Health and reproduction of red mullet, *Mullus barbatus*, in the western Mediterranean Sea

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Abstract The reproductive and general health of exploited fish stocks is an essential element of sustainable and profitable fisheries. The main purpose of this study was to assess the relationships between reproduction and two important parameters of fish health (parasitism and energy reserves) in female specimens of red mullet, *Mullus barbatus*, from the western Mediterranean Sea. We present new data for this species on (i) the prevalence and intensity of infection by metazoan parasites; (ii) the total lipid content in muscle and gonads as a measure of condition and (iii) fecundity and egg quality as a measure of their reproductive capacity. The results show that *M. barbatus* is a batch spawner with an income breeding strategy, an asynchronous development of oocytes and indeterminate fecundity. The results also indicate that the three most abundant and prevalent parasites significantly affect the condition and reproduction of *M. barbatus*. Specifically, the digenean, *Opecoeloides furcatus*, causes a reduction in the female’s energy reserves, while the nematodes, *Hysterothylacium fabri* and *H. aduncum*, produce a rise in egg production but impair egg quality. These implications of the relationships between parasitism, fish health and fish reproduction should be taken into consideration in the assessment and management of exploited species.

Keywords Egg quality · Energy reserves · Fecundity · Metazoan parasites · Reproductive strategy

Introduction

Parasitism is usually defined as a non-mutual symbiotic relationship between species, where the parasite benefits at the expense of the host. In theory, therefore, parasitism should produce deleterious effects on fish health because the parasites drain energy from the fish that would otherwise be used to sustain the fish’s condition, growth or reproduction. However, it appears that through the coevolution of host and parasite, a dynamic equilibrium has arisen between fish and their parasites (Barret, 1986). Indeed, in most cases,
parasites do not cause severe effects on their hosts because this would impair their chances of reaching the following stage of their life cycle or of reproducing.

Lipid storage and dynamics within the organism are an important aspect of fish health (Adams, 1999). The energy reserves of fish are a highly significant indicator of their condition and may have important implications in research into fish life-history traits and, consequently, for stock productivity and fisheries in general (reviewed by Lloret et al., 2012a, 2014). In particular, the maternal condition has been identified as an important factor in determining egg production and egg condition in a number of fish species, e.g. Atlantic cod, Gadus morhua L. 1758 and haddock, Melanogrammus aeglefinus L. 1758, (Saborido-Rey et al., 2003; Alonso-Fernández et al., 2009). However, little attention has been paid to the effects of parasitism on maternal condition or, consequently, on reproductive capacity. There are some studies on the relationships between parasitism and condition (e.g. Ondracková et al., 2010; Guidelli et al., 2011; Francová & Ondračková, 2013) and also some that include reproductive variables (e.g. Sasal et al., 2001; Bagamian et al., 2004; Bean & Bonner, 2009). However, most of these studies used simple morphometric indices of condition and have focused on freshwater fish species or on the effects of a single species of parasite.

With regard to marine exploited species, information on the effects of parasites on the energy reserves is limited to a few studies on northwestern Atlantic cod, Gadus morhua, (Khan & Lacey, 1986), young-of-year common sole, Solea solea L. 1758, in the northeastern Atlantic Ocean (Durieux et al., 2007), anchovy, Engraulis encrasicolus L. 1758, in the Black Sea (Shchepkina, 1980) and Japanese anchovy, E. japonicus Temminck & Schlegel 1846, (Sajiki et al., 1992). To our knowledge, the only study into the relationships between parasitism, energy reserves and reproductive capacity of an exploited marine species is our previous work on the European hake, Merluccius merluccius L. 1758, in the northwestern Mediterranean Sea (Ferrer-Maza et al., 2014). In short, little is known about the effects of parasites on the condition and the productivity of marine fishes.

Red mullet, Mullus barbatus L. 1758, is a demersal species that inhabits sandy and muddy bottoms of the continental shelf of the Mediterranean Sea, including the Black Sea, and also the eastern Atlantic from Scandinavia to Senegal (Fischer et al., 1987). This species is an important component of the Mediterranean demersal resources exploited by bottom trawling and small-scale fisheries (Fischer et al., 1987; Tserpes et al., 2002). The stocks of M. barbatus are highly vulnerable to recruitment changes (Tserpes et al., 2002), and all stocks in the Mediterranean Sea are currently considered overexploited (FAO, 2012). Despite the importance of energy reserves in estimating the productivity of exploited populations, the lipid content of M. barbatus has rarely been considered in the context of fishery ecology (Lloret et al., 2005, 2007; Roncarati et al., 2012). The available knowledge on the reproductive biology of M. barbatus is also very scarce and based mainly on macroscopic observations or simple morphometric indices (Desbrosses, 1935). There have been only a few attempts to estimate its fecundity (Tirasin et al., 2007; Aydın & Karadurmuş, 2013) and a single histological study describing the different stages of oocyte development (Kokokiris et al., 2014). Therefore, little information exists on the spawning strategy or the oocyte recruitment pattern. The metazoan parasites of M. barbatus have, in contrast, been widely reported, mostly in studies aimed at identifying a single species or group of parasites (e.g. Martínez-Vicaría et al., 2000; Ramdane et al., 2013) but also in the reporting of a list of the metazoan parasite fauna of this species (Carreras-Aubets et al., 2011, 2012).

The main purpose of this study was to assess the relationships between reproduction and two important parameters of fish health (i.e. parasitism and energy reserves) with regard to M. barbatus in the western Mediterranean Sea. By considering these relationships, this study also sheds light on several essential but undocumented aspects of the reproduction of this species, such as the oocyte recruitment pattern or the spawning strategy. The outcomes are discussed from a biological and ecological perspective in order to provide useful information for improving stock assessment and management of this economically valuable species.

