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Oocytes development, maturity classification, maturity size and spawning season of the red mullet (*Mullus barbatus barbatus* Linnaeus, 1758)

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Summary

The present study was designed to identify maturity of female red mullet, *Mullus barbatus barbatus,* using microscopic criteria, offering an accurate determination of the ovarian cycle and estimation of maturity size. Histological analysis of 499 females from 13 monthly samplings was applied (Thermaikos Gulf, N. Aegean Sea, Greece). Oocyte development was divided into five stages, with a mean oocyte size range from 56 to 363 μm in diameter. Ovarian maturity was defined by the maturation stage of the most advanced oocytes and divided into four phases. By using the monthly changes in the percentage frequency of ovarian phases, the ovarian cycle of *M. barbatus* was divided into three periods: a long period of early oogenesis (November to February), a short period of vitellogenesis (February to April) and a spawning period from April through June with peak activity in May. Both gonadosomatic (up to 7.5%) and hepatosomatic indices (up to 2%) can be used together to predict the spawning period. Red mullet should be classified as a multiple spawning species. Females reached 50% maturity (Lm_{50}) at 11.4 cm and 95% maturity (Lm_{95}) at 15.5 cm TL. The Lm_{50} values for female red mullet populations across the Mediterranean Sea tended to show differences ranging from 11 to 14.4 cm FL or 11.4–14 cm TL. The current European Union fisheries management plan stipulates a minimum landing size (MLS) of 11 cm TL for Mullidae spp. fisheries in the Mediterranean. This MLS is at the lower part of the Lm_{50} range, thus allowing an extensive removal of immature juveniles. A revision of this MLS value is recommended to help ensure that red mullet stocks remain within safe biological limits.

Introduction

The red mullet (*Mullus barbatus*, L. 1758) is common throughout the eastern Atlantic and the Mediterranean basin, including the Black Sea. Red mullet inhabits sandy and muddy bottoms (Lombarte et al., 2000). This fish has a high commercial value and is an important resource for the Mediterranean demersal fisheries (Tserpes et al., 2002 and references therein; Stergiou et al., 2007; Katsanevakis et al., 2010). Because these Mediterranean stocks are considered as heavily exploited, appropriate management schemes are needed (Tserpes et al., 2002 and references therein; Ozbilgin et al., 2004; Merino et al., 2007; Sieli et al., 2011).

Knowledge of several components of a stock's reproductive biology, such as spawning season, maturity size and spawning-stock biomass, are essential for fisheries management. These data form the basis of the biological reference points on the maximum fishing mortality that a population could sustain and the minimum biomass required for average recruitment. A review of reproductive characteristics relies on the accurate estimation of gonadal development and determination of maturity stages in individual fish. Determination is based either on a macroscopic (visual) examination or on a more accurate histological analysis of the gonad. Macroscopic examination is a quick and inexpensive method of estimating the reproductive state of many species *in situ* (Tomkiewicz et al., 2003). However, classifying individuals based on the method of visual examination of gonads has important limitations. The stage of oocyte development cannot be examined (needed to distinguish sexually active from sexually inactive females), the number of oocytes ready to be released remains unknown, and the presence of postovulatory follicles or atretic stages cannot be assessed (Costa, 2009; Núñez and Duponchelle, 2009). These limitations could hinder development of an effective management scheme, especially for multiple-spawning species, because the incorrect classification of maturity can lead to crucial over- or underestimations of fundamental reproductive parameters (Costa, 2009; Núñez and Duponchelle, 2009). Several studies reveal discrepancies between the macroscopic and histological examination of the gonads and emphasise the lack of accuracy using macroscopic inspection (García-Díaz et al., 1997; La Mesa et al., 2003; Vacchi et al., 2007; Costa, 2009; McBride et al., 2013). Clearly, histological examination is important to verify visual observations. Furthermore, the assessment of ovarian maturity should be based either on microscopic or macro- as well as microscopic observations.

To the best of our knowledge, research on red mullet reproduction focused exclusively on macroscopic evaluation of maturity. The present study provides a precise maturation scale based on histological evaluation of ovarian

maturity to accurately determine some of the most important life history traits for fisheries management. We have followed the proposed classification schemes for standardization of formats for reporting on oocyte staging for teleosts (Núñez and Duponchelle, 2009; Brown-Peterson et al., 2011) to make intra-specific comparisons easier. Our aim is twofold: to come to a deeper understanding of the reproductive biology of this species, and to contribute to developing a sound biological basis for red mullet fisheries management schemes.

Materials and methods

Fish sampling and histological analysis

A total of 499 *M. barbatus* individuals were randomly sampled on a monthly basis between November 2008 and November 2009. All fish were caught by commercial bottom trawl and artisanal fishery operating in the Thermaikos Gulf (40º23′00″N; 22º47′00″E).

For each specimen, total length (TL) to the nearest centimetre, as well as the total and gutted weight, and gonad and liver weights (accuracy of 0.01 g) were measured. Specimens ranged from 9.3 to 22.2 cm (TL).

Slices of the gonads were fixed in 10% buffered formaldehyde. After 24–48 h, a segment from the central part of the left lobe was dehydrated in increasing concentrations of ethanol and clarified in xylene. Each segment was then embedded in paraffin wax and cross-sections of 4–5 μm thickness were cut and stained with Mayers' haematoxylin-eosin.

