



Bathymetric gradients of fecundity and egg size in fishes: A Mediterranean case study



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ABSTRACT

There is a general hypothesis that species inhabiting deep-sea waters have lower fecundity and larger eggs than shallower species. However, there are few comparative studies which explore this trend because of the complexity of sampling in deep waters, especially in fishes. We present here the first analysis of fecundity and egg size with depth along an isothermal environment. We calculate the relative fecundity and egg size of 11 species of demersal deep-sea fish from the western Mediterranean and included in our analyses published data for an additional 14 species from the same geographic area. The results show that the relative fecundity (eggs per g of individual) of the analyzed fishes slightly decreased along the bathymetric gradient, whereas egg size increased with depth. When the analysis was conducted including only species from the order Gadiformes, the most speciose group in the region and with the widest depth range of distribution (50–2000 m), there was no relationship between relative fecundity and depth, while the deepest species had larger egg sizes than shallower ones. The finding of similar relative fecundities but larger egg sizes suggests that these deep-sea species are investing a higher amount of energy in the production of offspring than shallower water counterparts. The results are discussed in relation to the isothermal characteristics of the deep Mediterranean Sea and ecological adaptations for reproductive success.

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1. Introduction

In recent decades, commercial fishing is occurring at increasing depths around the globe (Gordon et al., 2003; Swartz et al., 2010; Norse et al., 2012) and many important fish stocks have collapsed (Coll et al., 2008; Pontecorvo and Schrank, 2014). To understand population productivity and thus increase our predictive power for the management and conservation of marine ecosystems, it is crucial to have precise knowledge of life-history strategies (Jorgensen et al., 2007; Clark et al., 2012; Hilário et al., 2015) and, in particular, the reproductive capacity of the species involved. Furthermore, the influence of environmental factors on fish fecundity and productivity is now relevant to improve our understanding of the effect of climate change in marine ecosystems (Kjesbu et al.,

1998; Morgan et al., 2013). Despite its importance, the reproductive capacity of many fish species is still unknown (Hutchings and Baum, 2005). This is especially true for deep-sea species for which there is a scarcity of reproductive data because of the extreme difficulties and cost in exploration and sampling (Bergstad, 2013).

The assessment of fecundity and egg size is essential to understand the life-history evolution and adaptation of species to their environment (Roff, 1992; Stearns, 1992). Several authors have studied the latitudinal and bathymetric patterns of fecundity and egg sizes between phylogenetically-related species as well as between higher taxonomic groups (Thresher, 1988; Fleming and Gross, 1990; Johnston and Leggett, 2002; Vila-Gispert et al., 2002; Laptikhovsky, 2006; Finotto et al., 2015). Additionally, studies on timing of spawning and fecundity are fundamental topics in the biology and population dynamics of organisms (Lambert, 2008; Wright and Trippel, 2009; Lowerre-Barbieri et al., 2011).

Fecundity and egg size have been recognized as two of the main variables in the life-history strategies for invertebrates

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(Chambers, 1997; Ramirez-Llodra, 2002; Marshall and Keough, 2007) and fishes (Winemiller and Rose, 1992). Several processes, such as ovarian organization and development, are mostly phylogenetically constrained (Eckelbarger and Watling, 1995). However, the number and size of eggs is not a conservative character and can vary depending on female characteristics and environmental factors (Ramirez-Llodra, 2002). In fish, several studies have demonstrated a positive relationship between total fecundity and fish length (Merrett, 1994; Rideout and Morgan, 2010) and mass (Johnston and Leggett, 2002; McBride et al., 2015) whereas the relationship between relative fecundity (i.e. fecundity standardized to the body mass) and size is not so clear and depends on the species. Both, total fecundity (standing stock of vitellogenic oocytes) and relative fecundity are modulated by environmental conditions, mainly by temperature and food availability. A decrease in food availability can cause a decrease in energy transfer to the gonads, resulting in a decrease either in yolk accumulation (e.g., smaller eggs) or the number of eggs produced (Kjesbu et al., 1991; Rideout et al., 2000; McBride et al., 2015). Higher fecundity has been related to physiological change during development caused by temperature (Kjesbu et al., 1998, 2010; Kraus et al., 2000; Pörtner et al., 2001; Marshall et al., 2010). Due to the usually low temperature and food scarcity in deep-sea ecosystems, Thorson (1950) predicted that benthic deep-sea invertebrates would show lower fecundity and larger egg sizes compared to shallow-water species. Indeed, this is the pattern most commonly observed in invertebrates, but it is not universal (Young, 2003; Ross et al., 2013). In fishes, the few intra- and inter-specific studies conducted to date also describe a negative correlation between maximum total fecundity and depth (Gibson, 2007; Drazen and Haedrich, 2012).

Because of the transitional nature of the continental slopes and their steep environmental and biological gradients, the study of bathyal species offers a good opportunity to understand the influence of a number of factors on the life histories of marine species (Company and Sardà, 1997; Levin and Dayton 2009). In addition, the Mediterranean Sea, offers a unique environmental setting for the study of ecological patterns along bathymetric gradients, where one of the most influential variables, temperature, remains constant at about 13 °C below the thermocline (Margalef, 1985; Sardà et al., 2004). Despite the low number of studied species in the deep Mediterranean, apparently similar bathymetric trends of fecundity are evident among invertebrate species (Company and Sardà, 1997; Sardà et al., 2009). For deep-sea Mediterranean fishes, only a few descriptive studies have analyzed fecundity (D'Onghia et al., 2008; Muñoz et al., 2010; Fernandez-Arcaya et al., 2012, 2013a) and evaluation of bathymetric patterns of fish fecundity and egg size at the community level is still lacking.

To address this knowledge gap, we compared the reproductive output of 25 fish species belonging to 9 orders in the western Mediterranean. We included also a specific analysis of the order Gadiformes, to evaluate phylogenetic effects, as this is the group with the widest depth range of distribution (50–2000 m). The objective of our study was to answer the following questions: (1) Does fish relative fecundity decrease and egg size increase along a bathymetric gradient in the Mediterranean Sea? and (2) What are the dominant forces driving the reproductive capacity of these species?

2. Materials and methods

2.1. Sampling

Data were obtained from 9 oceanographic cruises conducted in the western Mediterranean (41°34'N, 02°50'E–41°15'N, 02°48'E)

based on a seasonal sampling over two annual cycles in 2008–2009 and 2012–2013. In total, 95 benthic trawls were conducted between depths of 400 and 2750 m, at 150 m intervals, to obtain a representative bathymetric dataset of demersal fish from the study area (Fig. 1). The sampling was conducted seasonally to cover the reproductive period of the different species, with cruises in March, May, September and October (2009); March, May, October (2012) and April (2013). All sampling was conducted on board the R/V *García del Cid* using the otter-trawl Marieta system (OTMS) fitted with a codend mesh of 40 mm (Sardà et al., 1998). The OTMs mouth opening was estimated at 12.7 ± 1.3 m horizontally and 1.4 m vertically. The OTMS was trawled at 2.6–2.8 knots with bottom haul times of approximately 40–60 min.

