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Ege Üni. Su Ürünleri Fakültesi, 35100, Bornova, İzmir Ege Uni. Faculty of Fisheries, 35100, Bornova, Izmir, Turkey
Tel: +90 232 311 3838 Fax: +90 232 388 3685 http://www.egejfas.org info@egejfas.org

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Assessment of the ecological and trophic status of Lake Bafa (Turkey) based on phytoplankton

Bafa Gölü'nün (Türkiye) ekolojik ve trofik durumunun fitoplankton bazlı değerlendirilmesi

Atakan Sukatar[1] ● Alperen Ertaş[2]* ● Rıza Akgül[3] ● İnci Tüney Kızılkaya[4]

[1] Department of Biology, Faculty of Science, Ege University, 35100 Bornova, İzmir, Turkey https://orcid.org/0000-0002-1895-0165
[2] Department of Biology, Faculty of Science, Ege University, 35100 Bornova, İzmir, Turkey https://orcid.org/0000-0001-8510-6100
[3] Department of Crop and Animal Production, Mehmet Akif Ersoy University, Burdur, Turkey https://orcid.org/0000-0002-0280-2897
[4] Department of Biology, Faculty of Science, Ege University, 35100 Bornova, İzmir, Turkey https://orcid.org/0000-0003-0293-6964

*Corresponding author: alperenertas@hotmail.com
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Abstract: Phytoplankton groups are one of the major quality element to be used in the evaluation of the trophic and ecological state of freshwater ecosystems according to the EU Water Framework Directive. This research was made to assess the trophic and ecological status of Lake Bafa in Turkey, on the basis of phytoplankton communities. Büyük Menderes River is one of the most important factor that carries pollutants to Lake Bafa. The eight sampling station were assigned to evaluate the ecological and trophic state of the lake. Phytoplankton species were collected monthly for 2 years study period. Most commonly used phtoplankton indices Q index and Carlson's Trophic State Index (TSI), and different versions of diversity indices were used to estimate trophic and ecological state of the lake. Similarities between the sampling stations were clustured by using the unweighted pair group method using arithmetic average (UPGMA), based on phytoplankton communities. Correlations between the applied indices were determined by using Pearson Correlation. After the identification of collected phytoplanktons, total of 63 taxa which belong to classis of Cyanophyceae (11.2%), Bacillariophyceae (49.2%), Chlorophyceae (23.8%), Xanthophyceae (1.5%), Euglenophyceae (11.2%) and Dinophyceae (3.1%) were detected. The 1st and 2nd stations were the most similar stations to each other (88%) according to phytoplankton communities. Secchi disc depth (SD) and TP played an important role in the distribution of phytoplankton species in Lake Bafa. The highest significant positive correlation was determined between Q and TSI ($r = 0.987$, $p<0.01$). Considering the TDI values in the phytoplankton composition of the lake, it can be said that although the productivity status of the studied lake is still "*mesotrophic*", it has a tendency towards "*eutrophic*" state. According to the Q values, the first five stations reflect the *moderate* ecological state, while the 6th, 7th and 8th stations represent the *poor* ecological state.

Keywords: Lake Bafa, phytoplankton, Q index, Trophic State Index, Water Framework Directive

Öz: Fitoplankton, AB Su Çerçeve Direktifi'ne göre tatlı su ekosistemlerinin trofik ve ekolojik durumunun değerlendirilmesinde kullanılan en önemli kalite unsurlarından biridir. Bu araştırma, Bafa Gölü'nün trofik ve ekolojik durumunu fitoplankton grupları temel alarak değerlendirmek için yapılmıştır. Büyük Menderes Nehri, kirleticileri Bafa Gölü'ne taşıyan en önemli faktörlerden biridir. Gölün ekolojik ve trofik durumunu değerlendirmek için sekiz örnekleme istasyonu belirlenmiştir. Fitoplankton türleri 2 yıllık çalışma süresi boyunca aylık olarak toplanmıştır. Gölün trofik ve ekolojik durumunu tahmin etmek için en yaygın kullanılan fitoplankton indeksleri Q indeksi ve Carlson'un Trofik Durum İndeksi (TDI) ve çeşitlilik indekslerinin farklı versiyonları kullanılmıştır. Uygulanan indeksler arasındaki korelasyonlar Pearson Korelasyonu kullanılarak belirlenmiştir. Örnekleme istasyonları arasındaki benzerlikler, fitoplankton topluluklarına dayanan aritmetik ortalama ile ağırlıksız çift grup metodu (UPGMA) kümeleme yöntemiyle belirlenmiştir. Toplanan fitoplankton örneklerinin tanımlanmasından sonra, Cyanophyceae (11.2%), Bacillariophyceae (49.2%), Chlorophyceae (23.8%), Xanthophyceae (1.5%), Euglenophyceae (11.2%) ve Dinophyceae (3.1%) sınıflarına ait toplam 63 takson tespit edilmiştir. Fitoplankton topluluklarına göre 1. ve 2. istasyonlar (%88) birbirlerine en yakın istasyonlar olduğu görülmüştür. Secchi disk derinliği (SD) ve TP Bafa Gölünde fitoplankton türlerine dağılımında önemli rol oynamıştır. En yüksek anlamlı pozitif korelasyon Q ve TDI arasında belirlenmiştir ($r =0.987$, $p<0.01$). Gölün fitoplankton dağılımındaki TDI değerleri göz önüne alındığında, incelenen gölün verimlilik durumu halen "mezotrofik" olmakla birlikte, "ötrofik" duruma doğru bir eğilim gösterdiği söylenebilir. Q değerlerine göre, ilk beş istasyon orta ekolojik durumu yansıtırken, 6., 7. ve 8. istasyonlar kötü ekolojik durumu temsil etmektedir.

Anahtar kelimeler: Bafa Gölü, fitoplankton, Q indeksi, Trofik Durum İndeksi, Su Çerçeve Direktifi

INTRODUCTION

Increasing human population cause big pressure on the aquatic ecosystems. The oceans, lakes, rivers and streams are being "squeezed" by human activities such as demotechnical and industrial improvements, agricultural activities and other human impact. Thus, ecological quality associated with water quality deteriorates (Vollenweider and Kerekes, 1982).

The European Union (EU) Water Framework Directive (WFD) 2000/60/EC commits EU member states and is adopted in 2000 takes a pioneering approach to protecting aquatic systems. The directive aime to achieve good qualitative and quantitative status of all surface water bodies and groundwaters (European Commission, 2000).

According to the WFD, phytoplankton groups occupies an important place and it has a vital role in the aquatic systems, therefore, phytoplankton is one of the biological quality indicators required for evaluation of ecological status of freshwaters in Europe. Amount of the nutrient load, has direct effect on the community of the phytoplankton. Therefore many researchers have preferred, the usage of phytoplankton for both water quality and eutrophication impact assessment (Pasztaleniec and Poniewozik, 2010). In some cases, the accumulation of extra phytoplankton can cause undesirable situations such as decreasing the photosynthesis of submerged vegetation due to shading, and develop of anoxic conditions in aquatic ecosystems (Pasztaleniec, 2016). As a result of this kind of situations, phytoplankton composition shows alterations, which influence aquatic food web and can pose the risk of the harmful algal blooms in the aquatic ecosystems.

Biomonitoring of aquatic ecosystems based on phytoplanktons is a routine method in European countries, on the other hand, using the biotic index is the important part of the water quality assessment. The using of biotic indices are shows the differences according to the country, climate and topography. In Turkey, using the biometric approaches for determining water quality based on phytoplankton (Çelekli and Öztürk, 2014; Toudjani et al., 2017; Çelekli and Lekesiz, 2020) was not a common method but nowadays these kind of studies become prominent.

Lake Bafa has been faced with pollution pressure due to human activities. The inflow of the Büyük Menderes River into Lake Bafa causes pollutant accumulation in the lake. Büyük Menderes River is born Dinar district of Afyon province and flows 584 km to the Aegean Sea. One arm of Büyük Menderes River, that seperates from the river just before it goes into the Aegean Sea, and feeds the lake. Most of the industrial, agricultural and domestic wastes of the cities around lake, accumulates in the lake. In addition Büyük Menderes River, there are also many of olive oil facilities around the lake. Accidental leakage of black water from these factories reaches the lake and also overflowing of black water wells may happen after heavy rains. On the other hand, agricultural activities around the lake, using big amount of fertilizers, pesticides and chemicals are another pollution factors on the lake. All of this organic and inorganic pollutants increase salinity levels and caused the deterioration the biodiversity. Sarı et al. (1999) reported the fish fauna of the Lake Bafa changed mainly because of the increasing salinity levels. Balık and Ustaoglu (1989) also reported that one of the third endemic species in the basin, *Acanthobrama mirabilis* is extinct in Lake Bafa. Previous studies (Mermer, 1989; Balık et al., 1992; Balık, 1995) reported that *Cyprinus carpio*, *Cyprinus nasus*, *Barilius pectoralis*, *Silurus glanis* were also extinct in the lake due to increased salinity levels and decreased water quality.

With this study, it is aimed to reveal the phytoplankton composition and environmental condition of Lake Bafa. The trophic and ecological status of Lake Bafa was evaluated by using of Q index and TSI index based on phytoplankton and environmental data obtained monthly for two years.

MATERIAL AND METHODS

Study area

Lake Bafa, the largest lake in the Aegean Region, is located at 2 m a.s.l. in the inner parts of the Menteşe Mountains in the southeastern part of the Büyük Menderes basin. It is a brackish water lake with a deepest point of 21 mand a surface area of 65 km2. This lakeis part of the Aegean Sea in its initial formation, remains inside the coast for miles due to the alluviums carried by the Büyük Menderes River. Lake Bafa is an example of alluvial-set lakes in terms of formation.

Sampling method

The sampling was monthly carried out for 2 years in 8 sampling stations of Lake Bafa (Figure 1).

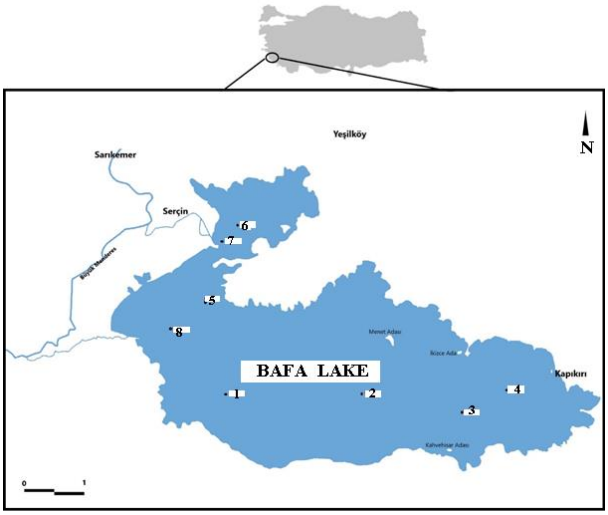

Figure 1. Sampling stations of Lake Bafa

Phytoplanton samples were collected monthly between July 2015 and June 2017 using plankton net (50 µm mesh size). Secchi disk visibility and chlorophyll a were measured in situ. Total phosphorus (TP) amount of stations were measured spectrophotometrically by using Merck Phosphate Cell Test Kit. Chlorophyll a was measured by using the BBE Moldaenke AlgaeTorch. In Lake Bafa, the water samples for quantitative analyses were fixed with lugol solution.

The quantification of microalgal cells, colonies and filamets were evaluated under inverted microscope according to the Uthermohl method (Utermohl, 1958). Counting and identification of algae were applied using a Palmer-Maloney counting cell (volume: 0.1 mL) and a inverted microscope equipped with water immersion lenses and a phase-contrast attachment (400× magnification). The final abundance of each algal species was considered to be the average from two replicates. Algal species were identified according to the most

updated literature. Taxonomy of algae was checked according to (Guiry and Guiry, 2020). Diatom identifications were performed after having prepared permanent slides following European standard NF EN 13946 (Feret et al., 2017). Diatom frustules were counted per slide at ×1,000 magnification, and diatoms were identified to the lowest taxonomic level possible using taxonomic literature from central Europe (Krammer and Lange-Bertalot, 1986; Sala et al., 1993; Round and Bukhtiyarova, 1996; Krammer, 1997; Compère, 2001; Bukhtiyarova, 2006; Potapova, 2006).

Statistical analysis

The diversity scores were obtained as by using PAST3 software program.

In order to determine the trophic and ecological status of Lake Bafa, we utilized to the phytoplankton assemblage index (Q) (Padisak et al., 2006) and the Carlson Trophic State Index (TSI) (Carlson, 1977). According to Reynolds et al. (2002), on the centre of the phytoplankton functional group concept, Padisak et al., (2006) improved the Q index. In order to determine ecological classes, Q index grouped the lakes into five classes (Padisak et al., 2006). Q index based on the factor F weights assigned to each functional group, lake type 2 (alkaline, average depth 1–3 m, and persistent) in Padisak et al., (2006) was selected because the characteristics of this type are similar to the Lake Bafa. According to Pasztaleniec and Poniewozik (2010), the Q index emphasized that relative shares (pi, where pi = ni/N) of functional groups (ni number of the i-th functional group; N: total number) in the total phytoplankton numbers, and a factor number (F) assessed for each functional group in each type of water body at the same time (Padisak et al., 2006).

$$Q = \sum_{i=1}^{n} pi.F$$

The ranges of the Q index is between the 0-5, and can be expressed into five degree classification procedure: 0-1 grade indicate bad ecological status, 1-2 grade indicate tolerable (poor) ecological status, 2-3 grade indicate medium (moderate) ecological status, 3-4 grade indicate good ecological status, 4-5 grade indicate high ecological status.

The TSI developed by Carlson (1977) is used to determine the productivity of lentic habitats in many limnological studies conducted today (Katip et al., 2015; Cigagna et al., 2016). According to Carlson (1977), Walker (1979), Swanson (1998) and Xu (2008), TSI proposed in order to determine the biochemical pattern of eutrophication and to powerful remove pollution related with the oligotrophic, mesotrophic and eutrophic trophic states. The ranges of the TSI is between the 0-100, and can be expressed into four degree classification procedure. If TSI values are <30 or 30-40, oligotroph, if 40-50 is mesotroph, 50-70 is eutroph, and 70-80 or> 80 is hyperutroph (Carlson and Simpson, 1996). (Table 1).

Table 1. Eutrophication state of Carlson Trophic State Iindex

Trophic state	SD (m)	Chl *a* (µg/L)	TP (µg/L)	TSI
Oligotrophic	>40	<2.6	<12	<40
Mesotrophic	20-40	2.6-7.2	12-24	40-50
Eutrophic	0.5-20	7.22-55.5	24-96	50-70
Hypertrophic	<0.5	>55.5	>96	>70

Secchi disk (SD), chlorophyll a (Chl a), and total phosphorus (TP) are used in the formulas for calculating the TSI (Saghi et al., 2014). The TSI index expressed by;

$$TSI = 60 - 14.43\ \ln(SD)$$
$$TSI = 3056 + 9.81\ \ln(Chl\ a)$$
$$TSI = 4.14 + 14.43\ \ln(TP)$$

The floral simiarity between the stations were evaluated by using Bray- Curtis similarity index (Somerfield, 2008; Yoshioka, 2008). UPGMA method was used to indicate existent clustering relationships based on phytoplanktons by using PAST3 software program.

Analysis of variance (One-Way ANOVA) was applied to data for determining the statistical differences in phytoplankton species and classis, physical parameters, Q index, TSI index and all diversity indices among the sampling stations of the Lake Bafa using SPSS 20.0. Pearson correlations between the physical parameters and the Q index, TSI index and all diversity indices were also determined using SPSS 20.0. Canonical Correspondance Analysis (CCA) was carried out using PAST3 software programme (Ter Braak and Šmilauer, 2002) to determine the relationships between the functional groups and physical variables. CCA was carried out on the log-normal transformed abundance data. Statistical significance of the all predictor variables was assessed by Kaiser-Meyer-Olkin (KMO) Sample Proficiency Test.

RESULTS

The physical variables and coordinate of the stations indicated in Table 2. The Chl a values varied between 6.00-82.8 µg/L, the TP values varied between 1.04-1.92 mg/L and the SD values varied between 0.50-3.60 m at the stations in the lake. SD depth is much lower in the 5th and 7th stations compared to other stations. Especially in the 5th stations, the turbidity is visibly more than the other stations. Although Chl a values are higher in 6th and 7th stations, there is no significant difference between Chl a values in other stations. There is no significant difference in TP values at all stations.

As a result, a total of 63 taxa were determined from the eight sampling stations in the lake. In this study, 7 taxa belong to Cyanophyceae, 31 taxa belong to Bacillariophyceae, 15 taxa belong to Chlorophyceae, 1 taxon belong to Xanthophyceae, 7 taxa belong to Euglenophyceae and 2 taxa belong to Dinophyceae.

Table 2. Mean and standart deviation values of the SD, TP, Chl-a and latitude and longitude at the stations

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Latitude	37°29' N	37°29' N	37°29' N	37°29' N	37°31' N	37°32' N	37°32' N	37°30' N
Longitude	27°24' E	27°27' E	27°29' E	27°31' E	27°24' E	27°23' E	27°24' E	27°22' E
SD (m)	3.60±0.30	3.40±0.50	3.30±0.20	3.00±0.15	0.50±0.01	2.00±0.15	1.50±0.01	2.50±0.19
TP (mg/L)	1.46±0.44	1.92±0.67	1.14±0.32	1.74±0.52	1.62±0.26	1.04±0.11	1.32±0.16	1.13±0.09
Chl *a* (µg/L)	12.4±2.33	15.4±2.47	14.5±1.95	19.1±3.66	6.50±0.43	82.8±9.56	69.0±7.98	6.00±0.38

Bacillariophyceae was the dominant group in the phytoplankton composition. As a result of diagnosed phytoplankton between the stations, the maximum amount of cells were counted at 7th station (871.778 cell/mL) while the minimum amount of the cells were counted at 8th station (20.833 cell/mL).

Percentage distribution of phytoplankton groups according to seasons is shown in Figure 2. As a result of the phytoplankton counting the dominant group is Bacillariophyceae (35%) in summer in Lake Bafa. The second dominant group is Dinophyceae (31%) while the third dominant group is Euglenophyceae (20%) in the summer. As a result of the phytoplankton counting, the dominant group is also Bacillariophyceae (95%) in the autumn, Dinophyceae (87%) in the winter, and Xanthophyceae (81%) in the winter in Lake Bafa. In Lake Bafa, distributions and relative occurence (%) of the diagnosed phytoplanktons were given at Table 3.

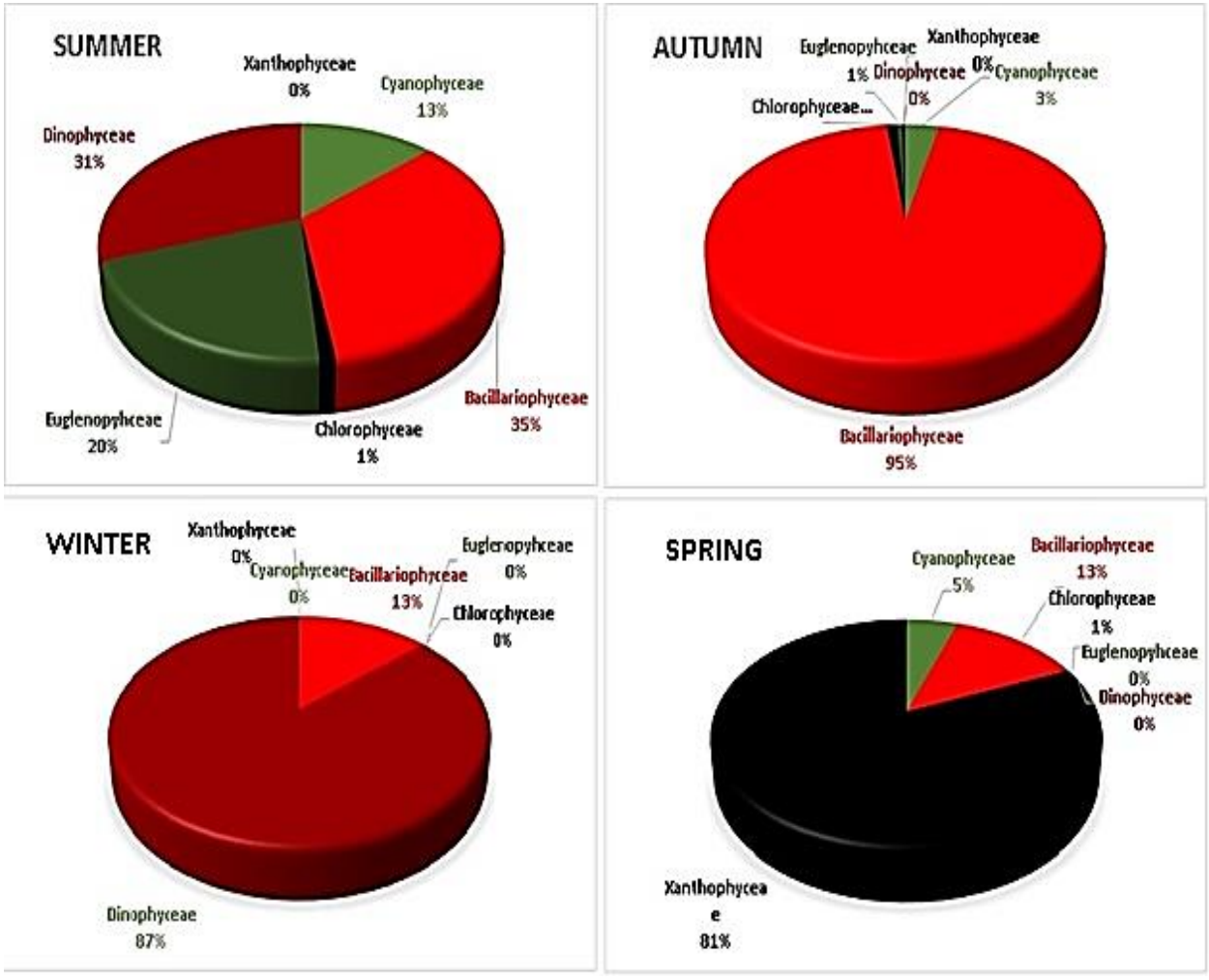

Figure 2. Proportional (%) distributions of phytoplankton groups in abundance basis according to seasons

Table 3. Distributions and relative occurence (%) of phytoplankton species at the stations

Code		Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
	Cyanophyceae								
1	*Komvophoron* sp.	-	-	-	-	-	0.01	-	-
2	*Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju	-	-	-	-	-	0.68	-	-
3	*Lyngbya sp.*	-	-	0.46	-	-	-	-	-
4	*Nodularia spumigena* Mertens ex Bornet and Flahault	0.03	-	0.03	3.87	0.52	1.97	5.94	-
5	*Oscillatoria spp.*	0.07	0.19	0.37	-	2.92	0.12	0.14	2.18
6	*Oscillatoria limosa* C.Agardh ex Gomont	-	-	-	-	0.14	0.13	0.08	-
7	*Phormidium spp.*	-	-	-	0.07	1.78	0.10	-	2.08
	Bacillariophyceae								
8	*Asterionella* sp.	-	-	-	-	-	0.39	0.10	-
9	*Biddulphia* sp.	-	-	0.07	0.74	-	0.01	0.03	-
10	*Caloneis amphisbaena* (Bory) Cleve	-	-	-	0.11	-	-	0.01	-
11	*Campylodiscus hibernicus* Ehrenberg	0.03	0.01	-	0.03	0.36	-	0.05	3.24
12	*Chaetoceros muelleri* Lemmermann,	17.9	13.7	5.32	2.61	0.76	-	0.48	9.06
13	*Cocconeis placentula var. euglypta* (Ehrenberg) Grunow	-	-	-	-	1.69	-	0.12	-
14	*Coscinodiscus granii* L.F.Gough	36.2	40.6	27.8	18.0	1.95	0.08	0.01	41.8
15	*Craticula cuspidata* (Kutzing) D.G.Mann	-	-	-	-	-	-	0.01	-
16	*Cyclotella sp.*	-	-	0.16	0.06	-	-	-	-
17	*Cyclotella meneghiniana* Kützing,	-	-	-	-	-	0.34	-	-
18	*Cymatopleura solea* (Brébisson) W.Smith	-	-	-	-	-	0.01	-	-
19	*Entomoneis paludosa* (W.Smith) Reimer in R.M.Patrick & Reimer	-	-	-	-	-	0.03	-	-
20	*Fragilariforma virescens var. exigua* (Grunow) M.Poulin in Hamilton & al.	-	-	-	-	-	1.44	-	-
21	*Gyrosigma acuminatum* (Kützing) Rabenhorst	-	-	-	-	0.34	0.05	0.01	-
22	*Gyrosigma attenuatum* (Kützing) Rabenhorst	0.06	-	0.04	2.59	1.18	0.14	0.16	0.47
23	*Licmophora sp.*	0.07	-	0.27	4.20	12.1	-	-	-
24	*Melosira nummuloides* C.Agardh	0.17	0.01	0.11	10.0	2.20	0.05	0.37	-
25	*Melosira varians* C.Agardh	0.08	-	0.10	0.05	0.27	0.07	0.66	0.39
26	*Navicula sp.*	-	-	-	0.04	-	-	-	-
27	*Nitzschia spp.*	12.1	16.5	32.7	8.09	9.13	2.79	1.27	0.17
28	*Nitzschia acicularis var. typica* A.Cleve	-	-	-	-	4.92	0.72	2.09	-
29	*Nitzschia closterium* (Ehrenberg) W.Smith	-	-	-	-	-	0.20	-	-
30	*Nitzschia incerta* (Grunow) M.Peragallo	-	-	-	-	-	0.03	-	-
31	*Nitzschia intermedia* Hantzsch	-	-	-	-	-	-	0.11	-
32	*Nitzschia lorenziana* Grunow in Cleve & Möller	-	0.02	0.04	-	4.11	0.16	0.29	-
33	*Nitzschia sigmoidea* (Nitzsch) W.Smith	-	-	-	-	-	0.08	0.12	-
34	*Surirella ovalis* Brébisson	-	0.01	-	0.06	0.19	0.01	0.37	0.65
35	*Stephanodiscus spp.*	0.29	-	0.29	0.47	4.34	2.82	0.49	1.66
36	*Ulnaria ulna* (Nitzsch) Compère	0.55	0.31	0.27	15.7	36.0	5.04	3.42	5.65
37	*Ulnaria capitata* (Ehrenberg) Compère	-	-	-	-	-	-	0.02	-
38	*Tryblionella littoralis* (Grunow) D.G.Mann	-	-	-	-	-	-	0.05	0.09
	Chlorophyceae								
39	*Chlamydomonas sp.*	-	0.54	-	-	-	-	-	-
40	*Cladophora glomerata* (Linnaeus) Kützing	0.02	-	0.12	-	-	-	-	-
41	*Desmodesmus communis* Hegewald	-	-	-	0.01	-	-	-	-
42	*Eudorina cylindrica* Korshikov	-	-	-	-	-	0.13	-	-
43	*Oedogonium sp.*	-	-	-	-	-	0.03	-	-
44	*Pandorina morum* (O.F.Müller) Bory	-	-	-	-	-	0.08	0.01	-
45	*Pseudopediastrum boryanum* (Turpin) E.Hegewald	-	-	-	-	-	0.24	0.18	-
46	*Pediastrum duplex* Meyen	-	-	-	-	0.02	0.06	0.06	-

Table 3. (Continued)

Code		Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
47	*Monactinus simplex* (Meyen) Corda	-	-	-	-	-	0.10	0.05	-
48	*Scenedesmus spp.*	-	-	-	-	-	0.14	0.03	-
49	*Tetradesmus obliquus (*Turpin) M.J.Wynne	-	-	-	-	-	0.08	-	-
50	*Tetradesmus lagerheimii* M.J.Wynne & Guiry	-	0.58	0.47	-	-	-	-	-
51	*Desmodesmus protuberans* (F.E.Fritsch & M.F.Rich) E.Hegewald	-	-	-	-	-	0.34	0.05	-
52	*Spirogyra spp.*	-	-	-	-	0.13	0.02	-	-
53	*Stauridium tetras* (Ehrenberg) E.Hegewald	-	-	-	-	-	0..01	-	-
	Xanthophyceae								
54	*Tribonema sp.*	-	-	-	-	0.09	0.09	-	-
	Euglenopyhceae								
55	*Lepocinclis acus* (O.F.Müller) B.Marin & Melkonian	-	-	-	-	-	3.85	0.09	-
56	*Euglena hemichromata* Skuja	-	-	-	-	-	0.40	0.11	0.04
57	*Lepocinclis oxyuris* (Schmarda) B.Marin & Melkonian	-	-	-	-	-	2.80	-	-
58	*Euglena texta* (Dujardin) Hübner	-	-	-	-	-	0.05	0.11	-
59	*Lepocinclis fusiformis* (H.J.Carter) Lemmermann	-	-	-	-	-	3.02	-	-
60	*Lepocinclis ovum* (Ehrenberg) Lemmermann	-	-	-	-	-	0.86	-	-
61	*Phacus acuminatus* A.Stokes	-	-	-	-	-	1.45	-	-
	Dinophyceae								
62	*Peridinium sp.*						0.74	1.26	
63	*Prorocentrum cordatum* (Ostenfeld) J.D.Dodge	32.2	27.4	31.3	33.1	14.9	68.0	81.6	32.4

As a result of the identification, it was determined that *Coscinodiscus granii*, *Prorocentrum cordatum* and *Chaetoceros muelleri* were dominant on station #1 and #8.

Coscinodiscus granii, *Prorocentrum cordatum* and *Nitzschia spp* were dominant on station #2 and #3. *Ulnaria ulna, Prorocentrum cordatum* and *Coscinodiscus granii* were dominant on station #4. *Ulnaria ulna, Prorocentrum cordatum* and *Licmophora sp.* were dominant on station #5. *Ulnaria ulna, Prorocentrum cordatum* and *Lepocinclis acus* were dominant on station #6. *Ulnaria ulna, Prorocentrum cordatum* and *Nodularia spumigena* were dominant on station #7 (Figure 3).

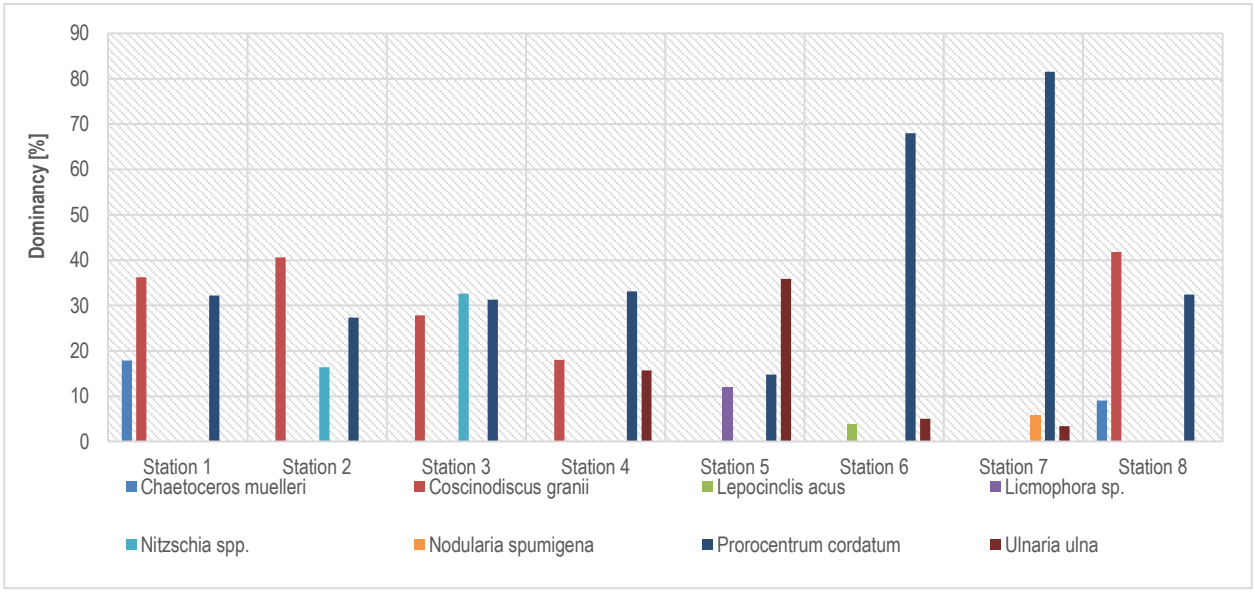

Figure 3. The most dominant phytoplankton species at the stations in Lake Bafa

On the basis of UPGMA, the classification of the sampling stations by phytoplankton composition was defined at Figure 4. Cluster analsis was used to classify the stations detected on Lake Bafa according to phytoplankton species. As a result of the UPGMA analysis, station #1 and station #2 were the most similar to each other (88%). Another high similarity was determined between the station #2 and station #3 (78%).

Two biometric approaches were used for evaluating the ecological quality of Lake Bafa are illustrated in Table 4. According to the Q index, the highest score value belong to station #3. The station #3 is determined as medium (moderate) ecological status. On the other hand, station #6, #7 and #8 determined as poor ecological status. In this study, trophic characterization of Lake Bafa was determined using the Carlson Trophic State Index concerning all the TSI components: SD, Chl *a* amount and TP, respectively. The TSI index diversified between the scores of 49 to 59 in the spring and summer period, in Lake Bafa. This result indicated that up to the limit of mesotrophic grade (50) in terms of the Carlson model, which classifies Lake Bafa as slightly eutrophic. TSI index varied between the values 42 to 49 on winter and autumn period.

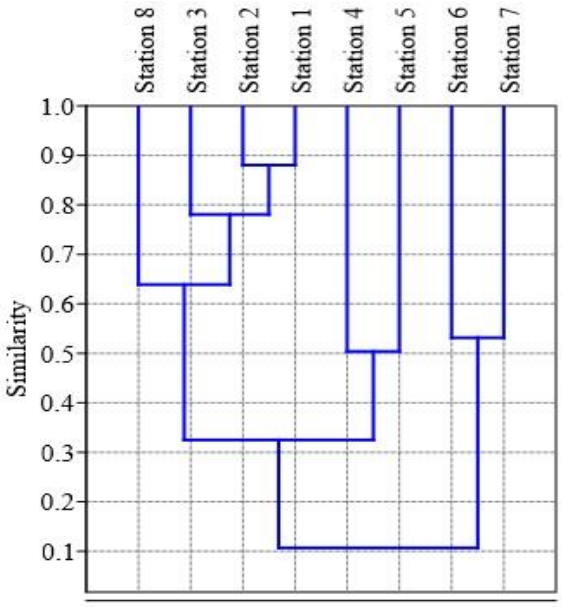

Figure 4. Classification of stations based on similarities of phytoplankton communities

Table 4. Score values of all indices and trophic status

İndices		Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Biotic indices									
Q Indice	Score	2.30±0.03	2.40±0.05	2.50±0.04	2.20±0.06	2.00±0.10	1.80±0.14	1.40±0.15	1.60±0.15
	Class	medium	medium	medium	medium	medium	poor	poor	poor
TSI	Score	45±2.27	43±1.65	42±1.14	46±1.76	49±2.04	51±1.56	59±1.88	53±1.03
	Class	mesotrophic	mesotrophic	mesotrophic	mesotrophic	mesotrophic	eutrophic	eutrophic	eutrophic
Species Diversity Indices									
SDI		0.72±0.17	0.71±0.15	0.71±0.13	0.81±0.16	0.82±0.14	0.53±0.05	0.33±0.04	0.71±0.12
SWDI		1.38±0.37	1.38±0.41	1.41±0.33	1.94±0.36	2.16±0.27	1.53±0.14	0.91±0.019	1.55±0.22
MDI		1.26±0.33	1.04±0.38	1.62±0.27	1.54±0.12	1.91±0.33	3.56±0.46	2.56±0.51	1.31±0.19
Menhinick		0.08±0.01	0.06±0.02	0.10±0.01	0.05±0.01	0.07±0.02	0.07±0.02	0.04±0.01	0.10±0.03
Fisher- Alpha		1.40±0.21	1.15±0.10	1.82±0.17	1.70±0.14	2.14±0.25	4.08±0.78	2.85±0.22	1.46±0.13
Brillouin		1.38±0.33	1.38±0.16	1.41±0.11	1.94±0.23	2.16±0.14	1.53±0.13	0.91±0.18	1.55±0.15
Evenness Index									
Evenness E1		0.28±0.07	0.33±0.08	0.23±0.08	0.37±0.09	0.38±0.06	0.10±0.01	0.07±0.01	0.34±0.06
Dominancy Index									
Berger- Parker		0.36±0.09	0.41±0.10	0.33±0.07	0.33±0.07	0.36±0.09	0.68±0.15	0.82±0.17	0.42±0.11

Average ± standard deviation
SDI: Simpson Diversity Index, SWDI: Shannon Weaver Diversity Index, MDI: Margalef Diversity Index

In this study, the SWDI, SDI, MDI, Menhinick, Brillouin and Fisher-Alpha diversity indices were calculated for each sampling station to assign species diversity. According to SWDI, the highest diversity score was found at station #5 (2.16), while the lowest diversity scores were found at station #1 and #2 (1.38). According to SDI, the highest diversity score was found at station #5 (0.82), while the lowest diversity score was found at station #7 (0.33). According to MDI, the highest diversity score was found at station #6 (3.56), while the lowest diversity score was found at station #2 (1.04). According to Menhinick Index, the highest diversity scores were found at station #3 and #8 (0.10), while the lowest diversity score were found at station #7 (0.04). According to Fisher-Alpha Index, the highest diversity score was found at station #6 (4.08), while the lowest diversity score was found at station #2 (1.15). According to Brillouin Index, the highest diversity score was found at station #5 (2.16), while the lowest diversity score was found at station #7 (0.91). According to Evenness E1 index, the highest evenness score was observed at station #5 (0.38), while the lowest evenness value was seen at station #7 (0.07).

According to Berger-Parker index, the highest dominancy value was observed at station #7 (0.82), while the lowest dominancy value were observed at station #3 and #4 (0.33) (Table 4).

Table 5, summarizes the correlations of Chl *a*, SD, TP, Q index, TSI index and species diversity indices. In this study, the random sample cases *(10% select case)* were made on the biotic and diversity indices to verify datasets and to determine that the data was entered without errors in the SPSS version 20.0. The correlation between Chl- *a* and SD (r = -0.305; p<0.01) was slightly strong. Between all biotic indices the highest significant correlation was determined between Q and TSI (r = 0.987, p<0.01). TSI showed positive correlastion with both TP and chl *a* while it showed significant negative correlation with SD. Among species diversity indices, evenness indice and dominancy indice, the highest significant correlation was found between MDI and Fisher- Alpha (r = 1.000, p<0.01), SWDI and Brillouin D (r = 1.000, p<0.01), SDI and Evenness E1 (r = 0.926, p<0.01). However, an increase in the results in Q index and TSI shows bad ecological quality.

Table 5. Correlation assesment between biotic and diversity indices used in Lake Bafa

	SD	TP	Chl-*a*	Q index	TSI	SDI	SWDI	MDI	Menhinick	Fisher_alpha	Brillouin	Evenness E1	Berger-Parker
SD	1	0.133	-0.305	0.634	-0.649*	0.247	-0.29	-0.539	0.268	-0.531	-0.29	0.162	-0.428
TP		1	0.431	0.439	0.399*	0.445	0.314	-0.545	-0.511	-0.551	0.314	0.598	-0.396
Chl-*a*			1	-0.543	0.577*	-.845**	-0.467	.906**	-0.477	.902**	-0.467	-.900**	.907**
Q index				1	.987**	0.657	0.267	-0.532	0.233	-0.522	0.267	0.465	-.759*
TSI					1	-.720*	-0.33	0.543	-0.328	0.531	-0.33	-0.524	.804*
SDI						1	.800*	-0.641	0.411	-0.631	.800*	.926**	-.959**
SWDI							1	-0.136	0.145	-0.131	1.000**	.722*	-0.62
MDI								1	-0.287	1.000**	-0.136	-.771*	.762*
Menhinick									1	-0.271	0.145	0.266	-0.493
Fisher_alpha										1	-0.131	-.767*	.753*
Brillouin											1	.722*	-0.62
Evenness E1												1	-.874**
Berger-Parker													1

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

Phytoplankton species and three environmental variables (SD, TP and Chl *a*) were only used in CCA analysis according to the Kaiser-Meyer-Olkin (KMO) Sample Proficiency Test (Figure 5). The obtained results of the KMO Sample Proficiency Test were calculated as 0.744 and show that the sample size is quite good and sufficient. The CCA analysis led to the explanation total of 72% variance according to phytoplankton species.

The distributions of *Lyngbya* sp., *Chaetoceros muelleri*, *Coscinodiscus granii, Nitzschia incerta* and *Cladophora glomerata* are positively correlated to the SD. The distributions of *Phormidium spp., Campylodiscus hibernicus,* and *Licmophora sp.* are positively correlated to the TP. The distributions of *Cylindrospermopsis raciborskii, Biddulphia* sp., *Navicula sp., Desmodesmus communis* and *Lepocinclis acus* are positively correlated to the chl *a*.

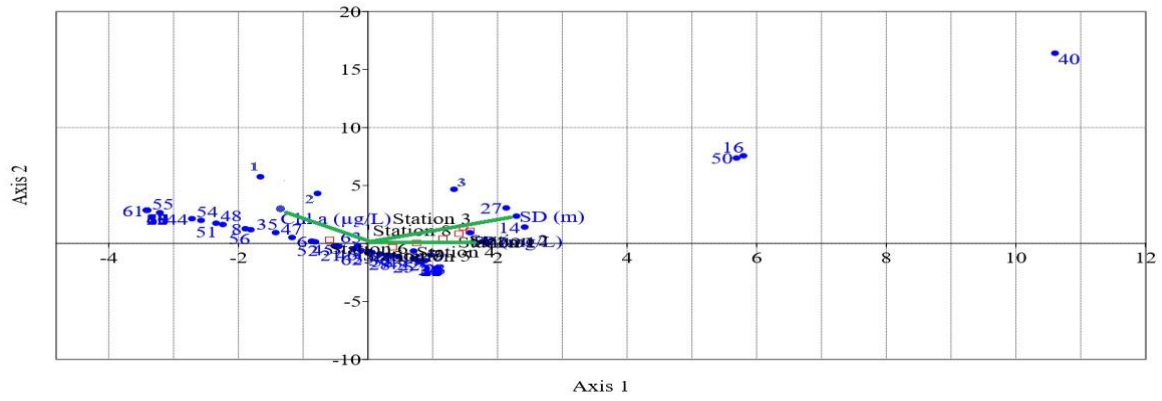

Figure 5. CCA plot of reference-, test-, and the phytoplankton species distributions with environmental variables

DISCUSSION

In this study, total of 63 taxa were revealed during the survey. After the classification of collected samples, Cyanophyceae (11.2%), Bacillariophyceae (49.2%), Chlorophyceae (23.8%), Xanthophyceae (1.5%), Euglenophyceae (11.2%) and Dinophyceae (3.1%) classis were determined. Cyanobacteria members were commonly detected in eutrophic lakes in our country (Cirik-Altındağ, 1982; Sömek and Balık, 2009) and they had excessive growth in eutrophic lakes rich in nutrients in summer months (Vaitomaa, 2006). In this study, a few taxa (*Cylindrospermopsis raciborskii, Lyngbya sp.* and *Nodularia spumigena*) were also frequently encountered in inland water ecosystems in Turkey (Taş and Gönülol, 2007; Çelekli et al., 2007). *Peridinium* sp., was detected at the 6th and 7th stations of Lake Bafa, which was also found in inland water ecosystems with mesotrophic or eutrophic character in our country (Sömek et al., 2005; Ongun-Sevindik, 2010).

The biological assessment based on phytoplankton metrics provides a more integrative approach to determine the ecological status for the conservation and restoration of the lentic ecosystems (Padisák et al. 2006; Ptacnik et al. 2009). Usages of phytoplankton indices in the evaluation of water quality have played a crucial role in water resource management worldwide (Padisák et al. 2006; Marchetto et al. 2009). Çelekli and Lekesiz (2020) reported the low cyanobacterial biovolume was found in Yapraklı Reservoir, Lake Gölhisar, and Çavdır Reservoir with good ecological conditions based on the results of the modified PTI and Med-PTI. Bacillariophyta showed the highest contribution (54.6%) to total phytoplankton community in Çavdır Reservoir. Low cyanobacteria and high diatom biovolume levels support good ecological conditions. Kazancı et al. (2008) were determined 15 species of phytoplankton from 4 different algal groups (Dinophyta, Chlorophyta, Bacillariophyta and Cyanophyta) in Lake Bafa on July 1996-1997. Although the number of Bacillariophyta species was higher than the other taxa, Dinophyta was the dominant group in the lake. According to Kazancı et al. (2008), all dominant species belong to Dinophyta. *Prorocentrum minimum* was the most dominant species with 78.8 % (11.000 org ml^{-1}, organism per mililitre) of total phytoplankton whilst second dominant species was *Prorocentrum micans* (19.37 % of total phytoplankton and 2704.43 org ml^{-1}). *Peridinium* spp was third dominant species with 1.78 % (248.29 org ml^{-1}). Kazancı et al. (2008) also calculated the diversity of the phytoplankton according to the SWDI. The diversity was found low (0.58) in the Lake Bafa because of the high quantity of *Prorocentrum minimum* (11 000 org ml^{-1} and 78.8% of total phytoplankton). In this study, all trophic components apparently were coherent in the determination of the trophic status of the Lake Bafa. Studies conducted in the lake, indicate the differences between the Chl *a* values and total phytoplankton which showed in some samples was likely due to high abundance of a broad algae masses in Lake Bafa on all seasons. Shekhar et al., (2008) reported that they determined the classis of Chrysophyceae (2%), Cyanophyceae (25%), Bacillariophyceae (20%), Chlorophyceae (36%) and Euglenophyceae (17%) in the Bhadta Reservoir. Shekhar et al., (2008) determined in polluted zones, percentage of Cyanophyceae and Euglenophyceae was higher when compared to Chlorophyceae and Bacillariophyceae. Ochocka and Pasztalenice (2016) reported that they determined the classis of Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae and Dinophyceae in the Masurian Lake. Ochocka and Pasztalenice (2016) stated that dominant group was filamentous cyanobacteria species (Planktothrix, Limnothrix, Planktolyngbya, Pseudoanabaena) in the lake.

Results of SD in the present study are relatively similar compared to mesotrophic lakes (Vollenweider and Kerekes, 1982). Similarly, the fact that chl a concentrations were determined to be moderate indicates that the moderately light transmission is not due to phytoplankton. In this study, the lakes were determined at the border of the mesotrophic state or slightly above the average TDI values calculated by using the chl a and SD measurement values.

In this study, the Chl *a* value varied between 6.00-82.8 µg/L at the stations in Lake Bafa. Sakamoto (1966) reported the degree of chlorophyll a as 5.00-140 µg/L for eutrophic lakes, 1.00-15.0 µg/L for mesotrophic lakes and 0.30-2.50 µg/L for oligotrophic lakes. The stations #1, #2, #3, #4, #5 and #8 are mesotrophic in Bafa Lake according to Sakamoto (1966) while the stations #6 and #7 are eutrophic in Bafa Lake.

In this study, it has been reported that the *Ulnaria ulna* which is frequently detected in Lake Bafa, is found in waters rich in nutritious minerals and high turbidity, and is a characteristic species of eutrophic lakes (Hustedt, 1930; Reynolds et al., 2002). Algae species, *Euglena* sp., *Oscillatoria* sp., *Scenedesmus* sp., and *Nitzschia* sp. was found on summer and autums in Lake Bafa, which are found in polluted waters (Nandan and Aher 2005; Çelekli and Lekesiz, 2020).

Çelekli et al. (2007) performed the multivariate approaches in three Aegean reservoirs. CCA analysis indicated that phytoplankton composition and distribution were mainly governed by environmental factors by TP, DO, TKN, BOD_5, TOC and temperature. The first two CCA axes explained 31% of cumulative percentage variance of species data with 97.7% between species-environment correlations during the study period. With regard to the ecological status, values of the Med-PTI indicated good quality waters for Ayvacık and Bayramiç Reservoirs, while Sevişler Reservoir had a moderate water quality. Based on the PTI, Bayramiç and Sevişler Reservoirs were classified as or moderate ecological status, while Ayvacık Reservoir indicated a good water quality. In Lake Bafa, The CCA analysis led to the explanation total of 72% variance according to phytoplankton species. The distributions of *Lyngbya* sp., *Chaetoceros muelleri*, *Coscinodiscus granii, Nitzschia incerta* and *Cladophora glomerata* are positively correlated to the SD. The distributions of *Phormidium spp.,*

Campylodiscus hibernicus, and *Licmophora sp.* are positively correlated to the TP. The distributions of *Cylindrospermopsis raciborskii, Biddulphia* sp., *Navicula sp., Desmodesmus communis* and *Lepocinclis acus* are positively correlated to the chl *a*.

Chlorococcales in Lake Bafa was the dominant order in the second dominant group of Chlorophyceae. Likely, *Scenedesmus* and *Pediastrum* species had been found abundantly in oligomesotrophic reservoirs and eutrophic lakes in Turkey (İşbakan-Taş et al., 2002; Kıvrak and Gürbüz, 2005; Ongun-Sevindik, 2010; Çelekli et al., 2018).

Wetzel (1975) reported that members of the Euglenophyceae classis were mostly found in shallow waters rich in organic matter. The *Lepocinclis fusiformis, Lepocinclis ovum* and *Phacus acuminatus* species identified in the research area are widely found in mesotrophic or eutrophic inland water ecosystems in our country (Cirik-Altındağ, 1982; Kılınç, 1998; Ersanlı and Gönülol, 2003).

The UPGMA is a simplest method in order to constructing a tree from distance matrix which has been used often in ecology, systematics and taxonomy. The clustering technique that used in this method based on arithmetic averages of the measures of dissimilarity and similarity (James and McCulloch, 1990). In this study, the station #1 and #2 were the most similar to each other according to phytoplankton community. These two stations are located in the middle of the lake and they are far from the drainage points of Büyük Menderes River. Other high similarities were determined between the station #2 and #3 while the lowest similarities was determined between the station #6 and #8. The station #2 and #3 are located relatively close to each other.and the station #3 is located close to the lake shore. The station #6 and #8 differ in depth and sediment structure. The lake is shallow and the bottom structure is muddy in the station #6 while, the lake is relatively deep and macrophytes are predominant in the station #8.

On the basis of diversity indices, species diversity values ranged from 0.04 to 4.08. According to Mason (2002), SWDI values ranges from >3 it indictes clean water, 1-3 shows moderate pollution, and 1< shows severe pollution, respectively. Ghosh and Biswas (2005) reported that the diversity value ranges from 0 (low density) to 1 at the SDI. Lake Bafa is oligotrophic state according to TP values while mesotrophic-eutrophic state according to chl *a* values. Lakes with a SD depth of more than 4 m are oligotrophic and 2-4 m are mesotrophs. Average SD depth in Lake Bafa is 2.47 m. When Lake Bafa is examined seasonally in terms of this parameter, it shows oligotroph-mesotroph lake characteristics. The TSI index results showed that Lake Bafa was from the limiting mesotrophic to slightly eutrophic. Sömek and Ustaoğlu (2016) stated that oligotrophic and mesotrophic indicatör species are found together in the phytoplankton composition of Saklıgöl, Karagöl, Gökçeova Pond and Kartal Lake, and considering the TSI values, the productivity status of the studied lakes is still oligotrophic but has a transition trend towards mesotrophic state. According to Akçaalan et al. (2007), in the research carried out in Sapanca Lake, which is an oligo-mesotrophic lake, diatom was reported to be predominantly found during the research period. According to Fakıoğlu and Demir (2011), the trophic level of Beyşehir lake is mesotrophic according to total phosphorus concentration, phytoplankton, chl *a* concentration value. The variation of the TP value of the Lake Bafa was examined and the TP value was found at the bottom depth and low on the surface. This is thought to be due to the release of phosphorus from the sediment. Lake Bafa is classified as mesotrophic according to the depth of Secchi. However, the low depth of Secchi may be caused by the increase of turbidity with the mixture from the bottom in shallow water. In addition, some eutrophic species have been found in the phytoplankton composition, Cyanophyceae have shown periodic increases, and the species and numbers of the Euglenophyceae groups which show organic contamination have increased.

Ongun-Sevindik et al. (2017) determined ecological status of two Mediterranean lakes with Q index. The average Q index was found as 3.05 for Lake Taşkısığı and 2.56 for Lake Akgöl, which reflect good and medium water qualities, respectively. The Q quality index generally varied between 2 and 4 (medium to good), and it was higher during winter in both of the lakes. Values were slightly higher in Lake Taşkısığı than Lake Akgöl during the studied period ($f = 11.13$, $P < 0.05$). In Lake Bafa, the highest Q index score values obtained from station #3, and the station #3 also is determined as a moderate ecological status. In this study, the sampling stations #6, #7 and #8 determined as a bad ecological status.

Considering the TDI values in the phytoplankton composition of the lake, it can be said that although the productivity status of the studied lake is still "*mesotrophic*", it has a tendency towards "*eutrophic*" state. According to the Q values, the first five stations reflect the *moderate* ecological state, while the 6th, 7th and 8th stations represent the *poor* ecological state.

CONCLUSION

The dataset obtained for two years study period in Lake Bafa indicated that the variability of trophic status of the lake. Q index and the TSI index classified Lake Bafa as moderate (medium) and meso-eutrophic, respectively. According to the obtained biological dataset from Lake Bafa, it is clear that due to the pollution factors as intense domestic and industrial wastes cause pollution presure on Lake Bafa. Industrial establishments located around the lake should be inspected frequently and prevented from giving their wastes to the system without treatment. Intermittent monitoring of Lake Bafa is benefical to prevent the negative effect of pollutants. As a result of this study, there is an importantly need for the constitute of biotic indices-based phytoplankton species to assess with high accuracy freshwaters.

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REFERENCES

Akçaalan, R., Albay, M., Gürevin, C. & Çevik, F. (2007). The influence of environmental conditions on the morphological variability of phytoplankton in an oligo-mesotrophic Turkish lake. *International Journal of Limmnology*, 43, 21-28. DOI:10.1051/limn/2007024

Balık, S. & Ustaoglu, M.R. (1989). Bafa golundeki Ulubat baligi (*Acanthobrama mirabilis* Ladiges, 1960)'nin biyoekolojik ve ekonomik yonlerden incelenmesi. *Turkish Journal of Zoology*, 13(3), 141–174.

Balık, S. Ustaoglu, M.R. & Sarı, H.M. (1992). Bafa gölü (Söke-Aydın) kababurun (*Chondrostoma nasus* l., 1758) populasyonunun biyolojik özelliklerinin incelenmesi. *Fırat Universitesi XI. Ulusal Biyoloji Kongresi*, 24–27 Haziran 1992, p. 49–58, Elazığ.

