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Reproductive biology characteristics of red mullet (*Mullus barbatus* L., 1758) in Southern Adriatic Sea and management implications

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Abstract – The main important reproductive aspects of *Mullus barbatus*, one of the most important target species for the fishery in the Mediterranean basin, have been studied on the basis of MEDITS trawl survey and sampling of commercial landing. The total length (TL) of females has been demonstrated to be positively correlated with egg sizes and plasmatic concentration of vitellogenin. The possible implications of these aspects on the management of red mullet resources are discussed in this work. Moreover, the relationship between TL and fecundity follows an exponential pattern with a temporal trend during the spawning season. These results represent an important input for more effective management measures, such as the seasonal closure of fishery in summertime. The frequency distribution of the oocytes' diameters showed an asynchronous ovarian organization with batch spawning pattern. Monthly variations in the gonadosomatic index and maturity stages percentage showed that males spawn during almost the entire year showed an increasing trend from November to June and a decreasing trend from July to October, while females in spawning phase are recorded from April to July, with peaks in May and June. The length at 50% maturity (L50) estimated respectively for females and males are 12.1 cm (±0.083) and 10.1 cm (±0.073).

Keywords: *Mullus barbatus* / reproduction period / eggs size / L50 / fecundity / vitellogenin

1 Introduction

Red mullet (*Mullus barbatus* L., 1758) is one of the most important resources for shelf fisheries of the Mediterranean basin (8817 tons) (STECF 2014), representing about 8% of the demersal species landings. This species is simultaneously exploited by different type of gears such as bottom trawl, trammel net, gillnet, traps and both beach and boat seine (Demestre et al. 1997). Red mullet is also one of the most important resources for shelf fisheries along the Italian coasts where it represents about 13% (5105 tons) of landings of the demersal species (STECF 2014). Reaching the target of a sustainable exploitation of the natural stocks of this species is a crucial objective for fishery management. Stock-recruitment analysis provides the basis for setting precautionary reference points for spawning stock sizes and utilization rates to prevent recruitment overfishing and an ultimate collapse of the stock. The stock-recruitment relationship is a key element for the stock-assessment models and long-term forecasts under

specific management strategy. Stock-recruitment relationships were originally based on the total egg production (Beverton and Holt 1957) as independent variable but, in case these data are not available, estimates of the spawning stock biomass could also be used as a proxy of reproduction potential, assuming that a direct proportionality between eggs production and spawning stock biomass exists. In recent years, this assumption was increasingly criticized (Mehault et al. 2010). Indeed, fishing mortality affects not only fish abundance, but also the size-age structure of fish populations, in particular the composition in length of the spawning stock. Moreover, several studies have described a positive effect of larvae survival in relation to female age-size (Trippel et al. 1997; Vallin and Nissling 2000; Nazzari et al. 2009), suggesting that the maternal effect on egg quality is very important for the recruitment success (Murawski et al. 2009). For this reason, the evaluation of the quality of the eggs in relation to fish length represents a reproductive characteristic with great relevance to stock management. These aspects of reproductive biology of red mullet are here analysed for the first time and discussed from a fishery management point of view.

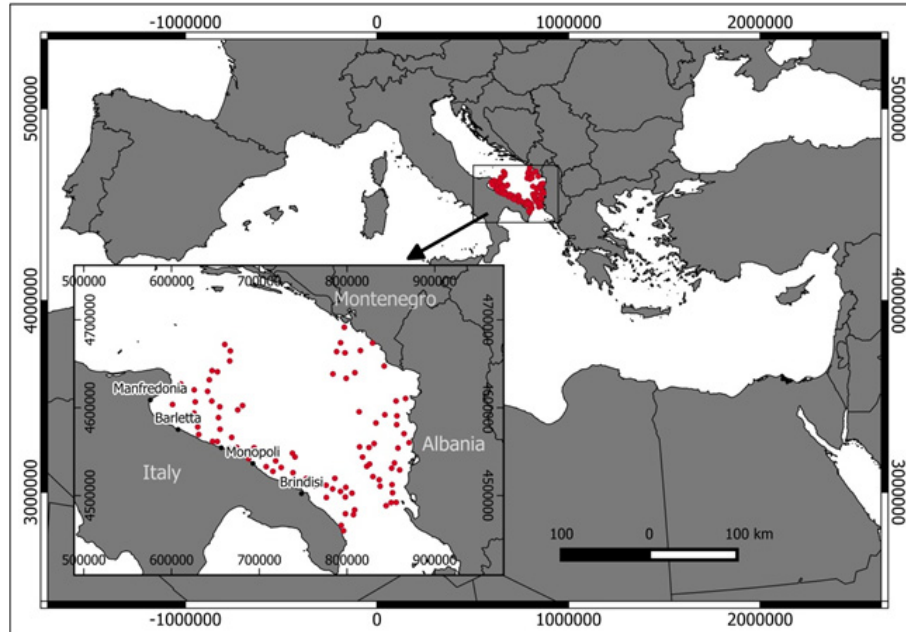[a] Corresponding author: carbonara@coispa.it

Fig. 1. Study area: the red points represent the hauls of the MEDITS trawl survey. The fishing sampling ports along the west coast (Italian) are indicated.

Moreover, the outdated biological data on reproductive biology of this species for the Southern Adriatic Sea represents an impediment to appropriate management measures. Indeed, stock-assessment models, particularly the analytical ones, require maturity parameters as important input data. The aim of the present study is also to fill this lack of knowledge.

Reproductive biology of the red mullet has already been studied in different areas of the Mediterranean basin (Vassilopoulou 1992; Tursi et al. 1994; Menini et al. 2001; Metin 2005; Layachi et al. 2007; Micale et al. 2007) and it is assessed here also for the Southern Adriatic sea. Hence, we estimated the length at 50% maturity (L50) and batch fecundity. In addition, the concentration of vitellogenin (VTG) in female plasma was also analysed. Vitellogenin is a sex specific protein produced by the liver under estradiol control, released in the blood and selectively incorporated into the oocytes and specifically cleaved into yolk proteins (Carnevali et al. 2008). Furthermore, relevant aspects of the red mullet reproductive biology, such as the period and the strategy of spawning and the maturity cycle in both sexes, were here also clarified.

