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Lipids in female northern bluefin tuna (*Thunnus thynnus thynnus* L.) during sexual maturation

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Abstract

Total lipids, lipid classes, fatty acids and vitamin E contents were measured in ovary, liver, dark muscle, white muscle and mesenteric perigonadal fat in four maturational stages of Atlantic female northern buefin tuna (*Thunnus thynnus thynnus* L.) during migration to Mediterranean spawning grounds through the Strait of Gibraltar. Ovary lipid content increased significantly by 37.6-fold from inmature to spawning stage, accumulating large quantities of steryl/wax esters, whereas the ratio of mesenteric perigonadal fat (primarily triacylglycerols) weight to ovary weight decreased linearly throughout maturation. In contrast, liver, dark red and white muscles lipid content did not show a major depletion during maturation compared to that of perigonadal fat. The fatty acid composition of ovary from bluefin tuna showed that docosahexaenoic acid (DHA; 22:6n-3) was the most abundant fatty acid and DHA: EPA: AA and DHA: EPA ratios were 19.3/4.3/1 and 4.5/1, respectively. Neutral lipids predominated over polar lipids (2-3:1) in ovary and triacylglycerol and steryl/wax ester were major lipid classes. In general, fatty acid composition of most tissues examined were similar to each other but, in contrast, the fatty acid profile of perigonadal fat was rich in monoenes such as 18:1n-9, 20:1n-9 and 22:1n-11, and DHA decreased significantly at spawning stage. The total lipid balance suggested for the disappearance of lipid from perigonadal fat depots and, to a lesser degree, from muscle and liver depots, reflected lipid utilization for gonadal development on the one hand and non-gonadal metabolism in the other. The former included lipid transferred to the gonad and lipid catabolized to provide metabolic energy for the biosynthesis of gonadal constituents and the later was likely to be lipid catabolism to provide energy for swimming during spawning migration.

Abbreviations: AA – all-*cis*-5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4(n-6)); DHA – all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6(n-3)); DMA – dimethyl acetal; DPA, all-*cis*-7,10,13,16,19-docosapentaenoic acid (22:5(n-3)); EPA – all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5(n-3)); FFA – free fatty acid; HUFA–highly unsaturated fatty acids ($\geq C_{20}$ and with $\geq$3 double bonds); LA – all-*cis*-9,12-octadecadienoic acid (linoleic acid, 18:2(n-6)); LNA – all-*cis*-9,12,15-octadecatrienoic acid (α-linolenic acid, 18:3(n-3)); PA – phosphatidic acid; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol; PS – phosphatidylserine; PUFA – polyunsaturated fatty acid(s); S – free sterol; SE – steryl ester; TAG – triacylglycerol; WE – wax ester.
Note: PC and PE represent diradyl (diacyl + alkenilacyl + alkylacyl) glycerophosphocholine and glycerophosphoethanolamine, respectively.

Introduction

Tuna comprises any of seven species of oceanic migratory endothermic fishes that constitute the genus *Thunnus* and are of great commercial value as food. They are related to mackerels and are commonly placed with them in the family Scombridae (order Perciformes). The giant of the group is the Northern bluefin tuna (*Thunnus thynnus thynnus* L.), which grows to a maximun length and weight of about 4.3 m and 800 kg, respectively. Tunas are of mayor importance in fisheries at high level of economic value and many organizations have much interest in studying the feasibility of developing the aquaculture technology for these species (Lee 1998; Iioka 1999) and protection, since natural stocks are low mainly because of overfishing. Moreover, at the meeting of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) held in 1992, there was a proposal that the bluefin tuna in the Atlantic Ocean should be listed as the endangered species.

The biology of bluefin tuna caught in the traps of the South Atlantic coast of Spain and Strait of Gibraltar was thoroughly studied from 1956 to 1961 by Rodríguez-Roda (1964). This study described in the reproductive migration two kinds of tuna in the expression of total weight, the tuna of 'derecho' (coming season) and the 'revés' (returning season). The first arriving in April, May and June (direction Atlantic to Mediterranean) very fat with gonads ripening or in the pre-spawning state, and the second during July and August (direction Mediterranean to Atlantic) very thin and with gonads in a post-spawning state. The tuna lost 14.73% of its weight between the coming and returning season (Rodríguez-Roda, 1964).

Seasonal variations in lipid levels in fish are fundamentally related to the reproductive cycle, since most marine fish generally accumulate large lipid deposits during sexual maturation prior to gonad development. This lipid reserve is subsequently used as metabolic energy during the spawning migration but in the female broodfish it is largely mobilised and transferred into the developing roe (Bell 1998). Therefore, egg lipids are derived from dietary lipids, lipids mobilized from body reserves and lipids synthesized *de novo*, and delivered to the oocyte by vitellogenin (rich in polar lipids) and other lipoproteins (rich in neutral lipids mainly TAG) (Wiegand 1996). The quantity and quality of lipid, in terms of energy provision and highly unsaturated fatty acids (HUFA) content, deposited in the eggs and subsequent larvae will be related to the nutritional input during the pre-spawning feeding period of the maturing fish, and derived directly from the broodstock lipid reserves in the period preceding gonadogenesis (Sargent 1995; Bell 1998). Lipid reserves in teleost eggs are stored in lipoprotein yolk and, in some species, a discrete oil globule. Lipoprotein yolk lipids are primarily polar lipids, especially phosphatidylcholine PC and phosphatidylethanolamine PE, and are rich in (n-3) HUFA, especially docosahexaenoic acid (DHA; 22:6n-3). Oil globule consists of neutral lipids (TAG, SE, WE) and is rich in monounsaturated fatty acids (Wiegand 1996). Thus, the essential fatty acids vital for early survival and development of newly hatched larvae, in both wild and farmed fish, are derived directly from the broodstock lipid reserves in the period preceding gonadogenesis (Sargent 1995; Wiegand 1996; Bell 1998).

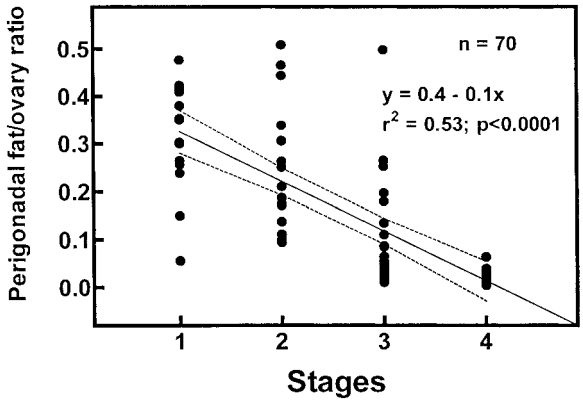

Figure 1. Ratio of mesenteric perigonadal fat weight to ovary weight throughout different maturational stages in female of bluefin tuna during spawning migration from Atlantic to Mediterranean spawning grounds.