Balık, S. (1995). Freshwater fish in Anatolia, Turkey. *Biological Conservation* (72), 213–223, Oxford. DOI: 10.1016/0006-3207(94)00084-4

Bukhtiyarova, L.N. (2006). Additional data on the diatom genus Karayevia and a proposal to reject the genus Kolbesia. *Nova Hedwigia Beiheft*, 130, 85–96.

Carlson, R.E. (1977). Atrophic state index for lakes. *Limnology and Oceanography*, 22(2), 361–369. DOI: 10.4319/lo.1977.22.2.0361

Carlson, R.E. & Simpson, J. (1996). A coordinator's guide to volunteer lake monitoring methods. *North American Lake Management Society*. 96 pp.

Çelekli, A., Albay, M. & Dügel, M. (2007). Phytoplankton (except Bacillariophyceae) Flora of Lake Gölköy (Bolu). *Turkish Journal of Botany*, 31, 49-65.

Çelekli, A. & Öztürk, B. (2014). Determination of ecological status and ecological preferences of phytoplankton using multivariate approach in a Mediterranean reservoir. *Hydrobiologia*, 740, 115–135. DOI:10.1007/s10750-014-1948-8

Çelekli, A., Toudjani, A.A., Lekesiz, H.Ö. & Çetin, T. (2018). Ecological quality assessment of running waters in the North Aegean catchment with diatom metrics and multivariate approach. *Limnologica*, 73, 20-27. DOI: 10.1016/j.limno.2018.09.001

Çelekli, A. & Lekesiz, Ö. (2020). Eco-assessment of West Mediterranean basin's rivers (Turkey) using diatom metrics and multivariate approaches. *Environmental Science and Pollution Research,* 27, 27796–27806. DOI:10.1007/s11356-020-09140-1

Cigagna, C., Bonotto, D.M., Camargo, A.F.M. & Sturaro, J.R. (2016). Trophic state index (TSI) and physico-chemical characteristics of a shallow reservoir in southeast Brazil. *Environmental Earth Sciences*, 75(2), 1-11. DOI:10.1007/s12665-015-4951-0

Cirik-Altındağ, S. (1982). Phytoplankton of Manisa-Marmara Lake, I-Cyanophyta (in Turkish with French abstract). *Doğa Bilim Dergisi*, 6(3), 67-81.

Compère, P. (2001). Ulnaria (Kützing) Compère, a new genus name for Fragilaria subgen. Alterasynedra Lange-Bertalot with comments on the typification of Synedra Ehrenberg In: R. Jahn, J.P. Kociolek, A. Witkowski, P. Compère (Eds), Lange-Bertalot-Festschrift: Studies on Diatoms. Dedicated to Prof. Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th Birthday. A.R.G. Gantner Verlag. K.G. pp. 97-102

Ersanlı, E. & Gönülol, A. (2003). Study on the phytoplankton and seasonal variation of Lake Simenit (Terme-Samsun, Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, 3, 29-39.

European Commission (2000). Directive 2000/60/EC of the European Parliament and of the council of 23rd October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities, L327/1. European Commission, Brussels.

Fakıoğlu, Ö. & Demir, N. (2011). The spatial and seasonal variations of phytoplankton biomass in Lake Beyşehir. *Ekoloji*, 20(80), 23-32. DOI:10.5053/ekoloii.2011.804

Feret, L., Bouchez, A. & Rimet, F. (2017). Benthic diatom communities in high altitude lakes: a large scale study in the French Alps. *Annales de Limnologie - International Journal of Limnology,* 53, 411–423. DOI:10.1051/limn/2017025

Ghosh, D. & Biswas, J. K. (2005). Macroinvertebrate diversity indices: A quantitative bioassessment of ecological health status of an Oxbow Lake in Eastern India. *Journal of Advances in Enviromental Health Research*, 3(2), 78- 90. DOI:10.22102/JAEHR.2015.40190

Guiry, M.D. & Guiry, G.M. (2020). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Retrieved from http://www.algaebase.org (25.12.2019).

Gürbüz, H. & Altuner, Z. (2000). Palandöken (Tekederesi) Göleti fitoplankton toplulugu üzerinde kalitatif ve kantitatif bir arastırma. *Turkish Journal of Biology*, 24, 13–30.

Hustedt, F. (1930). Bacillariophyta Diatome Heft: 10 A Pascher DieSusswasser Flore Mitteleuropas. Ed. Gustav Fischer Pub., Jena, 466p, Germany.

İşbakan Taş, B., Gönülol, A. & Taş, E. (2002). A study on the seasonal variation of the phytoplankton of Lake Cernek (Samsun-Turkey), *Turkish Journal of Fisheries and Aquatic Sciences*, 2, 1231-128.

James, F.C. & McCulloch, C.F. (1990). Multivariate analysis in ecology and systematics: Panacea or pandora's box. *Annual Review of Ecology*, 21, 129-66. DOI: 10.1146/annurev.es.21.110190.001021

Katip, A., İleri, S., Karaer, F. & Onur, S. (2015). Determination of the trophic state of Lake Uluabat (Bursa-Turkey). *Ekoloji*, 24(97), 24-35. DOI:10.5053/ekoloji.2015.07

Kazancı, N., Girgin, S. & Dügel, M. (2008). Research on the limnology of Lake Bafa in South-Western Turkey and climate change impacts, *Rewiew of Hydrobiology*, 2, 207-223.

Kılınç, S. (1998). A study in the seasonal variation of phytoplankton in Hafik Lake (Sivas, Turkey). *Turkish Journal of Botany*, 22, 35-41.

Kıvrak, E. & Gürbüz, H. (2005). Seasonal variations in phytoplankton composition and physical-chemical features of Demirdöven Dam Reservoir, Erzurum, Turkey. *Biologia Bratislava*, 60(1), 1-8.

Krammer, K. & Lange-Bertalot, H. (1986–1991). Bacillariophyceae 1. Teil: Naviculaceae; 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae; 3. Teil: Centrales, Fragilariaceae, Eunotiaceae; 4. Teil: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. Stuttgart: Fischer.

Krammer, K. (1997). Die cymbelloiden Diatomeen. Teil 2: Encyonema part., Encyonopsis und Cymbellopsis. Stuttgart: Cramer.

Marchetto, A., Padedda, B.M., Mariani, M.A., Luglie, A. & Nicola, S. (2009). A numerical index for evaluating phytoplankton response to changes in nutrient levels in deep mediterranean reservoirs. *Journal of Limnology*, 68, 106–121. DOI:10.4081/jlimnol.2009.106

Mason, C.F. (2002). Biology of freshwater pollution. 4th ed. New York, NY, USA: Prentice Hall.

Mermer, A. (1989). Gediz Nehrindeki Kababurun Baligi (*Chondrostoma nasus* Linnaeus, 1758) populasyonunun biyolojik yöndenden incelenmesi. – Ege Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dali, Yuksek Lisans Tezi, 48 pp, İzmir.

Nandan, S.N. & Aher, N.H. (2005). Algal community used for assesment of water quality of Haranbaree Dam and Mosam River of Maharashtra. *Journal of Environmental Biology*, 26, 223-227.

Ochocka, A. & Pasztalenice, A. (2016). Sensitivity of plankton indices to lake trophic conditions. *Environmental Monitoring Assessment*, 188, 622. DOI:10.1007/s10661-016-5634-3

Ongun-Sevindik, T. (2010). Phytoplankton composition of Çaygören Reservoir, Balikesir-Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 10, 295-304. DOI:10.4194/trjfas.2010.0301

Ongun-Sevindik, T., Tunca, H., Gönlüol, A., Yıldırım-Gürsoy, N., Küçükkaya, Ş.N. & Durgut-Kınalı, Z. (2017). Phytoplankton dynamics and structure, and ecological status estimation by the Q assemblage index: a comparative analysis in two shallow Mediterranean lakes. *Turkish Journal of Botany*, 41, 25-36. DOI:10.3906/bot-1510-22

Padisak, J., Borics, G., Grigorszky, I. & Soroczki-Pinter, E. (2006). Useofphytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. *Hydrobiologia*, 553,1–14. DOI:10.1007/s10750-005-1393-9

Pasztaleniec, A. & Poniewozik, M. (2010). Phytoplankton based assessment of the ecological status of four shallow lakes (Eastern Poland) according to Water Framework Directive – a comparison of approaches. *Limnologica*, 40, 251-259. DOI:10.1016/j.limno.2009.07.001

Pasztaleniec, A. (2016). An advanced phytoplankton trophic index: Test and validation with a nationwide lake survey in Poland. *International Review of Hydrobiology*, 101, 20-35. DOI:10.1002/iroh.201501799

Potapova, M. (2006). Achnanthidium zhakovschikovii sp. nov. (Bacillariophyta) and related species from rivers of Northwestern Russia. *Nova Hedwigia*, 82, 399–408. DOI:10.1127/0029-5035/2006/0082-0399

Ptacnik, R, Solimini, A.G. & Brettum P. (2009). Performance of a new phytoplankton composition metric along a eutrophication gradient in Nordic lakes. *Hydrobiologia*, 633, 75–82. DOI:10.1007/s10750-009-9870-1

Reynolds, C.S., Huszar, V., Kruk, C., Naselli-Flores, L. & Melo, S. (2002). Rewiew, towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research*, 24(5), 417-428. DOI:10.1093/plankt/24.5.417

Round, F.E. & Bukhtiyarova, L. (1996). Four new genera based on Achnanthes (Achnanthidium) together with a re-definition of Achnanthidium. *Diatom Research*, 11, 345–361. DOI: 10.1080/0269249X.1996.9705389

Saghi, H., Karimi, L. & Javid, A. H. (2014). Investigation on trophic state index by artificial neural networks (case study: Dez Dam of Iran). *Applied Water Science*, 5(2), 2190-5487. DOI:10.1007/s13201-014-0161-2

Sakamoto, M. (1966). The chlorophyll amount In the euphotic zone in some Japanese Lakes and its significace In thr photosynthetic production of phytoplankton community. *The botanical magazine* Tokyo, 79, 77-78. DOI: 10.15281/jplantres1887.79.77

Sala, S. E., Guerrero, J. M. & Ferrario, M. E. (1993). Redefinition of Reimeria sinuata (Gregory) Kociolek & Stoermer and recognition of Reimeria uniseriata nov. spec. *Diatom Research*, 8, 439–446. DOI: 10.1080/0269249X.1993.9705273

Sarı, H.M., Balık, S., Bilecenoğlu, M. & Türe, G. (1999). Recent changes in the fish fauna of Lake Bafa, Aegean region of Turkey. *Zoology in the Middle East*, 18, 67–76. DOI: 10.1080/09397140.1999.10637783

Shekhar, T.R., Kiran, B.R., Puttaiah, E.T., Shivaraj, Y. & Mahadevan, K.M. (2008). Phytoplankton as index of water quality with reference to industrial pollution. *Journal of Environmental Biology*, 29(2), 233-6.

Sömek, H., Balık, S. & Ustaoğlu M.R. (2005). Phytoplankton of Topçam Dam Lake (Çine-Aydın) and their seasonal variations (in Turkish with English abstract). *Süleyman Demirel University Eğirdir Journal of Fisheries Faculty*, 1(1), 26-32.

Sömek, H. & Balık, S. (2009). Seasonal variation of algal flora and environmental conditions of Karagöl (A Mountain Lake, İzmir-Turkey) (in Turkish with English abstract). *Ege Journal of Fisheries and Aquatic Sciences*, 26(2), 121-128. DOI: 10.12714/egejfas.2016.33.2.05

Sömek, H. & Ustaoğlu M.R. (2016). Summer phytoplankton composition and trophic state index values of some mountain lakes in Western Anatolia (Denizli-Muğla). *Ege Journal of Fisheries and Aquatic Sciences*, 33(2), 121-128. DOI:10.12714/egejfas.2016.33.2.05

Somerfield, P. J. (2008). Identification of the Bray-Curtis similarity index: Comment on Yoshioka (2008). *Marine Ecology Progress Series*, 372, 303-306. DOI: 10.3354/meps07841

Swanson, E. R. (1998). Trophic state index revisited. *Lake Line*, 18(4), 18–20.

Taş, B. & Gönülol, A. (2007). Planktonic algae of Derbent Dam Lake (Samsun, TURKEY). *Journal of FisheriesSciences.com*, 1(3), 111-123. DOI: 10.3153/jfscom.2007014

Ter Braak, C.J.F. & Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5). Biometris, Wageningen.

Toudjani, A.A., Çelekli, A., Gümüş, E.Y., Kayhan, S., Lekesiz, H.Ö. & Çetin, T. (2017). A new diatom index to assess ecological quality of running waters: a case study of water bodies in western Anatolia. *Annales de Limnologie - International Journal of Limnology*, 53, 333–343. DOI:10.1051/limn/2017012

Utermohl, H. (1958) Zur ver vollkommung der quantitativen phytoplankton-methodik. Mitteilung internationale vereinigung fuer theoretische unde amgewandte limnologie, 9, 39 p. DOI: 10.1080/05384680.1958.11904091

Vaitomaa, J. (2006). The effects of environmental factors on biomass and microcystin production by the freshwater cyanobacterial genera Microcystis and Anabaena. Edita, Helsinki, Finland, 56 p.

Vollenweider, R.A. & Kerekes J.J. (1982). Eutrophication of waters. Monitoring, assessment and control. OECD Cooperative programme on monitoring of inland waters (Eutrophication control), Environment Directorate, OECD, Paris. 154 p.

Walker, W.W. (1979). Use of hypolimnetic oxygen depletion rate as a trophic state index for lakes. *Water Research*, 15(6), 1463–1470. DOI: 10.1029/WR015i006p01463

Wetzel, R.G. (1975). Limnology. W,B. Sounders Conpany, Phiadelphia, 743p.

Yoshioka, P. M. (2008). Misidentification of the Bray-Curtis similarity index. *Marine Ecology Progress Series*, 368, 309-310. DOI:10.3354/meps07728

Xu, F.L. (2008). Trophic classification for lakes. In: Jørgensen SE, Fath BD (eds) *Ecological indicators*, Vol. 5 of encyclopedia of ecology, 5th edn., 359pp.

http://www.egejfas.org
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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Çakalburnu Lagünü (İzmir) sedimentlerinde real-time PCR ile bakteri ve arke düzeylerinin değerlendirilmesi

Assessment of bacteria and archaea levels in Çakalburnu Lagoon (İzmir) sediments by real-time PCR

Burcu Omuzbüken[1] ● Aslı Kaçar[2]*

[1] Dokuz Eylül Üniversitesi, Fen Bilimleri Enstitüsü, İzmir, Turkey
[2] Dokuz Eylül Üniversitesi, Deniz Bilimleri ve Teknolojisi Enstitüsü, İzmir, Turkey

https://orcid.org/0000-0002-6681-7174
https://orcid.org/0000-0002-8705-3695

*Corresponding author: asli.kacar@deu.edu.tr

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Öz: Kıyısal lagünler, bir veya daha fazla sayıda giriş ile su değişimine izin veren ve bir bariyer gibi denizden ayrılan sığ su kütleleridir. Bu kırılgan ekosistemler kendilerine özgü sediment yapılarına sahip olmaktadır. Büyük ölçüde bentik mikrobiyal döngü yoluyla yürütülen biyojeokimyasal süreçler; lagün ile bitişik kıyı bölgesi arasındaki ilişkiyi anlamak için oldukça önemlidir. Bu çalışma, İzmir Körfezi'nde bulunan ve 67 hektarlık bir alan kaplayan Çakalburnu Lagünü'nde gerçekleştirilmiştir. Çalışmanın amacını, lagün sedimentlerinde, farklı mikrobiyal toplulukların düzeylerinin belirlenmesi oluşturmaktadır. Araştırmada, lagünün 7 noktasından toplanan sediment örneklerinde, Genel Arke (ARC), Metanojenik Arke (MCRA), Anaerobik Metan Oksidasyonu yapan Arke (ANME 1, ANME 2a, ANME 2c), Genel Bakteri (BAC) ve Sülfat İndirgeyen Bakteri (SRB2) seviyelerinin tespitinde Real-Time qPCR analizleri gerçekleştirilmiştir. Çakalburnu Lagünü sedimentlerinde incelenen Genel Arke ve Genel Bakteri bollukların maksimum değerleri sırasıyla $2{,}66x10^{10}$ gen kopya sayısı/gr ve $3{,}89x10^{7}$ gen kopya sayısı/gr olarak belirlenmiş olup, bu çalışmada lagün sedimentlerinde arkeal bolluğun yoğun olduğu görülmüştür. Mikrobiyal çeşitliliğin karakterizasyonu, ekosistemin biyolojik temellerinin anlaşılması açısından önemlidir. Çalışmamızda sunulan veriler, lagünler gibi hassas ekosistemlerde, ekolojik ve mikrobiyolojik dengenin korunması ile biyojeokimyasal döngülerin belirlenmesine yönelik çalışmalara katkı sağlamaktadır. Gelecekteki çalışmalarda, mikrobiyal grupların seviyelerinin mevsimlere ve yıllara göre değişimlerinin izlenmesi yönünde çalışmalar yürütülecektir.

Anahtar kelimeler: Lagün, sediment, bakteri, arke, real-time PCR

Abstract: Coastal lagoons are shallow water masses, discredited from the marines as a barrier that permits water to change through one or more inputs. These fragile ecosystems have a specific type of sediments with their own characteristics. Biogeochemical processes, mostly intervened by the benthic microbial loop, are significant for understanding the relationships among the lagoon and the contiguous coastal partition. This study was conducted in the Çakalburnu Lagoon (İzmir) area, which is located at the Bay of İzmir and the area covers 67 hectares. The aim of the present study is to constitute of determining the number of different microbial communities in the lagoon sediments. We collected from lagoon sediments samples at 7 stations and we applied a Real-time qPCR assay to determine levels of archaea (ARC), methanogenic archaea (MCRA), anaerobic methane oxidation archaea (ANME 1, ANME 2a, ANME 2c), bacteria (BAC) and sulfate-reducing bacteria (SRB2) in the study. The amount of maximum abundance of archaeal and bacterial 16S rRNA gene in sediments are $2{,}66x10^{10}$ gene copy numbers/g and $3{,}89x10^{7}$ gene copy numbers/g, respectively. So, it was established that the archaeal abundance was intense in the lagoon sediments. The characterization of microbial diversity is significant for the comprehension of the biological fundamentals of the ecosystem. The data presented in our study contributes to the studies on preserving ecological and microbiological balance and determining biogeochemical cycles in sensitive ecosystems such as lagoons. The research will be conducted on studies to determine the abundance levels of seasonal and annual microbial groups in the future.

Keywords: Lagoon, sediment, bacteria, archaea, real-time PCR

GİRİŞ

Sulak alanlar, taşkınların önlenmesi, iklimsel döngülerin dengelenmesi, biyolojik çeşitliliğin korunması, kirliliğin kontrolü, kuraklığın etkilerinin en aza indirilmesi ve su kaynaklarının korunmasında vazgeçilmez bir rol oynamaktadır. Gezegendeki biyolojik olarak en verimli ekosistemlerden biri olduklarından "yaşamın beşiği" olarak adlandırılmışlardır (Li vd., 2020). Türkiye'nin de taraf olduğu Ramsar Sözleşmesi'nde sulak alanların tanımı; "Doğal ya da yapay, sürekli ya da mevsimsel, tatlı, acı ya da tuzlu, durgun ya da akan su kütleleri, bataklıklar, turbalıklar ve gelgitin çekilmiş anında derinliği altı metreyi aşmayan deniz suları" olarak belirtilmektedir (Ramsar Convention Bureau, 1992). Sulak alan ekosistemlerinin değeri hakkında genel bir anlayış eksikliği nedeniyle, bu alanlar, ülkelerin ekonomisinden ve yoğun kentsel nüfuslarından kaynaklanan sanayileşme ve kentleşme tehdidi altındadır (Yin, 2003).

Sucul ortamlardaki sedimentler ise küresel biyosferin önemli bileşenleridir ve çeşitli bitki ve hayvanlara yaşam alanı sağlamanın yanısıra, besin döngülerindeki önemli dönüşümleri destekleyen benzersiz biyojeokimyasal alanlar oluşturulmasına kadar çeşitli roller oynarlar (Obi vd., 2016). Lagün sistemleri, dünyanın doğal biyolojik kaynakları

olmalarının yanı sıra, bilimsel araştırmalar için doğal birer laboratuvar olarak, biyolojik çeşitliliğin korunması ve sürdürülmesinde büyük öneme sahip hassas ve kırılgan ekosistemlerdir (Yucel-Gier vd., 2018). Kıyısal lagünler, bir veya daha fazla sayıda giriş ile su değişimine izin veren ve bir bariyer gibi denizden ayrılan sığ su kütleleridir. Günümüz kıyı şeridinin %13'ünü sınırlayan bu alanlar, tipik olarak Holosen deniz seviyesinin yükselmesi sırasında, kıyı düzlüklerinin sular altında kalmasından kaynaklanan yaygın kıyı ekosistemleridir. Kıyısal lagünler biyojeokimyasal süreçler açısından oldukça dinamik ortamlardır ve kara-deniz geçiş yüzeyinde organik maddenin taşınmasında, dönüştürülmesinde ve birikmesinde önemli rol oynarlar. Bu sığ ortamlarda, su kolonu ile bağlantılı olduğu için (Oueslati vd., 2019) organik madde (OM) birikiminde bir havuz veya organik ve inorganik besin taşınımı yoluyla bitişik kıyı bölgelerini besleyebilen rezervuarlar olarak işlev görebilirler (Manini vd., 2003). Genellikle antropojenik aktivitelere önemli ölçüde maruz kalırlar ve böylece tarımsal, endüstriyel ve kentsel atıklarla taşınan çok çeşitli organik ve inorganik bileşiklerle kirlenirler (Ribeiro vd., 2016). Lagün sedimentlerinde organik madde mineralize edilir ve kara-deniz geçiş zonunda besin maddeleri ile oksijen konsantrasyonuna bağlı olarak sedimentlerde nitrifikasyon ve denitrifikasyon meydana gelebilir. Denitrifikasyon ile azot kaybı toplam mineralize azotun %25'ini oluşturabilir. Ayrıca, yüksek organik madde girdisiyle, mineralizasyon oranları yükselir ve sedimentlerin yüzeyinde oksijen hızla tükenir ve anoksik ortamlar gelişebilir (Glud, 2008). Büyük ölçüde bentik mikrobiyal döngü aracılığındaki bu süreçler, lagün ile bitişik kıyı bölgesi arasındaki ilişkiyi anlamak için oldukça önemlidir (Manini vd., 2003). Kentsel kanalizasyon sularıyla kirlenen birçok sığ kıyısal lagünde, sülfat redükleyici bakterilerin aktivitelerinin önemli oranlarda olduğu anoksik katmanlar (sular veya sedimentler) bulunmaktadır. Kara ve deniz arasındaki sınırlardaki konumlarından ötürü, hem organik malzemeli kıtasal tatlı suları, hem de tuzlu ve mineralce zengin deniz sularını aldıklarından; deniz suyundan gelen yüksek sülfat konsantrasyonu, yerinde birincil üretimden kaynaklanan organik maddenin mineralizasyonunda yüksek oranda yer alan anoksik katmanlarda sülfat redüklenmesini uyarır (Caumette, 1986).

Yine bentik Arkeler de biyojeokimyasal döngülerde ve besin ağlarında önemli bir rol oynar, ancak bu toplulukların kompozisyonu ve bolluğu kıyısal lagün sedimentlerinde çok detaylı araştırılmamıştır (Behera vd., 2020). Anaerobik metan-oksitleyici arke (ANME), metanojenik yolun tersine çevrilmesi yoluyla metanın anaerobik oksidasyonunu (AOM) gerçekleştirir. ANME tarafından gerçekleştirilen AOM'nin sülfat indirgemesine (SR) bağlandığı ilk olarak denizel sedimentlerde keşfedilmiştir. Burada ANME, Deltaproteobacteria'ya ait sülfat indirgeyen bakteriler (SRB) ile metabolik olarak birbirine bağlı konsorsiyumlar oluşturmuştur. Sülfata bağlı AOM (S-AOM) ile ilişkili ANME grupları, çoğunlukla çamur volkanlarında ve bazı sızıntı sedimentlerinde bulunan ANME-3 dışında birçok farklı deniz ortamında ortaya çıkmaktadır. Denizel ortamların yanı sıra, S-AOM'da yer alan ANME karasal ve tatlı su ekosistemlerinde bulunabilir (Timmers vd., 2017). Ayrıca sedimentlere sürekli organik madde girişi ve oksijen eksiklikleri sağlayan yüksek birincil üretim nedeniyle, sedimentlerin yüzey bölgesinde metan oluşturan arkeler (MA) için de uygun koşullar yaratılmış olur (Reindl ve Bolałek, 2017). SRB ve MA, karbon ve kükürt döngülerinde önemli bir rol oynadıkları haliçler ve kıyı lagünleri de dahil olmak üzere deniz ve kıyı sedimanlarındaki organik madde oksidasyonunun son aşamalarında aktiftir (Takii ve Fukui, 1991). SRB, sülfat indirgeme prosesi ile, sürekli bir sülfat girdisinin bulunduğu ortamlarda, %50'ye kadar bozulma kapasitesi olan organik maddenin anaerobik mineralizasyonundan sorumlu ana mikroorganizma grubudur (Jørgensen ve Bak, 1991). Kıyısal ve denizel ortamlar SRB'nin en karakteristik yaşam alanları olmasına rağmen, MA varlığı, haliçlerin tatlı su bölgelerinde ve MA için önemli bir substrat kaynağı sağlayan tuz bataklıklarında nispeten yüksek sayıda kaydedilmiştir. MA, metanogenez yoluyla, CH_4 ve CO_2'nin kıyı sistemlerinden atmosfere serbest bırakılmasında önemli bir rol oynayabilmektedir (Torres-Alvarado vd., 2016).

Dolayısı ile kıyısal lagünler, bentik mikrobiyal çeşitlilik açısından nadiren araştırılan oldukça değişken ve dinamik sistemlerdir (Manini vd., 2003). Genellikle bu sistemleri oldukça kararsız hale getiren ve öngörülemeyen dalgalanma koşullarına maruz kalan önemli fiziksel ve kimyasal değişkenlerle karakterize edilirler (Pusceddu vd., 1996). Bununla birlikte, lagün sedimentleri kirleticilerin depolama alanıdır ve bu nedenle kirleticilerin sediment üzerindeki etkilerinin anlaşılmasında ve mikrobiyal topluluklar ile ilişkilerinin belirlenmesinde, bir bütün olarak lagünlerin potansiyel etkilerini anlamak açısından önemlidir (Obi vd., 2016). Sedimentlerdeki besin maddeleri bileşimi ile ilişkili biyotik ve abiyotik faktörler, içerdiği mikrobiyal çeşitliliğini ve biyoaktif bileşik üretme potansiyelini yönlendirdiğinden (Al Amoudi, 2016), çalışmalar bu ortamların karakteristik özelliklerine sahip mikrobiyal çeşitliliğin tespitine odaklanmaktadır. Mikrobiyal açıdan bakıldığında Çakalburnu Lagünü sedimentleri nadir araştırılmış ve moleküler yöntemler kullanılarak bir inceleme yapılmamıştır. Genel olarak, doğal ve antropojenik streslerin lagün ekolojisi ve biyojeokimyasal fonksiyonlar üzerindeki etkisini değerlendirmek için bir gösterge olabilecek genel arke, metanojenik arke, anaeorobik metan oksidasyonu yapan arke, genel bakteri ve sülfat redükleyen bakteriyal toplulukların bileşimi hakkında mevcut bir bilgi yoktur. Çalışmamızda, antropojenik ve doğal faktörlerden dolayı yoğun bir baskı altında olan Çakalburnu Lagünü'nde bentik mikrobiyal toplulukların kompozisyonu ve bolluğu incelenmiştir.

MATERYAL VE METOT

Çalışma alanı ve örnekleme

Bu çalışma, İzmir Körfezi'nde (İzmir'in kuzeybatısında, 38.406 ° - 38.415 ° N ve 27.045 ° - 27.060 °E) bulunan 67 hektarlık bir alan kaplayan Çakalburnu Lagünü (İzmir) bölgesinde gerçekleştirilmiştir. Çakalburnu Lagünü'nün genişliği 752 m, uzunluğu 1054 m ve derinliği 0,5 ile 1 m arasında değişmektedir. Önemli ekonomik kalkınma ile teşvik edilen kentleşme, Türkiye'de son elli yılda hızla artmıştır. Sanayileşme ve kentleşme süreçlerinde (Esbah, 2004), sulak

alanlar Türkiye'nin tehdit altındaki ekosistemleri arasındadır (Kara, 2019). İzmir şehrinin toplam kentleşmiş bölgesi geniş sanayi bölgelerini kapsamakta olup, Türkiye'nin en büyük ihracat limanlarından birine sahiptir. Lagün, İzmir Körfezi'nin iç kısmında yer alır; kapalı ortamı, sığ derinliği ve sınırlı su sirkülasyonu nedeniyle ötrofik bir ortama sahiptir. Ayrıca Çakalburnu Lagünü kum midyesi (*Tapes decussatus* L., 1758) yetiştiriciliği açısından önemli bir alandır (Yucel-Gier vd., 2018).

Örnekleme, 7 adet kıyı istasyonundan (Şekil 1) toplanan sediment numunelerinin steril 50 mL'lik falcon tüplere alımı ile gerçekleştirilmiştir. Kıyı istasyonlarının seçiminde lagünün deniz ile bağlantı noktalarındaki sediment değişimi ve lagünün içindeki alan belirlenmiştir. Örneklemede asepsi-antisepsi kurallarına uyulmuş olup, sediment numuneleri laboratuvara 4°C'de transfer edilmiştir. Örnekler -20°C'de muhafaza edilmiş olup, 1 hafta içerisinde DNA ekstraksiyonu ve Real-time PCR analizleri gerçekleştirilmiştir.

Şekil 1. Çakalburnu Lagünü örnekleme noktaları
Figure 1. The sampling points of Çakalburnu Lagoon

DNA ekstraksiyonu

Toplam genomik DNA izolasyonu, DNeasy PowerSoil DNA İzolasyon (Qiagen) kiti kullanılarak, protokolde uygulanan bazı modifikasyonlar ile gerçekleştirilmiştir. Buna göre DNA ekstraksiyonu için her sediment numunesinden 0.25 g tartılarak DNA ekstrakte edilmiştir. Ekstrakte edilen toplam genomik DNA analiz edilene kadar -20°C'de saklanmıştır. Toplam genomik DNA konsantrasyonu, Synergy HTX multimod okuyucu (BioTek Instruments, Inc) kullanılarak A260nm / A280nm ölçümleriyle belirlenmiştir.

Primer setlerinin belirlenmesi ve Real-Time PCR

Çakalburnu Lagünü'nden elde edilen sediment örneklerindeki, Genel Arke (ARC), Metanojenik Arke (MCRA), Anaerobik Metan Oksidasyonu yapan Arkeler (ANME 1, ANME 2a, ANME 2c), Genel Bakteri (BAC) ve Sülfat İndirgeyen Bakterilerin (SRB2) seviyelerinin tespit edilebilmesi için gruplara özgü primer setleri literatürlerden seçilmiştir (Tablo 1). ANME'lerde üç farklı metanotrofik grup tanımlanmıştır: ANME-1 (alt gruplar a ve b), ANME-2 (alt gruplar a, b ve c) ve ANME-3. ANME-1 grubu Methanomicrobiales ve Methanosarcinales ile ilişkilidir. ANME-2, Methanosarcinales'in kültüre edilebilir üyeleriyle ilişkilidir ve ANME-3, daha çok *Methanococcoides* spp. ile ilişkilidir. ANME grupları birbirleriyle monofiletik değildir ve alt gruplar arasındaki filogenetik mesafe büyüktür, 16S rRNA gen dizisi benzerliği sadece %75-92'dir. ANME-2a ve ANME-2b alt grupları, ANME-2c'den ayırt edilen ve bu nedenle sıklıkla birlikte ANME-2a / b olarak gruplanan tutarlı bir küme oluşturur (Timmers vd., 2017). Sediment örneklerine uygulanan Real-time q-PCR analizleri, LightCycler 480 SYBR Green I Master Kit (Roche) kullanılarak, LightCycler 96 (Roche) Q-PCR cihazında gerçekleştirilmiştir.

ANME 1, ANME 2a, ANME 2c, MCRA ve SRB2 gruplarının tespiti için Q-PCR analizlerinde uygulanan koşullar; preincubation x 1 döngü (95°C – 300 saniye), 3 step amplification x 45 döngü (95°C – 10 saniye, 53°C – 15 saniye, 72°C – 15 saniye), melting x 1 döngü (95°C – 5 saniye, 65°C – 60 saniye, 97°C – 1 saniye), cooling x 1 döngü (40°C- 10 saniye) şeklindedir. BAC ve ARC gruplarının tespiti için Q-PCR analizlerinde uygulanan koşullar ise; preincubation x 1 döngü (95°C – 300 saniye), 3 step amplification x 45 döngü (95°C – 10 saniye, 50°C – 15 saniye, 72°C – 10 saniye), melting x 1 döngü (95°C – 5 saniye, 65°C – 60 saniye, 97°C – 1 saniye), cooling x 1 döngü (40°C- 10 saniye) şeklindedir.

Tablo 1. Real-time PCR analizlerinde kullanılan primerler
Table 1. The primers of Real-time PCR analyses

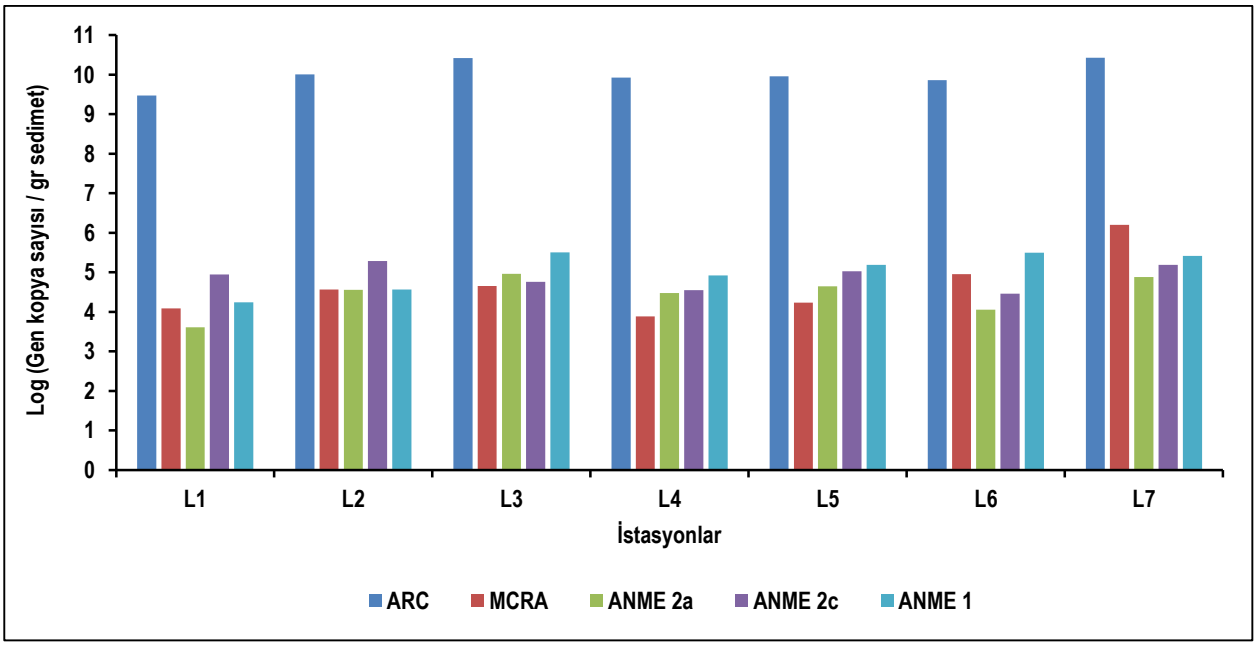
No	Primer Kodu	Hedef Grup	Fonksiyon	Sekans (5 → 3')	Kaynaklar
1	ARC –787F	Arke	Q-PCR - Forward primer	ATT AGA TAC CCS BGT AGT CC	(Yu vd.,2005; Vigneron vd., 2013)
2	ARC –1059R	Arke	Q-PCR - Reverse primer	GCC ATG CAC CWC CTC T	(Yu vd.,2005; Vigneron vd., 2013)
3	MCRA_Mreductase - subunit-mcrA genes	Metanojenik Arke	Q-PCR - Forward primer	GGT GGT GTM GGD TTC ACM CAR TA	(Steinberg ve Regan, 2009)
4	MCRA_rev	Metanojenik Arke	Q-PCR - Reverse primer	CGT TCA TBG CGT AGT TVG GRT AGT	(Steinberg ve Regan, 2009)
5	ANME 1 – 337F	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Forward primer	AGG TCC TAC GGG ACG CAT	(Timmers vd., 2017)
6	ANME 1 –724R	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Reverse primer	GGT CAG ACG CCT TCG CT	(Timmers vd., 2017)
7*	ANME 2a – 426F_1	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Forward primer	TGT TGG CTG TCC GGA TGA	(Timmers vd., 2015)
8*	ANME 2a – 426F_2	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Forward primer	TGT TGG CTG TCC AGA TGA	(Timmers vd., 2015)
9*	ANME 2a – 426F_3	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Forward primer	TGT TGG CTG TCC AGA TGG	(Timmers vd., 2015)
10	ANME 2a-1242R	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Reverse primer	AGG TGC CCA TTG TCC CAA	(Timmers vd., 2015)
11	ANME 2c_F	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Forward primer	TCG TTT ACG GCT GGG ACT AC	(Timmers vd., 2017)
12	ANME 2c_R	Anaerobik Metan Oksidasyonu yapan Arke	Q-PCR - Reverse primer	TCC TCT GGG AAATCT GGT TG	(Timmers vd., 2017)
13	BACT -1369F	Bakteri	Q-PCR - Forward primer	CGG TGA ATA CGT TCY CGG	(Vigneron vd., 2013; Suzuki vd., 2000)
14	BACT -1492R	Bakteri	Q-PCR - Reverse primer	GGW TAC CTT GTT ACG ACT T	(Vigneron vd., 2013; Suzuki vd., 2000)
15	SRB2 -649F	Sülfat Redükleyen Bakteri	Q-PCR - Forward primer	ACT TGA GTA CCG GAG AGG GA	(Timmers vd., 2015)
16	SRB2 -808R	Sülfat Redükleyen Bakteri	Q-PCR - Reverse primer	CCT AGT GCC CAT CGT TTA GG	(Timmers vd., 2015)

BULGULAR

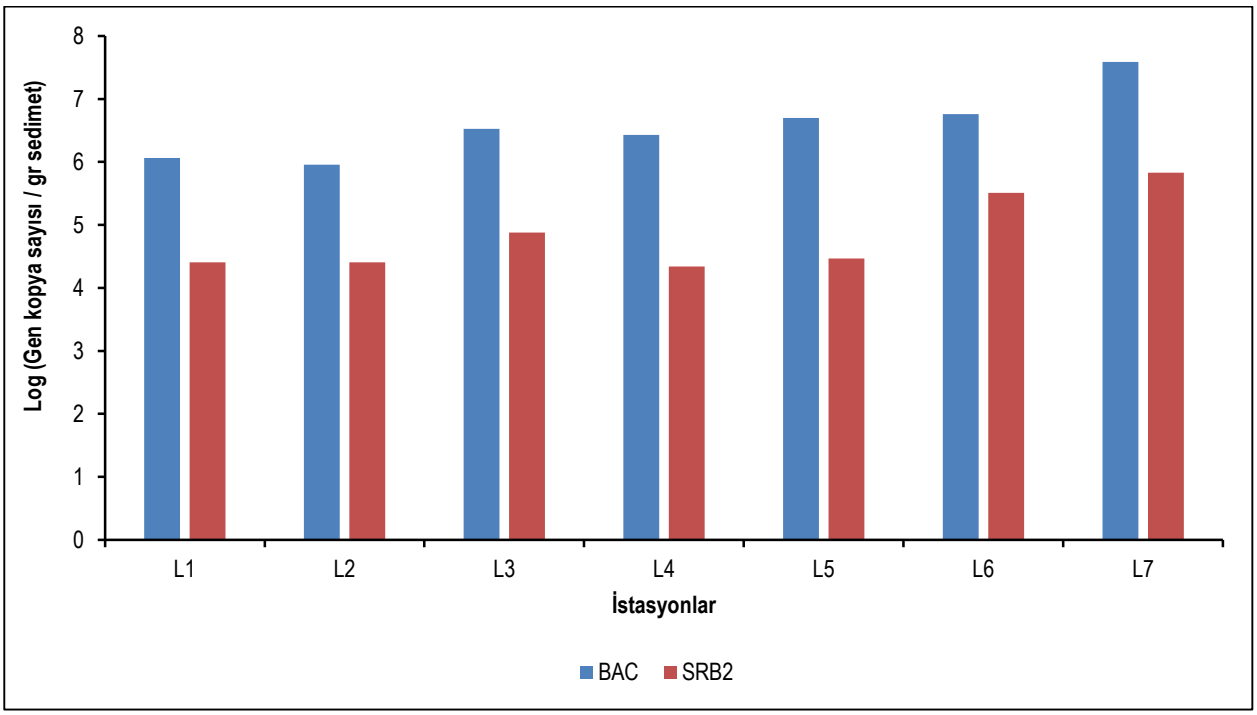
Çakalburnu Lagünü'nde belirlenen 7 istasyondan elde edilen sediment örneklerinden, ekstrakte edilen toplam genomik DNA'nın, Genel Arke (ARC), Metanojenik Arke (MCRA), Anaerobik Metan Oksidasyonu yapan Arke (ANME 1, ANME 2a, ANME 2c), Genel Bakteri (BAC) ve Sülfat İndirgeyen Bakteriler (SRB2)'in 16S rRNA genlerinin sayıca bolluğunun Real-time PCR analiz sonuçları Şekil 2, 3 ve 4 ile Tablo 2'de verilmiştir.

Tablo 2'de Çakalburnu Lagünü sedimentlerinde incelenen mikrobiyal bollukların değerleri belirtilmiştir. Bu değerlendirmeye göre incelenen arke ve bakteri bollukların maksimum değerleri sırasıyla $2,66x10^{10}$ gen kopya sayısı/gr ve $3,89x10^{7}$ gen kopya sayısı/gr olarak belirlenmiş olup, bu çalışmada lagün sedimentlerinde arkeal bolluğun yoğun

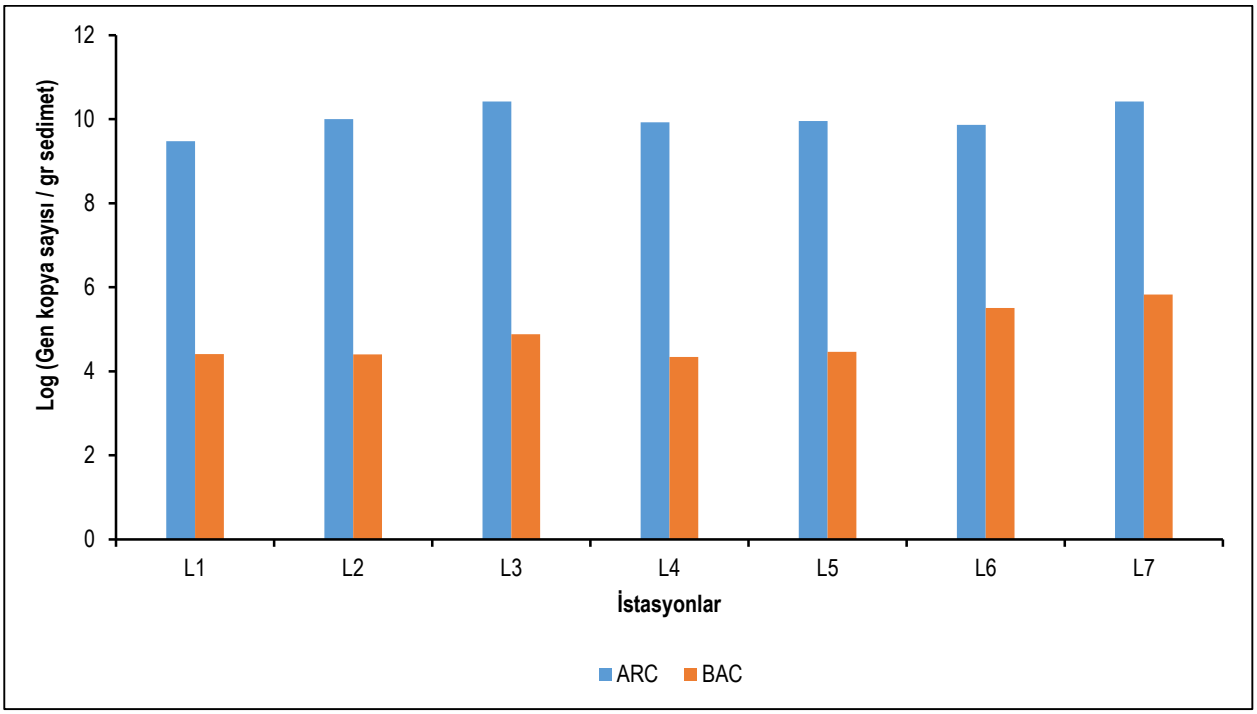
olduğu görülmüştür. ANME grupları kendi içinde değerlendirildiğinde, grupların bollukları arasında büyük bir fark gözlenmemiştir. Ayrıca incelenen mikrobiyal bollukta arkeal gruplar için minimum değer $4,06x10^3$ gen kopya sayısı/gr iken bakteriyal gruplar için bu sayı $2,18x10^4$ gen kopya sayısı/gr olarak tespit edilmiştir.



Şekil 2. Çakalburnu Lagünü sedimentlerinin arke bolluğu
Figure 2. Archeal abundance of Çakalburnu Lagoon sediments



Şekil 3. Çakalburnu Lagünü sedimentlerinin bakteriyal bolluğu
Figure 3. Bacterial abundance of Çakalburnu Lagoon sediments



Şekil 4. Çakalburnu Lagünü sedimentlerinin genel arke ve bakteri bolluğunun karşılaştırılması
Figure 4. The comparison of general archaea and bacterial abundance of Çakalburnu Lagoon sediments

TARTIŞMA VE SONUÇ

Elde edilen sonuçlara göre, sediment örnekleri arkeal ve bakteriyal bolluk açısından değerlendirildiğinde ARC ($2,66x10^{10}$ gen kopya sayısı/gr), MCRA ($1,60x10^{6}$ gen kopya sayısı/gr), BAC ($3,89x10^{7}$ gen kopya sayısı/gr) ve SRB2 ($6,75x10^{5}$ gen kopya sayısı/gr) grupları için en yüksek değerlere sahip olan istasyonun, L7 no'lu istasyon olduğu belirlenmiştir. Bu istasyon lagün içerisinde sucul bitkilerin yoğun olarak bulunduğu bir bölgede olup, sediment yapısı siyah renkli çamur şeklinde gözlenmiştir. Bu durumunda en yüksek anaerobik sediment yapısı bu bölgede tespit edilmiştir ve sucul bitkilerin varlığının sülfat redükleyen bakteriyel oluşumu ile desteklediği öngörülmektedir. ANME 2a ve ANME 1 grupları için en yüksek değerlerin $9,13x10^{4}$ gen kopya sayısı/gr ve $3,21x10^{5}$ gen kopya sayısı/gr olarak elde edildiği istasyonun L3, ANME 2c için ise $1,93x10^{5}$ gen kopya sayısı/gr ile L2 istasyonu olarak belirlenmiştir. L3 istasyonun lagünün dışında bulunan deniz sedimentlerinde, L2 istasyonun ise lagün ile deniz bağlantısında bulunması ve sediment yapılarının (kumlu-çakıllı) benzerlik göstermesi; ANME grubu organizmalar için denizel girdinin önemli olduğunu işaret etmektedir.

Ayrıca, elde edilen verilere değerlendirildiğinde; lagün sedimetleri genel arke ve genel bakteri açısından karşılaştırıldığında; Şekil 4'te de görülebileceği üzere mikrobiyal bolluk açısından Arke düzeyi, Bakterilerin yaklaşık 10^{4} katı üzerinde olup, sedimentteki baskın topluluğu oluşturmaktadır. Bu bakımdan lagün sedimentlerinin arkeal profilinin belirlenmesi oldukça önem taşımaktadır.

Tablo 2. Lagün sedimentlerindeki Real-time PCR sonuçlarının minimum ve maksimum değerleri
Table 2. The minimum and maximum values of Real-time PCR results from lagoon sediments

Primerler	Minimum (gen kopya sayısı/gr*)	Maksimum (gen kopya sayısı/gr*)	Ortalama (gen kopya sayısı/gr*)	Varyasyon Katsayısı
ARC	$2,97 \times 10^{9}$	$2,66 \times 10^{10}$	$1,29 \times 10^{10}$	73,08
MCRA	$7,70 \times 10^{3}$	$1,60 \times 10^{6}$	$2,58 \times 10^{5}$	229,30
ANME 2a	$4,06 \times 10^{3}$	$9,13 \times 10^{4}$	$4,19 \times 10^{4}$	76,66
ANME 2c	$2,90 \times 10^{4}$	$1,93 \times 10^{5}$	$9,45 \times 10^{4}$	64,57
ANME 1	$1,72 \times 10^{4}$	$3,21 \times 10^{5}$	$1,69 \times 10^{5}$	76,39
BAC	$9,05 \times 10^{5}$	$3,89 \times 10^{7}$	$8,24 \times 10^{6}$	165,29
SRB2	$2,18 \times 10^{4}$	$6,75 \times 10^{5}$	$1,68 \times 10^{5}$	148,13

* kuru ağırlık sediment

Torres-Alvarado vd. (2016), La Mancha Lagünü, Veracruz, Meksika Körfezi'nde 2016 yılında yaptığı benzer bir çalışmada, kuru ve yağışlı mevsimde metanojenik arke ve sülfat redükleyen bakterilerin sayıca bolluğunu, çevresel etmenler aracılığıyla incelemiştir. Sonuçlarında, kuru ve yağışlı mevsimlerde büyük değişiklikler tespit etmişlerdir. Sülfat redükleyen bakterilerin ve metanojenik arke, La Mancha Lagün sedimentlerinde organik maddenin mineralizasyonunda rol oynadığını, kuru mevsimde (kapalı giriş) sülfat redüklenmesi ve yağışlı mevsimde (açık giriş) ise metanojenezin ön plana çıktığını ortaya koymuşlardır. Bu lagündeki yağış ve nehir girdisindeki değişiklikler, sedimentlerdeki sülfat redükleyen bakterilerin ve metanojenik arke dinamiklerini düzenleyen ana faktörler olan tuzluluk ve sülfat içeriğini önemli ölçüde etkilediği yönünde görüş bildirmişlerdir. Bizim çalışmamızda yaz aylarında gerçekleştiğinden metanojenik arke ortalama bolluğu, sülfat redükleyen bakterilerin ortalamasından daha yüksek oluşu ile metanojenezin ön plana çıktığını görüşünü desteklemektedir.

Obi vd. 2016 yılında yaptıkları bir diğer çalışmada ise, Nijerya'da bulunan Lagos Lagünü sedimentlerinde mikrobiyal toplulukların belirlenmesi açısından incelenmiştir. Mikrobiyal topluluklar, Illumina sekanslama metoduna dayanarak 16S rRNA genleri ile araştırdıkları bu çalışmada, toplam 565 bakteriyel operasyonel taksonomik birim (OTU) (amplikonların % 97'si) ve 17 arkeal OTU (amplikonların % 3'ü) tanımlanmıştır. Apapa ve Eledu istasyonlarından alınan sedimentlerde mikrobiyal çeşitlilik (tür zenginliği) Ofin bölgesininkine kıyasla daha az olduğu ve her iki ikisinin de topluluklarına Helicobacteraceae (Epsilonproteobacteria) ailesine atanan tek bir OTU'nun hakim olduğunu tespit etmişlerdir. Ofin sedimentlerinde, Epsilonproteobacteria küçük bileşenleri kapsarken, büyük gruplar ise Apapa ve Eledu sedimentlerinde Siyanobakteriler, Bacteroidetes ve Firmicutes olarak belirlenmiştir. Çalışmamızda bu gruplar genel bakteri primeri kullanılarak değerlendirildiğinden OTU sekansları'ndaki gibi bir ayrım belirlenmemiştir. Ancak genel bakterilerin ortalama bolluğu 10^6 gen kopya sayısı/gr olarak tespit edilmiş ve bu oranın sedimentteki genel bakteriyel bolluğun da düşük olmayan düzeylerde olduğunu işaret etmektedir.

Tirez Lagünü'nde (İspanya), Montoya vd.'nin (2011) gerçekleştirdiği çalışma ile, su kolonundaki ve yüksek sülfatlı sedimentteki mevsimsel mikrobiyal çeşitlilik, hem moleküler hem de konvansiyonel mikrobiyolojik yöntemler kullanılarak rapor edilmiştir. Lagün sedimentinin, Alfa ve Deltaproteobacteria, Bacteroidetes, Firmicutes, Actinobacteria ve Arkeler açısından yüksek bir zenginlik gösterdiğini tespit etmişlerdir. Yine bizim çalışmamızda da arkeal ve bakteriyal bolluğun ve çeşitliliğin yüksek olduğu sonuçlar açısından bakıldığında, ortaya konmuştur.

Sonuç olarak, kıyısal bölgelerde, sedimentler biyojeokimyasal döngülerde önemli bir rol oynamaktadır (Pomeroy vd., 1965; Zeitzschell, 1980). Lagüner sedimentler detritik malzeme havuzu ve mineral besin kaynağı olarak hareket ederler (Lijklema, 1986). Sediment sistemlerinde sülfat redükleyen bakteriler ile metanojenik arkelerin biyokimyasal süreçlerdeki etkinlikleri de sıklıkla tespit edilmektedir (Montoya vd., 2011). Real-time PCR analizleri gerçekleştirilen fonksiyonel gen primerleri, Çakalburnu Lagünü sedimentlerinde bulunan mikrobiyal çeşitliliğinin bir tahminini sağlayabilmiştir. Çalışmamızdan elde ettiğimiz veriler ve literatürde gerçekleştirilen araştırmalarda da belirtildiği üzere lagün sedimentlerinde mikrobiyal çeşitliliğin tespiti, lagün ortamının daha detaylı aydınlatılması açısından büyük önem taşımaktadır. Ayrıca, lagün sedimentleri gibi hassas ekosistemlerde, ekolojik ve mikrobiyolojik dengenin korunması ile biyojeokimyasal döngülerin belirlenmesine yönelik çalışmalara sağlayacağı katkılar nedeniyle, bu ortamların düzenli (mevsimsel) olarak izleme programlarına dahil edilmesi önerilmektedir.