Materials and methods

Fish sampling

A total of 335 mature female specimens of M. barbatus were collected from January 2010 to May
2012, on a quarterly basis in order to obtain repre-
sentatives from each ovarian developmental phase. 
However, most of the sampling events (89%) were 
concentrated in the second quarter, i.e. the spawn-
ing period of the species, because this is when the 
reproductive capacity of females (fecundity and egg 
quality) can be measured. Moreover, as the females 
presumably need to allocate more of their energy to 
egg production during this period, they should be more 
sensitive to an eventual effect of parasites on their 
overall condition. Specimens were caught via bottom 
trawls (from MEDITS trawl survey and commercial 
trawlers) at depths ranging from 30 to 250 m along the 
western coast of the Spanish Mediterranean Sea 
(FAO-GFCM geographical sub-area GSA-06, North-
ern Spain) (Fig. 1). For each individual, the total body 
length ($L_T$) was recorded (±0.1 cm), as well as total 
body mass ($M_T$), eviscerated body mass ($M_E$) and 
gonad mass ($M_G$) (±0.1 g). The total lengths of the 
specimens sampled ranged from 12.5 to 26.9 cm 
(mean ± SD = 19.5 ± 2.5). All the muscle tissue 
from the left side of each specimen and one ovary lobe 
were removed and frozen at −20°C for subsequent 
lipid content determination, while the second ovary 
lobe was fixed in 4% buffered formaldehyde for 
histological processing and subsequent fecundity 
estimation.

Parasitism evaluation

All the specimens were examined for metazoan 
parasites prior to the removal of muscle and gonads 
for lipid content determination and histology. The 
entire viscera were removed from the body cavity, and 
the gills and internal organs (heart, oesophagus, 
stomach, intestine, spleen, gallbladder, liver and 
gonads) were examined using a stereomicroscope. 
The macroparasites were collected and washed with a 
saline solution (0.8% NaCl). They were first observed 
avive and then fixed in permanent preparations. 
Cestodes, nematodes and isopods were preserved in 
70% ethanol, whereas digeneans were fixed in Bouin’s 
solution under slight coverslip pressure. If necessary 
and depending on the taxonomic group, the specimens 
were cleared in lactophenol or stained with borax 
carmine and mounted in Canada balsam. Parasites 
were morphologically identified to the lowest taxo-
nomic level possible following the available keys and 
descriptions, such as Gibson et al. (2002), Jones et al. 
(2005) and Bray et al. (2008) for digeneans or 
Naidenova & Nikolaeva (1968), Petter et al. (1984) 
and Petter & Maillard (1987, 1988) for nematodes. 
Since the identification of each species of parasite is 
based on adult features, some parasite larvae could not 
be identified to the species level. These included
Immature digeneans, L3 larvae of *Contracaecum* sp., Tetraphyllidean plerocercoids and the praniza larvae of the gnathiid isopods (*Gnathia* sp.).

As described by Bush et al. (1997), the prevalence of parasites (*P*) was calculated as the proportion of fish infected with a particular parasite species and the individual intensity of the infection as the number of individuals of a particular species in a single infected host. The mean intensity was calculated as the average number of parasites of a particular species found in the infected hosts. The median intensity and its 95% confidence interval (CI) were also calculated.

Energy reserves determination

A subsample of muscles (*n* = 156) and ovaries (*n* = 87) large enough to perform lipid extraction (minimum required = 4 g of tissue) was selected to assess the condition (indicated by energy reserves) of *M. barbatus*. Females whose gonads were large enough to perform lipid extraction appeared only in May and June (spawning season). In that season, corresponding to a sensitive critical period of the fish life cycle, i.e. reproduction, more analyses of muscular lipid reserves were performed in order to optimise the detection of potential effects of parasitism on the lipid reserves. Furthermore, one ovary lobe from each *M. barbatus* ovary was fixed and sliced transversely in its midsection. The resulting slices were embedded in paraffin, cut into 8–10 μm sections and stained with both haematoxylin-eosin and Mallory’s trichrome stains. The latter staining method highlights the zona radiata and its continuity and facilitates the detection of degenerating oocytes which will not be spawned, i.e. atretic oocytes (Muñoz et al., 2010). To describe the developmental stage of the oocytes, we used the terminology employed by Lowerre-Barbieri et al. (2011): primary growth (PG), cortical alveolar (CA), vitellogenesis (VIT1 and VIT2), maturation (OM), hydrated oocytes (H) and atresia. On the other hand, we used the presence of specific histological markers to classify our specimens into four ovarian developmental phases, following Brown-Peterson et al. (2011): regenerating (REG)—sexually mature but reproductively inactive; developing (DEV)—gametes begin to develop; spawning capable (SC)—advanced, developed gametes ready for the spawning season and regressing (REGR)—massive atresia which indicates the end of the reproductive cycle.

The gonado-somatic index (*I*<sub>G</sub>), which is the relation between the gonad mass (*M*<sub>G</sub>) and the eviscerated mass (*M*<sub>E</sub>) of the females, was calculated as *I*<sub>G</sub> = 100 *M*<sub>G</sub> / *M*<sub>E</sub>. The mass of ovaries increases with vitellogenesis and hydration of eggs, thus this index provides information on the fish reproductive cycle. Furthermore, the prevalence of atresia (*P*<sub>A</sub>) was defined as the proportion of females with observed *α*-atretic oocytes, and the relative intensity of atresia (*I*<sub>A</sub>) was calculated for each female as the number of *α*-atretic oocytes divided by the total number of vitellogenic oocytes (α-atretic and normal). Three different fields from four different histological slides of each specimen were analysed.

Fecundity and egg quality estimations

Ovaries containing hydrated oocytes, but which did not have recent post-ovulatory follicles (POFs), were selected to assess the fecundity (*n* = 89). Slices from the central area of the ovaries were weighed.
dry mass of oocytes (M), and the oocytes were separated using a washing process, as described by Lowerre-Barbieri & Barbieri (1993), and sorted by size through several sieves (from 400 to 600 μm). The oocytes were counted, and their diameters were measured using a computer-aided image analysis system (Image-Pro® Plus 5.1, Media Cybernetics, Inc., Bethesda, MD, USA).

