Mar. Sci. Tech. Bull. (2019) 8(1): 13–22

e–ISSN: 2147–9666

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DOI: 10.33714/masteb.480957

RESEARCH ARTICLE

Determination of Usage Potential of Some Mediterranean Rays in Fish Oil Production

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ARTICLE INFO

Article History:

Received: 09.11.2018

Received in revised form: 19.03.2019

Accepted: 30.05.2019

Available online: 24.06.2019

Keywords:

Dasyatis pastinaca,
Raja radula,
Raja clavata,
Torpedo marmorata,
Heavy metal,
Fatty acid

ABSTRACT

In this study, it was determined the chemical composition of the four ray species (*Dasyatis pastinaca, Raja radula, Raja clavata* and *Torpedo marmorata*) caught from Mersin Bay in the Northeastern Mediterranean Sea. For this purpose, lipid levels, fatty acid profiles, macro-trace elements, and heavy metal levels in the liver and muscle tissues of the Mediterranean rays were investigated. Lipid levels of liver tissue of *D. pastinaca, R. radula, R. clavata, T. marmorata* were determined to be 80.21%, 53.73%, 45.57% and 45.26%, respectively; while lipid levels for muscle tissue were 1.62%, 1.31%, 1.20% and 1.43%, respectively. In the fatty acid composition of muscle tissues of the rays; ∑SFAs (total saturated fatty acids) levels were reported to be between 30.46% and 35.00%, ∑MUFAs (total saturated fatty acids) levels were 21.49% to 27.77%, ∑PUFAs (total polyunsaturated fatty acids) levels were 28.76% to 35.69%; while for liver tissues; ∑SFAs levels were reported to be between 25.76% and 31.15%, ∑MUFAs levels were 23.43% to 30.66%, ∑PUFAs levels were 21.86% to 30.54%. According to data of this current study, no potential toxic metals (Cr, As, Cd, Pb, Hg) were detected in the fish oils obtained from the tissues. Finally, it was showed that these fish had potential for fish oil production because of their having fat in the liver tissues and there were also no potential heavy metal in the both muscle oil and liver oil, being rather healthy.

Please cite this paper as follows:

Ayas, D., Erdoğan Eliuz, E.A., Peri, F., Bakan, M. (2019). Determination of Usage Potential of Some Mediterranean Rays in Fish Oil Production. *Marine Science and Technology Bulletin*, 8(1): 13–22.

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Introduction

Fish is a crucial source of food for human health and easily digestible because of its long muscle fibers. All fish species have fat, high quality protein, essential minerals and vitamins but, above all, significant amounts of omega-3 long-chain poly-unsaturated fatty acids (PUFAs) which are an essentially unique and very essential in human nutrition. PUFAs like eicosapentaenoic (EPA, C20:5n3), arachidonic (C20:4n6) and docosahexaenoic (DHA, C22:6n3) acids which need to be taken from the outside, are not synthesized in the human body (Kaur et al., 2012). The many studies have showed that PUFAs prevent and also potentially alleviate cancer, nervous system, reproductive system and cardiovascular diseases. These fatty acids are well reported mainly in the prevention of those diseases by protecting cell-membrane fluidity, improving functions of vascular endothelial cells, reducing of mild hypertension, decreasing susceptibility to ventricular abnormal heart rhythms, diminishing secretion of proinflammatory cytokines by macrophages, inhibiting blood platelet aggregation (Wiktorowska-Owczarek et al., 2015). Especially, EPA and DHA, critical for human health, both play a crucial role in the prevention of a wide range of disorders such as cancer, coronary heart and cardiovascular diseases (Chan et al., 2005; Navarro-García et al., 2009; Navarro-García et al., 2014). Therefore, there is increasing interest nowadays in fish lipids because of their PUFAs.

Heavy metals, macro and trace elements in commercial seafood are often subject to scientific researches in terms of public health (Fernandes et al., 2007). Especially, minerals such as sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), are very helpful for health and complementary nutritional factors of body metabolism (Ayas et al., 2016). While some metal and trace elements have a rather vital role for all living systems, toxic metals such as chromium (Cr), arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) have a directly harmful effect on human health in above acceptable limits and also are used indicator for the pollution level of the marine ecosystem (Mendil et al., 2010; Authman, 2015; Cresson et al., 2015). Because of the pollutant factors such as transportation ports, heavy marine traffic, industry waste, and other many factories, Northern-Eastern Mediterranean Sea is a risky region for metal pollution (Yılmaz et al., 2017). Therefore, the recent studies showed that fish oils can be a better alternative in terms of health nutrition due to fish tissues may contain relatively high levels of heavy metals (Başusta and Erdem, 2000; Foran et al., 2003; Çoğun et al., 2005).

D. pastinaca is a member of the Chondrichthyes represented by sharks, skates, and rays and most primitive living jawed aquatic vertebrates (Bouchaala et al., 2015). *R. clavata* and *R. radula*, named as thornback, live in sandy and muddy habitats, at depths ranging from shoreward region to 300 m (Saglam and Bascinar, 2008). *R. clavata*, a benthic feeder that prey mainly on crustaceans, is usually found on shelf and upper slope waters at depths between 10-60 m. These species are most common rays encountered by divers (Tufan et al., 2013). *T. marmorata*, known as marbled electric ray, also is a species of Torpedinidae family and commonly found in Mediterranean Sea (Başusta and Erdem, 2000).

Rays were reported to contain a high amount of fat and fatty acid, which is rich in nutrient by Pal et al. (1998), Colakoğlu et al. (2011), and Beckmann et al. (2014). However, there is a little information on the lipid level and fatty acid profiles of thornback rays (*R. radula, R. clavata*) and other rays (*D. pastinaca, T. marmorata*). Therefore, we determined the proximate composition of muscle and liver tissues of the rays caught from Mersin Bay. With the aim of making use of this important resources, the lipid levels of their tissues, fatty acid profiles of their lipids, macro and trace element levels and also the contamination levels with potential toxic heavy metals of lipids from extracted the liver and muscle tissues were investigated.