KAYNAKÇA

Al Amoudi, S. (2016). *Bioprospecting Sediments from Red Sea Coastal Lagoons for Microorganisms and Their Antimicrobial Potential*. King Abdullah University of Science and Technology Thuwal, Kingdom of Saudi Arabia, PhD thesis. DOI: 10.3390/md14090165

Behera, P., Mohapatra, M., Kim J.Y. & Rastogi, G. (2020). Benthic archaeal community structure and carbon metabolic profiling of heterotrophic microbial communities in brackish sediments. *Science of the Total Environment*, 706, 135709. DOI:10.1016/j.scitotenv.2019.135709

Caumette, P. (1986). Phototrophic sulfur bacteria and sulfate-reducing bacteria causing red waters in a shallow brackish coastal lagoon (Prevost Lagoon, France). *FEMS Microbiology Ecology*, 38, 113-124. DOI: 10.1016/0378-1097(86)90148-5

Esbah, H. (2004). Agricultural land loss due to urbanization. Proceedings of Agro-Environs, (pp. 231-238), Udine, Italy.

Glud, R.N. (2008). Oxygen dynamics of marine sediments. *Marine Biology Research*, 4, 243–289. DOI: 10.1080/17451000801888726

Jørgensen, B.B. & Bak, F. (1991). Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). *Applied and Environmental Microbiology*, 57, 847-856. DOI: 10.1128/AEM.57.3.847-856.1991

Kara, B. (2019). Agrarian and wetland areas under metropolitan threats: learning from the case of Inciralti, Izmir (Turkey*). Applied Ecology and Environmental Research* 17(6),15087-15102. DOI:10.15666/aeer/1706_1508715102

Li, W., Dou, Z., Cui, L., Zhao, X., Zhang, M., Zhang, Y., Gao, C., Yang, Z., Lei Y. & Pan X. (2020). Soil fauna diversity at different stages of reed restoration in a lakeshore wetland at Lake Taihu, China. *Ecosystem Health and Sustainability,* 6(1), 1722034. DOI:10.1080/20964129.2020.1722034

Lijklema, L. (1986). Phosphorus accumulation in sediments and internal loading. *Aquatic Ecology*, 20, 213-224. DOI:10.1007/BF02291164

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A. & Danovaro, R. (2003). Benthic microbial loop functioning in coastal lagoons: a comparative approach. *Oceanologica Acta*, 26, 27–38. DOI: 10.1016/S0399-1784(02)01227-6

Montoya, L., Lozada-Chavez, I., Amils, R., Rodriguez, N. & Marin, I. (2011). The sulfate-rich and extreme saline sediment of the ephemeral Tirez Lagoon: a biotope for acetoclastic sulfate-reducing bacteria and hydrogenotrophic methanogenic archaea. *International Journal of Microbiology*, 2011, 753758, 22. DOI:10.1155/2011/753758

Obi, C.C., Adebusoye, S.A., Ugoji, E.O., Ilori, M.O., Amund, O.O. & Hickey, W.J. (2016). Microbial Communities in Sediments of Lagos Lagoon, Nigeria: Elucidation of Community Structure and Potential Impacts of Contamination by Municipal and Industrial Wastes. *Frontiers in Microbiology*, 7, 1213. DOI: 10.3389/fmicb.2016.01213

Oueslati, W., Velde, S., Helali, M.A., Added, A., Aleya, L. & Meysman F.J.R. (2019). Carbon, iron and sulphur cycling in the sediments of a Mediterranean lagoon (Ghar El Melh, Tunisia). *Estuarine, Coastal and Shelf Science*, 221, 156–169. DOI: 10.1016/j.ecss.2019.03.008

Pomeroy, L.R., Smith, E.E. & Grant, C.M. (1965). The exchange of phosphate between estuarine water and sediments. *Limnology and Oceanography*, 10, 167-172. DOI: 10.4319/lo.1965.10.2.0167

Pusceddu, A., Serra, E., Sanna, O. & Fabiano, M. (1996*)*. Seasonal fluctuations in the nutritional value of particulate organic matter in a lagoon. *Chemistry and Ecology*, 12, 199–212. DOI: 10.1080/02757549608039082

Ramsar Convention Bureau. (1992). Ramsar Convention, Slimbridge, England.

Reindl A.R. & Bolałek, J. (2017). Biological factor controlling methane production in surface sediment in the Polish part of the Vistula Lagoon. *Oceanological and Hydrobiological Studies*, 46(2), 223-230. DOI:10.1515/ohs-2017-0022

Ribeiro, C., Ribeiro, A.R. & Tiritan, M.E. (2016). Occurrence of persistent organic pollutants in sediments and biota from Portugal versus European incidence: a critical overview. *Journal of Environmental Science and Health*, Part B, 51, 143–153. DOI: 10.1080/03601234.2015.1108793

Steinberg, L.M. & Regan, J.M. (2009). McrA-targeted real-time quantitative PCR method to examine methanogen communities. *Applied and Environmental Microbiology*, 75 (13), 4435–4442. DOI: 10.1128/AEM.02858-08

Suzuki, M.T., Taylor, L.T. & DeLong, E.F. (2000). Quantitative analysis of small-subunit rRNA genes in mixed 50- microbial populations via nuclease assays. *Applied and Environmental Microbiology*, 66, 4605–4614. DOI: 10.1128/AEM.66.11.4605-4614.2000

Takii, S. & Fukui, M. (1991). Relative importance of methanogenesis, sulfate reduction and denitrification in sediments of the lower Tama river. *Bulletin of the Japanese Society of Microbial Ecology*, 6, 1-8. DOI: 10.1264/microbes1986.6.9

Timmers, P.H.A., Gieteling, J., Widjaja-Greefkes, H.C.A., Plugge, C.M., Stams, A.J.M., Lens, P.N.L. & Meulepas, R.J.W. (2015). Growth of anaerobic methane-oxidizing archaea and sulfate- reducing bacteria in a high-pressure membrane capsule bioreactor. *Applied and Environmental Microbiology*, 82(4), 1286-1296. DOI: 10.1128/AEM.03255-14

Timmers, H.A., Welte, C.U., Koehorst, J.J., Plugge, C.M., Jetten, M.S.M. & Stams A.J.M. (2017). Reverse Methanogenesis and Respiration in Methanotrophic Archaea *Peer. Archaea*, 2017, 1654237, 22. DOI:10.1155/2017/1654237

Torres-Alvarado, M.R. Calva-Benítez, L.G., Álvarez-Hernández, S. & Trejo-Aguilar, G. (2016). Anaerobic microbiota: spatial-temporal changes in the sediment of a tropical coastal lagoon with ephemeral inlet in the Gulf of Mexico. *International Journal of Tropical Biology*, 64(4), 1759-1770. DOI: 10.15517/rbt.v64i4.22449

Vigneron, A., Cruaud, P., Pignet, P., Caprais, J.C., Cambon-Bonavita, M.A., Godfroy, A. & Laurent, T. (2013). Archaeal and anaerobic methane oxidizer communities in the Sonora Margin cold seeps, Guaymas Basin (Gulf of California*). ISME Journal*, 7, 1595–1608. DOI: 10.1038/ismej.2013.18

Yin, H. (2003). The Thoughts of Wetland Conservation in China. *Wetland Science*, 1, 68–72.

Yu, Y., Lee, C., Kim, J. & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnololgy and Bioengineering*, 89(6), 670-679. DOI:10.1002/bit.20347

Yucel-Gier, G., Kacar, A., Gonul, L.T., Pazi, I., Kucuksezgin, F., Erarslanoglu, N. & Toker, S.K. (2018). Evaluation of the relationship of picoplankton and viruses to environmental variables in a lagoon system (Çakalburnu Lagoon, Turkey). *Chemistry and Ecology*, 34 (3), 211–228. DOI: 10.1080/02757540.2018.1427230

Zeitzschell, B. (1980). Sediment-water interaction in nutrient dynamics. In: Tenore, K.R., & Coull, B.C. (Edit.), Marine Benthic Dynamics. University of South Carolina, Columbia., pp 195-218

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Feeding ecology of portunid crab, *Carcinus aestuarii* Nardo, 1847 in Çardak Lagoon (The Turkish Straits System)

Çardak Lagünü (Türk Boğazlar Sistemi)'nde bulunan portunid yengeç, *Carcinus aestuarii* Nardo,1847'nin beslenme ekolojisi

Seçil Acar[1*] ● A. Suat Ateş[2]

[1] Çanakkale Onsekiz Mart University Faculty of Marine Sciences and Technology 17100 Çanakkale, Turkey https://orcid.org/0000-0002-6426-8095
[2] Çanakkale Onsekiz Mart University Faculty of Marine Sciences and Technology 17100 Çanakkale, Turkey https://orcid.org/0000-0002-4682-1926

*Corresponding author: secilkolsal@gmail.com

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Abstract: The aim of the present study was to determine the feeding ecology of portunid crab, *Carcinus aestuarii* in Çardak Lagoon. For this purpose, a total of 533 crab stomachs were analyzed. Stomach contents were examined under a binocular stereomicroscope and 240 (45%) of the stomach samples were recorded as full (containing at least one food item) and 293 (55%) were recorded as empty. The occupancy rates were 25% in 127 stomachs, 50% in 69, and 100% of 44. The main diet of crab individuals was composed of fragments of fish species. Diatoms were the least consumed food by crabs. A total of 8494 diet fragments were found in the stomachs. Total stomach content was 26.39 g of fish vertebrates having the highest weight (3.58 g).

Keywords: *Carcinus aestuarii,* diet, lagoon ecosystem, Turkish Straits System

Öz: Bu çalışmanın amacı Çardak Lagünü'ndeki portunid yengeç *Carcinus aestuarii*'nin beslenme ekolojisini belirlemektir. Bu amaçla toplam 533 yengeç midesi analiz edilmiştir. Mide içerikleri binoküler mikroskop altında incelenmiş ve 240 (%45) mide örneği dolu (en az bir besin maddesi içeren) ve 293'ü (%55) boş olarak kaydedilmiştir. Doluluk oranları 127 midede %25, 69'unda %50 ve 44'ünde %100'dür. Yengeç bireylerinin ana besin maddesini balık türlerinin parçaları oluşturmuştur. Diatomlar yengeçler tarafından en az tüketilen besindir. Midede toplamda 8494 besin parçası bulunmuştur. Balık omurları en yüksek ağırlığa (3.58 g) sahip olarak toplam mide içeriği 26.39 g'dır.

Anahtar kelimeler: *Carcinus aestuarii,* diyet, lagün ekosistemi, Türk Boğazlar Sistemi

INTRODUCTION

Portunid crab, *Carcinus aestuarii* lives in estuaries and lagoon areas of the Mediterranean Ecosystem. Populations of *Carcinus aestuarii* can tolerate various environmental conditions (Abelló et al., 1997; Aydin, 2013). *Carcinus aestuarii* has both an omnivore and predator character with a high survival rate. *C. aestuarii*'s diets depend on local species in the tidal zones (Yamada and Hauck, 2001). These local species interact with benthos and may have a negative impact on the environment where they live.

Crustaceans are known to be important representatives of the trophic chain in many marine ecosystems. Thus, *C. aestuarii* is the main diet for commercial fish such as eel, sea bass, and sea bream in lagoon systems. They also migrate between the lagoons on the Mediterranean coast to feed. *C. aestuarii* is one of the potential species known as a biological indicator which can easily adapt to estuarine and lagoon areas (Mori et al., 1990). Additionally, it is the most common species of decapod crustaceans in the tidal zone, spreading from the shallow coastal zone to a depth of 60 m. This species is recorded in the estuaries and zones with low salinity as well (Lyons et al., 2012). Crab species include filter feeders, sand cleansers, mud, plant, and carrion feeders, predators, commensals, and parasites (Dall and Moriarty, 1983). Thus, stomach content analysis provides the most accurate information about the general diets of these species (Williams, 1981). Population dynamics and several biological variables of *C. aestuarii* were recently described from the coastal lagoons in Turkey (Can et al., 2004; Özcan et al., 2009; Koçak et al., 2011; Özbek et al., 2012), Tunisia (Baklouti et al., 2013), and Italy (Mori et al., 1990; Matozzo et al., 2011; Lumare et al., 2009; Cilenti et al., 2014), in Mediterranean ecosystems. *C. aestuarii* tolerating a wide range of physico-chemical variables is thought to be especially effective on benthic communities with their nutritional preferences. Therefore, the purpose of this study was to understand the feeding ecology of *C. aestuarii* in the Çardak Lagoon.

MATERIAL AND METHODS

The study was carried out in Çardak Lagoon which is a part of the Turkish Straits System. Samplings were carried out monthly between April 2015 and March 2016 by means of a static traditional trap used for the eel fishery. The traps were deployed during a 48-h period between the depths of 1.5 and 2 m from 6 different points in Çardak Lagoon (Figure 1).

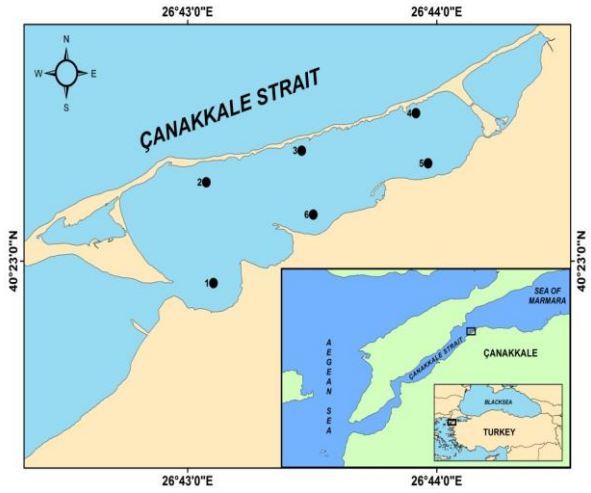

Figure 1. The map of the study area

After the crab samples were transferred to the laboratory, crab specimens were dissected and their stomachs were separated. The weights of the stomachs were measured on a precision scale of ± 0.001gr and then stored in Eppendorf tubes containing 70% ethanol. Stomach contents were examined under an OLYMPUS Brand SZX7 Model binocular stereomicroscope. Gastric occupancy rates were divided into 4 groups and were classified as empty (0%), low-filled (25%), half-filled (50%), and full-filled (100%). Relative abundance (F%), frequency of occurrence (N%), volumetric occupancy (V%) and IRI (Index of Relative Importance) values and vacuity index (Vacuity Index, VI) were also calculated. Chi-square ($\chi 2$) test was used to analyze the changes in Vacuity index by months. SIMPER and ANOSIM analysis were performed to determine the diet differences by month and sex. Statistical analysis were carried out using PAST 4.03.

IRI=(N+V)*F;

VI=Total of Empty stomach *100/ Total number of stomach

RESULTS

A total of 553 individuals were found in the study area between April 2015 and March 2016. The average length of carapace in crabs ranged between 23.36-73.61 mm. While the minimum carapace length in females was 23.36 mm, the maximum was 56.29 mm. In male crabs, the minimum carapace length was 26.2 mm and the maximum length was 73.61 mm (Figure 2). A total of 195 stomachs (115 empty and 80 full) in females and 338 stomachs in males were evaluated.

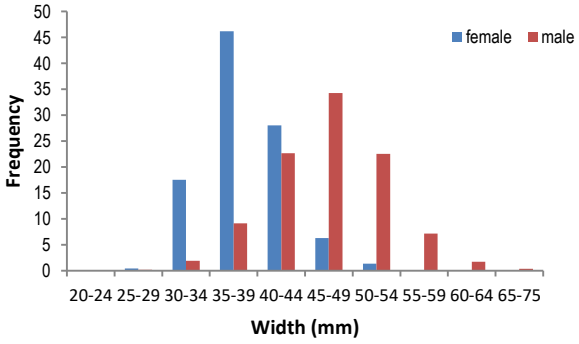

Figure 2. Proportional (%) distributions of phytoplankton groups in abundance basis according to seasons

Of the analyzed stomachs, 179 were empty and 159 were completely full. No diet fragments were found in 293 of the stomachs examined. The occupancy rates were 25%, 50% and 100% in 127, 69 and 44 of the crab stomachs, respectively. Main diet of the crab individuals was composed of fish fragments while the diatoms were the least consumed. A total of 8494 diet fragments were counted in all stomachs. Total weight of the stomach content was 26.39 g and 3.58 g of the total weight was composed of fish vertebrates. Additionally, fish vertebrates were observed in 171 of the stomachs. Besides the fish vertebrates, detritus was also a common food group with a total weight of 3.45 g in the stomach content (Table 1; Figure 3).

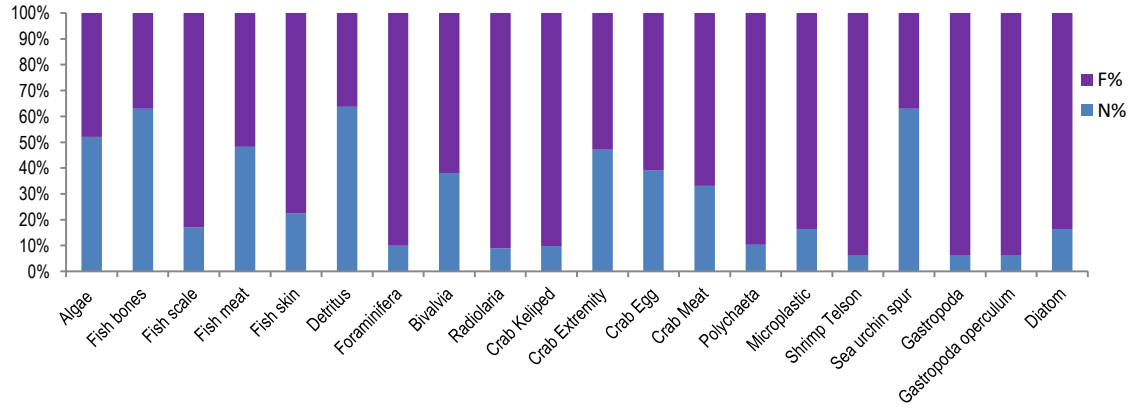

Figure 3. N% and F% values of stomach content

Table 1. Stomach content of *C. aestuarii* (N%, F% and IRI values)

Feeding Type Diet fragments	N%	F%	V%	IRI	IRI%
Photophilic algae	11.91	10.99	3.36	167.90	5.94
Fish bones	52.35	30.81	9.63	1909.72	67.60
Fish scale	3.04	14.77	7.13	150.17	5.32
Fish meat	16.25	17.48	8.74	436.71	15.46
Fish skin	0.62	2.16	4.30	10.65	0.38
Detritus	6.65	3.78	9.28	60.27	2.13
Foraminifera	0.26	2.34	1.21	3.44	0.12
Bivalvia	3.21	5.23	4.98	42.79	1.51
Radiolaria	0.11	1.08	0.54	0.70	0.02
Crab cheliped	0.29	2.70	6.05	17.15	0.61
Crab extremities	1.28	1.44	4.98	9.02	0.32
Crab egg	0.58	0.90	1.56	1.92	0.07
Crab meat	0.62	1.26	1.75	2.99	0.11
Polychaetes	0.08	0.72	0.40	0.35	0.01
Microplastic	0.11	0.54	0.97	0.58	0.02
Shrimp telson	0.04	0.54	0.67	0.38	0.01
Sea urchin spur	2.45	1.44	4.20	9.58	0.34
Gastropod	0.05	0.72	0.65	0.50	0.02
Gastropod operculum	0.06	0.90	0.27	0.30	0.01
Diatom	0.04	0.18	0.32	0.06	0,00

N%: Numerical percentage of food types, F%: Frequency percentage, V%: Weight percentage, IRI: Index of relative importance

The relationships between diets and amounts of diet by months were analyzed by t-test. The difference between the diet levels by months was statistically significant (p=0.000; p<0.05). Although main diets for both males and females were the fish meat and the vertebrates, fragments of photophilic algae were also found as important food sources. Generally being more aggressive cannibalism was observed in females as well. For example, eggs of other female individuals were found in the stomachs of several female individuals. The relationship between male and female diets was analyzed by a chi-square test and the relationship was found to be statistically significant (p=0.002; p<0.05) (Table 2)

Table 2. Values of diversity index belonging to stomach content by months

	April	May	June	July	August	September	October	November	December	January	February	March
Number of content types	7	7	8	7	4	7	4	9	10	10	17	17
Number of content	200	526	459	178	99	250	513	901	1300	1301	976	1791
Dominance (d)	0.29	0.389	0.465	0.547	0.636	0.338	0.421	0.259	0.302	0.354	0.271	0.405
Shannon_H'	1.465	1.137	1.022	0.952	0.745	1.387	0.954	1.488	1.549	1.393	1.773	1.443
Eveness	0.618	0.445	0.347	0.37	0.526	0.571	0.649	0.492	0.47	0.402	0.346	0.248

While the highest value (H' = 1.77) of diet diversity index was recorded in February and November (H' = 1.48) the lowest diet index value (H' = 0.95) was recorded in August. The highest amount of diet (number of individuals = 1791) was found in March. Shannon-weaver index showed that variety and amount of diet were higher in females than in males (H'=1.69) (Table 3).

ANOSIM analysis was used to determine the difference of feeding activity by months. The result of ANOSIM analysis showed that the type of the diet was important in different months (R=0.148; p<0.05). The most important contribution to diet depending on the prey groups were found to be fish vertebrates (43.18%) and fragments of photophilic algae (15.67%). Additionally, fish meat, detritus, and mussel fragments were the groups that contribute to diets the most. When the average abundances were examined according to the months, depending on the ratio of the fish vertebrate the highest values were observed in July (71.9%) and August (78.8%). Changes in IRI (%) values by months were calculated with Spearman Rank Correlation (rs). The relationship between % IRI values by months (rs = 0.214; p <0.05) was found to be statistically significant.

Fish vertebrates and fragments in crab stomachs were observed throughout the year. The highest consumed fragments of photophilic algae fragments were also found all through the year except the gastric samples obtained in August. Fragments of sea urchins were found mostly in stomach samples belonging to March, February and November (Figure 4).

Table 3. Values of diversity index belonging to stomach content by sex

	Female (♀)	Male (♂)
Number of content types	17	20
Number of content	2195	6299
Dominance	0.287	0.335
Shannon_H'	1.696	1.54
Eveness	0.32	0.233
Margalef	2.08	2.17

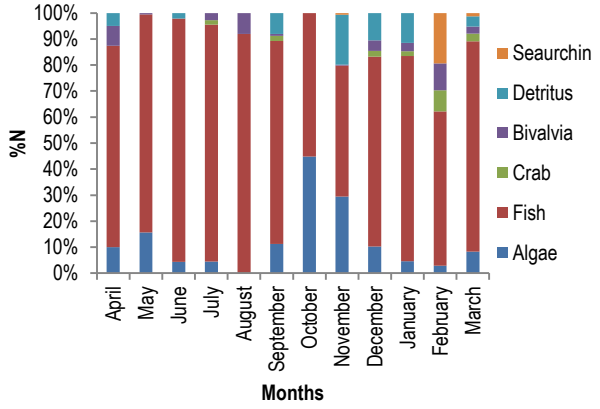

Figure 4. Diets in the stomachs of *C. aestuarii* specimens

DISCUSSION

Having information about the diets of a species in nature is very important in terms of understanding its feeding preferences and the relationships between the animal groups (Josileen, 2011). Although decapod crustaceans feed on macroscopic organisms, identification of the various food items as well as a reliable estimate of their (relative) quantity, are both very difficult (Josileen, 2011). The crabs use their mouthparts to cut the food into small pieces and then the gastric mill ossicles further reduce the food to unidentifiable fragments. Most researchers use the foregut contents to study the quantity and nature of the different diets consumed by crab species (Sukumaran and Neelakandan, 1997; Williams, 1981; Chande and Mgaya, 2003, 2004; Josileen, 2011). Adults of *C. aestuarii* individuals have an omnivore-predator feeding type, however, they are aggressively competitive. Crab species prefer mostly annelids, gastropods, mussels, fishes, photophilic algae and other crustaceans (e.i. shrimp, crab). Also, the cannibalism observed in this species is very important (Leignel et al., 2014). In this study, it was concluded that the behavior of cannibalism is high, especially in female individuals. For example, it has been determined in the stomach content that females consume crab eggs and limbs. On the other hand, one of the most important food sources of crab individuals is mussels. Mussel species along with their diets also vary according to the endemic/local species in the area where they live (Yamada and Hauck, 2001, Chen et al., 2004; Tepolt and Somero, 2014; Aydin, 2013). Baeta et al. (2006), studied the diet ecology of *C. maenas* in Mondego estuarine area (Portuguese coast). According to Baeta et al. (2006) shrimp, polychaete, and fish species are the most important diets of *C. maenas*. Recently, Chaves et al. (2010) analyzed, the feeding pattern of C. maenas in the same area and stated that *C. maenas* prefers predominantly the bivalves, polychaeta and photophilic algae fragments. Diet ecology of the Japanese population of the Mediterranean endemic *C. aestuarii* was also addressed by Chen et al. (2004). Chen et al. (2004) stated that individuals of *C. aestuarii* consumed 87 various fragments of peracarid and eucarid crustaceans (including cirriped, amphipod, decapod) and mostly bivalve species. Chen et al. (2004) indicated that Mediterranean mussel, *Mytilus galloprovincialis*, has been identified as a species commonly consumed by individuals of *C. aestuarii* (66.4%). Percentage of vegetative material in *C. aestuarii* stomachs is very low (1.9%) and both sex have no significant effect on diets ($p = 0.56$; $p > 0.05$). Yet, *C. aestuarii* was previously reported to be opportunistic and omnivorous (Chen et al., 2004). In Çardak Lagoon, the diets preferred by crabs were mostly fish species such as sea bream and goby species (IRI(%) = 52.35). Diatom and shrimp fragments were rarely observed in the stomach contents of crabs (IRI(%)= 0.04). Sex difference seemed to be effective on the crabs' diets in the study area ($p = 0.002$; $p < 0.05$). Additionally, crab specimens in the lagoon area showed similar feeding strategy as omnivorous or opportunistic type.

Changes in the diets of *C. aestuarii* with respect to months and localities were also associated with availability of food sources. As indicated in previous studies, crabs pursued different hunting strategies throughout the year and this behavior was realized for energy expenditure, growth and reproduction as well as for regeneration of shell with $CaCO_3$ (Rosas et al., 1994; Mantelatto and Fransozo, 1999; Jewett and Feder, 1982; Feder and Keiser, 1980; Chen et al, 2004). Therefore, mussels and crustaceans in shallow water have created an important part of the dietary component throughout the year since they contain abundant sources of $CaCO_3$.

Being opportunistic omnivores, Portunidae crabs rarely include motile forms such as fish and shrimp species in their diets (Ropes, 1968; Patel et al., 1979; Williams, 1982; Le Roux et al., 1990). In addition, it is known that omnivore feeding style minimizes the dependence of the animal on a specific type of food source and increases the variety of feeding (Chen et al., 2004). Although, *C. aestuarii* individuals also have a largely opportunistic omnivore diet as with other Portunidae crabs, they are more selective about their food preferences. In the present study conducted in Çardak Lagoon, it has been observed that the nutritional preferences of crabs were largely omnivorous alongside with mobile species such as fish. Thus, it can be concluded that especially with this type of diet, a negative effect on benthic fauna and flora can be expected. In addition, this study supported other studies conducted on nutrition ecology in similar aquatic systems.

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REFERENCES

Abelló, P., Aagaard, A., Warman, C.G. & Depledge, M.H. (1997). Spatial variability in the population structure of the shore crab *Carcinus maenas* (Crustacea, Brachyura) in shallow water tidal fjord. *Marine Ecology Progress Series*, 147, 97-103 DOI:10.3354/meps147097

Aydin, M. (2013). Length-Weight relationship and reproductive features of the Mediterranean Green Crab, *Carcinus aestuarii* Nardo, 1847 (Decapoda: Brachyura) in the Eastern Black Sea, Turkey. *Pakistan Journal of Zoology*, 45(6), 1615-1622. DOI: 0030-9923/2013/0006-1615

Baeta, A., Cabral, H.N., Marques, J.C. & Pardal, M.A. (2006). Feeding ecology of the green crab, *Carcinus maenas* (L., 1758) in a temperate estuary, Portugal. *Crustaceana*, 79(10), 1181-1193. DOI:10.1163/156854006778859506

Baklouti, S., Derbali, A., Dhieb, K. & Jarboui, O. (2013). Reproductive biology of the Mediterranean green crab, *Carcinus aestuarii* Nardo, 1847 (Crustacea: Portunidae), in the Gulf of Gabes (Tunisia, Central Mediterranean). *Cahiers de Biologie Marine*, 54, 411–417 DOI:10.21411/CBM.A.2E5C5488

Can E., Tıraşın, E.M. & Cihangir, B. (2004). Çakalburnu Dalyanı'nda *Carcinus aestuarii,* (Nardo, 1847) türü üzerine gözlemler. *Türk Sucul Yaşam Dergisi*, 2(3), 77-84

Chande, A.I. & Mgaya, Y.D. (2003). The fishery of *Portunus pelagicus* and the species diversity of portunid crabs along the coast of Dar es Salaam. Western *Indian Ocean Journal of Marine Science*, 2, 75-84. DOI:10.4314/wiojms.v2i1.28431

Chande, A.I. & Mgaya, Y.D. (2004). Food habits of the blue swimming crab *Portunus pelagicus* along the Coast of Dar es Salaam, Tanzania. *Western Indian Ocean Journal of Marine Science*, 3,37-42.

Chaves, M.L., Horta, M.S., Chainho, P., Costa, M.J. & Costa, J.L. (2010). New additions to the feeding ecology of *Carcinus maenas* (L., 1758) in a South-western Europe estuary (Portugal). *Cahiers de Biology Marine*, 51, 229-238 DOI: 10.21411/CBM.A.86A0CE1C

Chen, R.B., Watanabe, S. & Yokota, M. (2004). Feeding habits of an exotic species, the Mediterranean green crab *Carcinus aestuarii*, in Tokyo Bay. *Fisheries Science*, 70, 430–435 DOI: 10.1111/j.1444-2906.2004.00822.x

Cilenti, L., D'Errico, G., Scirocco, T., Manzo, C. & Fabbrocini, A. (2014). Spatial variability in the population structure of the *Carcinus aestuarii* in Varano lagoon. *Transitional Waters Bulletin*, 8 (1), 24-31. DOI: 10.1285/i1825229Xv8n1p24

Dall, W. & Moriarty, D.J.W. (1983). Functional aspects of nutrition and digestion. In: Bliss D.E. & Mantel L.H. (eds.), The biology of Crustacea, 5, Internal anatomy and physiological regulation: 255-261. (Academic Press, New York). DOI: 10.1016/B978-0-12-106405-1.50015-1

Feder, H. M., & Keiser, G. E. (1980). Intertidal biology. Port Valdez, Alaska: Environmental Studies 1976-1979, Chapter 8. *Occasional Publication*, (5), 1-373.

Jewett, S.C. & Feder, H.M. (1982). Food and feeding habits of the King Crab *Paralithodes camtschatica* near Kodiak Island. Alaska *Marine Biology*, 66, 243–250. DOI: 10.1007/BF00397029

Josileen, J. (2011). Food and feeding of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura) along the coast of mandapam, Tamil Nadu, India. *Crustaceana*, 84(10), 1169-1180. DOI:10.1163/001121611X590111

Koçak, C., Acarlı, D., Katağan, T. & Özbek, M. (2011). Morphometric characters of the Mediterranean green crab (*Carcinus aestuarii* Nardo, 1847) (Decapoda, Brachyura), in Homa Lagoon, Turkey. *Turkish Journal of Zoology,* 35 (4), 551-557 DOI:10.3906/zoo-0903-5

Leignel, V., Stilmann, J.H., Baringou, S., Thabet, R. & Metais, I. (2014). Overview on the European green crab *Carcinus spp*. (Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. *Environmental Science Pollution Research*, 21, 4929-9144. DOI: 10.1007/s11356-014-2979-4

Le Roux, P.J., Branch G.M. & Joska M.A.P. (1990). On the distribution, diet and possible impact of the invasive European shore crab *Carcinus maenas* (L.) along the South African Coast. *South African Journal of Marine Science*, 9, 85–93 DOI:10.2989/025776190784378835

Lumare, L., Lumare, D., Florio, M., Scirocco, T. & Lumare F. (2009). I crostacei decapodi del lago di acquatina: struttura delle popolazioni e ciclo riproduttivo. *Thalassia Salentina*, 31, 83- 102. DOI:10.1285/i15910725v31supp83

Lyons, L.J., O'riordan, R.M., Cross, T.F. & Culloty, S.C. (2012). Reproductive biology of the shore crab *Carcinus maenas* (Decapoda, Portunidae): a macroscopic and histological view. *Invertebrate Reproduction & Development*, 56 (2), 144-156. DOI:10.1080/07924259.2011.582693

Mantelatto, F.L.M. & Fransozo, A. (1999). Reproductive biology and moulting cycle of the crab *Callinectes ornatus* (Decapoda, Portunidae) from the Ubatuba region, Sao Paulo, Brazil. *Crustaceana*, 72, 63–76. DOI:10.1163/156854099502871

Matozzo, V., Gallo, C. & Marin, M.G. (2011). Can starvation influence cellular and biochemical parameters to the crab *Carcinus aestuarii*? *Marine Environmental Research*, 71, 207–212. DOI:10.1016/j.marenvres.2011.01.004

Mori, M., Mancon, R. & Fanciulli, G. (1990). Notes on the reproductive biology of *Carcinus aestuarii* Nardo (Crustacea, Decapoda) from the lagoon of San Teodoro (Island of Sardinia, Italy). *Rivista di Idrobiologia*, 29, 763–774.

Özbek, M., Koçak, C. & Acarlı, D. (2012). Reproductive biology of the Mediterranean Green Crab *Carcinus aestuarii* Nardo, 1847 (Crustacea, Brachyura, Portunidae) in Homa Lagoon, Aegean Sea, Turkey. *Oceanological and Hydrobiological Studies*, 41(4), 77-80 DOI:10.2478/s13545-012-0041-z

Özcan,T., Bakır, K. & Katağan, T. (2009). Length/width-weight relationships of the Mediterranean green crab *Carcinus aestuarii* Nardo, 1847 in the Homa Lagoon, Aegean Sea Turkey. *Journal of Fisheries Science.com*, 3, 1-4. DOI: 10.3153/jfscom.2009001

Patel, N.M., Chhaya, N.D. & Bhaskaran, M. (1979). Stomach contents of *Portunus pelagicus* (Linn.) from AD net catches. *Indian Journal of Marine Science*, 8, 48-49.

Ropes, J.W. (1968). The feeding habits of the green crab *Carcinus maenas*. *Fisheries Bulletin*, 67, 183-203.

Rosas, C., Lazaro-Chaves, E. & Buckle-Ramirez, F. (1994). Feeding habits and food niche segregation of *Callinectes sapidus*, *C. rathbunae*, and *C. similis* in a subtropical coastal lagoon of the Gulf of Mexico. *Journal of Crustacean* Biology, 14, 371–382 DOI:10.2307/1548915

Sukumaran, K.K. & Neelakantan, B. (1997). Food and feeding of *Portunus* (*Portunus) sanguinolentus (*Herbst) and *Portunus (Portunus) pelagicus* (Linnaeus) (Brachyura: Portunidae) along the Karnataka coast. Indian *Journal of Marine Science*, 26(1), 35-38.

Tepolt, C.K. & Somero, G.N. (2014). Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. *Journal of Experimental Biology*, 217, 1129–1138 DOI:10.1242/jeb.093849

Williams, M.J. (1981). Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *Journal of Experimental Marine Biology and Ecology,* 52, 103-113. DOI: 10.1016/0022-0981(81)90174-X

Williams, M.J. (1982). Natural food and feeding in the commercial sand crab *Portunus pelagicus* Linnaeus, 1766 (Crustacea: Decapoda: Portunidae) in the Moreton Bay, Queensland. *Journal of Experimental Marine Biology and Ecology*, 59, 165-176. DOI: 10.1016/0022-0981(82)90113-7

Yamada, S.B. & Hauck, L. (2001). Field identification of the European green crab species. *Carcinus maenas* and *Carcinus aestuarii Journal of Shellfish Research*, 20(3), 905-912.

http://www.egejfas.org
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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Heavy metal and Al bioaccumulation in the anemone *Actinia equina* Linnaeus, 1758 (Cnidaria: Actiniidae) from İskenderun Bay, North-Eastern Mediterranean, Turkey

İskenderun Körfezi (Kuzeydoğu Akdeniz, Türkiye) anemonu *Actinia equina* Linnaeus, 1758 (Cnidaria: Actiniidae)'da ağır metal ve Al birikimi

Önder Duysak[1]* ● Yavuz Mazlum[2] ● Erkan Uğurlu[3]

[1] İskenderun Technical University, Faculty of Marine Sciences and Technology, 31200 İskenderun, Hatay, Turkey https://orcid.org/0000-0002-7484-3102
[2] İskenderun Technical University, Faculty of Marine Sciences and Technology, 31200 İskenderun, Hatay, Turkey https://orcid.org/0000-0002-9547-0966
[3] İskenderun Technical University, Faculty of Marine Sciences and Technology, 31200 İskenderun, Hatay, Turkey https://orcid.org/0000-0001-8940-8421

*Corresponding author: onder.duysak@iste.edu.tr
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Abstract: The purpose of this study was to determine the accumulation levels of ten metals (Fe, Zn, Cd, Cu, Co, Ni, Al, Mn, Pb, and Cr) in the muscle tissues of *Actina equina* indvduals. A total of 120 individuals of *A. equina* were collected at six different stations in İskenderun Bay in spring 2013. The accumulation levels of heavy metals in the tissues were found to vary significantly among stations. The mean concentrations of Fe were the highest at Samandağ station (105.11 ± 74.28 mg/kg) whereas the lowest average value of Co (0.84 ± 0.10 mg/kg) was obtained at Dörtyol station. Heavy metal concentratıons in muscle tissue of *A. equina* were ordered as Fe>Zn>Mn>Al>Cr>Cu>Pb>Ni>Cd>Co, respectively. This study is the first detailed bioaccumulation study conducted with *A. equina* in İskenderun Bay.

Keywords: *Actinia equina*, heavy metal, İskenderun Bay, North-Eastern Mediterranean

Öz: Bu çalışmanın amacı, *Actinia equina* bireylerinin kas dokularında on metalin (Fe, Zn, Cd, Cu, Co, Ni, Al, Mn, Pb ve Cr) birikim düzeylerini belirlemektir. 2013 ilkbaharında İskenderun Körfezi'nde altı farklı istasyonda toplam 120 *A. equina* birey toplanmıştır. Dokulardaki ağır metal birikim seviyeleri istasyonlar arasında önemli farklılıklar göstermiştir. Ortalama Fe konsantrasyonu en yüksek Samandağ istasyonunda (105,11 ± 74,28 mg / kg), en düşük ortalama Co (0,84 ± 0,10 mg / kg) Dörtyol istasyonundan elde edilmiştir. *A. equina* kas dokusundaki ağır metal konsantrasyonları sırasıyla Fe> Zn> Mn> Al> Cr> Cu> Pb> Ni> Cd> Co şeklinde bulundu. Bu çalışma, İskenderun Körfezi'nde *A. equina* ile yapılan ilk ayrıntılı biyoakümülasyon çalışmasıdır.

Anahtar kelimeler: *Actinia equina,* ağır metal, İskenderun Körfezi, Kuzeydoğu Akdeniz

INTRODUCTION

Today, there is a continuous increase in the world population and intensive industrial development. This rapid growth gradually causes industrialization and population growth and consequently environmental pollution. Heavy metals, as one of the pollutants that are dangerous for marine environments and can harm human health, reach the sea as a result of various processes and cycles, and settle on the sea bottom. Sources of heavy metals that enter or exit in the marine environment are of natural or artificial origin. Natural contamination can be caused by rivers and erosions, as well as by volcanic movements on the seabed and atmospheric convection. The concentrations of artificially originated heavy metals in marine environments increase today as a result of industrial activities, agricultural activities, mining, refinery facilities, excessive consumption of fossil fuels, use of metal products in agriculture, and anthropogenic activities such as sea transportation (Anonymous, 2008).

İskenderun Bay is located in the eastern part of the Northeastern Mediterranean Sea and has an average depth of approximately 70 m when it has richer resources than other regions (Kosswing, 1953). İskenderun Bay is characterized having dense industrial establishments (iron steel factory, petrochemical industry, fertilizer industry, etc.), fishing, transportation, and urbanization. Due to its in and outer currents systems, pollutants resulting from above activities are spreading into bay and they lead it having a potential risk of pollution (Can et al., 2019). Also, in addition to the agricultural pollutants carried by the Seyhan and Ceyhan rivers, the increasing domestic wastes in the recreation areas in the summer season when the population doubles, thus increasing the pollution load (Anonymous, 1997; Duysak and Azdural, 2017).

The use of biological indicators to assess levels of pollutants especially trace metals in marine coastal ecosystems is very common nowadays. Marine organisms that receive dissolved and particulate metals can be used as an indicator of the bioavailability of a particular pollutant over time (Phillips and Segar, 1986; Rainbow and Phillips, 1993; Volterra and Conti, 2000). The animals living in aquatic areas

accumulate heavy metals 1.000–10.000 times more in their bodies when their living medium, the density of the water, and the nutrition chain are considered (Ekici and Yarsan, 2009). In temperate coastal communities, anemones are often the prominent members of the fauna. Actinia-type sea anemones are small-sized creatures and form large populations in a particular region (Sole-Cava and Thorpe, 1992). Members of this species are opportunistic omnivorous suspension feeders (Ormond and Caldwell, 1982). Since they have relatively short tentacles, they cannot actively search for prey and therefore feed on organisms or organic debris falling into their oral discs (Chintiroglou and Koukouras, 1992). This species is also highly tolerant of environmental variables such as extreme temperature and extreme salinity (Fish and Fish, 2011). The typical lifespan of *A. equina* under natural conditions is about three years (Fish and Fish, 2011). Sea anemone *Actinia equina* Linnaeus, 1758 which can be easily collected from their environment due to their sedentary life (Perrin et al. 1999), is used as a bioindicator species in heavy metal studies (Gadelha et al., 2010; Harland et al., 1990). Another reason for the use of marine anemones as bioindicators is their long life (Denny and Gaines, 2007).

In recent years, marine invertebrates have been used in the bioaccumulation studies conducted in the Bay of İskenderun. Mollusca (mollusca) is also observed in these studies (Turkmen and Türkmen, 2005; Yuzereroglu et al., 2010; Duysak and Ersoy, 2014; Duysak and Azdural, 2017). No bioaccumulation studies of *A. equina* species were found in the Bay of İskenderun. In this study, *A. equina* is also distributed in the coastal region of İskenderun to a depth of 1-5 meters in the coastal region. These animals are immobilized and are exposed to all discharges. Therefore, it is aimed to determine the accumulation of heavy metal levels in these species and to determine whether this species can be used in the biomonitoring studies carried out in İskenderun Bay which is exposed to various types of pollution. At the same time, this study is the most comprehensive heavy metal accumulation study ever made in the Bay of İskenderun for *A. equina* species.

MATERIAL AND METHODS

Sample preparation

Actinia equina individuals have been observed throughout the year from six different stations in İskenderun Bay (Figure 1), at a depth of 1-5 m, especially in ecosystems with a rocky substrate with high water movements. In the spring season (March-May 2013) when the anemone population was dense and the weather and sea conditions were suitable (since it can be collected by free diving), 20 samples were taken from each station and a total of 120 *A. equina* samples were collected. *A. equina* individuals were collected by scraping the hard rocks (1-5 m depth) with the help of plastic knives and spatulas. The collected samples were brought to the laboratory for storage in the cold chain. Morphometric measurements of the collected individuals were taken and their weights (g) were weighed (Table 1).

Figure 1. Map of the study area in İskenderun Bay, Turkey (modified from Simsek and Demirci, 2018)

Table 1. Average wet weight (g), pedal disc diameter (mm), and height from disc to tentacle (mm) measurements at different stations for *A. equina*

Stations	n	Wet weight (g)	Pedal disc diameter (mm)	Height from dics to tentacle (mm)
Yumurtalık	20	3.46±2.33	25.37±6.13	17.87±5.41
Dörtyol	20	4.42±2.57	28.22±7.69	18.96±5.04
Payas	20	2.37 ±1.30	21.01±3.92	15.85±5.22
İskenderun	20	8.01±3.32	27.17±5.38	12.38±0.33
Arsuz	20	2.8±1.06	16.48±3.01	23.75±4.45
Samandağ	20	2.04±1.08	15.19±2.24	12.06±0.46

İskenderun Bay, where the study was conducted, has various pollution discharges such as sea transport, domestic wastes, agricultural wastes, etc., in addition to intensive industrial activities. The pollution types of the 6 selected stations are given in Table 2.

Table 2. Sampling stations and pollution loads

Station name	Pollution types
Yumurtalık	Botaş pipeline, industrial waste, power plant, using chemical fertilizer, sea traffic, port activities, fishing activity
Dörtyol	Oil filling facilities, iron and steel industry, industrial waste, fertilizer industry, using chemical fertilizer, sea traffic, port activities, fishing activity, domestic waste
Payas	Iron and steel industry, industrial waste, Sea traffic, port activities, fishing activity, domestic waste
İskenderun	Sea traffic, tourism, industrial waste, Gübre Sanayi, port activities, fishing activity, domestic waste
Arsuz	Using chemical fertilizer, tourism, fishing activity, domestic waste
Samandağ	Using chemical fertilizer, tourism, fishing activity, domestic waste

Digestion procedures

This approach was modified from Tüzen (2003). A homogenized 2 g sample was placed in a 20 ml digestion tube, and 5 ml of high purity nitric acid (Merck) were added then the samples were heated to dissolve at 60˚C for 7 days. After digestion, the samples were filtered through Whatman-Quantitative (No: 42, 110 mm £) filter paper. The digested portion was then diluted to a final volume of 20 ml. A blank digest was carried out in the same way. All metals were determined against aqueous standards. Digested samples were analyzed three replicates for each metal.

Analytical procedures

Determination of all metal concentrations was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Varian model, Liberty Series II; Palo Alto, USA) equipment located at Mustafa Kemal University. High quality data was used for ICP-AES calibration and the absorption lines identified are given in Table 3. Purity Multi-Standard was used. Metal concentrations were calculated as mg/kg wet weight. The quality of the data was checked by the analysis of standard reference material DORM-2 (National Research Council of Canada; dogfish muscle and liver MA-A-2/TM fish flesh). Repeated analysis of reference materials showed good accuracy with recovery rates for metals between 91% and 104%. The results showed good agreement between the certified and the analytical values.

Table 3. Absorption line and detection limit of metals

Elements	Absorption line (nm)	Detection limit (ppm)
Fe	259.940	0.015
Zn	213.856	0.009
Cd	226.502	0.015
Cu	324.754	0.020
Co	242.5	0.15
Ni	231.1	0.20
Mn	257.610	0.003
Pb	220.353	0.14
Cr	267.716	0.040

Statistical analysis

All samples were collected and analyzed in duplicate and the results obtained as the mean ± standard deviation. One-way ANOVA and Tukey test were used to determine the significant difference between the means of metal concentration levels among the stations. All statistical calculations were done by SPSS 17.0 statistical software package. In all cases, the estimation was carried out at a significant level of 0.05 (Zar, 1984).

RESULTS

In this study, heavy metal accumulations in *A. equina* collected from six different stations in İskenderun Bay were investigated (Figure 1). The mean and comparison of heavy metal accumulation levels calculated from muscle tissue according to the six different stations was given in mg/kg wet weight in Table 4. We found that the mean concentration of heavy metals in tissues varied significantly among the stations ($p<0.05$).

Table 4. Mean concentrations of heavy metals in muscle tissue of *Actinia equina* at six different stations (mean ± SD. wet weight mg/kg)

	Stations					
Metals	**Yumurtalık**	**Dörtyol**	**Payas**	**İskenderun**	**Arsuz**	**Samandağ**
Fe	29.89±3.91^{a}	88.32±10.70^{b}	78.11±18.81^{a,b}	32.17±6.08^{a}	57.63±17.78^{a,b}	105.11±74.28^{a,b}
Zn	45.47±4.47^{a}	43.16±3.78^{a}	52.74±12.95^{a}	34.01±4.76^{a}	35.83±5.24^{a}	68.44±16.67^{b}
Cd	1.43±0.19^{a}	2.07±0.16^{a}	2.54±0.42^{a}	2.39±0.19^{a}	3.22±0.39^{a}	10.66±1.33^{b}
Cu	4.57±0.63^{a}	3.71±0.38^{a}	3.95±0.84^{a}	2.95±0.32^{a}	3.54±0.41^{a}	12.00±2.30^{b}
Co	0.95±0.15^{a}	0.84±0.10^{a}	1.70±0.36^{a}	2.27±0.19^{b}	3.21±0.39^{b}	8.66±1.76^{b}
Ni	2.84±0.94^{a}	5.52±3.86^{b}	4.09±1.72^{a,b}	1.76±0.60^{a}	3.87±1.40^{a}	6.22±2.32^{a,b}
Al	42.90±12.89^{a}	85.62±23.35^{a}	31.20±13.33^{a}	8.21±3.79^{a}	19.05±7.08^{a}	21.77±14.17^{a}
Mn	44.18±3.50^{a}	51.28±5.81^{a}	46.68±11.38^{a}	28.19±4.71^{a}	41.66±5.79^{a}	56.22±13.82^{a}
Pb	5.24±0.63^{a}	7.85±3.74^{a}	4.41±1.31^{a}	4.32±0.85^{a}	4.46±0.76^{a}	4.22±1.11^{a}
Cr	15.56±5.93^{a}	32.85±8.01^{a}	12.85±7.19^{a}	7.26±2.69^{a}	9.45±4.27^{a}	11.11±7.20^{a}

Letters a and b show differences among stations. Data shown with different letters are statistically significant at the differences p<0.05 level

In the study, the highest accumulation of Fe was found at Samandağ station, 105.11±74.28 mg/kg, while the lowest accumulation value for Co (0.84±0.10 mg/kg) was detected at Dörtyol station (Table 4). Although there was no significant difference in Fe concentration level between Yumurtalık and İskenderun stations (p>0.05), however, the difference in accumulation level at Dörtyol station was found significantly higher than Yumurtalık and İskenderun station (p <0.05).

The concentration of Zn was calculated in the highest concentrations at Samandağ station, followed by Yumurtalık, Dörtyol, Payas, Arsuz, and İskenderun with 45.47±4.47 mg/kg, 43.16±3.78 mg/kg, 52.74±12.95 mg/kg, 35.83±5.24 mg/kg, and 34.01±4.76 mg/kg, respectively (Table 4). During the study, Cd concentration was highest at Samandağ station (10.66±1.33 mg/kg), however, the concentrations of Cd determined were very low (1.43±0.19 mg/kg) in Yumurtalık station. The average of cadmium accumulation levels calculated from individuals in the stations are listed as follows: Samandağ> Arsuz> Payas> İskenderun> Dörtyol> Yumurtalık.

The Cu concentration ranged from 2.95±0.32 mg/kg at İskenderun station to 12.00±2.30 mg/kg Samandağ, followed by Yumurtalık (4.57±0.63 mg/kg), Payas (3.95±0.84 mg/kg), Dörtyol and Arsuz. The highest Cu accumulation was calculated at Samandağ station (12.00±2.30 mg/kg) and the lowest (2.95±0.32 mg/kg) at İskenderun station (Table 4). The Co concentration showed the lowest value at Dörtyol station with (0.84±0.10 mg/kg) and the highest amount (8.66±1.76 mg/kg) at the station of Samandağ. Co accumulation levels order in Anemones were Samandağ> Arsuz> İskenderun> Payas> Yumurtalık> Dörtyol. The highest Ni levels were recorded at Samandağ station (6.22±2.32 mg/kg), the lowest levels were at İskenderun station (1.76±0.60 mg/kg). Higher levels of Al were observed in the muscle tissue (85.62±23.35 mg/kg) at Dörtyol station and was the lowest (8.21±3.79 mg/kg) at İskenderun station. Comparison with the other station's Al metal levels are listed as Dörtyol> Yumurtalık> Payas> Samandağ> Arsuz> İskenderun (Table 4).

Mn accumulation in tissues of *A. equina* was in the following order: Samandağ> Dörtyol> Payas> Yumurtalık> Arsuz> İskenderun. The highest accumulation of Mn was recorded at Samandağ station. Pb was found to be the highest at Dörtyol station (7.85±3.74 mg/kg) and the lowest at Samandağ station (4.22±1.11 mg/kg). According to the accumulation levels of Pb at stations followed the order: Dörtyol> Yumurtalık> Arsuz> Payas> İskenderun> Samandağ (Table 4). Cr was most frequently found at Dörtyol (32.85± 8.01 mg/kg), and at least İskenderun station (7.26±2.69 mg/kg). The accumulation levels of Cr are listed as Dörtyol> Yumurtalık> Payas> Samandağ> Arsuz> İskenderun (Table 4). In our study, it was determined that the average difference of Al, Mn, Cr, and Pb accumulation was not significant (p> 0.05) among the stations, but Cd and Cu metal accumulation were found significantly different when compared to stations (p<0.05) (Table 4).

DISCUSSION

There is no information about bioaccumulation studies on the anemones in the İskenderun Bay and even in the Northeast Mediterranean until now. Therefore, this study was also discussed in comparison with the bioaccumulation studies performed in individuals belonging to anthozoa class, which have a resident life in the of the Meditrranean and other parts of different areas and seas.

Shiber (1981) observed that the most accumulated metal in the tissues of *A. equina* individuals distributed in Lebanon was Fe (428.5 µg/g dry weight). Similarly, in our study, it was determined that the most accumulated metal in the muscle tissues of *A. equina* individuals distributed in İskenderun Bay was also Fe (105.11 mg/kg wet weight).

Samawi et al. (2018) investigated Pb, Cd, and Cu accumulation levels in the tissues of the *Porites lutea* in 3 different regions of Indonesia. In their study, the ranges of concentrations of metal accumulation in the tissues of the *Porites lutea* were 72.85±24.22 µg/g-102.37±21.09 µg/g, 1.23±0.30 µg/g - 1.33±0.63 µg/g, and 2.04 ±0.57 µg/g - 2.75±0.33 µg/g (dry weight) for Pb, Cd, and Cu, respectively. In our study, the lowest and highest averages of metal accumulation in the muscle tissues of the *A. equina* individuals were 4.22±1.11 mg/kg and 7.85±3.74 mg/kg

for Pb, 1.43±0.19 mg/kg and 10.66±1.33 mg/kg for Cd and 2.95±0.32 mg/kg and 12.00±2.30 mg/kg (wet weight) for Cu were calculated. When these two studies were compared, it was found that Pb metal accumulated in anemones in Indonesia was higher than calculated in our study, and Cd and Cu accumulation levels were found lower. This difference is thought to be since the individuals used in both studies are not the same species and also the different pollution loads and amounts in their regions.

