IRI
Decapoda	57.14	36.67	26.66	3618.85	55.91
Natantia	27.86	9.52	3.58	364.97	11.71
<i>Processa</i> spp.	15.00	6.92	3.34	153.91	4.94
<i>Solenocera membranacea</i>	12.14	3.34	8.56	144.45	4.63
<i>Liocarcinus</i> spp.	7.86	2.22	4.69	54.30	1.74
<i>Philocheras sculptus</i>	2.86	6.30	0.52	19.49	0.63
Brachyura	5.00	1.11	0.99	10.53	0.34
Portunidae	2.14	0.99	0.91	4.06	0.13
Crangonidae	1.43	2.10	0.64	3.92	0.13
<i>Philocheras</i> spp.	1.43	2.10	0.14	3.21	0.10
<i>Processa canaliculata</i>	2.86	0.49	0.42	2.62	0.08
<i>Plesionika antigai</i>	0.71	0.12	1.94	1.48	0.05
<i>Galathea intermedia</i>	1.43	0.99	0.03	1.46	0.05
<i>Alpheus</i> spp.	0.71	0.12	0.40	0.38	0.01
<i>Pagurus</i> spp.	0.71	0.12	0.35	0.34	0.01
<i>Scyllarus pygmaeus</i>	0.71	0.12	0.13	0.18	0.01
Pandalidae	0.71	0.12	0.01	0.10	<0.01
Fish	19.29	8.40	55.57	1233.59	19.06
<i>Gymnamodytes cicereus</i>	17.86	6.80	39.17	820.84	26.33
Pisces	7.14	1.11	5.49	47.14	1.51
<i>Glossanodon leioglossus</i>	0.71	0.12	4.42	3.25	0.10
<i>Gadiculus argenteus</i>	0.71	0.12	3.64	2.69	0.09
<i>Trachurus trachurus</i>	0.71	0.12	2.68	2.00	0.06
<i>Pseudaphya ferreri</i>	0.71	0.12	0.24	0.26	0.01
Amphipoda	31.43	23.46	1.36	780.03	12.05
Gammaridae	31.43	22.25	1.10	733.76	23.54
Amphipoda	1.43	1.11	0.16	1.82	0.06
Hyperiididae	0.71	0.12	0.07	0.14	0.00
Isopoda	21.43	7.16	4.50	249.94	3.86
Isopoda	21.43	6.06	3.86	212.41	6.81
Cirolanidae	0.71	0.99	0.60	1.14	0.04
<i>Eurydice</i> spp.	0.71	0.12	0.04	0.12	<0.01
Lophogastrida	26.43	6.30	2.12	222.33	3.43
<i>Lophogaster typicus</i>	26.43	6.30	2.11	222.28	7.13
Mysida	17.14	10.00	0.55	180.85	2.79
Annelida	21.43	4.20	4.22	180.48	2.79
Polychaeta	15.71	2.97	2.01	78.14	2.51
Annelida	5.71	1.24	2.20	19.66	0.63
Other crustaceans	5.00	2.96	0.32	16.41	0.25
Unidentified Crustacea	4.29	2.84	0.31	13.50	0.43
Cumacea	0.71	0.12	0.01	0.10	<0.01
Mollusca	2.86	0.49	2.47	8.48	0.13
<i>Loligo forbesii</i>	0.71	0.12	2.23	1.68	0.05
Cephalopoda	1.43	0.25	0.24	0.69	0.02
<i>Cavolinia inflexa</i>	0.71	0.12	0.01	0.09	<0.01
Cnidaria	0.71	0.12	1.89	1.44	0.02
<i>Pelagia noctiluca</i>	0.71	0.12	1.90	1.44	0.05
Unidentified remains	0.71	0.12	0.28	0.29	<0.01
Algae	0.71	0.12	0.05	0.12	<0.01