On board, all fish individuals were identified to species level, measured to the nearest 0.1 cm and weighed to the nearest 0.1 g. The standard length was considered in all species, except in macrourids and nototheniids, where pre-anal fin-length was measured. Depth was estimated as the depth at which the population of a given species exhibits the highest abundance within the species' depth range. For the 11 species for which fecundity was calculated, maximum abundances depth (MAD) was extracted from the Deep Med Research Group© database, hosted at the Institute of Marine Sciences (ICM-CSIC, Barcelona, Spain). The results of bathymetric distribution obtained were in accordance with previously published data (e.g. Stefanescu et al., 1992, 1993; Massutí et al., 1995; Tecchio et al., 2013). The MAD for the other 14 species was obtained from the literature (see Table 3 for details).

The reproductive developmental stage of the ovaries was classified macroscopically based on ovary color, ovary size and egg size. For the fecundity, only mature females corresponding to the two following categories were used: spawning capable (with ovaries of considerable volume, in which some oocytes appear translucent) and actively spawning stage (when gonads fully occupy the body cavity with many visible eggs) (Brown-Peterson et al., 2011).

2.2. Histological and image analysis

The ovarian developmental reproductive phases, oocyte development stages and the absence of postovulatory follicles, which indicate that the spawning has not already started, were confirmed by histological screening in all the subsamples used for the fecundity calculation. The samples were embedded in paraffin blocks, cut into 7 µm sections and stained with Harris' haematoxylin and eosin. Histological sections were also used to determine the size of each oocyte developmental stage by measuring the diameter of 100 oocytes with Sigma Scan Pro4, following the Brown-Peterson et al. (2011) maturation scale.

Fecundity was determined using the gravimetric method (reviewed in Murua et al. (2003)). The ovaries were wet-weighed and then a small subsample of approximately 0.1 g from each ovary was weighed to the nearest 0.001 g, stained with Rose Bengal and then filtered through a 125 µm sieve. The filtered subsamples were photographed using a Canon camera attached to a binocular microscope (Leica MZ12). These photographs were analyzed by Image J software (Thorsen and Kjesbu, 2001) to determine the ovarian organization and count the oocytes in the sample. The oocyte-size frequency distribution of a species provides information on the ovarian developmental organization (asynchronous, synchronous or group-synchronous ovary organization) and partial information about the type of fecundity, either determinate or indeterminate (reviewed in Murua and Saborido-Rey (2003)). The species with group-synchronous ovarian organization were considered to have determinate fecundity (Murua and Saborido-Rey, 2003). In the species with group-synchronous ovary organization,

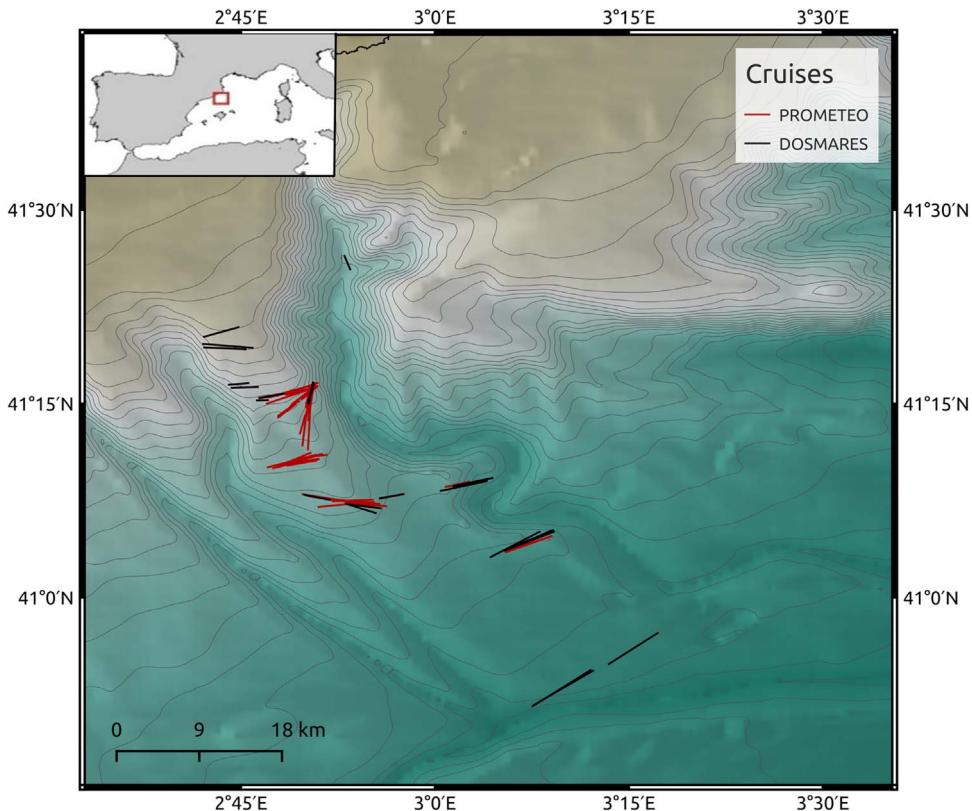


Fig. 1. Map of the sampling area in the western Mediterranean Sea, with detailed bathymetry, showing the location of the trawling lines during 2008–09 (in red, PROMETEO) and 2012–13 (in black, DOSMARES) cruises. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

total fecundity was calculated by counting all the oocytes larger than the mean diameter of early vitellogenic stage oocytes (EVtg) previously calculated from histological sections (Murua et al., 2003). In contrast, the species with asynchronous ovarian organization, which contain all stages of oocytes at the same time in the ovary of mature females (Ganias et al., 2013), mostly present indeterminate fecundity. For the calculation of total fecundity, all the oocytes larger than the mean diameter of the cortical alveoli (CA) were assumed to be developing and to be spawned during the following breeding season (Murua et al., 2003) (Table 2). As follows:

$$TF = [O_i/W_i] \times W_g$$

where O_i is the number of oocytes from the spawning capable phase, W_i is the mass of the ovary subsample, and W_g is the ovary weight. The relative fecundity (RF) was estimated by dividing total fecundity by the gonad-free weight of the fish. Because we were concerned about the artifact that might arise from the individual mass-specific effect in the fecundity comparison, we calculated RF adjusted to 346.6 g (RFM), the median value of the mass of the individuals used in the analysis. Following the equation used by Fleming and Gross (1990), $RFM = (RF^*W/W_0)^b$, where RF=relative fecundity, W=gonad-free median body mass of individuals, W_0 =observed body mass, and $b=-0.26$ was the slope of the linear regression (log scale) between the RF and the mass of all the species.

The egg size, estimated as the diameter of hydrated oocytes, was measured in individuals in the actively spawning phase (Brown Peterson et al., 2011) and in formaldehyde fixed tissue using image analysis because tissue shrinkage during histological processing may cause an underestimation of the oocyte diameter (Turner and Lawrence, 1979). A minimum of 20 hydrated eggs from different individuals were measured to calculate the mean

and maximum size of eggs for each species.

2.3. Statistical analysis

The data obtained during this study for the 11 species sampled during the cruises was combined with data available for other demersal western Mediterranean species from previously published studies. Fecundity and egg size can vary between species distributed at different latitudes (Fleming and Gross, 1990; Kock and Kellermann, 1991). Therefore, to avoid this possible bias, we decided to include only the data available from demersal species distributed along the western Mediterranean continental margin. Pelagic species, inhabiting a very different environment from the demersal fishes and Chondrichthyes, with dramatically different life-history characteristics compared to teleost fish (Rigby and Simpfendorfer, 2015), were excluded from this study.

