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Biochemical Composition of The Wild Long-Snouted Female and Male Seahorses (Hippocampus guttulatus Cuvier, 1829)

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ABSTRACT

The biochemical composition in wild long-snouted female and male individuals of *Hippocampus guttulatus* was investigated in Southeastern Black Sea. PUFA were the most important fatty acids in males (40%) and in females (41%). The main components of PUFA were DHA (20-22%) and EPA (9-11%). DHA was the most abundant fatty acids in *H. guttulatus*. Cholesterol was the most important sterol in the males and females (205.36 µg/g; 200.36 µg/g, respectively). α- tocopherols (vitamin E) was the most important lipophilic vitamins and α-tocopherol acetate was the highest amount (6.67 µg/g; 7.88 µg/g, respectively) in the females and males. Total protein was 13.87 mg/g in the male and 14.38 mg/g in the females. GSH (Glutathione) and GSSG (Oxidised Glutathione) levels were lower than MDA (Malondialdehyde). GSH was 99.66 µg/g in the male and 98.18 µg/g in the female. GSSG was 40.25 µg/g in the males and 40.18 µg/g in the females. It was thought that high MDA level (251.07-256.30 µg/g) occurred, because low vitamin E level did not prevent PUFA peroxidation. Our findings showed that biochemical composition of seahorses did not differ between male and female individuals except for fatty acids (p<0.01).

KEYWORDS: Seahorses, fatty acids, sterol, α- tocopherol, Southeastern Black Sea..

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1. Introduction

Seahorses are a member of animal kingdom, the Genus *Hippocampus* and Syngnathidae family, which consist of about 35 genera (Fritzsche, 1980). Syngnathidae are a dominant family in fishes around the World (Pollard, 1984). All of them is marine species and generally prefer to live among seagrasses, mangroves and corals in shallow temperate and tropical coastal waters (Vincent, 1996). They are distributed all over the Aegean Sea, Mediterranean Sea and Black Sea (Keskin et al., 2002).

Seahorses are rapacious predators which are relying completely on live, moving food. They can ingest anything that fits in to their mouth-mostly small crustacea and also small fishes. They ambush the prey by inhaling rapidly through their snout (Tipton and Bell, 1988). They are carnivores with excellent vision feeding on variety of benthic and pelagic organisms, mostly tiny zooplankton crustaceans and epibenthic isopods and worms. Although seahorses are unsuitable to consume as human food, they have international trade worldwide (Prein, 1995). In addition, they are used to produce such as seahorse wine and soup, pills and capsules for human consumption from them (Lin et al., 2008). Additionally, they are under high pressure of overfishing and seven million seahorses are traded each year (Evanson et al., 2011). Because, especially in China, they are used as raw material for souvenir, ornamental, drug in Traditional Chinese Medicine (TCM) and its derivates (e.g, Japan and Korea Traditional Medicine) (Vincent, 1996). Seahorses are in the Red List in IUCN (Faleiro and Narsico, 2010).

Lipids are one of the the most important biochemical compounds in trophic transfer in marine ecosystems because they provide the densest form of energy in the systems (Parrish, 2013). They are also important components of biomembranes and precursors of important hormone groups such as eicosanoids (Sargent et al., 2002). Lipid and fatty acid contents correlate with reproductive parameters such as egg quality, spawning, larval viability of many fish species (Rainuzzo et al., 1997; Yanes-Roca et al., 2009). Certain essential fatty acids and sterols in lipids are considered to be important determinants of ecosystem health and stability. Fatty acids and sterols are also susceptible to oxidative damage leading to cytotoxicity and a decrease in membrane fluidity (Parrish, 2013). In addition, they can use as biomarkers in ecology (Budge et al., 2006; Litzow et al., 2006). Especially, polyunsaturated fatty acids (PUFA) (e.g. docosahexaenoic acid-22:6ω3, DHA, eicosapentaneoic acid-20:5ω3, EPA and α-linolenic acid-18:3ω3, ALA) are among very important classes of fatty acids (FA) (Sargent et al., 1987; Caramujo et al., 2008; Lund et al., 2008). Proteins are another important biochemical components. They have the most important biological roles in some processes such as enzymatic catalysis, immune protection, generation and transmission of nerve impulses, and control of growth and differentiation (Zaia et al., 1998). Malondialdehyde (MDA) levels are good indicator of peroxidative damage to cell membranes (Gil et al., 2002). Thus, it is used as a marker for lipid peroxidation. In addition, it is a highly reactive three carbon dialdehyde. The reactive three carbon is produced as a by product of PUFA peroxidation (Janero, 1990) and also during arachidonic acid (ARA) metabolism for the synthesis of prostaglandins (Marnett, 1999). Glutathione (GSH) has an important role in protecting from oxidative damage (López-Barea and Gómez-Ariza, 2006) and is the most abundant antioxidant in aerobic cells. Cysteine thiol moiety gives antioxidant properties of GSH. The thiol is oxidized by cellular pro-oxidants, such as free radicals and reactive aldehydes, to form oxidized GSH disulfide (GSSG) (Owen and Butterfield, 2010). Because of these reasons, we investigated some biochemical dynamics of *H. guttulatus* in this study. Although, many studies have been published concerning the-long snouted seahorse (e.g. Curtis and Vincent, 2005; Curtis and Vincent, 2006; Curtis, 2007; Gürkan and Taşkavak, 2007; Gürkan et al., 2007; Faleiro et al., 2008; Kasapoglu and Duzgunes, 2014), these studies generally focused on seahorse culture, reproduction, biometric features, behavioural approach and population characteristics. Only some published studies about the biochemical content were observed by Faleiro and Narsico