Material and Methods

Collection and Measurements of Rays

Samplings (*D. pastinaca, R. radula, R. clavata, T. marmorata*) were carried out between Berdan River (36° 43' 31.8" N 34° 54' 27.0" E) and Yeşilovacık Bay (36° 08' 53.6" N 33° 39' 40.7" E) by using a commercial trawler (Figure 1). The sampling was conducted as one season between March 2016 and April 2016 in Mersin Bay, and ray samples were provided in sufficient quantities for each species. Fish were grouped according to species and their sizes (cm) and weights (g) were measured (Table 1).

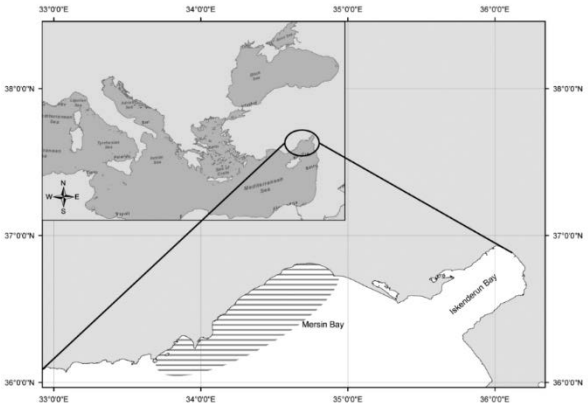

Figure 1. Map of the sampling location (The marked area is the sampling area)

Fat and Fatty Acids Analyses

Lipid content was measured by the method of Bligh and Dyer (1959). In extracted lipids, fatty acid methyl esters were obtained using the Ichihara et al. (1996) method. Fatty acid composition was analyzed using a Gas Chromatography (GC) Clarus 500 device (Perkin–Elmer, USA), one flame ionization detector (FID) and SGE (60 m × 0.32 mm ID BPX70 × 0.25 μm, USA or Australia) column. Injector and detector temperatures were set as 260°C and 230°C respectively. During this time, the furnace temperature was kept at 140°C for 8 minutes. After that, it was increased by 4°C per minute until 220°C, and from 220°C to 230°C by increasing the temperature 1°C per minute. It was kept at 230°C for 15 minutes to complete analysis. Sample scale was 1 μL and carrier gas was controlled at 16 ps. For split flow 40, 0 mL/minute (1:40) level was used. Fatty acids were determined using a comparison to the exit times of the FAME mix that contains 37 standard components.

Table 1. Length and weight of ray samples

Measurements	*D. pastinaca*	*R. radula*	*R. clavata*	*T. marmorata*
N	16	8	7	7
Mean DW (cm)	35.90	33.60	38.14	18.26
Range (cm)	30.0-43.5	32.2-35	37.5-39.0	17.7-19.0
Mean DL (cm)	31.83	32.95	31.51	19.93
Range (cm)	24.5-39.0	31-34.9	30.4-33.0	19.5-20.5
Mean TL (cm)	63.13	54.0	52.5	25.63
Range (cm)	54.0-73.0	51.5-56.5	51-54.5	24.6-27.0
Mean TW (g)	1849.38	895.5	899.86	386.43
Range (g)	955-3205	756-1035	824-1001	368-411
Mean LW (g)	140.16	26.21	27.03	12.30
Range (g)	58.3-265.3	23.01-29.40	21.06-35.0	10.65-14.5
LW/TW*100	7.58	2.93	3.00	3.18

Note: N: Total number of specimens, DW: Disc width, DL: Disc length, TL: Total length TW: Total weight LW: Liver weight.

Atherogenicity Index (AI) and Thrombogenicity Index (TI)

The AI and TI linked to the fatty acid composition were calculated according to Ulbricht and Southgate (1991).

$$AI=\frac{[(a\times 12:0)+(b\times 14:0)+(c\times 16:0)]}{[(d\times(\text{PUFA n-6+n-3}))+(e\times(\text{MUFA}))+(f\times(\text{MUFA-18:1}))]}$$

$$TI=\frac{[g\times(14{:}0+16{:}0+18{:}0)]}{[(h\times(\text{MUFA}))+(i\times(\text{MUFA-18:1}))+(m\times(n-6))+(n\times(n-3))+(n-3/n-6)]}$$

In these formulae; a, c, d, e, f=1; b=4; g=1; h, i, m=0.5; n=3.

Metal Analyses

The samples (0.1 g dry weight) used for metal analysis were dried at 105°C to reach constant weights, and then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and percholoric acid (2 mL, Merck, Darmstadt, Germany) were added to the samples, and they were put on a hot plate set to 150°C until all tissues were dissolved (Canli and Atli, 2003).

Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model, Japan) was used to determine metals. ICP-MS operating conditions were the following: radio frequency (RF) (W), 1500; plasma gas flow rate (L/min),15; auxiliary gas flow rate (L/min), 1; carrier gas flow rate (L/min), 1.1; spray chamber T (°C), 2; sample depth (mm), 8.6; sample introduction flow rate (mL/min), 1; nebuliser pump (rps), 0.1; extract lens (V), 1.5. The levels of macro (Na, Mg, P, K, Ca), trace element (Co, Cu, Zn, Mo, Ni, Se) and potential toxic metal (Cd, Pb) in samples were detected as μg metal g^{-1} dry weight. High Purity Multi Standard (Charleston, SC 29423) was used for determination of the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the macro and trace elements and potential toxic metals. Solutions have prepared for the toxic metals had a content of lead, cadmium, arsenic and chromium in the range of 1-50 ppb (0.001 to 0.050 mg/L), for the macro and trace elements had a content of copper, iron, and zinc in the range of 1-50 ppm (1 to 50 mg/L).

