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Lipid reserves of striped red mullet (*Mullus surmuletus*, L., 1758) from North-west Moroccan Mediterranean coast

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Abstract

Total lipid content has been analyzed for the first time in gonads, liver and muscle of striped red mullet (*Mullus surmuletus* L., 1758) from the Moroccan Mediterranean Sea during maturing and pre-spawning stage. This present study was conducted in order to evaluate the lipid reserves and to determine the correlation with length and water content for total lipid content in tissues of striped red mullet. Results showed that lipid content in the muscle ranged from 2. 93 to 22.33% and demonstrated that muscle was the major energy storage site in this species. The absolute lipid content in muscle (mean= 0.66 g±0.67) was higher than that in liver (mean= 0.058g±0.07). Moreover, it was highlighted that during reproduction period, liver relative lipid content did not vary. Mean values for percentage of lipid reserve in ovary and liver for maturing females were 21.33 and 10.84% respectively whereas they were 28.22 and 8.05% respectively for pre-spawning females. These results emphasize that the lipid stored in the muscle during the maturing stage is mobilized toward the gonad during pre-spawing. Regression analysis showed that the percentage of lipid in gonad and muscle revealed a significant relationship with length. An inverse linear relationship was obtained between percentage of water content and lipid content of muscle and gonad.

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Introduction

Lipids are the major energy store in teleosts (Shulman and Love, 1999). They are stored in the liver, viscera, and muscles. The concentration of lipid varies considerably in different parts of the body of the fish (Love, 1970; Sheridan and Kao, 1998). Lipid content in the body of the fish changes also depending on the time of the year (Chellapa, 1988), environmental conditions (Gill and Weatherly, 1984), state of nutrition (Elliot, 1976), age (Parker and Vanstone, 1966) and stage of maturity of the gonads (Craig, 1977; Gill and Weatherly, 1987). Due to their large influence on growth, reproduction and survival, lipids have come to attention (Sutharshiny and Sivashanthini, 2011). As a matter of fact, they are a particularly important aspect of fish health and population success (Lloret *et al.,* 2008). Indeed inadequate reserves have been implicated in the reduced reproductive potential of several fish species through reduced fecundity and quality of eggs and larvae or delayed maturation (Koops *et al.,* 2004). Moreover, deficiency can negatively affect gonad development, fecundity, fertilization and hatching rates (Adams, 1999; Morris and Culkin, 2000). Furthermore, lipids are also an indicator of seasonal cycles of reproduction (Clay, 1988), because they are often mobilized during reproductive periods (Shulman and 1999).

The Striped red mullet (*Mullus surmuletus*, L.) is a major target species of Mediterranean demersal fisheries and is exploited by more than one gear type (Stergiou *et al.,*1992; Renones *et al.,* 1995; Demestre *et al.,* 1997; Mehanna, 2009). This species occurs along the coast of Europe from the south of Norway (Wheeler, 1978) and the North of Scotland (Gordon, 1981) to Dakar. It is also encountered in the Mediterranean and Black Seas. It is a carnivorous species and its diet comprises of a wide range of animals like small fishes, crustaceans, molluscs and polychaetes (Bozzano *et al.,* 1997; Carpentieri *et al.,* 2005; Cherif et *al.,* 2008; Bautfista-Veg *et al.,* 2008; El Bakali *et al.,* 2010b; Mukadder and Ismen, 2013). Females and males striped red mullet attain sexual maturity in the second year of their life (Recasens, 1992; Chérif *et al.,* 2007; El Bakali *et al.,* 2010a). While Hatice Torcu-Koc, 2015 reported that *Mullus surmuletus* spawned between April and September with a peak in July on the north western Aegean coast of Turkey, Kousteni *et al.,* 2019 showed that spawning activity took place from March to July in the south of the Aegean Sea (eastern Mediterranean). In general, spawning occurred between April and June (N'Da and Deniel, 2005; El Bakali *et al.,* 2010a). Although several aspects of striped red mullet's biology in the Mediterranean have been studied, information on the energy reserves of this species is still lacking.

In Morocco, Striped red mullet, which is distributed along the entire Moroccan Mediterranean coast, is among the most valuable and highly priced fish species. In spite of their ecological and economic importance in the region, the research on biology and ecophysiology of the fish are still insufficient (El Bakali *et al.,* 2010a-2010b; El Bakali *et al.,* 2015; El Bakali *et al.,* 2016).