(2010), Lin et al. (2008) and Vite-Garcia et al. (2014). They generally investigated the biochemical composition in association with lipids and fatty acids. To our knowledge, this paper is the first report about biochemical dynamics such as fatty acids, sterols, lipophilic vitamins, total protein, MDA, GSH and GSSG of wild *H. guttulatus* from the natural environment. The main objective of this study is to show some biochemical composition of the wild seahorse (*Hippocampus guttulatus* Cuvier, 1829) which is an important predator and which has a special reproduction system in marine ecosystems. Eventually, it is aimed to show the biochemical differences and similarities between the male and female individuals of *H. guttulatus.*

2. Materials and Methods

2.1. Study Area and Sampling

The study was performed in the Southern part of the Black Sea at a coastal station with coordinates (40o57'12"N-40o9'30"E) (Fig. 1). Totally 18 individuals of H. guttulatus were sampled by trawl in 150 m depth. Samplings were made in 2012 autumn season with KTU's research vessel, Yakamoz. The samples were carrried to the laboratory, and were immediately separated female (n=8) and male (n=10) individials of H. guttulatus. They were kept in NUIIRE brand freezer (-80^{o}C) until analysis.

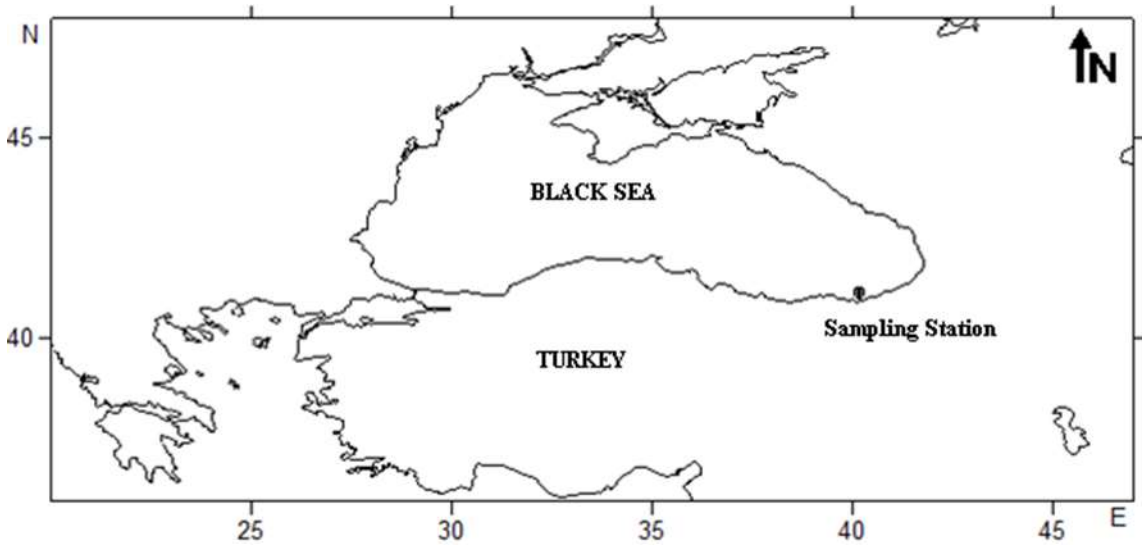

Fig. 1. Sampling station in the Southeastern Black Sea

2.2. Biochemical Analysis

The wet weight of frozen samples were homogenized with tissue fragmentation buffer. The homogenate was centrifuged and the supernatant part was used for GSH, MDA and GSSG analysis. In extraction the remaining homogenate pellets was used hexane: isopropanol (3:2 v/v) (Hara and Radin, 1978) and centrifuged for lipophilic vitamins and fatty acid analysis.

2.2.1. Derivatization and Analysis of Fatty Acid Methyl Esters (FAME)

5 ml H_2SO_4 (2%) solution was added in aliquot taking from the supernatant part of the sample pellet. The sample was vortexed and kept at 12 h in the oven (50°C). 5 ml NaCl solution (5%) was added and the sample was again vortexed. The FAME were esterified according to Christie (1990). 2×5 ml hexan was used for the extraction. Then, 5 mL $KHCO_3$ solution (2%) was treated on it. Nitrogen (N_2) was used for dry lipid by evaporating of hexan. Dry lipids were dissolved in 1 ml hexane.