Seasonal changes in the lipid composition in relation to gonad maturation (Henderson et al. 1984; Henderson and Almatar 1989) and/or the composition, accumulation and utilization of yolk lipids (Sargent 1995; Wiegand 1996; Bell 1998) have been studied in various fish species, but no studies about this subjects have been undertaken with regard to tunas. A few chemoecological studies, especially in relation to commercial exploitation, have dealt with the lipid and fatty acid contents in prey species and organs of tunids of commercial interest (Saito et al. 1995; Ishihara and Saito 1996; Murase and Saito 1996; Saito and Ishihara 1996; Saito et al. 1996, 1997) but little is known about the ecological biochemistry and energetics of bluefin tuna, especially in relation to sexual migration and maturation.

Table 1. Characterization of maturational stages by means of percentages of oocyte developmental stages, indicating fish weight, gonadosomatic and hepatosomatic indices and dry matter, total lipid and vitamin E contents in ovary, mesenteric perigonadal fat, liver, red and white muscle from Northern female bluefin tuna (*Thunnus thynnus thynnus* L.) during migration to Mediterranean spawning grounds through the Strait of Gibraltar

Stages	I	II	III	IV
Oocyte developmental stages (%)				
Previtellogenic	96.6	88.0	88.9	80.6
Early vitellogenic	3.4	8.4	7.9	14.3
Late vitellogenic	0.0	3.6	2.5	4.2
Migratory nucleus (hydrated)	0.0	0.0	0.7	0.9
Fish total mass (kg)	148	185	134	184
Gonado-somatic index (GSI)	0.66	2.01	4.24	4.68
Hepato-somatic index (HSI)	0.72	0.58	1.10	0.91
Ovary				
Dry mass (%)	21.2 ± 0.1^{a}	33.7 ± 2.2^{b}	34.7 ± 0.8^{b}	27.6 ± 0.6^{c}
Total lipid (dry mass %)	7.3 ± 0.3^{a}	17.2 ± 0.2^{b}	20.9 ± 0.8^{c}	23.9 ± 0.7^{d}
Vitamin E (ng/mg dry mass)	127.9 ± 12.5^{a}	296.2 ± 31.2^{b}	258.6 ± 17.5^{b}	250.3 ± 18.5^{b}
Mesenteric perigonadal fat				
Perigonadal fat weight/ovary weight ratio	0.31 ± 0.11^{a}	0.25 ± 0.14^{a}	0.10 ± 0.10^{b}	0.02 ± 0.01^{b}
Dry mass %	85.8 ± 5.7^{a}	82.6 ± 4.7^{a}	90.7 ± 1.5^{a}	48.5 ± 5.5^{b}
Total lipid (dry mass %)*	70.8 ± 3.3	78.4 ± 1.5	75.1 ± 2.9	75.0 ± 2.4
Liver				
Dry mass (%)	25.3 ± 1.0^{a}	17.8 ± 1.0^{b}	16.7 ± 0.4^{b}	21.8 ± 2.1^{c}
Total lipid (dry mass %)	20.2 ± 1.1^{a}	24.9 ± 0.3^{b}	18.4 ± 0.8^{a}	18.9 ± 0.3^{a}
Vitamin E (μg/mg dry mass)	2.6 ± 0.4^{a}	34.9 ± 1.3^{b}	12.3 ± 1.0^{c}	18.4 ± 0.3^{d}
Red muscle				
Dry mass (%)	21.1 ± 0.6^{a}	23.4 ± 1.5^{b}	20.5 ± 0.5^{a}	19.4 ± 0.2^{a}
Total lipid (dry mass %)	13.6 ± 0.7	14.3 ± 1.4	13.2 ± 0.5	12.6 ± 0.6
White muscle				
Dry mass (%)	21.1 ± 0.6^{a}	20.1 ± 0.7^{ab}	21.4 ± 1.1^{a}	18.5 ± 0.8^{b}
Total lipid (dry mass %)	3.9 ± 0.2^{a}	4.4 ± 0.5^{a}	4.5 ± 0.3^{a}	8.4 ± 0.6^{b}

Data are means $\pm$ SD ($n = 3$). Values within the same row and identical superscript letter are not significantly different ($P < 0.05$). *TAG > 76.9%

The present study was undertaken to examine and define the changes in the lipid content, lipid class and fatty acid compositions in ovary, liver, dark muscle, white muscle and fat deposists of female Atlantic bluefin tuna (*T. thynnus*) throughout the period when the fish are developing gonads during migration to the Mediterranean spawning grounds through the Strait of Gibraltar.

Materials and methods

Sample collection

The specimens used in the present study were collected during spring and early summer 1999 in waters off two Spanish locations. Bluefin tuna females at maturational stages I, II and III were caught by tuna traps ('almadrabas') off Barbate (Cádiz, Atlantic Southern Spain) and stage IV by means of purse seine in the Mediterranean spawning grounds (Balearic Islands). Shortly after capture (within 1–3 h) fishes were weighted to the nearest kg and the livers and ovaries (stripped of perigonadal fat) dissected out and weighted to the nearest kg. Small pieces of ovary, liver,

Table 2. Lipid class composition (percentage of total lipid) of red muscle and white muscle from female bluefin tuna (*Thunus thynus*) throughout sexual maturation during migration to Mediterranean spawning grounds

