Female reproductive biology of the bluemouth *Helicolenus dactylopterus dactylopterus* in the north-western Mediterranean: spawning and fecundity

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Running headline: spawning and fecundity of *Helicolenus*

17 Abstract

18 The aim of this study is to analyze the reproductive traits of the bluemouth (*Helicolenus*
19 *dactylopterus dactylopterus*) that are critical to the population dynamics of this deep-sea
20 species and the subsequent management of fisheries in the Mediterranean Sea. This is
21 a zygoparous species that spawns multiple batches of embryos enclosed within a
22 gelatinous matrix. Oocyte development is asynchronous and the recruitment of
23 secondary growth oocytes occurs continuously during the developing phase, but stops
24 prior to the start of the first spawning (i.e. fecundity is determinate). The number of
25 developing oocytes can be estimated as a function of the length of the fish, its ovary
26 weight and its gonadosomatic index. Only at the onset of spawning, when potential
27 fecundity is determined, does condition also have a significant effect on it. The low
28 levels of atresia detected during most of the spawning season show that this
29 mechanism does not substantially affect the process. There is variability both in the
30 spawning interval (with a mean of two days) and in the number of embryos conforming
31 every single batch (up to 37000). The expected impact from fisheries on behalf of the
32 reproductive characteristics of bluemouth is also discussed.

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34 Key Words: atresia, fecundity, *Helicolenus,* spawning, reproduction.

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37 Introduction

38 The bluemouth, *Helicolenus dactylopterus dactylopterus* (De La Roche, 1809), is a
39 benthic species widely distributed in the Atlantic Ocean and in the Mediterranean Sea,
40 inhabiting depths of between 100 and 1000 meters (Whitehead *et al.*, 1986). Although

there has been little commercial interest in this species, partly due to its low accessibility, it is now of growing commercial interest as new resources need to be found by fishing fleets due to the depletion of traditional resources. In this respect, the exploitation of deep-sea species has increased in the last ten years, particularly in the Spanish fishing industry. Among the 22 species caught by the Spanish fleet in Atlantic waters and analyzed by the "Working Group on the Biology and Assessment of Deep-Sea Fisheries Resources" of the International Council for the Exploration of the Sea (ICES, 2005), bluemouth captures increased by 200% between 2003 and 2004.

The risk of overexploitation of deep-sea species is high even at relatively low fishing mortality levels. This high risk is basically due to these species generally having slow rates of growth, late maturation and low fecundity (Gage & Tyler, 1991). Slow growth and late maturation are characteristics of the bluemouth (Heessen *et al*., 1996; Massutí *et al.*, 2000; White *et al*., 1998). As for its reproductive biology, a previous study has shown that it is an oviparous zygoparous species (Muñoz *et al*., 2002a), which implies a level of oviparity specialization. Fertilization is internal and the female is able to store sperm in the ovary for long periods of time, within specialized interlamellar structures (Muñoz *et al.*, 1999; 2000) where male sex cells are maintained in a viable state and protected from the female immune system (Muñoz *et al.*, 2002b; Vila *et al.*, 2007) until oocytes mature. Sperm is then released towards the ovarian lumen to fertilize mature oocytes.

Although several studies on bluemouth reproduction have also been published to show that spawning is multiple and consists of a gelatinous matrix that encloses eggs and

embryos at early stages of development (Sanchez & Acha, 1988; White *et al.*, 1998; Muñoz & Casadevall, 2002; Sequeira *et al.*, 2003), there are still many unknown characteristics of its reproductive biology that may affect the reproductive potential of the species – such as fecundity, atresia and spawning frequency – and that are critical to the population dynamics of this deep-sea species and the subsequent management of fisheries.

Incorporating aspects of reproductive biology into the management of fish stocks is increasingly important in world fisheries (reviewed by Jakobsen et al., 2009). For this reason, the aim of this work is to analyze the reproductive traits of the bluemouth that are important for the sustainability of fisheries where this species is exploited. We quantify the number of developing oocytes of the specimens and explore how this relates to the size and weight of the females, as well as to their condition and their gonadosomatic (GSI), hepatosomatic (HSI) and fat somatic (FSI) indices. The oocyte size-frequency distribution for each spawning female is also analyzed. Kjesbu (2009) believes oocyte size-frequency distributions can indicate at which point the female currently is in the maturation cycle (West, 1990; Murua & Motos, 2006) and can also demonstrate different maturation patterns and associated fecundities (Witthames *et al.*, 1995; Kjesbu *et al.*, 1998; among others).

The effect of atresia on the final number of developed oocytes is also analyzed, because the quantification of the process of reabsorption of oocytes is also a useful parameter to determine the oocyte recruitment process of the species. Finally, we determine the fraction of spawning females and the spawning interval, as well as the main characteristics of a single batch of spawn.

Materials and Methods

Samples

From January 2007 until October 2008 a total of 370 specimens of *H. dactylopterus* were obtained directly from Spanish bottom trawlers and longliners fishing on the continental shelf and submarine canyons, respectively, of the north-western Mediterranean, between 41º40´ and 42º30´ N and between 03º19´ and 06º23´ E. Sampling was conducted at different ports along the Costa Brava (Figure 1), just after the fishing vessels, which conduct one-day fishing trips, landed their catches. The number of samples varied monthly and increased during the peak of the reproductive season (between December and May).

The total length (TL), the total and eviscerated weights (TW and EW) and the weights of the gonad (GW), liver (LW) and perivisceral fat (PW) – adipose tissue surrounding the gastrointestinal tract – of all females were recorded. TL was measured in mm while all weights were measured to the nearest 0.01g for TW and EW, and to the nearest 0.0001g for the rest. Mean size and weight of the analyzed specimens are shown in Table I.

Histological analyses, fecundity and atresia

A histological analysis was performed in order to analyse the reproductive characteristics of the species. This is the most accurate way to determine the individual stage of sexual maturation of marine fish because it provides more consistent results

than a visual inspection of reproductive organs (Murua & Motos, 1998; Saborido-Rey & Junquera, 1998; Kjesbu *et al*., 2003; Tomkiewicz *et al*., 2003).

The ovaries of 230 individuals underwent histological analysis to determine the annual reproductive cycle of the species. Samples were embedded in blocks of paraffin, sectioned at between 3 and 8 µm – according to the state of maturity of the gonads – and stained with hematoxylin-eosin, Mallory's trichrome and the PAS-Schiff reaction. The last two staining methods highlight the *zona radiata* and its continuity, and facilitate the detection of atretic oocytes. The stages of development of the oocytes and the ovaries were determined using the criteria established by Wallace and Selman (1981) and West (1990). The mean cellular diameters during the stages of oogenesis of *Helicolenus dactylopterus* have been published in a previous work (Muñoz *et al*., 2002a). Fish reproductive cycles, according to Brown-Peterson *et al.* (2009), are divided into immature, developing, spawning-capable, actively spawning, regressing and regenerating stages.

The presence of hydrated oocytes, embryos and postovulatory follicles (POF) was analyzed to select suitable specimens for the calculation of potential fecundity. Alpha-atretic oocytes were quantified to evaluate the effect of atresia on the spawning process of the species.

The number of developing oocytes (NDO) (Dominguez-Petit, 2007), defined here as the total number of oocytes in the cortical alveoli stage and onwards (with a diameter ≥ 100µm), was calculated as an estimation of the potential fecundity of each specimen. The relative NDO (NDO/eviscerated weight) was also analyzed. These numbers and the oocyte-size frequency distributions were determined by the gravimetric method

combined with image analysis techniques (Dominguez-Petit, 2007). Slices from the central area of the ovary of the 81 females in late developing phases – spawning-capable or actively spawning (fish that will spawn in this cycle or with imminent, active or recent spawning, respectively) – were weighed. Oocytes were then separated using a washing process and sorted by size through several sieves (from 100 µm to 700 µm), which facilitated the next steps of counting and measuring oocytes using a computer-aided image analysis system (Image-Pro Plus 5.1). Homogeneity in oocyte distribution within the ovaries had already been established in Muñoz *et al.* (1999).