The objective of this study was to analyze and quantify lipid reserves of striped red mullet during maturing and pre-spawning stage for the first time in Morocco (Mediterranean Sea), but also to find out the correlation with length and water content for total lipid content in different body tissues of *Mullus surmuletus*.

Materials and methods

Fish sample collection

The samples for this study were obtained from commercial trawl fishery landed in the port of M'diq (e.g. Fig.1). A total of 156 red mullet were sampled between February and May 2010 for fat content determination. For each individual, the total length in cm and the total weight in g were measured. The fish samples were dissected, sex and stage of sexual maturity were identified macroscopically. Individuals were the eviscerated and their livers and gonads were removed and weighed. Samples of muscle were collected from each fish and weighed.

The collected tissues were then cleaned by removing skin, bones and scales. They were rinsed with seawater and immediately frozen at -24°C (Lloret *et al.*, 2007). For the validation of the stages of sexual maturity, samples of gonads were examined

histologically. In this study, fish in maturing (96 individuals) and pre-spawning stage (60 individuals) were used for lipid analysis. Percentage of lipid content in their muscles, livers and gonads were analyzed as a measurement of energy reserves.

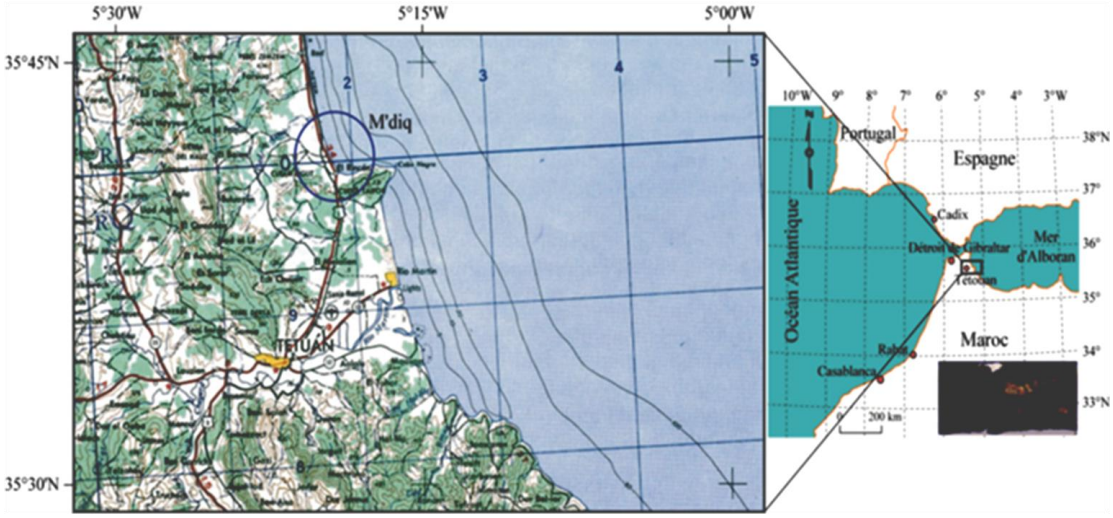

Fig. 1. Geographical situation of the study areas.

Determination of water content

After defrosting and drying on paper, tissues samples (liver, muscle and gonads) were cut into smaller pieces, placed in petri dishes and dried for 24 hours in a vacuum oven at 80°C until constant weight was reached (pelletier *et al.,* 1993). The percentage of water content was evaluated from the percentage difference between the wet-weight (WW) of samples tissues and the dry-weight (DW) of samples tissues.

Lipid extraction

Several procedures for total lipid extraction (Bligh and Dyer, Hara and Radin, Soxhlet, etc.) have been described in the literature (Booji and Van den Berg, 1994; Honeycutt *et al.,* 1995; Manirakiza *et al.,* 2001); however there is no unanimous opinion on the most convenient technique. The soxhlet method is one of the most popular techniques applied to extract lipid from fish (Tornaritis *et al.,* 1993; Clemens and tevens, 2003; Pothoven *et al.,* 2006; Llorret *et al.,* 2008; Martines *et al.,* 2009). In this study, total lipid content was extracted by soxhlet for 8 hours, by repeated washing with a volume of 200ml of hexane/ acetone (1:1 v/v) for 1g of sample. Then, the solvent was evaporated at 35°C using a rotary evaporator. Finally, the lipid content of samples was determined gravimetrically.

Statistical analyses

Linear regressions were used to identify relationships between lipid reserves and fish size. To compare lipid reserves among sexes and maturity stages, we used analyses of variance ANOVA.