Generalized additive mixed models (GAMM) were fitted to assess the potential effect of depth and fish mass on relative fecundity (RFM) and also to assess the relationship between depth and fish mass. Species was included as a random effect in mixed models, following the form: $(y_i) \sim \beta_i + s_1(x_1, k_1) + s_2(x_2, k_2) + e_i$, $random = 1/species$, where β_i is the intercept, s represents a polynomial smooth function, substituting the slope parameter in a linear regression (Wood, 2006), x represents explanatory variables, k represents the number of knots for the construction of the polynomial function and e_i is the error. To prevent model overfitting that may lead to spurious response vs explanatory relationships, restrictive small k values were applied (e.g., k values between 3 and 5). Generally, the response variable (e.g., RFM) was log-transformed to approach a normal distribution. The GAMM fitted to assess the effect of depth on RFM was $\log(RFM) \sim s_1(depth, k=3) + s_2(mass, k=3)$, $random = \sim(1/species)$. To assess the effect of the interaction between depth and mass on RFM the model was

fitted as $\log(RFM) \sim s_1(depth, mass, k=3)$, random = ~(1|species). Finally, generalized additive models (GAM) were fitted for the cases in which the effects of depth, mass and RFM on egg size were assessed because a single average egg size value for each species was used, thus no random species effect was needed. One order, Gadiformes, was represented across a wide bathymetric range on the western Mediterranean continental margin. Thus regression models were also conducted within the Order Gadiformes providing a unique opportunity to study bathymetric patterns of fecundity within a single phylogenetic group in an isothermal environment. Egg size in Gadiformes was not evaluated because of the relatively low number of data. The statistical package "gamm4" (Wood and Scheipl, 2014) in R was used to fit all the models.

3. Results

3.1. Ovarian organization and fecundity type

The number of individuals analyzed is summarized in Table 1. Despite the relatively high number of individuals captured for several species (i.e., *Phycis blennoides*, *Mora moro*, *Lepidion lepidion*, *Cataetyx alleni*, *Cataetyx laticeps* and *Coryphaenoides guentheri*) the number of mature females obtained was relatively low (Table 1). The oocyte size and type of ovarian organization for the species analyzed in the present study are detailed in Table 2 and Fig. 2.

The majority of the species analyzed (9 out of the 11) present group-synchronous ovarian organization. In these species, the ovaries of mature females had a small group of previtellogenic oocytes, which were the recruited oocytes for the following spawning period, and "clutches" of larger vitellogenic oocytes, which were the oocytes to be spawned during the current breeding season. The presence of previtellogenic and vitellogenic oocyte clutches was found in *N. bonaparte*, *N. melanorum*, *A. rosstratus*, *M. moro*, *L. lepidion* and *C. laticeps* (Fig. 2). In *B. mediterraneus*, *C. guentheri* and *C. alleni*, the previtellogenic oocyte population was not observed in the image analysis results. However, it is

Table 2

Basic statistics of oocyte size in different developmental stages (μm) for the species of NW Mediterranean deep-sea fish community. Following Brown-Peterson et al. (2011) oocytes were classified in four maturity stages: Primary growth (PG), Cortical alveoli (CA), Early Vitellogenic (EVtg), Advanced Vitellogenic (AVtg) and Germinal Vesicle Migration (GVM). Species name code as in Table 1.

Species	PG		CA		EVtg		AVtg		GVM	
	Mean	Std								
Not.bon	107.7	19.6	194.4	27.4	335.7	67.7	461.5	58.4		
Net.mel	124.3	34.0	157.7	2.5	255.6	30.6	465.5	83.7	630.4	75.2
Bat.med	76.7	17.6	133.7	27.6	196.6	51.1	421.9	75.7	555.9	46.9
Cor.gue	95.8	16.6			200.3	57.0	265.7	43.0		
Phy.ble	67.2	18.7	174.0	21.0	256.6	8.3	328.4	24.4	365.5	2.7
Mor.mor	89.2	12.9					447.4	37.2	471.7	34.5
Lep.lep	108.5	15.0			269.9	24.3			627.3	58.6
Cat.alle	92.0	26.5			233.5	34.7	380.5	45.1	489.1	71.1
Cat.lat	104.9	27.3	198.5	39.8	273.1	40.9	326.5	59.5	562.7	84.2
Epi.tel	78.9	14.4			245.9	13.2	353.1	63.2	391.2	25.9

quite possible that primary growth oocytes, due to their small diameter, could have escaped through the mesh ($< 125 \mu\text{m}$) used to process the samples for image analysis. This was corroborated by the histological characteristics of the females in stage IV (Fig. 3), in which primary growth oocytes were found.

In contrast, the oocyte size frequency distributions were continuous in *P. blennoides* and *E. telescopus*, suggesting asynchronous ovarian organization. Only when spawning was evident in *E. telescopus*, did the hydrated oocytes ($> 700 \mu\text{m}$) develop and form a separate group (Fig. 2).

The description of oogenesis from the histological analysis of mature females corroborated the oocyte distribution results. The mature females of 9 species classified as having group-synchronous ovarian development showed only two stages of oocytes: previtellogenic oocytes (PG) and advanced vitellogenic stage oocytes (AVtg) (Fig. 3A–D and G–J). In contrast, the mature ovaries of *P. blennoides* and *E. telescopus* had all stages of oocytes in the ovary at any single time: previtellogenic oocytes (PG), cortical alveoli (CA), early vitellogenic stage

Table 1

Total number of individuals measured, ovaries analyzed and females whose fecundity was calculated.

Species	Code	Individuals measured	Ovaries macroscopically analyzed	Ovaries microscopically analyzed	Fecundity measurements
Orden Notacanthiformes <i>Notacanthus bonaparte</i>	Not.bon	214	131	125	30
Orden Anguilliformes <i>Nettastoma melanurum</i>	Net.mel	52	25	25	9
Orden Osmeriformes <i>Alepocephalus rostratus</i>	Ale.ros	3980	1269	32	32
Orden Aulopiformes <i>Bathypterois mediterraneus</i>	Bat.med	3007	3007	63	35
Orden Gadiformes <i>Coryphaenoides guentheri</i>	Cor.gue	736	311	72	5
<i>Phycis blennoides</i>	Phy.ble	505	180	30	11
<i>Mora moro</i>	Mor.mor	654	307	30	1
<i>Lepidion lepidion</i>	Lep.lep	3211	1258	30	2
Orden Ophidiiformes <i>Cataetyx alleni</i>	Cat.all	53	24	24	6
<i>Cataetyx laticeps</i>	Cat.lat	47	13	13	7
Orden Perciformes <i>Epigonus telescopus</i>	Epi.tel	10	6	6	2
Total		12,469	6225	450	146