A single spawning batch was also analyzed. One of the females was captured just before spawning, so we could work with the whole gelatinous matrix that encloses the eggs and embryos. In this case no sub samples were taken, and the whole batch was analyzed. The number of hydrated oocytes and embryos of the most advanced spawning batch was also estimated from the largest modal group of oocytes of the oocyte size distribution (following Hunter *et al.,* 1985) of eight actively spawning females.

The prevalence of atresia (percentage of sexually mature females that show α-atretic oocytes -described for the species in Muñoz et al., 2002a- in their ovaries) and its relative intensity (percentage of α-atretic vitellogenic oocytes in relation to the total number of vitellogenic oocytes) were determined for the spawning-capable, actively spawning and regressing phases (from December to May). Three different fields from different histological slides of each specimen were analyzed. The fraction of spawning females (S) and the spawning interval (time between batches considering the percentage of spawning females in the population at a specific time) were determined

160 from the percentage of the sexually mature females showing signs of recent ovulation
161 (with day-1 POF). These indices were estimated following Murua & Motos (2006).
162
163 Fish condition and gonad size
164 To determine fish condition, we calculated several condition indices for each single
165 adult fish. First, Fulton's condition factor (K) was calculated using the formula K = 100
166 *(*EW/TL^3), where EW is the eviscerated wet body weight and TL is the total length. This
167 index assumes that a heavier fish than expected for a given length indicates a surplus
168 of energy stored in the body and hence a fish in better condition. However, the analysis
169 of length-weight relationships showed a b-value greater than 3, and so we also applied
170 the relative condition index (K_n, Le Cren, 1951), which was calculated using the formula
171 Kn = W / W', where W is the observed weight and W' is the estimated weight (estimated
172 from the weight-length relationship using all the collected data). This second
173 morphometric index was selected because it does not assume isometric growth and
174 thus compares the actual (observed) body weight of a given individual to a standard
175 predicted by the weight-length relationship based on the population from which the
176 individual was sampled.
177 The hepatosomatic index (HSI) was also calculated as a proxy for energy reserves
178 stored in the liver. The HSI was calculated as HSI = 100 (LW/EW), where LW
179 represents the liver weight.
180 Another estimate of condition was calculated using the fat somatic index (FSI), which
181 refers to the energy reserves stored in the perivisceral fat (the adipose tissue
182 surrounding the gastrointestinal tract). This index was calculated as FSI = 100
183 (PW/EW), where PW represents the weight of the perivisceral fat.

Finally, as a proxy of energy reserves invested in reproduction, we computed the gonadosomatic index (GSI = GW / EW *100) where GW is the gonad weight.

All these indices were calculated by separating the spawning-capable specimens that had still not started to spawn (ovaries without POF) from those that had already released at least one batch (ovaries with POF).

Statistical analyses

Linear regressions were used to analyze the relationship between the NDO (as a proxy of fecundity) and maternal attributes such as the total length, total body weight, the ovary weight, the gonadosomatic index (GSI), the hepatosomatic index (HSI), the fat somatic index (FSI) and both the K and Kn condition factors. Data for NDO, ovary weight, GSI and FSI were log-transformed to meet the assumption of normality; the rest of the variables were already distributed normally. The relationships were analyzed separately for individuals that had already started to spawn and those that had not (i.e. females with and without POF).

A partial least square regression (PLSR) analysis was also used to asses which maternal attributes (total length, GSI, HIS or K) are more decisive for defining the total number of developing oocytes. PLSR is a statistical technique that combines features of multiple regression and principal component analysis (PCA) (Abdi, 2003), and it is especially suitable when predictors are highly correlated. The strength of the relationships between each original predictor and the dependent variable (NDO) can also be evaluated through standardized coefficients (β). Data for NDO and GSI were again log-transformed to meet the assumption of normality.

We used a generalized linear model to test whether length of the specimens, month of the spawning season, developmental stage of the ovary and presence of POF in the ovary accounted for the change in the number of developing oocytes. The length of specimens was considered as covariate, and its influence on the potential fecundity was tested along with the three cited factors: month of the spawning season (six levels, since only spawning months, from November to April, were tested), the development stage of the ovary (five levels related to the most advanced developmental stage of the germinal cells) and presence of POF in the ovary (two levels: females that have already started to spawn and those that had not). The first two analyses were also done by separating ovaries with POF from those without them. The possible changes in the functional relationship between length of the specimens and the month of the spawning season, the developmental stage of the ovary or the presence of POF in the ovary were tested using homogeneity of slope tests (Garcia-Berthou & Moreno-Amich, 1993). As the interaction term from the homogeneity of slopes test was not always statistically significant ($p \geq 0.14$), it was deleted from the models, and standard ANCOVA analyses were run.

An analysis of variance was also used to test if the developmental stage of the ovary is related to the month.

Results

Oocyte development

The oocyte size-frequency distribution was continuous, without any evident hiatus between primary and secondary growth stage oocytes. In females that have not yet started to spawn – i.e. no POF were observed in the ovary – the oocyte size-frequency distribution is near normal, with a prominent stock of oocytes in early vitellogenic stages at 200-250 µm (Figure 2A). In January a low amount of late vitellogenic oocytes are observed (400-450 µm). When spawning has started and at least one batch has already been released (ovaries with POF), the oocyte size-frequency distribution changes and shows two clearly distinct modes (Figure 2B): one cohort of oocytes in early vitellogenesis and another one in advanced vitellogenesis or final maturation (migratory nucleus oocytes). Even at the end of the spawning season a stock of vitellogenic oocytes continues to exist. From this stock several batches can usually be observed, and a separate mode of hydrated oocytes and embryos in early stages of development can be differentiated (Figure 3).

Fecundity

The coefficients of determination and the significance levels of the linear regressions between the estimated fecundity and several maternal features are shown in Table II. There was always a positive significant ($p \leq 0.01$) relationship between the estimated fecundity and the total length of the specimens, but correlations were higher in females that had not started to spawn (i.e. POF were not present in the ovary) than in those that had started. The body weight, ovary weight and GSI of females are highly correlated with the number of developing oocytes before they start to spawn (ovaries without POF). The analysis of the partial least square regression (PLSR) (Table III) highlights the high incidence of the length of specimens and their GSI on this estimation of

fecundity. The relationship between estimated fecundity and any of the condition indices considered in this study was not significant (HSI, FSI, K; $p>0.01$), except in the case of Kn of females that had still not started to spawn. In those females, condition is significantly correlated with fecundity.

The total number of developing oocytes (estimated fecundity) in ovaries with and without POF is not significantly different (ANCOVA $\nu=1$, $F=0.14$, $p=0.7$). However, the total and the relative number of developing oocytes increases through the breeding season as the ovary develops until spawning starts (i.e. the presence of females with hydrated oocytes) as shown in Figure 4. Thus, specimens of the same size and captured in the same month, and that have still not started to spawn (ovaries without POF), showed significant differences in the number of developing oocytes, depending on the development stage of their ovaries (ANCOVA $\nu=4$, $F=4.59$, $p=0.0027$). Figure 5 shows three selected examples of females about the same size (239 mm) collected in January with considerable differences in NDO for females in the cortical alveoli stage – early vitellogenesis (17000 oocytes), middle-late vitellogenesis (77000 oocytes) and females close to spawning season, i.e. with already hydrated oocytes (143000 oocytes). Females that have already start to spawn (with POF in the ovary) show no significant differences in the estimated fecundity in relation to the developmental stage of the gonads (ANCOVA $\nu=1$, $F=1.02$, $p=0.3276$).

The NDO of females that have still not started to spawn (ovaries without POF) is also significantly related to the month (ANCOVA $\nu=3$, $F=4.88$, $p=0.0043$), because the stage of development of the ovaries advances as the breeding season progresses (ANOVA

$\nu=5$, F=5.5, p=0.0002). Again, differences are not significant when the analysis is done for females that have already started to spawn (ANCOVA $\nu=4$, F=4.98, p=0.0134).

As mentioned, both NDO and relative NDO increase from November to January, and during developmental stages in females that have still not started to spawn (Figure 4 A and B, respectively). However, as shown in this figure, there is a marked decrease in NDO in females in spawning condition (with hydrated oocytes and/or embryos) and during the months when spawning is common (from February to April). The maximum value for NDO was 428343 oocytes in development for a 380 mm-TL female; and the minimum number of oocytes detected in an actively spawning female was 31767 oocytes in a 292 mm-TL female (mean = 122984 ± 92197).