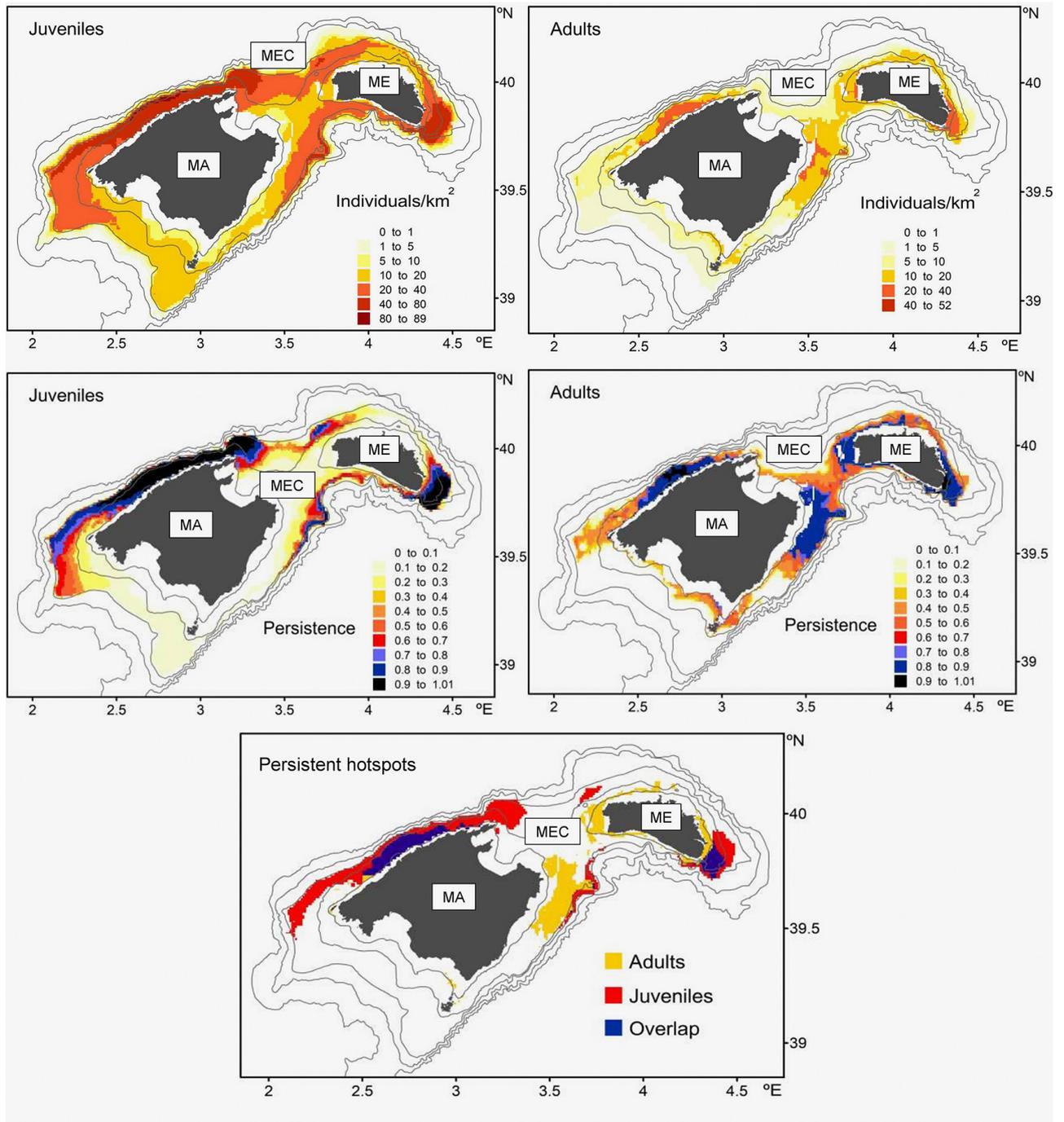


Fig. 8. – Maps showing the mean abundance throughout the time series of MEDITS surveys from 2003 to 2018 (as individuals-km⁻²) and the hotspot persistence for both adults and juveniles, and the overlap of persistent hotspots of adults and juveniles of *Raja polystigma* in the areas around Mallorca (MA), Menorca (ME) and the Menorca Channel (MEC). Isobaths represent 50, 100, 200, 500 and 800 m depth.

Geographic and bathymetric distribution

The mean predicted values of abundance and the persistence of hotspots showed that individuals are mainly found off the northwest coast of Mallorca, in the Menorca Channel and off the east coast of Menorca (Fig. 8). This distribution pattern was similar for both adults and juveniles, although the latter showed higher abundances and a deeper distribution. However, on

the northwest coast of Mallorca, high abundances and persistence of hotspots of juveniles were also detected on the shallow continental shelf. This area and the east of Menorca were the only ones where persistent hotspots for adults and juveniles overlapped (Fig. 8).

Raja polystigma individuals were captured between 46 and 375 m depth. GLMs showed a better fit to a unimodal bathymetric distribution model for the whole ($p < 0.05$) and juvenile ($p < 0.001$) populations, whereas

the adult population was better adjusted to a linear regression ($p < 0.001$).