FAME were analyzed on Gas Chromatography (GC) (SHIMADZU 17 Ver. 3). FAME were injected into a capillary column Machery-Nagel (Germany). The column was 25 m in length, 0.25 µm inner diameter, 25 µ film thickness. The column temperature was at 120-220°C. Column temperature ramped to 200°C at a rate 5°C/min and 220°C at a rate 4°C/min. The final temperature was held for 8 min at 220°C. The injector temperature stayed at constant 240°C, the detector temperature stayed constant 280°C. The carrier gas was N_2. Peaks identified using retention times from the FAME standard injected on the samples before the analysis. Then, fatty acid peaks were integrated making the necessary programming.

2.2.2. Derivatization and Analysis of Lipophilic Vitamins and Sterols

5 ml supernatant from the samples was put to 25 ml tubes. 5ml KOH: methanol (1: 10 v/v) was added. It was vortexed and kept in the oven at 85°C/15 min. After they were cooled until room temperature, 5 ml distilled water was added on them and shaked. In extraction of lipophilic vitamins 2×5 ml hexane was used. The hexane in the samples was evaporated under N_2. 1 ml acetonitrile:methanol (50 + 50% v/v) was used for dissolving process.

Sterols were analysed on a Shimadzu HPLC. LC-10 ADVP UV visible pump, SPD-10AVP detector, CTO-10ASVP column, SIL-10ADVP auto sampler, DGU-14A degasser unit and Class VP software (Shimadzu, Kyoto Japan) were used. The acetonitrile:methanol (60+40% v/v) was used in the mobile phase. UV detector and the Supelcosil LC 18 column (Sigma, USA) were used. 25 m in length, 0.25 µm inner diameter, 25 µ film thickness The column was 15×4.6 cm and 5 µm. Wave lenghts of the detection were 326 nm for vitamin A, 202 nm for vitamin E and 265 nm for D, K vitamins (Katsanidis and Addis, 1999).

2.2.3. Analyses of Lipid Peroxidation (MDA), Reduced (GSH), Oxidized Glutathione (GSSG) and Total Protein

The supernatant part was used for MDA, GSH and GSSG analyses. 1ml perchloric acid (10% v/v) was added to 1 ml supernatant and then centrifuged. After centrifuging, MDA, GSH and GSSG were analyzed on a Shimadzu HPLC. MDA was analyzed according to Karatepe et al. (2004). GSH was analyzed according to Yilmaz et al. (2009). Peaks were identified using retention times and spectra (multiwave length scan) of the standard and the samples. Total protein amount of wild *H. Guttulatus* was determined according to Lowry et al. (1951).

2.3. Statistical Analysis

Differences among data obtained from fatty acids, lipophilic vitamins and sterols in the wild female and male seahorses individuals were tested using multivariate statistical methods. Fatty acid, lipophilic vitamins, sterols and gender relationships were determinedin the Plymouth Routines in Multivariate Ecological Research (PRIMER-version 7.0.10). The software uses in analyzing of ecological data. Multivariate statistics that were used included: Analysis of similarities (ANOSIM), non-parametric multi-dimensional scaling (nMDS), and similarity of percentages analysis (SIMPER). The similarity percentages routine is a multivariate analysis that uses a resemblance matrix to give the average percentage similarity between groups. Also PERMANOVA (Permutational MANOVA) was used to examine average similarity between/within groups.

3. Results

3.1. Fatty Acid (FA) Composition

Up to 39 fatty acids were identified in each sample. FA composition of the female and male seahorses in the Southeastern Black Sea are shown in Table 1.

22 fatty acids were determined as levels >0.5% in the male seahorses, while 13 fatty acids were detected at levels >0.5% in the female seahorses. In results of SIMPER the total saturated fatty acids (∑SFA) contributed 34% in the male and 32% in the female of the total FA. The main component of SFA was 16:0 (20% and18%, respectively) followed by nearly the same proportion 18:0 (6%) in both of the males and the females. Similarly results shown in Tables 1. Total monounsaturated fatty acids (∑MUFA) were nearly the same proportion at 28% and the main component of the males and females was 18:1ω-9 (10% and 9%, respectively). PUFA

were the most important fatty acids. The total polyunsaturated fatty acids (∑PUFA) were 40% in the males and 41% in the females. The main components of PUFA were DHA (20-22%) and EPA (9-11%) and DHA was the most abundant fatty acid in *H. guttulatus.* Otherwise, the zooplankton fatty acids (∑20:1+∑22:1) were higher in the females (1.69%) than the males (1.21%).

