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# Biochemical composition of the Atlantic bonito *Sarda sarda* from the Aegean Sea (eastern Mediterranean Sea) in different stages of sexual maturity

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The content (% wet mass) in water, ash, lipid, crude protein, DNA and RNA of different tissues was determined during sexual maturation of bonitos *Sarda sarda* from the Aegean Sea. A total of 220 specimens were collected in the following stages of sexual maturity: immature, resting, developing, mature, spawning and spent. Highest lipid levels in the white muscle, red muscle and liver were measured in immature specimens, while lowest levels were found in spawning bonitos. The gradual percentage of lipid reduction from immature to spawning bonitos was relatively higher in the liver (females 71·2% and males 64·4%) than in the white (females 59·2% and males 53·5%) and red (females 62·1% and males 51·7%) muscle. Lipid levels in the gonads increased gradually from the immature to spawning stage. The decrease of lipid in the somatic tissues was more intense in females than in males, and gonadal lipid content was higher in females than in males. There was a strong reverse correlation between water and lipid percentage in all tissues. Protein content decreased significantly only in spawning bonitos. The percentage of protein reduction from immature to spawning stage was relatively higher in males than in females in both white (females 3·4% and males 4·6%) and red (females 4·6% and males 5·1%) muscles. Protein content in the liver was significantly lower than in the other tissues, being highest in mature females. Gonadal protein content in females increased with maturation and decreased after spawning. The content in ash exhibited considerable stability. The RNA:DNA ratio exhibited a similar pattern of variation in both muscles. The RNA:DNA ratio increased during gonadal development gradually from the developing to spent stage. It was concluded that in *S. sarda* during gonadal development, there was an increase in gonadal lipid accompanied by a decrease in somatic tissue lipid reserves. Thus, reproductive inactive bonitos have more lipid in their edible part and a higher nutritional value than active ones.


Key words: DNA; lipid; protein; RNA; *Sarda sarda*; sexual maturity.

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## INTRODUCTION

The bonito *Sarda sarda* (Bloch) is an epipelagic, neritic, schooling scombrid of the Atlantic Ocean, the Mediterranean Sea and the Black Sea and is a commercially important species. Females and males attain sexual maturity at the end of the first or second year of their life. The spawning grounds of this species in the eastern Mediterranean Sea are obscure. Very small preadults were found in the Black Sea, the Sea of Marmara and the Aegean Sea (Serbetis, 1955). Fertilized eggs have only been found in the Black Sea and the Sea of Marmara (Demir, 1963). Seasonal migration between the Aegean Sea and the Sea of Marmara has been verified by tagging experiments (Demir, 1963), but there are schools of bonito that remain the entire year in Greek waters (Yoshida, 1980). Demir (1963) believes that spawning in the Aegean Sea lasts from mid-May to the end of July. Genetic research has shown that the Aegean Sea population has little if any gene flow with the populations of the Ionian and the Ligurian Sea (Pujolar *et al.*, 2001).

Catches in Greek seas range around 2000 t per year (ICCAT, 2000). According to ICCAT (2000), very little is known about the biology of *S. sarda* and other small tuna species. Scientific studies on these species are rarely undertaken, largely because of difficulties in sampling landings from artisanal fisheries, which constitute a high portion of the fisheries exploiting small tuna resources. ICCAT (2000) recommends that studies should be carried out at the local or sub-regional level.

Studies on the biochemical composition of wild fish populations are rarely undertaken due to the difficulties in sampling, preservation of the samples and availability of a wide range of specimens from different ages, sizes, sexes and seasons from unpredictable catches of professional fisheries. The knowledge on biochemical composition of fishery species has fundamental importance in the application of different technological processes (Stansby, 1967; Connell, 1975; Huss, 1988). Moreover, biochemical composition is important as an aspect of quality of raw material, sensory attributes and storage stability (Sikorski, 1994) and is often dependent on sex (De Metrio *et al.*, 1989) and stage of sexual maturity (Connell, 1975; Huss, 1988, 1995).

The lipid and protein balance is important in assessing the flesh quality and as an indicator of seasonal cycles of reproduction and feeding (Clay, 1988). During starvation periods, the fish uses the energy depots in the form of lipids and also may utilize protein, thus depletion of these reserves results in a general diminution of biological condition (Huss, 1995). It also seems likely that principal constituents of developing gonads arise, at least in part, from other tissues of the body (Takama *et al.*, 1985).

Other biochemical measures, including the ratio of muscle RNA to DNA, have been strongly correlated with nutritional status in fishes (Lowery & Somero, 1990; Ferron & Leggett, 1994). Sex and stage of gonadal maturity, among other factors, were reported to affect nucleic acid levels in the different tissues examined (Buckley *et al.*, 1999).

Karakoltsidis *et al.* (1995) detected seasonal variations in the proximate biochemical composition of muscles of the bonito, but they did not analyse the effects of sex and stage of sexual maturity.

The objective of this study was to investigate the biochemical composition (water, ash, protein and lipid, and the RNA:DNA ratio) of the different tissues of *S. sarda* from the Aegean Sea and to detect eventual variations in relation to the stage of sexual maturity.

## MATERIALS AND METHODS

A total of 220 bonitos were sampled in the Aegean Sea from 1997 to 2003. Samples were obtained from commercial fisheries. Bonitos of different sizes were sampled throughout the year in order to collect specimens from all stages of sexual maturity. All samples were frozen immediately and kept frozen at −80° C until analyses. For each fish, total body mass in g (without removing the viscera) and fork length ($L_F$) in cm were measured. Each specimen was dissected, and organs including white muscle, red muscle, liver and gonads were taken. The gonadal mass ($M_G$) in g was also measured. The sex and stage of sexual maturity was identified macroscopically for all individuals. For the validation of the stages of sexual maturity, a number of samples were also examined histologically. A generalized classification of stages in fishes by Nikolsky (1963) was adopted: I = immature, II = resting, III = developing, IV = mature, V = spawning and VI = spent. The number of bonitos in each stage of sexual maturity, the mass and $L_F$ and the months of capture are shown in Table I.

For biochemical analysis, each tissue of each sample fish was homogenized. Dry matter was obtained by drying in an oven at 105° C for 24 h and ash by incineration in a muffle furnace at 550° C for 24 h. The rest of the homogenate was freeze-dried for lipid, protein, DNA and RNA analyses.