2 Material and method

Red mullet samples (2011–2012) were obtained monthly from commercial landings (Data Collection Framework-DCF, EU Reg. 1543/2000, 1639/2001, 1581/2004 and 199/2008) in the fishing ports along the Italian Southern Adriatic coasts (Fig. 1) and from the Mediterranean international trawl survey MEDITS (AAVV 2012). The total length (TL) at the nearest 0.5 cm and the total weight (TW) at the nearest 0.1 g were measured. Sex and the maturation stages were recorded for each sampled specimen, following the macroscopic scale reported in AAVV (2012) (Table 1). For a subsample of adult

Table 1. Maturity scale used in the present study (from MEDITS Handbook, AA.VV., 2012).

Stage	Description	
1	virgin	juveniles
2a	virgin developing	
2b	recovering	
2c	maturing	
3	mature/spawner	adults
4a	spent	
4b	resting	

red mullet specimens (see the maturity scale reported in Tab. 1) additional measurements were also taken at the nearest 0.01 g: gutted weight (GW) and gonad weight (GoW).

For 109 females in spawning phase (2 from April, 59 from May, 41 from June and 7 from July), three gonad samples (portions from anterior, central and rear parts) were collected, weighted (at the nearest 0.001 g) and fixed in 5% buffered formalin for the butch fecundity estimation (density of hydrated oocyte for the entire gonad) using the gravimetric method (Hunter et al. 1992). The number of hydrated oocytes was counted in a sub sample for each portion and the batch fecundity was extrapolated from the weight of the gonad according to the following relationship:

$$BF = \frac{\left(nP_{for} \times \left(P_{subform}\middle/P_{form}\right)\right)}{P_{fre}} \times GoW$$

where BF is the batch fecundity; nP_{for} is the number of hydrated oocytes counted in a subsample of the three portions fixed in formalin; $P_{subform}$ is the weight of the sub-sample (fixed in formalin) where the oocytes were counted; P_{form} is the weight of the whole gonad sample preserved in formalin;

P_{fre} is the fresh weight of the same gonad sample; GoW is the weight of the entire fresh gonad.

To estimate the relationship between batch fecundity and TL, a regression analysis was performed and the significance level was estimated by the analysis of variance of the regression. The models relative to samples of May and June were statistically compared by the Chen test (Chen et al. 1992).

Oocytes' diameters on fresh gonads were measured under a light microscope (at least 300 oocytes per gonad/specimen) for each adult and juvenile maturity stage. Maximum and minimum diameters were averaged for those oocytes that did not appear spherical in shape.

Moreover, morphometric measurements of hydrated oocytes (diameter of oocytes and diameter of oil globule) were taken on fresh samples of 62 active spawning females specimens (50 eggs, randomly selected for each specimen). A linear regression analysis was performed on both the mean diameter of oocytes and the oil globule diameter of the hydrated oocytes as a function of the TL. The linear relationships were statistically tested by the analysis of variance of the regression.

The gonado somatic index (GSI) was calculated according the following formulations: $GSI = (GoW/GW) \times 100$; where GoW is the gonad weight and GW the gutted weight.

The monthly trend of the GSI as well as the percentage of the maturity stages for both females and males were investigated to better understand the reproductive cycle of the species. The monthly means values of GSI were statistically tested by the Tukey's post hoc test of the ANOVA (R software).

The L50 (the length at which 50% of the population is mature) and the maturity range ($MR = L_{75\%}-L_{25\%}$) were estimated for both sexes (females $n = 3104$ and males $n = 3145$) using a binomial generalized linear model (GLM, R software) with logistic link (ICES 2008). In the analysis, the specimens classified as 2b, 2c, 3, 4a and 4b were considered adult while the specimens classified 1 and 2a were considered juveniles (Table 1). The ogive models of males and females were statistically compared using the Chen test (Chen et al. 1992).

On the 16 active spawning females of *M. barbatus* caught alive during the MEDITS trawl survey (2009), blood samples (about 1.5 ml) were taken from the caudal vein. The blood was immediately centrifuged at 2000 x g for five minutes to obtain the plasma, and then stored in liquid nitrogen.

The VTG assay was performed by Western blotting analysis. The plasma was electrophoresed and transferred to PVDF (Polyvinylidene fluoride membrane) (Maradonna et al. 2004). Briefly, 20 μg of each protein sample were separated using 4% stacking and 10% separating sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Leammli 1970), and electroblotted onto a filter using a mini trans-blotting electrophoretic transfer cell (all from Bio-Rad). Transfer was carried out for 30 min using Bio-Rad's Trans-Blot® Turbo™ Transfer System.

The membrane was soaked in 5% Nonidet-P40 for 1 h to remove SDS and incubated with 2% bovine serum albumin (BSA; Sigma) in PBSand then incubated with the αVTG$_{carpa}$ (1:10,000) (Carnevali and Belvedere 1991) in 3% milk-PBS for 2 h at room temperature. The second antibody solution (HRPconjugated anti-rabbit IgG; Bio-Rad) diluted 1:1000 in 2% BSA in PBS buffer was incubated for 1 h.
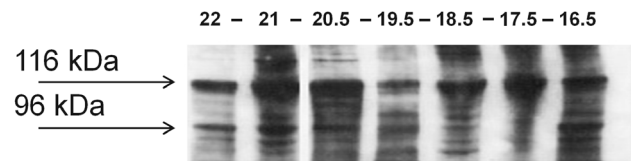

Fig. 2. Examples of Western blotting results for the plasmatic vitellogenin. The number on the top indicate the TL (cm) of specimens. Moreover the two components (116 kDa and 96 kDa) of vitellogenin taken into account in the densitometry analysis are indicated.

The reaction was visualized with ECL-PLUS (GE Healthcare, Milano, Italy) chemiluminescent reagent for Western blotting. Densitometric analysis was performed using ImageJ software for Windows. The two main bands, corresponding to the apparent molecular weight of 116 and 97 kDa (Fig. 2) were taken into account in this analysis.

A linear regression analysis (R software) was used to investigate the relationship between the plasmatic vitellogenin concentration and TL of each specimen. The linear relationships were statistically tested with the analysis of variance of the regression.

3 Results

The oocytes and the oil globule diameters resulted significantly correlated ($p < 0.05$) to TL following a linear relationship (Fig. 3).

The results of Western blotting analysis showed numerous forms of plasmatic vitellogenin (Fig. 2). The amount of plasma VTG (116 kDa and 97 kDa) was significantly ($p < 0.05$) correlated with the total length by a linear relationship (Fig. 4). The amount of plasmatic VTG ranges between 4.759 AU (14 cm of TL) and 9.014 AU for a females of 22 cm TL.