Table 1. Fatty acid composition (% total fatty acids ± 1 s.d.) of seahorses from Southeastern Black Sea

	***Hippocampus guttulatus*, Cuvier, 1829**	
FAs	**Male**	**Female**
14:0	4.33±0.52	4.89±1.22
14:1	-	0.12±0.09
15:0	0.77±0.29	0.76±0.25
15:1	0.39±0.04	0.16±0.17
16:0	20.00±2.08	18.03±0.37
16:1ω9	0.63±0.06	0.76±0.35
16:1ω7	9.54±1.21	10.05±1.57
16:1ω5	0.68±0.13	0.79±0.28
16:2ω4	0.51±0.08	0.48±0.12
17:0	1.09±0.19	1.23±0.29
16:3ω4	0.58±0.07	0.30±0.26
17:1	0.27±0.08	0.59±0.13
16:3ω3	0.43±0.09	-
18:0	5.81±0.88	5.07±0.80
18:1ω9	10.30±1.74	8.55±1.03
18:1ω7	2.27±0.39	2.33±0.37
18:1ω6	0.52±0.14	0.45±0.06
18:2a	0.52±0.07	0.48±0.10
18:2b	0.44±0.06	0.37±0.06
18:2ω6	2.36±0.33	2.05±0.49
18:3ω6	0.44±0.07	0.40±0.05
18:3ω4	0.69±0.16	0.40±0.36
18:3ω3	0.27±0.05	0.52±0.30
20:0	0.38±0.13	0.19±0.14
20:1ω9	0.96±0.15	1.33±0.30
20:2ω6	0.38±0.13	0.41±0.08
20:3ω6	1.70±0.31	0.82±0.73
20:4ω6	0.24±0.09	1.02±0.76

20:5ω3	8.73±1.26	11.05±1.02
22:00	0.17±0.04	0.12±0.09
22:1ω11	-	0.35±0.10
22:1ω7	0.25±0.08	-
22:2a	0.21±0.06	-
22:2b	0.45±0.06	0.27±0.20
22:5ω3	2.07±0.68	0.21±0.18
24:0	1.00±0.29	2.09±0.50
22:6ω3	19.83±1.74	22.29±1.74
24:1	0.83±0.21	1.07±0.30
∑SFA	33.54±2.25	32.37±1.45
∑MUFA	26.63±1.29	26.57±1.42
∑PUFA	39.82±1.44	41.06±2.21
∑HUFA	31.33±1.69	34.06±2.41
∑ω3	37.13±4.54	34.43±5.01
DHA/EPA	2.32±0.47	2.02±0.15
Zooplankton (∑20:1 + ∑22:1)	1.21±0.14	1.69±0.31
16:1ω7/16:0	0.48±0.10	0.56±0.09
18:1ω9/18:1ω7	4.53±0.81	3.67±0.82

Fig. 2 shows a two-dimensional configuration plot of MDS analysis of a resemblance matrix of fatty acid data. Multivariate analyses of FA were identified in 18 samples. The samples plotted were factored by gender. The samples in the MDS plot were identified by gender (as female and male). Stress is called as the degree to which the two dimensional configuration plotdistorts the sample relationship. The value 0.10 give a "potentially useful representation'', values below 0.05 give an "excellent representation,'' while values between 0.1 and 0.2 give "a potentiaaly useful 2-dimensional picture'' (Clarke and Warwick, 2001). Fig. 2. shows the importance of some fatty acids in gender groups of the seahorse. MDS plot showed that 18:0, 18:3ω4, 15:1, 20:3ω6, 22:1ω7, 16:3ω3 were more important in the female than the male of *H. guttulatus*. Although, 22:6ω3, 17:1, EPA and 20:4ω6 were important fatty acids in both of gender groups and, these fatty acids were more higher in the males than the females.

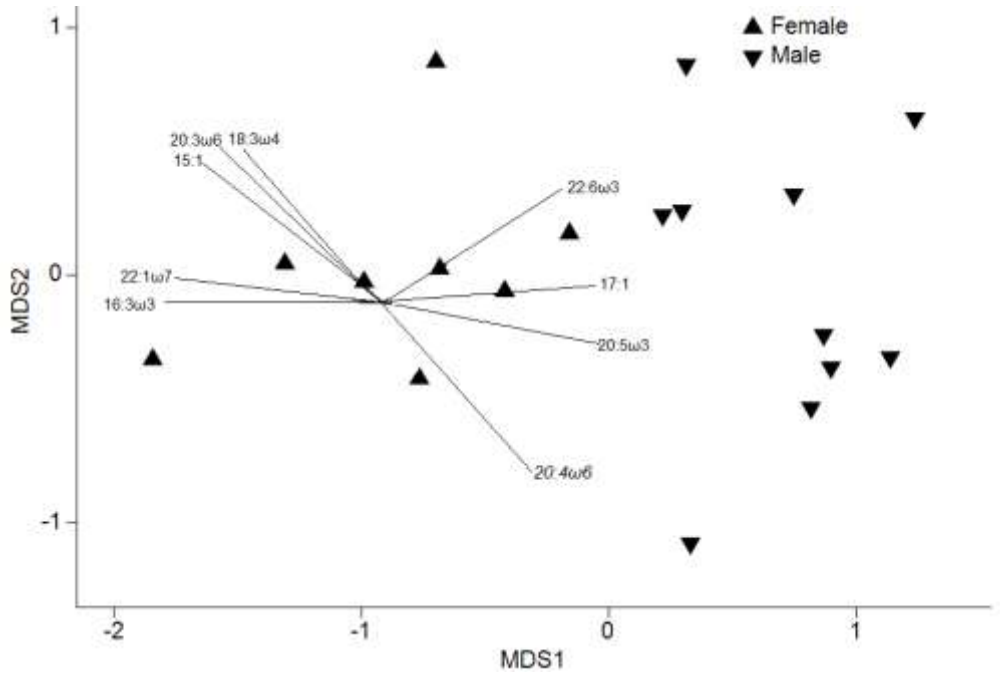

Fig. 2. Proportions of fatty acid of the wild seahorses (*H. guttulatus)* in MDS plot. The resemblance matrix was formed by Bray-Curtis similarity coefficients. Pearson correlations: >0.85. 2D stress 0.10.