In addition to obtaining the oocyte diameter–frequency distributions from the above procedure, we wanted to investigate this species’ spawning pattern (total spawner vs. batch spawner) and its oocyte recruitment pattern (synchronous vs. asynchronous oocyte development and determinate vs. indeterminate fecundity). To this end, 10 ovaries, identified as being from specimens in the spawning capable phase in the previous histological analysis, were randomly selected and used to measure the mean diameter of oocytes in the different stages of development. Oocytes in primary growth (PG), cortical alveolar (CA), vitellogenesis (VIT1 and VIT2) and maturation (OM) stages (n = 379) were measured individually in histological sections, whereas the hydrated oocytes (H) (n = 247) were measured using the aforementioned image analysis system, since they usually show an irregular shape in the histological preparations.

Investigation of the spawning pattern showed that M. barbatus is a batch spawner. Thus, in order to assess the reproductive capacity, we also estimated the batch fecundity (FB), defined as the number of eggs spawned per batch, and the relative batch fecundity (Frel), defined as the value of batch fecundity per gram of eviscerated female body mass.

Finally, the dry mass of hydrated oocytes was used as an indicator of egg quality. Samples of 200 oocytes in late maturation were removed from the ovaries mentioned above (n = 89) and weighed (±0.1 mg) after drying for 24 h at 110°C. The mean dry mass of oocytes (MDO) was calculated by dividing the sample’s dry mass by the number of oocytes in the sample.

Data analysis

The aggregated nature of parasite distributions leads to the concentration of a high proportion of parasites in a few host individuals. As argued by Rózsa et al. (2000), it is useful to report the confidence interval (CI) for the median intensity of infection. For this reason, a 95% CI was calculated, by the BCa method with 2000 bootstrap replications, using the free Quantitative Parasitology 3.0 software (Reiczigel & Rózsa, 2005). This software, which was developed to manage the particularly left-biased frequency distribution of parasites, was also used to compare the prevalences (Fisher’s exact test) and the median intensities (Mood’s median test) for each parasite species through the different ovarian developmental phases of the hosts. A false discovery rate (FDR) approach was used to counteract the problem of multiple comparisons (Benjamini & Hochberg, 1995; Verhoeven et al., 2005).

A general linear model (GLM) was used to determine the effects of total body length (LT), year, latitude and depth of capture, on the condition and reproduction response variables, i.e. lipid musculo-somatic index (ILM), lipid gonado-somatic index (ILG), relative batch fecundity (Frel) and the dry mass of oocytes (MDO). In order to satisfy normality assumptions, ILM was natural-log-transformed. Explanatory variables were removed by backward elimination and only those which contributed significantly to the model were kept. As shown in Table 1, some of the explanatory variables had significant effects on ILM, ILG and Frel although no effect on MDO was found. To eliminate this influence and focus on the effects of parasitism, all subsequent analyses were performed with the adjusted values of these response variables.

Once the normality of the data was tested through the different ovarian developmental phases and rejected, several non-parametric tests were performed to assess the possible effects of parasitism on M. barbatus condition (adjusted ILM and adjusted ILG) and reproduction (adjusted Frel and MDO). The Mann–Whitney U test was used to analyse possible differences between infected and uninfected M. barbatus specimens. The Spearman’s Rank Correlation coefficient was used to assess the possible relationships between the condition and reproduction parameters and the individual intensity of infection by parasites. These two analyses were performed for the specialist parasites, as well as for the generalist parasites provided the latter appeared in at least 10 specimens, i.e. with a prevalence of infection above 3% (Table 2). The level of statistical significance adopted was P < 0.05.
Results

Of the 335 specimens of *M. barbatus* examined, 276 (82.4%) were infected with at least one metazoan parasite species, with an individual intensity of infection that ranged from 1 to 81 parasites (mean ± SD = 8.38 ± 10.41). A total of 2,313 parasites belonging to at least 13 taxonomic groups were identified: six digeneans, five nematodes, one cestode and one isopod (Table 2). The real number of different species might be higher than reported as the groups of larvae classified as “immature digeneans”, *Contra-caecum* sp., *Tetraphyllidean plerocercoids* and *Gnathia* sp. may include several species. The most prevalent species was the nematode *Hysterothylacium fabri* (Rudolphi 1819), with a prevalence of 63.9% and a mean intensity of 3.95 ± 5.94 parasites. The species with the highest intensity of infection was the digenean *Opecoeloides furcatus* (Bremser in Rudolphi 1819), with a prevalence of 55.5% and a mean intensity of 6.97 ± 8.66 parasites (Table 2). The Fisher’s exact test showed differences in the prevalence of *O. furcatus* through the different ovarian developmental phases, being significantly higher (*P* = 0.002) in specimens in spawning capable phase (*P* = 61.8%) than in specimens in regenerating phase (*P* = 22.2%). No significant differences during different ovarian developmental phases were found in the prevalences of the other parasites. Neither were there any significant differences among the median intensities of any parasite species during the different ovarian developmental phases (Mood’s median test, *P* > 0.05).

In relation to condition, the *I*<sub>LM</sub> values of the 156 specimens analysed for lipids in the muscle ranged from 0.21 to 3.78 (mean ± SD = 1.03 ± 0.63), whereas the *I*<sub>LG</sub> values of the 87 specimens analysed for lipids in the ovaries ranged from 0.25 to 1.40 (mean ± SD = 0.93 ± 1.83). A Spearman’s rank correlation test revealed a positive correlation (*r*<sub>s</sub> = 0.31, *n* = 87, *P* = 0.004) between these two variables. All the specimens with ovaries large enough to perform the lipid extraction were in spawning capable phase. The *I*<sub>LM</sub> was analysed through the different ovarian developmental phases (Fig. 2), and a Kruskal–Wallis Test revealed that the distribution of *I*<sub>LM</sub> was the same during each ovarian developmental phases.