Determination of ovarian developmental phase

Development of the oocytes was described and maturity stages (MS) determined from microscopic observations of the ovarian histological slides. The ovaries consisted of ovarian lamellae containing oocytes at various stages of development. The oocyte development classification of Grier et al. (2009) in Carmen Uribe et al. (2012) was used to define stages of oogenesis. In this classification, oocyte development is divided into five stages: oogonia proliferation (OP), primary growth (previtellogenesis) (PG), secondary growth (vitellogenesis) (SG), final oocyte maturation (OM) and ovulation. The stages are divided into steps, identified with a lower-case abbreviation, and designed to identify the morphological changes in oocytes during growth and maturation. Ovarian maturity was defined by the maturation stage of the most advanced oocytes, and divided into four phases, following the conceptual models and the standardised terminology proposed by Núñez and Duponchelle (2009) and Brown-Peterson et al. (2011). The presence of postovulatory and atretic follicles was also recorded.

Seasonality of gonad development and spawning season

The spawning season of the red mullet was determined by monthly changes in the: (i) percent frequency of the maturity stages, (ii) gonadosomatic index (I_G), ($I_G = 100 \times$ gonad weight/gutted weight), (iii) hepatosomatic index (I_H), ($I_H = 100 \times$ liver weight/gutted weight, and (iv) condition factor (Kn = (total weight/TL^b) $\times$ 1000, b being the exponential of the regression $TW = aTL^b$, which is 3.35 (unpublished data). Changes of I_G, I_H and Kn in each maturity stage were also analysed in order to highlight changes of body indices with respect to various phases of ovarian development.

Length at sexual maturity

Total length (TL) of all individuals was used to estimate the size at first maturity and size at mass maturity. These are defined as the sizes (TL) at which 50% ($TL_{50\%}$) and 95% ($TL_{95\%}$) of all fish sampled are at the relevant maturity phase (developing, spawning capable, regressing ovarian phase). The proportions were estimated at length classes of 1 cm and the data fitted to the logistic curve (Pope et al., 1983): $P = 100/[1 + \exp(a + bTL)]$, where P = percentage of mature individuals as a function of size class (TL); and a and b = specific parameters that can change during the life cycle. A logarithmic transformation was applied to the equation to calculate the parameters *a* and *b* by means of linear regression.

Statistics

The significance of difference (at the 5% level) in I_G, I_H and Kn between monthly samplings and maturity stages was tested by analysis of variance (ANOVA) with a *post-hoc* Tukey test using the statistical package STATISTICA 9 (StatSoft® Incorporation). To meet the assumptions of ANOVA, the data were logarithmically transformed when deemed necessary.

Results

Reproductively inactive ovaries

Reproductively inactive ovaries were divided into sexually immature (immature subphase) and mature but reproductively inactive ovaries (regenerating subphase). Ovaries at both subphases contained oogonia and primary growth phase (PG) oocytes (mainly at the perinucleolus step, PGpn) possessing a spherical or ovoid germinal vesicle (Fig. 1a). Maximum diameter of the PGpn oocytes reached 56 ± 12 μm (n = 105). Regenerating subphase ovaries had more space between follicles, higher presence of interstitial tissue and a thicker ovarian wall.

Developing ovarian phases

Developing phase ovaries were divided into early and late developing subphase ovaries. Early developing ovaries were characterised by the appearance of oocytes in the yolk vesicles phase (PGyv), in which spherical inclusions (yolk vesicles) were dispersed throughout the ooplasm. Yolk vesicles were delimited by a membrane and could be clearly observed using light microscopy (Fig. 1b). The follicle cell layer was visible but the zona pellucida was not yet stained. PGyv oocytes reached a diameter of 124 ± 20 μm (n = 55).

Late developing phase ovaries included secondary growth stage oocytes (SG), which in turn enclosed within the oocyte periphery, spherical acidophilic yolk granules (yg) that pro-