The largest modal group of the oocyte size distribution of the eight females that were in imminent spawning phase is constituted by a variable number of embryos (between 3566 and 37097). From these, only one was collected immediately before spawning. The ovary of this female contained a gelatinous matrix with a few oocytes and more than 4750 embryos in early stages of development (Figure 6A). The ovary still showed an important stock of oocytes in early vitellogenic stages, as well a clutch of oocytes in the migratory nucleus, very likely the next batch to be spawned (Figure 6B).

Spawning

The spawning period for *H. dactylopterus* in the Gulf of Lion extends from the end of December to May (Figure 7), as shown by the presence of females with hydrated oocytes and/or embryos. From February to May there were no ovaries in the initial stages of the cycle (with only primary growth, cortical alveoli or early vitellogenic stages, i. e. ovaries in developing phase). From March onwards ovaries were only found in

actively spawning (late vitellogenic or hydrated oocytes and POF) and regressing phases (with the vitellogenic oocytes undergoing alpha or beta atresia, POF and some less-developed oocytes present). While in November there were no signs of spawning activity and the majority of females were in the developing phase (oocytes more developed were in early vitellogenesis), in December an important proportion of females were already in spawning condition, and even a few were in the regressing phase.

Of the analyzed females 54% were actively spawning at the time of their capture, i.e. spawning (presence of hydrated oocytes and/or embryos) or recently spawning (presence of recent POF). The mean spawning frequency estimated for actively spawning females indicates that the population spawns almost every two days (1/S = 1.98). The dynamic spawning interval throughout the spawning season is shown in Figure 8. During the peak of spawning most of the females produce almost one batch per day.

Atresia

The mean prevalence of atresia for the population analyzed is Pa=9.15% and its mean relative intensity is R_{Ia}=7.9%. The results for every month and the individual characteristics of the females with atresia are shown in Table IV. The relative intensity of the atresia is, in general, very low, but it increases as the spawning period advances, to 58% in the month of May, when ovaries are already in the regression phase.

The relative intensity of the atresia also varies in relation to the stage of development of the ovaries: spawning-capable (n=2; RIa=1.45%), actively spawning (just spawning n=1; RIa=5.56%) and recently spawning or with POF (n=8; RIa=3.04), and regressing (n=2; RIa=58.33%).

Discussion

We found relatively high estimated fecundity values in the bluemouth, bearing in mind its internal fertilization and zygoparity. This fecundity is comparable to, and in some cases higher than, that of other related species that fertilize externally and reproduce by the simpler oviparous mode (Muñoz et al*.,* 2003; 2005). However, the related viviparous genus *Sebastes* also shows high fecundities (Drevetnyak & Gusev, 1996): up to 300,000 larvae in a female measuring 55 cm (F. S-R own data). Zygoparous, embryoparous and viviparous species are able to produce more surviving young than do their strictly oviparous congeners from similar egg clutches, hence the evolution of viviparity and related strategies is often linked to a reduction in fecundity (Uribe *et al*., 2009). But, as noted by Wourms (1991), rockfish seem to have evolved an effective means of reproduction that combines the fecundity of oviparity with the enhanced chances of survival for embryos or larvae that are conferred by viviparity.

Some features suggest an indeterminate nature of the fecundity of this species (following Hunter *et al.* (1992), Greer-Walker *et al.* (1994) and Murua & Saborido-Rey (2003)), namely i) the absence of a hiatus between previtellogenic and vitellogenic oocytes in the oocyte-size distribution and ii) the increasing number of developing oocytes as the reproductive season progresses. However, this evidence only indicates that there is a continuous recruitment of oocytes throughout the season, which is typical of asynchronous development (Murua & Saborido-Rey, 2003; Korta *et al*., 2010). Thus, specimens of the same size and captured in the same month show significant differences in NDO depending on the development stage of their ovaries. An ovary in

earlier vitellogenesis had an estimated fecundity of between 30% and 50% of one that was in a more advanced stage of vitellogenesis.

On the contrary, NDO – as well relative NDO – decreases rapidly once females start to spawn, suggesting a continuous loss of oocytes with each spawning event and hence a determinate fecundity, i.e. NDO is an index of potential fecundity. If the fecundity were indeterminate, realized fecundity would have been considerably higher than estimated NDO, which is already very high, as mentioned above. The high NDO estimation is consistent, therefore, with a determinate fecundity.

The results presented here indicate that the potential fecundity of *H. dactylopterus* is a function of the length and weight of the fish, their ovary weight and GSI. However, our investigations did not reveal a major effect of condition on fecundity for this species. With the exception of Kn of females that had not yet started to spawn, the remaining condition indices evaluated in this study did not contribute significantly to explaining the observed variations in fecundity at an individual level (less than 10% of the variability in fecundity is accounted for by the indices considered). Condition indices of bluemouth normally show low variability during the peak of the spawning season (Muñoz & Casadevall, 2002), partially explaining the insignificant relationship between condition and fecundity of females. The positive and significant relationship between Kn of females that had not started to spawn and fecundity probably depicts a positive effect on subsequent fecundity of the energy stored in the muscle. Thus, this deep-sea species, which can be considered a warm-water species living in the coldest waters of the Mediterranean, apparently shows a mixture of capital and income breeding strategies, where recruitment of oocytes is partially dependent on female size, and

oocyte development, which is a rapid process, is more dependent on energy gained concurrently. In temperate fish species (Saborido-Rey *et al.,* 2010), fecundity is normally achieved using current energy income, i.e. income breeders (Houston *et al*., 2007), as in the case of hake (Domínguez-Petit and Saborido-Rey, 2009), whereas in cold-water species fecundity is more dependent on energy reserves (capital breeders), and therefore the effects of condition on fecundity are better detected (Kjesbu *et al.*, 1991; Lambert & Dutil, 2000; Oskarsson *et al.*, 2002). Examples of determinate fecundity with asynchronous development are often observed in temperate-water species such as dusky grouper (Reñones *et al*., 2010), mackerel (Greer-Walker *et al*., 1994), pouting (Alonso-Fernandez *et al*., 2009) or sole (Witthames & Greer-Walker, 1995).

The low levels of atresia during most of the spawning period suggest that this mechanism does not substantially affect the fecundity of this species during the reproductive season, and is therefore not used as a fine-tuning mechanism to regulate the number of eggs per batch as found by Bromley *et al.* (2000). This result suggests that potential fecundity is regulated through the mobilization of oocytes in cortical alveoli or early vitellogenesis towards oocyte final maturation in a rapid process that depends on surplus energy, and hence there is no need for follicular atresia to act as a regulatory mechanism. However, in many females the spawning season ends before all oocytes are developed, so there is a need to eliminate the underdeveloped non-ovulated oocytes, which is done through atresia, as shown by the sudden and marked increase in the relative intensity of atresia (up to 50%) detected in the ovaries at the end of the spawning season.

Development in the ovary, as defined by Wallace & Selman (1981), is asynchronous: oocytes of all stages of development are present in the ovary – histologically described by Muñoz *et al.* (1999) – and the oocyte size-frequency distribution is continuous. Only when hydration occurs is there a clearly differentiated stock of oocytes in terms of diameter. This oocyte size distribution confirms the bluemouth as a batch spawner, whose eggs are recruited and ovulated from the population of yolked oocytes in several batches over a protracted period during the annual spawning season. The number of developing oocytes is lower than the potential fecundity during most of the season, because secondary growth oocytes are recruited continuously in this period. Nevertheless, the potential fecundity becomes definitively fixed shortly before the spawning season, and all the successive batches will develop from the vitellogenic stock, without further oocyte recruitment.