The monthly percentageof maturity stage in females (Fig. 5) shows that the number of 2b stage females (recovering) with oocyte in pre-vitellogenic stage (Micale el al. 2007) progressively increases from July to January (from 19.5% to 100%). Females with gonads in maturation stage (2c) begin to appear in February (21.3%), until June (6.8%) with a peak in April (54.1%). The mature/spawner females present a peak that lasts two months: May (83.3%) and June (83.5%). Since the month of June it is possible to observe females in the post-reproduction phase: spent (4a) and resting (4b) with the higher concentration during July (36.2%) and August (35%). Moreover, it is possible to observe the presence of stages 4a/4b until December: during this month a new maturation cycle starts, with high presence of stage 2b.

The males' percentage of maturity stages (Fig. 5) shows a longer reproductive period compared with the females'. The resting males (2b) are present during all the year with an increasing trend since July to September and a decreasing trend starting in October. Males in maturation stage (2c) are caught since September (0.3%) until July (0.52%) showing two peaks, the first in February (87.1%) while the other is in June (52.1%). Males in spawning phase (stage 3) are present for almost the whole year with an increasing trend since November until June and a decreasing trend since July until October. Spent/resting
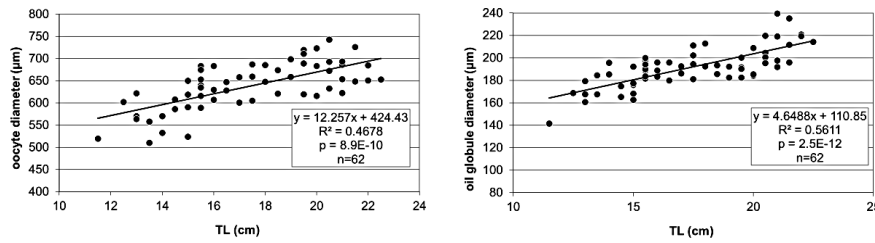
Fig. 3. Relationship between the hydrated oocyte diameters (left) and oil globule diameters (right) with total length in 67 spawning red mullet females. The equation and the relative statistics are also reported.

Table 2. Results of the Tukey's HSD ANOVA post-hoc test (R software). The grey cells indicate the existence of significant ($p < 0.05$) differences among the pairwise means comparison.
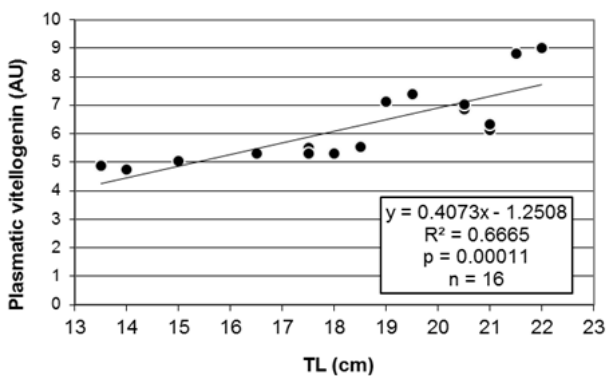

Fig. 4. Relationship between the plasmatic vitellogenin (AU: Arbitrary Unit – Western Botting Densitometric analysis) and the total length of spawning red mullet females. The equation and the relative statistics are also reported.

males (4a and 4b stages) occur since March until December with a peak in the period between July (76.2%) and August (76.4%).

A comparison between sexes shows that the males became mature earlier (October) than females (April). The females' spawning peak (May and June) corresponds to the elevated presence of males in the stage 3 of maturation (respectively 37.1% and 54.4%).

This maturation pattern is confirmed by the monthly analysis of the GSI trend (Fig. 6), both in males and females. In Table 2 are reported the results of the GSI mean comparison (Tukey's post hoc test of the ANOVA) for males and females. The females' GSI started to increase in March, reaching its highest peaks in May and June ($p < 0.05$), months in which the presence of spawning females is higher according to the maturation pattern. The GSI in males starts to increase earlier in October, and reaches the maximum in the period between March and May ($p < 0.05$).

The oocytes' diameters' frequencies distribution (ODF) of 10 female specimens by maturity stage and by month is reported in Figure 7, where the most representative maturity stage per month has been chosen. The stage 2a and 2b in March presented the same ODF pattern with a single mode around a $100 \mu m$ (previtellogenic oocytes) (Micale et al. 2007), but in the stage 2b the oocytes' diameters reach higher dimensions in comparison to maturity stage 2a. In April maturity stage 2c shows a polymodal pattern with three peaks respectively at $250 \mu m$, $500 \mu m$ and $750 \mu m$, corresponding to yolked oocytes at different stages of vitellogenesis (Micale et al. 2007). For the spawning maturity stage 3 (May–June) the ODF shows a poly-modal pattern, with maximum diameter in the range of classes between 1050 and 1100 μm. Moreover an evident lack between the diameter class of 900 and 1050 μm is present. Also during this stage three modes could be identified ($250 \mu m$, $500 \mu m$ and $750 \mu m$), representing oocytes in
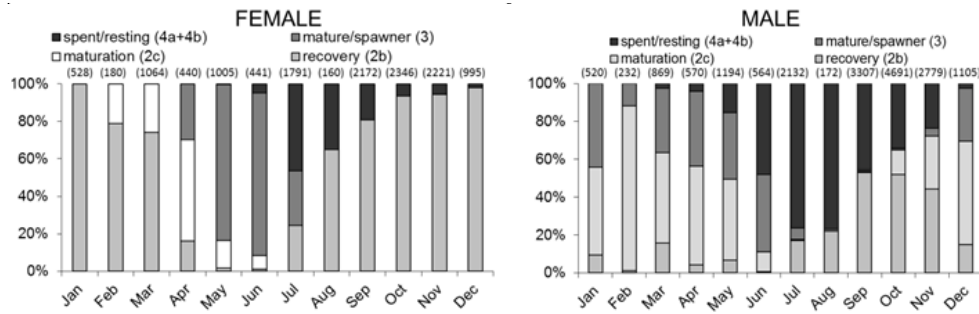
Fig. 5. Monthly trend of percentage of maturity stage of adult females and males. The number of the specimens analysed each month is reported in brackets.
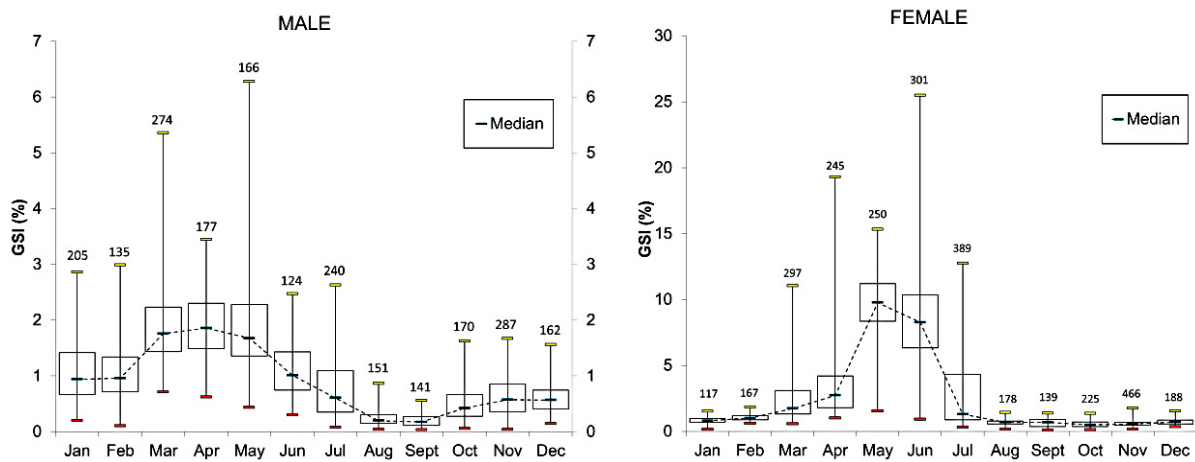