Corrias et al. (2020) conducted a study to determine the metal averages of Al, Cd, Cr, Cu, and Fe, which show accumulation in sea anemone *Anemonia sulcata* in 6 different stations of the Mediterranean region of Sardina. They calculated that the highest accumulation was Fe (80.828±24.108 mg/kg) and the lowest accumulation was Cd (0.002±0.003 mg/kg). Also, they reported that the metal order in descending order was Fe> Al> Cu> Cr> Cd. When compared with our study (Al, Cd, Cr, Cu and Fe), it was determined that the highest accumulation was Fe and the lowest accumulation was Cd in the muscle tissues of *A. equina* individuals. In our study, the metal ranking showing accumulation in muscle tissue was calculated as Fe> Al> Cr> Cu> Cd.

Escobar-Chicho et al. (2019) found the levels of metal accumulation in different tissues of an anemone (*Paraphelliactis pabista*) in the Gulf of California Guaymas basin at 501.15 mg/kg, 132.35 mg/kg, 9.94 mg/kg, 0.24 mg/kg, 3.21 mg/kg, 645.79 mg/kg, 136.28 mg/kg, 23.46 mg/kg, 8.86 mg/kg (dry weight) Fe, Zn, Cu, Co, Ni, Al, Mn, Pb, and Cr, respectively. When the accumulation levels of *A. equina* individuals distributed in the bay were compared, it was determined that Zn, Cu, Co, Ni, Mn, Cr levels were higher than other studies, whereas Fe, Al, Pb accumulation levels were lower than other studies. The reason for this is thought to be due to the difference in the anemone species used in the two studies and the geographical difference.

Lozano-Bilbao et al. (2020) investigated that the heavy metal accumulation levels in the muscle tissue of *Anemonia sulcata* individuals in 6 different regions of the Canary Islands. As a result of the study, the highest metal accumulation level of *A. sulcata* in muscle tissue was reported as 25.264±27.185 mg/kg and 0.058±0.050 mg/kg for Pb and Cd metals, respectively. As a result of our study, the highest metal accumulation levels calculated in muscle tissue were determined as 7.85±3.74 mg/kg and 10.66±1.33 mg/kg for Pb and Cd, respectively. When our study is compared with this study, the reason for the difference in the results is that the bays where the studies are conducted are exposed to different sources of pollution.

CONCLUSION

The accumulation levels of heavy metals (Fe, Zn, Cd, Cu, Co, Ni, Al, Mn, Pb, and Cr) in *A. equina*, which is a common anemone species for İskenderun Bay, were determined in six stations where different pollution discharges were observed in the İskenderun Bay. It was determined that the highest Fe accumulation level was obtained at Samandağ station, while the lowest accumulation value for Co was found at Dörtyol station. Metal concentrations accumulating in the muscle tissue of *A. equina* individuals are in descending order; Fe> Zn> Mn> Al> Cr> Cu> Pb> Ni> Cd> Co. In addition, this study is the first detailed bioaccumulation study conducted with *A. equina* in İskenderun Bay. In this study it is predicted that *A. equina* species can be used in biomonitoring studies due to reasons such as having a stable (silent) life somewhere, having a lifespan of about 3 years, accumulating metals in the environment and easily and to accumulate metals from the sea and to distribute the animal in the stations where the study is carried out in İskenderun Bay. In order to determine which metal contaminant is at which station, sea water samples should also be taken and analyzed as well as anemone samples. Another issue is that, since each station has more than one pollution source, it is very difficult to say which of the industrial activities that emit similar metals are more effective in these pollution values. Maybe another future study or studies investigating which of these different loads are more effective may be the subject of the new study.

REFERENCES

Anonymous, (1997). Türkiye Kıyılarındaki Lagünlerin Yönetim ve Geliştirilme Stratejileri ve Islahı. 1. Cilt. Tarım ve Köy İşleri Bakanlığı Tarımsal Üretim ve Geliştirme Genel Müdürlüğü, 578.

Anonymous, (2008). Toksikoloji. Trakya Üniversitesi Fen Fakültesi, Biyoloji Bölümü, Ders Notları.

Can, M.F., Yılmaz, A.B. & Mazlum, Y. (2019). A Meta-Analysis: Geo-statistical approach on the THQ values of Cu, Zn, and Fe accumulation in some fish species from İskenderun Bay, North-Eastern Mediterranean. *V. International Congress on Natural and Health Sciences* (ICNHS-2019), 69-89.

Chintiroglou, C.H. & Koukouras, A. (1992). The feeding habits of three Mediterranean sea anemone species, *Anemonia viridis* (Forskal), *Actinia equina* (Linnaeus) and *Cereus pedunculatus* (Pennant), *Helgol. Meeresunters*., 46, 53-68.

Corrias, F., Atzei, A., Addis, P., Secci, M., Russo, M. & Angioni, A. (2020). Integrated environmental evaluation of heavy metals and metalloids bioaccumulation in invertebrates and seaweeds from different marine coastal areas of Sardinia, *Mediterranean Sea. Environmental Pollution*, 266, 115048.

Denny, M.W. & Gaines, S.D. (2007). Encyclopedia of tidepools and rocky shores, Berkeley: University of California Press.

Duysak, Ö. & Ersoy, B. (2014). A biomonitoring study: heavy metals in *Monodonta turbinata* (Mollusca: Gastropoda) From Iskenderun Bay, North-Eastern Mediterranean. Pakistan *Journal of Zoology*, 46, 1317-1322. DOI: 0030-9923/2014/0005-1317

Duysak, Ö. & Azdural K. (2017). Evaluation of heavy metal and aluminium accumulation in a gastropod, *Patella caerulea* L., 1758 in Iskenderun Bay, Turkey. *Pakistan Journal of Zoology*, 49, 629-637. DOI: 10.17582/journal.pjz/2017.49.2.629.637

Ekici, H. & Yarsan, E. (2009). Akuakültür canlılarında zehirli etki oluşturabilecek maddeler. *E. Ü. Su Ürünleri Dergisi,* 26, 229–233.

Escobar-Chicho, M., Soto, L.A., Vanegas-Pérez, C. & Estradas-Romero, A. (2019). Heavy metal bioaccumulation in the anemone *Paraphelliactis pabista* Dunn, 1982 (Actiniaria: Hormathiidae) from the Hydrothermal System of Guaymas Basin, Gulf of California. *Bulletin of Environmental Contamination and Toxicology* 102, 486–491. DOI: 10.1007/s00128-019-02588-z

Fish J.D. & Fish, S. (2011) A student's guide to the seashore. Cambridge University Press. Cambridge.

Gadelha, J.R., Ferreira, V.A.M., Abreu, S.N., Soares, A.M.V.M. & Morgado, F.M.R. (2010). Experimental mercury bioaccumulation trends in sea anemone *Actinia equina* exposed to chlor-alkali ındustry effluent contaminated water. Interdisciplinary *Studies on Environmental Chemistry*, 3, 149-157.

Harland, A.D., Bryan, G.W. & Brown, B.E. (1990). Zinc and cadmium absorption in the symbiotic anemone *Anemonia viridis* and the non-symbiotic anemone *Actinia equina*. *Journal of the Marine Biological Association of the United Kingdom*, 70, 789-802. DOI: 10.1017/S0025315400059063

Kosswig, C. (1953). Türkiye balıkçılığının bazı biyolojik veçheleri. *Hidrobiyoloji Mecmuası*, 1(4), 145-153.

Lozano-Bilbao, E., Espinosa, J.M., Lozano, G., Hardisson, A., Rubio, C., González-Weller, D. & Gutiérrez, Á.J. (2020). Determination of metals in *Anemonia sulcata* (Pennant, 1777) as a pollution bioindicator. *Environmental Science and Pollution Research*, 23, 1-7. DOI: 10.1007/s11356-020-08684-6

Ormond, G.F.R. & Caldwell, S. (1982). The effect of oil pollution on the reproduction and feeding behaviour of the sea anemone *Actinia equina*. *Marine Pollution Bulletin*, 13,118-122.

Perrin, M.C., Thorpe, J.P. & Sole-Cava, A.M. (1999). Population structuring, gene dispersal and reproduction in the *Actinia equina* species group. *Oceanography and Marine Biology, An Annual Review*, 37, 129-152.

Phillips, D.J.H. & Segar, D.A. (1986). Use of bioindicators in monitoring conservative contaminants-programme design imperatives. *Marine Pollution Bulletin*, 17, 10-17. DOI: 10.1016/0025-326X(86)90797-6

Rainbow, P.S. & Phillips, D.J.H. (1993). Cosmopolitan biomonitors of trace metals. *Marine Pollution Bulletin*, 26, 593–601. DOI: 10.1016/0025-326X(93)90497-8

Samawi, M. F., Werorilangi, S., Tambaru, R. & Rastina, R. (2018). Accumulation of heavy metals within hard coral *Porites lutea* in Spermonde Archipelago, South Sulawesi. *Jurnal Ilmu Kelautan Spermonde*, 4(1), 39-42.

Shiber, J. G. (1981). Metal concentrations in certain coastal organisms from Beirut. *Hydrobiologi*a, 83(2), 181-195.

Simsek, E. & Demirci, A. (2018). Barotrauma treatment effects on survival rates for some discarded fish by trawl fishery. *Fresenius Environmental Bulletin*, 27 (7), 4867-4873.

Sole-Cava, A.M. & Thorpe, J.P. (1992). Thorpe, Genetic divergence between colour morphs in populations of the common intertidal sea anemones *Actinia equina* and *A. prasina* (Anthozoa: Actiniaria) in the Isle of Man. *Marine Biology*, 112(2), 243-252. DOI: 10.1007/BF00702468

Türkmen, A. & Türkmen, M. (2005). Seasonal and spatial variations of heavy metals in the spiny rock osyster, *Spondylus spinosus*, from coastal waters of İskenderun Bay, Northern East Meditteranean Sea, Turkey. *Bulletin of Environmental Contamination and Toxicology*, 75, 716-722. DOI: 10.1007/s00128-005-0810-0

Tüzen, M. (2003). Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chemistry*, 80: 119-123. DOI: 10.1016/S0308-8146(02)00264-9

Volterra, L. & Conti, M.E. (2000). Algae as biomarkers, bioaccumulators and toxin producers. *International Journal of Environment and Pollution*, 13, 92-125. DOI: 10.1504/IJEP.2000.002312

Yüzereroğlu, T.A., Gök, G., Çoğun, H.Y., Firat, O., Aslanyavrusu, S., Maruldalı, O. & Kargın, F. (2010). Heavy metals in *Patella caerulea* (Mollusca, Gastropoda) in polluted areas from the İskendeurn Gulf (Mediterranean Turkey). *Environmental Monitoring and Assessment*, 167, 257-264. DOI: 10.1007/s10661-009-1047-x

Zar, J.H. (1984). Biostatistical analysis. Second edition. Prentice Hall, Int., New Jersey, 718 p.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Microbiological quality of frozen black mussels (*Mytilus galloprovincialis*, Lamarck, 1819) purchased from markets in the İzmir Province of Turkey

Türkiye'nin İzmir ilinde marketlerden satın alınan dondurulmuş kara midyelerin (*Mytilus galloprovincialis, Lamarck*, 1819) mikrobiyolojik kalitesi

Bülent Kafa[1] ● Berna Kılınç[2]*

[1] Food Control Department, Izmir/Bornova Veterinary Control Institute, Bornova-İzmir, Turkey
[2] Ege University, Fisheries Faculty, Fish Processing Technology Department, Bornova-İzmir, Turkey

https://orcid.org/0000-0002-1402-894X
https://orcid.org/0000-0002-4663-5082

*Corresponding author: berna.kilinc@ege.edu.tr

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Abstract: The purpose of this study was to examine the microbiological quality and consumer safety of frozen black mussels purchased from four different markets (A, B, C, D) in the İzmir Province of Turkey. A total of 36 frozen black mussel packages, which contained approximately 50 mussels in each, were purchased from the markets. They were not only examined for total mesophilic, psychrotrophic, coliform and fecal coliform bacteria count but also investigated for pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* and *Vibrio spp*. According to the results of the mesophilic bacteria counts, frozen black mussels did not exceed the microbiological limits set for safe consumption. The findings showed that frozen black mussels purchased from (A, B, C, D) of markets were safe for human consumption because pathogenic bacteria species such as *E.coli, Salmonella spp., S. aureus, L. monocytogenes* and *Vibrio spp*. were not present in the frozen mussel samples.

Keywords: Frozen black mussel, microbiological quality, safety, pathogenic bacteria

Öz: Bu çalışmanın amacı Türkiye' nin İzmir ilinde dört farklı marketten (A, B, C, D) satın alınan dondurulmuş kara midyelerin güvenliğini ve mikrobiyal kalitesini belirlemektir. Bu amaçla dört farklı marketten her biri yaklaşık 50 adet dondurulmuş kara midye içeren toplam 36 paket satın alınmıştır. Dondurulmuş kara midyeler sadece toplam mezofilik, psikrotrofik, koliform, fekal koliform açısından değil, aynı zamanda *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes*, Vibrio türleri gibi patojenik bakteriler içinde incelenmiştir. Bulgularımız, Türkiye' nin İzmir ilindeki dört farklı marketten (A, B, C, D) satın alınarak incelenen dondurulmuş kara midyelerin, *E. coli*, *S. aureus*, *L. monocytogenes* ve *Vibrio spp*. gibi patojenik bakteri türlerini içermemesi nedeniyle, insan tüketimi için güvenli olduğunu göstermiştir.

Anahtar kelimeler: Dondurulmuş kara midye, mikrobiyolojik kalite, güvenlik, patojen bakteri

INTRODUCTION

Seafoods are main source of protein, vitamins, minerals and essential fatty acids. Many beneficial effects of seafoods on human health have been given by many authors (Lund, 2013; Tahergorabi and Jaczynski, 2016; Spiller et al., 2019). Chemical and microbiological contaminants in fisheries products could be a risk for human health (Jovic and Stankovic, 2014; Robert-Pillot et al., 2014; Nicolas et al., 2017; Zahelyazkov et al., 2018; Alvarez-Munoz et al., 2018; Liu et al., 2018; Farady, 2019; Hallström et al., 2019). Although hygienic and heath safety regulations are in place to minimize such risks, microbiological contaminants can still occur, either through lack of coastal marine environmental quality or through the processing and marketing chain (Kılınç and Besler, 2014). Mussels are benthic filter feeders that can accumulate chemical pollutants, microplastics, microorganisms and toxins from phytoplankton blooms etc. (Witte et al., 2014). Robert-Pillot et al. (2014) indicated that seafood consumption presented a potential risk to human health in France and also in their findings the authors highlighted the importance of tools for a preventive consumer protection policy. The safety of various seafoods varies according to the origin of the fishery products, microbiological ecology of the product, contamination level, handling, processing practices and preparations before consumption (Kılınç and Besler, 2014). Many studies have been done about the shelf life of mussels stored under refrigerated conditions (Manousaridis et al., 2005; Erkan 2005; Caglak et al., 2008; Bongiorno et al., 2018; Tosun et al., 2018). In addition to this; many studies have been done about the microbiological quality of seafood products (Huss, 1997; Ripabelli et al., 1999; Papadopoulou et al., 2007; Cruz-Romero et al., 2008; Lampila and McMillin, 2012; Robert-Pillot et al., 2014; Okpola, 2014; Turan et al., 2013;Turan and Onay, 2015; Kocatepe et al., 2016; Kılınç et al., 2018; Kocatepe et al., 2019). However, in the literature there is limited study found about examining the microbiological

quality and safe for human consumption of frozen mussels (Popovic et al., 2010; Georgescu et al., 2015; Angane et al., 2020). For this reason, the aim of this study was to examine the microbiological quality of frozen black mussels purchased from four different markets in the İzmir, Turkey.

MATERIAL AND METHODS

Frozen black mussel material

A total of 36 frozen mussel packages, including nine packages from each market were purchased from four different markets (A, B, C and D) in different locations in the İzmir Province. Following their collection from markets, samples were immediately brought to the laboratory under hygienic conditions in cooler box by using ice in approximately 30 minutes. The number of mussels contained in each frozen package ranged from 45 to 59 individuals. The total mussel weight in each package was 500 g (Table 1).

Table 1. The number of frozen black mussels in each package purchased from four different markets

Frozen Black mussel packages	A	B	C	D
1	53	52	52	47
2	47	49	47	51
3	45	52	47	50
4	52	48	51	49
5	53	49	47	49
6	51	52	47	52
7	46	49	47	59
8	59	47	48	49
9	57	49	49	49

Total number of mussels sampled: n=1800. The avarage number of mussels in all packages: n=50±3.32. The total weight of mussels in a per package: 500±0.00 g

Microbiological analyses

Each black mussel package was homonized by using stomacher (IUL, Barcelona, Spain) for 1 min, before microbiological evaluation. Microbiological tests were performed by using TS EN ISO 6887-2 method after the preparation of samples, initial suspension and decimal dilutions (Anonymus, 2001). Mesophilic and psychrotrophic aerobic bacteria counts were done according to the method described in ISO 4833-1:2013. Total mesophilic aerobic bacteria counts were done by using the pour plate method on plate count agar (PCA, Liofilchem, Italy). After the inoculation, petri dishes were incubated at 30±1°C for 72 h according to method of TS ISO 4833-1:2013. Thereafter all colonies on plate count agar were counted and converted into log cfu/g. For determining total psychrotrophic aerobic bacteria count, plate count agar (PCA, Liofilchem, Italy) was also used. The inoculated petri dishes were incubated at 2-8°C for 7 days were given as log cfu/g (Anonymous, 2013) Total coliform, fecal coliform and *E.coli* analyses were done according to TS ISO 7251:2015 procedure. Lauryl Sulfate Tryptose Broth (LSTB), EC Broth, and Tryptone Broth were used to determine of coliform, fecal coliform and *E.coli*, respectively, according to the Most Probable Number (MPN) method. Coliform bacteria growth and gas production were qualitatively determined (positive/negative). The tubes were incubated at 37°C±1°C for 24-48 h for coliform bacteria analysis. For confirmation, Brilliant Green Bile Broth (BGBB) was used as controls. Total coliforms were given as MPN/g. One loopful was taken from the positive tubes and inoculated into the EC Broth containing tubes. These tubes were incubated at 44°C±1°C for 24±2h for determining fecal coliform bacteria. After the incubation period, one loopful was taken from the positive tubes and inoculated into the Tryptone Broth. These tubes were incubated at 44°C±1°C for 48±2h for *E. coli* analysis. After the indol test, the tubes with positive results were given as *E. coli* MPN/g (Anonymous, 2015). The analysis of *Salmonella* species was investigated by using TS EN ISO 6579 method. Pre-enrichment was conducted from 25 g of samples were diluted in 225 ml of buffered peptone water and incubated at 37°C for 24 hours. Secondary selective enrichment was performed in Rappaport-Vassiliadis Soy Broth at 37°C for 24 h and Muller-Kaufmann tetrathionate broth with Novobiocin at 37°C for 24 h, and plating on XLD agar and Rambach agar, XLT-4 agar at 37°C for 24 h. (Anonymous, 2005). The analysis of *Listeria monocytogenes* was performed according to the method of ISO 11290-1. *Listeria* Enrichment Broth Base Fraser was prepared Fraser by adding the respective supplements. Pre-enrichment was conducted in Half Fraser Broth for 24 h at 30°C. Then, the primary and secondary enrichment tubes were inoculated onto Oxford and ALOA agar(Agar Listeria Ottaviani and Agosti) and incubated at 37°C for 48 hours (Anonymous, 1997). Baird parker agar with Rabbit Fibrinogen were used for the coagulase positive *Staphyloccocci* according to method of TS 6582-2 EN ISO 6888-2 (Anonymous, 2006). The analysis of the samples by Real-Time PCR for *Vibrio* species were done according to the method of Dupont Q7 BAX system analysis kit Dupont, BAX Part D12863877 (Anonymous, 2016).

Statistical analysis

All statistical analyses were carried out by using the SPSS 25.0 (IBM-SPSS, USA) Software Package Program of Social Sciences. The homegenicity of the bacterial counts of group variances were tested by using the Levene test (Gamgam and Altunkaynak, 2017) to distinguish differences between markets. The goodness of fit of the dependent variable to the normal distribution was controlled by Kolmogorov-Smirnov and Shapiro-Wilk methods, described in (Gamgam and Altunkaynak, 2017). Kruskal-Wallis test was performed from nonparametric tests (Gamgam and Altunkaynak, 2017) as the dependent variable for the market did not provide the normality assumption. Mann-Whitney test was also used for determining the differences of bacteria counts between the markets (Gamgam and Altunkaynak, 2017). The level of significance was represented as $p<0.05$.

RESULTS

Total mesophilic aerobic bacteria count (TMBC) (log cfu/g) of frozen black mussels purchased from four different markets are given in Table 2. The average mean values of TMBC of all packages (n=36) were found as 2.42 log cfu/g.

Group variances on the basis of markets for the TMBC as the dependent variable were found to be homogeneous according to the Levene test. The goodness of fit of the dependent variable to the normal distribution was tested by using the Kolmogorov-Smirnov and Shapiro-Wilk methods (Gamgam and Altunkaynak, 2017). Kruskal – Wallis test was also performed from nonparametric tests as the dependent variable for the markets did not provide the normality assumption and the test result was not statistically significant ($p>0.05$). According to the method of (Gamgam and Altunkaynak, 2017). The average value of TMBC did not differ significantly ($p>0.05$) between markets. According to ICMSF (1992); the upper acceptable limit for TMBC of mussels is 5.0 log cfu/g. In our study examined 36 packages from four different markets were not exceeded this limit.

Total psychrotrophic bacteria counts (TPBC) (log cfu/g) of the packages are given in Table 2.The average mean values of TPBC for all samples were found to be 2.57 log cfu/g. Group variances between markets were not homogeneous for the psychrotrophic dependent variable (Levene test, Kolmogorov-Smirnov and Shapiro-Wilk tests) described in (Gamgam and Altunkaynak, 2017). The assumption of homogenity was not found. So, Kruskal-Wallis test showed statistical significance (p-value=0.001). Thus, the average number of TPBC varies significantly between markets. According to the Mann-Whitney test, the psychrotrophic average value for market A differs significantly ($p<0.05$) from all other market averages (p-values: A and B= 0.031, p-values: A and C= 0.000, p-values: A and D= 0.000), the difference between other market averages was not significant ($p>0.05$). (p-values: B and C= 0.387, p-values: B and D= 0.094, p-values: C and D= 0.222). In another words, no significant differences ($p>0.05$) were determined in TPBC of mussel samples of B, C and D.

No significant differences ($p>0.05$) were determined in TMBC of frozen black mussels between the four different markets (A, B, C, D) as well as no significant differences ($p>0.05$) were determined in TPBC of frozen samples for the markets (B, C and D), but this difference was found statistically significant ($p<0.05$) for the market A.

In our study total coliform bacteria (TCB), fecal coliform bacteria (FCB), *S. aureus, E.coli, Salmonella spp., Listeria monocytogenes and Vibrio spp*. were not determined in any of frozen mussel samples which were purchased from four different markets in İzmir, Turkey (Table 2).

Table 2. Microbiological quality of frozen black mussels purchased from four different markets

	TMBC	TPBC	TCB	FCB
A				
1	2.34	2.65	<3.0	<3.0
2	2.38	2.66	<3.0	<3.0
3	2.36	2.62	<3.0	<3.0
4	2.34	2.63	<3.0	<3.0
5	2.46	2.67	<3.0	<3.0
6	2.47	2.64	<3.0	<3.0
7	2.34	2.66	<3.0	<3.0
8	2.38	2.63	<3.0	<3.0
9	2.46	2.64	<3.0	<3.0
B				
1	2.44	2.55	<3.0	<3.0
2	2.46	2.56	<3.0	<3.0
3	2.43	2.61	<3.0	<3.0
4	2.34	2.66	<3.0	<3.0
5	2.44	2.68	<3.0	<3.0
6	2.46	2.59	<3.0	<3.0
7	2.36	2.55	<3.0	<3.0
8	2.43	2.56	<3.0	<3.0
9	2.46	2.59	<3.0	<3.0
C				
1	2.38	2.55	<3.0	<3.0
2	2.44	2.55	<3.0	<3.0
3	2.43	2.59	<3.0	<3.0
4	2.46	2.61	<3.0	<3.0
5	2.38	2.56	<3.0	<3.0
6	2.44	2.62	<3.0	<3.0
7	2.41	2.50	<3.0	<3.0
8	2.46	2.53	<3.0	<3.0
9	2.49	2.63	<3.0	<3.0
D				
1	2.41	2.61	<3.0	<3.0
2	2.46	2.62	<3.0	<3.0
3	2.49	2.34	<3.0	<3.0
4	2.39	2.20	<3.0	<3.0
5	2.46	2.56	<3.0	<3.0
6	2.50	2.54	<3.0	<3.0
7	2.56	2.37	<3.0	<3.0
8	2.46	2.59	<3.0	<3.0
9	2.36	2.43	<3.0	<3.0

n=36; the mean value ± standard deviation of TMBC and TPBC of frozen samples: 2.42±0.05 and 2.57±0.09. *S. aureus, E.coli*, *Vibrio spp*., *L. monocytogenes*, *Salmonella spp*. were not present

DISCUSSION

Lampila and McMillin (2012) reported in their study that seafood had very highly perishable product. Another author also reported that they can be contaminated with pathogens such as *Staphylococcus aureus, Listeria monocytogenes, Salmonella* and *Vibrio* species etc. (Huss, 1997). For this reason, the importance of detection, identification and monitoring of these pathogenic bacteria in seafood was highlighted by the authors that was critical in achieving the seafood safety goals (Ripabelli et al., 1999; Cruz- Romero et al., 2008; Arvanitoyannis and Stratakos, 2010; Okpala, 2014;

Bavisett et al., 2018). According to The Turkish Food Codex Regulation on Microbiological Criteria No. 28157 dated December 29, 2011, *V. cholerae* and *V. parahaemolyticus* should not be present in fishery products that are grown/caught from salt water. Microbiological criteria for mussel samples in the notification criteria in Turkey also indicated that *Salmonella spp*. and *L. monocytogenes* should not be present in 25 grams of the sample according to (Communiqué on microbiological criteria in Turkey, 2011). In lights of above literatures, frozen black mussels purchased from the markets A, B, C, D in the İzmir Province of Turkey were safe for human consumption because pathogenic bacteria species such as *E. coli, Salmonella spp., S. aureus, L. monocytogenes and Vibrio* spp. were not present in the frozen samples.

Shelf-life studies were done about mussels which were packaged in different packages and stored at different temperatures. In one report the authors reported that under the current commercial practice, live mussels only had 10 days' shelf life (Odeyemi et al., 2018). Erkan (2005) indicated that the shelf-life of mussels at 4°C was limited to 4 days.

The authors reported in the below studies that the processing and packaging technologies applied on mussels could be extend the shelf-life of this product. In one study, the initial microbial load of the mussel samples reduced after the pasteurization process. The authors also observed that this reduction was determined not only in the total mesophilic and psychrophilic bacterial count, but also in the the yeast mold counts after pasteurization being 2.44, 2.07 and 2.37 log cfu/g, respectively (Tosun et al., 2018). (Cherifi and Sadok, 2016) reported that total mesophilic bacteria flora for marinated mussels (3.21 log CFU/g) had significantly lower level, when compared to the control sample (4.14 log CFU/g). In the study indicated by (Arcales and Nacional, 2018) that pretreatment using lactic acid could be used to improve the shelf-life of green mussels for 15 days, when compared with 6 days of uncontrolled samples (Arcales and Nacional, 2018). Turan et al. (2008) reported that the shelf-life of mussels smoked by hot smoking and stored at 4±1°C at refrigerator temperature determined as 12 days. The authors reported in another study that the sous-vide cook and chill method at 85°C for 10 minutes with or without salt brine resulted in being able to preserve the quality of mussels and extend their shelf-life to 21 storage days (Bongiorno et al., 2018). Turan et al. (2013) stated in their study that mussels in polystyrene plates wrapped with stretch film could be consumed until end of the 2nd day, whereas mussels placed in water in glass jar could be consumed until the end of the 3rd day. In another report; all black mussels retained desirable sensory characteristics during the first 8 days of storage, whereas the modified atmosphere packaged and vacuum packaged mussels exceeded the limit of consumption after 12 days of storage at 2°C (Caglak et al., 2008). The consumable period of mussel was found as 12 day and 18 day for MAP and vacuum group, respectively reported by (Turan and Onay, 2015). The effect of modified atmosphere packaging (MAP) on the keeping quality of green mussel stored at 4°C was investigated by (Masniyom et al., 2011). In this study the authors reported that MAP with 80% CO_2, 10% O_2 and 10% N_2 was determined as the best condition for extending the shelf-life of green mussel (Masniyom et al., 2011). In another report, 80%/20% CO_2/N_2 gas mixture was the most effective for black mussel preservation achieving a shelf-life of 14–15 days reported by (Goulas et al., 2005). Many studies have been done about the shelf-life of un processed and processed mussels stored under refrigerated conditions, which were decribed in the above studies, However very limited study have been found about examining the microbiological quality and safety of frozen mussels (Popovic et al., 2010; Georgescu et al., 2015; Angane et al., 2020). Popovic et al., (2010) reported in their study that the psychrophilic bacteria level of frozen 28 shellfish were determined between the 2-3 log cfu/g and only 2 samples were determined at above 3 log cfu/g. In our study the avarage value of TPBC of examined 36 frozen black mussel packages was found to be 2.57±0.09 log cfu/g. Our result was determined well correlated with the above study, which was determined the TPBC of frozen shellfish between the 2-3 log cfu/g.

In our study the avarage value of TMBC of examined 36 frozen black mussel packages was determined to be 2.42±0.05 log cfu/g. In one study; Georgescu et al. (2015) reported that the TMBC were higher for the mussels of locally harvested samples than frozen mussels. Popovic et al. (2010) also reported that total aerobic mesophilic bacteria level of 26 samples were determined as 5 log cfu/g, only 4 samples were indicated that below the level of 3 log cfu/g. In contrast to this study (Popovic et al., 2010), in our study TMBC of frozen black mussels were determined lower than total psychrotrophic bacteria counts. However, this study was very similar to our findings about not including pathogenic bacteria of frozen samples. In contrast to the frozen mussel samples, Papadopoulou et al. (2007) reported in their study that pathogenic microorganisms such as *E. coli* and *Listeria* spp. could be found at the ratio of 83.3 % (25/30) and 3.3 % (1/30) in examined raw mussels, respectively.

As a result, frozen black mussels taken from four different markets in İzmir Province of Turkey were determined as acceptable. The TMBC of all frozen black mussels were determined below the upper acceptable microbiological limit indicated as 5.0 log cfu/g according to ICMSF (1992).

The findings also showed that frozen black mussels bought from different markets in the İzmir Province of Turkey were determined as safe for consumption according to the Turkish Food Codex Regulation because pathogenic bacteria such as *E. coli*, *Staphylococcus aureus, Salmonella spp. Listeria monocytogenes, Vibrio* spp. were not present in any of the frozen mussel samples.

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REFERENCES

Alvarez-Munoz, D., Rodriguez-Mozaz, S., Jacobs, S., Serra-Compte, A., Caceres, N., Sioen, I., Verbeke, W., Barboso, V., Ferrari, F., Fernandez-Tejedor, M., Cunha, S., Granby, K., Robens, J., Kotterman, M., Marques, A. & Barcelo, D. (2018). Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European market: Concentrations and human exposure levels. *Environment International*, 119, 570-581. DOI: 10.1016/j.envint.2018.07.006

Angane, M., Gupta, S., Fletcher, G.C., Summers, G., Hedderley, D.I. & Quek, S.Y. (2020). Effect of air blast freezing and frozen storage on *Escherichia coli* survival, n-3 polyunsaturated fatty acid concentration and microstructure of Greenshell™ mussels. *Food Control*, 107284. DOI: 10.1016/j.foodcont.2020.107284

Anonymous. (1997). TS EN ISO 11290-1. Gıda ve yem maddelerinin mikrobiyolojisi-*Listeria monocytogenes*'in aranması ve sayımı metodu, Bölüm 1: Arama metodu, 50s.

Anonymous. (2001). TS 6235 EN ISO 6887-1. Gıda ve hayvan yemlerinin mikrobiyolojisi. Deney numunelerinin başlangıç süspansiyonun ve ondalık seyreltilerin hazırlanması için genel kurallar, 17s.

Anonymous. (2005). TS EN ISO 6579, Mikrobiyoloji gıda ve hayvan yemleri *Salmonella* türlerinin belirlenmesi için yatay yöntem, 30s.

Anonymous. (2006). TS EN ISO 6888-2/A1. Gıda ve hayvan yemlerinin mikrobiyolojisi- koagulaz- pozitif- stafilokokların (*Staphylococcus aureus* ve diğer türler) sayımı için yatay metot, Bölüm 2: Tavşan fibrinojeni agar besiyeri kullanarak belirlenmesi, 11s.

Anonymous. (2013). ISO 4833, Horizontal method for the enumaration of microorganism. Colony count technique at 30°C, 32s.

Anonymous. (2015). TS ISO 7251. Gıda ve hayvan yemleri mikrobiyolojisi - Muhtemel *Escherichia coli*'nin belirlenmesi ve sayımı için yatay yöntem - En muhtemel sayı tekniği, 22s.

Anonymous. (2016). BAX® System Real-Time PCR Assay for *Vibrio*. http://www.dupont.com/content/dam/dupont/products-and-services/food-protection/food-protection-landing/documents/bax_rt-vibrio_proddesc.pdf (10.12.2016).

Arcales, J.A.A. & Nacional, L.M. (2018). Effect of lactic and citric acid pretreatment on quality changes of green mussel (*Perna viridis*) during chilled storage. *Current Research in Nutrition and Food Science*, 6(3), 862-872. DOI: 10.12944/CRNFSJ.6.3.29

Arvanitoyannis, I.S. & Stratakos, A.C. (2010). Effect of Irradiation on fish and seafood In I.S. Arvanitoyannis (Ed.), *Irradiation of Commodities* (pp.287-365).

Bongiorno, T., Tulli, F., Comi, G., Sensidoni, A. & Andyanto, D. (2018). Sous-vide cook-chill mussel (*Mytilus galloprovincialis*): evaluation of chemical, microbiological and sensory quality during chilled storage 3°C. *LWT-Food Science and Technology*, 91, 117-124. DOI: 10.1016/j.lwt.2017.12.005

Caglak, E., Cakli, S. & Kılınç, B. (2008). Microbiological, chemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *European Food Research and Technology*, 226, 1293-1299. DOI: 10.1007/s00217-007-0657-1

Cherifi, H. & Sadok, S. (2016). Effects of marinating process on mussels physicochemicaland microbiological quality attributes during refrigerated storage. Bull. Inst. Natn. Scien. Tech. Mer de Salammbo, 43, 1-17.

Cruz-Romero, M., Kerry, J.P. & Kelly, A.L. (2008). Changes in the microbiological and physicochemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage. *Food Control*, 19, 1139-1147. DOI: 10.1016/j.foodcont.2007.12.004

Erkan, N. (2005). Changes in quality characteristics during cold storage of shucked mussels (*Mytilus galloprovincialis*) and selected chemical decomposition indicators. *Journal of Science of Food and Agriculture,* 85, 2625-2630. DOI: 10.1002/jsfa.2331

Farady, S.E. (2019). Microplatics as a new, ubiquitous pollutant: Strategies to anticipate management and advise seafood consumers. *Marine Policy*, 104, 103-107. DOI: 10.1016/j.marpol.2019.02.020

Gamgam, H. & Altunkaynak, B. (2017). Parametrik olmayan yöntemler, 6. Baskı, Seçkin Yayıncılık. 456 s.

Georgescu, M., Dobrea, M., Neagu, I., Popescu, A.A.D. & Dojana, R. (2015). Microbiological evaluation of imported frozen mussels and locally harvested Black Sea mussels. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine*, *72*(1), 179-180. DOI: 10.15835/buasvmcn-vm:10346

Goulas, A. E., Chouliara, I., Nessi, E., Kontominas, M.G. & Savvaidis, I.N. (2005). Microbiological, biochemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *Journal of Applied Microbiology,* 98(3), 752-760. DOI: 10.1111/j. 1365-2672.2004.02512.x

Hallström, E., Bergman, K., Mifflin, K., Parker, R., Tyedmers, P., Troell, M. & Ziegler, F. (2019). Combined climate and nutritional performance of seafoods. *Journal of Cleaner Production*, 230, 402-411. DOI: 10.1016/j.jclepro.2019.04.229

Huss, H.H. (1997). Control of indigenous pathogenic bacteria in seafood. *Food Control*, 8, 91-98. DOI: 10.1016/S0956-7135(96)00079-5

Jovic, M. & Stankovic, S. (2014). Human exposure to trace metals and possible public health risks via consumption of mussels *Mytilus galloprovincialis* from the Adriatic coastal area*. Food Chemistry Toxicology*, 70, 241-251. DOI: 10.1016/j.fct.2014.05.012

Kılınç, B. & Besler, A. (2014). The occurrence of enteric bacteria in marine environment and pollution. *Marine Science Technology Bulletin*, 3, 39-43.

Kılınç, B., Şen Yılmaz, B. & Gören, B. (2018). İzmir'in farklı bölgelerinde satışa sunulan midye dolmaların mikrobiyolojik kalitesi. *Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi,* 14(4), 276-290. DOI: 10.22392/egirdir.403570

Kocatepe, D., Taskaya, G., Turan, H. & Kaya, Y. (2016). Microbiological investigation of wild, cultivated mussels (*Mytilus galloprovincialis* L. 1819) and stuffed mussels in Sinop–Turkey. *Ukrainian Food Journal*, 5(2), 299-305. DOI: 10.24263/2304-974X-2016-5-2-9

Kocatepe, D., Keskin, İ. & Kaya, Y. (2019). The effect of frying–the chemical, microbiological, and acrylamide composition of mussels as a street Food. *Journal of Aquatic Food Product Technology*, 28(1), 117-128. DOI: 10.1080/10498850.2018.1563260

Lampila, L.E. & McMillin, K.W. (2012). Major microbial hazards associated with packaged seafoods. *Advances in Meat, Poultry and Seafood Packaging*, 891, 59-85. DOI: 10.1533/9780857095718.1.59

Liu, Q., Liao, Y. & Shou, L. (2018). Concentration and potential health risk of heavy metals in seafoods collected from Sanmen Bay and its adjacent areas, China. *Marine Pollution Bulletin*, 131, 356-364. DOI: 10.1016/j.marpolbul.2018.04.041

Lund, E.K. (2013). Health benefits of seafoods; Is it just the fatty acids? *Food Chemistry*, 140, 413-420. DOI: 10.1016/j.foodchem.2013.01.034

Manousaridis, G., Nerantzaki, A., Paleologos, E.K., Tsiotsias, A., Savvaidis, I.N. & Kontominas, M.G. (2005). Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. *Food Microbiology*, 22, 1-9. DOI: 10.1016/j.fm.2004.06.003

Masniyom, P., Benjama, O. & Maneesri, J. (2011). Extending the shelf-life of refrigerated green mussel (*Perna viridis*) under modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*, 33(2), 171-179.

Nicolas, J., Hoogenboom, R.A.L.P., Hendriksen, P.J.M., Bodero, M., Bovee, T.F.N., Rietjens, I.M.C.M. & Gerssen, A. (2017). Marine biotoxins and associated outbreaks following seafood consumption: Prevention and surveillance in the 21st century. *Global Food Security*, 15, 11-21. DOI: 10.1016/j.gfs.2017.03.002

Odeyemi, O.A., Burke, C.M., Bolch, C.J.S. & Stanley, R. (2018). Evaluation of spoilage potential and volatile metabolites production by *Shewanella baltica* isolated from modified atmosphere packaged live mussels. *Food Research International*, 103, 415-425. DOI: 10.1016/j.foodres.2017.10.068

Okpola, C.O.R. (2014). Investigation of quality attributes of ice-stored Pacific white shrimp (*Litopenaeus vannamei*) as affected by sequential minimal ozone treatment. *LWT-Food Science and Technology*, 57, 538-547. DOI: 10.1007/s00003-014-0904

Papadopoulou, C., Economou, E., Zakas, G., Salamoura, C., Dontorou, C. & Apostolou, J. (2007). Microbiological and pathogenic contaminants of seafood in Greece. *Journal of Food Quality*, 30(1), 28-42. DOI: 10.1111/j.1745-4557.2007.00104.x

Popovic, N.T., Skukan, B.A., Dzidara, P. & Rakovac, R.C. (2010). Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia. *Veterinarni Medicina*, 55, 233-241. DOI: 10.17221/2997-VETMED

Ripabelli, G., Sammarco, M.L., Grasso, G.M., Fanelli, I., Caprioli, A. & Luzzi, I. (1999). Occurrence of *Vibrio* and other pathogenic bacteria in *Mytilus galloprovincialis* (mussels) harvested from Adriatic Sea, Italy. *International Journal of Food Microbiology*, 49, 43-48. DOI: 10.1016/S0168-1605(99)00056-2

Robert-Pillot, A., Copin, S., Himber, C., Gay, M. & Quilici, M.L. (2014). Occurence of the three major *Vibrio* species pathogenic for human in sefood products consumed in France using Real-Time PCR. *International Journal of Food Microbiology*, 189, 75-81. DOI: 10.1016/j.ijfoodmicro.2014.07.014

Spiller, P., Hibbeln, J.R., Myers, G., Vannice, G., Golding, J., Crawford, M.A., Strain, J.J., Connor, S.L., Brenna, J.T., Kris-Etherton, P., Holub, B.J., Harris, W.S., Lands, B., Mcnamara, R.K., Tlusty, M.F., Salem Jr. N. & Carlson, S.E. (2019). An abundance of seafood consumption studies presents new apportunities to evaluate effects on neurocognitive development. *Prostaglandis, Leukotrienes and Essential Fatty Acids* 151, 8-13. DOI: 10.1016/j.plefa.2019.10.001

Tahergorabi, R. & Jaczynski, J. (2016). Seafood proteins and human health. In: S. Raatz, D. Bibus (Ed.), *Fish and Fish oil in Health and Disease Prevention* (pp.323-331). DOI: 10.1016/B978-0-12-802844-5.00029-4

Tosun, Ş.Y., Alakavuk, D.Ç. & Ulusoy, Ş. (2018). Quality changes of thermal pasteurized mussels (*Mytilus galloprovincialis*) during refrigerated storage at 4±1°C, *Aquatic Sciences and Engineering*, 33(4), 117-123. DOI: 10.26650/ASE2018428669

Turan, H., Sönmez, G., Çelik, M.Y., Yalçın, M. & Kaya, M. (2008). The effects of hot smoking on the chemical composition and shelf life of Mediterranean mussel (*Mytilus galloprovincialis* L. 1819) under chilled storage. *Journal of Food Processing and Preservation*, 32, 912-922. DOI: 10.1111/j.1745-4549.2008.00221.x

Turan, H., Kocatepe, D., Altan, C.O. & Erkoyuncu, İ. (2013). Soğukta saklanan midyelerin *Mytilus galloprovincialis* L. 1819 raf ömrünün belirlenmesi. *Akademik Gıda*, 11(3), 47-51.

Turan, H. & Onay, R.T. (2015). Modifiye atmosfer paketleme uygulanan midyelerin (*Mytilus galloprovincialis,* Lamarck 1819) buzdolabı (4±2° C) koşullarında raf ömrünün tespiti. *Journal of Food and Health Science,* 1(4), 185-198. DOI: 10.3153/JFHS15018

Witte, B.D., Devriese, L., Bekaert, K. & Hoffman, S. (2014). Quality assessment of the blue mussel (*Mytilus edulis)*: Comparison between commercial and wild types. *Marine Pollution Bulletin*, 85, 146-155. DOI: 10.1016/j.marpolbul.2014.06.006

Zahelyazkov, G., Yankovska-Stefanova, T., Mineva, E., Stratev, D., Vashin, I., Dospatliev, L., Valkova, E. & Popova, T. (2018). Risk assessment of some heavy metals in mussels (*Mytilus galloprovincialis*) and veined rapa whelks (*Rapana venosa*) for human health. *Marine Pollution Bulletin*, 128, 197-201. DOI: 10.1016/j.marpolbul.2018.01.024

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Retention probability of purse seine grating sieve based on fish morphology

Balık morfolojisine göre gırgır boylama eleğinin alıkoyma olasılığı

Zafer Tosunoğlu[1] ● Sinan Mavruk [2*] ● Nazlı Kasapoğlu[3]

[1] Ege University, Faculty of Fisheries, Izmir, Turkey
[2] Çukurova University, Fisheries Faculty, 01330, Balcalı, Adana, Turkey
[3] Central Fisheries Research Institute, Trabzon, Turkey

https://orcid.org/0000-0002-1168-9611
https://orcid.org/0000-0003-1958-0634
https://orcid.org/0000-0001-5526-778X

*Corresponding author: smavruk@cu.edu.tr

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Abstract: The retention-releasing patterns of the double-grid grating sieve placed on the deck of the purse seiners were revealed for four case species (*Sardina pilchardus, Sardinella aurita*, *Engraulis encrasicolus* and *Boops boops*) using a simulated data predicted from total length (TL) – maximum height (Hmax) and TL – maximum width (Wmax) regressions. To calculate these relationships, samples were collected during commercial purse seine operations between 3 April 2017 and 21 March 2018 in Izmir Bay, Aegean Sea (Turkey). Optimal Bar Spacing (OBS) values corresponding to minimum landing sizes or the length at first maturity were calculated separately for each species. OBS values were found 10.97 mm for sardine (*Sardina pilchardus*), 11.29 mm for round sardine (*Sardinella aurita*), 7.78 mm for anchovy *(Engraulis encrasicolus*) and 17.89 mm for bogue (*Boops boops*). The bar spacing regulations may constitute a promising management measure to release undersized fish for the purse seine fishery in the Aegean Sea.

Keywords: Aegean Sea, sieve selectivity, sardine, round sardine, anchovy, bogue

Öz: Gırgır teknelerinin güvertesine yerleştirilen çift ızgaralı eleğin alıkoyma-bırakma özellikleri dört tür (*Sardina pilchardus, Sardinella aurita, Engraulis encrasicolus* ve *Boops boops*) için toplam uzunluk (TL) - maksimum yükseklik (Hmax) ve TL - maksimum genişlik (Wmax) ilişkilerinden tahmin edilerek ele alınmıştır. Bu ilişkileri ortaya çıkarmak için, 3 Nisan 2017 ile 21 Mart 2018 tarihleri arasında İzmir Körfezi'nde (Türkiye) gırgır örneklemeleri yapılmıştır. Ele alınan türler için regresyon eşitlikleri tanımlandıktan sonra, Sechin'in seçicilik yaklaşımının bir uyarlaması kullanılarak, her bir türün farklı çubuk aralığındaki elekler tarafından yakalanma olasılıkları hesaplanmıştır. Sonrasında minimum yasal boy ya da ilk eşeysel olgunluk boyuna karşılık gelen optimal elek çubuk aralığı (OEÇA) değerleri, her tür için ayrı ayrı hesaplanmıştır. OEÇA değerleri sardalya (*Sardina pilchardus*) için 10,97 mm, yuvarlak sardalya (*Sardinella aurita*) için 11,29 mm, hamsi (*Engraulis encrasicolus*) için 7,78 mm ve kupes (*Boops boops*) için 17,89 mm olarak bulunmuştur. Çubuk aralığı düzenlemeleri, Ege Denizi'ndeki gırgır avcılığı için yakalama boyunun altındaki balıkların serbest bırakılması için umut verici bir yönetim önlemi olabilir.

Anahtar kelimeler: Ege Denizi, elek seçiciliği, sardalya, yuvarlak sardalya, hamsi, kupes

INTRODUCTION

Purse seine is one of the most efficient gear for catching pelagic species that shoaled (Ben-Yami, 1994), providing an important part of world's total fisheries production (FAO, 2020). Although this gear targets single species detected by a sonar or echo sounder it mostly captures various species under the light source in the Aegean Sea. Purse seine is usually known as a non-selective fishing gear. As it generally targets small pelagic fishes, it uses small mesh size in the main body and bunt of the net.

In the purse seine fishery in Aegean Sea, a shoal of fish is either aggregated to artificial light or detected on the sonar. Then the purse seine vessel spills the net around the gathered fish to quickly encircle them within the purse seine net. In this way, the fish shoal is completely trapped by a long wall of netting. After setting the net, the gear is closed from the lower part by hauling the purse line. Until the volume of the net becomes smaller, the net is pulled out of the water and stacked back on the deck of fishing boat with the aid of the hydraulic power block and the crew (hauling). In the final process, the crowded fish piles are pumped to the main deck of the seiner.

The main target species of purse seine fishery are sardine, round sardine, bogue and anchovy which constitutes the largest part of the catch in the Aegean Sea (Tosunoğlu et al., 2018a, 2020). On the other hand, over 70 species of different morphological and biological characteristics are captured by the net which is always in contact with bottom while operating. Although, this fishery has a considerable number of bycatch and discard species, the present legislation about purse seine net defines neither a minimum mesh size nor a mesh shape (Anonymous, 2020). However, there are Minimum Landing Size (MLS) regulations for sardines (11.0 cm), anchovy (9.0 cm) and horse mackerels (13.0 cm), and 15% of undersized catch is permitted in terms of total weight for each species.

Purse seiners use the double-layer grid system to separate fish species from each other and to sort market size

fish. Its usage and properties are not regulated by rules. Fishers started to use the system voluntarily about five years ago in the Aegean Sea. In general usage, 9 mm/8-7 mm bar spacing grids are used to separate sardine (*Sardina pilchardus*) from anchovy (*Engraulis encrasicolus*) and 13 mm/8 mm used for larger sizes of fish e.g. round sardine (*Sardinella aurita*), chub mackerel (*Scomber colias*), Atlantic mackerel (*Scomber scombrus*), bogue (*Boops boops*), bluefish (*Pomatomus saltatrix*) to separate from sardine (Tosunoğlu et al., 2020). The second grid (bar spacing 9-8-7 mm) are also used to select undersized fish, particularly sardine. So far, no research has been found that investigated the retention patterns in these sorting grids for grading of sizes of fish species in different morphologies.

The body shape of fish may be the most important factor, necessary to understand the consequences of changes in purse seine sieve grid size in retention patterns of different species. Depending on the shape, the maximum width or height of a fish is expected to be equal or larger to the gap between the two bars of the sorting grid to retain the fish. Although width or height of the fish determines grid selectivity of the device, minimum landing size regulations are based on the total length. Width or height is more difficult to measure compared to the total length and taking these measurements may take longer time. On the other hand, these morphometrics can be converted from the length and used in selectivity calculations. In this sense, Sechin (1969) showed how the girth-converted length values be used in the calculation of gill net selectivity directly based on fish body shape.

Apart from the bar spacing of the barrier trap (Tosunoğlu et al., 2018b), there are only a few studies investigated the relationship between fish body shape and trawl cod-end mesh shape in Turkey (Tosunoğlu et al., 2003a; 2003b; Tokaç et al., 2016). In these studies, it has been attempted to determine the most appropriate mesh shape, bar spacing and hanging ratio for the major commercial fish species in accordance with their body shape. The fish morphology and mesh shape studies are also performed (Efanov, 1987; Matsushita and Ali, 1997; Mendes et al., 2006; Stergiou and Erzini, 2002; Brčić et al., 2018a, b). However, there is no study investigating the grid selectivity based on fish body shape in purse seine.

In this study, we investigated the retention patterns of the purse seine grating device based on the fish morphometry. Passing between the two bars is not directly related to the total length of the fish species, but the width of the fish, and sometimes to the height. For this reason, firstly the relationship between total length and maximum body width and maximum body height were fitted for the four mostly landed fish species; sardine, round sardine, anchovy and bogue in Aegean purse seine fishery. Then, the total length of the four fish species from various bar spacing, and the bar spacing corresponding to the MLS of these species were estimated.

MATERIAL AND METHODS

Fish samples were collected during purse seining operations on board *Afala* (23.4 m LOA, 420 HP) between 3 April 2017 and 21 March 2018. Fishing was conducted in the outer bay of the İzmir Bay in the eastern Aegean Sea (Figure 1). A total of eight operations were conducted in eight days at depths ranging between 35 and 58 m (Table 1). The purse seine net was used by *Afala* consists of 5 bulk and 1 bunt, resulting in length of 750 m and depth of 164 m. Technical characteristics of the purse seine net used in this study was given by Tosunoğlu et al. (2018a). As the targeted species are small pelagic fishes such as sardine and anchovy, the mesh size of the purse seine net is 13 mm. Therefore, we assume that there is no size and species selectivity through the meshes. After the fish school were crowded during hauling the net (bunt) by hand, a fish pump directly transfers the catch to the sieve. Overall dimensions of the sorting grid panels in the grating sieve were 190 cm in length and 95 cm in width with a 10° angle attack. A vibrate motion was given to the sieve in order to perform successful selection process.

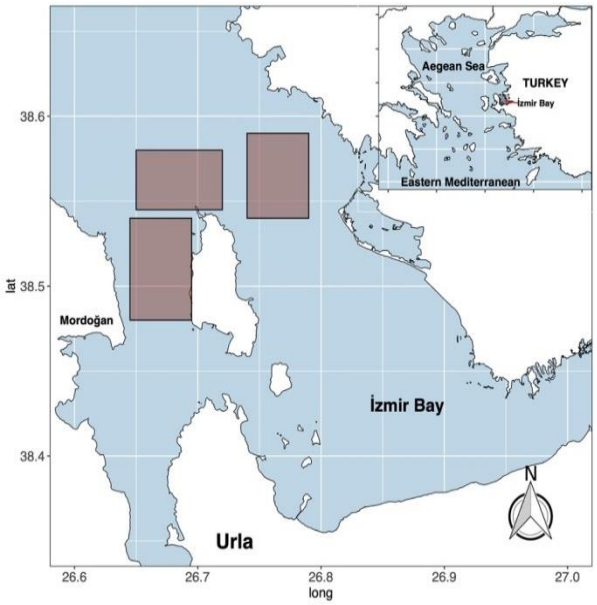

Figure 1. The map of sampling area

Table 1. Details of the purse seine operations where samplings were performed

Date	Coordinates	Depth (m)	Time
03.04.2017	38.64250° N- 26.79583° E	48	22:30-00:30
28.09.2017	38.62500° N- 26.76667° E	40	05:30-06:30
28.10.2017	38.62500° N- 26.77778° E	53	06:25-07:45
24.11.2017	38.61944° N- 26.80000° E	35	06:40-07:50
26.12.2017	38.60556° N- 26.78889° E	36	06:50-08:00
30.01.2018	38.65278° N- 26.68056° E	58	07:20-08:35
23.02.2018	38.58611° N- 26.75556° E	52	04:18-06:13
21.03.2018	38.58056° N- 26.72500° E	53	06:18-07:34

The grating sieve consists of a double layer sorting grid panel. Small-sized fishes passed through the both grid bar spaces were directly release into the sea. In these panels, fishers use 13 or 14 and 8 or 9 mm bar spacing in upper and lower compartments for larger (bogue, mackerel, bonito etc.) and smaller (sardine, anchovy etc.) fish species, respectively. However, these spaces are not precisely arranged, and their gaps may reveal small differences from the nominal classifications. For this reason, the bar spacing of the sorting grids of the sieve were measured by using a digital calliper from upper, middle and lower lines (Figure 2). Then the average and standard deviations of bar spacing were calculated for each nominal class (Table 2).