The histological analyses of gonads showed that *M. barbatus* maintained a reserve of primary growth (PG) oocytes, which is the first stage of oocyte growth and covers the chromatin nucleolar and the perinucleolar stages. The secondary growth of oocytes begins with the cortical alveolar (CA) stage, which was identified
Table 2 Taxonomic composition, number of infected hosts, prevalence \( (P_P) \) and intensity of metazoan parasites found in *Mullus barbatus* from the western Mediterranean Sea

| Parasite species                      | Stage | Site | Ovarian developmental phase | Infected hosts | \( P_P \) \( (n = 335) \) | Intensity |
|---------------------------------------|-------|------|-------------------------------|----------------|---------------------------|-----------|
|                                       |       |      |                               |                | Mean ± SD               | Median 95 % CI |
| Digenea                               |       |      |                               |                | Min–Max                  |           |
| *Derogenes latus* \( \text{Janiszewska, 1953} \) | A     | D    | *DEV, SC*                     | 6              | 1.79 (1–7)               | 2.50 ± 2.51 | 1.00–4.50 |
| *Lasiotocus mulli* \( \text{Stossich, 1883} \) \( a \) | A     | D    | *REG, REGR*                   | 2              | 0.60 (1–4)               | 2.50 ± 2.12 | 1.00–2.50 |
| *Lecithocladium excisum* \( \text{Rudolphi, 1819} \) \( \text{Lühe, 1901} \) | A     | D    | *DEV, SC*                     | 2              | 0.60 (2–3)               | 2.50 ± 0.71 | 2.00–3.00 |
| *Opecoeloides furcatus* \( \text{Bremser in Rudolphi, 1819} \) \( a \) | A     | D    | *REG, DEV, SC, REGR*          | 186            | 55.52 (1–53)             | 6.97 ± 8.66 | 5.88–8.41 |
| *Proctotrema bacilliovatum* \( \text{Odhner, 1911} \) \( f \) | A     | D    | *DEV, SC, REGR*               | 4              | 1.19 (1–7)               | 3.25 ± 2.63 | 1.50–5.75 |
| Unidentified immature specimens       | L     | D    | *SC, REGR*                    | 6              | 1.79 (1–15)              | 3.50 ± 5.65 | 1.00–10.33 |
| Nematoda                              |       |      |                               |                |                          |           |
| *Ascarophis mullusi* \( \text{Naidenova & Nikolaeva, 1968} \) \( a \) | A     | D    | *DEV, SC, REGR*               | 4              | 1.19 (1–5)               | 2.25 ± 1.89 | 1.00–4.00 |
| *Contracaecum* sp.                    |       |      |                               |                |                          |           |
| *Contracaecum* sp.                    |       |      |                               |                |                          |           |
| *Cucullanus longicollis* \( \text{Stossich, 1899} \) | A     | D    | *SC, REGR*                    | 13             | 3.88 (1–6)               | 1.85 ± 1.41 | 1.31–2.85 |
| *Hysterohylacium aduncum* \( \text{Rudolphi, 1802} \) | L3    | D, M | *SC, REGR*                    | 29             | 8.66 (1–4)               | 1.55 ± 0.78 | 1.28–1.83 |
| *Hysterohylacium fabri* \( \text{Rudolphi, 1819} \) | L3, L4 | A, D, M | *REG, DEV, SC, REGR*         | 214            | 63.88 (1–42)             | 3.95 ± 5.94 | 3.28–4.81 |
| Cestoda                               |       |      |                               |                |                          |           |
| Tetraphyllidean plerocercoids         | P     | D    | *REG, DEV, SC*               | 6              | 1.79 (1–3)               | 1.50 ± 0.84 | 1.00–2.17 |
| Isopoda                              |       |      |                               |                |                          |           |
| *Gnathia* sp. \( \text{praniza larvae} \) | L     | G    | *SC, REGR*                    | 9              | 2.69 (1–7)               | 2.67 ± 1.94 | 1.67–4.11 |

The parasite developmental stage, the site of infection and the ovarian developmental phase of hosts are also shown:

- \( n \) sample size.
- Stage: \( A \) adult, \( L \) immature larvae, \( L3 \) third-stage larvae, \( L4 \) fourth-stage larvae, \( P \) plerocercoid larvae.
- Site: \( D \) digestive tract, \( G \) gills, \( L \) liver, \( M \) mesenteries.
- Ovarian developmental phase of hosts: \( \text{REG} \) regenerating, \( \text{DEV} \) developing, \( \text{SC} \) spawning capable, \( \text{REGR} \) regressing.

\( a \) Indicates a specialist parasite of mullets (*Mullus* spp.)
by the formation of the chorion and the presence of small oil droplets and cortical alveoli in the periphery of the cytoplasm. The development of oocytes progressed through several substages of vitellogenesis (VIT1 and VIT2) and characterised by the presence of yolk globules and large oil droplets. The oocyte maturation (OM) stage was recognised by the fusion of the yolk material, the formation of a single large oil droplet and the migration of the germinal vesicle towards the plasma membrane. Finally, the last maturing event was the hydration (H) substage which is an uptake of fluid by the oocyte that leads to ovulation. As oocytes developed, their size increased gradually, with each stage sharing overlapping diameter ranges with the previous and subsequent stages, except in the case of the hydrated oocytes which had much larger diameters (Fig. 3).

The gonado-somatic index ($I_G$) presented a clear seasonal pattern. The highest values were observed in the second quarter, with the highest monthly means in
May (11.23 ± 3.62) and June (10.97 ± 2.03), while for the rest of the year, the \( I_G \) values remained low, with the lowest monthly mean in October (0.88 ± 0.24). Indeed, the histological examination of the ovaries showed that, overall, females spent the first and fourth quarter in either the regenerating phase (with only PG oocytes present) or the developing phase (with new CA and VIT1 oocytes present) (Fig. 4). All the females in the spawning capable phase (with PG, CA, VIT1, VIT2, OM and H oocytes present) appeared in the second and third quarter. Females in the regressing phase, which showed massive atresia, were captured in the second quarter, i.e. throughout the spawning season. Ovaries with atretic oocytes were not detected among the individuals in either the regenerating or the developing phases, but females in the spawning capable phase (\( P_A = 11.61\% \)) presented a moderate relative intensity of atresia (\( I_A = 12.15 ± 10.09\% \)) and, without exception, all the individuals in the regressing phase (\( P_A = 100\% \)) showed high levels of atresia (75% < \( I_A \leq 100\% \)).