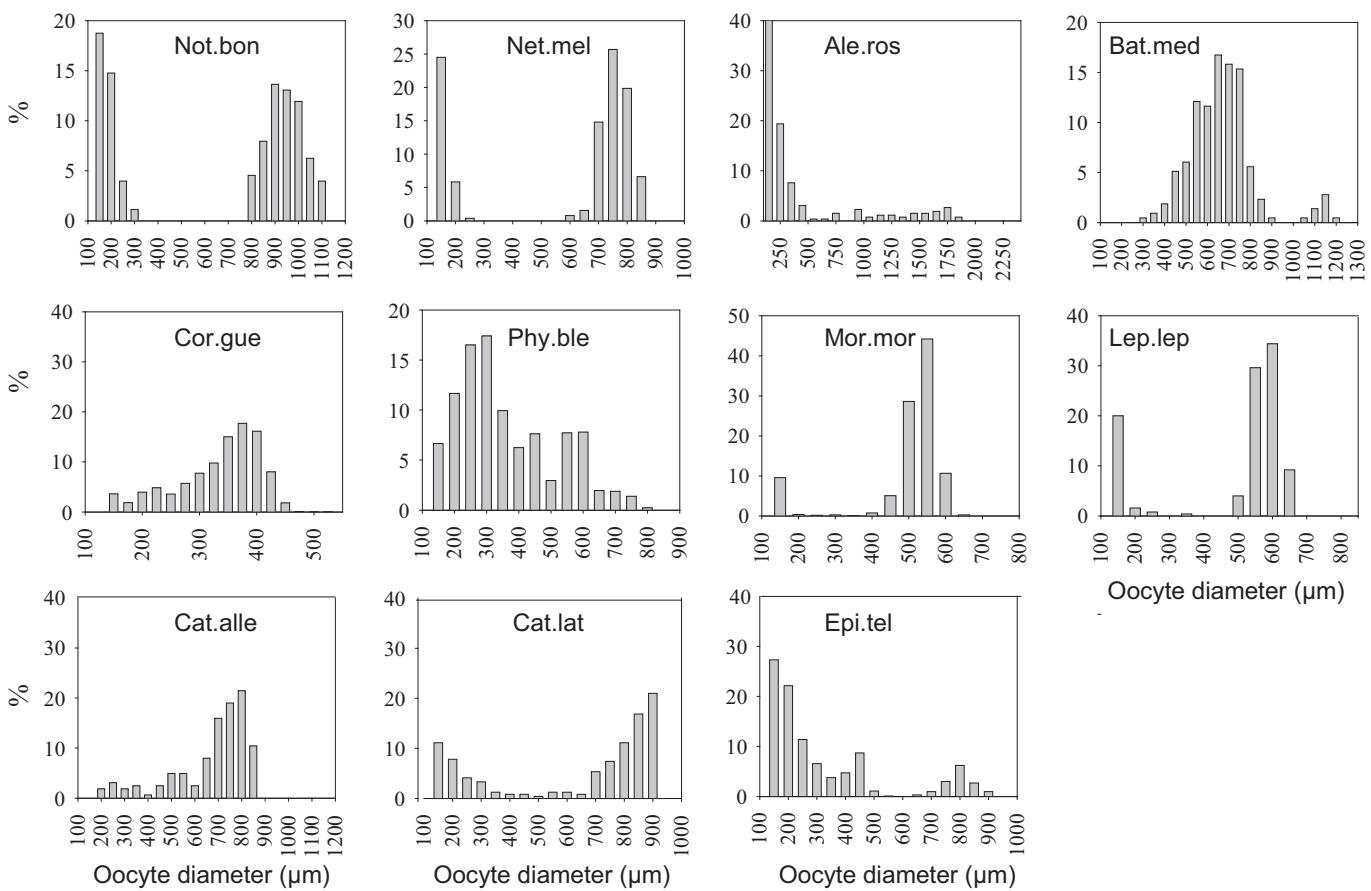


Fig. 2. Oocyte size frequency distribution in spawning capable females of 11 species from the NW Mediterranean deep-sea fish community. Species name codes are in Table 2.

oocytes EVtg and advance vitellogenic stage oocytes AVtg (Fig. 3K and F), suggesting asynchronous ovarian organization.

3.2. Bathymetric gradients of fecundity and egg size

The fecundity and egg size values of 25 species (11 species from the current study and 14 species from the literature) from the western Mediterranean are detailed in Table 3. Total fecundity (TF) was generally higher in the upper slope species (200–800 m) compared to the shelf (0–200 m), middle (800–1350 m) and lower slope (< 1350 m) species (Fig. 4). The values of relative fecundity (RFM) for the different species were similar across depths, with the exception of *A. rostratus* and *P. blennoides*, which had a RFM lower and higher than the average, respectively (Fig. 4C). Hydrated oocytes in the 25 species analyzed showed a wide range of sizes (0.5–2.3 mm) (Table 3).

The regression model results fitted for all 25 species analyzed are summarized in Table 4. RFM was significantly explained by depth ($R^2=0.14$, $p=0.04$) (Fig. 5A). No significant relationship between mass and RFM was found. Similarly, the model was no significant using the additive effect of depth and mass, as well as from the interaction effect of depth and mass (Model 1 and 2 in Table 4). Mass showed a significant decreasing pattern with depth, except at lowest sampled depths (i.e., 2500 m) where the presence of one species (i.e., *C. laticeps*) inverts the pattern (Model 5 in Table 4, Fig. 5B). Variation in egg size was explained by depth and mass and by their combined effect (Models 6–9 in Table 4 and Fig. 5C to E). The best model fit was obtained by the additive effect of depth and mass (Model 6 in Table 4) explaining 75% of egg size variability. Generally, the egg size (hydrated oocytes) significantly increased with depth ($R^2=0.46$) (Fig. 5C) and slightly with mass

($R=0.08$), except in the smaller individuals (Fig. 5D). The egg size was also significantly explained by RFM (Model 10 in Table 4 and Fig. 5F) that decreased with increasing egg size. The relationship between RFM and egg size was driven by the large egg size and low RFM of *A. rostratus* and the small eggs and higher RFM of *P. blennoides* (Fig. 5F). All the other species analyzed showed similar RFM values (e.g., log RFM between 4 and 6 egg/g), while egg size showed higher inter-specific variation (Fig. 5F). However, note that *P. blennoides* showed asynchronous ovarian organization and potential indeterminate fecundity. Thus, fecundity values are an approximation of the real fecundity (Murua et al., 2003).

The regression model results for the order Gadiformes are summarized in Table 5. The combination effects of depth and mass were significant (Models 1 and 2 in Table 5 and Fig. 6A), with RFM slightly decreasing with depth and increasing with mass. In contrast to the results for the whole community there was no significant relationship between RFM and depth (Model 3 in Table 5) while RFM increased significantly with mass (Model 4 in Table 5 and Fig. 6B). Additionally, a highly significant decreasing pattern of mass with depth was found with the model explaining 80% of the variability (Model 5 in Table 5 and Fig. 6C). The species distributed below 900 m depth (i.e., *N. aequalis*, *T. scabrus* and *C. mediterraneus*) had eggs larger than 1 mm, while the shallower-distributed species (i.e., *P. blennoides* and *M. merluccius*) had eggs equal or smaller than 1 mm (Table 3).