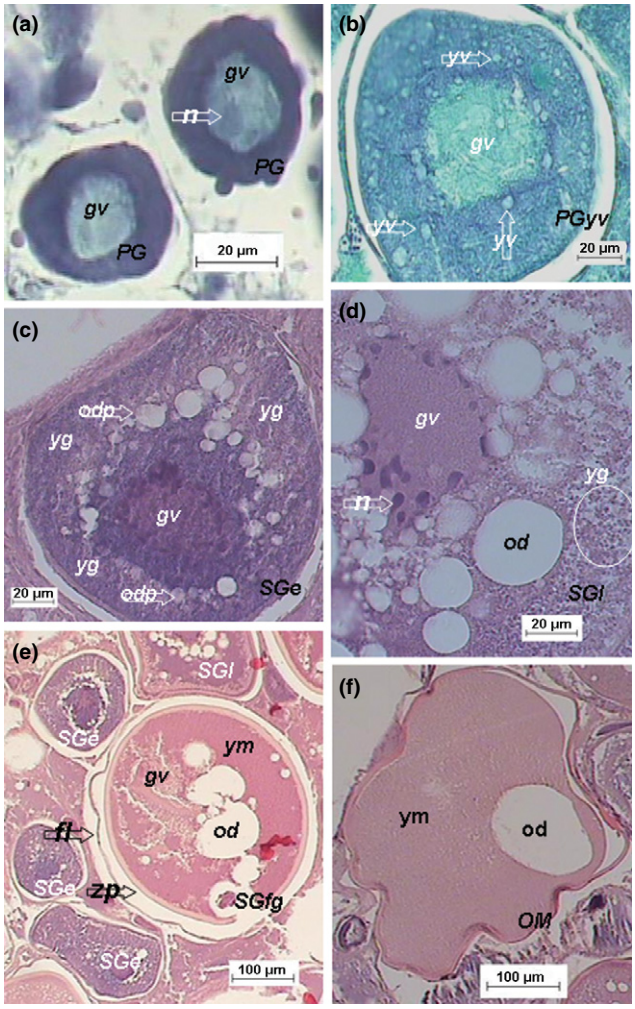

Fig. 1. *Mullus barbatus barbatus*. Ovarian sections stained by haematoxylin-eosin. a: Primary growth phase oocyte at the perinucleolus step (PGpn) possessing an ovoid germinal vesicle (gv). b: Primary growth phase oocyte at yolk vesicles phase (PGyv) with yolk vesicles (yv) dispersed throughout the ooplasm. c, d: Secondary growth stage oocytes (SG) with spherical acidophilic yolk granules (yg) and spherical oil droplets (odp). e: Full-grown vitellogenic oocyte (SGfg) with large regions of fluid yolk (ym) and a few oil drops (od). Also, secondary growth phase oocytes at early (SGe) or late vitellogenesis (SGl) phase. f: Oocyte at the final maturation phase (OM) with yolk material completely fused and an oil droplet. Germinal vesicle (gv) invisible due to disintegration and dispersion of nuclear membrane (GVBD). fl, follicular layer; n, nucleolus; ym, yolk mass; zp, zona pellucida

gressively increased in number and dispersed within the ooplasm (Fig. 1c,d). Additionally, spherical oil droplets (od) progressively appeared around the germinal vesicle (gv), increased in size and accumulated throughout the ooplasm (Fig. 1c,d). The zona pellucida (zp) remained large and was densely stained by eosin. SG oocytes are of various sizes and development stages, with few yolk globules (SG oocytes at early step, SGe) to moderate and many yolk globules (SG oocytes in the late phase, SGl). The diameter of SGe oocytes can be up to 177 ± 29 μm (n = 49), whilst SGl oocytes reach 268 ± 38 μm (n = 34).

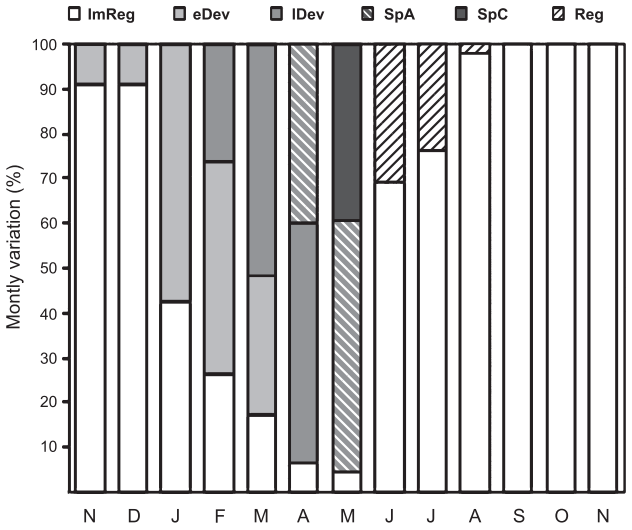

Fig. 2. Monthly variation (%) of ovarian maturity phases of female red mullet (n = 343), Thermaikos Gulf (N. Aegean Sea). ImReg, reproductively inactive ovaries; eDev, early developing ovaries; lDev, late developing ovaries; SpC, spawning capable ovaries; SpA, spawning active ovaries; Reg, regressing phase ovaries

Spawning capable ovaries

Ovaries capable of spawning were divided into two subphases: ovaries capable of spawning ('spawning capable') and actively spawning ('active spawning'). Spawning capable ovaries contained full-grown vitellogenic oocytes (SGfg), which were the most advanced oocytes. SGfg oocytes were distinguished by the formation of large regions of fluid yolk, formed by the progressive fusion of yolk granules. As a consequence, the ooplasm was displaced into the peripheral rim surrounding the yolk mass (Fig. 1e). During this step, the oil droplets concentrate around the nucleus, and then fuse into one or a few oil drops. The germinal vesicle has an irregular shape and its envelope becomes progressively folded. Numerous small and large nucleoli can also be seen and the zona pellucida reaches its maximum length. Some SGfg oocytes were shrunken and the zona pellucida, follicle cells and theca were separated from the oocyte. Such shrinkage was likely due to the histological preparation protocol. SGfg oocytes reached 363 ± 35 μm (n = 40) in size, close to maximum size.

During the final maturation phase (OM), spawning active ovaries are characterised by the presence of hydrated oocytes; in this phase, the yolk material is completely fused and a few or one oil drops are formed (Fig. 1f). The appearance of the oocyte is homogenous, finely granular, whilst the germinal vesicle is invisible due to disintegration and dispersion of the nuclear membrane (GVBD). The ooplasm, restricted to a narrow rim, lies next to the zona pellucida, which has become thinner. In some spawning-capable ovaries, empty ovarian follicles (post ovulatory follicles, POFs) were present, indicating that ovulation had occurred.

Regressing ovarian phase

Regressing phase ovaries were characterised by the presence of atretic SG oocytes at various stages of disintegration and

reabsorption, the presence of POFs and a few remaining SG oocytes, which are sparsely arranged among the stock of PG oocytes.

Seasonal ovarian cycle and variation of body indices

Reproductively inactive ovaries (immature or regenerating phase ovaries) were present almost all year round but with decreasing rates between November and May (Fig. 2). Early developing ovaries indicating the initiation of ovarian growth and oocyte development were initially seen from November to December but mainly from January onwards. The ovaries entered the late developing phase (vitellogenesis) from February onwards. Spawning-capable females were observed between April and May, peaking in May (100% spawning capable). Females probably finished spawning in June, as none capable of spawning and many fish with regressing phase ovaries were observed between June and August.