Statistical Analyses

Prior to the analyses, all data were checked for outliers and Levene's homogeneity of variance was also applied for variance homogeneity. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22 statistical program. ANOVA (Analysis of Variance) was used to evaluate the differences of metals levels of the species.

Results

Some Morphological Measurements of Mediterranean Rays

In this study, the ray samples caught in the Northeastern Mediterranean Sea (Figure 1) were grouped according to species. Four species of rays, *D. pastinaca, R. radula, R. clavata, T. marmorata* have been detected for some physical properties. A total of 38 individuals, total lengths (TL), total weight (TW), liver weight (LW), disc length (DL), disc width (DW) for each ray are presented in Table 1. The means of DW and DL are ranged from 18.26 to 38.14 cm, and from 19.93 to 32.95 cm in the rays, respectively. The means of the TL, TW, and LW for *D. pastinaca* were greater than those of *R. radula, R. clavata, T. marmorata.* The mean TL, TW, and LW were determined to be 63.13 cm, 1849.38 g, 140.16 g for *D. pastinaca*, 54.0 cm, 895.5 g, 26.21 g for *R. radula*, 52.5 cm, 899.86 g, 27.03 g for *R. clavata* and 25.63 cm, 386.43 g, 12.30 g for *T. marmorata*. The highest mean of LW/TW% was found *D. pastinaca* as 7.58%.

The Lipid Levels of the Tissues of Mediterranean Rays

The lipid levels of the liver and muscle tissues of Mediterranean rays are summarized in Table 2. Lipid levels of the liver tissues of *D. pastinaca, R. radula, R. clavata, T. marmorata* were determined to be 80.21%, 53,73%, 45,57% and 45.26%, while lipid levels for muscle tissue were determined to be 1.62%, 1.31%, 1.20% and 1.43%, respectively. The percentages of lipid levels in liver tissues of rays had a significantly higher than in their muscle tissues. Liver tissues of Mediterranean rays are more suitable for fish oil production.

The highest fish oil (80.21%) has been obtained from the liver of *D. pastinaca* (Table 2). The highest mean of LW/TW% was found *D. pastinaca* as 7.58% (Table 1). That's why, the most suitable tissue for fish oil production is the liver tissue of the *D. pastinaca*.

Table 2. The lipid levels of the liver and muscle tissues of Mediterranean rays

Measurements	*D. pastinaca*	*R. radula*	*R. clavata*	*T. marmorata*
Muscle lipid (%)	1.62	1.31	1.20	1.43
Range (%)	1.41-1.76	0.71-1.90	0.95-1.38	1.25-1.57
Liver Lipid (%)	80.21	53.73	45.57	45.26
Range (%)	74.32-85.10	41.90-59.17	40.63-49.28	44.12-46.12

Table 3. The fatty acid profiles of the muscle oil of Mediterranean rays (%)

Fatty Acid	*D. pastinaca* ($\overline{X \pm S_X}$)	*R. radula* ($\overline{X \pm S_X}$)	*R. clavata* ($\overline{X \pm S_X}$)	*T. marmorata* ($\overline{X \pm S_X}$)
C12:0	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{b}	0.00±0.00^{a}
C14:0	0.26±0.00^{a}	0.47±0.02^{b}	0.59±0.03^{c}	0.76±0.01^{d}
C15:0	0.27±0.00^{a}	0.51±0.00^{b}	0.52±0.02^{b}	0.56±0.01^{c}
C16:0	16.86±0.04^{a}	19.05±0.01^{b}	19.39±0.92^{b}	20.27±0.09^{c}
C17:0	0.84±0.00^{a}	1.69±0.04^{d}	1.40±0.06^{c}	1.10±0.01^{b}
C18:0	9.60±0.00^{c}	8.63±0.02^{b}	8.05±0.41^{a}	10.63±0.06^{d}
C20:0	0.11±0.00^{ab}	0.10±0.00^{a}	0.11±0.01^{b}	0.11±0.00^{b}
C22:0	0.08±0.01^{b}	0.17±0.00^{c}	0.03±0.00^{a}	0.03±0.00^{a}
C24:0	2.44±0.01^{c}	2.23±0.03^{b}	2.21±0.10^{b}	1.54±0.01^{a}
ΣSFA	30.46	32.83	32.31	35.00
C14:1	0.08±0.00^{a}	0.10±0.00^{b}	0.08±0.00^{a}	0.14±0.00^{c}
C15:1	0.12±0.00^{a}	0.18±0.00^{b}	0.13±0.01^{a}	0.28±0.00^{c}
C16:1	2.43±0.01^{c}	1.48±0.03^{a}	1.42±0.07^{a}	2.33±0.01^{b}
C17:1	0.53±0.04^{a}	0.61±0.00^{b}	0.55±0.02^{a}	0.82±0.01^{c}
C18:1n9t	0.49±0.00^{d}	0.33±0.01^{c}	0.24±0.01^{a}	0.28±0.01^{b}
C18:1n9c	7.02±0.00^{a}	7.78±0.02^{b}	9.33±0.47^{c}	10.39±0.06^{d}
C18:1n7	5.01±0.01^{d}	3.72±0.01^{b}	3.34±0.17^{a}	4.79±0.02^{c}
C20:1n9	0.74±0.00^{c}	0.37±0.00^{a}	0.62±0.03^{b}	0.93±0.01^{d}
C22:1n9	11.02±0.06^{c}	7.75±0.03^{b}	5.67±0.28^{a}	5.82±0.02^{a}
C24:1n9	0.33±0.00^{c}	0.15±0.00^{b}	0.11±0.01^{a}	0.10±0.00^{a}
ΣMUFA	27.77	22.47	21.49	25.88
C18:2n6t	0.19±0.00^{b}	0.13±0.00^{a}	0.14±0.01^{a}	0.14±0.00^{a}
C18:2n6c	0.95±0.02^{b}	1.02±0.01^{c}	1.05±0.05^{c}	0.90±0.01^{a}
C18:3n3	0.21±0.10^{b}	0.10±0.00^{a}	0.14±0.01^{a}	0.09±0.00^{a}
C18:3n6	0.40±0.00^{c}	0.25±0.00^{a}	0.31±0.04^{b}	0.52±0.00^{d}
C20:3n3	0.28±0.00^{d}	0.21±0.01^{c}	0.13±0.01^{a}	0.15±0.00^{b}
C20:3n6	0.08±0.00^{a}	0.12±0.00^{b}	0.14±0.01^{b}	0.13±0.01^{b}
C20:4n6	0.39±0.00^{b}	0.48±0.04^{c}	0.47±0.03^{c}	0.34±0.00^{a}
C20:5n3	2.28±0.00^{b}	3.82±0.02^{d}	3.45±0.18^{c}	2.06±0.01^{a}
C22:4n6	5.35±0.00^{d}	2.17±0.02^{c}	1.14±0.07^{b}	1.07±0.01^{a}
C22:6n3	19.84±0.01^{a}	27.05±0.12^{c}	28.39±1.46^{d}	23.03±0.07^{b}
C22:2cis	1.00±0.01^{c}	0.24±0.00^{a}	0.33±0.02^{b}	0.33±0.00^{b}
ΣPUFA	30.97	35.59	35.69	28.76
SFA/PUFA	0.98	0.92	0.91	1.22
Σn7	5.01	3.72	3.34	4.79
Σn6	7.36	4.17	3.25	3.10
Σn3	22.61	31.18	32.11	25.33
Σn9	19.60	16.38	15.97	17.52
n6/n3	0.33	0.13	0.10	0.12
n3/ n6	3.07	7.48	9.88	8.17
DHA/EPA	8.70	7.08	8.22	11.18
AI	0.24	0.31	0.33	0.36
TI	0.28	0.23	0.22	0.30
Unidentified	10.80	9.09	10.51	10.36