The single analyzed batch consisted of 4,750 embryos. The same ovary still has more than 150,000 oocytes in the secondary growth stage, which indicates that this female is able to, potentially, spawn up to 30 batches. Considering that the mean estimated spawning interval is around every two days, this suggests an individual spawning season for this female of nearly two months, which is consistent with the histological observations. However, we must bear in mind the variability obtained in the estimated fecundity, in the number of embryos per batch and in the spawning frequency through the reproductive season. Another constraint of this estimation is that batch fecundity was directly calculated in only one female. Although it was also estimated from the oocyte size distribution of more females, the wide range of obtained values indicates the need to continue analyzing this topic, since understanding temporal variability in egg

production is an essential component of the reproductive biology of multiple spawning fishes and can have important implications for the management of fished populations (Bushnell *et al.,* 2010). Therefore, more females should be collected in spawning condition, i.e. containing the gelatinous matrix with embryos just before its expulsion to the external environment. The absence of more captures in this stage is probably related to the behaviour associated with reproduction. Since spawners (big specimens) are mainly caught by longliners, if they stop feeding – as related species such as *Sebastes flavidus* do (Eldridge et al., 1991) – it becomes difficult to capture them. Another explanation could be related to the bathymetric distribution of the species – the largest specimens of *Helicolenus* are found at progressively deeper waters (Massutí et al., 2000) – together with the so-called Mediterranean reproductive fish refuge paradigm (Caddy, 1993). For the genus *Sebastes*, for instance, a clear migratory movement of mature fish from the recruitment area to the spawning area has been observed (Saborido-Rey *et al*., 2004). So, larger and actively reproductive *Helicolenus* females could have migrated to submarine canyons and abrupt bottoms that are not usually reached by longliners, making their capture more difficult.

In this work we found that the spawning period ran from the end of December to May, which was a little longer than the period defined for Greek waters (Terrats & Petrakis, 2001) or around the Azores in the north-east Atlantic (Mendonça *et al*., 2006). In the Celtic Sea, the spawning period has been ascertained to be in November and December by Quéro & Vayne (1997), and Allain (2001) states that spawning in the British Isles occurs from March to June. The strategy of releasing eggs over a long period of time can be advantageous because it increases the probability of offspring

446 survival (Lambert & Ware, 1984), but it can also be understood as a necessity in highly
447 fecund species, where a physical limitation occurs (Fordham & Trippel, 1999). This
448 second explanation is further enhanced in the *Helicolenus,* since the increase in the
449 volume of the ovaries is not only caused by the hydration of the oocytes, but also by the
450 accumulation of the gelatinous matrix that encloses the eggs, and the embryonic
451 development and subsequent increase in size of the fertilized eggs.
452
453 The reproductive information obtained in this study is relevant for estimating the stock
454 reproductive potential of the bluemouth, which will facilitate a measure of how far the
455 population can be exploited. The most widely accepted theory is that reproductive
456 potential decreases as mortality due to fishing increases, and that a decrease in
457 reproductive potential beyond acceptable limits can cause a stock collapse (Saborido-
458 Rey & Junquera, 1999). For this reason, attempts have been made to define the
459 biological limits or reference points that indicate the stock reproductive potential for
460 various species (Marshall *et al*., 2003), in which intraspecific variability has to be
461 accounted.
462 Although our results show that the bluemouth is a highly fecund species with low levels
463 of atresia and a relatively long spawning period, two factors may underpin the
464 reproductive potential of this species when fisheries are taken into account: (1) the
465 complex reproductive strategy of this species (zygoparous species with internal
466 fertilization, asynchronous reproductive cycles of males and females) and (2) the
467 relationship we observed between potential fecundity and the size of individuals. We
468 have already pointed out that the bluemouth in the NW Mediterranean is exploited by
469 trawlers and longliners. According to landings data of the year 2007 from the three most

important fishing ports of the Costa Brava where bluemouth is landed (Roses, Port de la Selva and Llançà, representing over half of all bluemouth landings in Catalonia), trawlers captured 70% of the total landings and longliners the remaining 30%. Longliners mainly target the larger individuals on the shelf break while trawlers exploit smaller individuals on the shelf (Ribas et al., 2006). This is a typical fishing pattern observed in the NW Mediterranean for other demersal species such as hake: larger specimens inhabit deep waters and smaller ones stay on the continental platform (Aldebert *et al.*, 1993). Therefore, longline fishing may have an impact on the reproductive potential of bluemouth in the NW Mediterranean by eliminating the larger, more fecund individuals. Selective fishing pressure on larger individual fishes over recent decades has caused the rapid evolution of decreased body size and fecundity of many harvested fishes (Olsten et al., 2004) and has highlighted the need to focus on protecting the larger individuals of long-lived fish species (Birkeland & Dayton, 2005).

As deep-water species are considered to be sensitive to exploitation because of their vulnerable biological characteristics (Clark, 2001; Morato *et al.*, 2006), and as the bluemouth is currently being targeted by deep-sea fishing operations in the north-west Mediterranean, the proper monitoring of its reproductive potential and the management of its exploitation to conserve sufficient reproductive potential must be addressed in future work to achieve sustainable exploitation.

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Table I. Mean size and weight of the sample specimens of *Helicolenus dactylopterus* for this study.

		n	Mean ± SE	range	standard deviation
Total Length (mm)	males	175	279.25 ± 3.06	151 - 380	40.41
	females	195	269.73 ± 2.76	163 - 380	38.48
Total Weight (gr)	males	175	389.19 ± 13.02	47 - 916	171.77
	females	195	364.95 ±12.03	64 - 1100	167.6

Table II. Determination coefficients and significance levels of the regressions between the logarithm of estimated fecundity and total length (mm), body weight (g) and logarithm of the gonadal weight (g) of the females, with and without postovulatory follicles (POF). The same regressions have been made versus the logarithms of the gonadosomatic index (GSI) and the fat somatic index (FSI), the hepatosomatic index (HSI), and versus the two condition factors (K and Kn). The symbols * and ** denote significance at p≤0.01 and p≤0.001, respectively.

			Fecundity (log)	
	POF	n	r^2	p
Total Length	Without	62	0.34	0.0000**
	With	18	0.31	0.008*
Total Weight	Without	62	0.86	0.0000**
	With	18	0.02	0.852
Ovary Weight (log)	Without	62	0.80	0.0000**
	With	18	0.22	0.026
GSI (log)	Without	62	0.70	0.0000**
	With	18	0.09	0.206
HSI	Without	62	0.01	0.433
	With	18	0.05	0.372
K	Without	62	0.04	0.062
	With	18	0.07	0.139
Kn	Without	62	0.46	0. 007*
	With	18	0.02	0.201
FSI (log)	Without	39	0.02	0.378
	With	7	0.00	0.947

Table III. Results of the Partial Least Square Regression (PLSR) analysis. The table shows the weights of the variables total length, GSI, HSI and K for the two first latent vectors extracted by the analysis (v1, v2) and the standardized regression coefficients (β). β-values larger than 0.40 are highlighted in bold.

	v1	v2	β
Total length	0.66	0.16	**0.41**
GSI	0.69	0.35	**0.49**
HSI	0.09	0.15	0.13
K	0.27	-0.9	0.01

Table IV. Data about atresia in *Helicolenus dactylopterus*. N: Number of the total analyzed specimens per month, Pa: Prevalence (%) of atresia per month, SM: fish reproductive phase of each specimen showing atresia (SC: spawning-capable; AS: actively spawning; R: regressing), TL: total length (mm) and TW: total weight (g) of each specimen, RIa: relative intensity (%) of atresia.

Month	N	Pa (%)	SM	TL	TW	RIa (%)
December	17	5.9	SC	270	349	1.56
January	39	5.1	AS	288	342	1.35
			AS	252	286	2.38
February	44	6.8	SC	308	427	1.35
			AS	264	345	1.82
			AS	281	460	4.12
March	5	60	AS	313	589	
			AS	365	844	2.17
			AS	302	525	3.85
April	14	14	AS	256	362	5.56
			AS	292	437	4.41
May	20	10	R	333	576	58.33
			R	318	591	

Figure captions

Figure 1. Map of the north-western Mediterranean showing the sampling area. Enlarged box corresponds to the Costa Brava.

Figure 2. Oocyte-size frequency distribution morphology in relation to whether females have still not started to spawn (ovaries without POF) (A) or have already started (ovaries with POF) (B). Both specimens have a similar length (293-294 mm TL).