4. Discussion

Several studies have proposed low fecundity with high parental investment in offspring as some of the life-history characteristics

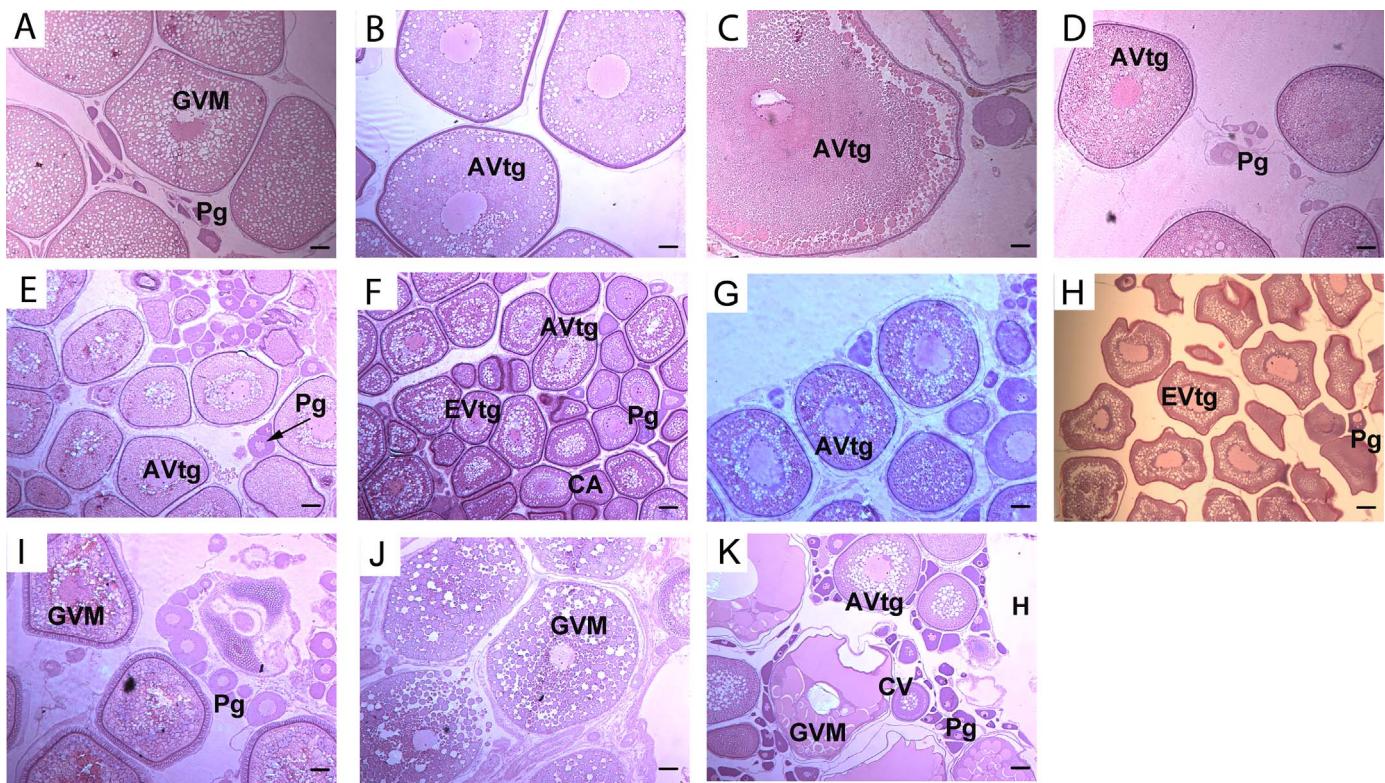


Fig. 3. Histological sections of mature females of the eleven analyzed species in the present study: A, *Notacanthus bonaparte*; B, *Nettastoma melanorum*; C, *Alepocephalus rostratus*; D, *Bathypterois mediterraneus*; E, *Coryphaenoides guentheri*; F, *Phycis blennoides*; G, *Mora moro*; H, *Lepidion lepidion*; I, *Cataetyx allenii*; J, *Cataetyx laticeps*; K, *Epigonus telescopus*. Pg, primary growth oocyte; CA, cortical alveoli; EVtg, early vitellogenic oocyte; AVtg, advanced vitellogenic oocyte; GMV, germinal vesicle migration oocyte. Scale bars=0.1 mm.

that result in a higher vulnerability to natural and anthropogenic stressors (Koslow, 1996; Jennings et al., 2001; Hutchings and Reynolds, 2004; Winemiller, 2005). These traits are the most commonly observed in the deep sea (Mead et al., 1964; Gage and Tyler, 1991; Allain, 2001; Young 2003), suggesting that deep-sea species might be highly vulnerable to impact such as overfishing (Merrett and Haedrich, 1997; Bailey et al., 2009; Norse et al., 2012). Drazen and Haedrich (2012) conducted a comparative approach to analyze bathymetric patterns of life history variables finding that maximum fecundity (fecundity of the largest females for a species) decreased gradually from the shelf to the abyssal plain. These authors suggest that the species' depth of distribution and its environmental covariates (e.g., temperature), explain a great deal of the variability in fecundity across diverse species as well as amongst species within a single order. In the Mediterranean Sea, our results showed a slight decrease in relative fecundity (RFM) with depth. The species inhabiting the middle and lower slopes (below 800 m depth) showed RFM values comparable with those of species distributed at shallower depths and the relationship between RFM and depth only explained 16% of the variability. Additionally, within the Gadiformes there was no relationship between RFM and depth. RMF was mainly related to mass of individuals, indicating that the higher reproductive output of females at shallower depths might be a consequence of their larger size. Similarly, King and Butler (1985) in a study focusing on five pandalid shrimp and later Van Dover and Williams (1991) studying 52 species of squat lobsters found that the fecundity was not correlated with depth and the higher reproductive output was a consequence of the individuals larger size. However, in contrast to these results, general patterns of fecundity decreasing with increasing depth have been reported for crustacean (Company and Sardà, 1997), solitary deep-sea corals (Waller and Tyler, 2011), fish (Drazen and Haedrich, 2012) and seastars species

(Tyler et al., 1984) often related to less favorable environments with lower energy availability, for example in the deep sea or Polar regions (Ramirez-Llodra, 2002).

Fecundity and egg size are intimately related in life-history strategies because the combination of these two parameters represents the maternal investment in reproduction. The evolution of life-history strategies in different species has resulted in different patterns of energy investment to maximize fitness (Ramirez-Llodra, 2002). Based on the different fish life-history strategies proposed by Winemiller and Rose (1992), most of the species analyzed in the present study appear to have adopted an intermediate strategy, lying between species with periodic and equilibrium strategies. At one end, species such as *P. blennoides* represent periodic reproductive species, with asynchronous ovarian organization, higher RFM, and smaller eggs than the average. At the other end, *A. rostratus* represents the equilibrium strategy (K-strategy adaptation), with lower RFM and larger eggs than the average. Note that our results suggest that *P. blennoides* has asynchronous ovarian organization and indeterminate fecundity, thus our values may be underestimates (Murua and Saborido-Rey, 2003) and further studies are needed to obtain the real fecundity values.

The production of large eggs that hatch into advanced larvae has been suggested as the most common pattern in deep-sea species, but it is not universal (Eckelbarger and Watling, 1995; Young, 2003; Pearse and Lockhart, 2004). Low temperature and limited food influence the life history of deep-sea species (Marshall, 1937). Between species, there is an increasing trend in egg size with a decrease in temperature (Clarke and Gore, 1993). Temperature driven pattern has been observed in different taxonomical groups mainly in crustacean and mollusks (Thatje and Hall, 2016). This rule, called the Thorson-Rass rule is based on the effect of temperature in the developmental mode and larval

Table 3

Reproductive characteristics of W Mediterranean fish species, including the depth distribution range, length range, total and relative fecundity ranges, maximum diameter of oocyte, ovarian organization and reproductive strategies: A, Asynchronous ovarian organization, GS, group synchronous ovarian organization; O, oviparous; V, viviparous; Z, zygotoparous.