Note: ($\overline{X \pm S_X}$) means Average ± Standard deviation

Fatty Acid Profiles of Mediterranean Rays

Detailed fatty acid profiles of muscle and liver oils of Mediterranean rays are listed in Table 3 and Table 4, respectively. The fatty acid profiles of muscle oil and liver oil obtained from *D. pastinaca, R. radula, R. clavata, T. marmorata* were compared.

The percentages of ΣSFAs in muscle tissues of the rays were reported to be between 30.46% and 35.00%, ΣMUFAs levels were 21.49% to 27.77%, ΣPUFAs levels were 28.76% to 35.69%; while for liver oils; ΣSFAs levels were reported to be between 25.76% and 31.15%, ΣMUFAs levels were 23.43% to 30.66%, ΣPUFAs level were 21.86% to 30.54% The lipidic fractions contained a high amount of PUFAs (up to 50% of the total), mainly composed of C22:6*n*3, C20:5*n*3, C22:4n6, C20:4*n*6, C20:3*n*3, C18:3*n*6, C18:3*n*3, C18:2*n*6, C18:2*n*6, in total fatty acid methyl esters (ΣFAMEs).

Table 4. The fatty acid profiles of the liver oil of Mediterranean rays (%)

Fatty Acid	*D. pastinaca* ($\overline{X} \pm S_X$)	*R. radula* ($\overline{X} \pm S_X$)	*R. clavata* ($\overline{X} \pm S_X$)	*T. marmorata* ($\overline{X} \pm S_X$)
C12:0	0.05±0.00^{b}	0.08±0.01^{c}	0.04±0.01^{b}	0.02±0.00^{a}
C14:0	1.56±0.01^{ab}	1.71±0.06^{b}	2.03±0.03^{c}	1.40±0.39^{a}
C15:0	0.98±0.01^{a}	1.15±0.04^{b}	0.87±0.01^{a}	0.92±0.23^{a}
C16:0	14.58±0.04^{a}	14.68±0.57^{a}	16.07±0.24^{a}	14.81±3.80^{a}
C17:0	1.20±0.00^{a}	1.88±0.11^{b}	1.32±0.02^{a}	1.26±0.32^{a}
C18:0	5.69±0.01^{a}	9.73±0.44^{c}	6.16±0.10^{ab}	6.52±1.62^{b}
C20:0	0.29±0.00^{c}	0.48±0.03^{d}	0.27±0.01^{bc}	0.22±0.06^{a}
C22:0	0.08±0.00^{b}	0.03±0.00^{a}	0.15±0.00^{c}	0.05±0.01^{a}
C24:0	1.33±0.00^{a}	1.41±0,06^{a}	1,53±0.02^{a}	1.91±0.49^{b}
ΣSFA	25.76	31.15	28.44	27.11
C14:1	0.28±0.01^{c}	0.22±0.01^{b}	0.26±0.01^{c}	0.15±0.04^{a}
C15:1	0.46±0.01^{c}	0.28±0.01^{b}	0.25±0.01^{b}	0.12±0.03^{a}
C16:1	6,03±0.02^{c}	3.75±0.15^{a}	4.64±0.07^{b}	3.46±0.93^{a}
C17:1	1.00±0.00^{c}	0.93±0.04^{ab}	0.80±0.01^{a}	0.97±0.24^{bc}
C18:1n9t	0.71±0.00^{d}	0.44±0.02^{c}	0.34±0.00^{b}	0.23±0.06^{a}
C18:1n9c	11.27±0.02^{a}	11.27±0.47^{a}	14.10±0.23^{b}	11.01±2.83^{a}
C18:1n7	4.49±0.01^{b}	3.95±0.17^{ab}	3.85±0.06^{ab}	3.83±0.99^{a}
C20:1n9	0.90±0.00^{b}	0.65±0.03^{a}	1.22±0.02^{c}	0.80±0.22^{ab}
C22:1n9	5.46±0.02^{c}	6.25±0.28^{d}	4.03±0.06^{b}	2.74±0.70^{a}
C24:1n9	0.06±0.00^{a}	0.11±0.01^{b}	0.05±0.00^{a}	0.12±0.03^{b}
ΣMUFA	30.66	27.85	29.54	23.43