The mean gonadosomatic index (I_G) increased significantly in April and May, peaking in May (7.5%, Fig. 4a, $P < 0.001$). The mean hepatosomatic index (I_H) had significantly increased from February to May, peaking in March (2%) and April (1.91%) during vitellogenesis and spawning (Fig. 3b, $P < 0.001$). The mean Kn values varied slightly from 0.98 (December) to 1.17% (November, Fig. 3c) tending to be lower from December to May during vitellogenesis and spawning.

Variation of body indices according to ovarian maturity phase

Mean I_G and I_H by ovarian stage were significantly higher in late developing (lDev), spawning capable and spawning active females (SpC, SpA), reaching the highest values in spawning active females (Fig. 4a,b, $P < 0.001$). I_G and I_H decreased to baseline values in regressed (Reg) and reproductively inactive females (ImReg). In contrast, the Kn values were significantly lower for late developing and spawning capable females (Fig. 4c, $P < 0.001$), with the lowest values for spawning active females (0.98%).

Maturity rates according to size of mature fish

Frequencies of immature and mature (developing, spawning capable and regressing phase ovaries) females during the gametogenic period (March to June) were divided into groups according to different total length (TL) (Fig. 5). The smallest individual with mature ovaries was 10.4 cm TL. The maturity curve showed a $TL_{50\%}$ of 11.4 cm and $TL_{95\%}$ of 15.5 cm (Fig. 5).

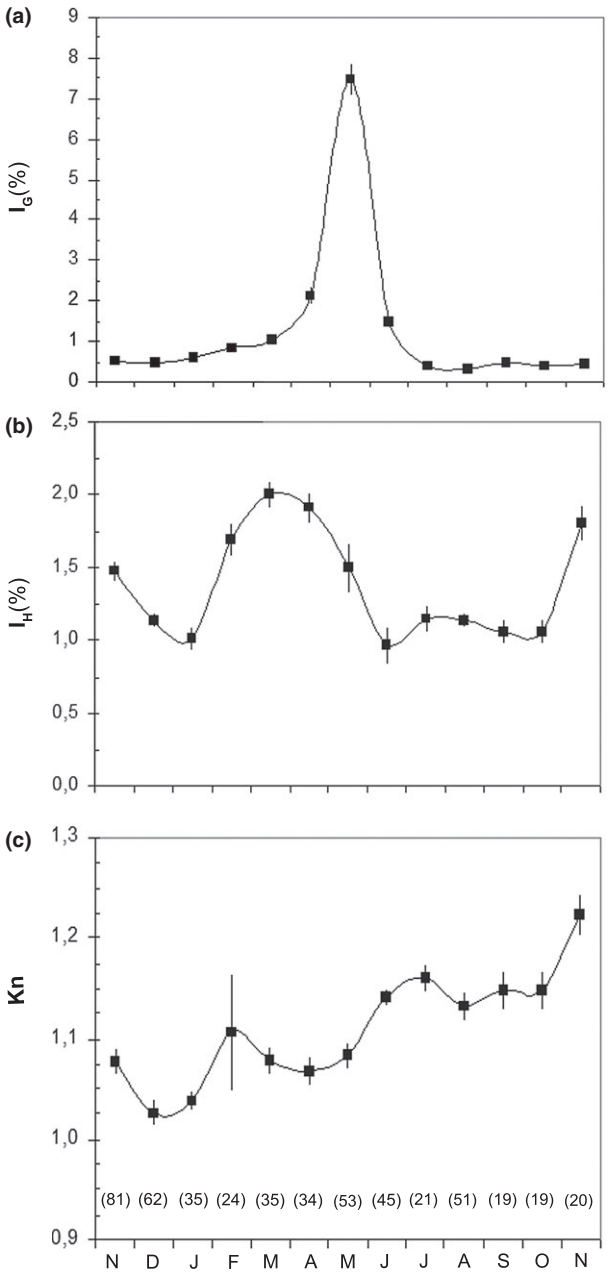

Fig. 3. Monthly variation of mean (±SE) gonadosomatic index (I_G) (a), hepatosomatic index (I_H) (b), and condition factor (Kn) (c), of female red mullet (n = 499), Thermaikos Gulf (N. Aegean Sea). In parentheses = size of monthly samples

Discussion

To our knowledge this is the first time histological analysis is being used to describe the reproductive pattern of red mullet (*M. barbatus*), an important target species for Mediterranean fisheries. Oocyte development during oogenesis shows similar characteristics to those described for other marine teleosts (Tyler and Sumpter, 1996; Núñez and Duponchelle, 2009; and references therein). The maturation scale defined in this study is well in line with the universal need for standardized and harmonized terminology on reproductive classification of fish, accompanied by manual-like features that can be used by researchers beyond the local area to allow for a better comparability of results.