Most females in the spawning capable phase showed a bimodal oocyte diameter–frequency distribution with a first component (smaller diameters) containing oocytes in different stages of development, with a gap at around 550 µm, and a separate second component (larger diameters) containing only oocytes in advanced stage of maturation (Fig. 5). This type of oocyte size distribution is prevalent in batch spawner species; therefore, those oocytes that were above 550 µm in size were considered as belonging to the next batch. The batch fecundity (\( F_B \)) ranged from 2,408 to 43,736 eggs (mean ± SD = 18,163 ± 9,778) and was positively related to the size of \( M. \)
The total length–batch fecundity points fitted a power function regression with the following equation $F_B = 0.33 \times L^{3.627}$ ($R^2 = 0.75$, $n = 89$, $P < 0.0005$), while the eviscerated mass–batch fecundity points fitted better to a linear regression, $F_B = 272.3 \times M_E - 2554.9$ ($R^2 = 0.76$, $n = 89$, $P < 0.0005$). The $F_{\text{BFrel}}$ ranged from 61 to 371 eggs g$^{-1}$ (eviscerated mass) (mean ± SD = 234 ± 63). Finally, the $M_{\text{DO}}$ ranged from 0.007 to 0.016 mg (mean ± SD = 0.012 ± 0.002) and did not present any correlation with the analysed variables.

No significant effect of parasitism was found on either the condition or the reproduction of red mullet in regenerating, developing or regressing phases. However, fish in spawning capable phase showed significant differences in their condition and reproduction that were related to parasitism variables (Table 3). Individuals that were infected by the

### Table 3

Results of Mann–Whitney $U$ test used to verify the existence of differences between infected and uninfected *Mullus barbatus* and Spearman’s rank correlation coefficient ($r_s$) used to evaluate possible relationships among the condition and reproduction variables and the individual intensity of parasitism

| Ovarian developmental phase $(n)$ | Parasite Variable | Mann–Whitney $U$ test | Spearman’s rank correlation |
|----------------------------------|------------------|-----------------------|-----------------------------|
|                                  |                  | $n$ Uninf. | $n$ Inf. | $Md$ Uninf. | $Md$ Inf. | $U$ | $p^a$ | $n$ | $r_s$ | $p$ |
| Spawning capable (267)           | *Opecoeloides forcatus* | $I_{LM}$ | 39 | 84 | −0.116 | −0.169 | 1126 | 0.005 | 123 | −0.278 | 0.002 |
|                                  | *Hysterothylacium aduncum* | $B_{\text{BFrel}}$ | 82 | 7 | 232 | 250 | 155 | 0.044 | 89 | 0.211 | 0.047 |
|                                  | *Hysterothylacium fabri* | $B_{\text{BFrel}}$ | 28 | 61 | 228 | 238 | 569 | 0.012 | 89 | 0.300 | 0.004 |
|                                  | *Hysterothylacium fabri* | $M_{\text{DO}}$ | 28 | 61 | 0.013 | 0.011 | 546 | 0.006 | – | – | – |

Only significant results ($P < 0.05$) are presented

$n$ subsample size, *Uninf.* uninfected fish, *Inf.* infected fish, *Md* variable median. Variables: $I_{LM}$ adjusted log-transformed lipid musculo-somatic index, $B_{\text{BFrel}}$ adjusted relative batch fecundity, $M_{\text{DO}}$ dry mass of oocytes

*a* Assymptotic significances (2-tailed) are displayed for Mann–Whitney $U$ tests with sample size above 10 in all groups, otherwise, exact significances [2 × (1-tailed Sig.)] are given
digenean *Opecoeloides furcatus* displayed lower median values of $I_{LM}$ compared with uninfected specimens (Mann–Whitney $U$ test, Table 3). There was also a negative correlation between the individual intensity of infection by this parasite and the individual value of $I_{LM}$ (Spearman’s rank correlation, Table 3). With regard to the reproduction variables, specimens infected by the nematode *Hysterothylacium aduncum* (Rudolphi, 1802) showed a higher median value of $F_{Bret}$ and a positive correlation between the individual intensity of infection by this nematode and the individual value of $F_{Bret}$. Similar relationships were found between $F_{Bret}$ and infection by *Hysterothylacium fabri*, i.e. individuals infected by this nematode displayed higher values of $F_{Bret}$. However, the individuals infected by *H. fabri* showed lower values of $M_{DO}$ than the uninfected specimens. We found no significant effects caused by the rest of the parasites that were analysed, i.e. *Lasiotocus mulli* (Stossich, 1883), *Proctotrema bacilliovatum* (Odhner, 1911), *Ascarophis mullusi* (Naidenova & Nikolaeva, 1968) and *Cucullanus longicollis* (Stossich, 1899).

**Discussion**

This study provides important new data on aspects of health and reproduction of red mullet, an exploited marine species, with particular focus on the links between parasitism, condition and reproductive capacity. We found that red mullet is a batch spawner with an income breeding strategy, an asynchronous development of oocytes and indeterminate fecundity. We also found that the parasites, *Opecoeloides furcatus* and *Hysterothylacium* spp., affect the condition and reproduction of this species.

The results of the histological observation of ovaries coupled with the gonado-somatic index analysis show that the spawning period of *M. barbatus* collected in the western Mediterranean Sea takes place in spring–summer, with a peak of spawning activity in May and June, which coincides with data provided by Tsiklaris et al. (2010). The fact that females in the regressing phase appeared throughout the spawning season may indicate that, although the spawning season for the species extends for several months, the individual spawning period might be much shorter.

The oocyte diameter–frequency distributions showed, for the first time, an asynchronous development of oocytes and that *M. barbatus* is a batch spawner. The stage-specific oocyte diameter showed a gradual increase and a slight overlapping between the diameters of cortical alveolar and vitellogenic oocytes. The absence of a hiatus separating the diameter of yolked oocytes from the one of unyolked oocytes indicates an indeterminate fecundity, i.e. the annual fecundity is not fixed before the onset of spawning and unyolked oocytes continue to mature and to be spawned during the spawning season (Murua et al., 2003). Despite the lack of published data on the oocyte recruitment pattern of this species, Anastasopoulou & Saborido-Rey (2011) showed that the recruitment of oocytes from primary growth to secondary growth stages continues throughout an individual’s spawning period, indicating an indeterminate strategy which is consistent with our observations. In addition, the high levels of atresia observed in the regressing phase of our specimens also indicated an indeterminate fecundity, since females have to resorb the over production of secondary growth oocytes (Murua & Saborido-Rey, 2003; Murua & Motos, 2006).