Fig. 6. Box-plot of the monthly GSI for females and males. The bars indicates the lower and the higher GSI values, while in brackets is reported the number of specimens used in the analysis.
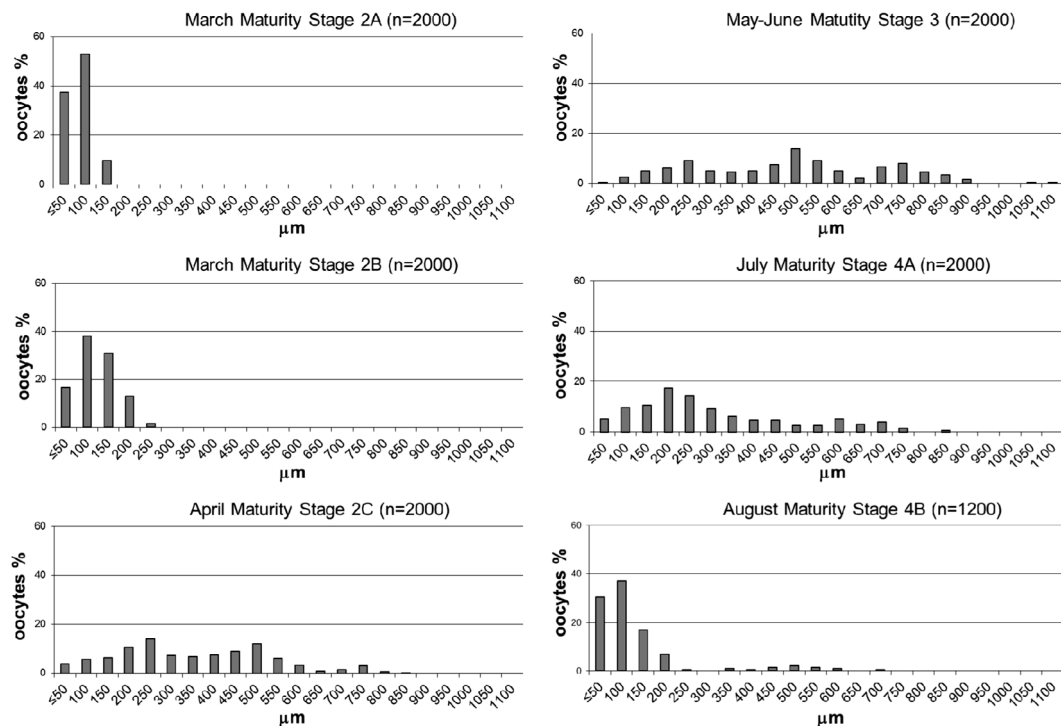

Fig. 7. Monthly frequency distribution of oocytes diameters by maturity stages. The number of measured oocytes is reported.
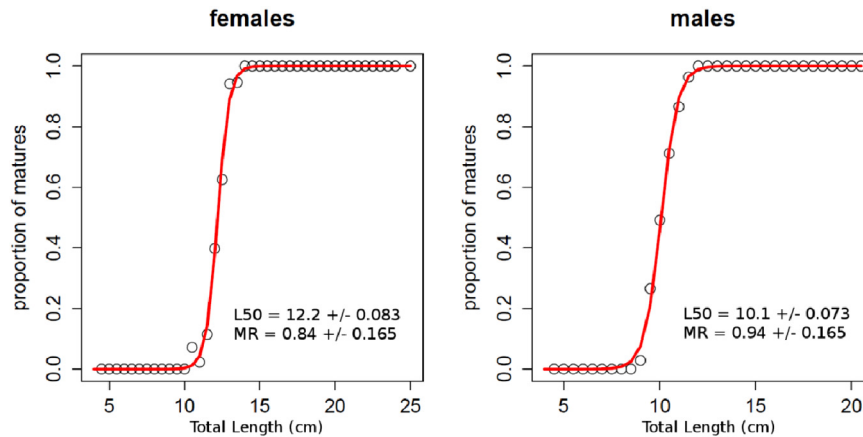
Fig. 8. Maturity ogives of females (left; $n = 3104$) and males (right; $n = 3145$) of red mullet. The Maturity Range (MR) and the lenght of 50% maturity (L50) is also reported.
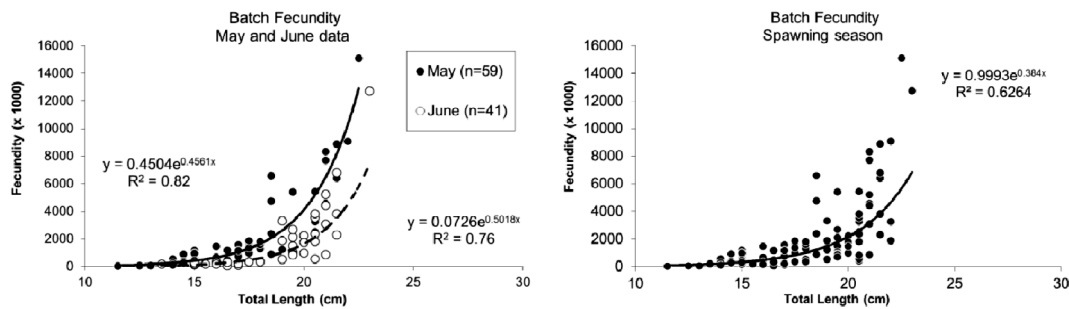