Early developing phase ovaries, indicating the initiation of gonadal growth and gamete development, were initially seen from November to December. The early oogenesis coincides well with the seasonal drop in water temperature and

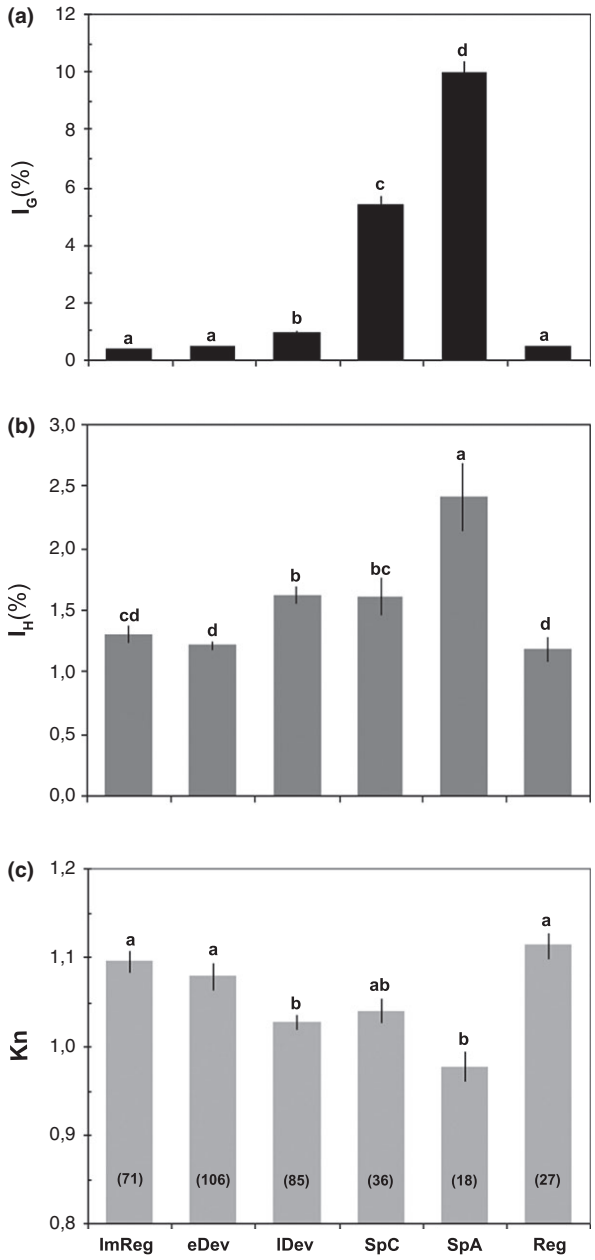

Fig. 4. Mean (±SE) gonadosomatic index (I_G) (a), hepatosomatic index (I_H) (b), and condition factor (Kn) (c) by ovarian phase of female red mullet (n = 343), Thermaikos Gulf (N. Aegean Sea). ImReg, reproductively inactive ovaries; eDev, early developing ovaries; lDev, late developing ovaries; SpC, spawning capable ovaries; SpA, spawning active ovaries; Reg, regressing phase ovaries. In parentheses = size of samples. Different letters indicate statistical differences ($P < 0.001$)

minimum range of day length. Photoperiod and temperature are among the key factors that control sexual maturity and spawning in fish and they could be the environmental correlatives to the initiation of ovarian growth, functioning as proximate cues in the case of red mullet. Similarly to the congeneric species *M. surmuletus* (N' Da and Deniel, 1993), the period of early oogenesis is long (November to February) but the period of vitellogenesis is short, from February to April.

Results of this study clearly indicate that spawning in the northern Aegean Sea extends from April to June, with a peak in May. The onset and duration of spawning across the Mediterranean are summarised in Table 1. Similar to data on most Mediterranean fish stocks (Tsikliras et al., 2010), the red mullet is a late spring – early summer spawner. The existence of such strong seasonality across its distribution indicates that seasonality is an important factor for spawning. Results suggest that the fertility of red mullet is synchronised with the summer peak of zooplankton abundance (Fernandez de Puelles et al., 2003), a condition that ensures optimum conditions for fish larval growth and survival (Winemiller and Rose, 1992). Warmer summer waters and stability of the water column maintain food patches, thus enhancing larval growth (Sabatés et al., 2007). Summer variation of environmental parameters and the accompanying changes in oceanographic factors (e.g. low level of offshore transport and turbulent mixing) may favour larval retention in spawning grounds (Sabatés et al., 2007) and survival of juveniles during their migration towards the nursery areas (Levi et al., 2003).

Spawning capable ovaries contained oocytes at different stages of development and POFs. The existence of several oocyte stages indicates ovarian development typical of a multiple spawning species. The congeneric species *M. surmuletus* has been identified as a determinate spawner (N' Da and Deniel, 1993) with a group-synchronous oocyte development. Data regarding the spawning pattern (group synchronous vs asynchronous) and fecundity estimates (determinate vs indeterminate) for red mullet are lacking. However, estimation of the fecundity pattern of a fish stock is of great importance for assessment of the spawning stock biomass (Ganias, 2013), which in turn is recognised as an essential parameter for fisheries assessment and management.

The I_H increases in mass during the spawning period in a manner positively associated with the I_G, supporting the assumption that I_H variations are related to energy storage for reproduction. Increasing hepatocyte numbers and size are linked to vitellogenesis in liver, because the precursors of the yolk and proteins of the egg chorion are synthesised in that organ (Hoar et al., 1983; N' Da and Deniel, 1993). Both indices (the I_G and I_H) can be used together to predict the spawning period of *M. barbatus*. However, the fact that the *Kn* index was not associated with the I_G may suggest that reproduction does not influence the condition of the fish (i.e. oocyte maturation is not reached at the expense of body muscle or lipids). Some species may compensate for inadequate energy deposits during gonadal development with the energy derived by feeding (Aristizabal, 2007). This is likely the case for female red mullet, which feed throughout their entire spawning period (Vassilopoulou and Papaconstantinou, 1993).