Results

Lipid reserves

A total of 156 individuals of striped red mullet sampled between February and May 2010 were used for lipid analysis. Tab.1 presents the mean (±SE) values of total length (TL), body weight (W) and percentage of lipid content of muscle, liver and gonads for different maturity stages of males and females. In mature individuals, muscle lipid content in females (n=56) ranged from 6.10 to 22.33% and in males (n=40) from 5.26 to 19.11%. Liver lipid content in females varied between 26.41 and 37.9 %whilst it varied between 23.93% and 36.43% in males. In pre-spawing individuals, muscle lipid content in females (n=43) ranged from 2.93 to 16.9% and in males (n=17) from 4.96 to 18.94%. Liver lipid content in females varied between 26.7 and 38.62% whilst it varied between 22.95 and 32.02% in males.The results showed that there was no significant differences between males and females in muscle relative lipid content (ANOVA, $p>0.05$).

Table 1. Mean values (±SE) of total length (TL, Body weight (W) and percentage of lipid content in muscle, liver and gonad for different maturity stages of *Mullus surmuletus* individuals.

TL (cm)± (SE) W (g) ± (SE)	Muscle(%)	Gonads (%)	Liver (%)
	Maturing Individuals (n =96)		
27.17 ± 3.76 194.2 ± 52.34	Femelles (n =56) (6.10-22.13) 10.84 ± 3.93	(8.54-30.9) 21.33 ±4.23	(26.41-37.9) 29.02 ± 1.33
23.76 ± 2.95 121.2 ± 40.21	Males (n = 40) (5,26-19,11) 10,42 ± 3,61		(23.93-36.43) 26.08 ± 1.68
	Pre-spawning Individuals (n = 60)		
28.22 ± 4.98 269.99 ± 97.30	Femelles (n =43) (2.93-16.9) 8.05 ± 3.04	16.64-41.28 28.22 ±4.99	26.7-38.62 29.65 ± 1.24
23.22 ± 4.45 199.02 ± 82.70	Males (n = 17) (4.96-18.94) 9.36 ± 2.01		22.95-32.02 25.84 ± 1.26

There was no significant differences (ANOVA, $p>0.05$) between the mean percentage of liver lipid reserves in pre-spawing individuals (mean =27.74±1.21) and that of mature individuals (mean= 27.55±1.43). Liver relative lipid content in males was found statistically different from that of females (ANOVA, $p<0.05$).

The mean percentage of gonads lipid reserves in pre-spawing females (mean=28.22±4.99) was higher than inmature females (mean=21.3±4.23). This difference was significant (ANOVA, $p<0.05$).

Relationship between length, percentage gonads lipid and percentage muscle lipid

The relationship obtained by regression analysis between length and lipid content of muscle of mature individuals irrespective of sexes (n=96) is illustrated in Fig. 2. That between length and lipid content in pre-spawning individuals (n=43) is given in Fig.3.

The percentage of lipid of all individuals is positively correlated to the length. Regression analysis of total length versus percentage of muscle lipid gave significant ($p< 0.05$) linear equations Y = 0.876x - 13.377. In individuals in pre-spawning, ovaries relative lipid content was inversely correlated with length (%lipid in ovaries = -0.4741 (TL) +41.33, R=0.47, $p<0.05$). Muscle relative lipid content among individuals in pre-spawing was found independent of length.

There was a minor correlation between relative lipid content of muscles in the analyzed striped red mullet and that of their ovaries (%lipid in ovaries = -0.4907 (% lipid in muscle) +41.33, R=0.47, $p<0.05$).

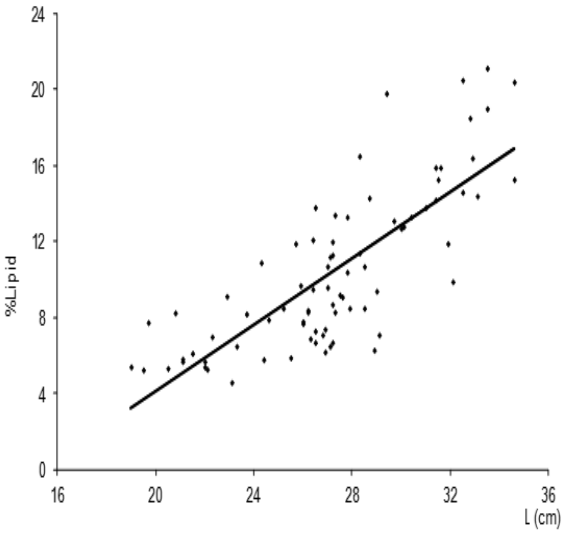

Fig. 2. Linear relationship between length and the lipid content (% dry weight) in the muscle. Y = 0.876x - 13.377, R = 0,79, *p<0,05*, n = 96.