Figure 2. Double layer sorting grids of purse seine sieve and measuring bar space (top on the right)

Morphometric data was collected to determine the relevant dimensions of fish body in relation with bar spacing of various purse seine sieves. Total length (TL), the maximum height (H_{max}), the maximum width (W_{max}) and the maximum girth (G_{max}) of sardine, round sardine, anchovy and bogue, which were the most landed species in Aegean purse seine fishery, were measured to the nearest mm using a digital calliper rule. Girth measurements were taken by means of a tape measure at area of maximum cross-section of the body without applying any extra force. As minimum landing size (MLS) was forced on basis of TL, linear regressions were fitted between the TL and the other measured dimensions of the fish using ordinary least-square regression (Zar, 1999).

Table 2. Descriptive statistics of the bar spacing of the purse seine sieve sorting grids

Nominal Bar Spacing	7 mm	8 mm	9 mm	11 mm	13 mm	14 mm
Measured average bar spacing	8.0	8.3	9.0	11.1	13.0	13.6
Standard deviation	0.62	0.52	0.47	0.70	0.83	0.60
Range	3.5	2.9	2.9	4.9	5.1	2.8
Minimum	6.7	7.0	7.4	8.5	10.2	12.4
Maximum	10.2	9.8	10.4	13.4	15.4	15.1
No. of measurement	147	144	135	126	111	105
± Confidence interval (95.0%)	0.10	0.09	0.08	0.12	0.16	0.12

The grid selectivity was calculated using an adaption of Sechins' morphometric approach (Sechin, 1969). Sechins' method originally suggested for calculating gill net selectivity based on an assumption of knife edge selection by the girth. Because of the variability in the girth at length relationship, this assumption cannot be directly applied to the length. To tackle this, Sechins' method calculates the probability of retention by length from the cumulative standard normal distribution (Hovgard and Lassen, 2000). In the case of grid selectivity, we rather used the fish width, the largest dimension of considered species and assumed that a fish is retained if the maximum width of body is larger than the grid size. The behaviour of the fishes passing through the sorting grids was not considered. For selectivity calculations, we predicted W_{max} and H_{max} values corresponding to a total length sequence from 0 to 25 cm using linear models, the details of which were given above. Then, the probability of retention ($P_{retained}$) by length was calculated using the following formula (Hovgard and Lassen, 2000);

$$P_{retained} = P(W_{max} \geq C) = 1 - \phi\left(\frac{C - K * D_{max}}{\sqrt{\sigma^2 + \sigma_c^2}}\right)$$

Here C is the grid spacing, K is the effect of compressibility of fish body and assumed as 1 in this study. D_{max} is the maximum dimension of fish which is W_{max} or H_{max}. s^2 is the mean squared error (MSE) of the Length- D_{max} relationship and s^2_c is the standard deviation of the grid size measurements. "fi" is the cumulative standard normal distribution function. After we calculated the selectivity curves, we calculated the L_{50} values and Optimum grid Bar Spacing (OBS) corresponding to minimum landing sizes (MLS) for *S. pilchardus* (11cm), *S. aurita* (11 cm) and *E. encrasicolus* (9 cm) and length at first maturity for *B. boops* (13 cm, Soykan et al., 2015) since there is no size limitation on the *B. boops* fishery. All calculations were performed in R Language and Environment for Statistical Computing (R Core Team 2019).

RESULTS

We measured six different sorting grid bar spacings of the purse seine grating sieve. The measured values of bar spacings were significantly different than the nominal bar spacings. The mean, minimum and maximum TL, H and W values for four fishes were given in the Table 3. The H_{max} values of the fishes were highly significantly higher than W_{max} values in all species (paired sample t-test, $p<0.001$). Based on one-way ANOVA and Tukey post hoc test, W/H values did not significantly ($p<0.05$) differed for the two sardine species, but it did for anchovy and bogue ($p<0.001$). TL-W_{max} and TL-H_{max} regressions were presented in Figure 3 and the regression parameters were given in Table 4.

Table 3. Descriptive statistics of the morphometric characters of fishes (TL: total length, H_{max}: maximum height, W_{max}: maximum width and G_{max}: maximum girth) in cm

		$\bar{x}$	sd	Min	Max	CI (95.0%)
Sardine (n=507)	**TL**	12.00	1.02	9.90	15.79	0.09
	H_{max}	2.00	0.19	1.60	2.79	0.02
	W_{max}	1.20	0.13	0.90	1.79	0.01
	G_{max}	5.70	0.72	4.20	7.89	0.06
	W/H	0.59	0.04	0.52	0.74	0.003
Round Sardine (n=55)	**TL**	20.00	0.92	22.4	17.60	0.25
	H_{max}	3.40	0.17	3.70	3.00	0.05
	W_{max}	2.00	0.14	2.20	1.70	0.04
	G_{max}	-	-	-	-	-
	W/H	0.58	0.03	0.52	0.64	0.01
Anchovy (n=41)	**TL**	12.20	1.23	10.00	14.60	0.39
	H_{max}	1.70	0.24	1.10	2.10	0.08
	W_{max}	1.20	0.18	0.80	1.60	0.06
	G_{max}	4.70	0.73	3.20	5.80	0.23
	W/H	0.70	0.05	0.60	0.76	0.02
Bogue (n=43)	**TL**	12.70	0.91	10.60	14.40	0.28
	H_{max}	2.70	0.28	2.00	3.30	0.09
	W_{max}	1.70	0.20	1.30	2.20	0.06
	G_{max}	7.60	0.80	5.20	9.20	0.25
	W/H	0.65	0.03	0.59	0.71	0.01

Table 4. Parameters of total length- maximum width and total length-maximum height regressions

	Species	Par.	Estimate	Std. Error	t value	Pr(>|t|)	$R^2_{adj.}$	MSE*
TL-W_{max}	*S.pilchardus*	a	0.063	0.048	1.300	0.194	0.52	0.009
		b	**0.094**	0.004	23.650	<0.001		
	S.aurita	a	0.095	0.340	0.280	0.781	0.36	0.013
		b	**0.094**	0.017	5.544	<0.001		
	E.encrasicolus	a	-0.284	0.163	-1.748	0.088	0.66	0.011
		b	**0.118**	0.013	8.905	<0.001		
	B. boops	a	-0.239	0.310	-0.771	0.445	0.49	0.021
		b	**0.156**	0.024	6.421	<0.001		
TL-H_{max}	*S.pilchardus*	a	**0.216**	0.058	3.740	<0.001	0.66	0.012
		b	**0.152**	0.005	31.640	<0.001		
	S. aurita	a	0.373	0.301	1.239	0.221	0.65	0.010
		b	**0.151**	0.015	10.033	<0.001		
	E.encrasicolus	a	0.127	0.293	0.435	0.666	0.4	0.035
		b	**0.126**	0.024	5.249	<0.001		
	B. boops	a	-0.350	0.372	-0.941	0.352	0.61	0.030
		b	**0.240**	0.029	8.227	<0.001		

*MSE: Mean Squared Error of the regression

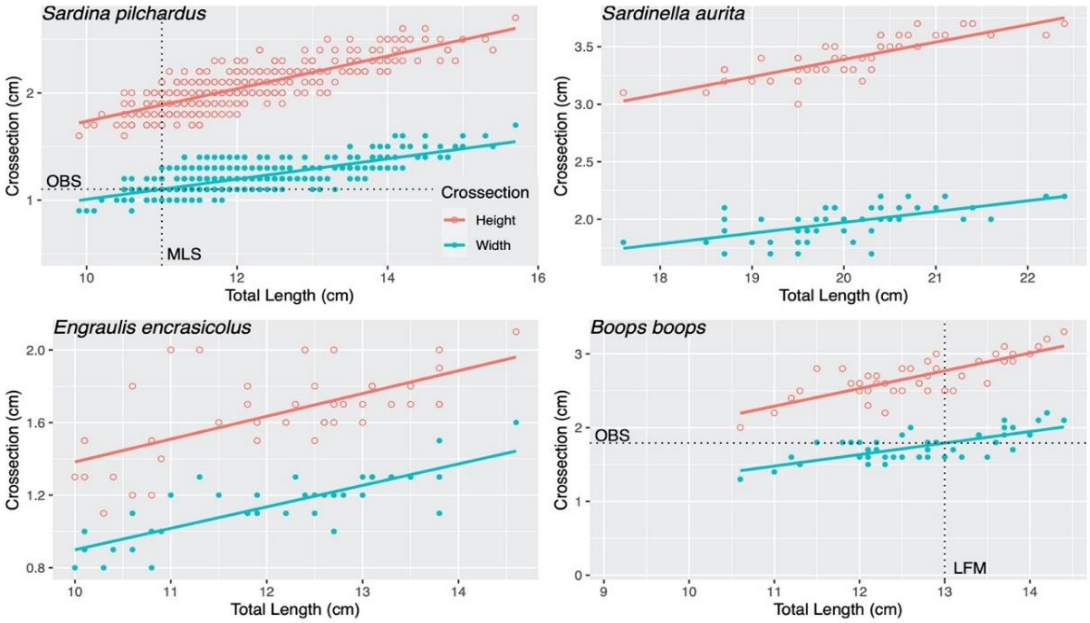

Figure 3. Total length-maximum width and total length-maximum height regressions (OBS: optimal bar spacing, MLS: minimum landing size, LFM: length at first maturity)

As the vibratory purse seine sieve eliminates the fish from its width, the maximum width of fish was taken as basis for estimating the selectivity parameters. L_{50} values of all investigated species were larger in width-based selectivity parameters compare to the height-based (Table 5). Therefore, all estimated selectivity curves for each bar spacings were given as width-based in Figure 4. From the table and figure, when the bar spacing of the purse seine sieves increases, the size selectivity of the fish also increases.

Optimal bar spacing values were found 10.97 mm for sardine, 11.29 mm for round sardine, 7.78 mm for anchovy and 17.89 mm for bogue. In our samples, all round sardine and anchovy individuals are larger than the length to be retained by their OBS. On the other hand, 9.7% for sardine and 39.5 % of bogue individuals were smaller than the OBS. However, commercial loss percentages were calculated as 35.1% for sardine and 39.5% for bogue.

Table 5. L_{50} values of investigated species in different grid sizes assuming the retention by width and height

	Grid	*S. pilchardus*	*S. aurita*	*E. encrasicolus*	*B. boops*
by Width	**s7**	7.81	7.51	9.17	6.65
	s8	8.12	7.83	9.42	6.85
	s9	8.86	8.57	10.01	7.29
	s11	11.09	10.81	11.79	8.64
	s13	13.1	12.83	13.39	9.86
	s14	13.73	13.47	13.90	10.24
by Height	**s7**	3.84	2.83	5.36	4.79
	s8	4.04	3.03	5.60	4.92
	s9	4.50	3.49	6.15	5.21
	s11	5.88	4.89	7.83	6.08
	s13	7.14	6.15	9.34	6.87
	s14	7.53	6.54	9.82	7.12

DISCUSSION

Sustainable fisheries management strictly anticipates that while fishing gears retain large fish in the catch, small juveniles are allowed to escape (Armstrong et al., 1990). Recently, there have been many studies aiming to improve towed gear selectivity by modifying gear design e.g. square mesh cod-end, escape panel and window, turned mesh and sorting grid (Lucchetti et al., 2015; STECF, 2015). Although, selectivity has been regulated by means of a legally defined minimum mesh size for many fishing gears conventionally, this is not convenient for the purse seine net. Therefore, use of sorting grids in the purse seine net (particularly in bunt) or sieve (on deck) is good solution for solving this problem.

Studies related to sorting grids mounted on the purse seine bunt shown that while these devices gave successful results for escaping undersized mackerel, operational conditions have made them very difficult to use (Misund and Beltestad, 1994). In the Aegean Sea, purse seine fishers use grating sieve consisting of double layer sorting grid on the deck of the seiner to separate fish species from each other and large sizes of the same species from small ones successfully. Although, the purse-seine fishers satisfied with the operating performance of the sieve, the scientific baseline has not yet been established about selectivity of this device and the survival rate of the fish escaped. Therefore, this study is initially important because it empirically determines the optimal bar spacing from fish body morphology for the most landed fish species in purse seine fishery.

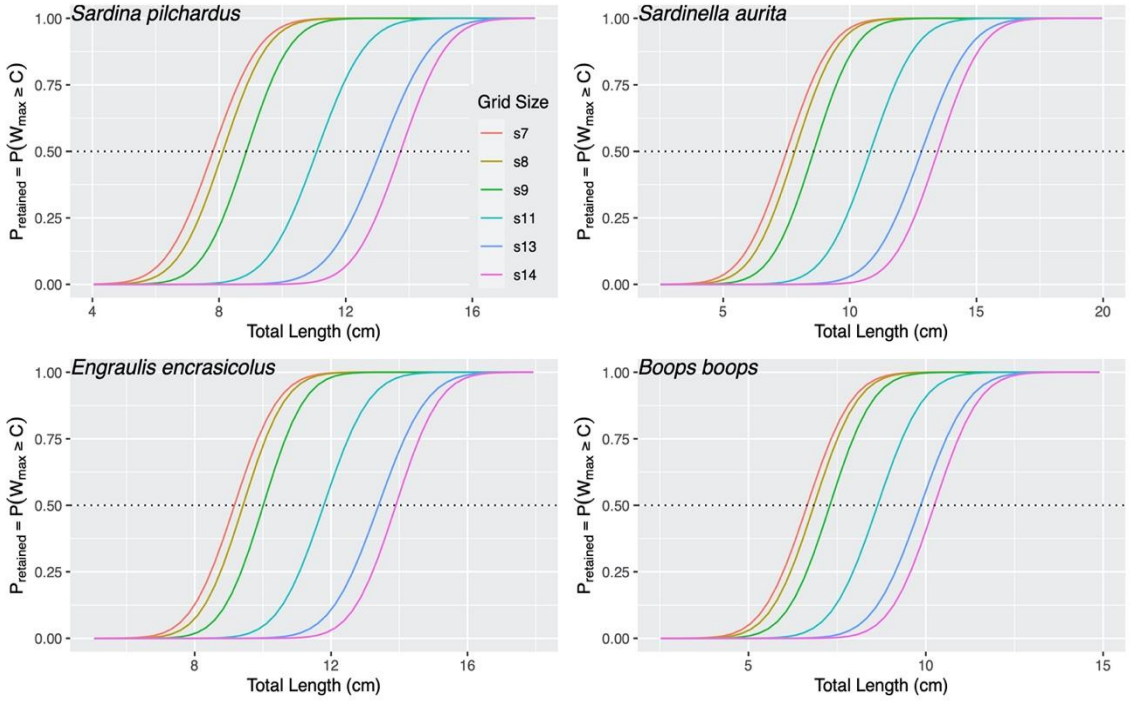

Figure 4. Selectivity curves of investigated species based on their maximum width

Diversity of fish caught in the purse seine is just like the demersal trawl which hinders the implementation of multi-species management (Tosunoğlu et al., 2018a, 2020). The reason is that there are many fish species that have different body shapes in the catch composition. For instance, sardine and gilthead sea bream have fusiform and laterally compressed body shapes, respectively. Width of sardine that corresponds to its enforced MLS is equal to 10.97 mm bar spacing. However, width of anchovy that correspond to its enforced MLS is equal to 7.78 mm. In this case, the bar spacing arranged for sardine will result in significant loss of commercial sizes of anchovy. Bar spacing of the purse seine grating sieve could be most important management measure for the exploitation of target fish since there is no selectivity in the net. The optimal bar spacing (10.97 mm) for sardine, most landed species (53.7%) in Izmir Bay purse seine fishery (Tosunoğlu et al., 2020), is thought to be suitable only for sardine with some commercial loses. However, this bar spacing is not suitable for anchovy (37.1%) and bogue (2.1%). In this situation, capture of all sizes of bogue and other larger size fishes e.g. mackerel and horse mackerels etc. below MLS is inevitable. Although other commercial fish

species such as mackerel, chub mackerel, horse mackerel and bluefish are caught in Aegean Sea purse seine fishery, these are larger sized fishes, and they all are retained on the first grid of sorting sieve. Therefore, their retention probabilities were not considered in this study.

Juveniles including mackerel, horse mackerel, round sardine and other species recruited to the fish stocks from the middle of March to end of the season (15 April) were caught by purse seiners in the Izmir Bay and other bays of the Aegean Sea (Cihangir and Ünlüoğlu, 2015). It is inevitable that the catch of these juveniles, especially those who move with sardine and anchovy shoal under the light vessel or detected by the sonar. For this reason, prevention of juvenile fish catch seems to be technically very difficult. Although it is possible to overcome this problem with the use of a grading sieve with suitable bar spaces in purse-seine fishing in the Aegean Sea.

Organizing and performing purse seine selectivity trials requires an important time, resources and effort. For this reason, we firstly used a morphometry-based approach to define the selectivity patterns of sorting sieve bar spacings in this preliminary study. This seems a promising approach in the management of data-poor fisheries. Although, this approach provided us quick exploratory results, further studies are required to understand which variables affect species and size selectivity as well as survival of selected individuals.

CONCLUSION

Every species investigated here shows a certain degree of difference in body shapes. For this reason, when the purse seine sieve selectivity is considered, maximum width of fish in relation to the maximum bar spacing could be a critical regulation measure. Regarding this issue, prediction with a simulation-based approach models developed by Herrmann et al. (2016) and Brcic et al. (2018a,b) have been applied to predict which sizes of fish are able to pass through the different bar spacing of the grating sieve in purse seine. As a result, determining or predicting the optimum bar spacing in the catching part of the purse-seine can be an effective fishing regulation method that provides maximum yield by avoiding juveniles.

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REFERENCES

Anonymous. (2020). Notification 5/1. The commercial fish catching regulations in 2020-2024 fishing period. Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture, Ankara (in Turkish).

Armstrong, D. W., Ferro, R. S. T., Maclennan, D. N. & S. A. (1990). Gear selectivity and the conservation of fish. *Journal of Fish Biology*, 37, 261-262. DOI:10.1111/j.1095-8649.1990.tb05060.x

Ben-Yami, M. (1994). *Purse seining manual*. Oxford, UK: A Division of Blackwell Scientific Publications Ltd.

Brčić, J., Herrmann, B. & Sala, A. (2018a). Predictive models for codend size selectivity for four commercially important species in the Mediterranean bottom trawl fishery in spring and summer: Effects of codend type and catch size. *PLoS One*, 13(10), e0206044, DOI:10.1371/journal.pone.0206044

Brčić, J., Herrmann, B., Mašanović, M., Šifner, S.K. & Škeljo, F. (2018b). CREELSELECT - A method for determining the optimal creel mesh: Case study on Norway lobster (*Nephrops norvegicus*) fishery in the Mediterranean Sea. *Fisheries Research*, 204, 433-440.

DOI:1016/j.fishres.2018.03.020

Cihangir, B. & Ünlüoğlu, A. (2015). Status of small pelagic fishes in the Aegean Sea. In T. Katağan, A. Tokaç, Ş. Beşiktepe, & B. Öztürk B (Ed.), *The Aegean Sea marine biodiversity, fisheries, conservation and governance* (pp. 303-323). Turkish Marine Research Foundation (TUDAV).

Efanov, S. F., Istomin, I.G. & Delmatov, A. A. (1987). Influence of the form of fish body and mesh on selective properties of trawls. ICES C.M. 13.

FAO, (2020). FAO Yearbook. Fishery and Aquaculture Statistics 2018. http://www.fao.org/documents/card/en/c/cb1213t, DOI: 10.4060/cb1213t

Herrmann, B., Larsen, R.B., Sistiaga, M.B., Madsen, N., Aarsæther, K.G., Grimaldo, E. & Ingolfsson, O. (2016). Predicting size selection of cod (*Gadus morhua*) in square mesh codends for demersal seining: simulation-based approach. *Fisheries Research*, 184, 36-46. DOI: 10.1016/j.fishres.2015.07.015

Hovgard, H. & Lassen, H. (2000). *Manual on estimation of selectivity for gillnet and longline gears in abundance surveys*. FAO Fisheries Technical Paper No. 397, 84 pp.

Lucchetti, A., Sala, A., Kholeif, S. & Notti, E. (2015). *Towards sustainable fisheries management*. Nova Science Publishers, Inc., New York, 276 pp.

Matsushita, Y. & Ali, R. (1997). Investigation of trawl landings for the purpose of reducing the capture of non-target species and sizes of fish. *Fisheries Research*, 29, 133-143. DOI: 10.1016/S0165-7836(96)00534-6

Mendes, B., Fonseca, P. & Campos, A. (2006). Relationships between opercula girth, maximum girth and total length of fish species caught in gillnet and trammel net selectivity surveys off the Portuguese coast. *Journal of Applied Ichthyology*, 22, 209-213. DOI: 10.1111/j.1439-0426.2006.00734.x

Misund, O. A. & Beltestad, A. K. (1994). Size selection of mackerel and saithe in purse seine. ICES CM, 1994/B:28.

Sechin, Y. T. (1969). A mathematical model for the selectivity curve of a gillnet. *Rbyn. Khoz*., 45, 56-58.

Stergiou, K. I. & Erzini, K. (2002). Comparative fixed gear studies in the Cyclades (Aegean Sea): Size selectivity of small-hook longlines and monofilament gillnets. *Fisheries Research*, 58, 25-40. DOI:10.1016/S0165-7836(01)00363-0

Soykan, O., İlkyaz, A. T., Metin, G. & Kınacıgil, H. T. (2015). Growth and reproduction of *Boops boops, Dentex macrophthalmus, Diplodus vulgaris* and *Pagellus acarne* (Actinopterygii: Perciformes: Sparidae) from East-Central Aegean Sea, Turkey. *Acta Ichthyologica et Piscatoria*, 45, 39-55. DOI:10.3750/AIP2015.45.1.05

STECF, (2015). Landing Obligation - Part 6 (Fisheries targeting demersal species in the Mediterranean Sea) (STECF-15-19). Publications Office of the European Union, EUR 27600 EN, JRC 98678, 268 p.

Tokaç, A., Herrmann, B., Gökçe, G., Krag, L. A., Nezhad, D. S., Lök, A., Kaykaç, H., Aydın, C. & Ulaş, A. (2016). Understanding the size selectivity of red mullet (*Mullus barbatus*) in Mediterranean trawl codends: A study based on fish morphology. *Fisheries Research*, 17, 81-93. DOI:10.1016/j.fishres.2015.09.002

Tosunoğlu, Z., Doğanyılmaz, Y. & Özbilgin, H. (2003a). Body shape and trawl codend selectivity for nine commercial fish species. *Journal of the Marine Biological Association of the United Kingdom*, 83, 1309-1313. DOI:10.1017/S0025315403008737

Tosunoğlu, Z., Doğanyılmaz, Y. & Özbilgin, H. (2003b). Determination of the appropriate hanging ratios to ease the escape of juvenile red mullet (*Mullus barbatus* L., 1758) and annular sea bream (*Diplodus annularis* L., 1758) from trawl cod-end. *Turkish Journal of Veterinary and Animal Sciences*, 27, 1193-1199.

Tosunoğlu, Z., Aydın, C., Metin, G., Kaykaç, M.H. & Düzbastılar, F. O. (2018a). Researches on Izmir Bay (Aegean Sea, Turkey) purse seine fishery (*in Turkish*). Ege University Scientific Research Project Coordination Unit, Project No: 2017/SUF/002, 75 p (Unpublished).

Tosunoğlu, Z., Karakuzu, R. O., Gökçe, G. & Kaykaç, M. H. (2018b). Fish morphology and barrier trap bar spacing in lagoon fisheries along the Aegean Coast of Turkey. *Mediterranean Fisheries and Aquaculture Research*, 1, 49-56.

Tosunoğlu, Z., Düzbastılar, F. O., Kaykaç, M. H., Aydın, C., Metin, G. & Güleç, Ö. (2020). Effect of Sorting Grid System on Izmir Bay Purse Seine Fishery: Species-Size Selectivity and Survival Rates (*in Turkish*). TUBITAK 118O317 Final Report, Ankara, 112 p (Unpublished).

Zar, J. H. (1999). *Biostatistical analysis*. 4 th ed. New Jersey, NJ, USA: Prentice- Hall, Upper Saddle River, New Jersey.

http://www.egejfas.org
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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effect of hook and bait type on catch per unit effort in the Aegean Sea demersal longline fishery

Ege Denizi dip paragat balıkçılığında iğne ve yem tipinin birim çabada av miktarına etkisi

Ozan Soykan[1] ● Cemil Sağlam[2]* ● İlker Aydın[3] ● Hasan Tuncay Kınacıgil[4]

[1] Ege University, Faculty of Fisheries, Bornova, İzmir, Turkey https://orcid.org/0000-0002-2227-1245
[2] Ordu University, Fatsa Faculty of Marine Sciences, Fatsa, Ordu, Turkey https://orcid.org/0000-0003-1430-1579
[3] Ege University, Faculty of Fisheries, Bornova, İzmir, Turkey https://orcid.org/0000-0003-1752-2780
[4] Ege University, Faculty of Fisheries, Bornova, İzmir, Turkey https://orcid.org/0000-0001-5707-1684

*Corresponding author: csaglam@odu.edu.tr
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Abstract: This study aimed to determine the impact of hook and bait type on the catch composition and catch per unit effort. Effects of hook and bait types on catch composition, catch per unit effort (CPUE), length and weight distributions in demersal longline fishery were determined by experimental surveys on demersal longline sets in the Aegean Sea. A total of 12 samplings corresponding to 4800 hook fishing effort were performed between April 2014 and September 2014. Two bait types; sardine (*Sardina pilchardus*) and grooved razor shell (*Solen marginatus*) and two hook types; J-hook and C-hook were tested. CPUE values were calculated for each species and assessed between different hook-bait combinations.

A total of 623 individuals were captured belonging to 3 families and 9 species. It was found that more than 60% of total catch was captured by grooved razor shell and more than 50% of the total catch was caught with J type hook. J hook was found to be close to significant (p=0.06) and grooved razor shell was found significant (p=0.02) for CPUE. The effect of bait type was found to be more significant than that of hook type for CPUE and length distribution. Hook-bait combination differed according to species and C hook baited with sardine was determined to be the best combination for *Sparus aurata* as the most targeted fish in the study area. Discard ratio was calculated to be 34% in terms of weight and 42.5% in terms of total number of individuals for pooled data. The condition value (K) of the species ranged from 1.05 to 1.68 and differed according to bait type.

Most of the high commercial value species caught with any hook-bait combination experimented within this study are larger than minimum fishing length according to minimum landing size regulations of Turkish fishery and maturity studies.

Keywords: Demersal longline, hook style, bait type, CPUE

Öz: Bu çalışmada iğne ve yem türlerinin, avlanan balık av kompozisyonu ve birim çabada av miktarı (BÇAM) üzerindeki etkisinin belirlenmesi amaçlanmıştır. İğne ve yem türlerinin av miktarına, birim çabada av miktarına (BÇAM), boy ve ağırlık dağılımlarına etkisi, Ege Denizi'nde kullanılan deneysel dip paragatları ile saptanmıştır. Toplam 12 adet tekrar ile 4800 adet iğne kullanılarak Nisan 2014 ve Eylül 2014 ayları arasında örnekleme yapılmıştır. İki yem tipi olarak Sardalya (*Sardina pilchardus* Walbaum, 1972) ve Sülünez (*Solen marginatus* Pulteney, 1799) ve iki iğne tipi olarak J-iğne ve C-iğne test edilmiştir. BÇAM her tür için ve farklı iğne-yem tiplerine göre hesaplanmıştır.

3 aileye ait farklı 9 türden 623 adet balık yakalanmıştır. Toplam avın %60'ından fazlasının sülünez ve %50'den fazlasının J tipi iğne ile yakalandığı bulunmuştur. BÇAM için J tipi iğne ile avcılık istatistiksel olarak önemli değere yakın (p=0.06) ve sülünez ile avcılık istatistiksel olarak önemli (p=0.02) bulunmuştur. Yem türünün BÇAM ve balık boyutu üzerindeki etkisi, iğne türünden daha önemli bulunmuştur. İğne-yem kombinasyonu türlere göre farklılık göstermektedir ve sardalya ile yemlenen C tipi iğne, çalışma alanında en çok hedeflenen balık olan *Sparus aurata* için en iyi kombinasyon olarak belirlenmiştir. Toplanan veriler için ıskarta oranı ağırlığa göre % 34.0 toplam tür sayısı açısından% 42.5 olarak hesaplanmıştır. Türlerin kondüsyon değeri (K) 1.05 ile 1.68 arasında değişmekte ve yem türüne göre farklılık göstermektedir. Bu çalışmada kullanılan iğne-yem kombinasyonu ile yakalanmış yüksek ticari değeri bulunan türlerin çoğu, yasal avlanma boyu ve cinsi olgunluk çalışmalarına göre, minimum avlanma boyundan büyüktür.

Anahtar kelimeler: Dip paragatı, iğne tipi, yem tipi, BÇAM

INTRODUCTION

A longline is composed of the principal line along which, at various places snoods equipped with baited hooks are attached. This fishing apparatus can be compared to a series of lines placed at regular intervals and left in the water for a few hours. Longlining, as a traditional fishing method, is perhaps one of the most ancient as it was used from the shore. Depending on the species of fish being sought, the longline can be set at different depths; demersal, pelagic or mixed (FAO, 1993).

Longline fishing is considered to be more selective than other fishing gears like gillnets or trawls (Gilman et al., 2006), even so, both demersal and pelagic longline fisheries experience by-catch of important species such as marine mammals, sea birds, sea turtles and sharks (Gilman et al 2006, 2008; Lewison et al., 2004; Soykan et al., 2008). Because of that some studies have been done on the effects of bait types and hook styles in the commercial and recreational fisheries (Coelho et al., 2012; Foster et al., 2012;

Santos et al., 2012, 2013; Fernandez-Carvalho et al., 2015; Amorim et al., 2015; Sistiga et al., 2018).

Longline fishery constitutes an important part of the Aegean and Mediterranean coasts of Turkish Seas. There have been 14479 commercial fishing vessels and 31842 fishing employees (fishermen) in Turkey and 3845 of those are longline vessels creating a population of 3714 fishermen in the field of longlining (Anonymous, 2017). Demersal longline fishery is an important fishing method for small-scale fishermen in İzmir Bay. Although there are some studies that have focused on different aspects of longline fishery for Turkey (Ulaş and Düzbastılar, 2001; Özyurt et al., 2003; Çekiç and Başusta, 2004, Erdem and Akyol, 2005; Özdemir et al., 2006; Akyol et al., 2007; Özgül et al., 2015; Gülşahin and Soykan, 2017; Güçlüsoy et al., 2020), information regarding the bait, hook size, catch composition, discard ratios and catch per unit effort (CPUE), are scarce and variable.

The aim of the present paper is to determine the hook and bait effects on the catch composition, length and weight distributions, catch per unit effort and discard ratios of demersal longline fishery.

MATERIAL AND METHODS

This study was carried out in Urla region located in the central Aegean Sea from a research boat named "Nereis" which belongs to Ege University Faculty of Fisheries during the period from April to September 2014 (Figure 1). Fishing trials were performed daily and 4 longline sets each including 100 hooks were used during operations. The experimental longline design consisted of two bait and two hook types in each longline set. Polyester material braided rope with a diameter of 0.45 mm was used as the main line, while the snoods were made of 0.30 mm diameter nylon monofilament. Traditional straight (J style) hooks were used together with alternative circle (C style) hooks on the experimental longline and hooks were prepared along the main line as one J hook and one C hook one after the other (J, C, J, C,…).

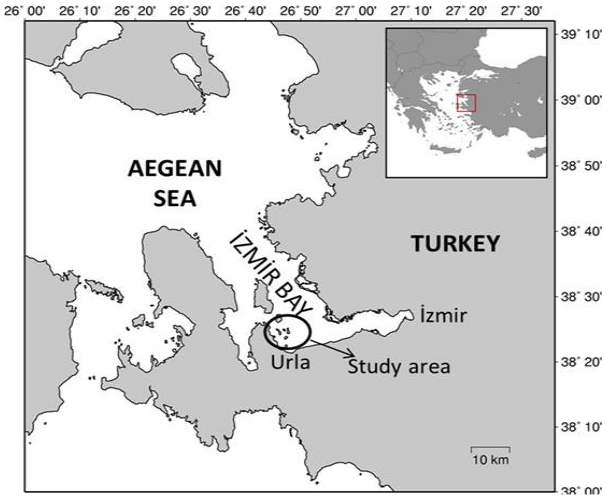

Figure 1. Study area

J style hook (Model: Owner 50560 Furansu, Figure 2) is being used traditionally in the Aegean Sea by small-scale fishermen in longline fishery and angling. C style hook (Model: Owner 50660 Mutu, Figure 2) was used alternatively in order to compare the efficiency of hook styles. Baits were sardine (*Sardina pilchardus*) and grooved razor shell (*Solen marginatus)* and they were placed alternately on each hook type. Only one bait type was used in each set-in order to avoid possible interaction effects as suggested by Watson et al. (2005). Bait pieces were standardized to 3 cm long in order to avoid the effect of bait size on fish length (Soykan et al., 2016). Point, barb and the bend of the hooks were totally covered with bait. Totally 4800 hooks were examined corresponding to 1200 hook/bait combinations. The period of fishing operations was set to noon to 2 hours between 5-20 m depths.

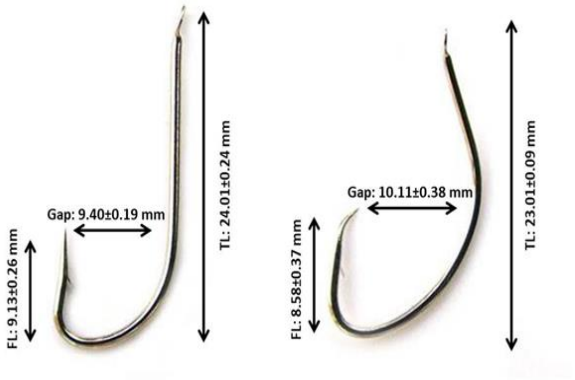

Figure 2. Hook styles and their measurements with standard deviation (TL = Total length, FL = Front length)

Captured specimens were brought to the laboratory and total length (TL) was measured in the natural body position to the nearest mm. Total weight (W) was measured to the nearest 0.1 g. Information such as geographical position, date, timing was recorded for each longline operation.

Catch per unit effort (CPUE) value was calculated in weight (g) per 100 hooks for each species in each fishing set (including sets with zero catches for those particular species) and the mean CPUE with the respective standard deviations for each hook-bait combination was calculated. CPUE data was tested for normality with Kolmogorov–Smirnov tests with Lilliefors correction (Lilliefors, 1969) and for homoscedasticity with Levene's tests. Normality and homoscedasticity assumptions were violated, so non-parametric test (Mann–Whitney) was employed to test for differences among the two baits and two hook types.

The size distribution of species and condition factor were compared according to different hook styles and bait types. The skewness and the kurtosis of the size data were calculated to assess departures from normality, and the results indicated that parametric tests were not appropriate to compare mean sizes among treatments, so for each hook-bait

combination, the mean size and the mean condition factor and its respective standard deviation were calculated and Mann–Whitney U test was used to compare the sizes between hooks styles and between bait types.

Fulton's condition factor:

$K = (W L^{-3}) 100$

where W, the weight and L, total length of the fish, was also used to calculate K (condition) of each fish.

Length-weight relationship (LWR) was calculated for all species. Parameters of LWR for six fish species were estimated by logarithmic transformation: log W= log *a* + *b* log L with *a* and *b* determined via least-squares regression.

Discard ratio (dr) was calculated with the formula given below (Kelleher, 2005):

$Dr = (Discards \times Total\ catch^{-1}) 100$

Accuracy of the growth parameters was examined by the t-test and the statistical analyses were performed using the R project for Statistical Computing Version 3.4.2 (R Core Team, 2017).

RESULTS

Longline operations resulted in 9 species belonging to 3 families. A total of 623 individuals corresponding to 36.8 kg were sampled and 58.1% belonged to sparids, 40.6% to serranids. *D. annularis* dominated the catch composition in terms of number with 28.2% and followed by *S. cabrilla* (27.6%) and *B. boops* (15.9%). *G. niger* was represented with the least number of individual (1.3%). Three species, *S. aurata, D. annularis* and *B. boops* composed more than the half of the total catch in terms of weight with 24%, 17%, 12% respectively. The contribution of the other sparid species, *P. erythrinus*, *L. mormyrus* and *D. vulgaris* on the total catch weight was calculated to be 8%, 5% and 1% respectively. Representatives of the family serranidae composed almost $^1/_3$ of total catch in weight and *S. cabrilla* and *S. scriba* had each 16% ratio within the total catch. *G. niger* represented 1% of the total weight.

While 39.4% of the total catch in terms of weight was captured by sardines, 60.6% was obtained by grooved razor shell. On the other hand, according to number-based distribution, 32.1% of the total catch belonged to sardine and the rest (67.9%) to grooved razor shell. 44.7% of the total catch weight was performed by C hook and 55.3% by J hook. Regarding the number-based distribution of the whole catch, 43.3% was done with C hook and 56.7% with J hook.

Discard ratio was calculated to be 34% in terms of weight and % 42.5 in terms of total number of individuals for pooled data. Moreover, weight-based discard ratio of C hook baited with sardine and with grooved razor shell were calculated to be 4.22% and 10.85%, J hook baited with sardine and with grooved razor shell were 5.84% and 11.75% respectively. Discard ratios in terms of number of captured fish according to hook and bait type also differed. C hook baited with sardine and with grooved razor shell were found to be 6.26% and 12.36%, J hook baited with sardine and with grooved razor shell were calculated as 6.58% and 17.34 % respectively.

The a and b parameters of the LWR are presented in Table 1. The b values varied from 2.40 (*B. boops*) to 3.31 (*G. niger*). Isometric growth was observed for *D. annularis*, *P. erythrinus*, *D. vulgaris*. Species with positive allometric growth were *S. cabrilla*, *G. niger* and negative allometric growth was observed for *B. boops*, *S. aurata*, *L. mormyrus*, *S. scriba*.

Table 1. Length-weight relationship parameters (95% CI), descriptive statistics (mean, min-max, standart error) and fish status (C: commercial, NC: noncommercial) (* Length-weight relationship parameters a and b range of min-max for 9 species of Fishbase data in Mediterranean Sea (Froese and Pauly, 2018))

	n	a	b	R^2	Length	Weight	Status	a*	b*
SPARIDAE									
Diplodus annularis (Linnaeus, 1758)	176	0.0130 (0.0097-0.0174)	3.09 (2.98-3.21)	0.94	12.6±0.13 (9.7-18.8)	35.1±1.21 (15.9-100.0)	NC	0.004-0.080	2.68-3.95
Boops boops (Linnaeus, 1758)	99	0.0589 (0.0444-0.0780)	2.40 (2.30-2.50)	0.96	16.3±0.24 (12.1-20.5)	49.4±1.68 (22.0-85.00)	C	0.002-0.17	2.81-3.52
Sparus aurata (Linnaeus, 1758)	41	0.0250 (0.0203-0.0307)	2.78 (2.72-2.84)	0.99	26.5±0.89 (18.7-36.0)	253.6±23.61 (87.2-570.0)	C	0.006-0.027	2.74-3.34
Pagellus erythrinus (Linnaeus, 1758)	28	0.0118 (0.0060-0.0231)	3.05 (2.81-3.30)	0.96	15.5±0.28 (13.5-18.5)	52.5±2.92 (32.6-85.9)	C	0.011-0.099	2.43-3.12
Lithognathus mormyrus (Linnaeus, 1758)	12	0.0588 (0.0175-0.1975)	2.51 (2.11-2.91)	0.95	21.1±0.79 (17.0-25.4)	127.0±12.4 (72.0-200.0)	C	0.006-0.026	2.47-3.45
Diplodus vulgaris (GeoffroySaint-Hilaire, 1817)	6	0.0375 (0.0134-0.1052)	2.66 (2.30-3.03)	0.99	17.3±0.36 (16.0-18.2)	74.3±4.07 (60.7-86.9)	C	0.003-0.086	2.43-3.59
SERRANİDAE									
Serranus scriba (Linnaeus, 1758)	172	0.0263 (0.0210-0.0329)	2.74 (2.65-2.82)	0.96	13.6±0.18 (9.0-17.7)	35.3±1.23 (10.4-66.2)	C	0.004-0.030	2.72-3.41
Serranus cabrilla (Linnaeus, 1758)	81	0.0088 (0.0063-0.0124)	3.18 (3.06-3.30)	0.97	16.1±0.35 (12.4-22.8)	69.4±5.06 (26.00-191.00)	NC	0.009-0.073	2.41-3.22
GOBIIDAE									
Gobius niger (Linnaeus, 1758)	8	0.0072 (0.0038-0.0136)	3.31 (3.05-3.56)	0.99	11.9±0.69 (9.7-14.7)	28.4±5.09 (12.50-51.00)	NC	0.005-0.017	2.84-3.39

Effects of the hook and bait type on the catch rates were assessed for each species. While the effect of bait was found to be significant for five species (*P. erthtinus, L. mormyrus, B. boops, S. scriba, D. vulgaris*) the effect of hook was differed significantly for only two species (*D. annularis, S. cabrilla*). Hooks baited with grooved razor shell had significantly higher CPUE than hooks baited with sardine for three commercial species *P. erythrinus*, *L. mormyrus*, *D. vulgaris* (Table 2). The catch of *Serranus cabrilla* had significantly higher CPUE values when using J hook in comparison to C hook. On the contrary, CPUE value of *D. annularis* differed significantly in favour of C hook. *B. boops* and *S. scriba* had significantly higher CPUE values with sardine than that of grooved razor shell (Figure 3, Table 2).

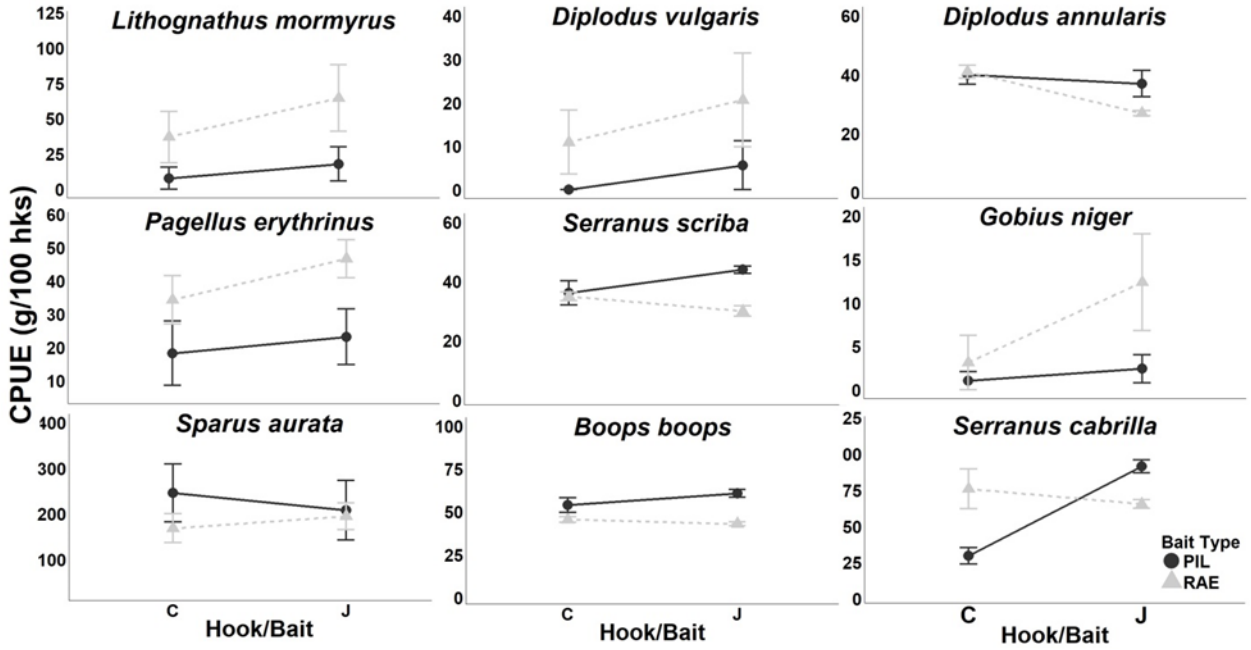

Figure 3. Catch per unit effort (CPUE, g/100 hooks) for target, bycatch and discard species. FAO codes for *Sardina pilchardus* (PIL) and *Solen marginatus* (RAE). Points refer to the means and error bars refer to the standard errors

Table 2. Mean CPUE (g/100 hooks) with respective standard deviation between parentheses, for the various hook-bait combinations. p-Values from non-parametric tests refer to Mann–Whitney U tests to compare bait types and hook styles (* sig. at the 10% level; ** sig. at the 5% level; *** sig. at the 1% level)

Species (n)	*Sardina pilchardus* J	*Sardina pilchardus* C	*Solen marginatus* J	*Solen marginatus* C	Bait	Hook
Sparus aurata	207.6 ±225.9 (7)	245.7 ±218.9 (8)	195.1 ±100.9 15	168.8 ±109.8 (11)	0.85	1.00
Pagellus erythrinus	23.2 ±28.9 (5)	18.3 ±33.4 (3)	46.7 ±19.9 (11)	34.3 ±25.2 (9)	0.03**	0.21
Lithognathus mormyrus	17.8 ±41.7 (2)	7.8 ±26.8 (1)	64.5 ±81.2 (5)	36.9 ±62.9 (4)	0.04**	0.36
Diplodus annularis	36.9 ±15.5 (27)	39.9 ±11.0 (29)	26.9 ±3.0 (68)	41.0 ±7.7 (52)	0.24	<0.01***
Boops boops	60.6 ±7.9 (25)	53.8 ±14.6 (12)	42.9 ±4.0 (31)	45.7 ±5.7 (31)	<0.01***	0.58
Serranus scriba	43.9 ±4.3 (37)	36.0 14.1 (19)	29.9 ±6.1 (62)	35.0 ±5.1 (54)	<0.01***	0.46
Serranus cabrilla	91.4 ±15.6 (12)	29.6 ±19.5 (9)	65.3 ±10.2 (36)	75.8 ±47.3 (24)	0.59	<0.01***
Diplodus vulgaris	5.6 ±19.4 (1)	0	20.6 ±37.4 (3)	10.9 ±25.5 (2)	0.08*	0.34
Gobius niger	2.4 ±5.6 (2)	1.0 ±3.6 (1)	12.4 ±19.3 (4)	3.1 ±10.8 (1)	0.33	0.12
Total	**54.4 ±96.3**	**48.0 ±102.2**	**56.0 ±68.6**	**50.1 ±64.7**	**0.02****	**0.06***

Effect of hook and bait type on fish size were determined. While the bait effect was found to be statistically significant for 6 species (*S. aurata, P. erythrinus, D. annularis, B. boops, S. scriba, G. niger*), hook effect was found to be distinctive for two species (*D. annularis, S. cabrilla*) (Table 3). Examination of hook type differences showed that the mean size of *D. annularis* caught with C hook was found to be slightly higher than that of J hook. In contrast, *S. cabrilla* captured by J hook were bigger than that of C hook. According to bait types, average size of fish captured by *S. pilchardus* were significantly higher for *S. aurata*, *P. erythrinus*, *D. annularis*, *B. boops*, *S. scriba* than grooved razor shell did. Hook and bait types were determined to be non-effective for *L. mormyrus* (Table 3). Maximum number of individuals for each species was captured by J hook-grooved razor shell combination.

Table 3. Mean sizes with the respective standard deviation (between parentheses) p-values refer to the Mann–Whitney tests to compare sizes with different baits and with different hooks (* sig. at the 10% level; ** sig. at the 5% level; *** sig. at the 1% level)

	Hook style		Bait type		Bait	Hook
	J	C	*Solen marginatus*	*Sardina pilchardus*		
Sparus aurata	26.3 ±5.8	26.7 ±5.8	24.3 ±4.4	30.4 ±5.7	<0.01***	0.81
Pagellus erythrinus	15.6 ±1.1	15.4 ±1.9	15.1 ±1.4	16.5 ±1.2	0.02**	0.45
Lithognathus mormyrus	22.3 ±1.4	19.5 ±3.4	21.4 ±3.1	20.2 ±0.8	0.48	0.11
Diplodus annularis	12.0 ±1.5	13.3 ±1.7	12.4 ±1.6	13.0 ±1.8	0.04**	<0.01***
Boops boops	16.5 ±2.3	16.0 ±2.5	15.5 ±2.3	17.7 ±1.8	<0.01***	0.61
Serranus scriba	13.5 ±2.5	13.7 ±2.2	13.1 ±2.4	14.6 ±1.9	<0.01***	0.52
Serranus cabrilla	16.5 ±2.6	15.5 ±3.7	16.1 ±3.4	16.1 ±2.3	0.61	0.03**
Diplodus vulgaris	17.6 ±0.7	16.5 ±0.7	17.4 ±1.0	16.6 ±0.0	0.66	0.26
Gobius niger	12.1 ±2.0	11.6 ± 2.5	13.2 ±1.2	9.9 ±0.2	0.04**	0.85

The condition value (K) of the species ranged from 1.05 to 1.68. It was considered that that hooks baited with grooved razor shell were more attractive for higher conditioned specimens of *B. boops* ($p<0.01$) and *S. aurata* ($p<0.05$). On the contrary, sardine bait was found to be more efficient for specimens of *S. cabrilla* which have higher conditions (Table 4).

L. mormyrus was the only species with a statistical difference on condition factor between the hook types. It can be declared that individuals in better conditions were captured by C type hook for this species. Consideration of bait types showed that better conditioned individuals of *B. boops S. aurata* and *G. niger* were captured by *S. marginatus*. Better conditioned specimen of *S. cabrilla* were caught by the bait *S. pilchardus* (Table 4).

Table 4. Condition factor of the species with the respective standard deviation (between parentheses) p-values refer to the Mann–Whitney tests to compare sizes with different baits and compare sizes with different hooks (* sig. at the 10% level; ** sig. at the 5% level; *** sig. at the 1% level)

	Hook style		Bait type		Bait	Hook
	J	C	*Solen marginatus*	*Sardina pilchardus*		
Serranus cabrilla	1.48±0.14	1.45±0.18	1.39±0.13	1.56±0.23	0.00***	0.28
Serranus scriba	1.35±0.19	1.30±0.11	1.33±0.17	1.29±0.10	0.21	0.11
Diplodus annularis	1.63±0.15	1.67±0.18	1.64±0.17	1.68±0.17	0.14	0.24
Boops boops	1.10±0.14	1.13±0.14	1.15±0.14	1.05±0.10	0.00***	0.31
Sparus aurata	1.22±0.08	1.24±0.06	1.24±0.07	1.21±0.08	0.03**	0.59
Pagellus erythrinus	1.36±0.08	1.37±0.07	1.36±0.08	1.38±0.06	0.82	0.98
Lithognathus mormyrus	1.26±0.08	1.40±0.16	1.34±0.15	1.25±0.03	0.58	0.07*
Diplodus vulgaris	1.43±0.03	1.45±0.04	1.43±0.03	1.47±0.00	0.67	0.80
Gobius niger	1.56±0.06	1.46±0.19	1.59±0.02	1.44±0.10	0.04**	0.64

DISCUSSION

Longline fishery has an important role in the small-scale fishery of the Aegean Sea. The main reason for this importance is low of fishing expenditures generally brings commercially valuable species. Moreover, small-scale fishermen and recreational anglers are using similar hook and bait types in longline and handline fisheries. For this reason, studies regarding the hook styles and bait types are important for both recreational and commercial fishermen. Although some studies were carried out on hook and bait types, commercial and discard ratios of longline fishery and length-

weight relationships of captured specimen in the study area, our paper covers the assessment of these parameters together for the first time.

When we examined CPUE with hook type, no significance was found for commercial species, but significant effect was detected for two discard species, *D. annularis* and *S. cabrilla*. CPUE value of C hook was slightly higher for *D. annularis*, conversely it was lower for *S. cabrilla*. which was reported as a discard species in many studies (Akyol, 2003; Aydın et al, 2008; Gökçe and Metin, 2007). On the other hand, Gülşahin and Soykan (2017) reported this species to be commercial in the South Aegean Sea. The reason for this case is attributable to dynamic structure of the word "discard" as it is affected by many factors and probably the most important ones are "regionality" and "consumer's preference". Total CPUE result showed that J hook has slightly higher than C hook for CPUEs. So, J hook is suggested for fishermen regardless of the quality of the catch. Beside the hook type, significant influence of hook size on the body size was also emphasized for white seabream and gilthead sea bream in Foça (Güçlüsoy et al., 2020).

Grooved razor shell and sardine are two of the most popular and cheap baits for commercial and recreational fishermen on the Turkish coast of the Aegean Sea. That's why scientific literature on the bait type of longline fishery in the study area was accumulated for the grooved razor and sardine. Özdemir et al (2006) used different bait types, squid (*Loligo vulgaris*) and sardine in Urla region and they caught 22% of the hall catch with sardine in demersal longline. Soykan et al (2016) reported that more than half of the catch by sardine (32%) and grooved razor (23%) in Urla and Çeşme region. Maktay (2012) examined the effects of deep water rose shrimp (*Parapenaeus longirostris*) and sardine as baits on the catch composition of longlining in Urla and reported the dominance of sardine with 77.4%. Çekiç and Başusta (2004) revealed that 55.3% of the total catch was captured by sardine and the rest by common cuttlefish (*Sepia officinalis*) in İskenderun Bay. In this study we found that catch amount for grooved razor shell was higher than that of sardine. Bait types were found significantly different for commercial species CPUEs, except *S. aurata.* CPUEs of grooved razor shell was slightly higher for *P. erythrinus*, *L. mormyrus* and *D. vulgaris* and CPUEs of sardine was slightly higher for *B. boops*, *S. scriba*. It was also observed that, commercial value of fish captured by grooved razor shell were higher than that of those captured with sardine depending on fish market prices in the region. Comparison of total CPUEs of the baits showed that grooved razor shell had significantly higher fishing effect. Therefore, grooved razor shell may be a better recommendation than sardine during bait choice in the study area.