Total lipid was extracted using the method of Folch *et al.* (1957), which does not underestimate the lipid content of tissues containing >2% lipid (Iverson *et al.*, 2001). Crude protein (nitrogen × 6·25) was determined using the method of Hach *et al.* (1985), a modified Kjeldahl procedure that uses $H_2SO_4$ and hydrogen peroxide for

TABLE I. Number of samples (*N*), months of capture, mean ± S.E. values and range of total body mass and fork length of *Sarda sarda* at different stages of sexual maturity (SSM)

| | | | Total body mass (g) | | $L_F$ (cm) | |
|---|---|---|---|---|---|---|
| SSM | *N* | Months | Mean ± S.E. | Range | Mean ± S.E. | Range |
| IM | 20 | 1, 2, 10 | 330 ± 318 | 280–460 | 29·5 ± 0·3 | 27·0–32·8 |
| FII | 20 | 2, 3, 8, 9, 10, 11, 12 | 1286 ± 171 | 329–2800 | 42·9 ± 1·0 | 29·0–62·0 |
| FIII | 20 | 3, 4 | 1681 ± 114 | 970–2300 | 49·7 ± 1·1 | 42·5–56·0 |
| FIV | 20 | 4, 5, 6 | 1536 ± 87 | 110–2100 | 48·3 ± 0·9 | 42·5–54·0 |
| FV | 20 | 5, 6 | 2067 ± 161 | 901–2800 | 53·8 ± 1·7 | 41·0–64·5 |
| FVI | 20 | 6, 7, 8 | 1006 ± 86 | 589–1700 | 40·4 ± 1·2 | 34·5–49·5 |
| MII | 20 | 2, 3, 4, 8, 10, 11, 12 | 1451 ± 229 | 332–3800 | 44·8 ± 2·3 | 29·0–61·7 |
| MIII | 20 | 4 | 1009 ± 114 | 437–2050 | 41·3 ± 1·2 | 35·0–53·2 |
| MIV | 20 | 4, 5, 6 | 2061 ± 110 | 1036–2500 | 53·1 ± 1·3 | 41·5–59·0 |
| MV | 20 | 5, 6 | 1928 ± 117 | 1062–2800 | 51·5 ± 1·2 | 41·5–62·0 |
| MVI | 20 | 6, 7, 8 | 1869 ± 209 | 1016–5400 | 49·7 ± 1·4 | 42·5–72·5 |

Months: 1, January; 2, February; 3, March; 4, April; 5, May; 6, June; 7, July; 8, August; 9, September; 10, October; 11, November; 12, December. Maturity: I, immature; II, resting; III, developing; IV, mature; V, spawning; VI, spent. Sex: F, female; M, male.

sample decomposition without added salts or metal catalysts. DNA and RNA determination was performed in the white and red muscle according to the microanalytical method of Holland & Gabbott (1971), modified by Holland & Hannant (1973), which uses colorometric procedures. From standard curves, the concentration of RNA (yeast RNA) and DNA (calf thymus DNA) was determined. All biochemical analyses were performed in triplicate.

In the white muscle, red muscle and liver, regression analysis was performed in order to investigate relationships of the biochemical variables with total wet mass ($M_T$) and $L_F$ within each stage of sexual maturity. In gonads, regression analysis was performed in order to investigate relationships between the biochemical variables and the $M_G$ within each stage of sexual maturity. A Kruskal–Wallis test was performed for the comparison of medians among different tissues, as well as for the comparison of medians among stages of sexual maturity within each tissue. Differences were considered significant when $P < 0{\cdot}05$. Means ± S.E. are presented. The least-squares method was used to obtain the regression curves for the percentage of lipid content as a function of the percentage of water content for each tissue. Statistical analysis was performed using the Statgraphics statistical package.

## RESULTS

In the white muscle, red muscle and liver, biochemical variables were not significantly related with $M_T$ or $L_F$ within each stage of sexual maturity ($P > 0{\cdot}05$). In gonads, biochemical variables were not significantly related with $M_G$ within each stage of sexual maturity ($P > 0{\cdot}05$).

The water content (% wet mass) of the white muscle, red muscle, liver and gonad was 74·21 ± 0·16%, 68·87 ± 0·28%, 64·61 ± 0·46% and 71·72 ± 0·26%, respectively (Fig. 1). Water content was significantly higher in the white muscle and gonads than in the red muscle and liver (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$).

The water content of the somatic tissues (white muscle, red muscle and liver) of bonitos was influenced by the stage of sexual maturity in both sexes (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$), being lowest in immature bonitos. In both females and males, the water content of somatic tissues increased with maturation of gonads (stages III, IV and V) and decreased after spawning (stage VI) (Table II and Fig. 2).

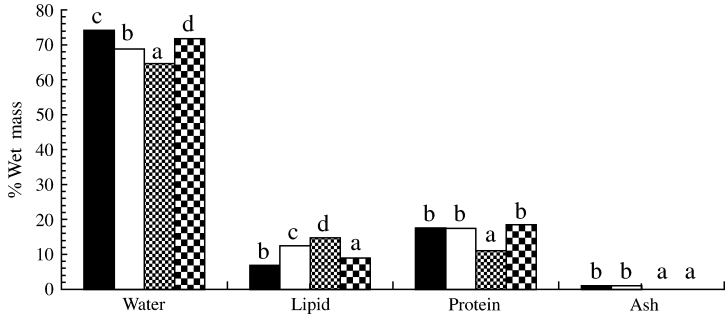


FIG. 1. Content (% wet mass) in water, lipid, crude protein and ash of the white muscle (■), red muscle (□), liver (▩) and gonads (▩) of *Sarda sarda*. Mean values with different lower case letters are significantly different ($P < 0{\cdot}05$).

TABLE II. Mean values of the content (% wet mass) in water, lipid, crude protein and ash of the white muscle, red muscle, liver and gonads of *Sarda sarda* at different stages of sexual maturity (SSM; see Table I)