Fig. 9. Relationship of batch fecundity and total length for the samples from May and June (left) and from all spawning season (right). Parameters and statistics of the exponential function are reported.

different vitellogenic phases. During post spawning maturity stages (4a and 4b), the percentage of oocytes in vitellogenic phase decreases and in August the ODF of maturity stage 4b shows only the residues of the vitellogenic oocytes in absorption. Most of the oocytes' ranges between ⩽50 to 250 μm as well as the previtellogenic oocytes (maturity stage 2b).

The estimated values of L50 for females and males (Fig. 8) are respectively 12.2 ± 0.083 cm and 10.1 ± 0.073 cm. The maturation ranges (MR L_{75}–L_{50}) are 0.84 ± 0.165 for females and 0.94 ± 0.144 for males. The comparison of the maturity ogives of males and females by the Chen test shows significant differences ($p < 0.05$). The smallest mature female observed was 10.5 cm in TL, while the smallest mature male was 9 cm of LT; moreover, the first length class with 100% of adult specimens are 14 and 13 cm respectively for the females and males.

The batch fecundity estimated with the specimens collected from April to July (spawning season) was significantly ($p < 0.05$) correlated with TL following an exponential relationship (Fig. 9). Also, the batch fecundity in May and June was significantly ($p < 0.05$) correlated with TL followingan exponential model (Fig. 9). The comparison of the batch fecundity curves of these two months by the Chen test ($p < 0.05$) shows a significantly higher batch fecundity in May samples.

4 Discussion

The diameters of oocytes measured in this work are comparable to data reported by Metin (2005) for the Aegean coast

of Turkey. In literature the diameter of hydrated oocyte and the dimension of oil globule was demonstrated in different species, such as *Dentex dentex* (Çoban et al. 2011), *Salmo salar* (Heinimaa and Heinimaa 2004) and *Merluccius merluccius* (Mehault et al. 2010), to be correlated with egg quality and, hence, with reproductive success. Therefore, the positive correlation between diameter of hydrated oocytes and the oil globule with TL, demonstrated here for the first time in red mullet, could indicate that females bigger in size may have a greater reproductive fitness, not only from a quantitative (fecundity) but also from a qualitative point of view (vitellogenin and eggs dimensions). The significant relations between TL and both the fecundity and size of the oocytes (expressed as the egg and the oil globule diameter) stand for animals with bigger size (TL) producing a greater number (Fig. 9) of larger eggs (Fig. 3).

As already demonstrated in other fish species (Trippel et al. 1997; Vallin and Nissling 2000; Berkeley et al. 2004; Heinimaa and Heinimaa 2004; Nazari et al. 2009), the bigger larvae are hatched from larger eggs, spawned by longer/older females. Studies on early life stage showed that a greater amount of yolk reserves are positively correlated with the survival of the larvae during the first days after hatching and with the growth rate (Mehault et al. 2010). Indeed the larger larvae may use a wider range of prey and therefore they have greater facility in finding food, wasting less energy (Miller et al. 1988). Moreover, the final egg diameter associated with the hydration process is one of the factor that influences buoyancy

(Vallin and Nissling 2000), determining their position in the water column. This aspect seems to play an important role in the fertilisation success of eggs as well as in larval survival (Nissling et al. 1994). The bigger eggs are placed more close to the water surface where there is better oxygenation and a higher abundance of plankton preys (Vallin and Nissling 2000). All these aspects are translated into higher chances of survival for the larvae generated by bigger/older females than the larvae hatched from eggs produced by smaller/younger females.

Fiorentino et al. (2008) in the Gulf of Castellammare (Southern Tyrrhenian Sea), where trawling has been banned since 1991, found that the effects of this management measure have determined the increase in average size of the spawning-stock of red mullet and an increase of the recruitment.

Although the oldest specimens are a small portion of the spawning-stock, they seem to have a great contribution to the total eggs production. Therefore, egg production is directly proportional to the spawning stock biomass, but the oldest specimens play a more influential role in reproduction fitness than younger ones, both in term of quality and quantity of egg/larvae produced.

However, further investigations need to be conducted to better understand some crucial steps influencing recruitment, as well as the factors that influence fertilization and larvae survival. Indeed, the mechanism occurring between red mullet spawning and recruitment remains poorly understood. Therefore the observed correlation between the eggs' quality and the size of females, suggests that in the stock assessment, spawning-stock should be taken into account not only in terms of biomass, but also in term of size/age structure composition (Mehault et al. 2010).

The presences of vitellogenin in the plasma during spawning phase was observed also in other species, such as Atlantic halibut (Methven et al. 1992), and seems linked to asynchronous ovary organization and to the consequent presences of oocytes in the vitellogenic phase also during the spawning period. Also the positive correlation between fish length and plasma vitellogenin concentration, found in this work, could be explained as an effect of higher reproduction fitness of larger females.

The monthly percentage of maturity stages and monthly trend of GSI seems to define a single reproductive event during the year extended from April to July with a bulk in May and June for the female. Mature/spawning males are present during almost the whole year (except in September) as well as in *M. surmuletus* (Mahé et al. 2013); GSI values tend to increase in October, reaching the highest values in January, remain high until July and then fall in late summer (Table 2). This maturation pattern supports the hypothesis that males anticipate the sperm emission phase to be ready for fertilization when the egg-spawning season starts. In literature few data (Hädar 1970; Layachi et al. 2007) are reported for the Mediterranean area about males' maturity and their reproduction cycle, but they are in agreement with an anticipation of the spawning phase in males in comparison to females. The higher male effort oriented in reproductive activity could explain the growth differences between the sexes in *Mullus barbatus*. Indeed, female specimens show higher growth rates compared

to male specimens, which accounts for a difference in size of about 2–4 cm of TL for the first two years of life (Tursi et al. 1994; Fiorentino et al. 1998; Voliani et al. 1998).