The length at 50% maturity (Lm_{50}) of female red mullet populations across the Mediterranean Sea are summarised in Table 1. The Lm_{50} values tend to show subtle differences (ranging from 11 to 14.4 cm FL or 13–14 cm TL). This

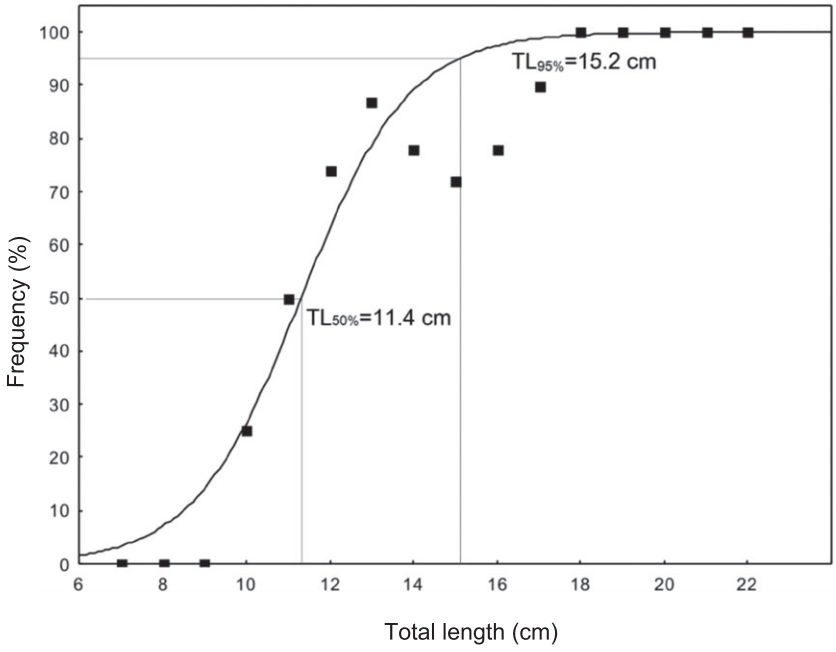

Fig. 5. Sexual maturity curve and size at maturity (thin lines = 50 and 90% TLs) for red mullet, Thermaikos Gulf (N. Aegean Sea)

Table 1
Spawning period and length at maturity ($Lm_{50,}$ cm) of female red mullet (*Mullus barbatus barbatus*) across the Mediterranean Sea indicated as fork (FL) or total length (TL).

	Months							Lm_{50} (cm)		
Area	M	A	M	J	J	A	S	FL	TL	Source
N. Aegean		•	•	•					11.4	Present study
N. Aegean		•	•	•	•			12–13		Papaconstantinou et al., 1981
Central Aegean			•	•	•			11,2		Vassilopoulou, 1992
N. Aegean			•	•	•					Celik and Torcu, 2000
Izmir Bay			•	•				14,2		Metin, 2005
Ligurian Sea			•	•	•					Orsi Relini and Arnaldi, 1986 in Sieli et al., 2011
NW Sicily		•	•	•	•	•				Sieli et al., 2011
Gulf of Tunis			•	•	•			13–14.4		Gharbi and Ktari, 1981 in Cherif et al., 2007
Gulf of Tunis			•	•	•				13.9	Cherif et al., 2007
Southern coast of France									13–14	Bougis, 1952 in Vassilopoulou, 1992
Moroccan Mediterranean		•	•	•	•	•		11		Slimani et al., 2003

reflects the existence of natural variations in length at maturity (i.e. in growth rates), fishery induced changes or the differences in the methodologies used to estimate size at maturity (i.e. sample size, gear selectivity, methodology to define maturity). Using histological criteria in this study, we defined Lm_{50} to be at the value of 11.4 cm TL, a value obviously lower than most of the values reported (see Table 1). This could be explained by the slower growth rate of the Thermaikos red mullet stock (Papaconstantinou et al., 1981), or it could possibly reflect a difference in the method used to define maturity (microscopic vs macroscopic) between the current study and previous studies.

Since 1996, the European Union fisheries management policy for Mullidae spp. (European Commission, 1994; Council Regulation No. 1626/94) in the Mediterranean has stipulated an MLS of 11 cm TL; however, this value is lower than most of the Lm_{50} values of Mediterranean populations (see Table 1). Regarding the Greek mixed fisheries, illegal sizes below MLS (Machias and Labropoulou, 2002; Tserpes et al., 2002; Stergiou et al., 2004; Tzanatos et al., 2008) and extensive removal of immature red mullet have been reported, indicating the ecological inefficiency of the existing MLS value for sustainable management (Stergiou et al., 2009). We therefore suggest increasing the current 11 cm TL MLS value, to ensure that red mullet stocks remain under maximum sustainable yield (MSY) and maximum economic yield (MEY). Sustainable fisheries management should be complemented by other ecosystem-based measures, such as large marine protected areas where fishing is totally prohibited (Stergiou, 2002), to ensure the survival of large and mature individuals (Pipitone

et al., 2000; Stergiou et al., 2004, 2009) and mediate the rebuilding of the overexploited red mullet stocks.

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References

Aristizabal, E. O., 2007: Energy investment in the annual reproduction cycle of female red porgy, *Pagrus pagrus* (L.). Mar. Biol. **152**, 713–724.

Bougis, P., 1952: Recherches biometriques sur les Rouges (*Mullus barbatus* L. et *Mullus surmuletus* L.). Thèses, Paris.