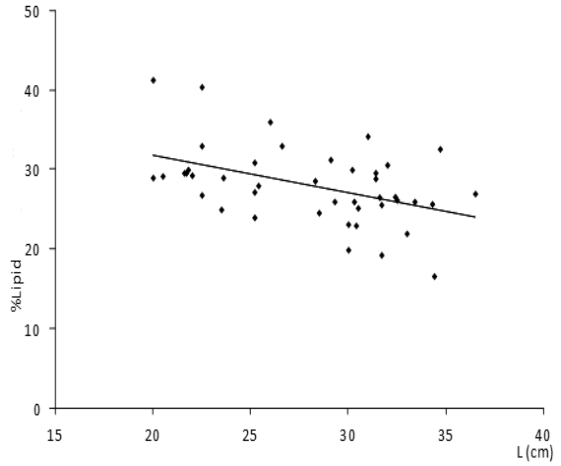

Fig. 3. Linear relationship between length and the lipid content (% dry weight) in the ovaries. Y =-0.474x + 41.34, R = 0.47, *p<0.05*, n = 43.

Relationship between lipid content and water content in different tissues

The relationship obtained by regression analysis between percentage of water content and lipid content of muscle of mature and pre-spawning individuals irrespective of sexes (n=156) is illustrated in Fig.4. Fig 5 shows this relationship between water content percentage and ovaries lipid content in pre-spawning individuals (n=43). There was a strong negative correlation (p<0.05) between lipid and water contents in these tissues. Regression analysis of water content versus percentage of muscle lipid and ovaries lipid gave linear equations of Y = -1.860x + 148.55 and Y = -0.996x + 101.07 respectively. %ML= -0.66 (%water) +55.42; n=43, R=0.84, p<0.001

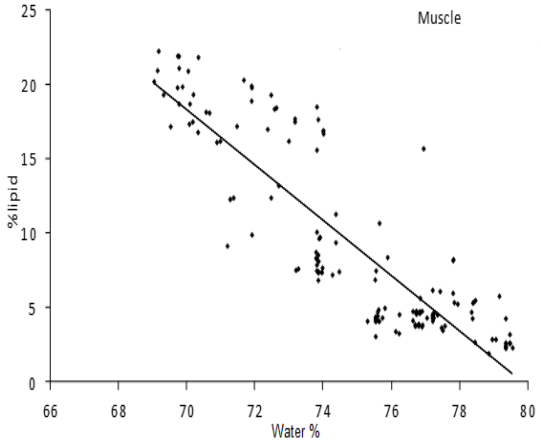

Fig. 4. Linear relationship between the lipid content (% dry weight) and water content in the muscle. Y = -1.860x + 148.55, R = 0,87, *p<0,05*, n = 156.

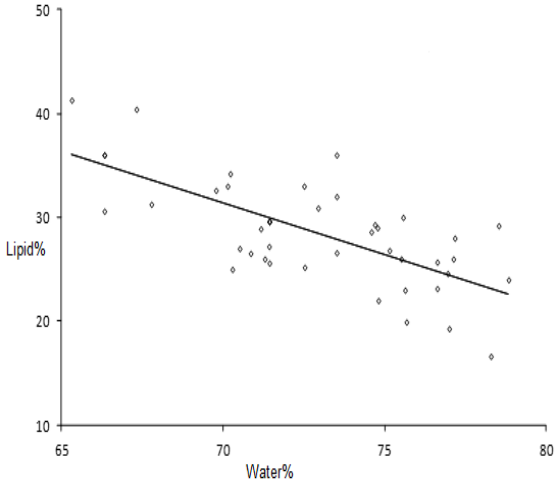

Fig. 5. Linear relationship between the lipid content (% dry weight) and water content in the ovaries. Y = -0.996x + 101.07, R = 0,69, *p<0,05*, n = 43.

Discussion

For the same species, muscle lipid content varies among individuals depending on several factors (age, sexual cycle and food diet). This variability is a common feature among all fishes from fishery (Shulman and Love, 1999). In our study, for *Mullus surmuletus*, the results showed that lipids constituted between 2.93% and 22.33% of dry muscle. These values are close to those of the study conducted by Lloret *et al.,* 2007 on lipid reserves of *Mullus barbatus*, which showed that lipid content varied from 1.77 to 26.18% of the dry muscle.