For red mullet females, even with some geographical differences, the various authors agree to indicate a spawning season included between the spring and early summer months (Table 2). The spawning season here estimated for the Southern Adriatic Sea, included in the period between April and July, is also reported for the Gulf of Thermaikos, North-West Sicily, and the North African coast (Ezzat et al. 1997; Gharbi and Ktari 1981; Micale et al. 2007; Kokokiris et al. 2014). A shorter period has been identified in the Ligurian Sea (Orsi Relini and Arnaldi 1986) (from June to July), as well as in the Gulf of Lion (Miramad et al. 1991) and the Antalya Gulf (Becer Özvarol et al. 2005). Other authors observed an extension of the spawning season from April to September for the North Adriatic Sea, the Catalan coast, the southern Tyrrhenian Sea and the Mediterranean coasts of Morocco (Jukic and Piccinetti 1981; Aguirre Villasenor 2000; Layachi et al. 2007; Sieli et al. 2011).

Through the oocytes' diameter frequency distribution in spawner females it is possible to define the reproductive behaviour of red mullet as a batch spawner, with asynchronous ovary organization (Murua and Saborido-Rey 2003). This result is in agreement with what has been described by other authors for different areas of the Mediterranean (Menini et al. 2001; Metin 2005; Layachi et al. 2007; Micale et al. 2007). The oocytes' diameter frequency distribution shows a polymodal pattern also in females in the spawning phase. Every mode could correspond to different oocytes' maturational vitellogenic stages (Menini et al. 2001; Micale et al. 2007), which would suggest a continuous maturation pattern of oocytes and spawning of eggs in successive batches (Murua et al. 2003; Kokokiris et al. 2014).

According to what was reported by other authors (Bougis 1952; Vassilopoulou 1992; Tursi et al. 1994; Metin 2005; Layachi et al. 2007), the L50 estimated in this work confirms that males reach sexual maturity at lower sizes ($L50 = 10.1$ cm) in comparison to females ($L50 = 12.2$ cm). This could be explained by the difference in growth pattern between males and females in *Mullus barbatus* described by several authors (Tursi et al. 1994; Fiorentino et al. 1998; Voliani et al. 1998; Joksimovic et al. 2008).

Concerning the $L50$ (Table 3), no previous estimates are reported in the literature for the Southern Adriatic Sea. Jukic and Piccinetti (1981) and Haïdar (1970) have reported for the Northern Adriatic Sea an estimated L50 coincident with those found in this study. While Joksimović (2005) reported for the Montenegrin shelf a L50 value of 13.54 mm, for both sexes combined, higher than those estimated in this work. Moreover, the values reported in this study are comparable with what is reported for the Southern Tyrrhenian Sea (Micale et al. 2007), the Mediterranean coast of Morocco (Layachi et al. 2007) and the south of France (Bougis 1952), while they are higher than those reported for the North Aegean Sea (Vassilopoulou 1992; Kokokiris et al. 2014) and Cyprus (Lividas 1988) and lower than those reported for the Ionian West Sea (Tursi et al. 1994), the Aegean Turkish coast (Metin 2005), the Northern Tyrrhenian Sea (Voliani et al. 1998) and the Tunisian coast

Table 3. The spawning period of *Mullus barbatus* in different Mediterranean areas.

Author	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Method	Area
Aguirre Villaseñor 2000													GSI	Catalan Coast
Becer Özvarol et al. 2005													GSI	Antalya Gulf
Cherif 2007													GSI	Tunis Gulf
Del Arbol et al. 2004													GSI	Alboran Sea
Ezzat et al. 1997													GSI	Alexandria Coast
Gharbi and Ktari 1981													GSI	Tunisian Coast
Haïdar 1970													GSI – Maturity stage	Northern Adriatic Sea
Miramad et al. 1991													GSI	Bay of Roquebrune (Gulf of Lion)
Jukic and Piccinetti 1981													GSI	Northern Adriatic Sea
Layachi et al. 2007													GSI	Nador Coast
Menini et al. 2001													GSI	Northern Tyrrenian Sea
Sieli et al. 2011													GSI	Castellamare Gulf (North-West Sicily)
Orsi Relini and Arnaldi 1986													Maturity stage	Ligurian Coast
Kokokiris et al. 2014													GSI – Maturity stage	Thermaikos Gulf
Vrantzas et al. 1992													GSI	Saronikos Gulf
Present work													GSI – Maturity stage	South-West Adriatic Sea

Table 4. The L50 values of *Mullusbarbatus* for the different areas of the Mediterranean.

Author	L50 (cm) Male	L50 (cm) Female	Area
Bougis 1952	11.5	13.5	Southern French coast
Gharbi and Ktari 1981	12.9–14.4		Tunisian coast
Jukic et al. 1981	10.5		Eastern Adriatic coast
Livadas 1988	9.5		Cyprus
Vassilopoulou 1992	9.4	10.4	Eubea (Aegean Sea)
Voliani et al. 1998		12.5	Tuscan coast
Joksimovic et al. 2005	13.5		Montenegro coast
Layachi et al. 2007	10.3	13.8	Moroccan Mediterranean coast
Cherif 2007	13.87	13.94	Gulf of Tunis
Del Arbor et al. 2003	10.8	11.5	North of Alboran Sea
Metin 2005	12.4	14.2	Izmir (Turkish Aegean Sea)
Micale et al. 2007	11–11.3	12–14	Gulf of Castellamare (North-West Sicily)
HaÏdar 1970	10.5	12	North-Eastern Adriatic coast
Tursi et al. 1994	12.5	13.5	Western Ionian Sea
Kokokiris et al. 2014		11.4	Gulf of Thermaikos
Present Work	10.1	12.2	Southern Adriatic Sea

(Gharbi and Ktari 1981). These differences could be explained by the phenotypic response to some environmental variation or by the evolutionary consequence of some selective pressure such as fishery efforts (Barot et al. 2004), as well as by the different estimation methods applied (ICES 2008).

The correlation between fecundity and TL by means of an exponential model has already been reported in the literature (Metin 2005; Menini et al. 2001; Micale et al. 2007; Tirasin et al. 2007). The data from this study provide a preliminary estimation of the reproductive potential of red mullet in the Southern Adriatic Sea.

The samples analysed in this study allowed an evaluation of changes in fecundity during the spawning season. The analysis of fecundity in the months of spawning peak showed a significantly higher red mullet fecundity during May rather than in June. Fishery management measures such as fishery interdiction in specific months of year, could be more effective taking into account the results of fecundity trend obtained in this study. These results further confirm the importance of knowing spawning dynamics for resource management.