Brown-Peterson, N. J.; Wyanski, D. M.; Saborido-Rey, F.; Macewicz, B. J.; Lowerre-Barbieri, S. K., 2011: A standardized terminology for describing reproductive development in fishes. Mar. Coast Fish. **3**, 52–70.

Carmen Uribe, M.; Grier, H. J.; Parenti, L. R., 2012: Ovarian structure and oogenesis of the oviparous goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (teleostei, Cyprinodontiformes). J. Morphol. **273**, 371–387.

Celik, O.; Torcu, H., 2000: Investigations on the biology of the red mullet (*Mullus barbatus*, Linnaeus, 1758) in Edremit Bay, Aegean Sea, Turkey. Turk. J. Vet. Anim. Sci. **24**, 287–295.

Cherif, M.; Zarrad, R.; Gharbi, H.; Missaoui, H.; Jarboui, O., 2007: Some biological parameters of the red mullet, *Mullus barbatus* L., 1758, from the Gulf of Tunis. Acta Adriat. **48**, 131–144.

Costa, A. M., 2009: Macroscopic vs. microscopic identification of the maturity stages of female horse mackerel. ICES J. Mar. Sci. **66**, 509–516.

European Commission, 1994: Council Regulation (EC) No. 1626/94 laying down certain technical measures for the conservation of fishery resources in the Mediterranean. Brussels, pp. 10.

Fernandez de Puelles, M. L.; Grás, D.; Hernández-Leó, S., 2003: Annual cycle of zooplankton biomass, abundance and species composition in the neritic area of the Balearic Sea, Western Mediterranean. PSZN Mar. Ecol. **24**, 123–139.

Ganias, K., 2013: Determining the indeterminate: evolving concepts and methods on the assessment of the fecundity pattern of fishes. Fish. Res. **138**, 23–30.

García-Díaz, M. M.; Tuset, V. M.; González, J. A.; Socorro, J., 1997: Sex and reproductive aspects in *Serranus cabrilla* (Osteichthyes: Serranidae): macroscopic and histological approaches. Mar. Biol. **127**, 379–386.

Gharbi, H.; Ktari, M. H., 1981: Biologie de *Mullus barbatus* Linnaeus, 1758 et *Mullus surmuletus* Linnaeus, 1758 des côtes tunisiennes, taille et age de première maturité sexuelle, cycle sexuel et coefficient de condition. Bull. Inst. Nat. Scien. Tech. Océanogr. Pêche Salambô **8**, 41–51.

Grier, H. J.; Carmen Uribe, M.; Aranzábal, M. C.; Patino, R., 2009: The ovary, folliculogenesis, and oogenesis in teleosts. In: Reproductive biology and phylogeny of fishes (Agnathans and Bony Fishes), Vol. 8A. B. G. M. Jamieson, (Ed.) Science Publishers, Enfield, pp 25–84.

Hoar, W. S.; Randall, D. J.; Donaldson, E. M., 1983: Fish physiology, Vol. IX (part B). Academic Press, New York, pp.477

Katsanevakis, S.; Maravelias, C. D.; Vassilopoulou, V., 2010: Otter trawls in Greece: landing profiles and potential métiers. Medit. Mar. Sci. **11**, 43–59.

La Mesa, M.; Caputo, V.; Rampa, R.; Vacchi, M., 2003: Macroscopic and histological analyses of gonads during the spawning season of *Chionodraco hamatus* (Pisces, Channichthyidae) off Terra Nova Bay, Ross Sea, Southern Ocean. Polar Biol. **26**, 621–628.

Levi, D.; Andreoli, M. G.; Bonanno, A.; Fiorentino, F.; Garofalo, G.; Mazzola, S.; Norrito, G.; Patti, B.; Pernice, G.; Ragonese, S.; Giousto, G. B.; Rizzo, P., 2003: Embedding sea surface temperature anomalies into the stock recruitment relationship of red mullet (*Mullus barbatus* L. 1758) in the Strait of Sicily. Sci. Mar. **67**, 259–268.

Lombarte, A.; Recasens, L.; Gonzalez, M.; de Gil Sola, L., 2000: Spatial segregation of two species of Mullidae (*Mullus surmuletus* and *M. barbatus*) in relation to habitat. Mar. Ecol. Prog. Ser. **206**, 239–249.

Machias, A.; Labropoulou, M., 2002: Intra-specific variation in resource use by red mullet, *Mullus barbatus*. Estuar. Coast. Mar. Sci. **55**, 565–578.

McBride, R. S.; Wuenschel, M. J.; Grace Thornton, P. N.; King, J. R., 2013: Latitudinal and stock-specific variation in size and age at maturity of female winter flounder, *Pseudopleuronectes americanus*, as determined with gonad histology. J. Sea Res. **75**, 41–51.

Merino, G.; Karlou-Riga, C.; Anastopoulou, I., 2007: Bioeconomic simulation analysis of hake and red mullet fisheries in the Gulf of Saronikos (Greece). Sci. Mar. **71**, 525–535.

Metin, G., 2005: Reproduction characteristics of red mullet (*Mullus barbatus* L., 1758) in Izmir Bay. Ege University. J. Fish. Aquat. Sci. **22**, 225–228.

N' Da, K.; Deniel, D. C., 1993: Sexual cycle and seasonal changes in the ovary of the red mullet, *Mullus surmuletus* from the southern coast of Brittany. J. Fish Biol. **43**, 229–244.