C18:2n6t	0.09±0.00^{a}	0.19±0.01^{bc}	0.21±0.00^{c}	0.15±0.04^{b}
C18:2n6c	1.22±0.01^{a}	1.55±0.07^{b}	1.49±0.03^{b}	1.09±0.28^{a}
C18:3n3	0.26±0.01^{b}	0.30±0.01^{c}	0.27±0.01^{bc}	0.20±0.06^{a}
C18:3n6	0.38±0.00^{ab}	0.47±0.02^{b}	0.35±0.02^{a}	0.45±0.13^{b}
C20:3n3	0.24±0.00^{b}	0.21±0.01^{ab}	0.18±0.00^{a}	0.21±0.06^{ab}
C20:3n6	0.31±0.01^{b}	0.25±0.01^{a}	0.39±0.01^{c}	0.31±0.08^{b}
C20:4n6	0.89±0.01^{c}	0.83±0.03^{c}	0.71±0.01^{b}	0.39±0.10^{a}
C20:5n3	5.30±0.01^{b}	9.14±0.40^{c}	5.72±0.10^{b}	2.64±0.68^{a}
C22:4n6	3.32±0.01^{c}	1.09±0.05^{b}	0.69±0.01^{a}	1.19±0.30^{b}
C22:6n3	8.79±0.02^{a}	13.69±0.62^{b}	20.21±0.33^{c}	17.71±4.57^{b}
C22:2cis	1.06±0.01^{b}	0.28±0.01^{a}	0.32±0.00^{a}	0.27±0.07^{a}
ΣPUFA	21.86	28.00	30.54	24.61
SFA/PUFA	1.18	1.11	0.93	1.10
Σn7	4.49	3.95	3.85	3.83
Σn6	6.21	4.38	3.84	3.58
Σn3	14.59	23.34	26.38	20.76
Σn9	18.40	18.72	19.74	14.90
n6/n3	0.43	0.19	0.15	0.17
n3/ n6	2.35	5.33	6.87	5.80
DHA/EPA	1.66	1.50	3.53	6.71
AI	0.32	0.32	0.34	0.33
TI	0.30	0.26	0.23	0.26
Unidentified	21.76	13.00	11.48	24.85

Note: ($\overline{X} \pm S_X$) means Average ± Standard deviation

Among the SFAs, those occurring at the highest proportions in muscle oil were palmitic acid (C16:0), 20.27% for *T. marmorata*, 19.39% for *R. clavata*, 19.05% for *R. radula*, 16.86% for *D. pastinaca* followed by stearic acid (C18:0) 10.63% for *T. marmorata*, 9.60% for *D. pastinaca,* 8.63% for *R. radula*, 8.05% for *R. clavata.* The most abundant MUFAs found in muscle oils of all samples were C22:1n9, 11.02% for *D. pastinaca,* 7.75% for *R. radula,* 5.82% for *T. marmorata*, 5.67% for *R. clavata*; C18:1*n*9c, 10.39% for *T. marmorata*, 9.33% for *R. clavata*, 7.78% for *R. radula*, 7.02% for *D. pastinaca* and C18:1*n*7, 5.01% for *D. pastinaca,* 4.79% for *T. marmorata,* 3.72% for *R. radula,* 3.34% for *R. clavata.* The percentages of PUFAs found in muscle of the studied ray species were important. EPA (C20:5n3), and DHA (C22:6*n*3), the major n-3 of PUFAs of the muscle tissues, were determined to be 2.06% and 23.03% for *T. marmorata*, 3.45% and 28.39% for *R. clavata*, 3.82% and 27.05% for *R. radula*, 2.28% and 19.84% for *D. pastinaca,* respectively.

AI values were between 0.24 and 0.36 in the muscle oils, and between 0.32 and 0.34 in the liver oils. The highest AI value was in the muscle of *T. marmorata* (0.44) and in the liver oil of *R. clavata* (0.34).

Table 5. Macro-trace elements and heavy metal levels of the tissues of the Mediterranean rays (μg g^{-1})