Adults and juveniles showed bathymetric segregation. Adults were related to the continental shelf (<200 m depth), with their maximum of occurrence at 50 m depth, whereas juveniles were found from the shelf to the upper slope, and showed their optimum depth on the shelf break around 160 m depth (Fig. 9).

Population trend

Frequency of appearance showed no significant trend, as it remained stable throughout the time series analysed (Fig. 10, $p = 0.135$). However, all the values remained above the baseline in the whole time series. Annual abundance showed no significant trend either. All of its values remained above the baseline, except for 2009 (Fig. 10, $p = 0.18$). Furthermore, the population PF showed no significant trend either (Fig. 11, $p = 0.058$).

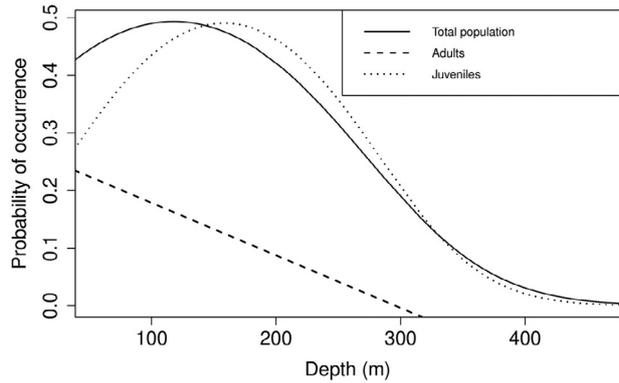


Fig. 9. – Bathymetric distribution of the total population of *R. polystigma* (continuous line) and of its adult (dashed line) and juvenile (dotted line) fractions. All the models applied were significant ($p < 0.05$).

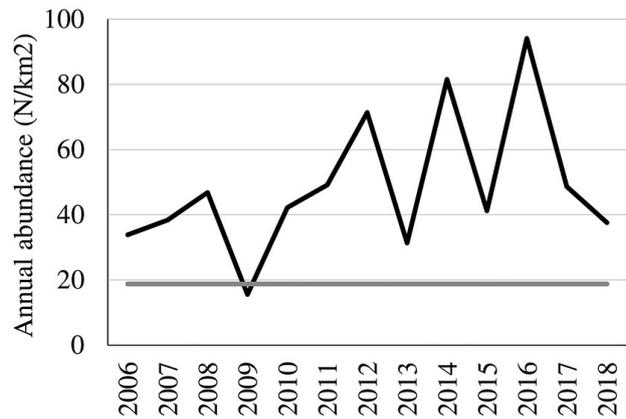
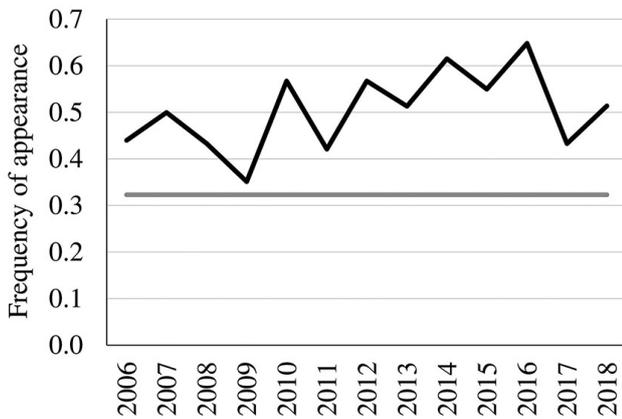


Fig. 10. – Frequency of appearance and mean annual abundances (black solid lines) of *R. polystigma* between 2006 and 2018. Grey lines indicate baselines (average frequency of appearance and mean annual abundance between 2003 and 2005).

DISCUSSION

Despite the critical situation of almost all Mediterranean batoid populations, little is known about their basic biological parameters. The present study estimated essential biological parameters (population structure, growth, diet, somatic condition, reproduction and geographic and bathymetric distribution) of *Raja polystigma*, an endemic species in the Mediterranean.