| | Water | | | | Lipid | | | | Protein | | | | Ash | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SSM | White muscle | Red muscle | Liver | Gonads | White muscle | Red muscle | Liver | Gonads | White muscle | Red muscle | Liver | Gonads | White muscle | Red muscle | Liver | Gonads |
| IM | 70·84 | 63·03 | 55·54 | 76·14 | 10·08 | 17·87 | 24·38 | 4·83 | 17·73 | 17·73 | 11·07 | 18·17 | 1·08 | 1·08 | 0·05 | 0·05 |
| FII | 71·98 | 66·26 | 60·15 | 74·90 | 9·05 | 15·98 | 19·16 | 5·78 | 17·74 | 17·27 | 11·12 | 18·24 | 1·08 | 1·08 | 0·05 | 0·05 |
| FIII | 72·77 | 65·23 | 57·16 | 70·74 | 8·47 | 15·94 | 20·25 | 9·64 | 17·51 | 17·33 | 11·19 | 17·78 | 1·07 | 1·08 | 0·05 | 0·05 |
| FIV | 76·63 | 72·32 | 67·34 | 66·02 | 4·43 | 8·72 | 10·07 | 14·00 | 17·59 | 17·59 | 11·25 | 19·20 | 1·08 | 1·08 | 0·05 | 0·05 |
| FV | 77·40 | 74·58 | 72·36 | 65·87 | 4·11 | 6·78 | 8·67 | 14·08 | 17·12 | 16·90 | 10·93 | 19·44 | 1·08 | 1·08 | 0·05 | 0·05 |
| FVI | 75·17 | 70·52 | 68·86 | 71·67 | 5·68 | 10·56 | 11·18 | 9·33 | 17·72 | 17·73 | 11·04 | 17·72 | 1·08 | 1·08 | 0·05 | 0·05 |
| MII | 72·24 | 65·59 | 60·37 | 75·30 | 8·80 | 15·93 | 18·93 | 6·34 | 17·59 | 17·80 | 11·23 | 17·54 | 1·08 | 1·07 | 0·05 | 0·05 |
| MIII | 73·25 | 67·33 | 60·45 | 73·02 | 7·67 | 13·59 | 19·48 | 7·98 | 17·78 | 17·71 | 11·04 | 18·25 | 1·08 | 1·08 | 0·05 | 0·05 |
| MIV | 75·22 | 70·82 | 67·73 | 72·48 | 5·62 | 10·65 | 12·07 | 8·67 | 17·67 | 17·20 | 10·89 | 18·33 | 1·08 | 1·08 | 0·05 | 0·05 |
| MV | 76·94 | 73·06 | 71·46 | 69·23 | 4·69 | 8·63 | 7·02 | 10·63 | 16·90 | 16·81 | 11·15 | 19·62 | 1·08 | 1·08 | 0·05 | 0·05 |
| MVI | 73·90 | 68·80 | 69·25 | 73·51 | 6·77 | 11·94 | 10·66 | 7·49 | 17·86 | 17·75 | 11·18 | 18·02 | 1·08 | 1·08 | 0·05 | 0·05 |
| Overall mean ± S.E. | 74·21 ± 0·27 | 68·87 ± 0·50 | 64·61 ± 0·90 | 71·72 ± 0·50 | 6·85 ± 0·25 | 12·42 ± 0·46 | 14·72 ± 0·74 | 8·98 ± 0·46 | 17·56 ± 0·02 | 17·44 ± 0·14 | 11·10 ± 0·11 | 18·39 ± 0·27 | 1·08 ± 0·00 | 1·08 ± 0·00 | 0·05 ± 0·00 | 0·05 ± 0·00 |

 

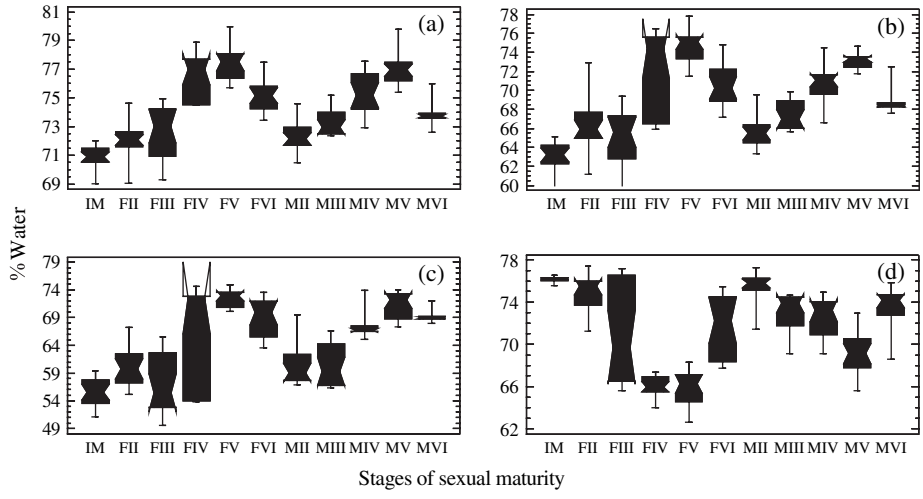


FIG. 2. Medians (box and whiskers plots) of the water content in the (a) white muscle, (b) red muscle, (c) liver and (d) gonads of *Sarda sarda* at different stages of sexual maturity (see Table I). Scale differs among graphs.

The water content of the gonads of bonitos in both sexes was influenced by the stage of sexual maturity (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$). In both females and males, the water content of the gonads decreased with maturation (stages III, IV and V) and increased after spawning (stage VI) (Table II and Fig. 2). The variation between stages was less in males than in females, and male gonads of most stages had higher water content than the respective female ones (Table II and Fig. 2). In immature specimens, water content in their gonads was comparable to that observed in females and males of stage II (Table II and Fig. 2).

The lipid content (% wet mass) of the white muscle, red muscle, liver and gonads was $6{\cdot}85 \pm 0{\cdot}15$, $12{\cdot}42 \pm 0{\cdot}27$, $14{\cdot}72 \pm 0{\cdot}43$ and $8{\cdot}98 \pm 0{\cdot}23$%, respectively (Fig. 1). Lipid content was significantly lower in the white muscle and gonads than in the red muscle and liver (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$).

The lipid content of the somatic tissues of bonitos was influenced by the stage of sexual maturity in both sexes (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$), being highest in immature bonitos. In both females and males, the lipid content of the somatic tissues decreased with the maturation of gonads (stages III, IV and V) and increased after spawning (stage VI) (Table II and Fig. 3). Lipid content in the muscles of mature, spawning and spent bonitos (stages IV, V and VI, respectively) was lower in females than in males, particularly in the red muscle. Lipid content in the liver was lower in females than in males, only in mature bonitos (stage IV). Furthermore, the percentage of lipid reduction from immature to spawning bonitos was relatively higher in the liver (females 71·2% and males 64·4%) than in the white (females 59·2% and males 53·5%) and red (females 62·1% and males 51·7%) muscle.