The results of this study indicate that the total length and the eviscerated mass of *M. barbatus* originating from the western Mediterranean Sea are good predictors of its batch fecundity, as also reported in Turkish waters, such as the Aegean and Levantine Sea (Tirasin et al., 2007) or the eastern Black Sea (Aydın & Karadurmuş, 2013). It was observed that the number of eggs increases as a power of the length of the fish and linearly with its mass. It can therefore be assumed that large specimens have a higher reproductive capacity than smaller ones. This result provides further support for the hypothesis that, for many fishes, the removal of large individuals can adversely affect the reproductive potential of species (Birkeland & Dayton, 2005; Lloret et al., 2012b). Concerning egg quality, as measured by the hydrated oocyte dry mass, no previous studies were found with which to compare the results; thus, we consider that future studies should take this variable into account.

The results of this study concur with the comprehensive study by Carreras-Aubets et al. (2012) and indicate that although *M. barbatus* can harbour a wide range of helminths, the most prevalent and abundant parasites are the digenean *Opecoeloides furcatus* and
the nematode *Hysterothylacium fabri*. Our analysis of
the parasitism throughout the different ovarian develop-
mental phases of the host showed that there were no
changes in the intensities of infection of any of the
parasites through the reproductive cycle (although
differences were found in the prevalence of *O. furcatus*,
which was higher in specimens in the spawning capable
phase compared to other phases would indicate that
red mullet continues to feed during reproduction since,
in most cases, fish become infected when actively
feeding. If this is so, it would imply an income
breeding strategy. In relation to this, while it is true
that some authors found that *M. barbatus* curtails
feeding during the spawning season in spring-summer
(Vassilopoulou & Papaconstantinou, 1993; Chérif
feeding during the spawning season in spring-summer
that some authors found that
*M. barbatus* actually feeds intensely during the reproductive period
(Bizsel, 1987).

The results of this study show that there is no
decrease in the lipids stored in the muscle as the
gonads develop, indicating that reproduction is fuelled
by direct food intake. Moreover, the positive correla-
tion found between lipid content in muscle and in the
gonads reinforces the hypothesis that *M. barbatus*
does not mobilise lipids from the muscle to the gonads.
Similarly, Lloret et al. (2007) analysed the total lipid
content in muscle and gonads of pre-spawning females
of *M. barbatus* and also found that, although *M. barbatus*
allocates lipid reserves to the gonad during
the reproductive period, there was no depletion of the
muscle lipids. These results provide further evidence
that *M. barbatus* is an income breeder rather than a
capital breeder.

The evidence that *M. barbatus* continues feeding
throughout the spawning season coupled with their
income breeding strategy is consistent with their
indeterminate fecundity. That is to say, the number of
eggs is not fixed before the onset of the spawning
season but, instead, is adjusted to food intake, without
reliance on stored energy (Rijnsdorp & Witthames,
2005). However, it should be noted that some authors
have pointed out that the boundaries between capital/
income breeding and determinate/indeterminate fe-
cundity are not as clear as had previously been
assumed (Saborido-Rey et al., 2010; Ganias, 2013).

For example, Domínguez-Petit et al. (2010) argued
that for the European hake, *Merluccius merluccius*, in
the northeastern Atlantic, gonadal development was at
the expense of food intake, whereas Ferrer-Maza et al.
(2014) have shown that, in the northwestern Medi-
erranean, this species is a capital breeder most of the
year except in summer, when the abundance of its prey
is at its peak in the Mediterranean. Thus, spawning
strategies may be flexible within species and may
depend on the availability of food. There is a well-
documented distinction between capital breeders and
income breeders, but there are also species that use a
variety of mixed-breeding strategies (Houston et al.,
2006; McBride et al., 2013). It seems probable that
some species of fish may be capable of adopting either
a capital or income strategy or a blend of both in
response to internal or external conditions.

The study of the relationships between parasitism,
condition and reproduction of fish has highlighted that
the specimens of *M. barbatus* in the spawning capable
phase infected by the digenean *Opecoeloides furcatus*
showed a lower lipid musculo-somatic index than
uninfected specimens. *O. furcatus*, like most dige-
neans, is a parasite of the digestive tract where it feeds
on mucus, epithelial cells and probably some of the
host’s gut content. This result might indicate a
negative effect of *O. furcatus* on the energy reserves
of *M. barbatus*, especially in the spawning season
when fish are dividing their energy consumption
between maintaining fitness and reproduction. With
regard to the latter, parasitism seems to affect repro-
duction by causing quantitative and qualitative
changes in the spawning. Although specimens infect-
ed by the nematodes *Hysterothylacium aduncum* and
*H. fabri* showed higher fecundity than uninfected ones,
it was found—at least in the case of those
infected by *H. fabri*—that there was a lower mass of
hydrated oocytes. Accordingly, it can be hypothesised
that nematodes belonging to the genus *Hysterothy-
lacium* cause a reduction in the quality of the eggs, but
in response, female *M. barbatus* tries to compensate
for this by producing a higher number of eggs.

As mentioned in the introduction, a limited number
of studies have dealt with the effects of parasitism on
the energy reserves or the reproduction of marine
species. Fogelman et al. (2009) found that female five-
lined cardinalfish, *Cheilodipterus quinquelineatus*
Cuvier 1828, infected by the isopod *Anilocra*
apogonae Bruce 1987, had fewer and smaller eggs than uninfected fish. Sasal et al. (2001) also found that the females of the gobid fish Gobius bucichhii Steindachner 1870, infected by the acanthocephalan Acanthocephaloides propinquus Dujardin 1845, showed a reduction in egg production. With regard to exploited fish, there are contradictory results about the effects of nematodes on energy reserves. For example, while Shchepkina (1980) found that the anchovy, Engraulis encrasicolus, infected by Contracaecum sp. showed lower lipid content, Sajiki et al. (1992) found that E. japonicus infected by Anisakis sp. did not experience a depletion in the total lipid content, but there was an increase in the free fatty acids in their viscera. Finally, Ferrer-Maza et al. (2014) have recently revealed that the anisakid nematodes, especially Anisakis pegreffii Campana-Rouget & Biocca 1955, negatively affect the condition of the European hake, Merluccius merluccius, mainly when these fish are allocating their energy reserves to gonadal development, while infections by other parasites do not affect them significantly.