Elements	*D. pastinaca* ($\overline{X} \pm S_X$)	*R. radula* ($\overline{X} \pm S_X$)	*R. clavata* ($\overline{X} \pm S_X$)	*T. marmorata* ($\overline{X} \pm S_X$)	*Tissue*
Na	3950.64±373.50^{a,y}	3365.60±842.70^{a,x}	10627.43±449.97^{c,y}	7720.04±100.03^{b,x}	*Muscle*
	1338.30±330.94^{a,x}	2051.93±268.19^{ab,x}	3028.69±50.01^{b,x}	7214.95±50.23^{c,x}	*Liver*
Mg	759.20±96.59^{a,y}	1350.39±250.94^{b,y}	822.85±22.11^{a,y}	800.76±10.07^{a,y}	*Muscle*
	0.35±0.01^{a,x}	0.32±0.01^{a,x}	0.29±0.01^{a,x}	0.32±0.02^{a,x}	*Liver*
P	7570.53±426.70^{a,y}	6500.16±734.76^{a,y}	10356.34±301.45^{b,y}	5828.01±99.87^{a,y}	*Muscle*
	2823.01±299.85^{a,x}	3134.10±99.14^{a,x}	3082.65±53.24^{a,x}	3277.31±147.09^{a,x}	*Liver*
K	14095.17±1397.87^{a,y}	9559.93±373.51^{a,y}	28546.95±500.27^{b,y}	11107.98±478.83^{a,y}	*Muscle*
	4231.31±507.93^{a,x}	4789.51±122.84^{a,x}	6182.47±97.08^{a,x}	7201.11±476.43^{a,x}	*Liver*
Ca	608.65±25.50^{a,x}	474.16±161.71^{a,x}	492.63±9.78^{a,x}	829.09±23.89^{b,x}	*Muscle*
	757.81±53.79^{a,x}	614.18±198.02^{a,x}	543.52±15.44^{a,x}	1378.97±98.99^{b,y}	*Liver*
Cr	2.96±0.43^{b,x}	2.96±0.97^{b,x}	4.76±0.15^{b,y}	0.35±0.02^{a,x}	*Muscle*
	1.94±0.75^{a,x}	0.50±0.38^{a,x}	0.91±0.05^{a,x}	1.52±0.05^{a,y}	*Liver*
Mn	0.62±0.06^{a,x}	0.83±0.05^{a,x}	0.83±0.03^{a,x}	0.85±0.05^{a,x}	*Muscle*
	1.82±0.25^{a,y}	2.25±0.12^{a,y}	2.98±0.14^{b,y}	1.87±0.10^{a,y}	*Liver*
Cu	2.42±0.31^{b,x}	0.74±0.10^{ab,x}	0.35±0.03^{a,x}	0.60±0.05^{ab,x}	*Muscle*
	3.24±0.31^{a,x}	13.08±2.24^{d,y}	10.37±0.35^{c,y}	6.25±0.09^{b,y}	*Liver*
Zn	12.61±0.53^{bc,x}	14.23±0.50^{c,x}	9.24±0.49^{a,x}	11.83±0.95^{ab,x}	*Muscle*
	13.83±1.75^{a,x}	24.60±4.99^{b,x}	22.30±1.57^{b,y}	14.26±0.26^{a,x}	*Liver*
As	123.07±25.74^{a,y}	138.98±34.00^{a,x}	800.56±19.78^{b,y}	64.52±2.56^{a,x}	*Muscle*
	41.06±6.44^{a,x}	69.74±18.60^{ab,x}	119.14±9.77^{b,x}	43.73±0.99^{a,x}	*Liver*
Se	9.94±1.23^{b,x}	2.93±0.56^{a,x}	5.26±0.20^{b,x}	2.90±0.26^{a,x}	*Muscle*
	8.00±1.08^{c,x}	2.74±0.41^{a,x}	5.38±0.29^{bc,x}	4.57±0.33^{ab,x}	*Liver*

Note: Values in same rows and columns in each metals with different letters are significantly different ($p<0.05$)

($\overline{X} \pm S_X$) means Average ± Standard deviation

Table 6. The levels of the potential toxic metal in the muscle and liver oils (μg g^{-1})

Elements	*D. pastinaca* ($\overline{X} \pm S_X$)	*R. radula* ($\overline{X} \pm S_X$)	*R. clavata* ($\overline{X} \pm S_X$)	*T. marmorata* ($\overline{X} \pm S_X$)	*Oil*
Cr	ND	ND	ND	ND	*Muscle*
	ND	ND	ND	ND	*Liver*
As	ND	ND	ND	ND	*Muscle*
	ND	ND	ND	ND	*Liver*
Cd	ND	ND	ND	ND	*Muscle*
	ND	ND	ND	ND	*Liver*
Pb	ND	ND	ND	ND	*Muscle*
	ND	ND	ND	ND	*Liver*
Hg	ND	ND	ND	ND	*Muscle*
	ND	ND	ND	ND	*Liver*

Note: ($\overline{X} \pm S_X$) means Average ± Standard deviation; ND means not detected

The Macro Elements, Trace Elements and Toxic Heavy Metal Contents of the Ray Samples

Macro (Na, Mg, P, K and Ca), trace element (Cu, Mn and Se) contents of livers and muscles from the studied species (*D. pastinaca, R. radula, R. clavata, T. marmorata*) are illustrated in Table 5. The relationship between the amounts of macro elements in muscle tissue of *D. pastinaca* and *R. radula* were determined to be K>P>Na>Mg>Ca and K> Na> P >Mg>Ca for *R. clavata, T. marmorata*. In addition, in liver tissues, the relationship between the macro element levels of *D. pastinaca*, *R. radula* and *R. clavata* were measured to be K>P>Na>Ca>Mg and Na >K> P>Ca>Mg for *T. marmorata*. K was the most abundant macronutrient for all ray species and the highest values (14095.17±1397.87, 9559.93±373.51, 28546.95±500.27, 11107.98±478.83 µg/g) were observed for muscle tissues. The other macro elements were also detected at much higher levels in muscle from the fishes compared to that of liver.

Discussion

In the present study, the TL (total length) of *D. pastinaca* ranged from 54.0 to 73.0 cm, and W (weight) was between 955 and 3205 g. Its measurements were reported by Yeldan et al. (2009), TL and W ranged 14.6 to 100.9 cm and 22.5 to 6800 g, respectively. Ismen (2003) reported that the total length of *D. pastinaca* females ranged from 20.5 to 88 cm, and of its males from 20 to 73 cm caught in Iskenderun Bay. Maximum total length observed in our study for *R. clavata* (54.5 cm) were slightly smaller than found in a study carried out (73.2 cm) in the Southeastern Black Sea (Demirhan et al., 2005). Kadri et al. (2013) reported that the TL of *R. radula* collected from the Gulf of Gabes (southern Tunisia, central Mediterranean Sea) ranged from 13.4 to 65 cm, similar to our report (51.5 to 56.5 cm). The average total length and weight of *T. marmorata* collected from North-Eastern Mediterranean were detected to be between 12.26 to 40 cm, 40.40 to 1062.00 g, respectively by Duman and Basusta (2013). According to the study of Filiz and Bilge (2004), *T. marmorata* ranged between 9.2 to 34.3 cm in TL and 14.88 to 862.11 g in W; between 20.5 to 99.0 cm in TL and 28.86 to 2614.28 g in W for *R. clavata*; and 37.3 to 74.2 cm in TL and 333.23 to 2955.0 g in W for *D. pastinaca* in the North Aegean Sea. These measurements were close to our results (mean TL=25.63, mean TW=386.43).