Figure 3. Several batches of oocytes or embryos of different diameters that will be spawned can be detected in the oocyte size-frequency distribution of this female. Note the dominance of stock in the early vitellogenic phase.

Figure 4. Means with their standard deviation of the number of developing oocytes by month (A) and by ovary developmental stage (B). Both NDO (squares) and relative NDO (rhombus) are represented. Most advanced developmental stage in the ovary: perinucleolar oocytes (PN), cortical alveoli oocytes (CA), vitellogenesis (VIT), maturation (M) and hydration (H).

Figure 5. Oocyte size-frequency distributions of three females of similar size (238-240 mm TL), captured in the month of January, and that had not yet started to spawn (ovaries without POF). Note the variations in their estimated fecundity (NDO) in relation to the maturation stage of their ovaries: cortical alveoli (CA) and initial vitellogenesis (VIT. I) in the first (A), more developed vitellogenic oocytes (VIT. II and III) in the second (B) and hydrated oocytes in the third (C).

Figure 6. Oocyte-size frequency distribution of the spawning batch analyzed (A) and that of the ovary that had recently spawned it (B).

Figure 7. Fish reproductive stage of the specimens of *Helicolenus dactylopterus* in relation to the month of their capture.

25 Figure 8. Evolution of the spawning fraction and the spawning interval of *H.*
26 *dactylopterus* from November to May.

Figure

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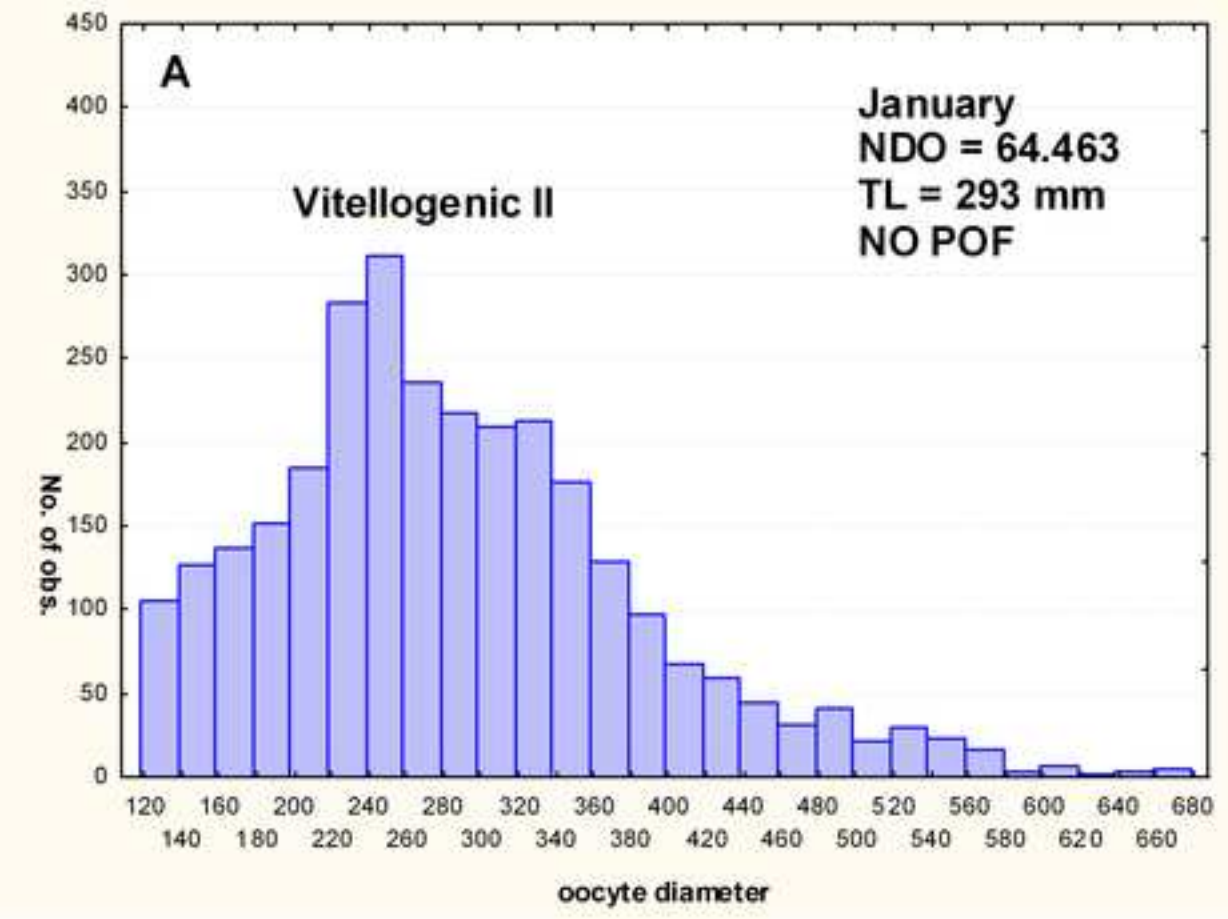