The lipid content of the gonads of bonitos in both sexes was influenced by the stage of sexual maturity (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$), being lowest in immature bonitos. In females, the lipid content of the gonads increased with maturation (stages III, IV and V) and decreased after spawning (stage VI)

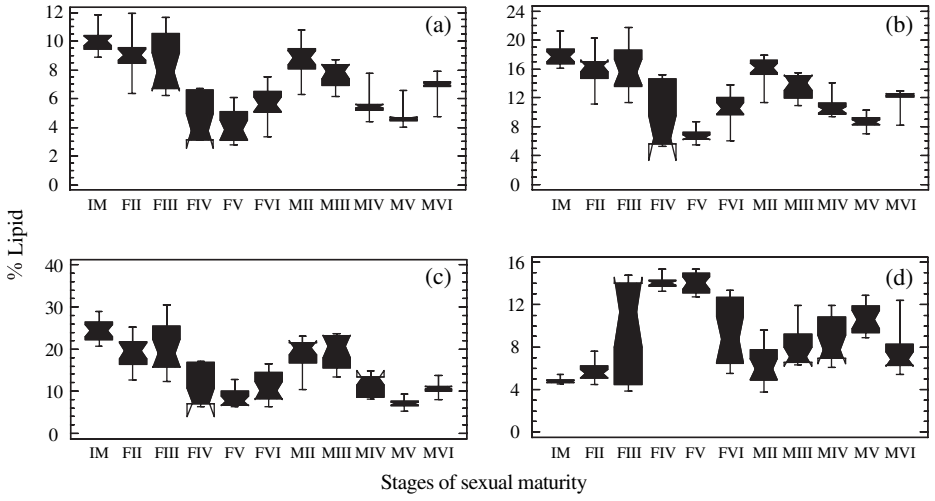


FIG. 3. Medians (box and whiskers plots) of the lipid content (% wet mass) in the (a) white muscle, (b) red muscle, (c) liver and (d) gonads of *Sarda sarda* at different stages of sexual maturity (see Table I). Scale differs among graphs.

(Table II and Fig. 3). A similar trend was observed in the gonads of male bonitos, although the variation between stages was less, and male gonads of most stages had lower lipid content than the respective female ones (Table II and Fig. 3).

There was a strong negative correlation between the lipid and the water content in all four tissues examined (Fig. 4).

The crude protein content (% wet mass) of the white muscle, red muscle, liver and gonads was 17·59 ± 0·04, 17·44 ± 0·05, 11·10 ± 0·03 and 18·50 ± 0·09, respectively (Fig. 1). Protein content was significantly lower in liver than in all other tissues examined (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$).

The protein content of the somatic tissues of bonitos was influenced by the stage of sexual maturity (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$). The protein content of the white and red muscle was significantly lower in spawning females and males than in immature, resting, developing and spent ones (Table II and Fig. 5). Protein content decreased significantly only in spawning bonitos. The percentage of protein reduction from immature to spawning stage was relatively higher in males than in females in both white (females 3·4% and males 4·6%) and red (females 4·6% and males 5·1%) muscles. The protein content of the liver was significantly higher in mature females (stage IV) than in resting, developing and spent ones (stages II, III and VI, respectively) and was almost stable in males (Table II and Fig. 5).

The protein content of the gonads of female bonitos was influenced by the stage of sexual maturity (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$). Specifically, it increased with maturation (stages IV and V) and decreased after spawning (stage VI) (Table II and Fig. 5).

The ash content (% wet mass) of the white muscle, red muscle, liver and gonads was 1·08 ± 0·00, 1·08 ± 0·00, 0·05 ± 0·00 and 0·05 ± 0·00, respectively (Fig. 1). Ash content did not differ significantly in the two muscles. Liver

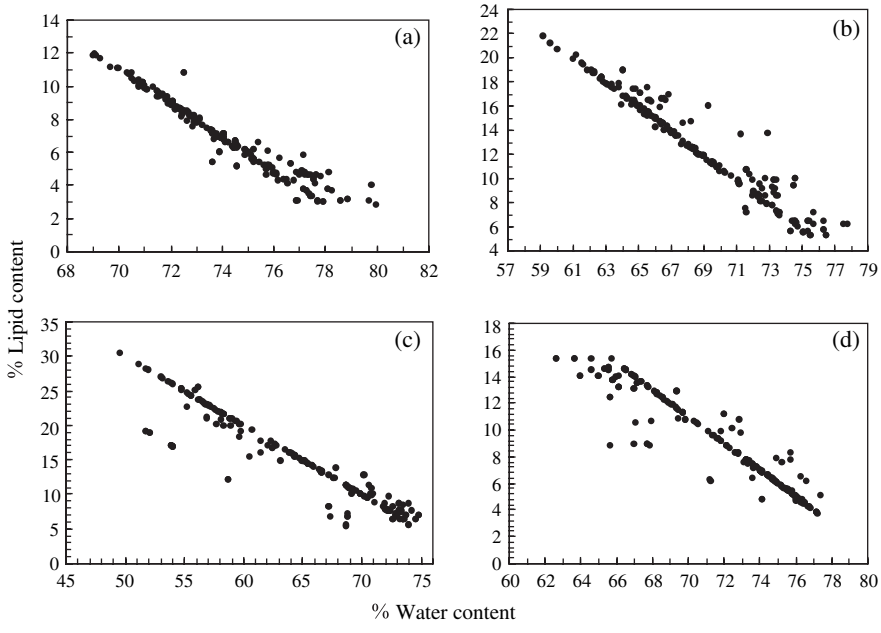


FIG. 4. Lipid content (% wet mass) regressed on water content in the (a) white muscle, (b) red muscle, (c) liver and (d) gonads of *Sarda sarda*. Scale differs among graphs. The curves were fitted by: (a) $y = 74{\cdot}7202 - 0{\cdot}9143x$ ($r^2 = 0{\cdot}92$, $P < 0{\cdot}001$, $n = 220$), (b) $y = 77{\cdot}7983 - 0{\cdot}9491x$ ($r^2 = 0{\cdot}94$, $P < 0{\cdot}001$, $n = 220$), (c) $y = 70{\cdot}9079 - 0{\cdot}8695x$ ($r^2 = 0{\cdot}89$, $P < 0{\cdot}001$, $n = 220$) and (d) $y = 69{\cdot}9397 - 0{\cdot}8498x$ ($r^2 = 0{\cdot}92$, $P < 0{\cdot}001$, $n = 220$).

and gonads had significantly lower ash content than both muscles (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$). The ash content of the white muscle, red muscle, liver and gonads of bonitos was not influenced by the stage of sexual maturity (Kruskal–Wallis, d.f. = 219, $P > 0{\cdot}05$).

The DNA content (% wet mass) of the white and red muscle was $0{\cdot}57 \pm 0{\cdot}00$ and $0{\cdot}57 \pm 0{\cdot}00$, respectively. The RNA content (% wet mass) of the white and red muscle was $0{\cdot}69 \pm 0{\cdot}00$ and $0{\cdot}70 \pm 0{\cdot}00$, respectively. Both DNA and RNA content did not differ significantly between the white and the red muscle (Kruskal–Wallis, d.f. = 219, $P > 0{\cdot}05$).