It was determined that, hook type did not affect the condition of the captured individuals. On the other hand, bait was found to be significant for two commercial (*B. boops, S. aurata*) and two discard (*S. cabrilla, G. niger*) species. Aydın (2011), reported condition factor values of *B. boops* and *D. annularis* captured by sardine and razor shell to be 1.18±0.13, 1.07±0.09 and 1.53±0.07, 1.72±0.14 respectively in the same area. On the contrary, in this study, condition factor of *B. boops* captured by razor shell was greater than Aydın's (2011) result. The difference is attributable to sampling technique. This may lead to make a further consideration that different fishing gears may have different effects on condition of the same species. In addition, while bigger individuals of *S. aurata* and *B. boops* were captured with sardine bait, more conditioned individuals of the same species were caught with grooved razor shell. This case showed that bait choice could differ between different size individuals of the same species. It was also reported that many factors affect the condition of fish such as sex and reproduction period, water temperature and salinity, sex and food availability (Aydın, 2011; Tesch, 1971; Moutopoulos and Stergiou, 2002)

Among the species composition of the study, *S. aurata*, *P. erythrinus*, *L. mormyrus*, *B. boops*, *D. vulgaris,* and *S. scriba* are of commercial importance in the Aegean Sea longline fishery. In this study discarded catch composition composed of 3 species (*D. annularis, S. cabrilla, G. niger*) and number-based discard ratio was 42.5%. Discard ratios of the previous studies (Özdemir et al., 2006; Maktay, 2012; Odabaşı, 2013; Aydın and Bolat, 2014; Soykan et al., 2016; Gülşahin and Soykan, 2017) performed in Turkish coast of Aegean Sea ranged from 6.5% to 55.5% and our result is between the limits. The reason for this big range is due to quality and quantity of discards in longline fishery depends on several factors such as region, technical features of the gear, bait type and target species (Gülşahin and Soykan, 2017).

The relation between length and weight is an informative instrument in fisheries science. However, parameters of the length-weight relationship, even for the same species, differ between regions, sampling methods and the measurement accuracy of the researchers (Gülşahin and Soykan, 2017). When we compare our results on length-weight relationship parameters with those of other studies conducted in the Mediterranean basin; "b" values are within the general range except for *B. boops* (b=2.40) which is lower than other studies. Furthermore "a" value is also within the general range except for *L. mormyrus* (higher than compared studies) and *S. cabrilla* (slightly lower than previous studies) (Table 4). Various factors may be responsible for the differences on LWR parameters between seasons and years, such as temperature, salinity, food (quantity, quality, and size), sex, time of year and stage of maturity (Dulcic and Kraljevic, 1996). Also bait type affects the LWR in the longline fishery as the juvenile and mature individuals of the same species may have different feeding preferences (Gülşahin and Soykan, 2017).

Among 9 species obtained from the present study, only 3 of them were emphasized in the minimum landing size (MLS) regulation of the Turkish fishery legislation. While the total

length of *S. aurata* was found to be higher for any hook-bait combination than 20 cm (Anonymous, 2016) and *P. erythrinus* was found to be higher for any hook-bait combination than 15 cm (Anonymous, 2016) given limit for Turkey, size of *D. vulgaris* for all hook-bait combinations was below 18 cm (Anonymous, 2016) MLS regulation. Therefore, hook style, bait type and their combination mentioned in this work is considered to be convenient for sustainable fishery of *S. aurata* and *P. erythrinus.* Absence of MLS regulation in Turkish fishery for other sparid and serranid species of the present study prevents making a comprehensive evaluation on the sustainability for those 6 species. However, we determined the length range of *P. erythrinus* in between 13.5 and 18.5 cm with a mean length of 15.5 cm. Metin et al (2011) reported the length at maturity of females and males of *P. erythrinus* as 11.30 and 15.08 cm, respectively. It was found higher than that of Metin et al (2011) indicating a sustainable fishing trend for the species. Soykan et al (2015), stated the length at maturity of *B. boops* (♀) to be 12.96 cm. In this study, it was found 16.3 cm for *B. boops* that is greater than the reported maturity length, which supports the sustainable fishery for the species.

CONCLUSION

This study focused on hook and bait effects on catch composition, size distribution, discard ratio and condition of the captured species in demersal longline fishery on the Turkish coast of the Aegean Sea. Investigated criterions are of crucial importance for fisheries management in terms of providing the sustainability of demersal longline gear which is one of the eco-friendly fishing gears. Therefore, further and comprehensive studies on suitable and sustainable hook-bait combinations are required not only for catching high commercial value fishes and reduction of discards but also for protecting the coastal fish stocks.

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REFERENCES

Akyol, O. (2003). Retained and trash fish catches of beach-seining in the Aegean coast of Turkey. *Turk Journal of Veterinary and Animal Sciences*, 27, 1111-1117.

Akyol, O., Kınacıgil, H.T. & Şevik, R. (2007). Longline fishery and length-weight relationships for selected fish species in Gökova Bay (Aegean Sea, Turkey). *International Journal of Natural and Engineering Sciences*, 1, 1-4.

Amorim, S., Santos, M., Coelho, R. & Fernandez-Carvalho, J. (2015). Effects of 17/0 circle hooks and bait on fish catches in a southern Atlantic swordfish longline fishery. Aquatic Conservation: *Marine and Freshwater Ecosystems*, 25(4), 518-533, DOI: 10.1002/aqc.2443

Anonymous (2016). 4/1 Numaralı Ticari Amaçlı Su Ürünleri Avcılığının Düzenlenmesi Hakkında Tebliğ [Notification 4/1 on recreational fisheries.] Gıda, Tarım ve Hayvancılık Bakanlığından. [Ministry of Food, Agriculture and Livestock.] Tebliğ No: 2016/35 [Notification No. 2016/35]

Anonymous (2017). 2017 Su Ürünleri İstatistikleri [Fisheries Statistics in 2017]. https://www.tuik.gov.tr (accessed date: 12 Sept. 2017).

Aydın, İ., Gökçe, G. & Metin, C. (2008). The effects of netting twine on discard rates of commercial red mullet gillnets in Izmir Bay. *Turkish Journal of Fisheries and Aquatic Sciences*, 8, 373-376.

Aydın, I. (2011). Is natural bait type a stochastic process for size and condition of fishes in the recreational fishery of Izmir Bay? *Mediterranean Marine Science*, 12(2), 390-400. DOI: 10.12681/mms.39

Aydın, C. M. & Bolat, Y. (2014). The selectivity of different hook size in the bottom longline which used for catching white seabream (*Diplodus sargus* L., 1758) in Finike Bay. *Journal of Fisheries Sciences*, 8, 186-193. DOI: 10.3153/jfscom.201423

Çekiç, M. & Başusta, N. (2004). Effect on the species selectivity of different bait and hook size in longline in Iskenderun Bay, Eastern Mediterranean Sea. *Ege Journal of Fisheries and Aquatic Sciences*, 21, 73-77.

Coelho, R., Santos, M. N. & Amorim, S. (2012). Effects of hook and bait on targeted and bycatch fishes in an equatorial Atlantic pelagic longline fishery. *Bulletin of Marine Science*, 88(3), 449-467. DOI: 10.5343/bms.2011.1064

Dulcic, J. & Kraljevic, M. (1996). Weight-length relationships for 40 fish species in the eastern Adriatic (Croatian waters). *Fisheries Research*, 28, 243-251. DOI: 10.1016/0165-7836(96)00513-9

Erdem, M. & Akyol, O. (2005). A preliminary study on longlining of swordfish (*Xiphias gladius* L., 1758) in Fethiye Region (Mediterranean). *Ege Journal of Fisheries and Aquatic Sciences*, 22, 201-204.

FAO (1993). Longline fishing. J.P. George (ed.) Fao training series, no:22 42 pp. FAO Fisheries Department, Rome.

Fernandez-Carvalho, J., Coelho, R., Santos, M.N. & Amorim, S. (2015). Effects of hook and bait in a tropical northeast Atlantic pelagic long line fishery: part II- target, by-catch, and discard fish. *Fisheries Research*, 164, 312-321. DOI: 10.1016/j.fishres.2014.11.009

Foster, D.G., Epperly, S.P., Shah, A.K. & Watson, J.W. (2012). Evaluation of hook and bait type on the catch rates in the western North Atlantic Ocean pelagic longline fishery. *Bulletin of Marine Science*, 88, 529–545. DOI: 10.5343/bms.2011.1081

Froese, R. & Pauly, D. (Ed.) (2018). Fish Base. World Wide Web electronic publication. www.fishbase.org, (06/2018).

Gilman, E., Zollet, E., Beverly, S., Nakano, H., Davis, K., Shiode, D., Dalzell, P. & Kinan, I. (2006). Reducing sea turtle by-catch in pelagic longline fisheries. *Fish and Fisheries*, 7(1), 2–23. DOI: 10.1111/j.1467-2979.2006.00196.x

Gilman, E., Clarke, S., Brothers, N., Alfaro-Shigueto, J., Mandelman, J., Mangel, J., Peterson, S., Piovano, S., Thomson, N., Dalzell, P., Donoso, M., Goren, M. & Werner, T. (2008). Shark interactions in pelagic longline fisheries. *Marine Policy*, 32(1), 1–18. DOI: 10.1016/j.marpol.2007.05.001

Gökçe, G. & Metin, C. (2007). Landed and discarded catches from commercial prawn trammel net fishery. *Journal of Applied Ichthyology*, 23, 543-546. DOI: 10.1111/j.1439-0426.2007.00832.x

Güçlüsoy, H., Öndes, F. & Tosunoğlu, Z. (2020). The catch characteristics of a set longline fishery in the Foça Special Environmental Protection Area, Turkey. Acta Ichthyology Piscatoria, 50 (3), 247–255. DOI: 10.3750/AIEP/02809

Gülşahin, A. & Soykan, O. (2017). Catch composition, length-weight relationship and discard ratios of commercial longline fishery in the Eastern Mediterranean. *Cahiers de Biologie Marine*, 58, 1-7. DOI: 10.21411/CBM.A.B3268672

Kelleher, K. (2005). Discards in the world's marine fisheries. An update. FAO Fisheries Technical Paper # 470. FAO: Rome. 131 pp.

Lewison, R.L., Freeman, S.A. & Crowder, L.B. (2004). Quantifying the effects of fisheries on threatened species: the impact of pelagic longlines on loggerhead and leatherback sea turtles. *Ecology Letters*, 7(3), 221–231. DOI: 10.1111/j.1461-0248.2004.00573.x

Lilliefors, H.W. (1969). On the Kolmogorov–Smirnov test for the exponential distribution with mean unknown. *Journal of the American Statistical Association*, 64, 387–389. DOI: 10.1080/01621459.1969.10500983

Maktay, C.B. (2012). Effect on catching efficency of different natural bait in Longline fisheries. Master's Thesis, Science Institute of Ege University, İzmir, Turkey. 38 pp.

Metin, G., Ilkyaz, A.T., Soykan, O. & Kınacıgıl, H.T. (2011). Biological characteristics of the common pandora, *Pagellus erythrinus* (Linnaeus, 1758), in the central Aegean Sea. *Turkish Journal of Zoology*, 35, 307-315. DOI: 10.3906/zoo-0904-4

Moutopoulos, D.K. & Stergiou, K.I. (2002). Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). *Journal of Applied Ichthyology*, 18, 200-203. DOI: 10.1046/j.1439-0426.2002.00281.x

Odabaşı, O.B. (2013). Investigation of bycatch composition in longline fisheries in Çanakkale Region. Master's Thesis, Science Institute of Onsekiz Mart University, Çanakkale, Turkey.

Özdemir, S., Ayaz, A., Gurbet, R. & Erdem, Y. (2006). Catch efficiency of bottom long-line used with different hook size and different type bait at dawn and daytime. *Anadolu University Journal of Science and Technology*, 7, 405-411.

Özgül, A., Ulaş, A., Lök, A., Düzbastılar, F.O. & Metin, C. (2015). A comparison of alternative circle hook (Kahle Hook) and J style hook performance in experimental pelagic longline fishery in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 19-27. DOI : 10.4194/1303-2712-v15_1_03

Özyurt, C. E., Sarıhan, E. & Akar, M. (2003). The possibilities of using longline at pike-perch (*Sander lucioperca*, Bogustkaya & Naseka, 1996) fishing at Seyhan Dam Lake. E.U. *Journal of Fisheries & Aquatic Sciences*, 20, 43-50.

R Core Team (2017). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/

Santos, M.N., Coelho, R., Fernandez-Carvalho, J. & Amorim, S. (2012). Effects of hook and bait on sea turtle catches in an Equatorial Atlantic pelagic longline fishery. *Bulletin of Marine Science*, 88(3), 683–701. DOI: 10.5343/bms.2011.1065

Santos, M.N., Coelho, R., Fernandez-Carvalho, J. & Amorim, S. (2013). Effects of 17/0circle hooks and bait on sea turtles bycatch in a Southern Atlantic swordfish longline fishery. Aquatic Conservation: *Marine and Freshwater Ecosystems*, 23(5), 732–744. DOI: 10.1002/aqc.2324

Sistiaga, M., Herrmann, B., Rindahl, L. & Tatone, I. (2018). Effect of bait type and bait size on catch efficiency in the European Hake *Merluccius merluccius* Longline Fishery. Marine and Coastal Fisheries: Dynamics, *Management, and Ecosystem Science*, 10(1), 12-23. DOI: 10.1002/mcf2.10007

Soykan, C.U., Moore, J.E., Zydelis, R., Crowder, L. B., Safina, C. & Lewison, R. L. (2008). Why study bycatch? An introduction to the theme section on fisheries bycatch. *Endangered Species Research*, 5, 91–102. DOI: 10.3354/esr00175

Soykan, O., İlkyaz, A.T., Metin, G. & Kınacıgil, H.T. (2015). Growth and reproduction of *Boops boops*, *Dentex macrophthalmus, Diplodus vulgaris*, and *Pagellus acarne* (Actinopterygii: Perciformes: Sparidae) from East-Central Aegean Sea, Turkey. *Acta Ichthyologica et Piscatoria*, 45(1), 39–55. DOI: 10.3750/AIP2015.45.1.05

Soykan, O., Aydın, İ. & Kınacıgil, H.T. (2016). A preliminary study on the potential use of an alternative bait for demersal longline fishery; Sepietta sp. *Ege Journal of Fisheries and Aquatic Sciences*, 33, 163-167. DOI: 10.12714/egejfas.2016.33.2.11

Tesch, F.W. (1971). Age and growth. p. 98-130. In: Methods for assesment of fish production in fresh waters. W.E. Ricker (Ed). Blackwell Scientific Publications, Oxford.

Ulaş, A. & Düzbastılar, O.F. (2001). Comparison of catch efficiency of different longlines, *Ege Journal of Fisheries and Aquatic Sciences*, 18, 175-186.

Watson, J.W., Epperly, S.P., Shah, A.K. & Foster, D.G. (2005). Fishing methods to reduce sea turtle mortality associated with pelagic longlines. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 965–981. DOI:10.1139/F05-004.

http://www.egejfas.org
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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Micronucleus formation in mussel' (*Mytilus galloprovincialis, Lamarck 1819*) haemolymph, liver, and gill cells as a biomarker in the assessment of genotoxicity in İzmir Bay (Aegean Sea, Turkey)

İzmir Körfezi (Ege Denizi, Türkiye) genotoksisitenin değerlendirilmesinde biyobelirteç olarak Midye (*Mytilus galloprovincialis*) hemolenf, karaciğer ve solungaç hücrelerinde mikronükleus oluşumu

Özlem Çakal Arslan[1*] ● Meltem Boyacıoğlu[2] ● Beyza Nalbantlar[3] ● Gizem Gülsever[4] Muhammet Ali Karaaslan[5]

[1] Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0001-7777-3886
[2] Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0002-9871-6132
[3] Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0003-2206-9589
[4] Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0001-7526-6207
[5] Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0003-3737-2361

**Corresponding author: ozlem.cakal@ege.edu.tr*

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Abstract: The sea has involved a large variety of environmental contaminants and plays a crucial role in aquatic ecosystems. The Izmir Bay, which has been rapidly polluted since the 1960s, was one of the intensely polluted areas in the Mediterranean. Organic materials, hydrocarbons, metals, and pathogenic organisms that are accumulated in the region, caused high pollution and threaten health and aesthetics. Because of this, the aim of this paper was focus on to investigations of the genetic damages in mussels in Izmir Bay. Investigations of mutagenic/carcinogenic potential have an advantage in genotoxicity studies because biomarker for pollution exposure in mussel is the early detection of possible long-term effects such as cancer. Therefore, genotoxicity was the focus of the biomarker investigations in mussel during the investigations. Thus, micronucleus tests were afforded to determine genetic damage in the *haemolymph*, liver, and gills of *Mytilus galloprovincialis* living in Izmir Bay (Western Coast of Turkey). In the present study, results showed that the frequency of MN was found at a high level in station 4 and station 5 where wastes from dockyard existed contributed to the high level of pollution. The recommendation is to standardize procedures for assessment of the toxic impact of pollutants at the cellular level in aquatic species by using micronucleus assays for biomonitoring of environmental pollution.

Keywords: Micronucleus test, pollution, genotoxicity, mussel

Öz: Denizler, çok çeşitli çevresel kirleticileri içermekte ve su ekosistemlerinde önemli bir rol oynamaktadır. 1960'lı yıllardan itibaren hızla kirlenen İzmir Körfezi, Akdeniz'deki kirliliğin en yoğun olduğu alanlardan biridir. Bölgede biriken organik maddeler, hidrokarbonlar, metaller ve patojen organizmalar, estetik ve sağlık açısından yüksek derecede tehdit oluşturmaktadır. Bu nedenle, bu çalışmanın amacı İzmir Körfezi' nde bulunan midyelerde meydana gelen genetik hasarların araştırılmasına odaklanmıştır. Mutajenik / karsinojenik potansiyelin araştırılması, genotoksisite çalışmalarında bir avantaj sağlamaktadır, çünkü kirliliğe maruz kalan midyelerde kullanılan, biyobelirteçler; kanser gibi olası uzun vadeli etkilerin erken saptanmasına olanak sağlamaktadır. Biyoizleme çalışmalarında; midyede yapılan biyobelirteçlerin merkezinde genotoksisite bulunmaktadır. Bu amaçla çalışmamızda; İzmir Körfezi'nde (Türkiye'nin Batı Sahili) yaşayan *Mytilus galloprovincialis'in* hemolenf hücrelerinde, karaciğer hücrelerinde ve solungaç hücrelerinde genetik hasarı belirlemek için mikronükleus testleri gerçekleştirilmiştir. Bu çalışmada elde edilen sonuçlar, tersaneden gelen atıkların bulunduğu 4. istasyon ve 5. istasyonda MN frekansının (‰ micronucleus) yüksek bulunmasına paralel olarak, kirliliğin bu bölgelerde yoğun olduğunu göstermiştir. Elde ettiğimiz verilerle, çevresel kirliliğin biyolojik olarak izlenmesi için, kirleticilerin suda yaşayan türlerde hücresel düzeyde toksik etkilerinin belirlenebilmesini, mikronükleus testlerinin kullanımının yaygınlaştırılmasını ve bu yöntemlerin standartlaştırılmasının gerekliliğini ortaya koymaktayız.

Anahtar kelimeler: Mikronükleus testi, kirlilik, genotoksisite, midye

INTRODUCTION

Chemicals remain a long time in the water, leading to increased environmental pollution; were caused by bioaccumulation of toxic compounds in aquatic organisms. Increases in the human population and the development of the industry are reasons for industrial pollution. All aquatic organisms are affected by chemicals in the water. However, the degree of exposure varies by species. Of these, benthic organisms are the ones with the highest degree of exposure. On the other hand, mussels and similar benthic creatures filter more water than their body weight and take them into their bodies, and all the contaminants in the water are transferred to the creatures. In this way, toxic effects of

pollution occur. This pollution is caused by the accumulation of genotoxic and carcinogenic compounds that fixing their physiologies or causing to carcinogenesis and inhibiting the survival of the organisms in the aquatic environment. Bolognesi and Hayashi, (2011) reviewed that among the various carcinogenic and mutagenic compounds are the most dangerous as they hold their activities for several generations. Many hazardous substances exist in the water and sediment, and they are accumulated by aquatic organisms and trigger DNA or cellular damage and affect the ecosystem by the trophic chain (Izquierdo et al., 2003). It is useful to measure the number of chemical pollutants in the living body and to determine how much it is stored and taken in the environment. However, this information reflects very little biological and ecological effects, but it does not allow us to understand biochemical interactions within the organism. For this reason, it is necessary to develop Environmental Protection System or "early warning system." This system is based on the biological response of the organism in the environment against the pollutant. This is necessary to limit ecological damage. Developing this early warning system is seen as a very important points to try to determine the result if possible and to prevent cell damage at a later stage. It is not possible, not economic, and time-consuming to determine the concentration of such substances in the tissues analytically with available chemical methods. Thus, the biological methods and those based on screening for carcinogenic and mutagenic substances in the tissues of indicator organisms have gained importance (Arslan et al., 2010; 2015).

Genotoxic substances show the initial effect and even if the agent disappears, the effect continues. In fact, this effect can continue until the next generation. Genotoxic effects cause several changes that affect the morphological and chemical structure of DNA. However, the molecular biological changes that pollutants cause at first glance are on DNA. Lately, several standards in vitro genotoxicity experiments have been studied for the determination of the genotoxic potential of contaminated waters on aquatic species (Vahl et al., 1997; Harvey et al., 1999). In genetic toxicology damage at the level of chromosome play, a critical role since mutation of chromosome like chromosome breakage plays the most important role in cancer formation (Fenech, 2000). Micronuclei (MN) test is one of the most reliable techniques used to determine genetic changes in the organisms in contaminated waters and complex mixtures. In recent years, this test has been improved using many aquatic organisms (Hayashi et al., 1998, Arslan et al., 2010; 2015; Dailianis et al., 2003, Tsarpali and Dailianis, 2012). The MN assay is today applied in laboratory and field studies using hemocytes and gill cells from bivalves, mainly from the genera *Mytilus*. These represent 'sentinel' organisms because of their ability to survive under polluted conditions and to accumulate both organic and inorganic pollutants (Bolognesi and Fenech, 2012). Mussels are the main indicators of the health of the aquatic environment.

The Izmir Bay, which has been rapidly polluted with organic materials, hydrocarbons, metals, and pathogenic organisms accumulated in the region posed a high degree of threat to aesthetics and health. Of these basic pollutants affecting water quality, 50% were caused by industrial waste, 15% by rain, 10% by rivers, 10% by agricultural resources, and 15% by other causes. In the first evaluation, although the receiving environment of the Bay is highlighted by the intensive industrialization and rapid increase of population in the eastern and northern regions of the city, there are many natural and man-made material wastes determining the ecological status of Izmir Bay. To take the precautions of domestic and industrial pollutants mentioned in the first two articles above, all industrial wastes flowing to the Bay are treated and 70% of the domestic wastes are treated and delivered to the Bay. Because of the high pollution of Izmir Bay, biomonitoring studies are needed sensitive and practical techniques such as biomarkers (micronucleus) for detecting early warning signals for aquatic health. The MN frequency test has generally been applied to organisms where other biological effects, techniques, and contaminant levels are well documented. In general, indigenous, ecologically and economically important mollusk species could serve as indicator species for biomonitoring of environmental genotoxicity levels, for screening of Geno toxins distribution, or for assessments of genotoxicity effects from contaminant spills or effluent discharges waters.

MATERIALS AND METHODS

Izmir Bay is located in the eastern Aegean Sea between latitudes of 38°20' and 38°42' N and longitudes of 29°25'–27°10' E. Izmir which is the third largest city in Turkey is located at the eastern end of Izmir Bay and it has the second biggest harbor in Turkey (Sinem Atgin et al., 2000) (Figure 1). Izmir Bay, as a study area has an extensive domestic and industrial pollution load (Sinem Atgin et al., 2000). Izmir Bay has a highly disturbed environment due to the rapid increase of the population and development of industry (Sinem Atgin et al., 2000).

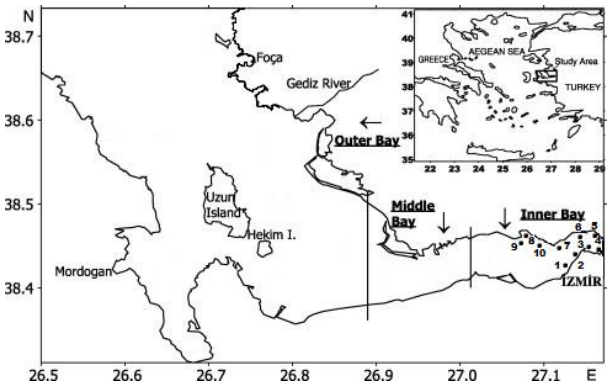

Figure 1. Study area and stations

The study area physic-chemical properties were measured as; temperature range from 13 °C to 14.5°C, salinity range from 37.50 to 38 PSU, 6.91 (pH), 5.5 mg/lt (dissolved oxygen).

Mytilus galloprovincialis micronucleus test

For the MN test, 15 individuals of *M. galloprovincialis* 6 ± 3 cm in size obtained from harbor feet in 10 stations of Izmir Bay [Stat. 1 (Konak Pier), Stat. 2 (Üçkuyular), Stat. 3 (Göztepe), Stat. 4 (Konak), Stat. 5 (Pasaport), Stat. 6 (Alsancak), Stat. 7 (Bayraklı), Stat. 8 (Karşıyaka), Stat. 9 (Bostanlı) and Stat. 10 (Turan)], were used (Figure 1) The diagnostic criteria for micronucleus identification were used (Baršiene et al., 2006). These are, a) the micronucleus is<1/3 of the size of the main nucleus, b) micronuclei are round- or ovoid-shaped, non-refractive chromatin bodies located in the cytoplasm of the cell and can, therefore, be distinguished from artifacts, c) micronuclei are not connected to the main nuclei, and the micronuclear boundary should be distinguishable from the nuclear boundary. One thousand cells were examined per slide, with tree replicate slides per sample. The MN and BN index for the sample was determined as the mean number of micronuclei per thousand cells over the tree slides.

Haemolymph

For the MN test, *haemolymph* taken with a thin-tipped syringe is mixed with a fixative (3:1 methanol: acetic acid) and centrifuged at x1000 rpm. The supernatant is removed after the fixation. The pellet is smeared on the slide then fixed with methanol for 10 minutes. It is allowed to dry, then stained with 5% Giemsa followed by then rinsed with distilled water and closed with a cover slide. A total of 1000 cells were counted for each individual and MN/BN frequency was calculated as ‰ number of MN and BN. A total of 45.000 cells were counting for micronucleus (MN) and binucleus (BN) frequency from each station.

Gill

For the micronuclei test, gills dissected by scissors were fixed in acetic acid. The samples were centrifuged at x2000 rpm for 10 minutes to obtain epithelial cells shed from gill tissue by using acetic acid and pipette. The pellet obtained by this procedure is smeared on the slide and fixed by methanol. It is allowed to dry. Then, it is stained with 5% Giemsa and the slide is covered with entelan after. As mentioned before, 3 preparations were made for each individual, and 1000 cells were examined for each preparation. A total of 45.000 gill cells were counting for micronucleus (MN) and binucleus (BN) frequency from collected mussels in each station.

Hepatopancreas

For the MN test, hepatopancreas tissue was removed from 15 animals from each station and placed into Carnoy's fixative. For preparation hepatopancreas were transferred into vials containing 45% acetic acid solution for 30 min. After that, tissues were gently minced and filtered to obtain a cell suspension. The obtained cells were smeared on a clean slide, air-dried, and fixed in Carnoy's fixative for 20 min. Finally, they were stained with 5% Giemsa solution for 30 minutes. Three slides per animal and 45 slides per station were prepared. From each slide, 1000 cells were scored under 1000· magnification to determine the frequencies of bi-nucleated and micronucleated cells.

Statistical analysis

The frequency of micronuclei and binuclei in the samples obtained from Izmir Bay were calculated based on 1000 cells. Kruskal-Wallis test, Ftest and p (ANOVA), and Student's t-test were used to compare nuclear abnormalities (BN and MN frequencies) between the sampling locations. All statistical analyses were performed by Statistica 6.0 statistics software.

RESULTS

The present study attempted to determine whether genotoxic potential existed in the environment of Izmir Bay using the micronuclei test of indicator organisms. The frequency of BN and MN was determined. During the examinations, other nuclear abnormalities were observed such as nuclear bats, but they were not included in the calculations since their numbers are not statistically significant. MN and BN frequencies were calculated based on microscopic examinations of slides with Haemolymph, gill, and Hepatopancreas cells of *Mytilus galloprovincialis*. Result data were compared statistically.

Table 1 and Figures 2, 3 reports the results related to the application of the MN assay in biomonitoring study in the field. carried out in Izmir Bay (Turkey). Table 1 shows the nuclear abnormalities in the gills of mussels from Izmir Bay. As shown in the table. MN frequency in mussel hemolymph cells obtained from 10 stations varies between 23.7 - 38.5 ‰ and BN frequency varies between 0.2 and 0.8 ‰. The frequencies of MN and BN were higher in mussel in the station 9 region which is highly polluted due to terrestrial inputs. Statistically, a significant difference was found between MN and BN values when micronuclei and binuclei frequencies of the locations ($p<0.05$). According to Table 1 nuclear abnormalities in Hepatopancreas cell from sampling locations. Micronuclei averages ranged between 22.9-37.7‰ from the bay. MN frequencies were found at a higher level in fish from stat.8 and 10. A significant difference was found when frequencies of micronuclei found in cells were statistically compared between the stations (Figure 1). With the effect of density of pollution. the presence of MN in a cell increases or the formation of BN became observed.

Table 1. Nuclear abnormalities observed in mussels (mean ± standard error)

	Gill cell (N=1000)			Hemolymph cell (N=1000)			Hepatopancreas cell (N=1000)		
Stations	N	MN	BN	N	MN	BN	N	MN	BN
1	966.3±6.9	32.77±7.3	0.97±1.9	976.4±0.5	28.9±0.3	0.8± 0.2	971.8±0.6	28.1±0.1	0.1± 0
2	969.57±4.4	30±4.5	0.43±1.0	970.3±0.4	29.85±0.3	0.5± 0.2	971±1	29.13±0.2	0± 0
3	974.43±5.8	25.23±5.8	0.33±0.7	969.65±0.5	24.55±0.4	0.15± 0.1	971.1±0.5	28.37±0.4	0.6± 0
4	959.2±15	35.6±11.5	5.27±3.9	975.3±0.52	23.7±0.4	0.00±0.0	968.7±0.8	30.3±1.3	0.3± 0
5	974.03±3.8	25.8±3.7	0.17±0.4	963.05±1.5	36.35±1.3	0.6± 0.3	976.6±0.4	22.9±0.7	0.4± 0
6	965.87±11.4	31.53±9.5	2.43±2.6	964.72±0.7	34.83±0.6	0.4± 0.2	970.03±0.5	29.5±0.7	0.5± 0
7	965.23±6.2	29.83±6.1	5.2±2.8	963.6±0.4	36±0.4	0.5± 0.2	964.5±0.6	35.2±0.5	0.3± 0
8	968.77±7.2	29.7±5.4	1.27±2.3	961.2±0.4	36.05±1.1	0.00± 0.0	961.97±0.6	37.7±0.5	0.3± 0
9	968.47±7.9	29.4±8.6	1.27±1.5	65.1±1.1	38.55±0.3	0.3± 0.1	973.1±0.9	26.9±0.5	0.00±0
10	956.87±8.5	39.33±7.9	3.8±2.1	963.95±1.1	35.25±1.0	0.2± 0.0	963.05±1.5	36.35±1.3	0.1± 0

*P<0.05

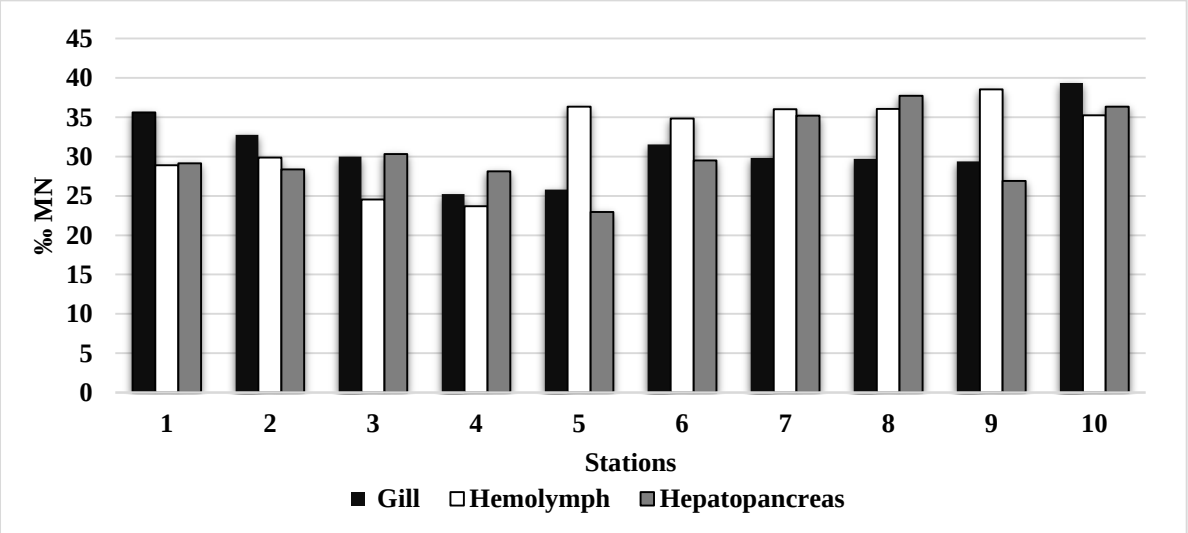

Figure 2. Distribution of MN frequencies in gill. hemolymph and hepatopancreas cells by 10 stations mussel

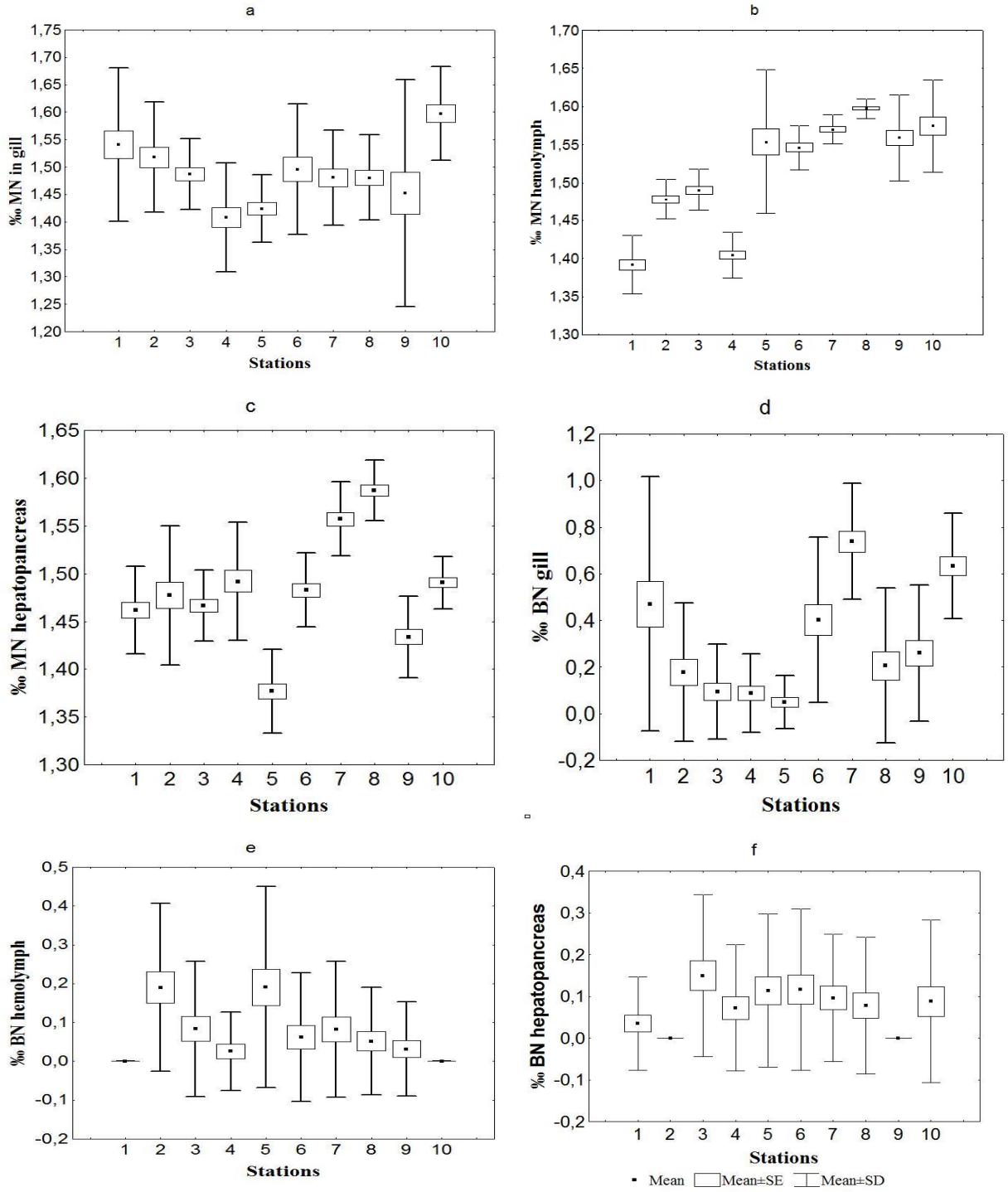

Figure 3. Statistically comparison of MN and BN frequencies between stations after logarithmic transformation. KW-H: Kruskal-Wallis test. Ftest and p (ANOVA) a) gill cells [MN: KW-H (9;300) = 65.2608; p = 0.0000; F(9;290) = 7.3252; p = 0.000000001]. b) MN hemolymph: KW-H(9;295) = 204.8336; p = 00.0000; F(9;285) = 75.7703; p = 00.0000. c) MN hepatopancreas: KW-H(9;300) = 177.8579; p = 00.0000; F(9;290) = 48.9195; p = 00.0000. d) BNgill: KW-H(9;300) = 109.5615; p = 00.0000; F(9;290) = 18.9162; p = 00.0000. e) BN hemolymph: KW-H(9;295) = 45.6443; p = 0.0000007; F(9;285) = 5.5943; p = 0.0000004. f) BN hepatopancreas: KW-H(9;300) = 29.3676; p = 0.0006; F(9;290) = 3.1736; p = 0.0011

According to examinations of mussel gills. MN frequency ranged between 25.60 - 39.33 ‰ and BN frequency between 0.17 and 5.27 ‰. As can be seen in Table 1. higher MN and BN frequencies were found in mussel gills taken from stations 10 and 4 than those taken from others. The station has four shipyard areas in which shipyard removal and repair operations are made, but it is also located near the Izmir port. As mentioned previously considering that micronuclei and binuclei frequencies vary depending on pollution one may argue that station 4 is more polluted than others. Determined MN frequencies showed statistically significant differences when micronuclei and binuclei frequencies were compared statistically between locations ($p<0.005$). A statistically significant difference was found between MN values when micronuclei frequencies of the locations ($p< 0.05$) (Figure 3). Results show that frequencies of MN in gill cells higher than hemolymph and hepatopancreas. Such studies are recommended because gill cells as well exhibit high sensitivity to the agents promoting the formation of micronuclei (Hayashi et al., 1998). Generally, gills are metabolically active tissues because their cells are under the influence of the aquatic circulation system.

DISCUSSION

In this paper, we investigate genotoxicity in the Mediterranean mussel (*Mytilus galloprovincialis*) from Izmir Bay (Turkey). Because the Bay is generally exposed to chemical, industrial, domestic and urban pollution whose causes a biological impact especially DNA level. Therefore, these effects were evaluated, using biomarker approaches on sentinel species. Native mussels were used to the identification of sensitiveness of specific biomarker responses. Frequencies of MN are an indirect marker of numeric and structural chromosomal abnormality in the cells by many agents. The aquatic organisms are highly exposed to various toxic, carcinogenic, and mutagenic agents that are caused to negative effects parallel to increasing environmental pollution. To determine the presence of mutagen / carcinogenic pollution based on cells in Izmir Bay tests were performed using the mussel hemolymph, gill, and hepatopancreas cells from 10 stations.

The Bay of Izmir has been the subject of many scientific types of research in the last 30-35 years. The research has been conducted in almost all branches of marine sciences (physical, chemical, biological, geological oceanography, marine ecology, satellite oceanography, coastal area management. etc.) (Kontas et al., 2004; Arslan et al., 2010). These monitoring studies have demonstrated the importance of eutrophication in the Gulf and that nutrient concentrations have increased from the Outer Gulf to the Central and Inner Gulf, which is due to terrestrial inputs. The concentrations of mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), and zinc (Zn) in sediment were measured between 1997-2007 and no significant change was observed in metal levels. It is stated that chromium (Cr), manganese (Mn), lead (Pb), zinc (Zn), copper (Cu), and arsenic (As) measurements made in sediment samples collected in 2009 are mostly from anthropogenic sources. Heavy metal bioaccumulation was also analyzed in our previous study (Gulsever and Arslan, 2019). In this study, Heavy metal contents of sediment samples taken from 12 stations of Izmir inner Bay were examined. The results of the analysis showed that as pollution progressed to the inner parts of the gulf. The lowest heavy metal accumulation in the sediment samples taken was at Station 2 station except manganese (Pb: 84.95, Zn:211, Cd: 0.37, Cu: 64.48, Cr:91.8, Hg:0.326, Mn: 424, Fe: 27200.0, Al: 17000.0 mg/kg sediment). The highest concentration of Pb, Zn, Cd, Cr and Cu for Station 10 (35.14; 138.9; 0.23; 82.4; 41.22 mg/kg) while the Hg concentration in station 3 (0.436 mg/kg). Mn and Fe concentration station 8 (463, 29700.0 mg/kg). It was observed that there was not a big change in heavy metal accumulation in sediment after the big canal project in Izmir inner bay. According to the enrichment factor calculations, Station 6 compared to all other stations the accumulation of Pb, Cd, and Cu elements in sediments from the region is quite high such as 6.16 - 5 and 3.69. respectively. When these metal levels and MN frequencies are comparing results show that MN frequencies parallel with pollution. Weak mutagenicity was found in a study by Boyacıoğlu (2001; 2004) on sediment samples to detect mutagenic substances in Izmir Bay. In addition, in a study showing that Izmir inner Bay sediment is not suitable for living life. Alaybey stations where the shipyard wastes are found to be high and the amount of pollution is high (Arslan et al., 2010). Based on the results of BN and MN counting on epithelial gill, hemolymph, and hepatopancreas cells from the *Mytilus galloprovincialis* showed that Izmir Bay has mutagenic potential. Furthermore, t, the difference between the stations was statistically significant ($p<0.005$). There are no reference values because we are not sure about the situation of pollution of the area; thus, results from the mussels were evaluated to make comparisons with those from the study with the same species. (MN frequency in the control group: 0-1 ‰). MN assays with mussel also have shown potential as an in-situ biomonitoring tool for detecting genotoxic agents in the marine environment (Bolognesi and Fenech, 2012).

According to our previous study (Arslan et al., 2010); examinations of mussel gills, MN frequency detected from 1.500 gill cells ranged between 30.56–89.76‰ and BN frequency between 6.19‰ and 33.40‰. As can be reported higher MN and BN frequencies were found in mussel gills taken from stations 10 and 5 than those taken from others. As

mentioned previously considering that micronuclei and binuclei frequencies vary depending on pollution one may argue that these two locations are more polluted than others. Micronuclei and their frequency found as a consequence of microscopic examinations in haemolymph cells of *M. galloprovincialis* from each location were showed that MN frequency ranged between 29.13‰ and 47.55‰ and BN frequency between 13.73‰ and 29.9‰. When the frequencies were compared with the previous and present study (MN: 25.2-39.3 in gill, MN: 23.7-38.5 in hemolymph, and MN: 22.9-36.3 in hepatopancreas) showed that effects of pollution on induction of micronuclei at *M. galloprovincialis* decrease. Those of the gill cells with higher BN and MN frequencies indicate that gills are a better marker in micronuclei tests comparing to mussels. Statistically, a significant difference was found between MN and BN values when micronuclei and binuclei frequencies of the locations ($p < 0.05$) (Figure 3).

Bolognesi and Cirillo (2014) reported that MN assay has revealed the effects of exposure to different classes of pollutants (e.g., polycyclic aromatic hydrocarbons, heavy metals, organochlorinated compounds) showing good discrimination power and allowing the identification of genotoxicity events along a pollution gradient and facilitating recovery effects after accidental pollution events. The study of Magni et al. (2006) *M. galloprovincialis* was carried out in selected sites along the Gulf of Oristano (Sardinia, Italy) including a commercial port and lagoon areas characterized by intensive agricultural and mining activities. They are reported that the extent of increase of MN frequency shows changes parallel to pollution input level and composition or seasonal physiological changes. Also indicates that the use of biomarkers of DNA and chromosomal damage together with biochemical markers could provide a comprehensive indication of the impact of chemical pollutants in coastal marine ecosystems.

Klobučar et al. (2008) performed genotoxicity assessment via MN in Kaštela Bay and the neighboring Trogir Bay using the micronucleus test and Comet assay with mussel (*M. galloprovincialis*) haemocytes. Native and caged mussels were included in the studies. Their results confirmed that mussels in Kaštela and Trogir Bays are affected by genotoxic contaminants. The study performed by Venier and Zampieron (2005) to determine genetic damage in *M. galloprovincialis* and Zosterisessor ophiocephalus, in Venice Lagoon in Italy, reported that genetic damage existed by examining MN and cellular abnormalities in haemolymph and gill tissues (5 individuals from each location). In this study, MN frequency was reported to range between 33‰ and 37‰. Furthermore, it was reported that the species naturally existing in the Lagoon were subjected to pollution-causing genetic damage. Three different species of fish were used for micronuclei tests in the monitoring study to determine environmental mutagens in different areas of the Baltic Sea by Baršiene et al. (2006). A result of the study showed that the pollutants causing genetic damage existed in the environment as understood from observation of MN formations. Meanwhile where more mutagenic-carcinogenic pollutants existed was determined by finding changes in MN frequencies among the locations. Barsiene et al. (2006) were reported that an increase was observed in micronuclei formation due to an increase in pollution. In a study by Dolcetti and Venier (2002) MN frequencies in Mediterranean mussel *M. galloprovincialis* was examined to determine genetic damage in both the individuals collected from its natural environment and those subjected to benzopyrene in the laboratory setting. And also, researchers were reported that MN formation was observed in the mussels (12 alive) collected from different areas in different periods and that micronuclei frequency found in the gills (about 8.5 ‰) was higher than that found in the haemolymph. And it was noted that MN frequency increased parallel to the increase in pollution for long years.

In the study of Izquierdo et al. (2003) micronuclei test was performed in brachial cells of *Mytilus edulis* to determine pollution on two different coastal areas (location of discharging domestic waste Gijon and Pueto Madryn and on locations of discharging industrial waste Puerto Madryn). According to this study results, the MN test by the mussel was shown to be sensitive in monitoring domestic pollution and proposed as a bioindicator in routine pollution investigations in coastal ecosystems. Dailianis et al. (2003), performed MN assessment in haemolymph and gills of *Mytilus galloprovincialis* collected from Thermaikos and Strymonikos Bays (South Greece) between July and October 2001. As a result of the MN assessment, either gill or haemolymph tissues showed no significant difference existed between seasons when samplings in June and October were compared.

Study Touahri et al. (2016) the mussels *Mytilus galloprovincialis* collected from a no contaminated site (Chaib Rasso) were transplanted during one, three, and six months at Ghazaouet harbor (GH) areas with a strong gradient of pollution. The micronucleus test (MN) was selected to monitor the impact of contamination along with physiological indexes (condition index CI and organo-somatic indexes RI and GSI).

In this research, they have reported a negative correlation of MN variation in gill cells with CI but a positive correlation with transplantation duration and also indicate that MN in the hemolymph and gills of transplanted mussels for one, three, and six months at GH are significantly higher than those of the reference site. However, no significant differences were noted between the three transplants at the two organs.

Harvey et al. (1999) were examined the environmental genotoxicity and cytotoxicity along the Spanish Mediterranean coast was investigated through the determination of levels of micronuclei (MN) and other nuclear abnormalities (NAs) such as nuclear buds (NB) and binucleated cells (BN) in gills of wild mussels *M. galloprovincialis* from 17 study sites. Their study obtained that the highest MN and NB levels were found in mussels from metal-polluted sites such as Cartagena (MN: 11.6‰, NB: 4.6‰) and Portman (MN: 8.0‰, NB: 3.5‰). Furthermore, MN levels at sites highly polluted by organic contaminants such as Barcelona, Vallcarca, Tarragona, and Valencia were lower than expected (ranging from 3.8 to 5.8‰). This data compares with the recent study result showed that Izmir Bay is highly polluted and has a mutagenic character.

The research of Kalpaxis et al., (2004) *M. galloprovincialis* were placed in bow nets and immersed at 3–10 m depth in a clean coastal region (reference area). Itea and two marine stations along Gulf of Patras, N. Peloponnesus Greece. One site is near the estuaries of the Glafkos River which are influenced by local industrial and urban sources (Station 1), the second site Agios Vasilios has no evident organic pollution but is enriched in metals (Station 2). One month after immersion gill cells were isolated and their micronuclei content was determined. Compared with the reference samples the micronuclei frequency values observed in mussels transplanted to Station 1 (Gulf of Patras) showed ranged between 7±1 and 12±3 parts per thousand (ppt). These values were significantly higher ($P<0.01$) than those measured in specimens collected from Station 2. Nevertheless, the micronuclei frequency in both cases was much higher than 2.4±1‰ recorded in reference samples. The investigators reported that MN frequency increased parallel to the increase in exposure time and concentration in the gill cells although both micronuclei and nuclear abnormalities decreased as subjection time and concentration in hemolymph increased. They noted that the difference between two types of the cell was related to cellular kinetics and cellular renewal and as a second option they noted that the difference was due to the fact that gill cells were affected by directly and continuously contaminated waters. Our results didn't show similarities with previous studies that have found lower MN frequencies in *M. galloprovincialis* in contaminated areas. Our results are in agreement with previous studies that have found elevated MN frequencies in mussel inhabiting contaminated areas. Micronuclei frequencies at gill, hemolymph, and hepatopancreas cells of *M. galloprovincialis* compared with previous studies. Izmir Bay was found polluted by mutagenic and genotoxic compounds comparing the knowledge based on scientific literature information. Aquatic ecosystems should be protected against all kinds of adverse activities which may lead to dramatic changes. Studies aimed that determining the effects of various pollutants on the organisms takes a long time and requires expensive analytic operations. More practical biological tests have many advantages such as saving time and money and obtaining reliable results and conclusions. As well as the other aquatic organisms' mussel a very suitable experimental organism for bio tests and toxicity assays.

CONCLUSION

Environmental conditions and xenobiotic exposure can be sources of stress to living organisms. Biological markers are a measurable indicator of changes that may happen at any biological level and can be considered an early warning signal of some biological or environmental state or condition. Species of the genus Mytilus sp. are systematically used as biological models for assessing the presence of pollutants in the surrounding coastal waters (Tsarpali and Dailianis, 2012). Exposure of mussels to toxicants. performed normally through direct contact of the organisms with contaminated water could result in observable structural and/or functional changes. The micronucleus (MN) test represents a sensitive indicator of both organic and inorganic mutagens and clastogens. The purpose of the present work was to compare the biological effect on the shellfish at the site assessed as being exposed to differing levels of sewage and chemical pollutants under a range of environmental conditions.

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REFERENCES

Arslan, Ö., Parlak, H., Katalay, S., Boyacioglu, M., Karaaslan, M.A. & Guner, H. (2010). Detecting micronuclei frequency in some aquatic organisms for monitoring pollution of Izmir Bay (Western Turkey). *Environmental Monitoring and Assessment,* 165(1–4), 55–66. DOI:10.1007/s10661-009-0926-5

Arslan, Ö., Boyacioğlu, M., Parlak, H., Katalay, S. & Karaaslan, M.A. (2015). Assessment of micronuclei induction in peripheral blood and gill cells of some fish species from Aliağa Bay Turkey. *Marine Pollution Bulletin*, 94(1–2),48–54. DOI:10.1016/j.marpolbul.2015.03.018

Barsiene, J., Schiedek, D., Rybakovas, A., Syvokiene, J., Kopecka, J. & Förlin, L. (2006). Cytogenetic and cytotoxic effects in gill cells of the blue mussel *Mytilus* spp. from different zones of the Baltic Sea. *Marine Pollution Bulletin*, 53(8–9), 469–478. DOI: 10.1016/j.marpolbul.2005.11.015

Bolognesi, C. & Hayashi, M. (2011). Micronucleus assay in aquatic animals. *Mutagenesis,* 26(1), 205–213. DOI:10.1093/mutage/geq073

Bolognesi, C. & Fenech, M. (2012). Mussel micronucleus cytome assay. *Nature Protocols*, 7(6), 1125–1137. DOI: 10.1038/nprot.2012.043

Bolognesi, C. & Cirillo, S. (2014). Genotoxicity biomarkers in aquatic bioindicators. *Current Zoology,* 60(2), 273–284. DOI: 10.1093/czoolo/60.2.273

Boyacıoğlu, M. & Parlak, H. (2001). Mutagenicity of River Sediments Draining into Izmir Bay. *Ege Journal of Fisheries and Aquatic Sciences*, 18(3), 23-36.

Boyacıoğlu, M. (2004). Determination of direct mutagens in sediment samples of Izmir Bay. *Ege Journal of Fisheries and Aquatic Sciences*, 21(1), 11-18.

Dailianis, S., Domouhtsidou, G.P., Raftopoulou, E., Kaloyianni, M. & Dimitriadis, V.K. (2003). Evaluation of neutral red retention assay, micronucleus test, acetylcholinesterase activity, and a signal transduction molecule (cAMP) in tissues of *Mytilus galloprovincialis* (L.), in pollution monitoring. *Marine Environmental Research*, 56(4), 443–470. DOI: 10.1016/S0141-1136(03)00005-9

Dolcetti, L. & Venier, P. (2002). Susceptibility to genetic damage and cell types in Mediterranean mussels. *Marine Environmental Research,* 54(3–5), 487–491. DOI: 10.1016/S0141-1136(02)00142-3

Fenech, M. (2000). The in vitro micronucleus technique. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 455(1–2), 81–95. DOI: 10.1016/S0027-5107(00)00065-8

Gulsever G. & Çakal Arslan, O. (2019). İzmir iç körfezi sediment kirliliğinin kimyasal ve biyolojik entegre yöntemler ile incelenmesi. 215 sayfa, Doktora Tezi, E.Ü Fen Bilimleri Enstitüsü, İzmir.