The analysed population of *R. polystigma* showed similar maximum lengths (around 60 cm TL) to those reported for this species in the central Mediterranean (Porcu et al. 2020), around 60 cm TL. *Raja polystigma* probably reproduces throughout the year in the Balearic Islands, as males in the “spawning” stage and “regenerating” females have been observed throughout the year. However, a peak was observed in the spawning activity in winter and spring. The highest percentage of individuals with egg capsules was detected in winter, and maximum GSI values of females were observed in spring, suggesting that reproduction in this

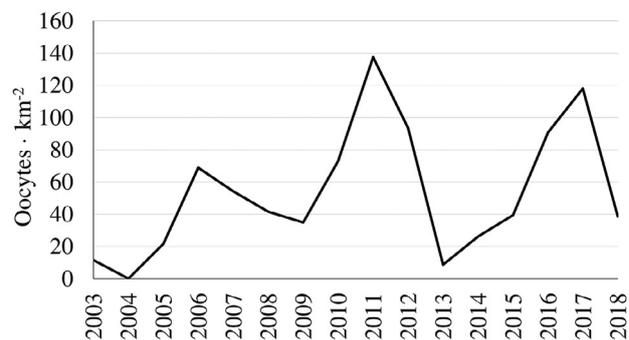


Fig. 11. – Average of oocytes · km⁻² (population potential fecundity, black solid line) for *R. polystigma* between 2003 and 2018.

species occurs mainly during the first half of the year in the study area. This finding is similar to that of Capapé and Quignard (1978), who also report the winter as the main reproduction period. However, Porcu et al. (2020) observed that the reproduction period in individuals col-

lected off Sardinia was mainly in summer. The spawning peak in winter and spring in the Balearic Islands is also coincident with the seasonal variations in the length-frequency distributions. The highest number of egg capsules was recorded in winter, and was followed in spring by a cohort of recruits, individuals of 16 to 25 cm TL, which corresponds to the TL reported for newborns by Porcu et al (2020). These recruits were not detected in summer but could correspond to the mode of 26 to 28 cm TL detected in autumn.

The sex ratio showed a significantly higher proportion of females in individuals larger than 45 cm TL. These differences could be due to the greater longevity reported in females as opposed to a faster growth, because males and females show similar growth rates, $k = 0.32$ and $k = 0.28$, respectively (Porcu et al. 2020).

Males and females showed significant differences in their length-weight relationships, females being heavier than males at a given TL. These differences cannot be related to the gonad weight since the same results were also found when the length-eviscerated weight relationship was analysed. According to Orlov and Cotton (2011) differences in body shape caused by sexual dimorphism could be the reason for the observed differences in weight by sex.

The L_{50} values of *R. polystigma* showed a difference of 9 cm TL between females (47.7 cm TL) and males (38.4 cm TL). These differences are lower than those reported in the central Mediterranean, where L_{50} is estimated at 50.6 and 48.8 cm TL for females and males, respectively (Porcu et al. 2020). A similar pattern in size differences at maturity related to sex has been observed in other skate species such as *Raja brachyura* from Sardinia waters ($L_{50}=87$ cm for females and 81 cm for males; Porcu et al. 2015) and *Raja clavata* from Tunisia ($L_{50}=81$ cm for females and 67 cm for males; Kadri et al. 2014) and from the Balearic Islands ($L_{50}=76.4$ cm for females and 67.3 cm for males; Ramírez-Amaro et al. 2020).

PF of *R. polystigma* increases with size, as previously suggested by Capapé and Quignard (1978). The PF estimated here ranged between 11 and 45 yolked follicles per individual per year. A wide range of between 20 and 62 yolked follicles per year was also reported by Capapé and Quignard (1978). In contrast, Porcu et al. (2020) reported a shorter range of between 14 and 19 yolked follicles per year. Means of PF by season matched with the estimated reproductive season (winter-spring), because the maximum values of PF were found in winter and autumn, just before the detected egg-laying episodes. However, the presence of females in the “regenerating” stage throughout the year and the unknown duration of this stage indicate that egg-laying females could be present throughout the year. They could therefore also be present in summer, a season during which we did not detect them, although regenerating females were found during autumn.

In view of the results obtained in the condition indices, we can consider *R. polystigma* as a capital breeder. Capital breeders are species that acquire resources in advance and store them until they need them for reproductive investment (Jönsson 1997). Hepatosomatic

index and DSI were used in this study as approximate measures of the energy stores because of the important role played by the liver in lipid storage (Lloret et al. 2014) and food intake, respectively. Mature individuals showed an increase in HSI and DSI during summer and a decrease afterwards during their spawning season (winter). This may indicate that individuals of *R. polystigma* increase their feeding activity when preparing for spawning and, as a result, the resources stored in the liver increase so that they can be finally used during the spawning season. This also happens in other elasmobranchs, such as *Mobula eregoodoo*, although increased food intake has also been observed during reproduction (Lawson et al. 2022).