Various factors such as seasons, years, climate, temperature, nutrient, reproductive cycle period, salinity, geographical location, sex and maturity may be responsible for the differences in parameters of oil yield, length and weight of the fish (Olusoji et al., 2010). In our study, we reported that the lipid levels were to be 80.21%, 53,73%, 45,57%, 45.26% in liver and 1.62%, 1.31%, 1.20%, 1.43% in muscle tissue for *D. pastinaca, R. radula, R. clavata, T. marmorata*, respectively. Compared to the lipid content of muscle, the livers of all rays contains significant lipid content. Colakoglu et al. (2011) reported that total lipid content of *R. clavata* was 3.39%. The liver oil level for *D. brevis* was reported 25–50% by Navarro-Garcia et al. (2004). The lipid levels of liver of *D. bleekeri* were reported as 63.4% from the coastal region of West Bengal, India (Pal et al., 1998). Liver oil level for *D. americana* from the Gulf of Mexico (Navarro-Garcia et al., 2009) and *D. dipterura* from Sinaloa of Mexico (Navarro-Garcia et al., 2014) were 38.2% and 46.41%, respectively. In the present study, liver oil of *D. pastinaca* was 80.21%. This level is higher than the results obtained in other studies. This may be due to differences of the ray species and regional differences.

The most abundant SFAs found in liver oil of all rays were palmitic acid, 16.07% for *R. clavata,* 14.81% for *T. marmorata,* 14.68% for *R. radula,* 14.58% for *D. pastinaca and* stearic acid (C18:0), 9.73% for *R. radula,* 6.52% for *T. marmorata,* 6.16% for *R. clavata,* 5.69% for *D. pastinaca.* Among the MUFAs, those occurring at the highest proportions in liver oil were oleic acid, 14.10% for *R. clavata,* 11.27% for *R. radula,* 11.27% for *D. pastinaca,* 11.01% for *T. marmorata* followed by C18:1n7 and C22:1n9, 4.49% and 5.46% for *D. pastinaca,* 3.95% and 6.25% for *R. radula,* 3.85% and 4.03% *R. clavata,* 3.83% and 2.74% for *T. marmorata*. EPA (C20:5n3) and DHA (C22:6n3), the major n-3 of PUFAs, in the liver oils of fishes were determined to be 5.30% and 8.79% for *D. pastinaca,* 9.14% and 13.69% for *R. radula,* 5.72% and 20.21% for *R. clavata,* 2.64% and 17.71% for *T. marmorata*, respectively.

The primary SFA was palmitic acid (C16:0; 26.45%) in muscle oil of *R. clavata*, palmitic acid was followed by stearic acid (C18:0; 10.62%) in *R. clavata* (Turan, 2007). Fernandez-Reiriz Pastoriza and Sampedro, (1992) previously had reported that the main SFAs in *R. clavata* was stearic acid followed by palmitic acid. The palmitic acid and stearic acid were found to be the major SFAs in different fish species (Jabeen and Chaudhry, 2011). Their study demonstrated *Oreochromis mossambicus*, *Cyprinus carpio* and *Labeo rohita* from the Indus River contained reasonable amounts of essential PUFAs such as eicosapentaenoic, docosahexaenoic, and arachidonic acids (Jabeen and Chaudhry, 2011).

The percentages of total SFA and PUFA levels in muscle oil of rays were observed to be higher than those in liver oil. Differences were also determined in the fatty acid profiles. The percentages of ΣMUFAs in liver oil of *D. pastinaca, R. radula, R. clavata* were higher than those in the muscle oil, whereas the ΣMUFAs level in liver oil of *T. marmorata* was lower than in the muscle oil. Moreover, the fatty acid composition of these fishes showed a relatively high ratio of SFA/PUFA in muscle and liver oils; for *D. pastinaca*: 0.98 and 1.18, *R. radula*: 0.92 and 1.11, *R. clavata:* 0.91 and 0.93, *T. marmorata:* 1.22 and 1.10, respectively. The fatty acid profiles of both muscle and liver oils showed to be predominant C16:0 (14.58–20.27%), followed by C22:6*n*−3, (8.79–28.39%).

With regard to n-3 PUFAs, we reported that C20:5*n*3 and C22:6*n*3, the major n-3 of PUFAs, in the liver oils of rays were determined to be 5.30% and 8.79% for *D. pastinaca,* 9.14% and 13.69% for *R. radula,* 5.72% and 20.21% for *R. clavata,* 2.64% and 17.71% for *T. marmorata*, respectively. In another studies, the concentration of the EPA and DHA were found 5.3 and 4.8 g/100 g liver oil in *Dasyatis brevis*, 5.9 and 10.0 g/100 g liver oil in *Gymnura marmorata*. G. (Navarro-Garcia et al., 2004). Similar levels with our study of EPA had been reported in liver oil of *R. clavata.* (Ozyilmaz, 2016). High proportions of DHA

were detected in muscle and liver of *Dasyatis marmorata* (11.1% and 16.1%, respectively) caught from the East Tropical Atlantic Ocean (Ould El Kebir et al., 2003).