Sample	I	II	III	IV
Red muscle				
Total polar lipid	40.6 ± 1.0^{ab}	29.4 ± 2.5^{bc}	46.1 ± 4.2^{a}	27.3 ± 5.2^{c}
Phosphatidylcholine	15.6 ± 0.3^{ab}	12.1 ± 1.3^{b}	17.9 ± 0.7^{a}	11.8 ± 2.1^{b}
Phosphatidylethanolamine	8.4 ± 0.1^{ab}	4.8 ± 1.2^{c}	9.5 ± 1.0^{a}	5.4 ± 1.2^{bc}
Phosphatidylserine	1.5 ± 0.1^{ab}	0.8 ± 0.2^{b}	1.8 ± 0.4^{a}	0.7 ± 0.1^{b}
Phosphatidylinositol	2.2 ± 0.2	2.0 ± 0.4	2.4 ± 0.4	2.5 ± 0.3
Phosphatidic acid/cardiolipin	7.9 ± 0.6^{a}	4.8 ± 0.6^{b}	8.6 ± 0.9^{a}	1.5 ± 0.8^{c}
Sphingomyelin	1.1 ± 0.0	1.2 ± 0.5	1.4 ± 0.1	1.1 ± 0.3
Others	4.0 ± 0.5	3.7 ± 1.4	4.5 ± 0.7	4.4 ± 0.7
Total neutral lipid	59.4 ± 1.0^{bc}	70.6 ± 2.5^{ab}	53.9 ± 4.2^{c}	72.4 ± 5.3^{a}
Sterol	7.7 ± 0.7^{a}	6.1 ± 0.4^{ab}	7.5 ± 0.8^{ab}	5.5 ± 0.7^{b}
Free fatty acid	9.7 ± 0.4^{a}	5.4 ± 0.6^{b}	5.7 ± 0.2^{b}	5.7 ± 1.3^{b}
Triacylglycerol	39.1 ± 0.4^{b}	57.1 ± 2.8^{a}	37.3 ± 5.3^{b}	59.8 ± 5.6^{a}
Steryl/wax ester	2.2 ± 0.1	1.4 ± 0.6	2.2 ± 0.5	1.4 ± 0.2
Others	0.6 ± 0.1^{b}	0.5 ± 0.0^{b}	1.2 ± 0.2^{a}	n.d.
White muscle				
Total polar lipid	6.3 ± 1.8^{d}	54.7 ± 2.7^{a}	46.6 ± 0.9^{b}	31.0 ± 2.4^{c}
Phosphatidylcholine	7.1 ± 0.6^{d}	25.9 ± 1.3^{a}	21.8 ± 0.2^{b}	9.9 ± 0.6^{c}
Phosphatidylserine	0.8 ± 0.2^{b}	2.5 ± 0.1^{a}	2.5 ± 0.6^{a}	2.8 ± 0.5^{a}
Phosphatidylinositol	1.3 ± 0.2^{c}	4.7 ± 0.3^{a}	4.9 ± 0.4^{a}	2.7 ± 0.4^{b}
Phosphatidic acid/cardiolipin	1.1 ± 0.4^{b}	4.2 ± 0.1^{a}	3.3 ± 0.5^{a}	1.7 ± 0.5^{b}
Phosphatidylethanolamine	3.2 ± 0.6^{c}	10.4 ± 0.1^{a}	8.6 ± 0.3^{a}	5.6 ± 0.4^{b}
Sphingomyelin	0.8 ± 0.1^{c}	3.3 ± 0.3^{a}	3.4 ± 0.2^{a}	2.1 ± 0.2^{b}
Others	2.0 ± 0.5	3.8 ± 0.9	2.2 ± 0.7	6.2 ± 1.1
Total neutral lipid	83.7 ± 1.8^{a}	45.2 ± 2.7^{d}	53.4 ± 0.9^{c}	68.7 ± 2.4^{b}
Sterol	3.6 ± 0.3^{d}	9.5 ± 0.3^{a}	8.1 ± 0.5^{b}	6.1 ± 0.1^{c}
Free fatty acid	1.6 ± 0.6^{c}	3.5 ± 0.4^{b}	1.8 ± 0.1^{c}	6.0 ± 0.6^{a}
Triacylglycerol	76.7 ± 3.0^{a}	27.3 ± 3.4^{d}	42.5 ± 1.1^{c}	54.6 ± 2.1^{b}
Steryl/wax ester	1.1 ± 0.1^{b}	4.2 ± 1.6^{a}	1.0 ± 0.5^{b}	1.6 ± 0.7^{ab}
Others	0.7 ± 0.4	0.7 ± 0.2	n.d.	tr

Results are mean ± SD ($n = 3$). Mean values within the same row and identical superscript letter are not significantly different ($P < 0.05$). n.d., not detected; tr, trace <0.5. Totals include some minor components not shown.

dark muscle, white muscle and mesenteric perigonadal fat were obtained and blotted in filter paper before being frozen in liquid nitrogen and stored in the freezer at −80 °C until analysis. In parallel, 0.5 cm cross sections from the broadest part of one of the lobes of the ovary (situated at about one third of its length from the rostral end) were used for histological examination. The classification of developing oocytes and ovaries was based upon the scheme used by Farley and Davies (1998) for Southern bluefin tuna (*Thunnus maccoyii*). Four oocyte developmental stages were disthinguished: previtellogenic, early vitellogenic, late vitellogenic and migratory nucleus (hydrated). According to the presence (%) of each oocyte type 4 different maturational stages were stablished for the present study. The gonadosomatic and hepatosomatic indices (GSI and HSI) were calculated according to the formula: organ index (%) = (organ weight/total body weight) ×100. Perigonadal fat weight to ovary weight ratios were also recorded for the different stages of maturation.

Dry weight determination

Replicates of preweighed samples (approximately 500 mg wet weight) were maintained at 110 °C for

Table 3. Lipid class composition (percentage of total lipid) of ovary and liver from female bluefin tuna (*Thunus thynus*) throughout sexual maturation during migration to Mediterranean spawning grounds

Stage	I	II	III	IV
Ovary				
Total polar lipid	35.5 ± 1.1^{a}	23.2 ± 0.6^{b}	26.8 ± 3.4^{b}	23.8 ± 1.1^{b}
Phosphatidylcholine	12.2 ± 0.7	11.8 ± 0.6	14.7 ± 1.8	12.3 ± 0.3
Phosphatidylethanolamine	7.6 ± 0.1^{a}	4.0 ± 0.4^{b}	5.0 ± 0.4^{b}	4.2 ± 0.2^{b}
Phosphatidylserine	3.0 ± 0.2^{a}	1.1 ± 0.3^{b}	1.0 ± 0.2^{b}	0.8 ± 0.1^{b}
Phosphatidylinositol	3.4 ± 0.1^{a}	2.2 ± 0.1^{ab}	2.2 ± 0.6^{ab}	1.7 ± 0.3^{b}
Phosphatidic acid/cardiolipin	2.0 ± 0.3	1.4 ± 0.2	1.4 ± 0.4	1.5 ± 0.2
Sphingomyelin	3.0 ± 0.2^{a}	1.7 ± 0.2^{ab}	1.1 ± 0.1^{b}	1.2 ± 0.0^{ab}
Others	4.2 ± 0.4^{a}	1.1 ± 0.4^{b}	1.5 ± 0.3^{b}	1.9 ± 0.7^{b}
Total neutral lipid	64.4 ± 1.2^{b}	76.8 ± 0.6^{a}	73.2 ± 3.4^{a}	76.2 ± 1.1^{a}
Sterol	10.6 ± 0.3^{a}	7.2 ± 0.9^{b}	7.5 ± 0.4^{b}	6.4 ± 0.6^{b}
Free fatty acid	4.2 ± 0.6^{a}	1.1 ± 0.2^{c}	1.1 ± 0.2^{c}	2.4 ± 0.3^{b}
Triacylglycerol	30.1 ± 0.5^{a}	27.7 ± 0.5^{ab}	24.0 ± 1.0^{c}	25.8 ± 1.6^{bc}
Steryl/wax ester	18.6 ± 0.1^{a}	40.9 ± 0.8^{a}	40.6 ± 2.8^{a}	41.0 ± 0.2^{a}
Others	0.9 ± 0.2	n.d.	n.d.	0.5 ± 0.7
Liver				
Total polar lipid	33.5 ± 2.0^{c}	37.0 ± 1.2^{bc}	47.4 ± 1.7^{a}	39.6 ± 2.0^{b}
Phosphatidylcholine	11.4 ± 0.8^{b}	13.0 ± 0.2^{b}	18.3 ± 0.5^{a}	16.9 ± 0.5^{a}
Phosphatidylethanolamine	6.7 ± 0.2^{b}	7.1 ± 0.6^{ab}	8.7 ± 0.4^{a}	6.9 ± 0.8^{b}
Phosphatidylserine	2.8 ± 0.3^{a}	2.0 ± 0.2^{bc}	2.3 ± 0.1^{ab}	1.7 ± 0.1^{c}
Phosphatidylinositol	2.8 ± 0.3^{c}	3.6 ± 0.1^{b}	5.2 ± 0.2^{a}	3.9 ± 0.3^{b}
Phosphatidic acid/cardiolipin	2.2 ± 0.1^{b}	3.4 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.3^{a}
Sphingomyelin	2.6 ± 0.2^{a}	1.9 ± 0.1^{b}	2.4 ± 0.2^{ab}	1.3 ± 0.1^{c}
Other	5.1 ± 0.5	6.1 ± 0.7	7.0 ± 1.1	5.9 ± 0.6
Total neutral lipid	66.5 ± 2.0^{a}	63.0 ± 1.2^{ab}	52.0 ± 0.9^{c}	60.4 ± 2.0^{b}
Sterol	9.1 ± 0.4	7.7 ± 0.1	9.7 ± 0.6	9.2 ± 1.6
Free fatty acid	5.1 ± 0.8^{b}	13.7 ± 1.1^{a}	10.1 ± 0.8^{a}	11.6 ± 1.8^{a}
Triacylglycerol	40.2 ± 1.8^{a}	18.9 ± 0.1^{b}	11.8 ± 1.4^{c}	18.3 ± 1.6^{b}
Steryl/wax ester	11.9 ± 2.1^{b}	22.3 ± 2.3^{a}	19.9 ± 1.7^{a}	19.9 ± 3.8^{ab}
Others	tr	tr	0.5 ± 0.1	1.4 ± 0.2