The RNA:DNA ratio of both white and red muscle was influenced by the stage of sexual maturity in both sexes (Kruskal–Wallis, d.f. = 219, $P < 0{\cdot}001$). Specifically, the RNA:DNA ratio increased gradually from stage III to VI (Fig. 6).

## DISCUSSION

There are a number of classifications that divide fishes into groups according to their lipid content. According to Ackman (1989), *S. sarda* belongs to the group of high-fat fishes having an average muscle lipid content of 8–15%. Tanakol *et al.* (1999) came to the same conclusion after measuring the lipid content of the edible muscle of one specimen of *S. sarda* caught in the Sea of Marmara and compared it with other species from the same area. The high

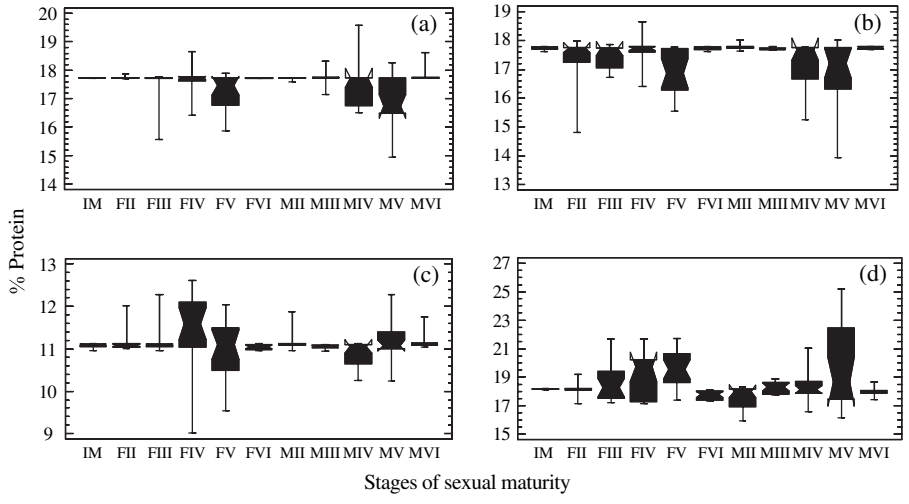


FIG. 5. Medians (box and whiskers plots) of the crude protein content (% wet mass) in the (a) white muscle, (b) red muscle, (c) liver and (d) gonads of *Sarda sarda* at different stages of sexual maturity (see Table I). Scale differs among graphs.

lipid content in the tissues of the bonito may be attributed to its diet, which consists mainly of small pelagic fish species like sardines *Sardina pilchardus* (Walbaum) and European anchovies *Engraulis engrasicholus* L. (Demir, 1963). The above species are also found to be rich in lipid (Tanakol *et al.*, 1999).

Lipid content is measured in many other species of the Scombridae, like yellowfin tuna *Thunnus albacares* (Bonnaterre) (Saito *et al.*, 1996), albacore *Thunnus alalunga* (Bonnaterre) (Murase & Saito, 1996), northern bluefin tuna *Thunnus thynnus* L. (Clay, 1988), Pacific bluefin tuna *Thunnus thynnus orientalis* (Temminck & Schlegel) (Murai *et al.*, 1982), kawakawa *Euthynnus affinis* (Cantor) and striped bonito *Sarda orientalis* (Temminck & Schlegel) (Saito *et al.*, 1999) (Table III). In almost all these species, lipid content in all tissues was lower than that of the *S. sarda*.

Scombrids stand out from most other fishes by their ability to cruise at relatively high speeds and migrate long distances. To sustain fast, continuous locomotion, they have evolved a suite of morphological specializations that

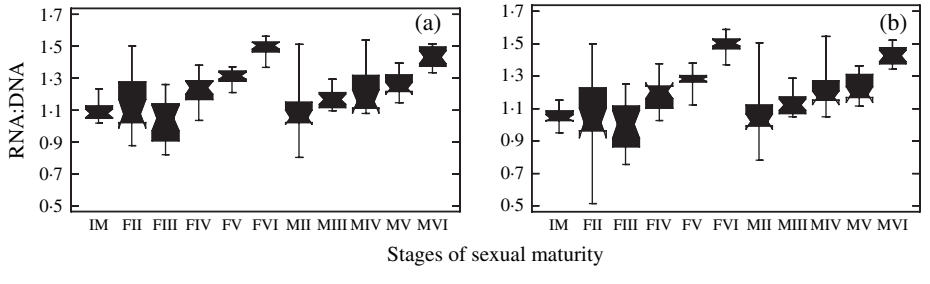


FIG. 6. Medians (box and whiskers plots) of the RNA:DNA ratio in the (a) white and (b) red muscle of *Sarda sarda* at different stages of sexual maturity (see Table I). Scale differs among graphs.

 

TABLE III. Mean values of the content (% wet mass) in lipid and crude protein of the white muscle, red muscle and liver of scombrid species caught in different areas

| | | | Lipid | | | Protein | | |
|---|---|---|---|---|---|---|---|---|
| Author | Area | Species | White muscle | Red muscle | Liver | White muscle | Red muscle | Liver |
| Saito *et al.*, 1996 | East Caroline Basin | *Thunnus albacares* | 0·5 ± 0·3[a] | 4·3 ± 1·3[a] | 6 ± 1·3[a] | 25·8 ± 0·5[a] | | |
| | Pacific coast of Japan | *T. albacares* | 0·8 ± 0·1[a] | 4·2 ± 1·4[a] | 1·5 ± 1·3[a] | 25·6 ± 0·9[a] | | |
| Murase & Saito, 1996 | Japanese coast | *Thunnus alalunga* | 1·5 ± 1·9[a] | 2·3 ± 0·8[a] | 8·7 ± 4·6[a] | 27·7 ± 1·1[a] | 24·7 ± 1·5[a] | |
| | Emperor Seamount Chain | *T. alalunga* | 8·5 ± 3·3[a] | 7·6 ± 2·7[a] | 21·6 ± 3·8[a] | 26·2 ± 1·2[a] | 22·1 ± 1·9[a] | |
| Clay, 1988 | Gulf of St Lawrence | *Thunnus thynnus* | 17 ± 8[a] | | | 18 ± 2[a] | | |
| Murai *et al.*, 1982 | Japanese coast | *Thunnus thynnus orientalis* | 0·6 ± 0·0[a] | 1·7 ± 0·1[a] | 4·0 ± 2·6[a] | 24·9 ± 0·2[a] | 20·3 ± 0·5[a] | 20·1 ± 1·1[a] |
| Saito *et al.*, 1999 | East China Sea | *Euthynnus affinis* | 5·5 ± 1·1[b] | | 14·1 ± 2·9[b] | | | |
| | East China Sea | *Sarda orientalis* | 3·3 ± 0·4[b] | | 12·3 ± 1·0[b] | | | |
| Saito *et al.*, 1997 | Philippine Sea | *Katsuwonus pelamis* | 0·6 ± 0·1[b] | 4·0 ± 0·6[b] | 10·9 ± 2·3[b] | | | |
| | East China Sea | *K. pelamis* | 0·7 ± 0·1[b] | 3·5 ± 0·1[b] | 5·7 ± 1·3[b] | | | |
| | Pacific coast of Japan | *K. pelamis* | 1·6 ± 0·5[b] | 5·2 ± 0·3[b] | 7·4 ± 1·7[b] | | | |
| Present study | Aegean Sea | *Sarda sarda* | 6·8 ± 0·1[b] | 12·4 ± 0·3[b] | 14·7 ± 0·4[b] | 17·6 ± 0·0[b] | 17·4 ± 0·0[b] | 11·1 ± 0·0[b] |