5 Conclusion

One of the main contributions of this work was to demonstrate, in red mullet female specimens, the significant correlations between total length with both egg dimensions (oocytes and oil globule diameter) and plasma vitellogenin concentration. Several studies have already described that the maternal effect (eggs dimension) is important on the recruitment success. Hence, for more effective fishery management the stock assessment should take into account the spawning stock not only in terms of biomass, but also in term of size/age structure composition.

The fecundity, during the spawning season, shows for the red mullet in the southern Adriatic Sea a significantly time differences. Also, this result has important implications for the conservation of this important resource for the Mediterranean basin, mostly in the application of some management measures such as fishery closures in some periods of years. Moreover, the monthly data of the GSI and maturity stage define a single reproductive event during the year extended from April to July with a bulk in May and June for the female, while mature/spawning males are present during almost the whole year. The results on the reproductive pattern and L50 seem in accordance with the knowledge on this species for the Mediterranean basin.

References

Aguirre Villaseñor H., 2000, Aspectos Biològicos y Ecològicos del Salmonete de Fango *Mullus barbatus* L., 1758 y del Salmonete de Roca *Mullus surmuletus* L., 1758, del Meditèrraneo Noroccidental. PhD thesis, Universitat Politecnica de Catalunya, Barcelona, 218 p.

AA.VV., 2012, MEDITS (International bottom trawl survey in the Mediterranean)-Handbook Revision No. 6, April 2012. MEDITS Working Group, 92 p.

Barot S., Heino M., O Brien L., Dieckman U., 2004, Estimation reaction norm for age and size at maturation when age at first reproduction is unknown. Evol. Ecol. Res. 6, 659–678.

Becer Özvarol Z.A., Balci B.A., Özbaş M., Gökoğlu M., Gülyavuz H., 2005, Antalya Körfezinden Avlanan Barbunya (*Mullus barbatus* L., 1758) Balıklarının Eşfeysel Olgunluk Yaşı Ve Boyu Ile Üreme Zamanının Belirlenmes. Türk Sucul Yaşam Dergisi 3(4), 85–88.

Berkeley S.A., Chapman C., Sogard S.M., 2004, Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops.* Ecology 85, 1258–1264.

Beverton R.J.H., Holt S.J., 1957, On the dynamics of exploited fish populations. Fishery Investigations Series 2: Sea Fisheries 19, 1–533.

Bougis P., 1952, Recherches biométriques sur le rouget (*Mullus barbatus* L. et *Mullus surmuletus* L.). Archives de Zoologie Expérimentale et Générale 89, 59–174.

Carnevali O., Belvedere P., 1991, Comparative studies of fish, amphibian and reptilian vitellogenins. J. Exp. Zool. Part A 259, 18–25.

Carnevali O., Cionna C., Tosti L., Cerdà J., Gioacchini G., 2008, Changes in cathepsin gene expression and relative enzymatic activity during gilthead sea bream ovarian follicle maturation. Mol. Reprod. Dev. 75, 97–104.

Chen Y., Jackson D.A., Harvey H.H., 1992, A comparison of Von Bertalanffy and polynomial functions in modelling fish growth data. Can. J. Fish. Aquat. Sci. 49, 1228–1235.

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(2), 131–144.

Çoban D., Kamacı H.O., Suzer C., Yıldırım Ş., Arda G, Yıldırım, Korkut A., Saka Ş., Fırat, K., 2011, Effect of Some Morphometric Characteristics on Egg Quality in Common *Dentex, Dentex dentex* (Linnaeus, 1758). Turk. J. Fish. Aquat. Sci. 11, 425–431.

Del Arbol P.J., Gil de Sola L., 2003, Fishery and population of red mullet in the north Alboran sea (W. Mediterranean). Working Paper. GFCM-SAC Sub-committee on Stock Assessment (SCSA).

Demestre M., Sbrana M., Alvarez F., Sánchez, 1997, Analysis of the interaction of fishing gear in *mullus barbatus* fisheries of the Western Mediterranean. J. Appl. Ichthyol. 13, 49–56.

Ezzat A., Hosny C.F. Osman A., 1997, Reproductive strategy of *Mullus barbatus* and *Mullus surmuletus* from Alexandria Waters, Egypt. In Özhan, E. (Ed.) Proceedings of the third International Conference on the Mediterranean Coastal Environment. Medcoast 97, Qawra, Malta, pp. 135–149.

Fiorentino F., Zamboni A., Rossi M., Relini G., 1998, The growth of the Red Mullet (*Mullus barbatus*, L. 1758) during the first years of life in the Ligurian Sea (Mediterranean). CHIEAM-Options Medit. 35, 65–78.

Fiorentino F., Badalamenti F., D'Anna G., Garofalo G., Gianguzza P., Cristina M., Pipitone C., Rizzo P., Fortibuoni T., 2008, Changes in spawning-stock structure and recruitment pattern of red mullet, *Mullus barbatus*, after a trawl ban in the Gulf of Castellammare (central Mediterranean Sea). ICES J. Mar. Sci. 65, 1175–1183.

Gharbi H., Ktari M.H., 1981, Biologie de *Mullus barbatus* L.1758 et *Mullus surmuletus* L., 1758 (Poissons, Téléostéens, Mullidae) des regions tunisiennes. Taille et âge de première maturité sexuelle, cycle sexuel et coefficient de condition. Bull. Inst. Natl. Sci. Tech. Oceanogr. Peche Salammbo 8, 41–51.

Häidar Z., 1970, L'oecologie du rouget (*Mullus barbatus* L.) en Adriatique orientale. Acta Adriat. 14, 88–92.

Heinimaa S., Heinimaa P., 2004, Effect of female size on egg quality and fecundity of the wild Atlantic salmon in the sub-artic river Teno. Boreal Environ. Res. 9, 55–62.

Hunter J.R., Macewicz B.J., Kimbrell C.A., 1992, Fecundity, spawning and maturity of female Dover Sole, *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90, 101–128.

ICES, 2008. Report of the Workshop on Maturity Ogive Estimation for StockAssessment (WKMOG), 3–6 June 2008, Lisbon, Portugal. ICES CM2008/ACOM: 33, 72 p.

Joksimović A., 2005, Population dynamic of Red mullet *Mullus barbatus*, Linneaus, 1758 in the Montenegrin shelf. Doctoral dissertation. Faculty of biology, University of Belgrade, p. 93.