Regarding the diet of this skate, our results suggest that the main prey of *R. polystigma* are decapod crustaceans, specially natantians, in agreement with previous reported studies in the Balearic Islands (Valls et al. 2011). Nonetheless, it is also a highly piscivorous species. Among fish prey, *R. polystigma* showed a preference for a single species (*G. cicereus*, representing 26%IRI), whose behaviour consists of burrowing itself completely in the sand sediment (Wheeler 1979). This could make it more accessible to *R. polystigma* than other fishes that are less in contact with the substrate. Also, the fact that *G. cicereus*, *S. membranacea* (Demestre and Abelló 1993, Cartes et al. 2007) and *Lophogaster typicus* (Cartes et al. 2011) are among its most frequent prey might indicate a preference for muddy and sandy-muddy bottoms for feeding.

Adults of *R. polystigma* are mainly found on the continental shelf from 45 to 180 m depth, whereas juveniles are mainly found on the deep shelf from 50 to 360 m depth, with an optimum depth of around 160 m, which agrees with the finding by Porcu et al. (2020). It also agrees with the general bathymetric distribution described in Tunisia (from 50 to 400 m depth; Capapé and Quignard, 1978) and in Sicily (Geraci et al. 2021). However, Porcu et al. (2020) suggest that *R. polystigma* completes its life cycle in shallow waters. Although our data are limited to samplings carried out from 50 m depth to the slope, the abundance of *R. polystigma* decreases at depths of less than 50 m in the Balearic Islands, where bottoms are dominated by *Posidonia oceanica*. *Raja polystigma* is known to mainly inhabit soft bottoms (Porcu et al. 2020). Moreover, catches of *R. polystigma* from the artisanal fleet operating shallower than 50 m depth in the Balearic Islands are negligible (pers. comm.).

The bathymetric segregation of adults and juveniles may avoid intraspecific competition. This kind of segregation has also been observed in other elasmobranch species, such as *Scyliorhinus canicula* (Massutí and Moranta 2003). Nonetheless, this segregation often happens the other way around. For instance, juveniles of the shark *Galeus melastomus* are found shallower, on the upper slope (300-500 m), than adults, which inhabit the middle slope (500-700 m) (Massutí and Moranta 2003). The deep shelf and the upper slope have recently been described as a hotspot of recruitment and community nekto-benthic species richness and density in the Mediterranean (Tugores et al. 2019). This seems also to be the case of *R. polystigma*, for which the deep

shelf, shelf break and beginning of the upper slope are very important areas where recruits aggregate and may represent an essential fish habitat for this species.

Raja polystigma seems to be widely distributed geographically in the study area and also occupies a wide bathymetric range. Our results show that it is widespread around the islands of Mallorca and Menorca. The whole study area seems suitable for this species and what really determines its abundance is the availability of its preferred bathymetric range (from 45 m to 400 m depth). However, fishing activity might also exert some effect on their abundance. Elasmobranchs are particularly vulnerable to fishing pressure (Stevens et al. 2000). The Balearic Islands show both lower levels of fishing exploitation and higher diversity of demersal elasmobranchs than adjacent waters off the Iberian Peninsula (Massutí and Moranta 2003). Our data seem to indicate that fishing exploitation also affects the abundance distribution of *R. polystigma* in the Balearic Islands, since although the southeast and southwest of Mallorca have its preferred bathymetric range, this area is subjected to higher exploitation than other areas in the archipelago (Farriols et al. 2017), and consequently the abundances are lower.

Raja polystigma has never been assessed previously in the Balearic Islands. Because of its low abundance and the fact it is not a target species, information on the size structure of the catches is scarce, so structural models cannot be used to assess its state of exploitation. Therefore, we conducted a preliminary assessment of this species based on the trends of its frequency of appearance, its annual abundance and its PF in the Balearic Islands, which resulted in stable trends. Stability has also been reported for other skate species in the area, *R. miraletus*, *R. radula*, *Leucoraja naevus* and *Dipturus oxyrinchys*, and even recovery in the case of *R. clavata* (Ramírez-Amaro et al. 2020). In addition, from commercial catch data, Guijarro et al. (2012) observed that elasmobranchs showed a stable trend or even some recovery on the shelf of the Balearic Islands. The stable trend could be related to the reduction of the bottom trawl fishing effort on the shelf and its transfer to greater depths (Quetglas et al. 2016, Farriols et al. 2019, Ramírez-Amaro et al. 2020).

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