Lipids are one of the main sources of energy reserves in fish and their transfer from their storage tissues to other tissues to fulfill physiological actions is known to be influenced by the biological condition of the fish (Kandemir *et al.,* 2007). The maturation and the enrichment of gonads in lipid coincided with a decline in muscle lipid content. The production of very large numbers of gametes, during the relatively short period of reproduction is very energy intensive (Tocher, 2003). In our study, we found that lipid content decreased in muscle (mean= 8.05±3.04), whereas it increased in gonads (mean = 28.22±4.99). These results are consistent with those obtained by Lloret *et al.,* 2007 for *Mullus barbatus*. These studies also concluded that muscle was the storage site for lipids in these species (Family: Mullidae).

Many studies show that during maturation period, lipids are mobilized and transferred to gonads (Chelappa *et al.,* 1989, Wiegand, 1996, Brooks *et al.,* 1997; Adams, 1999; Morris and Culkin, 2000; Okuda, 2001; Zudaire *et al.,* 2014; McBride, 2015; Dhurmeea *et al.,* 2018). Some studies established evidence that muscle lipid content in salmonidae decreases of 40 to 60% prior to spawn (Aksnes *et al.*,1986; Nassour and Léger 1989). Female pre-spawners seem to expend much energy on reproductive activities since they presented lower muscle lipid reserves (mean= 8.05 ± 3.04) than those of maturing females (mean= 10.84 ± 3.93). Therefore, it could be emphasized that lipids stored in the muscle of striped red mullet during the maturing stage are mobilized toward the gonad during pre- spawning. The increase of lipid content in the muscle of maturing *M. surmuletus* with size indicates a progressive accumulation of energy in muscle as fish grows until sexual maturation. This increase might be accounted for by ontogenic variations occurring in the diet of striped red mullets (El Bakali *et al.,* 2010b). These results concur well with previous findings in *Mullus barbatus* (Lloret *et al.,* 2007).

The level of corporal lipid reserves can be used as an indicator of fish population's well-being (Shulman, 1974). Accordingto Nikolsky, 1963 the increase in somatic lipid in wild fish reveals a greater availability of food biomass. Conversely, the decrease of lipid content may hence have been provoked by a decrease in the quality and/or the quantity of food. The results of this study show that the average muscle lipid content (8.55%±2.94, n=60) in individuals in pre-spawing remains high. However, no significant relationship between the muscle lipid reserves and the gonad lipid reserves of has been discovered (R=0.39). That means that although striped red mullet allocates lipid reserves to the gonad during the reproductive period, it does not experience depletion in muscle lipid. This can be explained by the fact it feeds intensely during that time (Bizsel, 1987; Lloret *et al.,* 2007; El Bakali *et al.,* 2010b) and the energy invested in eggs production is obtained directly from food instead of the muscle.

Water is one of the most important components that determine the quality of food matrices including fish muscle. Water influences quality attributes such as appearance, storage stability and texture (Andersen and Rinnan, 2002). In fat fishes, the relationship between lipid and water is linear (love, 1970) and the percentages of lipid and water are inversely related (Brandes and Dietrich en 1953 (cites par Hardy and Keay, 1972; Clay, 1988; Shulman and Love, 1999; Shulman et *al.,* 2005; Lloret *et al.,* 2007; Lloret *et al.,* 2008).

In our study, the lipid to water relationship was strong in the muscle and ovaries of the striped red mullet. This suggests that the equations presented in Fig 3 and Fig.4 could be used with confidence to predict the fat levels of striped red mullet by simple and low-cost water content analysis. These results concur well with previous findings by Yeannes and Almandos 2003 and Zaboukas *et al.,* 2006.

Conclusion

The analysis of liver, muscle and gonads lipid reserves in striped red mullet from the Moroccan Mediterranean Sea in this study underlined the role of muscle as the principal lipid-storing organ in this species. Lipid content fluctuated between 2.60 and 22.27% of muscle dry weight. Our results show that despite the allocation of lipid reserves into gonads, muscle lipid in striped red mullet does not undergo any significant decrease. Intense feeding during that period accounts for this lack of depletion, so the energy invested in eggs production is obtained directly from it instead of the muscle. On the other hand, a negative correlation between percentage of water content and lipid content was observed. This might be employed as a predictor of fat levels of striped red mullet with only recourse to inexpensive water content analysis.

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