Núñez, J.; Duponchelle, F., 2009: Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes. Fish Physiol. Biochem. **35**, 167–180.

Orsi Relini, L.; Arnaldi, D., 1986: Note di biologia della triglia di fango, *Mullus barbatus* L., 1758, del Mar Ligure; riproduzione e reclutamento. Boll. Mus. Ist. Biol. Univ. Genova **52**(Suppl.), 237–250.

Ozbilgin, H.; Tosunoglu, Z.; Bilecenoglu, M.; Tokac, A., 2004: Population parameters of *Mullus barbatus* in Izmir Bay (Aegean Sea), using length frequency analysis. J. Appl. Ichthyol. **20**, 231–233.

Papaconstantinou, C.; Tsimenidis, N.; Daoulas, C., 1981: Age, growth and reproduction of red mullet (*Mullus barbatus* L., 1758) in the Gulfs of Saronikos and Thermaikos. Thalassographica **4**, 39–66.

Pipitone, C.; Badalamenti, F.; D'Anna, G.; Patti, B., 2000: Fish biomass increase after a four-year trawl ban in the Gulf of Castellammare (NW Sicily Mediterranean Sea). Fish. Res. **48**, 23–30.

Pope, J. A.; Marggets, A. R.; Hamley, J. M.; Akyuz, E. F., 1983: Manual of methods for fish stock assessment. Pt. 3. Selectivity of fishing gear. FAO Fish. Tech. Pap. **41**, 1–65.

Sabatés, A.; Olivar, M. P.; Salat, J.; Palomera, I.; Alemany, F., 2007: Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. Progr. Oceanogr. **74**, 355–376.

Sieli, G.; Badalucco, C.; Di Stefano, G.; Rizzo, P.; D'Anna, G.; Fiorentino, F., 2011: Biology of red mullet, *Mullus barbatus* (L. 1758), in the Gulf of Castellammare (NW Sicily, Mediterranean Sea) subject to a trawling ban. J. Appl. Ichthyol. **27**, 1218–1225.

Slimani, A.; Elouamari, N.; Hamdi, H., 2003: Etat des stocks des principales ressources en Méditerranée marocaine. Document présenté au groupe de travail du Sous comité d'évaluation des stocks (SCSA–SAC-GFCM) Tanager, Morocco, 12–14 March 2003, pp. 32.

Stergiou, K. I., 2002: Overfishing, tropicalization of fish stocks, uncertainty and ecosystem management: resharpening Ockham's razor. Fish. Res. **55**, 1–9.

Stergiou, K. I.; Moutopoulos, D. K.; Krassas, G., 2004: Body size overlap in industrial and artisanal fisheries for five commercial fish species in the Mediterranean Sea. Sci. Mar. **68**, 179–188.

Stergiou, K. I.; Moutopoulos, D. K.; Tsikliras, A. C.; Papaconstantinou, C., 2007: Hellenic marine fisheries: a general perspective from the national statistical service data. In: State of Hellenic fisheries. C. Papaconstantinou, A. Zenetos, V. Vassilopoulou

and G. Tserpes (Eds). Institute of Marine Biological Resources, Hellenic Centre for Marine Research, Athens, pp. 132–150.

Stergiou, K. I.; Moutopoulos, D. K.; Armenis, G., 2009: Perish legally and ecologically: the ineffectiveness of the minimum landing sizes in the Mediterranean Sea. Fish. Manag. Ecol. **16**, 368–375.

Tomkiewicz, J.; Tybjerg, L.; Jespersen, A., 2003: Micro- and macroscopic characteristics to stage gonadal maturation of female Baltic cod. J. Fish Biol. **62**, 253–275.

Tserpes, G.; Fiorentino, F.; Levi, D.; Cau, A.; Murenu, M.; Zamboni, A. D. A.; Papaconstantinou, C., 2002: Distribution of *Mullus barbatus* and *M. surmuletus* (Osteichthyes: Perciformes) in the Mediterranean continental shelf: implications for management. Sci. Mar. **66**(Suppl. 2), 39–54.

Tsikliras, A. C.; Antonopoulou, E.; Stergiou, K. I., 2010: Spawning period of Mediterranean marine fishes. Rev. Fish. Biol. Fish. **20**, 499–538.

Tyler, C. R.; Sumpter, J. P., 1996: Oocyte growth and development in teleosts. Rev. Fish Biol. Fish. **6**, 287–318.

Tzanatos, E.; Somarakis, S.; Tserpes, G.; Koutsikopoulos, C., 2008: Catch length analysis, relation to minimum landing sizes and management implications from a Mediterranean small-scale fishery (Patraikos Gulf, Greece). Fish. Res. **93**, 125–134.

Vacchi, M.; Bottaro, M.; Pisano, E.; Eastman, J. T.; Eakin, R. R., 2007: Aspects of gonadal morphology in the South Georgian plunderfish *Artedidraco mirus* (Perciformes: Artedidraconidae). Polar Biol. **30**, 125–131.

Vassilopoulou, V., 1992: Biological aspects of red mullet, *Mullus barbatus* off the coasts of Central Greece. Bull. Mar. Biol. Res. Centre **9**, 62–81.

Vassilopoulou, V.; Papaconstantinou, C., 1993: Feeding habits of red mullet (*Mullus barbatus*) in a gulf in western Greece. Fish. Res. **16**, 69–83.

Winemiller, K. O.; Rose, K. A., 1992: Patterns of life-history diversification in North American fishes: implications for population regulation. Can. J. Fish. Aquat. Sci. **49**, 2196–2218.

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