Results are mean ± SD ($n = 3$). Mean values within the same row and identical superscript letter are not significantly different ($P < 0.05$). tr, trace < 0.5. Totals include some minor components not shown.

24 h. The dry weights were determined after cooling *in vacuo* for at least 1 h.

Total lipid extraction, lipid class separation and quantification

Total lipid was extracted after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957), and gravimetrically quantified. Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) on silica gel 60 plates, using a single-dimension double-development method described previously (Tocher and Harvie 1988; Olsen and Henderson 1989). The classes were quantified by charring (Fewster et al. 1969) followed by calibrated densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (Olsen and Henderson 1989).

Fatty acid analyses

Fatty acid methyl esters (FAME) from total lipids were prepared by acid-catalyzed transmethylation for 16 h at 50 °C, using tricosanoic acid (23:0) as internal standard (Christie 1989). FAME were extracted and purified as described previously (Tocher and Harvie 1988) and were separated in a Hewlett-Packard 5890A

Table 4. Total lipid fatty acid composition (mass %) of ovary from female bluefin tuna (*Thunus thynus*) throughout sexual maturation during migration to Mediterranean spawning grounds

Fatty acid	I	II	III	IV
14:0	0.9 ± 0.1	1.9 ± 0.4	1.1 ± 0.4	1.4 ± 0.2
15:0	1.9 ± 0.8	3.1 ± 0.1	2.1 ± 0.9	2.1 ± 0.4
16:0	17.4 ± 0.5^{a}	11.9 ± 0.4^{c}	13.0 ± 0.3^{c}	15.0 ± 0.3^{b}
18:0	5.6 ± 0.2^{a}	3.7 ± 0.1^{c}	4.2 ± 0.2^{c}	4.9 ± 0.2^{b}
Total saturated	26.4 ± 1.2^{a}	20.9 ± 0.3^{c}	20.7 ± 0.7^{c}	23.6 ± 0.6^{b}
16:1(n-7)	1.9 ± 0.1^{b}	2.7 ± 0.0^{a}	2.1 ± 0.1^{b}	2.7 ± 0.2^{a}
18:1(n-11)	0.1 ± 0.2^{b}	1.2 ± 0.1^{a}	0.8 ± 0.6^{ab}	0.9 ± 0.2^{ab}
18:1(n-9)	11.6 ± 0.3^{d}	13.6 ± 0.4^{c}	20.2 ± 0.5^{ab}	18.7 ± 0.5^{b}
18:1(n-7)	2.6 ± 0.0^{a}	2.5 ± 0.0^{a}	2.3 ± 0.1^{b}	2.5 ± 0.1^{a}
20:1(n-9)	1.4 ± 0.2^{ab}	1.9 ± 0.1^{a}	1.4 ± 0.1^{ab}	0.9 ± 0.3^{b}
22:1(n-11)	0.1 ± 0.1^{b}	0.8 ± 0.1^{a}	0.3 ± 0.0^{ab}	0.1 ± 0.0^{b}
Total monoenes	18.1 ± 0.6^{c}	23.3 ± 0.7^{b}	27.5 ± 0.3^{a}	26.0 ± 1.0^{a}
18:2(n-6)	1.0 ± 0.0^{b}	1.4 ± 0.1^{a}	1.0 ± 0.0^{b}	1.2 ± 0.1^{b}
18:3(n-6)	n.d.	1.1 ± 0.1^{a}	0.6 ± 0.4^{ab}	0.1 ± 0.1^{b}
18:3(n-3)	0.4 ± 0.1^{b}	0.8 ± 0.3^{a}	0.5 ± 0.0^{ab}	0.6 ± 0.0^{ab}
18:4(n-3)	0.8 ± 0.4^{b}	3.0 ± 0.1^{a}	3.6 ± 0.2^{ab}	3.5 ± 0.5^{a}
20:2(n-6)	1.6 ± 0.7	1.3 ± 0.1	1.5 ± 0.8	0.6 ± 0.1
20:4(n-6)	3.5 ± 0.1^{a}	1.9 ± 0.1^{b}	1.2 ± 0.0^{c}	1.2 ± 0.0^{c}
20:4(n-3)	0.7 ± 0.2	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
20:5(n-3)	7.8 ± 0.6^{a}	7.2 ± 0.2^{a}	4.8 ± 0.1^{b}	5.2 ± 0.3^{b}
22:5(n-6)	1.1 ± 0.0	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.4
22:5(n-3)	1.4 ± 0.1^{a}	0.8 ± 0.2^{b}	1.3 ± 0.0^{a}	1.6 ± 0.1^{a}
22:6(n-3)	25.1 ± 0.8^{a}	21.7 ± 1.2^{b}	23.4 ± 0.6^{ab}	23.2 ± 0.4^{ab}
Total polyenes	47.6 ± 1.6^{a}	44.3 ± 0.9^{ab}	42.1 ± 1.1^{b}	40.9 ± 1.1^{b}
Total (n-9)	13.3 ± 0.3^{d}	16.1 ± 0.5^{c}	22.5 ± 0.6^{a}	20.3 ± 0.8^{b}
Total (n-7)	4.9 ± 0.1^{b}	5.9 ± 0.1^{a}	4.9 ± 0.1^{b}	5.5 ± 0.2^{a}
Total (n-6)	9.0 ± 0.9^{a}	7.5 ± 0.3^{a}	5.7 ± 0.1^{b}	4.4 ± 0.5^{b}
Total (n-3)	38.3 ± 0.8^{a}	36.2 ± 0.8^{ab}	35.3 ± 1.1^{b}	35.8 ± 0.6^{ab}
HUFA(n-6)	5.2 ± 0.6^{a}	3.4 ± 0.3^{b}	2.3 ± 0.4^{b}	2.2 ± 0.4^{b}
HUFA(n-3)	35.6 ± 0.7^{a}	31.5 ± 1.0^{b}	30.5 ± 0.9^{b}	31.1 ± 0.3^{b}
DHA/EPA	3.2 ± 0.3^{b}	3.0 ± 0.2^{b}	4.9 ± 0.1^{a}	4.5 ± 0.3^{a}

Results are mean $\pm$ SD ($n = 3$). Mean values within the same row and identical superscript letter are not significantly different ($P < 0.05$). tr, trace < 0.5. Totals include some minor components not shown.