Species	Depth range (m)	Length range (mm)	Total fecundity (eggs/females)		Relative fecundity (egg/g)		Egg size (mm)	Ovarian organization	Reproductive strategies	Source
			Range	Mean \pm std	Range	Mean \pm std				
O. Notacanthiformes										
Not.bon	300–2250	69–125	1378–12,387	3870 \pm 2188	110–402	119 \pm 71	1.3	GS	O	This study
O. Anguilliformes										
Net.mel	700–1400	222–830	2025–53,132	35,459 \pm 15,474	96–325	222 \pm 64	0.9	GS	O	This study
Oph.ruf	90–130	320–540	1426–23,605	22,676 \pm 4100	103–489	259 \pm 90.5	0.7	GS	O	Casadevall et al. (2001)
Gna.mys	80–400	288–388	2951–26,522	13,325 \pm 6148	73–403	228 \pm 96.5	0.7	GS	O	Casadevall (1991)
O. Osmeriformes										
Ale.ros	300–2300	270–380	1003–3869	1687 \pm 693	2–9	4 \pm 1	2.1	GS	O	This study
O. Aulopiformes										
Bat.med	900–3300	69–180	1829–15,528	6780 \pm 3670	109–564	326 \pm 139		GS	O	This study
O. Gadiformes										
Nez.aeq	300–1500	42–60	3408–20,552	10,630 \pm 4850	78–454	215 \pm 105	1.7	A	O	Fernandez-Arcaya et al. (2013a,b)
Tra.sca	400–1500	130–181	4189–43,644	14,191 \pm 8998	22–174	82 \pm 52	1.1	GS	O	Fernandez-Arcaya et al. (2012)
Coe.med	1000–2200	55–98	2127–17,377	7693 \pm 3692	31–476	244 \pm 102	1.4	GS	O	Fernandez-Arcaya et al. (2013a,b)
Cor.gue	1200–3000	52–71	1835–14,975	4657 \pm 5162	123–412	203 \pm 107	**0.55	GS	O	This study
Mer.mer	50–750	300–780	17,296–681,489	126,035 \pm 100,547	53–462	204 \pm 88	1.0	A	O	Recasens et al. (2008)
Phy.ble	300–1300	266–540	684,505–4,209,000	1,643,898 \pm 115,944	1036–3960	1942 \pm 882	0.81	A	O	This study
Mor.mor	400–1500	304	181,256		622		*0.66	GS	O	This study
Lep.lep	400–2300	212–285	1609–9733	5670 \pm 4062	26–49	37 \pm 12	*0.85	GS	O	This study
O. Ophidiiformes										
Cat.alie	700–2000	75–123	101–1507	844 \pm 636	7–273	61 \pm 52	*1.11	GS	V	This study
Cat.lat	1200–3000	302–490	6445–28,029	20,107 \pm 19,904	22–174	45 \pm 26	*1.1	GS	V	This study
Oph.bar	90–500	139–187	539–3814	1696 \pm 1064			1.1	A	O	Casadevall (1991)
Oph.roc	0–150	155–240	273–17,003	5968 \pm 3645			1.1	A	O	Casadevall (1991)
O. Lophiiformes										
Lop.bud	100–800	460–650	87,569–398,986		65–149		1.38	GS	O	Colmenero et al. (2013)
O. Scorpaeniformes										
Asp.obs	30–170	131–207	6819–22,525	12,038 \pm 3485	80–334	230 \pm 85	1.0	GS	O	Muñoz et al. (2003)
Hel.dac	100–400	163–224	11,274–87,438	35,748 \pm 18,478	93–330	330 \pm 169	0.64	A	Z	Muñoz et al. (2003)
Sco.not	50–700	94–151	6026–32,756	15,194 \pm 6070	70–397	201 \pm 72	0.5	A	Z	Muñoz et al. (2005)
Sco.scr	50–300	199–356	152,949–428,057	247,407 \pm 98,050	237–481	357–98	0.6	GS	Z	Sabat (2005)
Sco.por	50–800	104–356	34,421–428,057	147,417 \pm 96,508	228–1071	501–254	0.6	GS	Z	Sabat (2005)
O. Perciformes										
Epi.tel	800–1200	309–405	100,312–603,599	351,955 \pm 251,643	72–571	321 \pm 249	0.95	A	O	This study

Oph.ruf, *Ophichthus rufus*; Gna.mys, *Gnathophis mystax*; Nez.aeq, *Nezumia aequalis*; Tra.sca, *Trachyrincus scabrus*; Coe.med, *Coelorinchus mediterraneus*; Mer.mer, *Merluccius merluccius*; Oph.bar, *Ophidion barbatum*; Oph.roc, *Ophidion rochei*; Lop.bud, *Lophius budegassa*; Asp.obs, *Aspitrigla obscura*; Hel.dac, *Helicolenus dactylopterus*; Sco.not, *Scorpaena notata*; Sco.por, *Scorpaena porcus*.

* Diameter from oocyte in initial migration phase.

** Diameter from oocyte in advance vitellogenic phase.

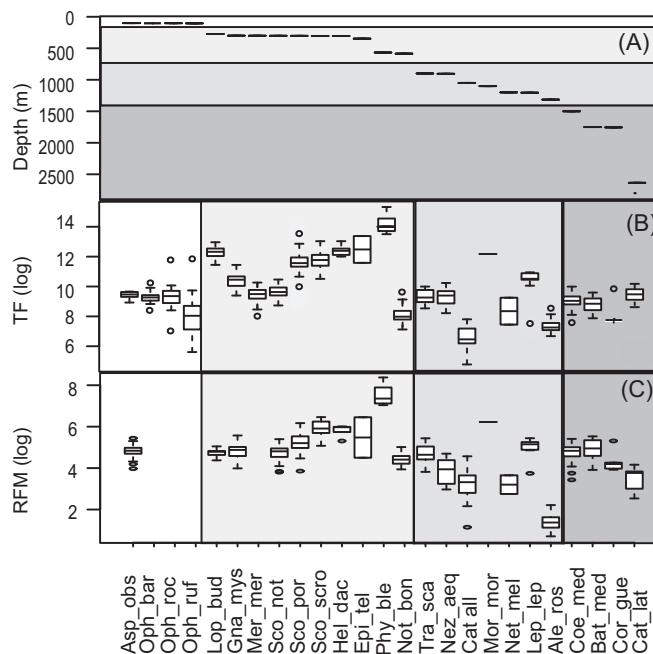


Fig. 4. Bathymetric distribution of the 25 species analyzed in the present study ordered by their maximum abundance depth. The bathymetric groups are represented by shades of the grey scale: the continental shelf in white (0–200 m); the upper slope in light grey (200–800 m); the middle slope in dark grey (800–1350 m); and the lower slope in light black (1350–2850 m). (A) Bathymetric distribution. (B) Total fecundity (TF), and (C) relative fecundity (RFM) are shown by bathymetric group. Boxes are the lower to the upper quartile, and the centerline is the median. Species name codes are below the Table 3.