One-way SIMPER analysis showed the average similarity of fatty acids in the female and the male seahorses (Resemblance: Bray Curtis similarity, cut off for low contributions: 70%). The average similarity was nearly at the same level in the males (91.60%) and the females (91.99%) and they had a high similarity level. The highest contributor was DHA (20% and 23%, respectively). In both groups, average similarity was 88% and DHA has the highest contribution (12%). The PUFA contribution was evenly highest in the both of seahorses at 28% in the female and male. However, when female and male seahorses were compared, ω3 (omega3) had the highest contribution (28%) and the PUFA was the second contributor at 20%.

The PERMANOVA the same add-on to PRIMER v7 was used to test to test significant differences among the groups. There was difference in pairwise comparisons among the gender groups (p=0.001) (p<0.01). Average similarity between/within groups were generated from same add-on. Average similarity between groups (female and male) was 88%, average similarity with in groups was equal with 92%. SIMPER also showed that 22:6ω3 (DHA) and 16:0 were always among the top two contributors to the similarity in each of the female and male seahorses (DHA, 20-23%; 16:0, 19-20% respectively). However, 18:1 ω9 was the fifth contributer in the males while 18:1ω9 was the third contributer in the females. EPA was the third contributor to the similarity at 11% in the males, fifth contributor at 9% in the females.

3.2. GSH, MDA, GSSG and Total Protein

Table 2 shows GSH, MDA, GSSG and total protein content of male and female seahorses. Total protein was nearly the same amount in the males (13.87 mg/g) and females (14.38 mg/g). Similarly, there was not an important difference in GSH, MDA and GSSG amount of the female and the male seahorses. Only, MDA was 251 µg/g in the males, while MDA 256 µg/g in the females. Total protein, MDA and GSSG were higher in the females than the males. Conversely, GSH was higher in the males than the females.

Table 2. GSH, MDA, GSSG (µg/g ± 1 s.d.) and total protein (mg/g ± 1 s.d.) content of seahorses from Southeastern Black Sea

	***Hippocampus guttulatus,* Cuvier, 1829**	
	Male	**Female**
GSH (µg/g)	99.66±6.93	98.18±10.20
MDA(µg/g)	251.07±12.44	256.30±20.01
GSSG (µg/g)	40.25±1.34	40.80±5.68
Total Protein (mg/g)	13.87±0.12	14.38±1.74

3.3. Lipophilic Vitamins and Sterols

Table 3 shows the lipophilic vitamin and sterol content (µg/g vitamin ± 1 s.d.) of male and female seahorses. One-way SIMPER analysis showed the average similarity of lipophilic vitamins and sterols in the wild female and the male seahorses (Resemblance: Bray Curtis similarity, cut off for low contributions: 90%). The average similarity of lipophilic vitamins was higher in the females (95%) than the males (79%). The highest contributor was α-tocopherol acetate (70% and 65%, respectively) in the females and males. In the sterols, the average similarity was higher in the females (97%) than the males (93%).

Table 3. Lipophilic vitamin and sterol content (µg/g vitamin and sterol ± 1 s.d.) of seahorses from Southeastern Black Sea

	***Hippocampus guttulatus* Cuvier, 1829**	
Vitamins	**Male**	**Female**
K_2	0.5±0.13	0.26±0.03
R-tocopherol	0.44±0.19	0.37±0.04
D_2	0.16±0.06	-
α- tocopherol	2.02±0.77	2.83±0.50
α- tocopherol acetate	6.67±2.29	7.88±0.53
Retinol	0.35±0.16	0.11±0.02
	205.36±27.46	200.36±11.43
	47.13±3.17	48.34±2.37
Sterols	5.16±1.55	5.06±0.17

PERMANOVA was used to test differences among the groups in lipophilic vitamin. There was no difference in pairwise comparisons among the gender groups (p=0.07) ($p<0.01$) in lipophilic vitamins. In the sterols, there was no difference in pairwise comparisons among the gender groups

(p=0.75) (p<0.01). Average similarity between/within groups were generated from same add-on. However, identical data can be obtained from a SIMPER analysis. Average similarity between groups was 88%, while average similarity within groups was 95% within sterols of the females, and 79% of the males. SIMPER showed that α-tocopherol acetate and α-tocopherol were always the top two contributors to the similarity in each of the females and males both between and within groups. In the sterols, the cholesterol and stigmasterol were the main contributors. Cholesterol and stigmasterol had the same contribution value (79% and19%, respectively) within groups. Between groups, while cholesterol was 85%, stigmasterol was 11%. D_2 vitamin did not exist in the female seahorses and it was the lipophilic vitamin that had the least amount (0.16 µg/g). Generally, lipophilic vitamin amounts were less in the female than male except for the α-tocopherol group vitamins (vitamin E). Cholesterol was higher in the males (205 µg/g), than the females (200 µg/g), while stigmasterol was higher in the females (48 µg/g) than in the males (47 µg/g). However, β-sterol was nearly equal (5%) in both of them.