Series II gas chromatograph equipped with a chemically bonded (PEG) Supelcowax-10 fused silica wall coated capillary column (30 m $\times$ 0.32 mm i.d., Supelco Inc., Bellefonte, USA), 'on column' injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50 °C to 180 °C at 25 °C/min and then to a final temperature of 235 °C at 3 °C/min. The final temperature was maintained for 10 min. Individual FAME were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard ChemStation software.

Determination of vitamin E content

Vitamin E concentrations (as tocopherol plus α-tocopheryl esters) were measured in tissue samples using high-performance liquid chromatography (HPLC). Samples were weighed, homogenized and saponified as described by Bieri (1969), but using a single-step hexane extraction (Bell et al. 1987). HPLC analysis was performed using a 250 $\times$ 2 mm reverse phase

Table 5. Total lipid fatty acid composition (mass %) of liver from female bluefin tuna (*Thunus thynus*) throughout sexual maturation during migration to Mediterranean spawning grounds

Fatty acid	I	II	III	IV
14:0	0.7 ± 0.0^{b}	0.9 ± 0.1^{a}	0.6 ± 0.0^{b}	1.0 ± 0.0^{a}
15:0	1.7 ± 0.3	1.9 ± 0.3	1.8 ± 0.4	1.5 ± 0.3
16:0	14.9 ± 0.2^{d}	21.3 ± 0.1^{a}	19.0 ± 0.1^{c}	20.5 ± 0.2^{b}
17:0	0.5 ± 0.0^{a}	0.2 ± 0.1^{b}	0.3 ± 0.1^{ab}	0.4 ± 0.0^{ab}
18:0	9.0 ± 0.2^{c}	10.0 ± 0.1^{b}	11.7 ± 0.1^{a}	11.4 ± 0.2^{a}
Total saturated	26.9 ± 0.6^{b}	34.8 ± 0.3^{a}	33.6 ± 0.3^{a}	35.0 ± 0.7^{b}
16:1(n-7)	2.3 ± 0.0^{a}	2.2 ± 0.1^{a}	1.4 ± 0.0^{c}	1.8 ± 0.0^{b}
18:1(n-9)	16.7 ± 0.2^{a}	15.7 ± 0.1^{b}	15.1 ± 0.3^{c}	14.1 ± 0.0^{d}
18:1(n-7)	3.9 ± 0.1^{a}	3.6 ± 0.1^{b}	2.9 ± 0.0^{c}	2.9 ± 0.0^{c}
20:1(n-9)	2.1 ± 0.1^{b}	4.0 ± 0.0^{a}	1.5 ± 0.1^{d}	1.8 ± 0.2^{c}
22:1(n-11)	0.9 ± 0.1^{b}	2.5 ± 0.1^{a}	0.4 ± 0.1^{c}	0.6 ± 0.1^{c}
24:1(n-9)	0.6 ± 0.0^{c}	1.1 ± 0.1^{a}	0.8 ± 0.0^{b}	0.6 ± 0.0^{c}
Total monoenes	27.1 ± 0.4^{b}	29.8 ± 0.5^{a}	22.4 ± 0.1^{c}	22.0 ± 0.1^{c}
18:2(n-6)	1.2 ± 0.0^{a}	1.3 ± 0.0^{a}	0.9 ± 0.0^{b}	1.0 ± 0.0^{b}
18:3(n-3)	0.7 ± 0.0^{a}	0.5 ± 0.0^{c}	0.5 ± 0.0^{c}	0.6 ± 0.2^{b}
20:2(n-6)	0.8 ± 0.0	tr	0.9 ± 0.0	0.9 ± 0.1
20:4(n-6)	2.5 ± 0.1^{b}	2.2 ± 0.1^{c}	2.9 ± 0.1^{a}	2.7 ± 0.1^{ab}
20:4(n-3)	1.1 ± 0.0^{a}	0.4 ± 0.0^{b}	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
20:5(n-3)	9.2 ± 0.1^{a}	3.1 ± 0.1^{d}	4.5 ± 0.0^{c}	5.5 ± 0.1^{b}
22:5(n-6)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	tr
22:5(n-3)	2.1 ± 0.1^{a}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}
22:6(n-3)	20.4 ± 0.5^{c}	18.2 ± 0.3^{d}	25.8 ± 0.3^{a}	23.8 ± 0.4^{b}
Total polyenes	42.0 ± 0.9^{a}	29.7 ± 0.5^{c}	39.5 ± 0.7^{b}	38.9 ± 0.5^{b}
Total (n-9)	19.6 ± 0.2^{b}	20.9 ± 0.2^{a}	17.9 ± 0.1^{c}	17.0 ± 0.3^{d}
Total (n-7)	6.5 ± 0.2^{a}	6.2 ± 0.2^{a}	4.3 ± 0.0^{b}	4.6 ± 0.0^{b}
Total (n-6)	6.0 ± 0.1^{a}	5.0 ± 0.1^{b}	5.7 ± 0.1^{a}	5.7 ± 0.2^{a}
Total (n-3)	35.7 ± 0.7^{a}	24.5 ± 0.5^{c}	33.3 ± 0.6^{b}	32.7 ± 0.7^{b}
HUFA(n-6)	3.5 ± 0.1^{ab}	3.1 ± 0.1^{c}	3.7 ± 0.0^{a}	3.3 ± 0.1^{bc}
HUFA(n-3)	33.4 ± 0.7^{a}	23.3 ± 0.4^{c}	31.9 ± 0.4^{ab}	30.9 ± 0.5^{b}
DHA/EPA	2.2 ± 0.0^{c}	5.8 ± 0.0^{a}	5.7 ± 0.0^{a}	4.3 ± 0.0^{b}

Results are mean $\pm$ SD ($n = 3$). Mean values within the same row and identical superscript letter are not significantly different ($P < 0.05$). tr, trace <0.5. Totals include some minor components not shown.

Spherisorb ODS2 column (Sigma Chemical Co, St. Louis MO, USA) essentially as described by Carpenter (1979). The mobile phase was 98% methanol pumped at 0.2 ml/min, the effluent from the column was monitored at a UV wavelength of 293 nm and quantification achieved by comparison with ($\pm$)-α-tocopherol (Sigma Chemical CO, St. Louis, MO, USA) as external standard (10 μg/ml).

Statistical analysis

Results are presented as means $\pm$ SD ($n = 3$). The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analysis. Differences between mean values were analyzed by one-way analysis of variance (ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$. The relation between perigonadal fat/ovary ratios and maturational stages was studied by means of linear regression analysis (Zar 1984).

Materials

Potassium bicarbonate, potassium chloride, BHT and tricosanoic acid (>99% pure) were from Sigma Chemical Co. (Alcobendas, Madrid, Spain). HPTLC (10 × 10 cm ×0.15 mm) plates precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid, sulphuric acid and all solvents (HPLC grade) were purchased from Fluka Chemicals Co. (Alcobendas, Madrid, Spain).