biology, as well as changes in the species physiology (i.e., yolk composition and accumulation patterns) (Rass, 1935; Thorson 1936, 1950). Low metabolic rate of females at lower depths reduces the somatic cost allowing a higher increase of energy allocation on the vitellogenetic process (Shearer, 1996). We observed that deep Mediterranean fish, although inhabiting isothermal waters, follow this general rule, with deeper species investing more energy into individual eggs. The unique conditions of the Mediterranean Sea, with a constant and relatively high temperature (13 °C) below 200 m depth, suggest that rather than temperature, other environmental (i.e., food-limited environment) or phylogenetic variables may be driving the observed patterns of egg size in the deep-sea fishes studied. Larvae hatching from larger eggs appear to hatch at a more advanced developmental stage and have longer dispersal periods than larvae from smaller eggs (Marshall and Keough, 2007), which increases their survival probability (Vance, 1973; Smith and Fretwell, 1974; Stearns, 1992). Several authors have suggested ontogenetic vertical migration

patterns in many deep-sea species (Stein and Pearcey, 1982; Adams et al., 2012; Priede and Froese, 2013; Longmore et al., 2014). Several studies suggest that some species of grenadiers, for example, spawn near the seafloor and the buoyant eggs float to the thermocline where the larvae hatch. The larvae grow near the thermocline and then descend to adult-living depths (Lin et al., 2012; Marshall, 1973). These patterns have been associated with the higher temperature (Werner et al., 1996), higher food availability (Lin et al., 2012) and recently also to the decrease of pressure (Smith et al., 2015) at shallower depths. In our study, the presence of eggs with large oil droplets (which contribute to buoyancy), the description of larvae of some of these species in the first 200 m depth (Bianco et al., 1931; Sabates and Fortuño, 1987) and the occurrences of juveniles at the shallowest depth sampled (i.e., 400–600 m) (Fernandez-Arcaya et al., 2013b), suggest also a pelagic larval development and ontogenetic vertical migration of these fish species in the W Mediterranean. Larval vertical distributions may enhance also larval transport and dispersal of deep-sea species (Arellano et al., 2014; Hilário et al., 2015). From a phylogenetical point of view, many deep-sea species colonized the Mediterranean through larval dispersal over the Gibraltar sill from the Atlantic Ocean (Bouchet and Taviani, 1989). Thus egg size patterns might reflect the species' Atlantic and cold ancestral history (Eckelbarger and Watling, 1995). In fact, theory suggests that selection exerts stronger control on egg size than upon fecundity (Johnson and Legett, 2002).

The finding of similar relative fecundities but larger egg sizes in the fish species studied compared to shallower counterparts here suggests that these deep-sea species are investing a higher amount of energy in the production of offspring than shallower water counterparts. This is surprising given the typical reduction in food availability with depth, particularly strong in the Mediterranean (Margalef, 1985), which might constrain energy investments in reproduction. There are some results that suggest that non-annual reproduction could be a strategy used by some of these species, potentially allowing for a higher investment per offspring in a food-limited environment; (1) Despite the high number of females of *Phycis blennoides*, *Mora moro*, *Lepidion lepidion*, *Cataetyx allenii*, *C. laticeps* and *Coryphaenoides guentheri* sampled in this study, and the fact that the sampling was conducted during the reproductive period of the studied species, only a small number of spawning females were collected, with most of the females reproductively inactive, (2) the developmental phase was corroborated by the microscopic analysis of the gonads, which confirmed that the ovaries were not at the post-spawning or early oogenesis stages of the reproductive cycle, (3) this phenomenon was also observed in a previous study conducted in the Mediterranean on the same fish species, where, although high numbers of individuals of *L. lepidion*, *M. moro* and *P. blennoides* were found, very few mature females were captured (Rotllant et al., 2002). A

Table 4

Summary of regression models results assessing the effects of the maximum abundance depth, length and weight of all the fish species on reproductive variables in the Mediterranean Sea. RFM=relative fecundity; Species=random effect; n=number of samples, edf=estimated degree of freedom; AIC=Akaike Information Criteria.

Model	Response variable	Explanatory variable	n	edf	AIC	R ²	p-value
1. GAMM	RFM	~s(Depth)+s(Mass) Species	388	1.00:2.17	600	0.15	0.05; 0.14
2. GAMM	RFM	~s(Depth, Mass) Species	388	2	599	0.14	0.10
3. GAMM	RFM	~s(Depth) Species	388	1.00	598	0.16	0.04*
4. GAMM	RFM	~s(Mass) Species	388	2.30	600	0.00	0.36
5. GAMM	Mass	~s(Depth) Species	391	2.17	525	0.17	0.049*
6. GAM	Egg size	~s(Depth)+s(Mass)	442	3; 2.97	15	0.75	< 0.01*; < 0.01*
7. GAM	Egg size	~s(Depth, Mass)	356	2.72	223	0.43	< 0.01*
8. GAM	Egg size	~s(Depth)	442	2.97	221	0.46	< 0.01*
9. GAM	Egg size	~s(Mass)	356	2.94	467	0.08	< 0.01*
10. GAM	Egg size	~s(RFM)	353	1.91	270	0.34	< 0.01*

* Statistically significant.