Jukić S., Piccinetti C., 1981, Quantitative and qualitative characteristics of demersal resources in the Adriatic Sea with some population dynamics estimates. General Fisheries council for the Mediterranean.Report of the second Technical Consultation on Stock Assessment in the Adriatic, Ancona, 18–22. May 1981. FAO. Fish. Rep. 253, 73–91.

Kobayashi K., Tamotsu S., Yasuda K., Oishi T., 2005, Vitellogenin-immunohisto-chemistry in the liver and the testis of the medaka, *Oryzias latipes*, exposed to 17β-estradiol and p-nonylphenol. Zool. Sci. 22, 453–461.

Kokokiris L., Stamoulis S., Monokrousos N., Doulgeraki S., 2014, Oocytes development, maturity classification, maturity size and spawning season of the red mullet (*Mullus barbatus barbatus* Linnaeus, 1758). J. Appl. Ichthyol. 30, 20–27.

Laemmli U.K., 1970, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Layachi M., Melhaoui M., Srour A., Ramdani M., 2007, Contribution à l'étude de la reproduction et de la croissance du Rouget-barbet de vase *(Mullus barbatus* L., 1758) de lazone littorale méditerranéenne de Nador (Maroc). Bull. l'Institut Sci. (Rabat) Sect. Sci. Vie 29, 43–51.

Livadas R.J., 1988, A study of the growth and maturity of striped mullet (*Mullus barbatus* L., 1758) in waters of Cyprus. FAO Fish. Rep. 412, 44–51.

Mahé K., Coppin F., Vaz S., Carpentier A., 2013, Striped red mullet (*Mullus surmuletus*, Linnaeus, 1758) in the eastern English Channel and southern North Sea: growth and reproductive biology. J. Appl. Ichthyology 29, 1067–1072.

Maradonna F., Polzonetti V., Bandiera S.M., Migliarini B., Carnevali O., 2004, Modulation of the hepatic CYP1A1 system in the marine fish *Gobius niger*, exposed to xenobiotic compounds. Environ. Sci. Technol. 38, 6277–6282.

Mehaulta S., Domínguez-Petita R., Cerviño S., Saborido-Rey F., 2010, Variability in total egg production and implications for management of the southern stock of European hake. Fish. Res. 104, 111–122.

Menini S., Pirone A., Fabiani O., Volani A., Baino R., 2001, Osservazioni su maturità gonadica e fecondità in Mullus barbatus. Biol. Mar. Medit. 8, 736–740.

Methven D.A., Crim L.W., Norberg B., Brown J.A., Goff G.P., Huse I., 1992, Seasonal reproduction and plasma levels of sex steroids and vitellogenin in Atlantic halibut (*Hippoglossus hippoglossus*). Can. J. Fish. Aquat. Sci. 49, 754–759.

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

Micale V., D'Anna G. Di Stefano G., Guerrera M.C., Muglia U., Sieli G., Fiorentino F., 2007, Oocyte development, spawning pattern and batch fecundity of the red mullet (*Mullus barbatus*, L. 1758) from a Mediterranean area subjected to a trawling ban. XII European Congress of Ichthyology. Cavtat (Croatia).

Miramand P., Laufaurie M., Fowler S.W., Lemaire P., Guary J.C., 1991, Reproductive cycle and heavy metals in organs of red mullet, *Mullus barbatus* (L.), from the northwestern Mediterranean. Sci. Total Environ. 103, 47–56.

Murawski S.A., Rago P.J., Trippel E.A., 2001, Impacts of demographic variation in spawning characteristics as reference points forfishery management. ICES J. Mar. Sci. 58, 1002–1014.

Murua H., Saborido-Rey F., 2003, Female reproductive strategies of marine fish and their classification in the North Atlantic. J. Northwest Atl. Fish. Sci. J. 33, 23–31.

Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera, J., 2003, Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. J. Northwest Atl. Fish. Sci. J. 33, 33–53.

Nazari R.M., Sohrabnejad M., Ghomi M.R., Modanloo M., Ovissipour M., Katantarian H., 2009, Correlation between egg size and dependent variables related to larval stage in Persian sturgeon *Acipenser persicus*. Mar. Freshw. Behav. Physiol. 42, 147–155.

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. Zool. Anat. Comp. R. Univ. Genova 52, 237–250.

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, MediterraneanSea) subject to a trawling ban. J. Appl. Ichthyol. 27, 1218–1225.

Scientific, Technical and Economic Committee for Fisheries (STECF) – Assessment of Mediterranean Sea stocks - part 1-2 (STECF-14-17. 2014. Publications Office of the European Union, Luxembourg, EUR 26955 EN, JRC 93120, 393 p.

Tirasin E.M., Unluoglu A., Cihangir, B., 2007, Fecundity of red mullet (*Mullus barbatus* L., 1758) along the Turkish coasts of the Mediterranean Sea. Rapp. Comm. int. Mer Médit. 38, 614.

Trippel E.A., Kjesbu O.S., Solemdal P., 1997, Effects of adult age and size structure on reproductive output in marine fishes. In Chambers, R.C., Trippel E.A. (Eds.), *Early Life History and Recruitment in Fish populations*. Chapman and Hall, New York, pp. 31–62.

Tursi A., Matarrese A., D'Onghia G., Sion L., 1994, Population biology of red mullet (*Mullu barbatus* L.) from the Ionian Sea. Marine Life 4, 33–43.

Vallin L., Nissling A., 2000, Maternal effects on egg size and egg buoyancy of the Baltic Cod, *Gadus morhua*; implications for stock structure effects on recruitment. Fish. Res. 49, 21–37.

Vassilopoulou V., 1992, Biological aspects of red mulet. *Mullus barbatus* off the coasts of central Greece. Bull. Mar. Biol. Res. Cent. 9, 61–81.

Voliani A., Abella A., Serena F., 1998, Problematiche inerenti la valutazione dello stato di sfruttamento di *Mullus barbatus*. Biol. Mar. Medit. 5, 169–183.

Vrantzas N., Kalagia M., Karlou C., 1992, Age, growth and state of stock of red mullet (*Mullus barbatus* L., 1758) in the Saronikos gulf of Greece. Annex 4 to: Fourth session of the technical consultation on stock assessment in the Eastern Mediterranean. Thessaloniki, Greece, 7–10 October 1991. FAO Fish. Rep. 477, 51–56.