[a]S.D.; [b]S.E.

distinguish them from other fishes. The bonito is among the scombrid species with the fastest recorded cruising speeds (Altringham & Shadwick, 2001). The high lipid content of its tissues may reflect the need for high-energy biomolecules to perform the energy-consuming locomotion.

The high lipid content in the liver of *S. sarda* could be expected because, as Sheridan (1994) pointed out, the liver of fishes contain a considerable amount of lipid. Lipid percentage in *S. sarda* was significantly higher in the red muscle than in the white muscle, a usual characteristic in many fishes (Tocher, 2003). The energy requirements of the red muscle in order to perform sustained swimming are provided largely by fatty acids (Tocher, 2003). The similar lipid content in liver and red muscle of the high-fat and active bonito is in accordance with: high-fat fishes use their muscle as a lipid reserve (Hodder *et al.*, 1973) and skeletal muscle is an important storage site in active species (Braekken, 1959).

Similar fluctuations in lipid percentage among the white muscle, the red muscle and the liver were also observed in the skipjack tuna *Katsuwonus pelamis* L. caught in the Pacific Ocean (Saito *et al.*, 1997). The lipid content of the gonads of *S. sarda* was in similar levels with the ones measured by Hiratsuka *et al.* (2004) in the gonads of *K. pelamis*.

The lipid to water relationship was strong in all four tissues of the bonitos. This suggests that the equations presented in Fig. 4 could be used with confidence to predict the fat levels of bonitos by a simple and low-cost water content analysis. A similar strong relationship was found in the white muscle of *T. thynnus* by Clay (1988). Love (1970) stated that in high-fat fishes, the relationship between lipid and water is linear and their sum is almost always stable. Additionally, Shearer (1994) concluded that percentages of lipid and water are inversely related. Yeannes & Almandos (2003) stated that measuring water content allows the estimation of flesh (edible part) lipid content for high-fat content families like the Scombridae, with the corresponding cost savings upon performing one instead of two analytical determinations.

The maturation and the enrichment of gonads in lipid coincided with a decline of the lipid content in muscles and liver. The production of very large numbers of gametes, particularly eggs, during the relative short period of reproduction is very energy intensive (Tocher, 2003). Jobling (1994) stated that although the energetic investment in eggs is greater than that in sperm, the energetic costs of engaging in reproductive behaviour may be markedly higher for the males, and the overall investment in reproduction may be similar for the two sexes. In the present study, the more intense reduction in the lipid content in the somatic tissues of females compared to males indicate that the reproductive cost is higher in females than in males in *S. sarda*.

There is little information on the energetic costs of reproduction in scombroids (Schaefer, 2001). Studies on other families of marine and freshwater fishes suggest, however, that the energetic costs associated with reproduction are significant and can have important consequences affecting growth and mortality rates after sexual maturation (Wootton, 1990). Jobling (1994) believes that somatic and gonadal growth can be considered to be in competition for limited resources, and a decrease is commonly seen in somatic growth rate when fishes mature. In the present study, lipid of the somatic tissues gradually decreased from immature to spawning stage in order to supply gonads with lipid and

possibly to fulfil energetic requirements associated with the period of sexual maturation. Seasonal variations in lipid levels in fishes are fundamentally related to the reproductive cycle since most marine fishes generally accumulate large lipid deposits prior to gonadal development (Mourente *et al.*, 2002). This lipid reserve is subsequently used as metabolic energy during the spawning migration, but in the female broodfish, it is largely mobilized and transferred into the developing ovary (Bell, 1998). In agreement, an increased gonadal lipid was observed in female bonitos.

The lipid reduction in the somatic tissues of fishes during gonadal development has been described for many species, indicating an interspecific differentiation in the degree of lipid utilization in muscle and liver reserves (Krivobok, 1964; Love, 1970, 1980; Lapin, 1973; Lewander *et al.*, 1974; Shatunovsky, 1980; Dabrowski, 1982; Henderson *et al.*, 1984; Haug & Gulliksen, 1988; Kozlova, 1997). In *S. sarda*, a higher percentage of lipid reduction was observed in liver compared to muscles, indicating the use of liver reserves in a greater extent than the muscle reserves.

Protein content has been measured in few other species of Scombridae (Table III). The protein content of most tissues of *T. albacares* (Saito *et al.*, 1996), *T. alalunga* (Murase & Saito, 1996), *T. thynnus orientalis* (Murai *et al.*, 1982) and *K. pelamis* (Saito *et al.*, 1997) was found to be higher than that of the respective tissues of the bonito. The protein content of *T. thynnus* (Clay, 1988), however, was similar to that of the bonito.

The protein content of the somatic tissues in *S. sarda* was less dynamic than the lipid content. Similarly to the present results, Brown & Murphy (2004) found that muscle protein was the least dynamic tissue fraction compared to muscle lipid, liver lipid and gonadal lipid of largemouth bass *Micropterus salmoides* (Lacèpede). The protein content in the red and the white muscle decreased only during the spawning stage in both sexes. This reduction of protein content after the depletion of lipid in the muscles may reflect a strong need for energy supply in the gonads or the need for structural biomolecules. The increased protein content in the liver of female bonitos may be due to vitellogenin, a lipoprotein which is synthesized in the liver and is transported to the ovary (Wallace, 1985). Besides, gonadal protein content increased with maturation only in female bonitos.