Harvey, J.S., Lyons, B.P., Page, T.S., Stewart, C. & Parry, J.M. (1999). An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 441(1), 103-114. DOI: 10.1016/S1383-5718(99)00037-6

Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Saotome, K. & Ojima, Y. (1998). Development of genotoxicity assay systems that use aquatic organisms. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 399(2), 125-133. DOI: 10.1016/S0027-5107(97)00251-0

Izquierdo, J.I., Machado, G., Ayllon, F., d'Amico, V.L., Bala, L.O., Vallarino, E. & Garcia-Vazquez, E. (2003). Assessing pollution in coastal ecosystems: a preliminary survey using the micronucleus test in the mussel *Mytilus edulis. Ecotoxicology and Environmental Safety,* 55(1), 24-29. DOI: 10.1016/S0147-6513(02)00041-6

Kalpaxis, D.L., Theos, C., Xaplanteri, M.A., Dinos, G.P., Catsiki, A.V. & Leotsinidis, M. (2004). Biomonitoring of Gulf of Patras, N. Peloponnesus, Greece. Application of a biomarker suite including evaluation of translation efficiency in *Mytilus galloprovincialis cells. Environmental Research,* 94(2), 211–220. DOI: 10.1016/S0013-9351(03)00048-3

Klobučar, G.I.V., Štambuk, A., Hylland, K. & Pavlica, M. (2008). Detection of DNA damage in haemocytes of *Mytilus galloprovincialis* in the coastal ecosystems of Kaštela and Trogir bays, Croatia. *Science of the Total Environment,* 405(1–3), 330–337. DOI: 10.1016/j.scitotenv.2008.05.015

Kontas, A., Kucuksezgin, F., Altay, O. & Uluturhan, E. (2004). Monitoring of eutrophication and nutrient limitation in the Izmir Bay (Turkey) before and after Wastewater Treatment Plant. *Environment International*, 29(8), 1057–1062. DOI: 10.1016/S0160-4120(03)00098-9

Magni, P., De Falco, G., Falugi, C., Franzoni, M., Monteverde, M., Perrone, E. & Bolognesi, C. (2006). Genotoxicity biomarkers and acetylcholinesterase activity in natural populations of *Mytilus galloprovincialis* along a pollution gradient in the Gulf of Oristano (Sardinia, western Mediterranean). *Environmental Pollution*, 142(1), 65–72. DOI: 10.1016/j.envpol.2005.09.018

Sinem Atgin, R., El-Agha, O., Zararsiz, A., Kocataş, A., Parlak, H. & Tuncel, G. (2000). Investigation of the sediment pollution in Izmir Bay: trace elements. *Spectrochimica Acta, Part B: Atomic Spectroscopy*, 55(7), 1151–1164. DOI:10.1016/S0584-8547(00)00231-7

Touahri, H.G., Boutiba, Z., Benguedda, W. & Shaposhnikov, S. (2016). Active biomonitoring of mussels *Mytilus galloprovincialis* with integrated use of micronucleus assay and physiological indices to assess harbor pollution. *Marine Pollution Bulletin*, 110(1), 52-64.

Tsarpali, V. & Dailianis, S. (2012). Investigation of landfill leachate toxic potency: An integrated approach with the use of stress indices in tissues of mussels. *Aquatic toxicology,* 124, 58-65. DOI: 10.1016/j.aquatox.2012.07.008

Vahl, H.H., Karbe, L. & Westendorf, J. (1997). Genotoxicity assessment of suspended particulate matter in the Elbe river: Comparison of *Salmonella microsome* test, arabinose resistance test, and umu-test. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis,* 394(1–3), 81–93. DOI: 10.1016/S1383-5718(97)00129-0

Venier, P. & Zampieron, C. (2005). Evidence of genetic damage in grass gobies and mussels from the Venice lagoon. *Environment International*, 31(7), 1053-1064. DOI: 10.1016/j.envint.2005.05.016

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Optical characterization of chromophoric dissolved organic matter at eutrophic and oligotrophic parts of a semi-enclosed bay (İzmir, Aegean Sea)

Yarı-kapalı bir körfezin (İzmir, Ege Denizi) ötrofik ve oligotrofik kısımlarında kromoforik çözünmüş organik maddenin optik karakterizasyonu

Hakan Alyürük[1*] ● Aynur Kontaş[2]

[1] Dokuz Eylül University, Institute of Marine Sciences and Technology, 35340, İnciraltı, İzmir, Turkey https://orcid.org/0000-0001-8632-4281
[2] Dokuz Eylül University, Institute of Marine Sciences and Technology, 35340, İnciraltı, İzmir, Turkey https://orcid.org/0000-0002-6273-1568

*Corresponding author: hakan.alyuruk@deu.edu.tr

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Abstract: Optical characterization of chromophoric dissolved organic matter (CDOM) from İzmir Bay (Aegean Sea) waters was investigated. For sampling, surface and subsurface seawater from 7 stations were collected in summer 2015. Excitation-emission matrix (EEM) spectra of each sample were recorded on a fluorescence spectrophotometer. The results showed that dissolved organic carbon (DOC) concentrations and EEM peaks were increased from the outer bay to inner bay stations. EEM peaks indicated the presence of both humic-like and protein-like components which were higher at middle-inner bays than outer bay. Spearman's rank correlation coefficients for EEM peak intensities and DOC concentrations were highly positive ($p<0.05$). HIX found between 0.73-3.51, whereas BIX ranged from 0.31 to 0.96 in the bay. Humification degree of CDOM in the middle-inner bays were higher compared to outer bay stations. High HIX values in the middle-inner bays could be linked to the presence of Melez stream (heavily polluted), other streams, rain run-offs and maritime activities at İzmir Bay. High BIX values in the middle-inner bays indicated presence of freshly produced DOM from bacterial origin. Optical characterization of CDOM could be used for tracing fluorescent DOM components and determining different DOM sources (autochthonous or allochthonous) in further studies.

Keywords: Chromophoric dissolved organic matter, dissolved organic carbon, fluorescence, seawater, İzmir Bay

Öz: Bu çalışmada, İzmir Körfezi (Ege Denizi)'nden alınan deniz suyunda kromoforik çözünmüş organik madde (KÇOM)'nin optik karakterizasyonu araştırılmıştır. Deniz suyu örnekleri 7 istasyonda yüzey ve yüzey-altı derinliklerden 2015 yılı yaz mevsiminde toplanmıştır. Her bir örneğin uyarma-emisyon matris (UEM) spektrumu bir floresans spektrofotometresi aracılığıyla kaydedilmiştir. Sonuçlar, çözünmüş organik karbon (ÇOK) ve UEM pik şiddetlerinin dış körfezden iç körfeze doğru arttığını göstermiştir. UEM pikleri, hem hümik asit benzeri hem de protein benzeri organik madde bileşenlerinin orta-iç körfezlerde dış körfeze kıyasla daha yüksek olduğunu ortaya koymuştur. Spearman'ın sıralama korelasyonu testleri sonucunda UEM pik şiddetleri ve ÇOK konsantrasyonları arasında yüksek pozitif ilişki bulunduğu saptanmıştır ($p<0.05$). Körfez genelinde hümikleşme indeksi (HI) 0.73-3.51 aralığında, biyolojik indeks (BI) ise 0.31-0.96 aralığında değişim göstermiştir. KÇOM'a ait hümikleşme derecesi orta-iç körfezlerde dış körfeze kıyasla daha yüksek tespit edilmiştir. Orta-iç körfezlerde gözlenen yüksek HI değerlerinin yüksek kirlilik yükü taşıyan Melez çayının varlığı, iç körfeze ulaşan diğer yüzey sularının varlığı, yağmur ile denize sürüklenebilen karasal organik maddeler ve İzmir Körfezi'ndeki denizcilik aktiviteleri ile ilişkili olduğu düşünülmektedir. Orta-iç körfezlerde gözlenen yüksek BI değerleri ise özellikle bakteriyel kökenli ve yeni üretilmiş çözünmüş organik madde üretimi ile ilişkilendirilebilir. KÇOM'un optik karakterizasyonu, gelecek çalışmalarda çözünmüş organik madde içerisindeki floresans özellikteki bileşenlerin takip edilebilmesi ve çeşitli organik madde kaynaklarının (otokton veya allokton) belirlenmesi amacıyla kullanılabilir.

Anahtar kelimeler: Kromoforik çözünmüş organik madde, çözünmüş organik karbon, floresans, deniz suyu, İzmir Körfezi

INTRODUCTION

Dissolved organic matter (DOM) is a heterogeneous compound pool that is composed of complex organic molecules within seawater. DOM could be originated from terrestrial processes (allochthonous) or *in situ* marine (autochthonous) sources (Hedges, 2002; Libes, 2009). DOM pool contains vast amounts of biomolecules at different chemical structures and molecular sizes. Humic acids, fulvic acids, lignins, amino acids, carbohydrates, lipids, fatty acids, and sterols are such examples to most abundant biomolecules in DOM. Primary production and exudates of phytoplankton, sloppy feeding of metazoan grazers, viral cell lysis, egesta of protists and metezoans, and extracellular hydrolysis of POM by bacteria are the main sources of *in situ* DOM production (Aparicio et al., 2016; Brussaard, 2004; Motegi et al., 2009; Ortega-Retuerta et al., 2009; Ridgwell and Arndt, 2015; Romera-Castillo et al., 2011a; Sala and Güde, 2004; Sarmento et al., 2013; Zeri et al., 2014). Following its release to the marine environment, DOM is constantly circulated, transferred,

and subjected to transformations within the marine environment. The factors controlling its transformations could be photochemical processes (Mopper et al., 1991; Santos et al., 2014; Sulzberger and Durisch-Kaiser, 2009; Vähätalo and Wetzel, 2004; Zhang et al., 2013), sorption in sediments by flocculation (Cauwet, 2002), sorption by sinking particles (Carlson and Hansell, 2015; Hansell et al., 2009), sorption by metal-oxides (Couturier et al., 2016), physical processes (Boyd and Osburn, 2004; Cauwet, 2002; Dixon et al., 2014), and bacterial processes (Boyd and Osburn, 2004; Nelson et al., 2004; Santos et al., 2014; Vähätalo and Wetzel, 2004). Significant contributions of anthropogenic inputs to the DOM pool in coastal waters were also reported (Hong et al., 2005; Sun et al., 2014; Tedetti et al., 2011; Tzortziou et al., 2015; Wang et al., 2014), which in turn, these may lead to eutrophication, hypoxia or harmful algal blooms (Anderson et al., 2002; Cloern, 2001; Conley et al., 2009; Davidson et al., 2012; Davidson et al., 2014; Heisler et al., 2008; Jessen et al., 2015; Korpinen and Bonsdorff 2015; Sellner et al., 2003). Therefore, understanding and monitoring of DOM sources and its transformations in coastal waters is of great importance for elucidating the fates of DOM components and the assessment of ecological status of the marine environment.

In recent studies, optical characterization of DOM has been used frequently as it provides information about autochthonous or allochthonous sources and its transformations by applying rapid and inexpensive methods (Coble et al., 1990; Kowalczuk et al., 2010; Kowalczuk et al., 2015; Lonborg et al., 2015; Lu et al., 2015; Nieto-Cid et al., 2006; Romera-Castillo et al., 2011b; Romera-Castillo et al., 2013; Yang et al., 2016; Zeri et al., 2014). The fraction of DOM that is able to absorb and/or emit light is called as chromophoric dissolved organic matter (CDOM). The light spectra of CDOM is recorded by applying simultaneous (or three dimensional) excitation-emission matrix (EEM) spectroscopy with a fluorometer. Parallel factor analysis (PARAFAC) is used to extract the characteristic peaks of humic-like or protein-like compounds (Murphy et al., 2013; Stedmon et al., 2003). Fluorometric characterization of CDOM, when combined with absorption measurements, not only provides information about its sources and transformation but also helps to understand its relations with bacterial and photochemical processes (Guo et al., 2007; Kowalczuk et al., 2003; Lu et al., 2015; Murphy et al., 2008; Yamashita and Tanoue, 2003).

İzmir Bay is located at the Eastern coast of the Aegean Sea. It has an L shaped structure, and its entrance is oriented to the north with its longer part. The hydrography of the İzmir Bay is influenced from several factors: exchanges between the atmosphere and the sea, exchange of water masses with the Aegean Sea, freshwater inputs with anthropogenic loads, topography of the bay, sea level changes, wind-driven circulations of water masses and winter convection (Sayin, 2003). Under the influence of these factors, water masses in the İzmir Bay could be divided into three different parts: Inner Bay water (anthropogenically polluted), Outer Bay water (the water mass influenced from Gediz River and Aegean Sea, the upwelling water at Gülbahçe Bay, and the water mass located at salt production area), and Middle Bay water (connects Outer Bay to Inner Bay) (Sayin, 2003). Remarkable differences for DOC, Chl-a, dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) levels were reported at outer, middle and inner bay stations in the previous studies (Kontas et al., 2004; Kucuksezgin et al., 2005; Sunlu et al., 2012). Also, algal blooms and eutrophication have been observed in the inner bay that is under the influence of anthropogenic inputs (Ozkan et al., 2008; Sunlu et al., 2011). For example, temporarily, high abundances of *Ceratium furca* var. *eugrammum*, *Cylindrotheca closterium, Prorocentrum micans* and *Noctiluca scintillans* biomasses have been observed in the range of 2-5 µM C (Sunlu et al., 2007). However, there are no studies on the optical characterization of DOM, and its possible sources in the İzmir Bay. The aim of this study was to investigate the optical characteristics of CDOM in middle-inner (eutrophic) and outer (oligotrophic) parts of İzmir Bay with dissolved organic carbon (DOC) levels, humification index (HIX) and biological index (BIX).

MATERIAL AND METHODS

Seawater sampling

The seawater samples were collected from surface and subsurface (5 m) depths at 7 stations in İzmir Bay (Figure 1) in summer 2015. The sampling stations were selected according to previous observations of physical and chemical characteristics of water masses in the bay (Kontas et al., 2004; Kucuksezgin et al., 2005; Sayin, 2003). The samples were collected with a 1.7 L Nansen bottle. The samples were immediately filtrated from 47 mm Whatman GF/F (0.7 µm) glass fiber filters and filtrated samples were stored frozen at −20 °C until the analyses in the laboratory.

Dissolved organic carbon analysis

The detection principle of DOC (measured as CO_2) was based on the discoloration of buffered phenolphthalein solution proportionally to the CO_2 concentration (Gershey et al., 1979; Schreurs, 1978). The analysis was performed on a continuous flow nutrient analyzer (San Plus, Skalar) according to instructions of the manufacturer (Cat.No: 311-412). First, 0.06 N sulfuric acid was added to the sample. Then, digestion reagent was added, and UV digestion was applied. Following the digestion, hydroxylammonium chloride solution was introduced and the CO_2 was separated from reaction mixture with a gas dialysis membrane. The liberated CO_2 was reacted with the 1% phenolphthalein buffer solution. Colorimetric reading was performed at 550 nm. Potassium hydrogen phthalate was used as organic carbon standard. Accuracy of the method was checked using potassium hydrogen phthalate at every 10 sample readings. Synthetic seawater including NaCl, $MgSO_4$ and Milli-Q water was used as blank. The system was washed with Milli-Q water until the low and stable instrumental blank. The detection limit of the method was 16 µM C.

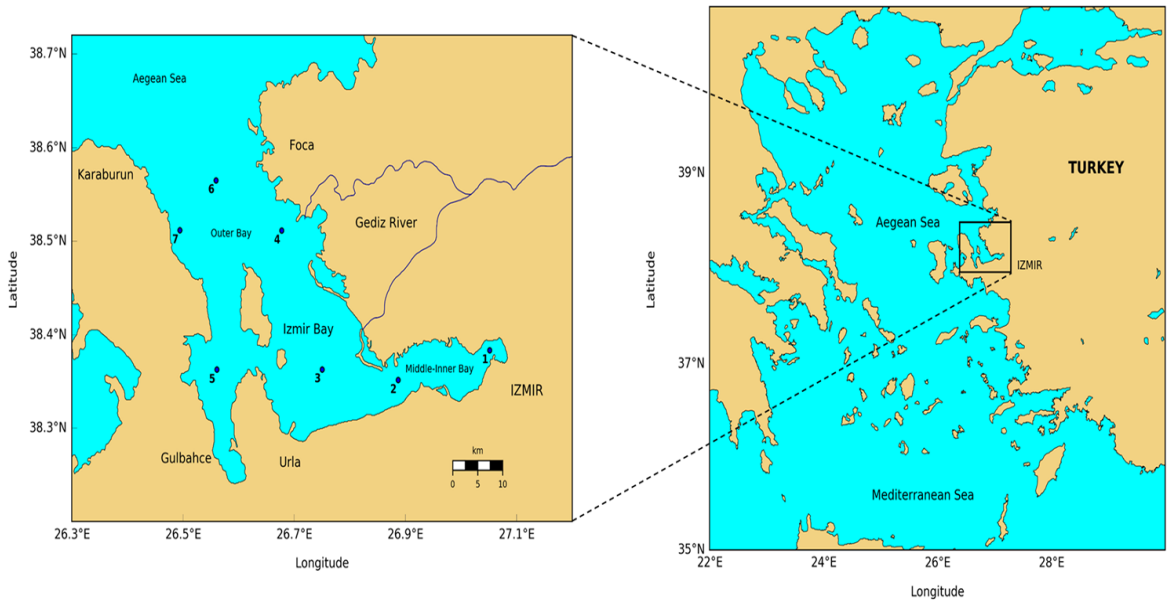

Figure 1. Sampling stations at İzmir Bay, Aegean Sea

Characterization of CDOM

EEM spectrums were recorded with an Agilent Cary Eclipse Fluorescence Spectrophotometer. EEM spectra were collected at excitation wavelengths of 230-500 nm and emission wavelengths of 250-600 nm with a spectral resolution of 2 nm. EEM spectra of Milli-Q water was subtracted from the EEM spectra of samples to remove scattering effects of water (Chari et al., 2012). Raman normalization was applied to normalize the data for comparability (Chari et al., 2012; Murphy et al., 2010; Stedmon et al., 2003) and fluorescence intensities were represented as Raman Units (RU). Raman normalization was performed by using Raman peak area (Eq. 1, λex=350 nm, λem=381-421 nm) and dividing fluorescence intensity to Raman peak area (Eq. 2). EEM peaks of samples were determined according to Coble (1996) (Table 1). Post-processing of EEM spectrums were performed with modified PLOTEEM script in R (Lapworth and Kinniburgh, 2009; R Core Team, 2016). EEM peaks were extracted according to "algorithm–based approach" by selecting the peak points with maximum fluorescence intensities at defined emission regions (Korak et al., 2014).

$$A_R^{350} = \int_{381}^{426} I(\lambda_{em})d\lambda_{em} \quad (1)$$

$$I(\mathrm{RU}) = \frac{1}{A_R^{350}} I(\mathrm{AU}) \quad (2)$$

where A_R^{350} indicates area under emission spectrum between 381 and 421 nm at excitation wavelength of 350 nm, $I(\lambda_{em})$ represents fluorescence intensity as a function of emission wavelength, I(RU) represents fluorescence intensity of samples in RU, I(AU) represents raw fluorescence intensity of samples in Arbitrary Units (AU).

Table 1. Determination criteria of EEM peaks according to Coble (1996)

EEM peaks	Peak name (Wavelength range)
A	UV humic-like (Ex:260, Em:380-460)
B	Tyrosine-like, protein-like (Ex:275, Em:310)
C	Visible humic-like (Ex:350, Em:420-480)
M	Marine humic-like (Ex:312, Em:380-420)
T	Tryptophan-like, protein-like (Ex:275, Em:340)

Calculations of fluorescence indexes

HIX and BIX are used to extract quantitative information on contributions of humic matter and autochthonous production to the fluorescence intensities of seawater samples. HIX was first introduced by Zsolnay et al. (1999) for estimating the humic matter content of DOM in soil samples. As a result of higher humification degree, C/H ratio and aromaticity was increased (Lüttig, 1986; Stevenson, 1982) and a shift to longer emission wavelengths was observed (Chen et al., 2011; Huguet et al., 2009; Tam and Sposito, 1993; Zsolnay et al., 1999). HIX is defined as the ratio of spectral area under emission wavelengths of 435-480 nm to emission wavelengths of 300-345 nm at excitation wavelength of 254 nm. HIX can be formulated as below (Eq. 3):

$$\mathrm{HIX} = \frac{\int_{435}^{480} I(\lambda_{em})d\lambda_{em}}{\int_{300}^{345} I(\lambda_{em})d\lambda_{em}} \quad (3)$$

where I represents fluorescence intensity as a function of emission wavelength (λ_{em}).

BIX was introduced by Huguet et al. (2009) for determination of the autochthonous biological activity and freshly produced DOM in natural water samples. While large BIX values indicate diagenetically unaltered DOM, numerator part of the fraction corresponds to microbially produced DOM, and denominator part represents highly decomposed DOM (Fellman et al., 2010; Lu et al., 2015; Wilson and Xenopoulos, 2009). It is calculated by dividing the fluorescence intensity at excitation wavelength of 310 nm and emission wavelength of 380 nm to the maximum emission intensity in the range of 420-480 nm at excitation wavelength of 310 nm (Eq. 4).

$$BIX = \frac{I_{\lambda_{ex310}/\lambda_{em380}}}{max_{420\leq\lambda_{em}\leq480} I_{\lambda_{ex310}}} \quad (4)$$

where *I* represents fluorescence intensity.

Statistical analyses

Statistical analyses were performed with R Statistical Computing Software, v3.3.1 (R Core Team, 2016). The Spearman's rank correlation test was performed between EEM peaks, HIX, BIX and DOC. Also, relationships between EEM peaks, HIX, BIX and DOC were investigated with linear regression analyses.

RESULTS AND DISCUSSION

The results of DOC, EEM peak intensities of CDOM components and fluorescence indexes (HIX and BIX) were given in Table 2. DOC concentrations were found in the range of 35.3-244.2 µM throughout the bay. DOC levels in the middle-inner bays (stations 1 and 2) observed higher compared to outer bay (stations 3-7) (Figure 2a). DOC concentrations found in this study were similar to the previous reports (56.1-121 µM) for İzmir Bay (Kucuksezgin et al., 2005)

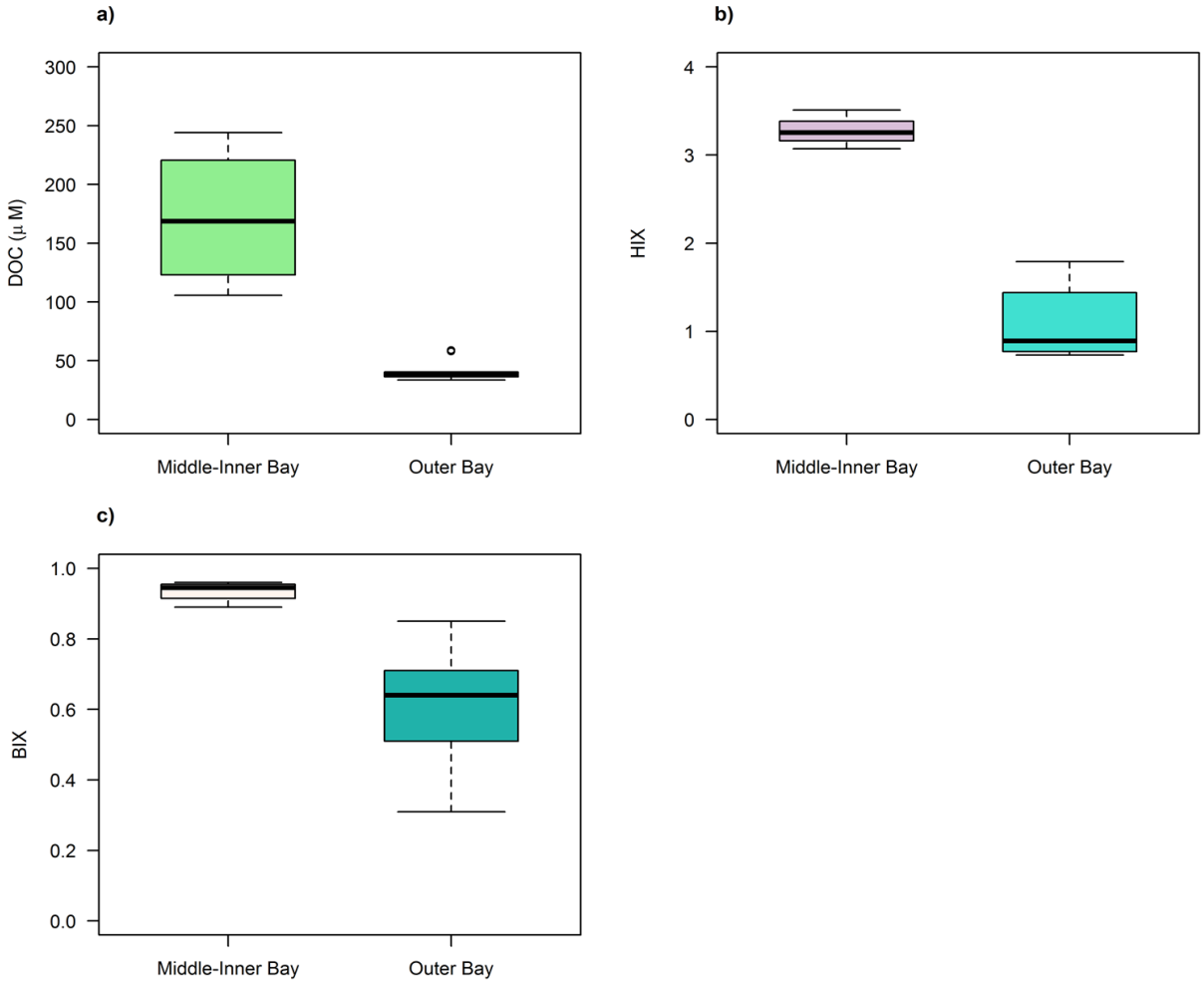

Figure 2. Distributions of DOC, HIX and BIX in the middle-inner and outer bays: a) DOC, b) HIX, and c) BIX

Table 2. DOC concentrations, fluorescent components of CDOM and descriptive fluorescence indexes (S: Surface, SS: Subsurface)

Station	DOC (µM)	Peak M	Peak A	Peak C	Peak T	Peak B	HIX	BIX
St1-S	244.2	0.13	0.18	0.11	0.11	0.05	3.51	0.94
St1-SS	206.6	0.14	0.18	0.10	0.09	0.06	3.26	0.95
St2-S	140.3	0.09	0.14	0.08	0.07	0.05	3.25	0.89
St2-SS	105.6	0.11	0.14	0.09	0.09	0.05	3.07	0.96
St3-S	58.9	0.03	0.05	0.04	0.04	0.02	1.79	0.85
St3-SS	42.7	0.03	0.05	0.02	0.02	0.02	1.58	0.68
St4-S	45.4	0.01	0.02	0.02	0.02	0.01	0.77	0.37
St4-SS	42.7	0.02	0.02	0.02	0.01	0.02	0.87	0.31
St5-S	38.6	0.03	0.04	0.02	0.03	0.02	1.08	0.60
St5-SS	46.7	0.03	0.03	0.02	0.02	0.01	1.44	0.73
St6-S	58.9	0.02	0.03	0.02	0.02	0.01	0.79	0.68
St6-SS	37.7	0.03	0.03	0.02	0.03	0.04	0.73	0.51
St7-S	38.6	0.02	0.03	0.02	0.01	0.01	0.77	0.57
St7-SS	35.3	0.01	0.02	0.02	0.01	0.01	0.91	0.71

Raman normalized fluorescence intensities of EEM peaks decreased from inner to outer bay stations (Table 2). EEM spectrums of the samples were given in Figures 3 and 4. Intensities of EEM peaks decreased in the order of A, M, C, T, and B at station 1 and 2. The intensities of peak A at station 3 were slightly higher compared to other outer bay stations. On the other hand, EEM peak intensities were very similar to each other at outer bay stations. Results indicated the presence of higher humic-like (peaks A, C, and M) and protein-like (peaks T and B) components at middle-inner bays than outer bay stations.

HIX and BIX indexes decreased from inner to outer bay stations (Figure 2b,c). HIX was between 0.73-3.51, whereas BIX ranged from 0.31 to 0.96 in the bay. According to scales of HIX defined by Huguet et al. (2009), HIX values in this study showed that the DOM in middle-inner bays (3.07-3.51) might be originated from biological or aquatic bacterial processes, but this character was weaker at outer bay stations (0.77-1.79). According to BIX values (Huguet et al., 2009), DOM has strong autochthonous character at middle-inner bays (0.89-0.96) whereas DOM showed autochthonous character from low to strong levels at a wide range (0.31-0.85) in outer bay.

Observation of relatively high HIX values in the middle-inner bays could be linked to the presence of Melez stream (heavily polluted), other streams, rain run-offs and maritime activities at İzmir Bay. High HIX values at middle-inner bays might be related with humic matter inputs (humification degree) and stabilities of CDOM components (Bai et al., 2014; Chari et al., 2012; Huguet et al., 2009). Also, high BIX values indicated presence of freshly produced DOM from bacterial origin (Fellman et al., 2010; Lu et al., 2015; Wilson and Xenopoulos, 2009). According to HIX vs. BIX plot in Figure 5, it was possible to distinguish CDOM characteristics of middle-inner bay stations from outer bay. High HIX and BIX values for CDOM might be resulted from high anthropogenic inputs, physico-chemical characteristics and biological processes in middle-inner bays.

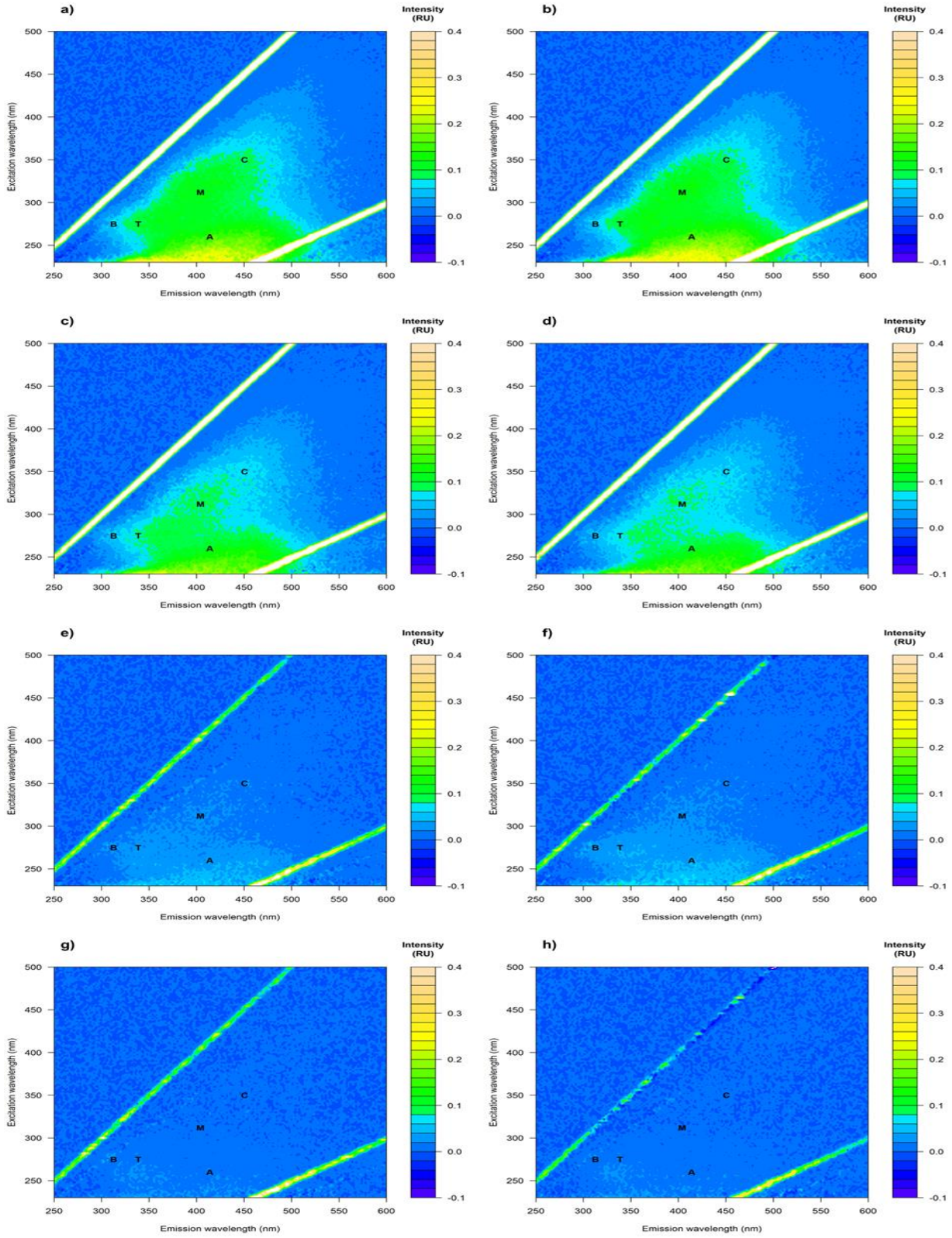

Figure 3. EEM plots for Station 1 to 4: a) St1-S, b) St1-SS, c) St2-S, d) St2-SS, e) St3-S, f) St3-SS, g) St4-S, and h) St4-SS (S: Surface, SS: Subsurface)

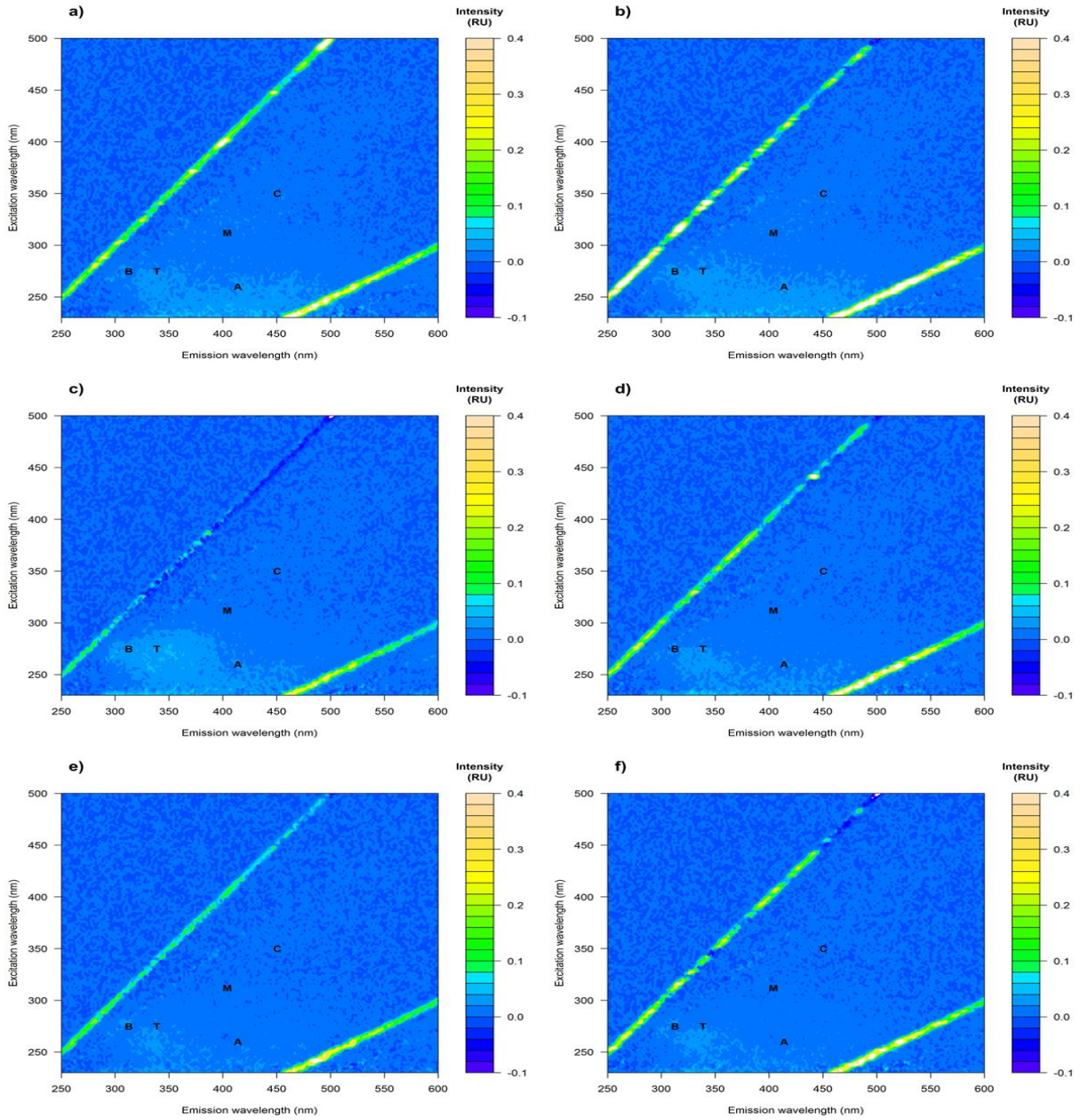

Figure 4. EEM plots for Station 5 to 7: a) St5-S, b) St5-SS, c) St6-S, d) St6-SS, e) St7-S, and f) S7-SS (S: Surface, SS: Subsurface)

Spearman's rank correlations between EEM peak intensities, HIX, BIX, total fluorescence intensities (ΣFL) and DOC concentrations were found highly positive ($p<0.05$, Table 3). Linear relationships between EEM peaks, HIX, BIX and DOC concentrations at middle-inner and outer bays were given in Figure 6 and Table 3.

According to linear regression between DOC and ΣFL, the fluorescent fraction of DOM in the surface and subsurface waters of İzmir Bay were composed of highly fluorescent and slightly fluorescent fractions (Figure 6c). Also, humic-like (A, C, M) and tryptophan-like (T) EEM peaks were able to explain more than 85% of variation in DOC concentrations. Linear relationships between HIX and DOC concentrations (explaining 80% variation in DOC) indicated that humic matter has an important contribution to DOM.

On the other hand, correlation ($\rho=0.719$, $p=0.004$) and linearity ($r^2=0.484$, $p=0.005$) between BIX and DOC represented weaker contribution of autochthonous processes to DOM pool. As a result, DOM composition in İzmir Bay could be linked to humic matter inputs and *in situ* marine production processes.

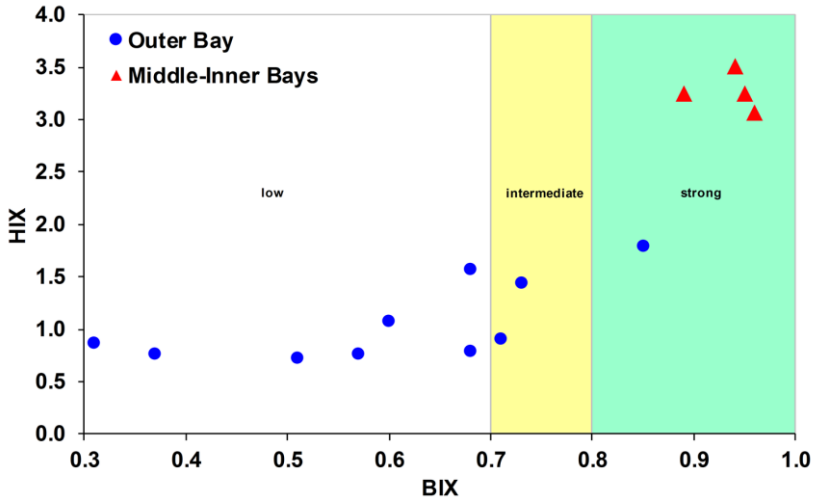

Figure 5. Relationships between HIX and BIX. Low, intermediate and strong indicates autochthonous character of DOM (Huguet et al. 2009)

Spectral ranges of EEM peaks found in this study were very similar to the peaks of CDOM components previously reported in the literature (Penru et al., 2013; Retelletti Brogi et al., 2015; Su et al., 2015; Zeri et al., 2014). Excitation and emission wavelengths of peak regions and corresponding CDOM components were compared with other studies in Table 4. In the North Aegean Sea and Marmara Sea, Zeri et al. (2014) have identified three PARAFAC components within CDOM corresponding to UV and visible humic-like (C1), marine humic-like (C2) and tyrosine-like (C3) peaks. Similar humic-like and protein-like peaks were also present in studies at the Northwestern Mediterranean Sea (Penru et al., 2013) and Tyrrhenian Sea (Retelletti Brogi et al., 2015). Humic-like peaks have been reported at coastal environments under the influences of terrestrial inputs and low salinity waters (Coble, 1996; Stedmon and Markager, 2005). Marine humic-like peaks have been associated with increased phytoplankton activity and autochthonous production (Coble, 1996; Murphy et al., 2008). Tyrosine and tryptophan-like peaks have been shown to originate from bacterial degradation of organic matter and these components are widely distributed along coastal waters (Coble, 1996; Stedmon et al., 2003; Yamashita et al., 2008).

Due to the low velocities of water masses inflowing and outflowing, inner part of İzmir Bay has been more heavily influenced from anthropogenic inputs. According to Sayin (2003), water mass renewal time for inner part was found as 3 months, whereas renewal time for outer bay was around 1-1.5 months. Similarly, Jiang et al. (2008) have reported the influence of water circulation on DOM transport. In middle-inner and outer bays, variations of EEM peak intensities, HIX and BIX (Figures 2-4) could be related to the influences of anthropogenic inputs and physico-chemical characteristics of water masses.

Table 3. Results of Spearman's rank correlation tests and linear regression analyses between EEM peaks, Σ FL, HIX, BIX and DOC

	Correlations with DOC	Linear Regression Equations
Peak A	ρ = 0.744, p = 0.002	[DOC] = 1044.9 [A] + 9.9, r^2 = 0.896, p = 0.000
Peak B	ρ = 0.575, p = 0.031	[DOC] = 2968.0 [B] + 1.0, r^2 = 0.654, p = 0.000
Peak C	ρ = 0.843, p = 0.000	[DOC] = 1826.9 [C] + 3.3, r^2 = 0.888, p = 0.000
Peak M	ρ = 0.701, p = 0.005	[DOC] = 1382.3 [M] + 12.5, r^2 = 0.873, p = 0.000
Peak T	ρ = 0.751, p = 0.002	[DOC] = 1828.4 [T] + 7.1, r^2 = 0.846, p = 0.000
Σ FL	ρ = 0.727, p = 0.003	[DOC] = 332.6 [ΣFL] + 5.8, r^2 = 0.877, p = 0.000
HIX	ρ = 0.774, p = 0.001	[DOC] = 56.1 [HIX] – 13.9, r^2 = 0.796, p = 0.000
BIX	ρ = 0.719, p = 0.004	[DOC] = 226.1 [BIX] – 75.9, r^2 = 0.484, p = 0.005

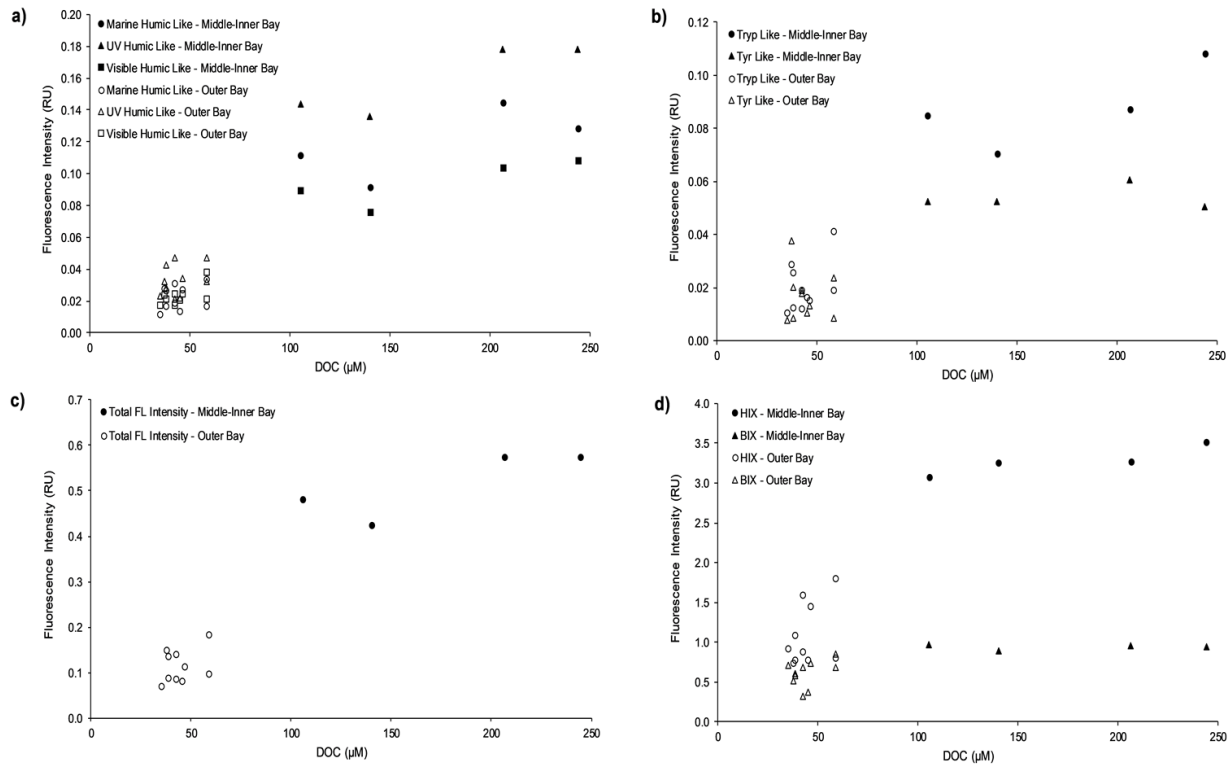

Figure 6. Linear relationships between DOC and a) Marine Humic-like, UV Humic-like and Visible Humic-like peaks, b) Tryptophan-like and Tyrosine-like peaks, and c) ΣFL, and d) HIX and BIX

Table 4. Characteristics of the EEM peaks identified in this study and their comparison with those of other studies

EEM Peaks (Ex/Em) (This Study)	Marmara Sea; North Aegean Sea (Zeri et al., 2014)	Northwestern Mediterranean Sea (Penru et al., 2013)	Tyrrhenian Sea (Retelletti Brogi et al., 2015)	Southern Yellow Sea; East China Sea (Su et al., 2015)
A (260/380–460)	**C1** (<260 (330)/464)	**III** (220–250/380–580)	**P1** (250/400–500)	**C2** (335/400)
B (275/310)	**C3** (270/308)	**I** (220–250/280–332)	**P4** (270/315)	-
C (350/420–480)	**C1** (<260 (330)/464)	**V** (250–470/380–580)	**P3** (350/450)	**C1** (360/440)
M (312/380–420)	**C2** (<250, 285/364)	**IV** (250–470/280–380)	**P2** (315/419)	-
T (275/340)	-	**II** (220–250/332–380)	**P5** (280/341)	**C4** (280/360)

CONCLUSION

Optical characterization of chromophoric dissolved organic matter in İzmir Bay was studied by excitation-emission matrix spectroscopy in this study. Fluorescence measurements indicated the presence of higher humic-like (peaks A, C, and M) and protein-like (peaks T and B) components at eutrophic middle-inner bays. DOC concentrations, EEM peak intensities and HIX/BIX values increased from outer to inner bay. High HIX and BIX values in the middle-inner bays could be explained by the presence of higher humification degrees and freshly produced DOM with bacterial origin. In middle-inner bays, CDOM composition might be influenced from nutrient and DOM rich terrestrial inputs, and water renewal times. In conclusion, optical characterization of CDOM could be used for tracing fluorescent DOM components and determining different DOM sources (autochthonous or allochthonous) in further studies.

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REFERENCES

Anderson, D.M., Glibert, P.M. & Burkholder, J.M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, 25(4), 704-726. DOI:10.1007/BF02804901

Aparicio, F.L., Nieto-Cid, M., Borrull, E., Calvo, E., Pelejero, C., Sala, M.M., Pinhassi, J., Gasol, J.M. & Marrasé, C. (2016). Eutrophication and acidification: Do they induce changes in the dissolved organic matter dynamics in the coastal Mediterranean Sea? *Science of the Total Environment*, 563-564, 179-189. DOI:10.1016/j.scitotenv.2016.04.108

Bai, Y., Su, R. & Shi. X. (2014). Assessing the dynamics of chromophoric dissolved organic matter in the southern Yellow Sea by excitation–emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Continental Shelf Research*, 88, 103-116. DOI:10.1016/j.csr.2014.07.011

Boyd, T.J. & Osburn, C.L. (2004). Changes in CDOM fluorescence from allochthonous and autochthonous sources during tidal mixing and bacterial degradation in two coastal estuaries. *Marine Chemistry*, 89(1–4), 189-210. DOI:10.1016/j.marchem.2004.02.012

Brussaard, C.P.D. (2004). Algae, mortality rates, photosynthetic protists, Phycodnaviridae, virus. *Journal of Eukaryotic Microbiology*, 51(2), 125-138. DOI:10.1111/j.1550-7408.2004.tb00537.x

Carlson, C.A. & Hansell, D.A. (2015). DOM Sources, Sinks, Reactivity, and Budgets. In D.A. Hansell & C.A. Carlson (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter* (pp. 65–126). Florida, USA, Academic Press, 712 pp. DOI:10.1016/B978-0-12-405940-5.00003-0

Cauwet, G. (2002). DOM in the Coastal Zone. In D.A. Hansell & C.A. Carlson (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter* (pp. 579–609). Florida, USA, Academic Press, 774 pp. DOI:10.1016/B978-012323841-2/50014-2

Chari, N.V.H.K., Sarma, N.S., Pandi, S.R. & Murthy, K.N. (2012). Seasonal and spatial constraints of fluorophores in the midwestern Bay of Bengal by PARAFAC analysis of excitation emission matrix spectra. *Estuarine Coastal and Shelf Science*, 100, 162–171. DOI:10.1016/j.ecss.2012.01.012

Chen, H., Zheng, B., Song, Y. & Qin, Y. (2011). Correlation between molecular absorption spectral slope ratios and fluorescence humification indices in characterizing CDOM. *Aquatic Sciences* 73(1), 103-112. DOI:10.1007/s00027-010-0164-5

Cloern, J.E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, 210, 223-253. DOI:10.3354/meps210223

Coble, P.G., Green, S.A., Blough, N.V. & Gagosian, R.B. (1990). Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature,* 348(6300), 432-435. DOI:10.1038/348432a0

Coble, P.G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, 51(4), 325–346. DOI:10.1016/0304-4203(95)00062-3

Conley, D.J., Carstensen, J., Vaquer-Sunyer, R. & Duarte, C.M. (2009). Ecosystem thresholds with hypoxia. In J.H. Andersen & D.J. Conley (Eds.), *Eutrophication in Coastal Ecosystems, Towards better understanding and management strategies* (pp. 21-29). Dordrecht, Netherlands, Springer, 264 pp. DOI: 10.1007/978-90-481-3385-7_3

Couturier, M., Nozais, C. & Chaillou, G. (2016). Microtidal subterranean estuaries as a source of fresh terrestrial dissolved organic matter to the coastal ocean. *Marine Chemistry*, 186, 46-57. DOI:10.1016/j.marchem.2016.08.001

Davidson, K., Gowen, R.J., Tett, P., Bresnan, E., Harrison, P.J., McKinney, A., Milligan, S., Mills, D.K., Silke, J. & Crooks, A.M. (2012). Harmful algal blooms: How strong is the evidence that nutrient ratios and forms influence their occurrence? *Estuarine Coastal and Shelf Science*, 115, 399-413. DOI:10.1016/j.ecss.2012.09.019

Davidson, K., Gowen, R.J., Harrison, P.J., Fleming, L.E., Hoagland, P. & Moschonas, G. (2014). Anthropogenic nutrients and harmful algae in coastal waters. *Journal of Environmental Management*, 146, 206-216. DOI:10.1016/j.jenvman.2014.07.002

Dixon, J.L., Osburn, C.L., Paerl, H.W. & Peierls, B.L. (2014). Seasonal changes in estuarine dissolved organic matter due to variable flushing time and wind-driven mixing events. *Estuarine Coastal and Shelf Science*, 151, 210-220. DOI:10.1016/j.ecss.2014.10.013

Fellman, J.B., Hood, E. & Spencer, R.G.M. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography*, 55(6), 2452-2462. DOI:10.4319/lo.2010.55.6.2452

Gershey, R.M., Mackinnon, M.D., Williams, P.J. & Moore, R.M. (1979). Comparison of three oxidation methods used for the analysis of dissolved organic carbon in seawater. *Marine Chemistry*, 7, 289-306. DOI:10.1016/0304-4203(79)90017-3

Guo, W., Stedmon, C.A., Han, Y., Wu, F., Yu, X. & Hu, M. (2007). The conservative and non-conservative behavior of chromophoric dissolved organic matter in Chinese estuarine waters. *Marine Chemistry*, 107(3), 357-366. DOI:10.1016/j.marchem.2007.03.006

Hansell, D.A., Carlson, C.A., Repeta, D.J. & Schlitzer, R. (2009). Dissolved Organic Matter in the Ocean: A Controversy Stimulates New Insights. *Oceanography*, 22, 202-211. DOI:10.5670/oceanog.2009.109

Hedges, J.I. (2002). Why Dissolved Organics Matter. In D.A. Hansell & C.A. Carlson (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter* (pp. 1–33). Florida, USA, Academic Press, 774 pp. DOI: 10.1016/B978-012323841-2/50003-8

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K. & Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, 8(1), 3-13. DOI:10.1016/j.hal.2008.08.006

Hong, H., Wu, J., Shang, S. & Hu, C. (2005). Absorption and fluorescence of chromophoric dissolved organic matter in the Pearl River Estuary, South China. *Marine Chemistry*, 97(1-2), 78-89. DOI:10.1016/j.marchem.2005.01.008

Huguet, A., Vacher, L., Relexans, S., Saubusse, S., Froidefond, J.M. & Parlanti, E. (2009). Properties of fluorescent dissolved organic matter in the Gironde Estuary. *Organic Geochemistry*, 40(6), 706–719. DOI:10.1016/j.orggeochem.2009.03.002

Jessen, C., Bednarz, V.N., Rix, L., Teichberg, M. & Wild, C. (2015). Marine Eutrophication. In H.R. Armon & O. Hänninen (Eds.), *Environmental Indicators* (pp. 177-203). Dordrecht, Netherlands, Springer, 1068 pp. DOI: 10.1007/978-94-017-9499-2_11

Jiang, F., Lee, F.S.C., Wang, X. & Dai, D. (2008). The application of Excitation/Emission Matrix spectroscopy combined with multivariate analysis for the characterization and source identification of dissolved organic matter in seawater of Bohai Sea, China. *Marine Chemistry*, 110(1-2), 109-119. DOI:10.1016/j.marchem.2008.02.010

Kontas, A., Kucuksezgin, F., Altay, O. & Uluturhan, E. (2004). Monitoring of eutrophication and nutrient limitation in the Izmir Bay (Turkey) before and after Wastewater Treatment Plant. *Environment International*, 29(8), 1057–1062. DOI:10.1016/S0160-4120(03)00098-9

Korak, J.A., Dotson, A.D., Summers, R.S. & Rosario-Ortiz, F.L. (2014). Critical analysis of commonly used fluorescence metrics to characterize dissolved organic matter. *Water Research,* 49, 327–338. DOI:10.1016/j.watres.2013.11.025

Korpinen, S. & Bonsdorff, E. (2015). Eutrophication and hypoxia: impacts of nutrient and organic enrichment. In T.P. Crowe & C.L.J. Fridin (Eds.), *Marine Ecosystems: Human Impacts on Biodiversity, Functioning and Services* (pp. 202–243). Cambridge, UK, Cambridge University Press, 416 pp. DOI: 10.1017/CBO9781139794763.008

Kowalczuk, P., Cooper, W.J., Whitehead, R.F., Durako, M.J. & Sheldon, W. (2003). Characterization of CDOM in an organic-rich river and surrounding coastal ocean in the South Atlantic Bight. *Aquatic Sciences*, 65(4), 384-401. DOI:10.1007/s00027-003-0678-1

Kowalczuk, P., Cooper, W.J., Durako, M.J., Kahn, A.E., Gonsior, M. & Young, H. (2010). Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Relationships between fluorescence and its components, absorption coefficients and organic carbon concentrations. *Marine Chemistry*, 118(1-2), 22-36. DOI:10.1016/j.marchem.2009.10.002

Kowalczuk, P., Sagan, S., Zabłocka, M. & Borzycka, K. (2015). Mixing anomaly in deoxygenated Baltic Sea deeps indicates benthic flux and microbial transformation of chromophoric and fluorescent dissolved organic matter. Estuarine Coastal and Shelf Science, 163(B), 206-217. DOI:10.1016/j.ecss.2015.06.027

Kucuksezgin, F., Kontas, A., Altay, O. & Uluturhan, E. (2005). Elemental composition of particulate matter and nutrient dynamics in the Izmir Bay (Eastern Aegean). *Journal of Marine Systems,* 56(1), 67–84. DOI:10.1016/j.jmarsys.2005.01.002

Lapworth, D.J. & Kinniburgh, D.G. (2009). An R script for visualising and analysing fluorescence excitation–emission matrices (EEMs). *Computers and Geosciences*, 35(10), 2160–2163. DOI:10.1016/j.cageo.2008.10.013

Libes, S.M. (2009). Introduction to Marine Biogeochemistry. California, USA, Academic Press, 928 pp.