Results

Deatails of the characterization of maturational stages in ovary by means of percentages of oocyte developmental stages, fish weight, gonadosomatic and hepatosomatic indices and dry matter percentages, total lipid and vitamin E contents in ovary, mesenteric perigonadal fat, liver, dark muscle and white muscle from female bluefin tuna (*T. thynnus*) during migration to Mediterranean spawning grounds are presented in Table 1. The ovaries varied from inmature at Stage I (where only previtellogenic and early vitellogenic oocytes are present), through Stages II and III that can be considered as mature (with late vitellogenic oocytes but no evidence of spawning activity) with Stage III being a more advanced stage of maturation, to Stage IV that could be considered as spawning although few postovulatory follicles were observed and so it may be more correct to asign a pre-spwaning stage to Stage IV. Compared to Stage I, the gonadosomatic index increased by 3-fold at Stage II, by 6.4 at Stage III and by 7 at Stage IV where the ovary comprised 4.7% of fish total body weight. On the other hand, the hepatosomatic index showed little variations and values were around 1 in most cases.

The ovary dry matter content increased significantly, by approximately 38%, from Stage I to Stages II and III and then decreased significantly by 20.5% at Stage IV as a result of the hydratation process that occurs at spawning. Ovary total lipid content increased significantly by 57.5% from Stage I to Stage II, by 17.7% from Stage II to Stage III and by 12.5% from Stage III to Stage IV. This represent a 3.3-fold increase overall from inmature to spawning ovary and, in absolute terms, a 37.6-fold increase, reaching values over 500 g of total lipids per ovary at spawning. Vitamin E content in the ovary increased significantly from Stage I to Stage II (2.3-fold) and then remained constant till spawning. Tha ratio of mesenteric perigonadal fat weight to ovary weight decreased linearly ($y = 0.4 - 0.1x$; $r^2 = 0.53$, $P < 0.0001$; $n = 70$) throughout maturation from values of over 30% to almost nothing at spawning (Figure 1). The dry mass content of the perigonadal fat did not change during the first 3 maturational stages (ranging from 82.6% to 90.7%) but decreased significantly at Stage IV (only 48.5%). Total lipid content of the perigonadal fat on a dry mass basis was about 75% during all stages and with a minimun triacyglycerol content of 76.9% (Table 1).

In contrast, liver dry matter content decreased significantly, by approximately 32%, from Stage I to Stages II and III and then increased significantly by aproximately 21% at Stage IV. Liver total lipid content increased significantly by 19% from Stage I to Stage II, and then decreased significantly by 25% to Stages III and IV. Liver vitamin E content was about one thousand times more concentrated than in ovary and increased by 93% from Stage I to Stage II, then decreased by 65% to Stage III, and finally increased by 33% from Stage III to Stage IV.

Female bluefin tuna dark (or dark red) muscle dry matter content showed a slight but significantly increase by 9.8% from Stage I to Stage II but then returned to the same level as at Stage I during Stages III and IV. Total lipid content in dark muscle did not show any variation during maturation and its value was about 13% on a dry matter basis. Lipid class composition of dark red muscle showed that neutral lipids (53.9% to 72.4%) predominated over polar lipids (27.3% to 46.1%). Triacylglycerol (37.3% to 59.8%) was the primary lipid class with the proportion of free fatty acids also being relatively high (5.4% to 9.7%) (Table 2).

White muscle (or semi red) showed values of dry matter content of about 20% from Stages I to III and then decreased signeficantly by 13.5% from Stage III to Stage IV. On the other hand, total lipid content did not vary from Stage I to III and then increased significantly at Stage IV, from 3.9–4.5% to 8.4% (Table 1). Lipid class composition in tuna white (or semi red) muscle showed that total neutral lipids (45.2% to 83.7%) usually predominated over polar lipids (16.3% to 54.7%), and was mostly triacylglycerol (27.3% to 76.7%) (Table 2).

Lipid class composition (as percentages of total lipid) of ovary from bluefin tuna (*T. thynnus*) throughout sexual maturation is described in Table 3. Total neutral lipid predominated over total polar lipids and

Table 6. Total lipid fatty acid composition (mass %) of mesenteric perigonadal fat from female bluefin tuna (*Thunus thynus*) throughout sexual maturation during migration to Mediterranean spawning grounds

Fatty acid	I	II	III	IV
14:0	2.1 ± 0.1^{b}	2.4 ± 0.1^{a}	1.9 ± 0.1^{b}	1.1 ± 0.1^{c}
15:0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.2
16:0	13.8 ± 0.1^{a}	13.7 ± 0.2^{a}	14.6 ± 0.1^{a}	9.5 ± 0.5^{b}
17:0	tr	tr	0.5 ± 0.0	0.5 ± 0.1
18:0	4.5 ± 0.1^{c}	5.1 ± 0.1^{b}	5.3 ± 0.1^{b}	9.1 ± 0.1^{a}
Total saturated	21.8 ± 0.2^{b}	22.7 ± 0.3^{a}	23.4 ± 0.1^{a}	21.8 ± 0.3^{b}
16:1(n-7)	3.4 ± 0.0^{a}	3.4 ± 0.1^{a}	3.5 ± 0.1^{a}	1.7 ± 0.1^{b}
18:1	28.7 ± 0.1^{b}	25.4 ± 0.2^{c}	25.9 ± 0.2^{a}	31.6 ± 0.4^{a}
20:1(n-9)	6.0 ± 0.1^{c}	8.3 ± 0.2^{b}	5.4 ± 0.2^{c}	11.6 ± 0.4^{a}
22:1(n-11)	5.2 ± 0.1^{c}	8.5 ± 0.4^{c}	4.5 ± 0.2^{c}	13.0 ± 0.7^{a}
24:1(n-9)	0.7 ± 0.3^{b}	0.9 ± 0.0^{b}	0.7 ± 0.4^{b}	2.5 ± 0.2^{a}
Total monoenes	44.0 ± 0.5^{c}	46.5 ± 0.4^{b}	40.1 ± 0.8^{d}	60.4 ± 0.9^{a}
18:2(n-6)	1.2 ± 0.0^{a}	1.2 ± 0.0^{a}	1.3 ± 0.0^{a}	1.0 ± 0.1^{b}
18:3(n-3)	0.6 ± 0.0^{b}	0.6 ± 0.0^{ab}	0.7 ± 0.0^{a}	tr
18:4(n-3)	0.7 ± 0.0	0.6 ± 0.3	0.8 ± 0.0	0.5 ± 0.0
20:4(n-6)	0.9 ± 0.0^{a}	0.8 ± 0.0^{b}	1.0 ± 0.0^{a}	0.8 ± 0.1^{b}
20:4(n-3)	0.7 ± 0.0^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{a}	tr
20:5(n-3)	6.5 ± 0.1^{a}	5.0 ± 0.0^{a}	5.8 ± 0.1^{b}	1.9 ± 0.2^{d}
22:5(n-6)	tr	tr	0.5 ± 0.0^{b}	0.6 ± 0.0^{a}
22:5(n-3)	1.8 ± 0.0^{b}	1.7 ± 0.0^{ab}	1.7 ± 0.0^{a}	1.5 ± 0.1^{a}
22:6(n-3)	14.6 ± 0.4^{b}	13.3 ± 0.3^{b}	17.3 ± 0.6^{ab}	5.2 ± 0.4^{c}
Total polyenes	29.6 ± 0.3^{b}	26.3 ± 0.5^{c}	32.1 ± 0.6^{a}	13.8 ± 0.8^{d}
Total (n-9)	35.4 ± 0.4^{b}	34.6 ± 0.0^{b}	32.1 ± 0.7^{a}	45.7 ± 0.3^{a}
Total (n-7)	3.4 ± 0.0^{a}	3.4 ± 0.1^{a}	3.5 ± 0.1^{c}	1.7 ± 0.1^{b}
Total (n-6)	3.1 ± 0.1^{b}	3.0 ± 0.0^{bc}	3.6 ± 0.0^{a}	2.9 ± 0.0^{c}
Total (n-3)	26.5 ± 0.3^{a}	23.3 ± 0.5^{b}	28.4 ± 0.6^{a}	10.9 ± 0.8^{c}
HUFA(n-6)	1.4 ± 0.0^{bc}	1.3 ± 0.0^{c}	1.6 ± 0.0^{a}	1.5 ± 0.1^{ab}
HUFA(n-3)	24.1 ± 0.3^{a}	21.1 ± 0.3^{b}	25.9 ± 0.6^{a}	9.1 ± 0.7^{c}
DHA/EPA	2.3 ± 0.1^{c}	2.7 ± 0.1^{b}	3.0 ± 0.1^{a}	2.7 ± 0.1^{b}