4. Discussion

Fatty acids are indispensable to living organisms. They are energy resources and essential nutrients for survival and growth. Also, they have important roles in the structure of cell membranes (Alfaro et al., 2006). Fatty acids are transferred from primary producers to higher trophic levels with out change (Parrish et al., 2000), they are useful markers to track the flow of materials through food webs. FA have been used as biomarkers to trace food web relationships (Iverson, 2009). Similarly, sterols are be important drivers for ecosystem health and stability. FA and sterols are sensitive against oxidative damage. Oxidative damage is an unwanted situation in cell because of lead to toxicity and decrease of membrane fluidity. The cell can be defended from the influence of temperature, pressure, or lipid peroxidation by the physical structures of biological membranes (Parrish, 2013).

In this study, the biochemical parameters, which are fatty acids, lipophilic vitamins, sterols, total protein, MDA, GSH and GSSG in female and male individuals of *H. guttulatus* have been investigated. In ecological studies, DHA has been used as a dietary marker of dinoflagellates (Kelly and Scheibling, 2012), whereas EPA has been used as diatoms marker in marine environments 2012). Therefore, DHA/EPA reflects the relative proportions of dinoflagellate to diatoms in the diets of herbivorous and omnivorous copepods (Viso and Marty, 1993). Additionally, the ratios of 16:1ω7/16:0 (Graeve et al., 1994; Auel et al., 2002; Parrish et al., 2015), DHA/EPA and 18:1ω9/18:1ω7 point out the relative contributions of different phytoplankton groups (Dalsgaard et al., 2003). Also, 16:1ω7/16:0 is used to discriminate diatom *versus* dinoflagellates feeding, 18:1ω9/18:1ω7 is markers of trophic position and a carnivore index (Parrish et al., 2015). Therefore, in the present study, ratios of DHA/EPA, 18:1ω9/18:1ω7 and 16:1ω7/16:0 were used in approach of *H. guttulatus* food preferences**.** In the present study, the major fatty acids in the seahorses were the dinoflagellate fatty acid trophic marker 22:6ω3 (DHA), the diatom marker 20:5ω3 (EPA), followed by 16:0, 16:1ω7, 18:1ω9. When the male and female seahorses are compared, DHA/EPA and 18:1ω9/18:1ω7 as carnivore biomarkers are higher on average in the male (2.32 and 4.53, respectively) than in the female (2.02 and 3.67, respectively). Conversely, 16:1ω7/16:0 as herbivore biomarker were higher on average in the females (1.69) than in the males (0.48). Also, it was observed that zooplankton (∑20:1 + ∑22:1) fatty acids (∑20:1 + ∑22:1) were higher in the females (1.69%) than in the males (1.21%). Our all the findings suggest that *H. guttulatus* prefer carnivore food sources rather than herbivore food source, because 16:1ω7/16:0 as herbivore index was less than carnivore indexes as 18:1ω9/18:1ω7 and DHA/EPA and zooplankton who are a carnivore food source. Our findings sopport that seahorses are carnivores feeding on variety of benthic and pelagic organisms, mostly tiny zooplankton (Prein, 1995).

It was shown that *H. guttulatus* had high 16:0, EPA and DHA content. 16:0, DHA and EPA seem to have an important energetic role by Faleiro and Narciso (2010). Lin et al. (2008) indicated that the most abundant fatty acid was 16:0 in six seahorse species, *Hippocampus* sp, from China Coast. 16:0 ranged from 15.04±0.67-31.04±4.32. They found

that ∑SFA ranged 31.75±1.47-49.36±2.13% in these species. ∑MUFA and ∑PUFA ranged from 14.89±0.75-25.70±2.12, 19.97±1.08-32.96±1.56 in their study. Also, they reported that EPA and DHA amounts of the seahorses were approximately 20% of the total fatty acids. There was difference in pairwise comparisons between the gender groups (p=0.001) ($p<0.01$). Lin et al. (2009) showed that 16:0 was the highest among all the fatty acids in wild seahorses, 27.13±4.32% in *Hippocampus kuda* and 25.99±3.68% in *Hippocampus trimaculatus*, and it was significantly higher than those in cultured seahorses ($F_{3,12}$=28.44, $P<0.05$). Additionally, they determined that 18:0 and 18:1 were the major fatty acids in wild *H. kuda* (13.35±2.53 and 7.74±0.93%, respectively) and *H. trimaculatus* (16.91±1.79 and 14.19±0.82%, respectively). However, DHA was the most abundant fatty acid in cultured *H. kuda* (27.23±3.44%) and *H. trimaculatus* (22.41±3.10%) (Lin et al., 2009). We found that 18:0 was second major fatty acid in SFA and had not at a major level (5.81% in the males; 5.07% in the females) in all fatty acids. The composition and nutritional content of wild seahorses are often valiable (Kattner and Krause, 1989; Delbare et al., 1996; Payne and Rippingale, 2000). For culturing seahorses, the same diets may not be used in different seahorse species (Payne and Rippingale, 2000). Therefore, biochemical compositions of wild and cultured seahorse species provide both a valuable foundation for culturing seahorse species and for assessing the medical value of seahorses (Lin et al., 2009).