Along with those of previous studies, our results have demonstrated that parasitism, condition and reproduction are closely linked. The physical health of exploited stocks, which depends on the health and reproductive capacity of the individuals, is an essential element of sustainable and profitable fisheries (Lloret et al., 2012a). Therefore, the results of this study, especially those concerning the effects of parasites on condition and reproduction, may have an important impact on the development of management strategies and research involving other species, whether wild or captive-bred.

In summary, the present study not only provides basic knowledge on the reproduction, condition and parasitism of Mullus barbatus in the western Mediterranean Sea, but also some important implications of their relationships. The results lead us to suggest that M. barbatus should be classified as an iteroparous batch spawner species with an income breeding strategy, an asynchronus development of oocytes and indeterminate fecundity. This study also showed that larger females have a higher fecundity, confirming that older spawners contribute heavily to reproduction. We have also concluded that although M. barbatus can harbour a wide range of helminths, only the most abundant and prevalent ones (i.e. the digenean Hysterothylacium spp. and the nematodes Hysterothylacium spp.) significantly affect the condition and reproduction of M. barbatus during the spawning season. Furthermore, the effects are complex: the digenean O. furcatus causes a reduction in the energy reserves of fish, whereas infection by the nematodes Hysterothylacium spp. results in a rise in egg production but impaired egg quality. This shows that the relationships between parasitism, condition and reproduction are not always evident in income breeder fish. We consider, therefore, that further research regarding the role of the energy reserves and the nutrient acquisition would be of great help in understanding the energy cost of reproduction and parasitism.

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References

Adams, S. M., 1999. Ecological role of lipids in the health and success of fish populations. In Arts, M. T. & B. C. Wainmann (eds), Lipids in Freshwater Ecosystems. Springer, New York: 132–153.

Alonso-Fernández, A., A. C. Vallejo, F. Saborido-Rey, H. Murua & E. A. Trippel, 2009. Fecundity estimation of Atlantic cod (Gadus morhua) and haddock (Melanogrammus aeglefinus) of Georges Bank: application of the autodiametric method. Fisheries Research 99: 47–54.

Anastasopoulos, A. & F. Saborido-Rey, 2011. Reproductive ecology of Mullus barbatus in eastern Mediterranean Sea. In Book of Abstracts of the Fish Reproduction and Fisheries (FRESH) Final Conference, 16–20 May 2011, Vigo. http://freshcost.quadralia.net/fresh-final-conference/.

Aydın, M. & U. Karadurmus, 2013. An investigation on age, growth and biological characteristics of red mullet (Mullus barbatus ponticus, Essipov, 1927) in the Eastern Black Sea. Iranian Journal of Fisheries Sciences 12: 277–288.

Bagamian, K. H., D. C. Heins & J. A. Baker, 2004. Body condition and reproductive capacity of three-spined
stickles (Mullus barbatus) respond to small-scale variation in the feeding ground. Marine Biology Research 10: 241–250.

Jones, A., R. A. Bray & D. I. Gibson, 2005. Keys to the Mediterranean. FAO, Rome, 768 pp.

Khan, R. A. & D. Lacey, 1986. Effect of concurrent infections of Lernaeocera Branchialis (Copepod) and Trypanosoma murmanensis (Protozoa) on Atlantic cod, Gadus morhua. Journal of Wildlife Diseases 22: 201–208.

Kokokiris, L., A. Stamoulis, N. Monokroussos & S. Doukeraki, 2014. Oocytes development, maturity classification, maturity size and spawning season of the red mullet (Mullus barbatus barbatus) Linnaeus, 1758. Journal of Applied Ichthyology 30: 20–27.

Lloret, J., M. Demestre & J. Sánchez-Pardo, 2007. Lipid reserves of red mullet (Mullus barbatus) during pre-spawning in the northwestern Mediterranean. Scientia Marina 71: 269–277.

Lloret, J., E. Faliex, G. E. Shulman, J. A. Raga, P. Sasal, M. Muñoz, M. Casadevall, A. E. Ahuir-Baraja, F. E. Montero, A. Repullés-Albelda, M. Cardinale, H. J. Rätz, S. Vila & D. Ferrer-Maza, 2012a. Fish health and fisheries, implications for stock assessment and management: the Mediterranean example. Reviews in Fisheries Science 20: 165–180.
Lloret, J., R. Galzin, L. Gil de Sola, A. Souplet & M. Demestre, 2005. Habitat related differences in lipid reserves of some exploited fish species in the north-western Mediterranean continental shelf. Journal of Fish Biology 67: 51–65.

Lloret, J., M. Muñoz & M. Casadevall, 2012b. Threats posed by artisanal fisheries to the reproduction of coastal fish species in a Mediterranean marine protected area. Estuarine, Coastal and Shelf Science 113: 133–140.

Lloret, J., G. Shulman & R. M. Love, 2014. Condition and Health Indicators of Exploited Marine Fishes. Wiley Blackwell, Oxford, 262 pp.

Lowerre-Barbieri, S. K. & L. R. Barbieri, 1993. A new method of oocyte separation and preservation for fish reproduction studies. Fishery Bulletin 91: 165–170.

Lowerre-Barbieri, S. K., N. J. Brown-Peterson, H. Murua, J. Tomkiewicz, D. M. Wyanski & F. Saborido-Rey, 2011. Emerging issues and methodological advances in fisheries reproductive biology. Marine and Coastal Fisheries 3: 32–51.