The RNA:DNA levels of the muscles increased from developing to spawning females and males, reflecting increased rate of protein synthesis (Buckley *et al.*, 1999), related to the requirements of sexual maturation and spawning. The highest RNA:DNA ratio observed in the muscles of spent females and males may indicate enhanced protein synthetic activity after the loss in protein during spawning. As it is shown in Table I, spent specimens occur exclusively from June to September, when water temperatures and food availability are high. The elevated RNA:DNA levels in spent bonitos are possibly due to the effort of fish to restore their energy reserves, after the exhausting function of reproduction, while food is still abundant. Fish RNA:DNA and food availability are highly correlated in a wide variety of species in both laboratory and field studies (Buckley, 1980; Buckley *et al.*, 1984; Buckley & Lough, 1987; Theilacker *et al.*, 1996). Buckley *et al.* (1999) stated that as the fish mature, seasonal cycles of temperature and gonadal development have large effects on nucleic acid

levels in different tissues. In addition, seasonal variations in the lipid content were related to reproduction and food availability (Takama *et al.*, 1985). This may explain the increase of the lipid content in the somatic tissues of spent bonitos.

The pattern of variation of RNA:DNA ratio is very similar for both muscles. This similarity may reflect the similar rate of protein synthesis in both muscles. Besides, protein content was similar in both red and white muscle of *S. sarda* during sexual maturity. Stickland (1983) found a different pattern of growth among the white and red muscle of the rainbow trout *Oncorhynchus mykiss* (Walbaum). As far as is known, development of the muscles has not been studied in *S. sarda*. Present results, however, indicate a similar rate of development in both muscles.

The ash content of the muscles of the bonitos was in similar levels to the ones found by Karakoltsidis *et al.* (1995) and comparable to those measured in other scombroids like *T. thynnus* (Clay, 1988) and *T. thynnus orientalis* (Murai *et al.*, 1982). Love (1970) stated that ash levels fall when fishes are starved or suffering depletion. In the present study, the content in ash of all four tissues examined did not show any significant fluctuation. Thus, the 220 bonitos from the Aegean Sea are not considered to be under any extreme stress.

*Sarda sarda* during gonadal development used mainly lipid that was derived mostly from the liver. The lipid reduction was more intense in females than in males, particularly in muscles. Somatic protein was more conservative than lipid, in relative terms. Scombroids are more attractive to the consumer when they are rich in lipid. Especially in bluefin tuna, the price of its meat is dependent on its lipid content. The data of the present study suggest that the edible part of the bonito is low in lipid during spawning season. This new information can be used for the determination of the fishing season for the species when it is not reproductively active and has high lipid content and therefore high nutritional value.

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## References

Ackman, R. G. (1989). Nutritional composition of fats in seafoods. *Progress in Food Nutrition Science* **13,** 161–241.

Altringham, J. D. & Shadwick, R. E. (2001). Swimming and muscle function. In *Tuna: Physiology, Ecology and Evolution* (Block, B. & Stevens, D., eds), pp. 319–344. San Diego, CA: Academic Press.

Bell, J. G. (1998). Current aspects of lipid nutrition in fish farming. In *Biology of Farmed Fish* (Black, K. D. & Pickering, A. D., eds), pp. 114–145. Sheffield: Sheffield Academic Press Ltd.

Braekken, O. R. (1959). A comparative study of vitamins on trunk muscles of fishes. *Report on Technological Research Concerning Norwegian Fish Industry* **3,** 1–15.

Brown, M. L. & Murphy, B. R. (2004). Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacèpede). *Ecology of Freshwater Fish* **13,** 123–136.

Buckley, L. J. (1980). Changes in RNA, DNA, and protein content during ontogenesis of the winter flounder (*Pseudopleuronectes americanus*) and the effect of starvation. *Fishery Bulletin* **77,** 703–708.

Buckley, L. J. & Lough, R. G. (1987). Recent growth, biochemical composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. *Canadian Journal of Fisheries and Aquatic Sciences* **44,** 14–25.

Buckley, L. J., Turner, S. I., Halavik, T. A., Smigielski, A. S., Drew, S. M. & Laurence, G. C. (1984). Effects of temperature and food availability on growth, survival and RNA–DNA ratio of larval sand lance (*Ammodytes americanus*). *Marine Ecology Progress Series* **15,** 91–97.

Buckley, L. J., Caldarone, E. & Ong, T. L. (1999). RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* **401,** 265–277.

Clay, D. (1988). Fat, water, protein and ash composition on bluefin tuna collected in the Gulf of St. Lawrence. *ICCAT Collective Volume of Scientific Papers* **28,** 196–202.

Connell, J. J. (1975). *Control of Fish Quality*. Farnham: Fishing News Books Ltd.

Dabrowski, K. (1982). Seasonal changes in chemical composition of fish body and nutritional value of the muscle of the pollan (*Cyprinus pollan* Thompson) from Lough Neagh, Northern Ireland. *Hydrobiologia* **87,** 121–141.

De Metrio, G., Ciruzzi, B., Megalofonou, P., Montemurro, O. & Lauda-Dio, V. (1989). Early results of chemical composition, according to sex and size of *Spicara smaris* L. (Osteichthyes - Centarcanthidae) of the gulf of Taranto. *Atti della Societa Italiana delle Scienze Veterinarie* **43,** 669–674.

Demir, M. (1963). Synopsis of biological data on bonito *Sarda sarda* (Bloch) 1793. *FAO Fisheries Report* **6,** 101–129.

Ferron, A. & Leggett, W. C. (1994). An appraisal of condition for marine fish larvae. *Advances in Marine Biology* **30,** 217–303.

Folch, J. M., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipid from animal tissue. *Journal of Biological Chemistry* **226A,** 497–509.

Hach, C. C., Scott, V. B. & Kopelove, A. B. (1985). A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. *Journal of Agricultural and Food Chemistry* **33,** 1117–1123.

Haug, T. & Gulliksen, B. (1988). Variations in liver and body condition during gonad development of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Fiskeridirektoratets Skrifter Serie Havundersøkelser* **18,** 351–363.

Henderson, R. J., Sargent, R. J. & Hopkins, C. C. E. (1984). Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. *Marine Biology* **78,** 255–263.

Hiratsuka, S., Kitagawa, T., Matsue, Y., Masasyuki, H. & Wada, S. (2004). Lipid class and fatty acid composition of phospholipids from the gonads of skipjack tuna. *Fisheries Science* **70,** 903–909.

Hodder, V. M., Parsons, L. S., Winters, G. H. & Spencer, K. (1973). Fat and water content of herring in Newfoundland and adjacent waters, 1966-1971. *Fisheries Research Board of Canada Technical Report* **365**.

Holland, D. L. & Gabbott, P. A. (1971). A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. *Journal of the Marine Biological Association of the United Kingdom* **51,** 659–668.

Holland, D. L. & Hannant, P. J. (1973). Addendum to a micro-analytical scheme for the biochemical analysis of marine invertebrate larvae. *Journal of the Marine Biological Association of the United Kingdom* **53,** 833–838.