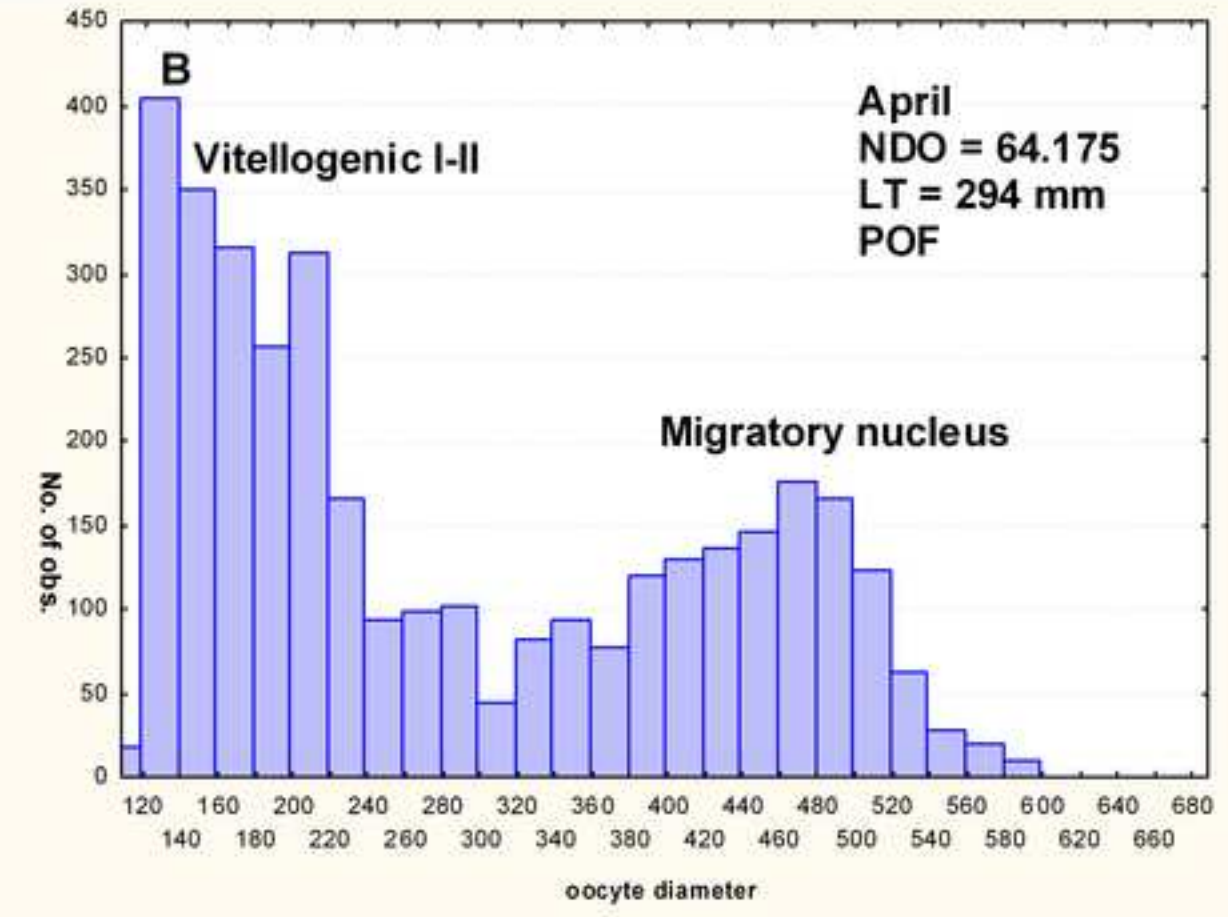

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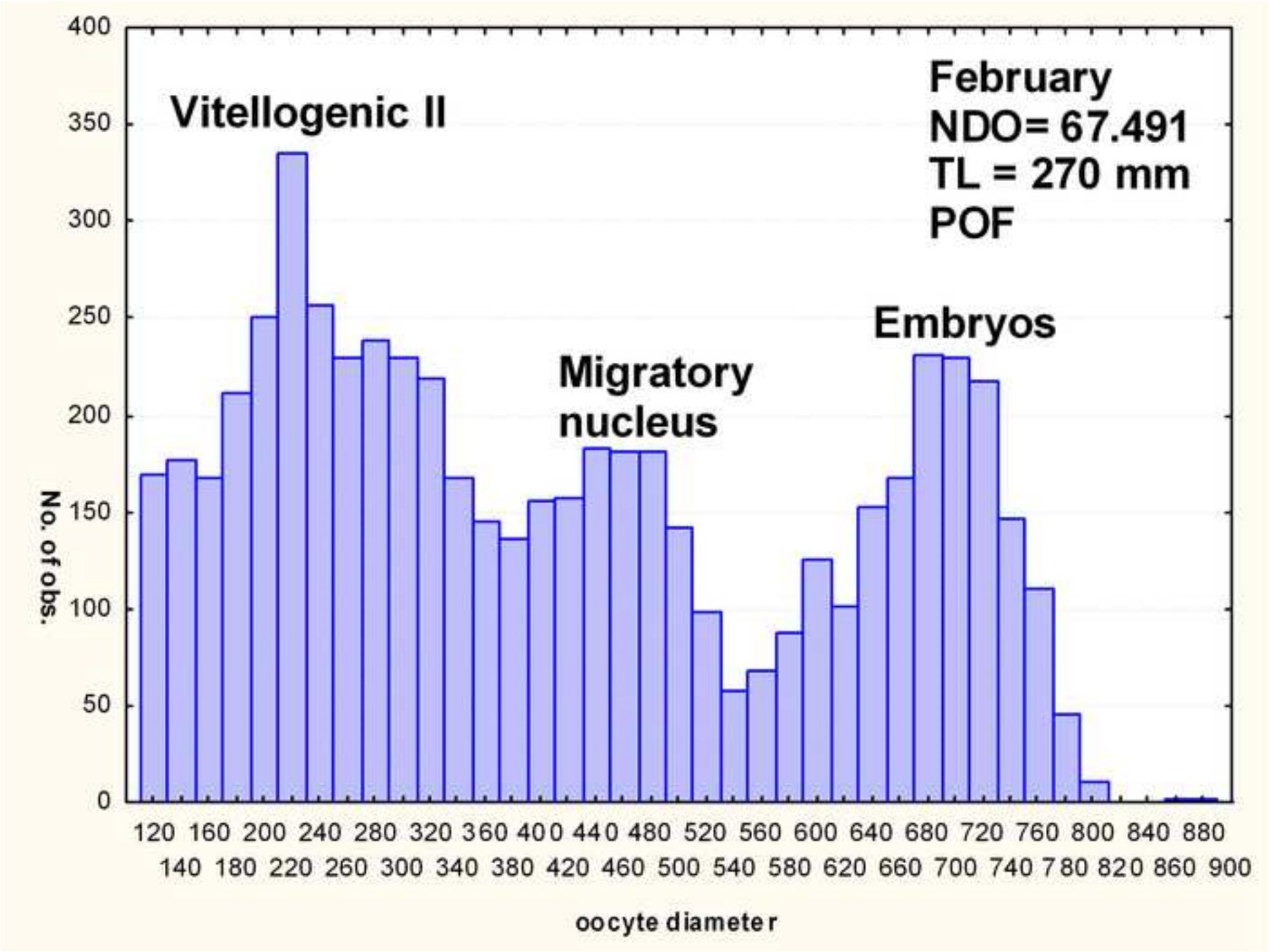

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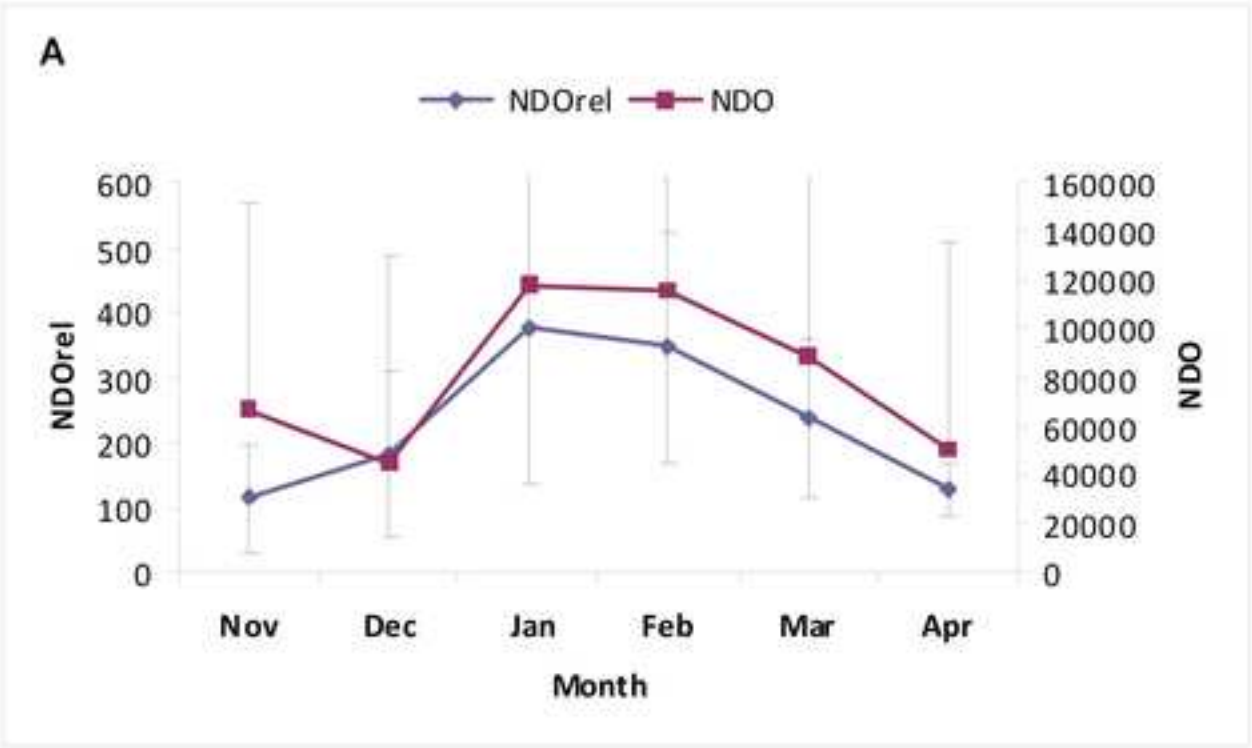