Martínez-Vicaría, A., J. Martín-Sánchez, P. Illescas, A. M. Lara, M. Jiménez-Albarrán & A. Valero, 2000. The occurrence of two opecoelid digeneans in Mullus barbatus and M. surmuleius from the Spanish south-eastern Mediterranean. Journal of Helminthology 74: 161–164.

McBride, R. S., S. Somarakis, G. R. Fitzhugh, A. Albert, N. A. Yaragina, M. J. Wuenschel, A. Alonso-Fernández & G. Baslione, 2013. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries. doi:10.1111/faf.12043.

Muñoz, M., C. Dimitriadis, M. Casadevall, S. Vila, E. Delgado, J. Lloret & F. Saborido-Rey, 2010. Female reproductive biology of the bluemouth Helicolenus dactylopterus dactylopterus: spawning and fecundity. Journal of Fish Biology 77: 2423–2442.

Murua, H. & L. Motos, 2006. Reproductive strategy and spawning activity of the European hake Merluccius merluccius (L.) in the Bay of Biscay. Journal of Fish Biology 69: 1288–1303.

Murua, H. & F. Saborido-Rey, 2003. Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fishery Science 33: 23–31.

Murua, H., G. Kraus, F. Saborido-Rey, P. R. Witthames, A. Thorsen & S. Junquera, 2003. Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science 33: 33–54.

Naidenova, N. N. & V. M. Nikolaeva, 1968. Nematode fauna of some benthic fish of the Mediterranean Basin. Biologiya Morya 14: 63–82.

Ondracková, M., K. Francová, M. Dávidová, M. Polacik & P. Jurajda, 2010. Condition status and parasite infection of Neogobius kessleri and N. melanostomus (Gobiidae) in their native and non-native area of distribution of the Danube River. Ecological Research 25: 857–866.

Petter, A. J., C. Lèbre & B. M. Radukovic, 1984. Nematode parasites of oesteichthyen fish of the southern Adriatic. Acta Adriatica 25: 205–221.

Petter, A. J. & C. Maillard, 1987. Ascarids of fishes from Western Mediterranean sea. Bulletin du Muséum National d’Histoire Naturelle. Section A, Zoologie, Biologie et Écologie Animales 9: 773–798.

Petter, A. J. & C. Maillard, 1988. Larval Ascarids parasites of fishes from western Mediterranean sea. Bulletin du Muséum National d’Histoire Naturelle. Section A, Zoologie, Biologie et Écologie Animales Muséum national d’histoire naturelle 10: 347–369.

Ramdane, Z., J. P. Trilles, K. Mahé & R. Amara, 2013. Meta-zoa ectoparasites of two teleost fish, Boops boops (L.) and Mullus barbatus barbatus L. from Algerian coast: diversity, parasitological index and impact of parasitism. Cybium 37: 59–66.

Reiczigel, J. & L. Rózsa, 2005. Quantitative Parasitology 3.0. Budapest. Distributed by the authors. Available at http://www.zoologia.hu/wpq/wp.html.

Rijnsdorp, A. D. & P. R. Witthames, 2005. Ecology of reproduction. In Gibson, R. N. (ed.), Flatfishes: Biology and Exploitation. Fish and Aquatic Resources Series 9. Blackwell Science, Oxford: 68–93.

Roncarati, A., G. Brambilla, A. Meluzzi, A. L. Iamiceli, R. Fanelli, I. Moret, A. Ubaldi, R. Miniero, F. Sirri, P. Melotti & A. di Domenico, 2012. Fatty acid profile and proximate composition of fillets from Engraulis encrasicholus, Mullus barbatus, Merluccius merluccius and Sarda sarda caught in Tyrrenhenian, Adriatic and Ionian seas. Journal of Applied Ichthyology 28: 545–552.

Rózsa, L., J. Reiczigel & G. Majoros, 2000. Quantifying parasites in samples of hosts. Journal of Parasitology 86: 228–232.

Saborido-Rey, F., O. S. Kjesbu & A. Thorsen, 2003. Buoyancy of Atlantic cod larvae in relation to developmental stage and maternal influences. Journal of Plankton Research 25: 291–307.

Saborido-Rey, F., H. Murua, J. Tomkiewicz & S. Lowerre-Barbieri, 2010. Female reproductive strategies: an energetic balance between maturation, growth and egg production. In Wyanski, D. M. & N. J. Brown-Peterson (eds), Proceedings of the 4th Workshop on Gonadal Histology of Fishes, 16–19 June 2009, El Puerto de Santa María: 15–18. http://hdl.handle.net/10261/24937.

Sajiki, J., K. Takahashi, Y. Hayashi, Y. Ando, M. Kaneda & T. Hamazaki, 1992. Fatty acid composition in anchovy (Engraulis japonicus) infected with Anisakis simplex. Japanese Journal of Toxicology and Environmental Health 38: 361–365.

Sasal, P., E. Falici, I. De Buron & S. Morand, 2001. Sex discriminatory effect of the acanthocephalan Acanthocephaloides parvipinexus on a gobid fish Gobius bucchichii. Parasite 8: 231–236.

Shahidi, F., 2001. Extraction and measurement of total lipids. In Wrolstad, R. E. (ed.), Current Protocols in Food Analytical Chemistry. Wiley, New York: 1–11.

Shchejkina, A. M., 1980. Lipid composition of the tissues of Engraulis encrasicholus during its annual cycle and in infection with larvae of the nematode Contracaecum aduncum. Ekologiya Morya 3: 33–39.

Tirasin, E. M., A. Unluoglu & B. Cihangir, 2007. Fecundity of red mullet (Mullus barbatus L., 1758) along the Turkish coasts of the Mediterranean sea. Rapport Commission International pour l‘Exploration Scientifique de la Mer Méditerranée 38: 614.

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

Tsikiras, A. C., E. Antonopoulou & K. I. Stergiou, 2010. Spawning period of Mediterranean marine fishes. Reviews in Fish Biology and Fisheries 20: 499–538.

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

Verhoeven, K. J. F., K. L. Simonsen & L. M. McIntyre, 2005. Implementing false discovery rate control: increasing your power. Oikos 108: 643–647.