DHA, EPA and 16:0 are the main fatty acids to supply the energetic demands of seahorse embryos (Faleiro and Narciso, 2010). Also, catabolism of EPA and DHA are necessary for energy during the early life of many marine fish. EPA and DHA have important structural and physiological roles in the life history. Indeed, they are major energetic resource in ω-3HUFA (Tocker, 2003). In this study, total high unsaturated fatty acids (∑HUFA) were higher male (37.13±5.01) than female (34.43±4.54). Fatty acids is used as egg quality indicators in fish eggs including the seahorse eggs, and are charecterized via a high ω-3 HUFA amount (Sargent et al., 1989, 1999; Faleiro and Luis, 2010). HUFA expecially comes to forefront in the fish eggs (Faleiro and Narciso, 2010) and essential proporties in marine fish (Sargent et al., 1989, 1999). HUFA levels of the broodstock is affected by their diets (Rainuzzo, 1993). Planas et al. (2010) determined that level of ω-3 HUFA was 18.5 (38.4mg/g dry weight). They indicated that fatty acid profile of eggs was similar to the broodstock diet enriched with adult Artemia. SFA, MUFA and PUFA can be metabolized for energy during development of marine eggs. However while selective retention of HUFA are quite consistent during development of marine fish, MUFA are preferred for energyuse. Conversely, PUFA including HUFA aren't selectively retained during embryogenesis of *H. guttulatus*. HUFA creates the major source of metabolic energy (Faleiro and Narciso, 2010). It was observed that Seahorse *Hippocampus kuda* fed with frozen mysis without any enrichment had a higher content of some SFA (22:0 and 24:0) by Saavedra et al. (2014). However, they indicated that when the enrichment was added to the diet, a better fatty acid profile of the eggs was obtained. Also, they reported that the eggs from females fed with the enriched diet (frozen mysis) showed a higher content of PUFA and sum of ω-3 HUFA fatty acids was higher. Saavedra et al. (2016) researched effect of fatty acid enrichment in diet on the egg quality of *Hippocampus hippocampus* during the spawning season. They showed that the enrichment diet of *H. hippocampus* improved egg viability and obtained an increase in quality of eggs by maintaining PUFA content in the last of the spawning season.

Our study showed that α- tocopherol (vitamin E) are the most important lipophilic vitamins and α-tocopherol acetate was the highest amount (6.67µg/g and 7.88µg/g, respectively) among the females and males of *Hippocampus guttulatus*, following α-tocopherol (2.02µg/g and 2.83µg/g, respectively). α-tocopherols are among the most important lipid-soluble antioxidants in living beings. Especially, they protect to biological membranes against lipid peroxidation (Yamamota et al., 2001). Vitamin E is a constituent of tocopherols or reduced coenzyme Q (ubiquinols), that reside within cellular membranes. MDA is decomposition products of tocopherols, and unstable. They can damage vital cellular components, including DNA, which becomes toxic if not removed or repaired (Marnett, 1999). In this study, MDA level was found as 251.07 µg/g in the

male and 256.30 µg/g in the female of *Hippocampus guttulatus***.** GSH and GSSG levels were lower than MDA. GSH was 99.66 µg/g in the males and 98.18 GSSG µg/g in the females. GSSH was 40.25 µg/g in the males and 40.18 µg/g in the females. MDA is lipid peroxidation product (Ji et al., 2011) and is used as a marker of lipid peroxidation, and protein carbonyl content, which provides an indication of protein oxidative damage. It is a highly reactive three carbon dialdehyde and produced asperoxidation roduct of PUFA (Janero, 1990). Moderate dietary HUFA supplementation significantly reduces peroxidation products of lipids and is beneficial to a higher survival and growth (Ji et al., 2011). High MDA production is a result of high dietary HUFA level (Du et al., 2008; Ji et al., 2011). Excess HUFA fortification may probably cause adverse effects due to oxidative stress (Ji et al., 2011). This situation is effects on the enzymatic activities of juvenile of *Hippocampus erectus* (Yin et al., 2012). Yin (2012) found that highest MDA content occurred in the treatment of 54.0 µL/L HUFA in *Hippocampus erectus*, fed with HUFA enriched *Artemia nauplii*. GSH contributes importantly to maintenance of the intracellular redox environment and the antioxidant defense system for protection of tiol disülfide redoks situation of protein. Decrease of GSH/GSSG ratio reflects that GSH oxidation to GSSG cause in intracellular redox imbalance. This imbalance is connected with oxidative stress (Dalle-Donne et al., 2008). In our study, GSH/GSSG was measured 2.48 in the males and 2.41 in the females. It was shown that MDA level was quite higher than GSH and GSSG levels. Also, GSH/GSSG ratio was quite low. When animals ingest high levels of PUFA, high concentrations of α-tocopherol require to protect tissue lipids from free radical attack (Lammi-Keefe and Jensen, 1984; Debier et al., 2002; Machlin, 1991). Additionally, α-tocopherol can prevent PUFA peroxidation by acting as quenchers of singlet oxygen. An increase in membrane PUFA causes deficient in vitamin E (Mourente et al., 2007). In our study, it was thought that high MDA level occurred because low vitamin E level did not prevent PUFA peroxidation.