Results are mean $\pm$ SD ($n = 3$). Mean values within the same row and identical superscript letter are not significantly different ($P < 0.05$). tr, trace <0.5, Totals include some minor components not shown.

its proportion increased significantly from Stage I to Stage II and its value (approximately 75%) remanined constant at Stages III and IV. Total polar lipid proportion showed a concomitant decrease from Stage I to Stage II and then remained constant at Stages III and IV. The significant increase presented by total neutral lipids was due to SE/WE which increased by approximately 55% from Stage I to Stage II and then remained constant thoughout the rest of maturation. In contrast, proportions of TAG and S both showed decreasing trends throughout sexual maturation. The major polar lipid class in ovary was PC, and its proportion remained constant at about 12.7% of total lipids during maturation. In contrast, PE decreased significantly from Stage I to Stage II and then remained constant. The proportions of PS and PI and other minor polar classes also showed downward trends during maturation (Table 3).

The lipid class composition of liver from female bluefin tuna during sexual maturation is also presented in Table 3. Total neutral lipids predominated over total polar lipids and its proportion decreased significantly from Stage I to Stage III and then increased significantly from Stage III to Stage IV due mainly to similar significant changes in the proportions of the major lipid class, TAG. In contrast, total polar

lipids increased significantly from Stage I to Stage III, mainly due to significant increases of 38%, 23% and 46% of PC, PE and PI, respectively. Free fatty acids in tuna liver were higher than the values normally found for this fraction in other tissues and/or organisms. Liver S only accounted for about 10% of total lipids and showed few variations throughout maturation, whereas the proportion of SE/WE increased by 47% from Stage I to Stage II and then remained at this higher level (Table 3).

The total lipid fatty acid composition of ovary from female bluefin tuna throughout maturation is showed in Table 4. The proportion of total saturated fatty acids decreased significantly from Stage I to Stages II and III and then increased from Stage III to Stage IV, mainly due to variations in 16:0 and 18:0. Total monoenes, increased significantly from Stages I to III, remaining at this level during Stage IV, due to increased proportions of 18:1(n-9). The proportion of polyunsaturated fatty acids (PUFA) remained more or less stable, with minor decreases, troughout maturation and accounted for over 40% of total fatty acids with DHA accounting for over half this value. However, in absolute terms arachidonic acid (AA; 20:4n-6) increased 16.7-fold, eicosapentaenoic acid (EPA; 20:5n-3) increased 31.1-fold and DHA 42.3-fold from Stage I to Stage IV, reaching values of 5.0, 21.8 and 97.4 g per ovary, respectively, with a DHA/EPA ratio of up to 5.

Total lipid fatty acid composition of liver from female bluefin tuna during maturation is presented in Table 5. The proportion of total saturated fatty acids increased significantly during maturation due to increased 16:0 and 18:0. Total monoenes , in contrast, decreased significantly due to decreased proportions of 16:1(n-7), 18:1(n-9) and 18:1(n-7). The proportion of PUFA decreased significantly from Stage I to Stage II but increased again at Stages III and IV. This trend was followed by the major PUFA such as DHA, EPA and AA.

The total lipid fatty acid composition of mesenteric perigonadal fat (primarily TAG) is presented in Table 6. Saturated fatty acids accounted for about 20% of total fatty acids, increasing significantly from Stage I to Stage III before decreasing. This trend was shown by 16:0 the major saturated fatty acid but, in contrast, 18:0 showed a significant upward trend throughout all Stages. Total monoenes, predominantly 18:1(n-9), increased from Stage I to Stage II, then decreased from Stage II to Stage III and then finally increased at Stage IV. The proportions of 20:1(n-9) and 22:1(n-11) showed significan increases from Stage I to Stage II, significant decreases from Stage II to Stage III and finally significant increases from Stage III to Stage IV. Total PUFA decreased by 11.1% from Stage I to Stage II, increased by 18% from Stage II to Stage III and then decreased by 57% from Stage III to Stage IV. The major PUFA, DHA and EPA showed similar trends with decreases from Stage III to Stage IV by 70% and 83%, respectively.

Discussion

Tunas have been described as ‘energy speculators’ based on their high rates of energy turnover in a nutrient poor pelagic environment, where prey are patchily distributed and feeding success depends upon the ability to find, capture, and process food items as rapidly as possible. This elevated metabolic rate is sustained by a high aerobic capacity and tunas consume oxygen at 2 to 5 times the rate of other active teleosts, since they must swim continuosly for both ram gill ventilation and to provide the hydrodynamic lift required to maintain stability and position in the water column (Korsmeyer et al. 1996). A certain percentage of ingested energy is lost due to the costs of the mechanical and biochemical processes associated with digestion and assimilation, since tunas can consume up to 30% of their body mass per day (Kitchell et al. 1978). Digestion rates are also high and gut clearance (emptying of gut in about 12 h) is 4 to 5 times faster than for other piscivores of comparable size (Olson and Boggs 1986). Bluefin tuna, which may elevate visceral temperature with vascular retia, may also increase digestive activity to consume large amounts of food and rapidly clear the gut, enabling them to take advantage of encounters with patchily distributed prey aggregations and still meet their high metabolic demands (Olson and Boggs 1986; Korsmeyer et al. 1996). Tunas also have relatively high rates of growth and sexually mature females can devote considerable energy into egg production (Korsmeyer et al. 1996).