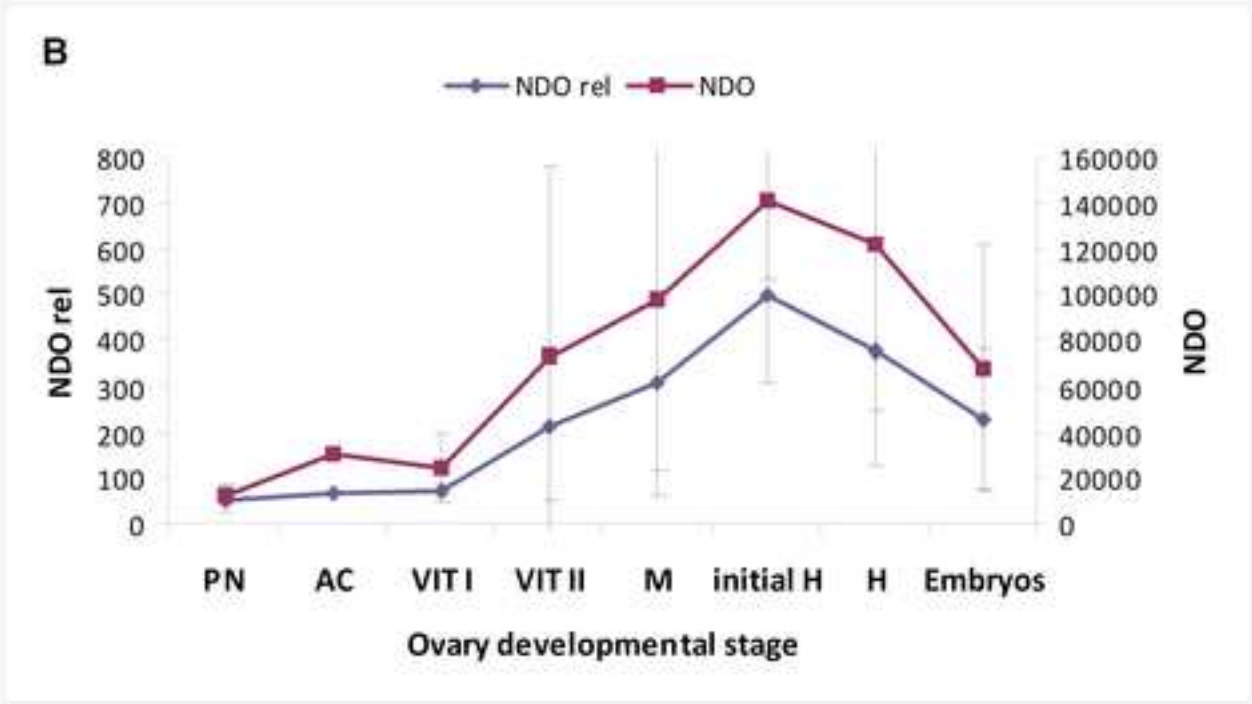

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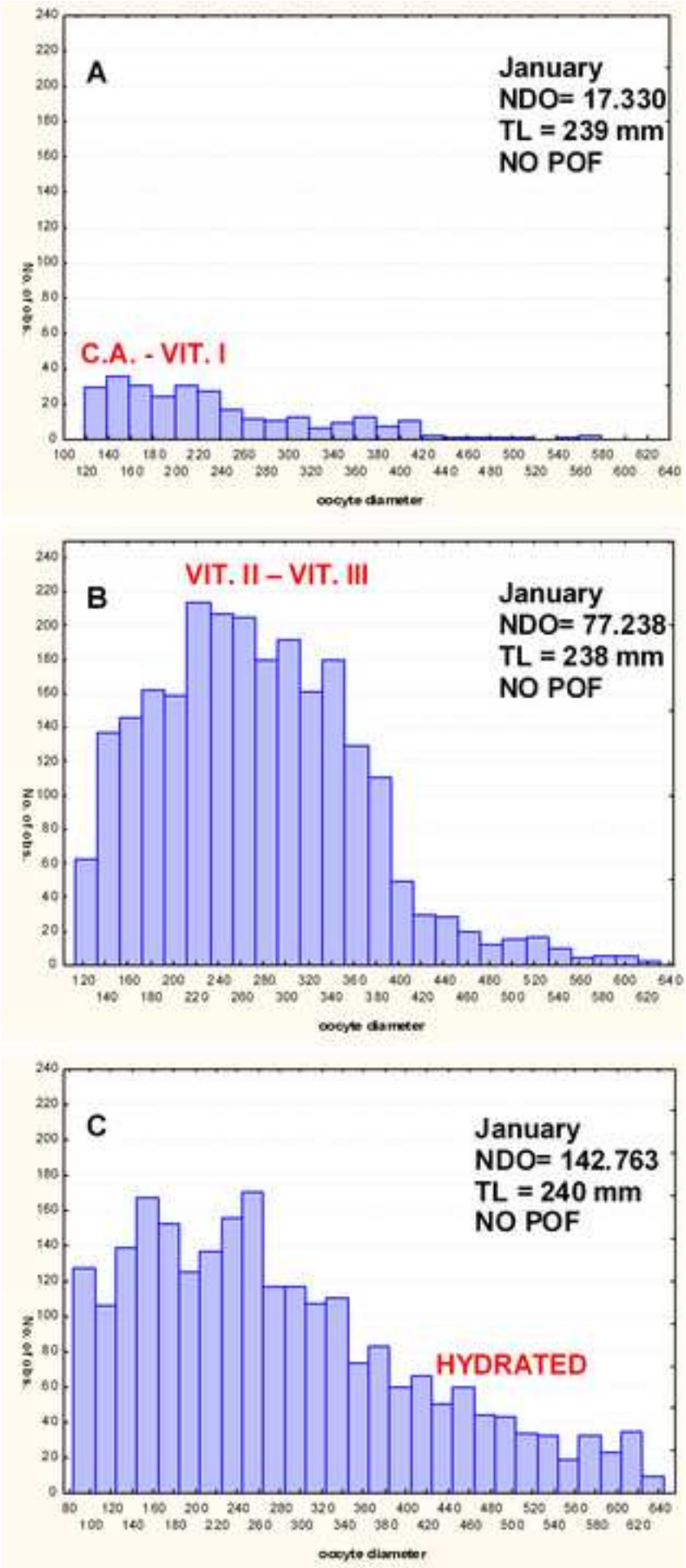

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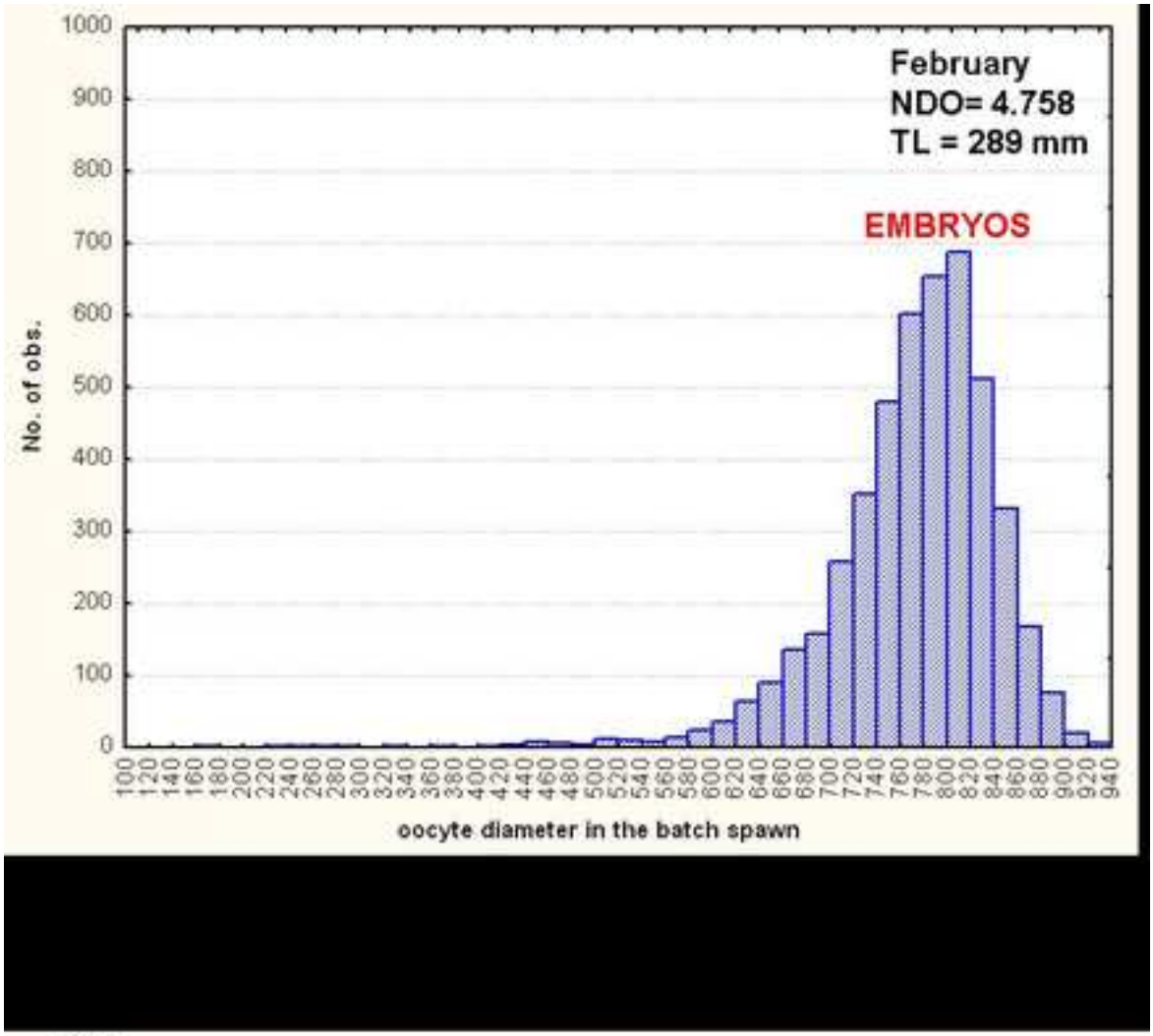