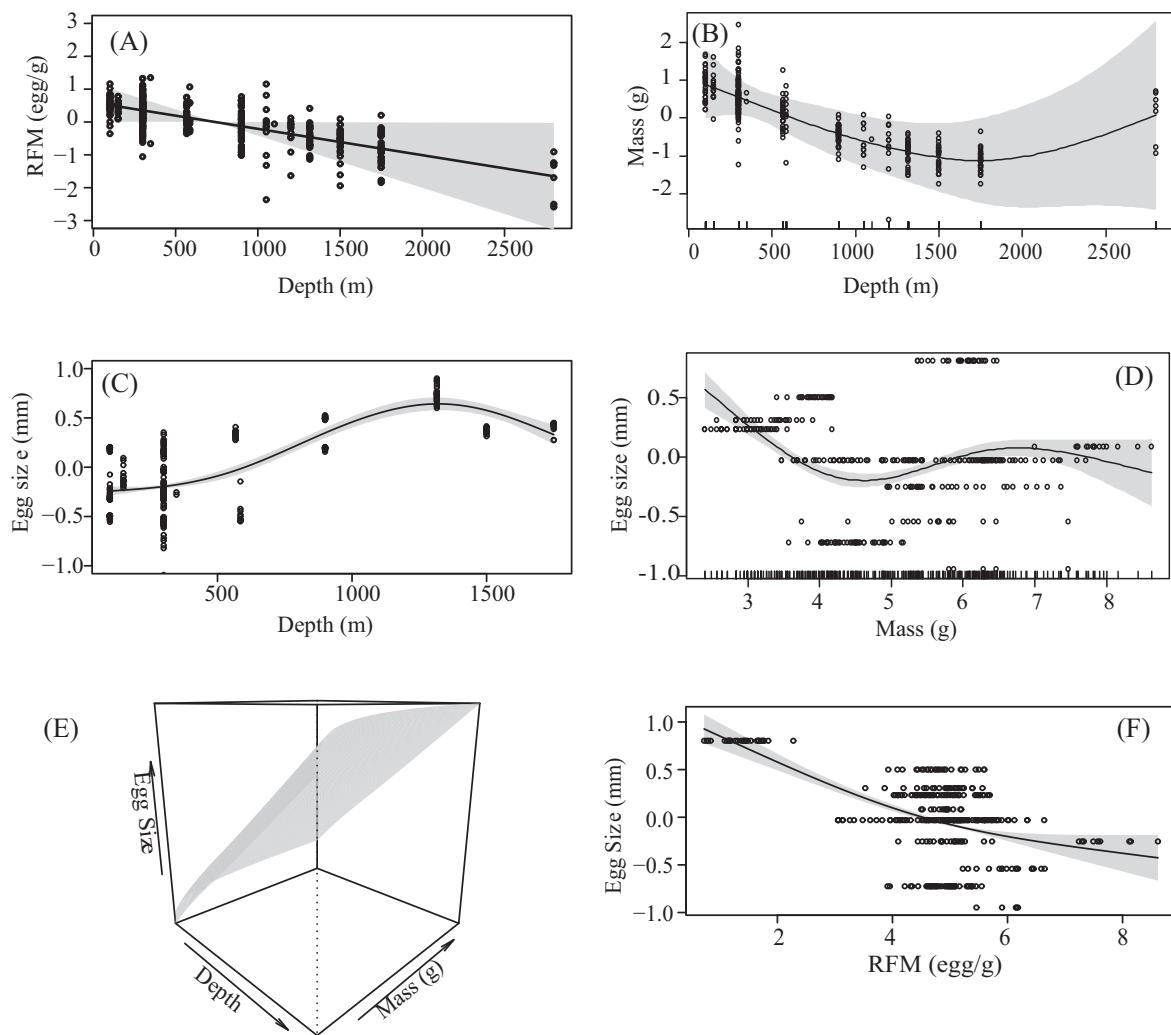


Fig. 5. Results of significant regression models, fitted for all the fish species to assess the connection of relative fecundity (RFM) with depth (A) mass of individuals and depth (B), egg size with depth (C), egg size with mass (D), egg size with the interaction of depth and mass (E) and egg size with relative fecundity (RFM) (F). Shadowed areas represent 95% confidence intervals. The statistical summary of plots is summarized in Table 4.

counterargument is that migration of spawning females to spawning grounds could explain the lack of reproductively active females in our samples. However, the region was well sampled bathymetrically and because of the low energy availability of the deep Mediterranean Sea it is more probable that species delay spawning for a longer period, rather than consume a high proportion of their energetic budget to migrate for spawning. In fact, several authors have suggested semi-annual spawning for certain deep-sea fish species (i.e. *Coryphaenoides acrolepis* and *Hoplostethus atlanticus*) (Bell et al., 1992; Drazen, 2002; Minto and Nolan, 2006), as well as episodic recruitment periods (Koslow et al., 2000; Norse et al., 2012). This phenomenon could be an adaptive response to the low food availability in the deep sea, especially in the oligotrophic Mediterranean Sea, where the species might need to invest energy into egg development for a period longer than one year to maximize fitness (Jorgensen et al., 2006). As it has been noted by some authors (i.e., Rideout, 2005; Alonso-Fernandez, 2011), the shortage of historical data on non-annual spawning of fish in the wild may reflect the rarity of this condition, or may simply reflect the difficulty of identifying non-reproductive individuals. This phenomenon is even more critical in the deep sea, where long-term and seasonal data on reproduction is still very scarce and such analyses are in their infancy. Thus, our results are still preliminary and further investigations are needed in order to

corroborate the occurrence of skipped spawning in these deep Mediterranean fishes.

In conclusion, we present here the first study on the bathymetric patterns of fish fecundity and egg size in an isothermal deep-sea environment. Egg size increased with depth, suggesting

Table 5

Summary of regression models results assessing the effects of depth, length and weight on reproductive variables of Gadiformes species in the Mediterranean Sea. RFM=relative fecundity; Species=random effect; n=number of samples, edf=estimated degree of freedom; AIC=Akaike Information Criteria.

Model	Response variable	Explanatory Variable	n	edf	AIC	R ²	p-value
1. GAMM	RFM	~s(Length)+s(Mass) Species	145	1.00; 1.00	245	0.12	0.6; 0.01*
2. GAMM	RFM	~s(Depth, Mass) Species	145	2.00	243	0.12	0.03*
3. GAMM	RFM	~s(Depth) Species	145	1.00	245	0.07	0.24
4. GAMM	RFM	~s(Mass) Species	145	1.00	243	0.18	0.01*
5. GAMM	Mass	~s(Depth) Species	146	1.00	241	0.80	<0.01*

* Statistically significant.

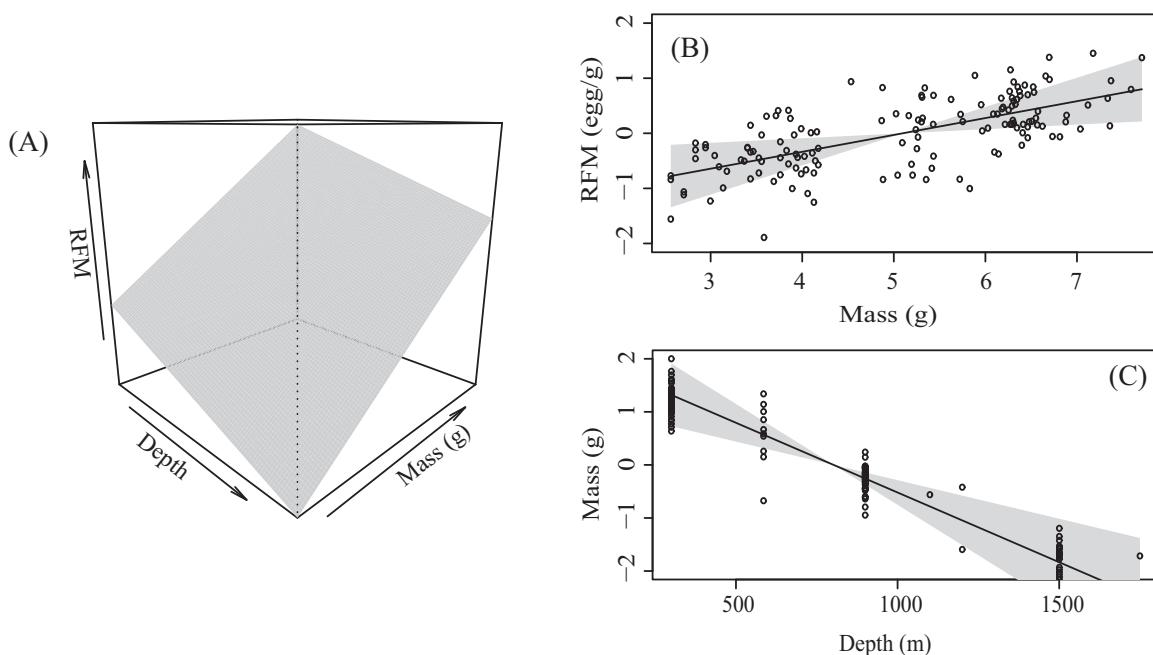


Fig. 6. Results of significant regression models, fitted for species of the Order Gadiformes to assess the link of relative fecundity (RFM) with the interaction of depth and mass (A), RFM with fish mass (B) and fish mass with depth (C). Shadowed areas represent 95% confidence intervals. The statistical summary of plots is summarized in Table 5.

that rather than temperature, environmental (e.g., food supply) or phylogenetic factors are driving this trend. The results also showed that Mediterranean deep-sea fishes are investing a high amount of energy in the production of offspring, showing similar relative fecundities but larger egg sizes than the shallower distributed species. The present study provides a step forward in our knowledge of reproductive output of deep-sea fishes. Nevertheless, it highlights also the need for further investigations, including: (1) fecundity and egg size data of a number of marine fishes, including commercial and non-commercial species; (2) early life-history characteristics and larval ecology of deep-sea species, to better understand evolutionary pressures, dispersal and connectivity; (3) long-term time series data to assess the possible significance of skipped spawning (non-annual reproduction) in a population. Such knowledge is necessary to anticipate the recruitment variability of deep-sea species and, therefore, to contribute to understanding the factors that shape their vulnerability to natural and anthropogenic change and their resilience and potential for recovery.

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