In our study, the major *n*-3 of PUFAs, were determined to be 2.06% and 23.03% for *T. marmorata*, 3.45% and 28.39% for *R. clavata*, 3.82% and 27.05% for *R. radula*, 2.28% and 19.84% for *D. pastinaca,* respectively. In the other hand, the concentration of the DHA (C22:6n3) in all the rays was seven times higher than the EPA (C20:5n3). Colakoglu et al. (2011) detected that *n*-3 and *n*-6 PUFAs of *R. clavata* were higher compared to those of spiny dogfish. Beckmann et al. (2014) reported that fish oil or poultry oil-fed *Heterodontus portusjacksoni* sharks showed significant differences in the muscle and liver FA profiles in different times (6, 12, 18 weeks). In control groups, the SFA, PUFA and MUFA were detected 32.5%, 36.0%, 31.6% in liver oils and 36.3%, 35.7%, 28.0% in muscle oils, respectively. When compared to other studies, the most abundant fatty acids in liver and muscle oils of *Salmo trutta macrostigma* caught from Tohma River were found to be palmitic acid, stearic acid, oleic acid, EPA and DHA (Akpinar et al., 2009). Navarro-Garcia et al. (2010) reported that liver oils from both ray species (*Rhinoptera bonasus* and *Aetobatus narinari*) had similar EPA + DHA contents (13.2 and 8.0, respectively).

It was suggested that the *n*3/*n*6 (1:1) ratio is a sufficient index in comparing relative nutritional value of fatty acids of different fish species (Turan, 2007). As our study, the *n*3/*n*6 ratio of the rays was found between 3.07 and 9.88 in muscle oils, while between 2.35 and 6.87 in liver oils.

The lowest TI value determined in the muscle oil of *R. radula* and both muscle and liver oils of *R. clavata* were 0.23. The highest TI value was in the liver oil of *D. pastinaca* (0.30). From these results, we detected that both muscle and liver oils had greatly atherogenic and thrombogenic indices. In another study, Jankowska et al. (2010) reported the AI values were between to be 0.38 and 0.70, while TI values were between to be 0.24 and 0.34 in the muscle, liver, and mesenteric fat of *Perca fluviatilis.*

When comparing with the present study, Türkmen et al. (2014) measured similar Pb, Mn, Cu, and Cd in livers of *T. marmorata, D. pastinaca,* and *R. radula*. Heavy toxicity metals such as As, Cd, Hg and Pb are among the most serious elements of marine pollution worldwide, given their persistency in the environment and bioaccumulation. These elements are released to the nature through natural and/or artificial processes, including agriculture, mining, industrial and urban discharges (Ansari et al., 2004). When it reaches human body, several toxicological such as immunotoxicity, teratogenicity, endocrine toxicity and carcinogenetic promotion occurred. (Ahlborg et al., 1994). When metal varies that accumulated by aquatic organisms are excessively intake to human body, they cause diseases in metabolism due to their toxicity (Foran et al., 2003). In our study, we reported that there is no information about Cd, Pb, Cr, As, Hg metals in any oil samples extracted from liver and muscle. Similarly, Rubio-Rodríguez et al. (2012) reported that heavy metals (Cd, Hg, Pb, As) in fish oil extracted from livers of *Merluccius paradoxus*, *Hoplostethus atlanticus*, and *Salmo salar* were to be negligible. In the study of Foran et al. (2003), they have shown that the levels of mercury in the 5 commercial brands of fish oil ranged from non-detectable (6 mg/L) to negligible (10–12 mg/L). Also, they have expressed that consumption of fish oil were safer alternative to fish preparations (Foran et al., 2003).

In the studied on tissues, according the study of Canli and Atli (2003), the concentrations of Cu (202.8), Cd (4.50), Cr (17.1) and Pb (41.2) were detected in liver tissues of *Mugil cephalus, Trigla cuculus*, *Sardina pilchardus* and *Atherina hepsetus*, respectively. Bat and Arici (2016) presented that toxic element limit values of Atlantic bonito (*Sarda sarda*) caught in the Black Sea were edible levels. Gümgüm et al. (1994) were not detected Co, Mo, Pb and V accumulation in *Cyprinion macrostomus* and *Garra rufa* from Tigris River by Ergani Copper Plant and the geochemical structure of this region. Studies have reported that Pb and Cd accumulation levels of marine fish were in the following orders: Gonads > Skin > Gill > Liver > Muscle according to Masoud et al. (2007) and Spleen > Kidney > Liver > Gill > Muscle according to Çoğun et al. (2005). Anyway, mean value of As levels in *Sardinia lascaris* were Liver > Muscle > Skin, and in *Trachurus lucerna*, Muscle > Liver > Skin (Juresa and Blanusa, 2003).

The liver and muscle tissues of fish are generally contaminated with toxic metals. However, in this study we have reported that heavy metals in the oils extracted from liver and muscle tissues from *D. pastinaca, R. radula, R. clavata, T. marmorata* were to be below the limit of detection and not contain a significant amount of them. Therefore, the oils extracted from these species are suitable for human consumption in terms of metal contamination and risk of toxicity.

Conclusion

This study compared the fat and fatty acid composition, macro and trace element levels of the muscle and liver tissues of *R. radula*, *R. clavata*, *D. pastinaca*, *T. marmorata* and also the contamination levels with potential toxic heavy metals of oils of their tissues. Lipid contents of all the species were reported as sufficient for human health. The results show that rays are potential resources of polyunsaturated fatty acids (PUFAs) and should be used in the diet of local populations. They contained essential macro and trace elements, fatty acids, which are very crucial for health. Finally, these fish have potential for fish oil production because of their having no toxic heavy metal in the oils extracted their liver and muscle tissues. Especially, It was determined that the most suitable tissue for fish oil production is liver tissue of *D. pascinata* in terms of its oil level, size or weight and also having none heavy metal risk of its oils.

Acknowledgements

This study was supported by the Research Fund of Mersin University in Turkey with Project Number: 2017-1-TP2-2173.

Conflict of Interest

The authors declare that there is no conflict of interest.

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