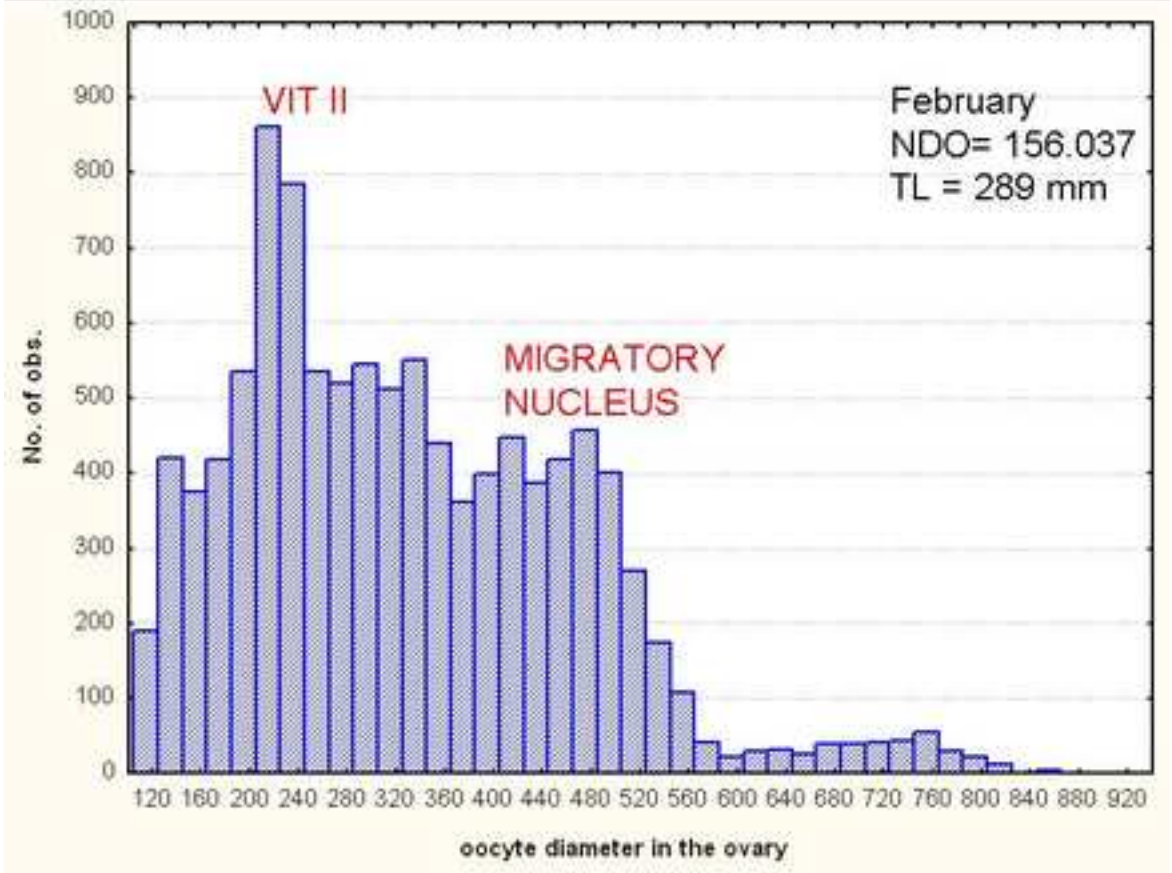

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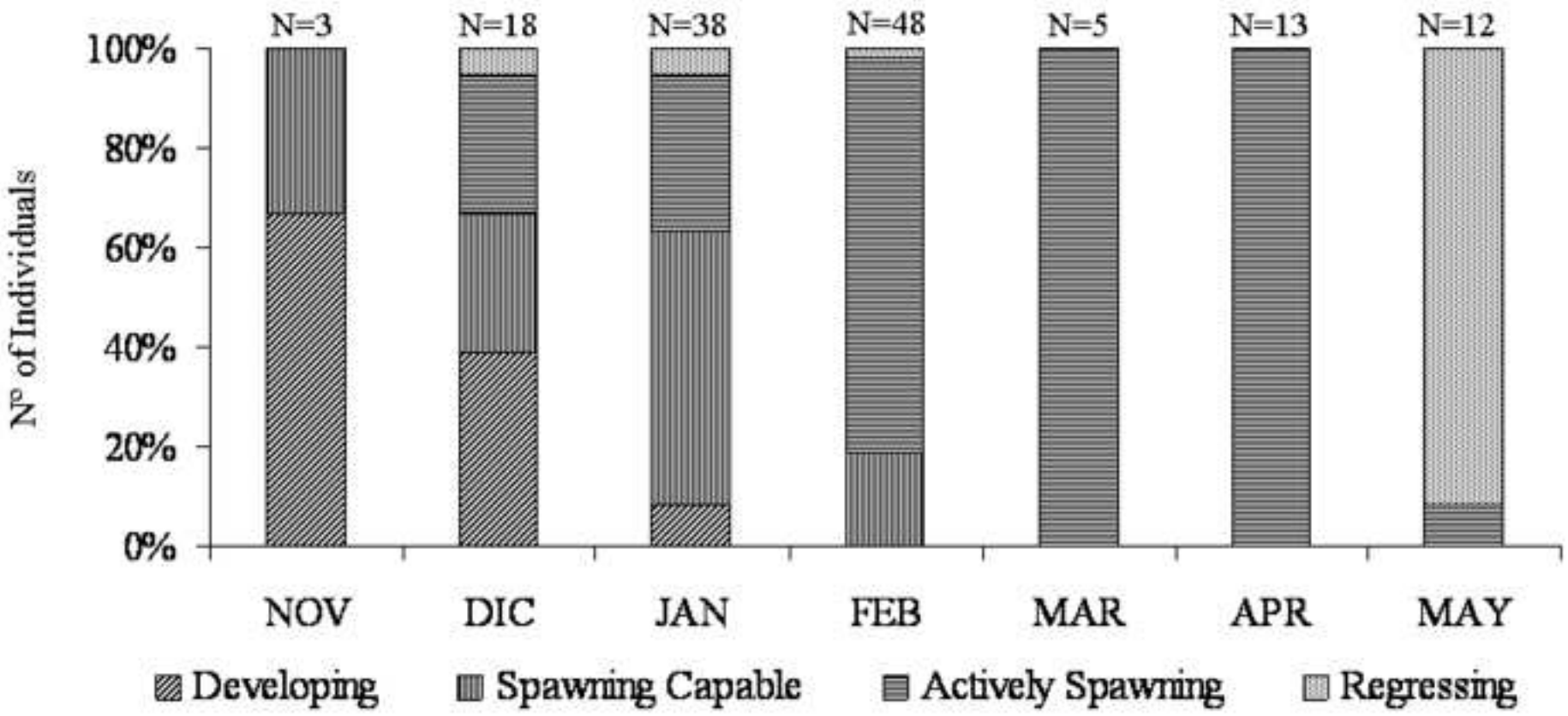

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