Many biochemical parameters such as lipoprotein lipase (LPL), superoxide dismutase (SOD), malate dehydrogenase (MHD), glutathione peroxidase (GPX) and Chloramphenicol acetyltransferase **(**CAT) are used as oxidative stress indicators. For example, increase of HUFA level is increased SOD activity, consistent with MDA content, indicating that higher HUFA levels induce oxidative stress (Ji et al., 2011). Yin et al. (2012) emphasized that GPX activity was dramatically stimulated in high HUFA level, implying the role of CAT in protection of *Hippocampus erectus* against the harm by hydroperoxide. Additionally, oxidative stress may be responsible for adaptation of organisms to broad range of environmental stressors (e.g. temperature, salinity, oxygen level, transition metal ion) (Lushchak, 2011). E.g temperature, different mechanisms are responsible for induction of free radical attack with the changing of in the environmental temperature. The temperature increase enhances oxygen consumption. Therefore, It may be increased reactive oxygen species. The increase of environmental temperature indice oxidative stress in fish (Heise et al., 2006; Bagnyukova et al., 2007).

Fishes are known to be a valuable source of protein (Sikorski, 1994; Andrew, 2001) They are among the most important source of animal protein (Andrew, 2001). Especially, proteins with marine fishes origine area key component for a healthy diet for human diet (Rahman et al., 1995). Seahorses are an important source of food in countries such as China and intensively comsumed. They are used in products such as seahorse wine and soup, capsules, and pills for human consumption (Lin et al., 2008). Boran and Karaçam (2011) reported that average protein amount was 14.78% in horse macharel, 16.43% in shad, 16.19% in golden mullet and 16.89% in garfish from the Black Sea. Lin et al.(2009) determined that the crude protein contents in dry weight (DW) of wild seahorses were 72.7±2.5% in *H. kuda* and 78.5±4.2% in *H. trimaculatus.* We found that total protein was 13.87% in the male and 14.38% in the female seahorses. The results showed that the total ptotein amount of the seahorses are almost close the fish consumed in the Black Sea Region.

Sterols are a source of oxygenated lipids. They have a variety of biological properties including cytotoxicity and effects on specific enzymes

(Parrish, 1991). In the present study cholesterol was the most important sterol. The chelosterol amount was 205.36 µg/g in the male and 200.36 µg/g in female seahorses. Cholesterol is predominant sterol in most animals. It is a starting point for the other sterols.

Consequently, our findings showed that in biochemical dynamics used except for fatty acids was not different between wild males and females of *H. guttulatus* (p<0.01). Foods of seahorses have an effect on their biochemical composition. Adult seahorses feed basically on small shrimps, such as *Acetes* sp. and *Mysis* sp. (Woods, 2003; Lin et al., 2006). In addition, It is known that fishes food composition strongly affects to the nutritional composition (Henderson and Tocher, 1987; Orban et al., 2007; Velu and Munuswamy, 2007). Besides other factors such as species, genetic, size, reproductive status, and the environmental characteristics (temperature, season, and geographical location) effects the nutritional composition of fishes (Özparlak, 2013; Harlioglu, 2012; Orban *et al.*, 2002). Therefore, the diets of the seahorses may influence their proximate biochemical composition (Orban et al., 2002).

5.Conclusion

Seahorses have international commercial value in world wide (Prein, 1995). They are under high pressure of overfishing, because they are used as raw material for souvenirs, ornaments, drugsin Traditional Chinese Medicine (TCM) (Vincent, 1996) and in products such as seahorse wine and soup, capsules, and pills for human consumption (Lin et al., 2008). In this study some biochemical parameters of wild male and female seahorses (*H. guttulatus*) were investigated. Consequently, we determined that only for fatty acids there was a difference between wild males and females of *H. guttulatus* (p<0.01). Additionally, we can say that in females and males of *Hippocampus guttulatus*, PUFA and α-tocopherol were the most important biochemical parameters. This study supports that seahorses are carnivores feeding on a variety of benthic and pelagic organisms, mostly tiny zooplankton.

6.Conflict of interest

All authors report no conflict of interest.

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