Studies during seven years on stomachal content of ‘derecho’ migrating bluefin tuna (coming season) catches in the tuna traps of the Strait of Gibraltar have shown empty stomachs in the migration to the Mediterranean spawning grounds (Rodríguez-Roda 1964). On the other hand, not all migrating fish have the same amount of stored lipid for energy reserves as shown in Table 1 and Figure 1. In fish, lipid storage is partioned among mesenteric fat, liver and muscle. In migrating tuna breeders, the main lipid storage sites

are large depot of perigonadal mesenteric fat together with fat acumulated in muscle. Liver is not so important as a lipid storage organ in this species, but the focal point of organismal lipid processing with a high capacity for *de novo* lipid synthesis. Therefore, the liver plays a major role in processing fatty acids mobilised from other fat depots (mesenteric fat and muscles) prior to their transfer to ovary, and this accounts for the marked transient increase in liver lipid prior to the period of rapid increase in ovary weight. Generally in fish, mesenteric fat is the primary lipid depot and is well suited for long term storage of lipid, whereas lipid storage in liver and muscle is secondary and is probably influenced by life history patterns to a greater extent than that in mesenteric fat (Sheridan 1994). It is not documented if female bluefin tuna feed during their reproductive migration (maturational Stages I, II and III) from the Strait of Gibraltar to the Mediterranean spawning grounds (Stage IV) or only depend on fat stores to obtain the energy required for reproduction.

Medina et al. (2002) have determined an average batch fecundity of 92.8 oocytes per g of body mass indicating an average female bluefin tuna of 150 kg can produce about 8.6 kg of mature ovaries. Taking into account that bluefin tuna can lose about 15% of its weight in passing from 'derecho' (maturation) to 'revés' (post spawn) we can calculate that, in a 'revés' post-spawning female, approximately 50% of the lost weight correspond to spawned ovary and the other 50% to lipid stores, mostly mesenteric perigonadal fat and muscle fat, used for energy.

The female bluefin tuna red muscle comprises only a small portion of the total body mass but has a high fat content ($>12\%$ on a dry matter basis, primarily triacylglycerol), high capillarity, high mitochodrial protein concentration and high myoglobin content which results in the dark red colour (Korsmeyer et al. 1996). Tuna red muscle uses lipids as an energy source via fatty acid oxidation and has a high aerobic potential and metabolic performance to maintain sustained swimming during migration or 'basal' swimming, and maintain elevated temperatures with heat production a by-product of locomotion (Ballantyne 1995).

In contrast, tuna white muscle accounts for about 50% of total body mass and is predominantly anaerobic and fueled by glycogen. However, when compared to the white muscle of other fishes it also has a relatively high aerobic capacity, as reflected in a high intracellular lipid content (about 4% primarily TAG), high capillarity, mitochondrial abundance, and high concentrations of aerobic enzymes (Korsmeyer et al. 1996). The high anaerobic potential of tuna white muscle, suported by a high lactate deshydrogenase activity and buffereing capacity allow extremely high, short duration, 'burst' swimming speed. This anaerobic activity produces large amounts of lactate in white muscle, most of which is resynthesized to glycogen *in situ*, a process that requires ATP by mitochondrial oxidative metabolism. This suggests that the high aerobic capacity of tuna white muscle may be a specialization for rapid repayment of oxygen debt (Korsmeyer et al. 1996). In consequence, since female bluefin tuna mature while migrating, when sustained and 'burst' swimming also have to be maintained, red and white muscles integrity has also to be maintaned during migration, leaving only mesenteric perigonadal fat as the major source of lipids for maturing ovary during migration. Liver lipids did not show a major depletion during maturation compared to that of perigonadal fat. Although little is known about lipid metabolism in tunas, this is likely to be important, given their relatively high-fat diet, high reproductive rates and ability to store and mobilize large amounts of lipid (Dickson 1996).

The fatty acid composition of ovary from female bluefin tuna at Stage IV (spawning) shows that DHA is the most abundant fatty acid, with DHA:EPA:AA ratio of 19.3/4.3/1 and DHA:EPA ratio 4.5/1. The egg spawned by bluefin tuna female is of the high lipid content type (23.9% on a dry matter basis; Table 1) (Finn 1994), with neutral lipids predominating over polar lipids (approx. 3:1), and containing an oil globule (Katabami et al. 1997) with TAG and SE/WE, similar to those of other marine fish species of temperate waters such as sea bream, sole or common dentex (Wiegand 1996; Bell 1998; Mourente et al. 1999). It is really remarkable the great proportion of SE/WE (40% of total lipids) in ovary from Stage II onwards. In liver the proportion of these classes is still high (20%) and very low (about 2%) in red or white muscle. WE have been found in large proportions in roe of scombroid fish (Kaitaranta and Ackman 1981). However, it is not clear why marine fish store in roe large quantities of neutral lipids as both TAG and SE/WE instead of only TAG. WE formation may be a biochemical mechanism for elaborating lipids at unusually high rates from amino acids and glucose precursors. The possible functions of wax esters are for transport and storage molecules (particularly when carbon skeletons are limiting), buoyancy (lower specific gravity than TAG), permeability control and as energy reserve and

fatty acid reserve for modifying structural lipids after egg fertilization. In any case, it seems clear that the nature of the lipid deposited in the ovary, specifically its high percentage of neutral lipid, implies that bluefin tuna egg lipid is designed for combustion to provide metabolic energy as well as for the formation of biomembranes in the developing embryo.

The fatty acid profiles of ovary and liver are very similar to those reported for juvenile bluefin tuna by Ishihara and Saito (1996), who found that the fatty acid composition of most tissues examined were generally similar to each other. It is evident that tuna accumulates DHA in almost all its tissues from a very early stages of sex maturation. Similar results have been found in other tunids (Murase and Saito 1996; Saito and Ishihara 1996; Saito et al. 1995, 1996, 1997). Thus, high DHA levels may be considered as an essential character of tuna family and may result from the unceasing accumulation of DHA originated from their prey fish, as the tuna is a top predatory fish in the marine food chain.

In contrast, the fatty acid profile of mesenteric perigonadal fat was somewhat different. It contained a large proportion of 18:1(n-9) (over 25%), a fatty acid that may be considered a product of fatty acid synthesis *de novo* in fish (Henderson and Tocher 1987). It also contained large proportions of the monoenes 20:1(n-9) and 22:1(n-11), derived from the corresponding fatty alcohols in wax esters of copedods, indicating predation of prey species of zooplankton-consuming fish in Northern latitudes (Sargent 1995) during Atlantic trophic migrations previous to spawning migration. Moreover, the DHA content decreased significantly at Stage IV (spawning), suggesting that hormone sensitive lipase liberates DHA into the serum to be delivered to ovary, *via* the liver, as components of egg-specific lipoproteins, consistent with the high leves of DHA accumulated in bluefin tuna ovary at spawning. In consequence, there is a strong selection against monoenes such as 20:1(n-9) and 22:1(n-11) during gonadogenesis, which are not common in structural phospholipids for steric reasons and may be a preferred substrate for oxidation in muscle to be used as fuel in reproductive migration and spawning activity (Wiegand 1996).

In summary, the total lipid balance suggested for the disappearance of lipid from mesenteric perigonadal fat depots and, to a lesser degree, from muscle and liver depots, reflects lipid utilization for gonadal development on the one hand and non-gonadal metabolism on the other. The former includes lipid transferred to the gonad (with or without modification) and lipid catabolised to provide metabolic energy for the biosynthesis of gonadal constituents. A major component of non-gonadal metabolism is likely to be lipid catabolism to provide energy for swimming muscles during migration.

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