Huss, H. H. (1988). El pescado fresco: Su calidad y cambios de calidad. *Colección FAO Pesca* **29**.

Huss, H. H. (1995). Quality and quality changes in fresh fish. *FAO Fisheries Technical Paper* **348**.

ICCAT (2000). *Small Tunas Executive Summary*. Madrid: International Commission for the Conservation of Atlantic Tuna.
Iverson, S. J., Lang, S. L. & Cooper, M. H. (2001). Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* **36,** 1283–1287.
Jobling, M. (1994). *Fish Bioenergetics*. London: Chapman & Hall.
Karakoltsidis, P. A., Zotos, A. & Constantinides, S. M. (1995). Composition of the commercially important Mediterranean fin fish, crustaceans, and molluscs. *Journal of Food Composition and Analysis* **8,** 258–273.
Kozlova, T. A. (1997). Seasonal cycles in total chemical composition of the two Lake Baikal benthic-pelagic sculpins (*Cottocomephorus*, Cottoidei). *Journal of Fish Biology* **50,** 734–743.
Krivobok, M. N. (1964). On the role of the liver in the maturation of the ovaries of the Baltic herring, *Clupea harengus membras* L. *Vosposy Ikthiologii* **4,** 483–494.
Lapin, V. I. (1973). Seasonal variations in biochemical composition of organs and tissues of flounder *Platichthys flesus bogdanovi* (Sanderberg) from White Sea. *Vosposy Ikthiologii* **13,** 313–327.
Lewander, K., Dave, G., Johanson, M. L., Larson, A. & Lidman, U. (1974). Metabolic and hematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L. Carbohydrate, lipid, protein and inorganic ion metabolism. *Comparative Biochemistry and Physiology* **47B,** 571–581.
Love, R. M. (1970). *The Chemical Biology of Fishes*, Vol. 1. New York: Academic Press.
Love, R. M. (1980). *The Chemical Biology of Fishes*, Vol. 2. New York: Academic Press.
Lowery, M. S. & Somero, G. N. (1990). Starvation effects on protein synthesis in red and white muscle of the barred sand bass *Paralabrax nebulifer*. *Physiological Zoology* **63,** 630–648.
Mourente, G., Megina, C. & Díaz-Salvago, E. (2002). Lipids in female northern bluefin tuna (*Thunnus thynnus thynnus* L.) during sexual maturation. *Fish Physiology and Biochemistry* **24,** 351–363.
Murai, T., Hirasawa, Y., Akiyama, T. & Nose, T. (1982). Free ninhydrin reactive substances in the white muscle, dark muscle, and liver of cultured and wild bluefin tuna juvenile *Thunnus thynnus orientalis*. *Bulletin of the Japanese Society of Scientific Fisheries* **48,** 1633–1637.
Murase, T. & Saito, H. (1996). The docosahexaenoic acid content in the lipid of albacore *Thunnus alalunga* caught in two separate localities. *Fisheries Science* **62,** 634–638.
Nikolsky, G. V. (1963). *The Ecology of Fishes*. London: Academic Press.
Pujolar, J. M., Roldán, M. I. & Pla, C. (2001). Allozyme differentiation of bonito in the Mediterranean Sea. *Journal of Fish Biology* **59,** 169–174. doi: 10.1006/jfbi.2001.1597
Saito, H., Ishihara, K. & Murase, T. (1996). Effect of prey fish lipids on the docosahexaenoic acid content of total fatty acids in the lipid of *Thunnus albacares* yellowfin tuna. *Bioscience Biotechnology and Biochemistry* **60,** 962–965.
Saito, H., Ishihara, K. & Murase, T. (1997). The fatty acid composition in tuna (Bonito, *Euthynnus pelamis*) caught at three different localities from tropics to temperate. *Journal of the Science of Food and Agriculture* **73,** 53–59.
Saito, H., Yamashiro, R., Ishihara, K. & Changhu, X. (1999). Lipids of three highly migratory fishes: *Euthynnus affinis*, *Sarda orientalis* and *Elagatis bipinnulata*. *Bioscience Biotechnology and Biochemistry* **63,** 2028–2030.
Schaefer, K. M. (2001). Reproductive biology of tunas. In *Tuna Physiology, Ecology and Evolution* (Block, B. & Stevens, D., eds), pp. 319–344. San Diego, CA: Academic Press.
Serbetis, C. (1955). Migrations des pelamides et des maquereaux de la Mer Egee. *Technical Papers of the General Fisheries Council of the Mediterranean FAO* **3,** 381–385.
Shatunovsky, M. I. (1980). *Ecological Regularities of Metabolism in Sea Fishes*. Moscow: Nauka.
Shearer, K. D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture* **11,** 63–88.

Sheridan, M. A. (1994). Regulation of lipid metabolism in poikilothermic vertebrates. *Comparative Biochemistry and Physiology* **107B,** 495–508.
Sikorski, Z. E. (1994). *Tecnología de los Productos del Mar: Recursos y Composición Nutritiva*. Zaragoza: Acribia.
Stansby, M. E. (1967). *Tecnología de la Industria Pesquera*. Zaragoza: Editorial Acribia.
Stickland, N. C. (1983). Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). *Journal of Anatomy* **137,** 323–333.
Takama, K., Love, R. M. & Smith, G. L. (1985). Selectivity in mobilisation of stored fatty acids by maturing cod, *Gadus morhua* L. *Comparative Biochemistry and Physiology* **80B,** 713–718.
Tanakol, R., Zeliha, Y., Erdal, S. & Ergin, S. (1999). Fatty acid composition of 19 species of fish from the Black Sea and the Marmara Sea. *Lipids* **34,** 291–297.
Theilacker, G. H., Bailey, K. M., Canino, M. F. & Porter, S. M. (1996). Variations in larval walleye pollock feeding and condition: a synthesis. *Fisheries Oceanography* **5,** 112–123.
Tocher, D. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* **11,** 107–184.
Wallace, R. A. (1985). Vitellogenesis and oocytes growth in nonmammalian vertebrates. In *Developmental Biology: A Comprehensive Synthesis*, Vol. 1 (Browder, R. W., ed.), pp. 127–177. New York: Plenum Press.
Wootton, R. J. (1990). *Ecology of Teleost Fishes*. London: Chapman & Hall.
Yeannes, M. I. & Almandos, M. E. (2003). Estimation of fish proximate composition starting from water content. *Journal of Food Composition and Analysis* **16,** 81–92.
Yoshida, H. O. (1980). Synopsis of biological data on bonito of the genus *Sarda*. *NOAA Technical Report NMFS Circular 432, FAO Fisheries Synopsis* **118**.