Lønborg, C., Yokokawa, T., Herndl, G.J. & Álvarez-Salgado, X.A. (2015). Production and degradation of fluorescent dissolved organic matter in surface waters of the eastern north Atlantic ocean. *Deep Sea Research Part I*, 96, 28-37. DOI:10.1016/j.dsr.2014.11.001

Lu, Y.H., Edmonds, J.W., Yamashita, Y., Zhou, B., Jaegge, A. & Baxley, M. (2015). Spatial variation in the origin and reactivity of dissolved organic matter in Oregon-Washington coastal waters. *Ocean Dynamics*, 65(1), 17-32. DOI:10.1007/s10236-014-0793-7

Lüttig, G. (1986). Plants to peat: the process of humification. In C.H. Fuchsman (Eds.), Peat and Water, Aspects of water retention and dewatering in peat (pp. 9-19). Amsterdam, Netherlands, Springer, 374 pp.

Mopper, K., Zhou, X., Kieber, R.J., Kieber, D.J., Sikorski, R.J. & Jones, R.D. (1991). Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature*, 353(6339), 60-62. DOI:10.1038/353060a0

Motegi, C., Nagata, T., Miki, T., Weinbauer, M.G., Legendre, L. & Rassoulzadegand, F. (2009). Viral control of bacterial growth efficiency in marine pelagic environments. *Limnology and Oceanography*, 54(6), 1901-1910. DOI:10.4319/lo.2009.54.6.1901

Murphy, K.R., Stedmon, C.A., Waite, T.D. & Ruiz, G.M. (2008). Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. *Marine Chemistry*, 108(1-2), 40-58. DOI:10.1016/j.marchem.2007.10.003

Murphy, K.R., Butler, K.D., Spencer, R.G.M., Stedmon, C.A., Boehme, J.R. & Aiken, G.R. (2010). Measurement of Dissolved Organic Matter Fluorescence in Aquatic Environments: An Interlaboratory Comparison. *Environmental Science and Technology*, 44(24), 9405-9412. DOI:10.1021/es102362t

Murphy, K.R., Stedmon, C.A., Graeber, D. & Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods,* 5(23), 6557-6566. DOI:10.1039/C3AY41160E

Nelson, N.B., Carlson, C.A. & Steinberg, D.K. (2004). Production of chromophoric dissolved organic matter by Sargasso Sea microbes. *Marine Chemistry*, 89(1-4), 273-287. DOI:10.1016/j.marchem.2004.02.017

Nieto-Cid, M., Álvarez-Salgado, X.A. & Pérez, F.F. (2006). Microbial and photochemical reactivity of fluorescent dissolved organic matter in a coastal upwelling system. *Limnology and Oceanography*, 51(3), 1391-1400. DOI:10.4319/lo.2006.51.3.1391

Ortega-Retuerta, E., Frazer, T.K., Duarte, C.M., Ruiz-Halpern, S., Tovar-Sánchez, A., Arrieta, J.M. & Rechea, I. (2009). Biogeneration of chromophoric dissolved organic matter by bacteria and krill in the Southern Ocean. *Limnology and Oceanography*, 54(6), 1941-1950. DOI:10.4319/lo.2009.54.6.1941

Ozkan, E.Y., Kocatas, A. & Buyukisik, B. (2008). Nutrient dynamics between sediment and overlying water in the inner part of Izmir Bay, Eastern Aegean. *Environmental Monitoring and Assessment*, 143(1), 313–325. DOI:10.1007/s10661-007-9984-8

Penru, Y., Simon, F.X., Guastalli, A.R., Esplugas, S., Uorens, J. & Balg, S. (2013). Characterization of natural organic matter from Mediterranean coastal seawater. *Journal of Water Supply: Research and Technology* – AQUA, 62(1), 42–51. DOI:10.2166/aqua.2013.113

R Core Team. (2016). R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org.

Retelletti Brogi, S., Gonnelli, M., Vestri, S. & Santinelli, C. (2015). Biophysical processes affecting DOM dynamics at the Arno river mouth (Tyrrhenian Sea). *Biophysical Chemistry*, 197, 1–9. DOI:10.1016/j.bpc.2014.10.004

Ridgwell, A. & Arndt, S. (2015). Why Dissolved Organics Matter: DOC in Ancient Oceans and Past Climate Change. In D.A. Hansell & C.A. Carlson (Eds.*), Biogeochemistry of Marine Dissolved Organic Matter* (pp. 1–33). Florida, USA, Academic Press, 712 pp. DOI: 10.1016/B978-0-12-405940-5.00001-7

Romera-Castillo, C., Sarmento, H., Álvarez-Salgado, X.A., Gasol, J.M. & Marrasé, C. (2011a). Net Production and Consumption of Fluorescent Colored Dissolved Organic Matter by Natural Bacterial Assemblages Growing on Marine Phytoplankton Exudates. *Applied and Environmental Microbiology,* 77(21), 7490–7498. DOI:10.1128/AEM.00200-11

Romera-Castillo, C., Nieto-Cid, M., Castro, C.G., Marrasé, C., Largier, J., Barton, E.D. & Álvarez-Salgado, X.A. (2011b). Fluorescence: Absorption coefficient ratio — Tracing photochemical and microbial degradation processes affecting coloured dissolved organic matter in a coastal system. *Marine Chemistry*, 125,(1-4), 26-38. DOI:10.1016/j.marchem.2011.02.001

Romera-Castillo, C., Álvarez-Salgado, X.A., Galí, M., Gasol, J.M. & Marrasé, C. (2013). Combined effect of light exposure and microbial activity on distinct dissolved organic matter pools. A seasonal field study in an oligotrophic coastal system (Blanes Bay, NW Mediterranean). *Marine Chemistry*, 148, 44-51. DOI:10.1016/j.marchem.2012.10.004

Sala, M.M. & Güde, H. (2004). Ectoenzymatic activities and heterotrophic bacteria decomposing detritus. *Archives of Hydrobiology*, 160(3), 289-303. DOI:10.1127/0003-9136/2004/0160-0289

Santos, L., Santos, E.B.H., Dias, J.M., Cunha, A. & Almeida, A. (2014). Photochemical and microbial alterations of DOM spectroscopic properties in the estuarine system Ria de Aveiro. *Photochemical and Photobiological Sciences,* 13(8), 1146-1159. DOI:10.1039/C4PP00005F

Sarmento, H., Romera-Castillo, C., Lindh, M., Pinhassi, J., Sala, M.M., Gasol, J.M., Marrase, C. & Taylor, G.T. (2013). Phytoplankton species-specific release of dissolved free amino acids and their selective consumption by bacteria. *Limnology and Oceanography*, 58(3), 1123–1135. DOI:10.4319/lo.2013.58.3.1123

Sayin, E. (2003). Physical features of the Izmir Bay. *Continental Shelf* Research, 23(10), 957–970. DOI:10.1016/S0278-4343(03)00083-9

Schreurs, W. (1978). An automated colorimetric method for the determination of disolved organic carbon in seawater by U.V. destruction. *Hydrobiological Bulletin Amsterdam*, 12, 137–142. DOI:10.1007/BF02260715

Sellner, K.G., Doucette, G.J. & Kirkpatrick, G.J. (2003). Harmful algal blooms: causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology*, 30(7), 383-406. DOI:10.1007/s10295-003-0074-9

Stedmon, C.A., Markager, S. & Bro, R. (2003). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, 82(3-4), 239-254. DOI:10.1016/S0304-4203(03)00072-0

Stedmon, C.A. & Markager, S. (2005). Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. *Limnology and Oceanography*, 50(5), 1415-1426. DOI:10.4319/lo.2005.50.5.1415

Stevenson, F.J. (1982). Humus Chemistry. New York, USA, Wiley, 443 pp.

Su, R., Bai, Y., Zhang, C. & Shi, X. (2015). The assessment of the spatial and seasonal variability of chromophoric dissolved organic matter in the Southern Yellow Sea and the East China Sea. *Marine Pollution Bulletin*, 100(1), 523–533. DOI:10.1016/j.marpolbul.2015.09.002

Sulzberger, B. & Durisch-Kaiser, E. (2009). Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability. *Aquatic Sciences*, 71(2), 104-126. DOI:10.1007/s00027-008-8082-5

Sun, Q., Wang, C., Wang, P., Hou, J. & Ao, Y. (2014). Absorption and fluorescence characteristics of chromophoric dissolved organic matter in the Yangtze Estuary. *Environmental Science and Pollution Research*, 21(5), 3460-3473. DOI:10.1007/s11356-013-2287-4

Sunlu, U., Buyukisik, H.B., Koray, T., Brockel, K., Sunlu, F.S., Sever, T.M., Aydın, H., Aksu, M., Aydın, A. & Orçun, E. (2007). The effects of Izmir Big Channel waste water treatment project to the lower trophic level of Izmir Bay (Aegean Sea, Turkey). The Scientific and Technical Research Council of Turkey (TUBITAK-CAYDAG), Project No:102Y116, Final Report. 253p. (in Turkish).

Sunlu, F.S., Sunlu, U., Buyukisik, B., Kukrer, S. & Aksu, M. (2011). Effects of Wastewater Treatment Plant on Water Column and Sediment Quality in Izmir Bay (Eastearn Aegean Sea). In F.S.G. Einschlag (Eds.), *Waste Water - Evaluation and Management* (pp. 253–268). InTech, 482 pp.

Sunlu, F.S., Sunlu, U., Buyukisik, B., Kukrer, S. & Uncumusaoglu, A. (2012). Nutrient and Chlorophyll a Trends after Wastewater Treatment Plant in Izmir Bay (Eastern Aegean Sea). *Journal of Animal and Veterinary Advances,* 11(1), 113–123. DOI:10.3923/javaa.2012.113.123

Tam, S.C. & Sposito, G. (1993). Fluorescence spectroscopy of aqueous pine litter extracts: effects of humification and aluminium complexation. *Journal of Soil Science*, 44(3), 513-524. DOI:10.1111/j.1365-2389.1993.tb00473.x

Tedetti, M., Cuet, P., Guigue, C. & Goutx, M. (2011). Characterization of dissolved organic matter in a coral reef ecosystem subjected to anthropogenic pressures (La Réunion Island, Indian Ocean) using multi-dimensional fluorescence spectroscopy. *Science of the Total Environment,* 409(11), 2198-2210. DOI:10.1016/j.scitotenv.2011.01.058

Tzortziou, M., Zeri, C., Dimitriou, E., Ding, Y., Jaffé, R., Anagnostou, E., Pitta, E. & Mentzafou, A. (2015). Colored dissolved organic matter dynamics and anthropogenic influences in a major transboundary river and its coastal wetland. *Limnology and Oceanography*, 60(4), 1222-1240. DOI:10.1002/lno.10092

Vähätalo, A.V. & Wetzel, R.G. (2004). Photochemical and microbial decomposition of chromophoric dissolved organic matter during long (months–years) exposures. *Marine Chemistry*, 89(1-4), 313-326. DOI:10.1016/j.marchem.2004.03.010

Wang, Y., Zhang, D., Shen, Z., Chen, J. & Feng, C. (2014). Characterization and spacial distribution variability of chromophoric dissolved organic matter (CDOM) in the Yangtze Estuary. *Chemosphere,* 95, 353-362. DOI:10.1016/j.chemosphere.2013.09.044

Wilson, H.F. & Xenopoulos, M.A. (2009). Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geosciences*, 2(1), 37-41. DOI:10.1038/ngeo391

Yamashita, Y., Jaffé, R., Maie, N. & Tanoue, E. (2008). Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnology and Oceanography*, 53(5), 1900-1908. DOI:10.4319/lo.2008.53.5.1900

Yamashita, Y. & Tanoue, E. (2003). Distribution and alteration of amino acids in bulk DOM along a transect from bay to oceanic waters. *Marine Chemistry,* 82(3-4), 145-160. DOI:10.1016/S0304-4203(03)00049-5

Yang, L., Chen, C.T.A., Lui, H.K., Zhuang, W.E. & Wang, B.J. (2016). Effects of microbial transformation on dissolved organic matter in the east Taiwan Strait and implications for carbon and nutrient cycling. *Estuarine Coastal and Shelf Science,* 180, 59-68. DOI:10.1016/j.ecss.2016.06.021

Zeri, C., Besiktepe, S., Giannakourou, A., Krasakopoulou, E., Tzortziouc, V., Tsoliakos, D., Pavlidoua, A., Mousdis, G., Pitta, E., Scoullos, M. & Papathanassiou, E. (2014). Chemical properties and fluorescence of DOM in relation to biodegradation in the interconnected Marmara–North Aegean Seas during August 2008. *Journal of Marine Systems*, 135, 124–136. DOI:10.1016/j.jmarsys.2013.11.019

Zhang, Y., Liu, X., Osburn, C.L., Wang, M., Qin, B. & Zhou., Y. (2013). Photobleaching Response of Different Sources of Chromophoric Dissolved Organic Matter Exposed to Natural Solar Radiation Using Absorption and Excitation–Emission Matrix Spectra. *PLoS ONE,* 8(10), 1-14. DOI: 10.1371/journal.pone.0077515

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B. & Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, 38(1), 45–50. DOI:10.1016/S0045-6535(98)00166-0

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The evaluation of seasonal fatty acid composition and food sources of *Pileurobrachia pileus* (Ctenophora) in terms of trophic marker fatty acids in the Southeastern Black Sea

Güney Doğu Karadeniz'de *Pleurobrachia pileus'un* (Ctenophora) trofik işaret yağ asitleri açısından mevsimsel yağ asiti kompozisyonu ve besin kaynaklarının değerlendirilmesi

Nurgül Şen Özdemir[1*] ● Ali Muzaffer Feyzioğlu[2] ● Fatma Caf[3]

[1] Department of Veterinary Medicine, Vocational School of Technical Sciences, Bingöl University, 12000, Turkey https://orcid.org/0000-0001-6656-822X
[2] Department of Marine Sciences and Technology Engineering, Surmene Faculty of Marine Sciences, Karadeniz Technical University, 61530, Trabzon, Turkey https://orcid.org/0000-0003-1171-5493
[3] Department of Veterinary Medicine, Vocational School of Technical Sciences, Bingöl University, 12000, Turkey https://orcid.org/0000-0002-0363-4848

*Corresponding author: nsozdemir@bingol.edu.tr

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Abstract: Seasonal changes of the lipid and fatty acid composition of *Pleurobrachia pileus* investigated monthly from March 2012 to February 2013. Average total lipid content was determined as percentage (%) and per individual (mg ind-1). It was highest in February (1.48 %; 3.55 mg ind-1). However, it was proportionally the lowest in April (0.40 %), and per individual in August (0.33 mg ind-1). Major fatty acids of *P. pileus* were identified as 16:0, 14:0, 11:1 n-9c, 20:5 n-3, and 22:6 n-3. *P. pileus* had on average 27.27 % ∑SFA, 25.04 % ∑MUFA and 47.63 % ∑PUFA content. EPA and DHA were the major fatty acids from PUFA. Seasonal changes of DHA were more obvious than EPA ($p<0.05$). Herbivore calanoid zooplankton trophic markers; 20:1 n-9 and DHA/EPA and herbivory trophic markers; EPA and DHA content were high in *P. pileus* fatty acids. It showed that herbivory fatty acids were taken by feeding from herbivory zooplankton and phytoplankton. Diet was an important factor in seasonal fatty acid changes of *P. pileus*. In addition, we revealed that *P. pileus* has a rich lipid content and fatty acid composition and plays an important role in the Southeastern Black Sea ecosystem functionalities between herbivory and carnivory species.

Keywords: DHA, EPA, fatty acid, Southeastern Black Sea, *Pileurobrachia pileus*, trophic marker

Öz: *Pileurobrachia pileus*'un lipit ve yağ asiti kompozisyonunun mevsimsel değişimleri Mart 2012'den Şubat 2013'e kadar aylık olarak araştırılmıştır. Ortalama toplam lipit içeriği yüzde (%) ve birey başına (mg ind-1) olarak belirlenmiştir. Ortalama toplam lipit miktarı en yüksek şubat ayında bulunmuştur (% 1,48; 3,55 mg ind-1). Bununla birlikte, oransal en düşük miktar Nisan ayında (%0,40) ve birey başına Ağustos ayında (0,33 mg ind-1) belirlenmiştir. *P. pileus*'taki en önemli yağ asitleri 16: 0, 14: 0, 11: 1 n-9c, 20: 5 n-3 ve 22: 6 n-3 olarak tespit edilmiştir. *P. pileus*'da ortalama % 27,27 ∑SFA, % 25,04 ∑MUFA ve % 47,63 ∑PUFA belirlenmiştir. EPA ve DHA'nın, başlıca PUFA yağ asitleri olduğu ve. DHA'da mevsimsel değişikliklerin EPA'dan daha belirgin olduğu saptanmıştır ($p<0,05$). *P. pileus* yağ asitlerinde, herbivor kalanoid otçul zooplankton trofik işaretleri; 20: 1 n-9 ve DHA / EPA ve herbivor trofik işaretleri; EPA ve DHA içeriğinin yüksek olduğu tespit edilmiştir. Otçul yağ asitlerinin otçul zooplankton ve fitoplanktondan beslenerek alındığı görülmüştür. *P. pileus*'un mevsimsel yağ asiti değişiminde besinin önemli bir faktör olduğu saptanmıştır. Bunun yanında, *P. pileus*'un zengin bir lipit içeriğine ve yağ asiti bileşimine sahip olduğu ve Güneydoğu Karadeniz ekosisteminde, herbivor ve karnivor türler arasında önemli bir rol oynadığı ortaya konulmuştur.

Anahtar kelimeler: DHA, EPA, yağ asiti, Güney Doğu Karadeniz, *Pileurobrachia pileus,* trofik işaret

INTRODUCTION

Genus Pleurobrachia tentaculate ctenophores is common in coastal and neritic seas around of the world (Fraser, 1970; Frank, 1986; Mutlu et al., 1994, Mutlu and Bingel, 1999). Their diets have a wide range of prey (Hirota, 1974; Reeve & Walter, 1978). Although their diets usually reflects the ambient in environment (Fraser, 1970; Mazlum et al., 2018), prey are likely to be selected on the basis of size and escape response (Greene et al., 1986). *Pleurobrachia pileus* from Pleurobrachia genus is a gelatinous planktonic carnivore Ctenophora, Tentaculata, Cydippida and a special tentacle feeder (Reeve and Walter, 1978). Copepods appear to be the main prey for ctenophores (Gibbons and Painting, 1992). It is known a little information about the role of ctenophores and other gelatinous zooplankton species in the energy flow in the marine ecosystems (Greene et al., 1986). In order to fully understand the energy flux through the foodwebs, detailed information about the bioenergetics of the organism is required (Møller et al., 2010). Lipids are important biochemical compounds in energy flows in marine food webs because they are rich in carbon with very high energy values (Parrish, 1988). Already, it was reported firstly by Lee et al.(1971) that fatty acids (FA) are an important lipid group are transferred from one

trophic level to the next. Sargent and Whittle (1981); Sargent and Falk-Petersen (1988); Falk Petersen et al. (1990); Graeve et al. (1994); Stübing et al. (2003) suggested the potential of dietary fatty acids as food chain indicators and trophic markers. Similarly, fatty acids in consumer tissues can provide dietary information (Sargent et al., 1987; Graeve et al., 1994; Stübing et al., 2003), and some have been used successfully as trophic markers to monitor energy transfer to investigate prey-predator relationships (Falk-Peterson et al., 2004; Litzow et al., 2006). However, there are limited lipid studies about lipid amount and fatty acid composition of *P. pileus*. Therefore, we investigated seasonal changes of lipid amount and fatty acid composition of *P. pileus*, to determine the role of *P. pileus* in the functions between herbivorous and carnivorous species of the Southeastern Black Sea ecosystem. Additionally, it was thought that *P. pileus* generally carnivorous and feed on copepods in the Black Sea (Mutlu and Bingel, 1999; Mutlu, 2001; Birinci Özdemir et al., 2018). However, firstly, it was reported that phytoplankton was dominant group in the diet of *P. pileus* and zooplankton were the second dominant group in the Southeastern Black Sea by Mazlum et al. (2018). The fatty acids 16:l n-7 and 20:5 n-3 (Eicosapentaenoic Acid, EPA) as well as 18:4 n-3 and 22:6 n-3 are characteristic of diatoms and dinoflagellates, respectively. 16:l n-7 and 18:4 n-3 fatty acids proved best-suited for trophic analyses, since their occurrence is most closely associated with the different phytoplankton groups ingested (Graeve et al., 1994). Therefore, we also aimed to evaluate the dietary composition of *P. pileus* using trophic marker fatty acids.

MATERIAL AND METHODS

The study was performed in the southern part of the Black Sea (Çamburnu Bay) at a station with coordinates 40º 57' 12" N' - 40º 9' 30" E. Samplings was made monthly from March 2012 to February 2013 aboard KTU's research vessel Yakamoz. Zooplankton samples were taken with a vertical haul with a 200 µm mesh Hydro-Bios net with a mouth diameter of 110 cm from the depth of the upper border of the anoxic layer 130 m up to the surface layer.

Lipids were quantitatively extracted from the samples using chloroform/methanol with a mixing ratio of 2:1 Folch et al., (1957). Chloroform (2 mL) and 2N NaOH solution in methanol (2 mL) were used to determine fatty acid methyl esters (FAME). Then, 2 mL hexane was added on the restored dry lipid and the sample was transferred to a vial (Kates, 1986). The FAME were detected by gas Chromatography (Shimadzu GC-17 version 3). For the analysis, Capillary coumn had with a length of 25 m, an inner diameter of 0.25 mm and a thickness of 25 mm (Permabond). 20 µl samples were used to inject into the GC. The column temperature was set to 120-220 ºC using increments of 5ºC min^{-1} until 200ºC and reached and 4ºC min^{-1} to 220ºC. The column was kept for 8 min at 220ºC and the total time was determined 35 min. The injection temperature was 240 ºC and detector temperature was 280ºC. Nitrogen (N_2) was used as the carrier gas (Christie, 1990).

Statistical analysis

Datas were analyzed in STATISTICA 8.0. One-way variance analysis (ANOVA) was used in datas analysis. The comparisons among averages of the samples were carried out by using TUKEY test. TUKEY test created by post-hoc, homogenous groups ($p<0.05$). Spearman Rank Correlation was used in definition of the statistical differences.

RESULT AND DISCUSSION

Fatty acid composition and total lipid amount of *P. pileus* were examined monthly from March 2012 to February 2013 in Southeastern Black Sea. Total lipid was determined in wet weight (WW) as percent (%) and weight (mg ind^{-1}). Lipid amount for most gelatinous zooplankton is low and they have about 95 % water (Nelson et al., 2000). According to Larson and Harbison (1989) the average lipid amount of Antarctic gelatinous zooplankton is approximately 3% (0.4-6%), whereas Arctic gelatinous zooplankton have on average (8 %) lipid as a percent 1.5-22 % in dry weight. In the study, the highest total lipid (% ; mg ind^{-1}) was obtained in February (1.48%; 3.55 mg ind^{-1}, respectively). However, total lipid (%) was the lowest in April (0.40 %), while total lipid mg ind^{-1} was the lowest in August (0.33 mg ind^{-1}) (Figure 1). Also, the average individual weight of *P. pileus* was the highest in September (220.39 mg ind^{-1}) and, the lowest in December (44.49 mg ind^{-1}).

It was reported that total lipid amount of *P. pileus* was 17 mg g^{-1} in dry weight in North Sea by Hoeger (1983) and 3.4 mg g^{-1} in the Black Sea by Anninsky et al. (2005). Nelson et al. (2000) emphasized that the total lipid amount in wet weight was very low in 153 jelly zooplankton (0.1-5 mg g^{-1}) in Antarctic during January 1997 and February 1998. Also, they reported that the total lipid amount of *P. pileus* was 3.6 mg/g in wet weight and 7.1 % in dry weight. In this study, the average total lipid of *P. pileus* was 1.26 mg ind^{-1}. If we used gram instead of individual, total lipid amount will be 7.47 mg g^{-1} in wet weight. We thought that the differences in results of the studies can be derived from regional, seasonal and environmental differences. Especially, we thought that prey/diet in the environment can cause these differences. Phleger et al. (1998) indicated that lower lipid levels of gelatinous predators probably reflect lower lipid in their prey in Antarctic. Gelatinous macrozooplankton group mainly feeds on zooplankton, fish eggs and larvae in the Black Sea (Mutlu, 2001; Birinci Özdemir et al., 2018). Copepods were the most important group among of the digested food of *P. pileus*. Copepods have 47.2% of the total number of the digested food (Yip, 1984). Mutlu and Bingel (1999) reported that the stomach contents of *P. pileus* consisted mainly of Copepoda 90%. However, Mazlum et al. (2018) indicated that seasonal diet of *P. pileus* is mainly phytoplankton in autumn and winter periods in Southeastern Black Sea. *Calanus euxinus* has the highest proportion 39% in copepoda of the Southeastern Black Sea. Therefore, especially C. *euxinus* lipids can be an important effective on lipid levels of *P. pileus*. Also, phytoplankton lipids consumed directly by *P. pileus*; and phytoplankton lipids that

the calanus often prefer as diet can affect lipids of *P. pileus*. Total lipid amount of *C. euxinus* from copepoda is the highest in February 7.03% (WW). Also, C. *euxinus* reaches the highest abundance in February (847 ind m^{-3}) between March 2012 and February 2013 in the Southeastern Black Sea (Sen Ozdemir et al., 2017). We determined the highest lipid amount in the same season and sampling period for *P. pileus* (Figure 1).

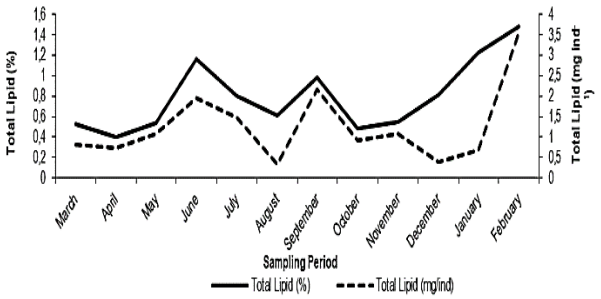

Figure 1. Total lipid variations (% ; mg ind^{-1}) of *P. pileus* during the sampling period

In this study, average total saturated fatty acids (∑SFA) were 27.27% in the during sampling period. 16:0 (palmitic acid), 14:0 (myristic acid) and 18:0 (stearic acid) were the major SFA in *P. pileus*. 14:0, 16:0 and 18:0 were the lowest in November (2.08%; 9.97%; 3.13%, respectively) (Table 1). However, while 16:0 and 18:0 were the highest in September (19.29%; 14.64%, respectively), 14:0 was the highest in December (8.17%). There was no statistically significant difference between seasons for 16:0 whereas there was statistically significant difference between seasons for 14:0 and 18:0 ($p<0.05$) (Table 2). Whereas, 16:0 was the lowest in spring (11.76%) and 18:0 was the lowest in winter (3.93%). 14:0 was the highest in winter (6.83%) and 18:0 was the highest in autumn (7.66%). Additionally, 14:0 was the lowest in autumn (4.02%), whereas 16:0 was the highest in autumn (14.00 %). Nelson et al. (2000) found the same results for the major SFA of *P. pileus*. In their results, 14:0 was 5.4%, 16:0 was 25.8% and 18:0 was 20.0%.

Average total monounsaturated fatty acids (∑MUFA) were 25.04% during the sampling period. 18:1 n-9c (oleic acid) was the dominant MUFA and on average 14.19% for *P. pileus*. followed by palmitoleic acid (16:1 n-7) on average 4.85%. Palmitoleic acid was the highest in April (7.56 %) and the lowest in July (3.27 %). Oleic acid was the highest in November (29.68%) and the lowest in January (6.68%) (Table 3). It increased in autumn (21.57%) and immediately decreased in winter (7.53%) (Table 4). 16:1 n-7 is generally of diatom origin and together with 20:1 n-9 can be found in high proportions in planktonic systems (Reinhardt and Van Vleet, 1986; Pakhomov and Perissinotto, 1996). 20:1 n-9 is the herbivorous calanoid marker (Dalsgaard et al., 2003; Falk-Peterson et al., 2009; Şen Özdemir et al., 2019). It shows herbivor copepods comprised an important part of the predator's diet (Stowasser et al., 2012). The MUFA herbivorous calanoid fatty acid markers were associated most strongly with pelagic species and vertical migrators (Şen Özdemir et al., 2019).

Ctenephoras were characterized by moderate to high levels of polyunsaturated fatty acids (PUFA) (20-47%) with exception of *P. pileus* (Nelson et al., 2000). Nelson et al., (2000) showed 21.1% PUFA in *P. pileus*. Average PUFA of *P. pileus* was found 47.63% in the study. The high level can depend on PUFA content of prey. DHA and EPA were the most important fatty acids in PUFA. Seasonal changes of DHA was statistically important ($p<0.05$). DHA and EPA were the highest in March (30.49%; 16.68%. respectively). However. DHA and EPA were the lowest in September (17.85%; 7.98%. respectively) (Table 5). EPA showed a significant decrease only in autumn (9.15 %). while the other seasons did not differ with similar values (Table 6). It was found that seasonal changes in DHA were more obvious than EPA ($p<0.05$). When these findings show a parallelism with Nelson et al., (2000) in terms of the variety of fatty acids that have the highest portion in *P. pileus*. Carnivorous zooplankton are rich in PUFA than herbivorous crustaceans zooplankton (Cripps and Atkinson, 2000; Stevens et al., 2004). Yet another index of carnivory is the DHA/EPA (Dalsgaard et al., 2003). DHA/EPA is used to explain trophic relations. EPA is typically found in higher proportions in diatoms whereas dinoflagellates contain higher DHA relative EPA (Nelson et al., 2000). However this ratio also reflects the relative proportions of dinoflagellate to diatoms in the diets of herbivorous and omnivorous copepods (Viso and Marty, 1993). In the study, the average DHA/EPA reached the highest value in autumn (2.37) and summer (2.08) hot seasons and the lowest value in winter (2.01) and spring (1.76) cold seasons (Table 6). While dinoflagellate blooms are generally observed in summer, diatom blooms are observed generally in mid spring in the Southeastern Black Sea (Sahin et al., 2007). According to Mazlum et al., (2018) phytoplankton is the dominant food group in their diet followed by zooplankton. They did not mention the presence of diatoms in dietary analysis for *P. pileus*. However, diatoms marker EPA was one of the major fatty acids during all the sampling seasons in our results. This may be a proof that they received EPA via herbivorous zooplankton in the Southeastern Black Sea food chain. EPA can be transferred from herbivor zooplankton to *P. pileus* by food web. *P. pileus* had high proportion DHA/EPA. This can be show as a proof that *P. pileus* is mostly a carnivorous because DHA/EPA carnivory index. Additionally, we think that the presence of *Calanus euxinus* which constitutes the main food source of *P. pileus* can be effective on the nutritional habit of *P. pileus*. Sen Ozdemir et al., (2017) reported that *C. euxinus* was the lowest abundance in the spring and summer and highest abundance winter and chlorophyll-a which is as an indicator of phytoplankton abundance was the high especially in late spring and early summer in the same sampling station and during the same time periods. Also, *Sagitta setosa* and copepod species were the others food sources of *P. pileus* in the Southeastern Black Sea. Şen Özdemir et al., (2020) indicated that *S. setosa* and copepod species (nauplii and copepodites) had the highest abundance in spring and summer in the same station and same sampling periods. *P. pileus* may be preferred abundant phytoplankton as food rather than scarce copepod in these seasons. We understand that food sources changes depending on season and this effects food preference of *P. pileus* in the Southeastern Black Sea. Diet was an important factor in seasonal fatty acid changes of *P. pileus*.

Table 1. SFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
14:0	5.12±0.25^{bcd}	4.31±0.16^{def}	5.51±0.68^{bcd}	5.93±0.75^{abcd}	5.65±0.17^{bcd}	4.67±1.01^{cde}	2.68±0.51^{ef}	7.32±0.83^{ab}	2.08±0.07^{f}	8.17±0.88^{a}	5.51±0.18^{bcd}	6.80±1.40^{abc}
15:0	-	-	1.14±0.12^{a}	-	-	-	-	-	-	-	-	0.84±0.10^{a}
16:0	12.52±0.77^{bc}	11.56±0.14^{bc}	11.21±1.14^{bc}	10.94±1.05^{bc}	13.98±0.53^{b}	11.37±1.32^{bc}	19.29±1.14^{a}	12.76±0.06^{bc}	9.97±1.02^{bcd}	13.09±0.66^{bc}	11.81±0.73^{bc}	12.58±0.50^{bc}
17:0	-	-	-	-	-	-	1.11±0.06^{a}	-	-	-	0.81±0.14^{a}	0.95±0.02^{a}
18:0	5.09±0.24^{b}	4.41±0.24^{bc}	3.74±0.29^{bc}	4.56±0.96^{bc}	5.24±0.21^{b}	5.07±0.71^{b}	14.64±2.45^{a}	5.22±0.21^{b}	3.13±0.14^{c}	3.75±1.17^{bc}	4.09±0.03^{bc}	3.94±0.52^{bc}
20:0	-	1.04±0.05^{a}	-	-	-	-	-	-	-	-	1.42±0.60^{a}	-
22:0	6.35±0.38^{b}	3.24±0.80^{cd}	5.57±0.37^{b}	6.03±0.38^{b}	-	-	2.58±0.48^{d}	-	8.71±0.65^{a}	5.32±0.43^{bc}	-	6.00±0.12^{b}
∑SFA	29.08±0.94^{bc}	24.55±0.69^{de}	27.17±0.39^{bcde}	27.46±0.96^{bcd}	24.87.±0.90^{cdef}	21.11±2.01^{f}	40.30±4.08^{a}	25.29±0.59^{cdef}	23.89±1.46^{ef}	30.33±0.08^{b}	23.64±0.33^{def}	31.11±0.80^{b}

Means followed by different letters and letter groups in the same row are significantly different t $p<0.05$. n=3. values are means±SD

Table 2. Seasonally SFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	Spring	Summer	Autumn	Winter
14:0	4.98±0.61^{ab}	5.41±0.66^{ab}	4.02±2.87^{b}	6.83±1.33^{a}
15:0	0.38±0.66^{a}	-	-	0.28±0.49^{a}
16:0	11.76±0.68^{a}	12.10±1.65^{a}	14.00±4.78^{a}	12.56±0.64^{a}
17:0	-	-	0.37±0.64^{a}	0.59±0.51^{ab}
18:0	4.42±0.68^{ab}	4.96±0.35^{ab}	7.66±6.13^{a}	3.93±0.17^{b}
20:0	0.35±0.60^{a}	-	-	0.47±0.82^{a}
22:0	5.06±1.61^{a}	2.01±3.48^{a}	3.76±4.47^{a}	3.78±3.46^{a}
∑SFA	26.95±2.14^{a}	24.48±1.46^{a}	29.28±2.06^{a}	28.36±2.09^{a}

Means followed by different letters and letter groups in the same row are significantly different t $p<0.05$. n=3-9. values are means±SD

Table 3. MUFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
15:1	-	-	1.56±0.16^{a}	1.66±0.40^{a}	-	-	-	-	-	-	0.95±0.081^{b}	1.02±0.21^{b}
16:1 n-7	4.8±0.4^{bcd}	7. 56±0.17^{a}	5.93±0.41^{ab}	5.28±0.27^{bc}	3.27±0.27^{de}	5.03±0.95^{bcd}	3.41±0.47^{de}	5.31±0.52^{bc}	2.57±0.50^{e}	4.97±1.01^{bcd}	3.74±0.64^{cde}	6.38±0.50^{ab}
17:1	-	-	-	-	-	-	0.97±0.05^{a}	-	-	-	0.63±0.04^{b}	1.03±0.12^{ab}
18:1 n-9c	9.29±0.32^{d}	14.68±0.41^{c}	13.35±1.57^{c}	15.55±1.51^{c}	14.66±0.86^{c}	15.48±1.32^{c}	20.91±1.45^{b}	14.10±1.18^{c}	29.68±2.15^{a}	7.69±0.64^{d}	6.68±1.28^{d}	8.24±0.58^{d}
20:1 n-9	4.35±0.68^{a}	2.25±0.16^{c}	2.24±0.41^{c}	2.53±0.13^{bc}	3.11±0.13^{abc}	4.43±0.60^{a}	2.67±0.26^{bc}	3.14±0.39^{abc}	1.72±0.25^{b}	3.84±0.30^{ab}	3.07±0.18^{abc}	2.76±0.22^{bc}
20:1 n-X	-	-	4.74±0.64^{abc}	4.38±0.06^{bc}	-	-	-	3.66±0.19^{c}	-	5.66±0.58^{a}	4.92±0.69^{ab}	4.69±0.47^{abc}
∑MUFA	18.44±1.32^{g}	24.49±0.19^{cdef}	27.76±3.12^{bc}	29.40±3.87^{ab}	21.04±1.17^{efg}	24.93±2.80^{bcde}	27.95±0.11^{bc}	26.22±0.81^{bcd}	34.64±1.9^{a}	22.16±1.14^{defg}	19.99±2.36^{fg}	24.12±0.75^{cdef}

Means followed by different letters and letter groups in the same row are significantly different p<0.05. n=3. values are means±SD

Table 4. Seasonally MUFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	Spring	Summer	Autumn	Winter
15:1	0.52±0.90^{a}	0.55±0.96^{a}	-	0.66±0.57^{a}
16:1 n-7	6.07±1.39^{a}	4.53±1.09^{ab}	3.76±1.07^{b}	5.03±1.32^{ab}
17:1	-	-	0.32±0.56^{b}	0.59±0.52^{a}
18:1 n-9c	12.44±2.81^{b}	15.23±0.49^{b}	21.57±7.81^{a}	7.53±0.79^{c}
20:1 n-9	2.95±1.22^{a}	3.35±0.97^{a}	2.51±0.71^{a}	3.22±0.56^{a}
20:1 n-X	1.58±2.73^{b}	1.46±2.53^{b}	1.22±2.11^{b}	5.09±0.51^{a}
∑MUFA	23.56±4.73^{b}	25.12±4.18^{ab}	29.38±4.45^{a}	22.09±2.07^{b}

Means followed by different letters and letter groups in the same row are significantly different t p<0.05. n=9. values are means±SD

Table 5. PUFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
18:2 n-6*t*	-	-	-	-	-	-	-	-	-	-	-	0.90±0.02
18:2 n-6*c*	5.31±0.17^{def}	3.71±0.65^{f}	5.70±0.55^{def}	6.32±1.20^{cdef}	13.64±0.86^{b}	8.87±1.53^{c}	6.88±1.37^{cde}	7.53±0.84^{cd}	17.29±1.56^{a}	3.79±0.17^{f}	4.41±0.09^{ef}	5.42±0.83^{def}
18:3 n-3*c*	-	1.57±0.07^{a}	1.63±0.19^{a}	1.53±0.21^{a}	-	-	-	0.96±0.51^{b}	-	-	1.28±0.13^{ab}	1.02±0.03^{b}
20:2 n-6	-	4.25±0.23^{a}	-	-	-	-	-	-	-	-	3.65±1.03^{b}	-
20:3 n-6	-	1.35±0.26	-	-	-	-	-	-	-	-	-	-
20:4 n-3	-	2.78±0.26^{b}	-	-	-	5.52±0.56^{a}	-	-	-	-	1.36±0.31^{c}	-
20:4 n-6	-	1.41±0.04^{e}	-	-	-	-	-	-	-	-	-	-
22:2	-	-	-	-	-	-	-	-	-	-	3.10±0.62	-
20:5 n-3(EPA)	16.68±0.51^{a}	13.40±0.22^{b}	13.72±0.48^{b}	12.54±1.29^{b}	12.51±0.99^{b}	12.38±0.70^{b}	7.98±1.55^{c}	12.68±0.45^{b}	6.80±1.57^{c}	14.07±0.84^{ab}	13.21±0.85^{b}	14.32±0.98^{ab}
22:6 n-3(DHA)	30.49±2.10^{a}	22.50±0.60^{cde}	23.99±1.13^{bcd}	22.73±2.52^{cde}	27.96±1.81^{abc}	27.20±1.18^{abc}	17.85±3.62^{e}	27.33±0.83^{abc}	18.05±1.51^{de}	29.60±1.50^{ab}	29.36±0.77^{ab}	23.77±1.34^{bcde}
∑PUFA	52.48±2.28^{ab}	50.96±0.53^{abc}	45.04±1.84^{cde}	43.12±2.95^{de}	54.11±2.01^{a}	53.96±0.85^{a}	32.71±6.32^{f}	48.50±0.31^{abcd}	42.14±3.20^{e}	47.50±1.06^{bcde}	56.38±2.44^{ab}	46.77±1.48^{de}
DHA/EPA	1.82±0.13^{b}	1.68±0.02^{b}	1.75±0.04^{b}	1.81±0.13^{b}	2.23±0.03^{ab}	2.20±0.07^{ab}	2.24±0.15^{ab}	2.16±0.09^{ab}	2.72±0.23^{a}	2.11±0.22^{ab}	2.22±0.04^{ab}	1.67±0.20^{b}

Means followed by different letters and letter groups in the same row are significantly different t p<0.05. n=3. values are means±SD

Table 6. Seasonally PUFA composition of *P. pileus* during the sampling period (% total defined FAME)

FA	Spring	Summer	Autumn	Winter
18:2 n-6*t*	-	-	-	0.30±0.17
18:2 n-6*c*	4.91±1.05^{b}	9.61±3.69^{a}	10.57±5.83^{a}	4.54±0.82^{b}
18:3 n-3*c*	1.07±0.04^{a}	0.51±0.21^{a}	0.32±0.51^{a}	0.76±0.18^{a}
20:2 n-6	1.42±0.23^{a}	-	-	1.22±1.03^{a}
20:3 n-6	0.45±0.26	-	-	-
20:4 n-3	0.93±0.26^{a}	1.84±0.56^{a}	-	0.45±0.31^{a}
20:4 n-6	0.47±0.27	-	-	-
22:2	-	-	-	1.03±0.59^{a}
20:5 n-3(EPA)	14.60±1.81^{a}	12.48±0.09^{a}	9.15±3.11^{b}	13.87±0.58^{a}
22:6 n-3 (DHA)	25.66±4.25^{ab}	25.96±2.83^{ab}	21.08±5.42^{b}	27.59±3.30^{a}
∑PUFA	49.50±3.93^{a}	50.40±6.30^{a}	41.12±7.94^{b}	49.50±5.35^{a}
DHA/EPA	1.76±0.07^{b}	2.08±0.24^{ab}	2.37±0.30^{ab}	2.01±0.29^{a}

Means followed by different letters and letter groups in the same row are significantly different t p<0.05. n=3-9. values are means±SD

CONCLUSION

P. pileus has a rich lipid content and fatty acid composition. It plays an important role in the Southeastern Black Sea ecosystem functionalities between herbivory and carnivory species. Calanoid herbivory markers 20:1 n-9 and DHA/EPA and herbivory marker EPA and DHA content were high in fatty acids of *P. pileus*. We showed that although *P. pileus* mostly carnivorous, it had herbivorous fatty acid markers. Herbivory fatty acids were taken by feeding from herbivory species and phytoplankton (dinoflagellates and diatoms) especially when zooplankton are low abundance in the Southeastern Black Sea ecosystem.

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REFERENCES

Anninsky, B.E., Finenko, G.A., Abolmasova, G.I., Hubareva, E.S., Svetlichny, L.S., Bat, L. & Kideys, A.E. (2004). Effect of starvation on the biochemical compositions and metabolic rates of ctenophores *Mnemiopsis leidyi* and *Beroe ovata* in the Black *Sea. Journal of Marine Biology Association United Kingtom,* 85, 549–561. DOI: 10.1017/S0025315405011471

Birinci Özdemir, Z., Erdem, Y. & Bat, L. (2018). Food composition and distribution of gelatinous macrozooplankton in the southern Black Sea. *Indian Journal of Geo Marine Sciences*, 4712, 2541–2548.

Christie, W.W. (1990). Gas chromatography and lipids. (pp 302-320). The Oil Press. Glasgow.

Cripps, G.C. & Atkinson, A. (2000). Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba. Canadian Journal of Fisheries Aquatic Sciences,* 57(S3), 31–37. DOI:10.1139/f00-167

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D. & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances Marine Biology*, 46, 225–340. DOI: 10.1016/s0065-2881(03)46005-7

Falk-Petersen, S., Hopkins, C.C.E. & Sargent, J.R. (1990). Trophic relationships in the pelagic arctic food web. In M. Barnes. R.N. Gibson (Eds). Trophic relationships in the marine environment (pp 315-333). Proc. 24th Europ Mar Biol Symp. Aberdeen: Aberdeen University Press.

Falk-Petersen, S., Haug, T., Nilssen, K.T., Wold, A. & Dahl, T.M. (2004). Lipids and trophic linkages in harp seal *Phoca groenlandica* from the eastern Barents Sea. *Polar Research*, 23, 43–50. DOI: 10.1111/j.1751-8369.2004.tb00128

Falk-Petersen, S., Mayzaud, P., Kattner, G. & Sargent, J.R (2009). Lipids and life strategy of Arctic Calanus. *Marine Biology Research*, 5(1), 18-39. DOI: 10.1080/17451000802512267

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957). A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *The Journal of Biological Chemistry,* 226, 497-509. DOI: 10.1016/S0021-9258(18)64849-5

Frank, K.T. (1986). Ecological significance of the ctenophore *Pleurobrachia pileus* off southwestern Nova Scotia. *Canadian Journal of Fisheries and Aquatic Sciences*, 431, 211–222. DOI: 10.1139/f86-024

Fraser, J.H. (1970). The ecology of the ctenophore *Pleurobrachia pileus* in Scottish waters. *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer,* 332, 149–168. DOI: 10.1093/icesjms/33.2.149

Gibbons, M.J. & Painting, S.J. (1992). The effects and implications of container volume on clearance rates of the ambush entangling predator *Pleurobrachia pileus* Ctenophora: Tentaculata. *Journal of Experimental Marine Biology and Ecology,* 163, 199–208. DOI: 10.1016 / 0022-0981 (92) 90049-G

Graeve, M.G., Kattner, G. & Hagen, W. (1994). Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology,* 182, 97–110. DOI: 10.1016/0022-0981(94)90213-5

Greene, C.H., Landry, M.R. & Monger, B.C. (1986). Foraging behaviour and prey selection by the ambush entangling predator *Pleurobrachia bachei. Ecology*, 67, 1493–1501. DOI: 10.2307/1939080

Hirota, J. (1974). Quantitative natural history of *Pleurobrachia bachei* in La Jolla Bight. *Fishery Bulletin-Washington*, 722, 295–335.

Hoeger, U. (1983). Biochemical composition of ctenophores. *Journal of Experimental Marine Biology and Ecology,* 72, 251–261. DOI: 10.1016/0022-0981(83)90110-7

Kates, M. (1986). Techniques of lipidology: isolation, analysis, and identification of lipids, 2nd edn. Amsterdam. New York: Elsevier. New York : Elsevier Science Pub *Co Laboratory Techniques in Biochemistry and Molecular Biology*, 32, 464.

Larson, R.J. & Harbison, G.R. (1989). Source and fate of lipids in polar gelatinous zooplankton. *Arctic*, 42, 339-346. DOI: 0.14430/arctic1675

Lee, R.F., Hirota, J. & Barnett, A.M. (1971). Distribution and importance of wax esters in marine copepods and other zooplankton. *Deep Sea Research,* 18, 1147-1165. DOI: 10.1016/0011-7471(71)90023-4

Litzow, M.A., Bailey, K.M., Prahl, F.G. & Heinz, R. (2006). Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. *Marine Ecology Progress Series*, 315, 1–11. DOI: 10.3354 / meps315001

Mazlum, R.E., Aytan, U. & Agirbas, E. (2018). The feeding behaviour of *Pleurobrachia pileus* (ctenophora: tentaculata) in the Southeastern Black Sea: in relation to area and season. *Fresenius Environmental Bulletin*, 27(2), 871-879.

Mutlu, E., Bingel, F., Gücü, A.C., Melnikov, V.V., Niermann, U., Ostr, N.A. & Zaika, V.E. (1994). Distribution of the new invader *Mnemiopsis sp.* and the resident *Aurelia aurita and Pleurobrachia pileus* populations in the Black Sea in the years 1991–1993. ICES *Journal of Marine Sciences*, 51, 407–421. DOI: 10.1006/jmsc.1994.1042

Mutlu, E. & Bingel, F. (1999). Distribution and abundance of ctenophores. and their zooplankton food in the Black Sea. I. *Pleurobrachia pileus. Marine Biology.* 135. 589-601. DOI: 10.1007/s002270050661

Mutlu, E. (2001). Distribution and abundance of moon jellyfish *Aurelia aurita* and its zooplankton food in the Black Sea. *Marine Biology,* 138, 329-339. DOI: 10.1007 / s002270000459

Møller, L.F., Canon, J.M. & Tiselius, P. (2010). Bioenergetics and growth in the ctenophore *Pleurobrachia pileus. Hydrobiologia*, 645, 167–178. DOI: 10.1007 / s10750-010-0219-6

Nelson, M.M., Phleger, C.F., Mooney, B.D. & Nichols, P.D. (2000). Lipids of Gelatinous Antarctic Zooplankton: Cnidaria and Ctenophora. *Lipids*, 35 (5), 551–559. DOI: 10.1007/s11745-000-555-5

Pakhomov, E.A. & Perissinotto, R. (1996). Trophodynamics of the hyperiid amphipod *Themisto gaudichaudii* in the South Georgia region during late austral summer. *Marine Ecology Progress Series*, 134, 91–100. DOI: 10.3354/meps134091

Parrish, C.C. (1988). Dissolved and particulate marine lipid classes: a review. *Marine Chemistry*, 23, 17–40. DOI: 10.1016/0304-4203(88)90020-5

Phleger,C.F., Nichols, P.D. & Virtue, P. (1998). Lipids and trophodynamics of Antarctic zooplankton. *Comparative Biochemistry and Physiology*, 120B, 311–323. DOI: 10.1016/S0305-0491(98)10020-2

Reeve, M.R. & Walter, M.A. (1978). Nutritional ecology of ctenophores – a review of recent research. *Advances in Marine Biology*, 15, 249–287. DOI: 10.1016/S0065-2881(08)60406-X

Reinhardt, S.B. & Van Vleet, E.S. (1986). Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. *Marine Biology*, 91, 149–159. DOI: 10.1007/BF00569431

Sahin, F., Bat, L., Ustun, F., Birinci Ozdemir, Z., Satilmis, H. H., Kideys, A.E. & Develi, E.E. (2007). The Dinoflagellate- Diatom ratio in the Southeastern Black Sea off Sinop in the Years 1999-2000. *Rapp. Comm. Int. Mer Médit,* 38, 388.

Sargent, J.R, & Whittle, K.J. (1981). Lipids and hydrocarbons in the marine food web. In A.R Longhurst. (Ed). Analysis of marine ecosystems (pp 491-533). London: Academic Press.

Sargent, J.R., Parkes, R J., Mueller-Harvey, I. & Henderson, R.J. (1987). Lipid biomarkers in marine ecology. In M.A. Sleigh (Ed). Microbes in the Sea (pp 119–138). Harwood: Chichester.

Sargent, J.R. & Falk-Petersen, S. (1988). The lipid biochemistry of calanoid copepods. *Hydrobiologia,* 167-168, 101-114. DOI: 10.1007/BF00026297

Sen Ozdemir, N., Feyzioglu, A.M., Caf, F. & Yildiz, I. (2017). Seasonal changes in abundance. lipid and fatty acid composition of *Calanus euxinus* in the South-eastern Black Sea. I*ndian Journal of Fisheries*, 643, 55-66. DOI:10.21077/ijf.2017.64.3.62172-09

Stevens, C.J., Deibel, D. & Parrish, C.C. (2004). Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis Jaschnov. Journal of Experimental Marine Biology and Ecology*, 303, 135–156. DOI: 10.1016 / j.jembe.2003.11.008

Stowasser, G., Pond D.W. & Collins, M.A. (2012). Fatty acid trophic markers elucidate resource partitioning within the demersal fish community of South Georgia and Shag Rocks Southern Ocean. *Marine Biology*, 159, 2229-2310. DOI: 10.1007/s00227-012-2015-5

Stübing, D., Hagen, W. & Schmidt, K. (2003). On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill. *Euphausia superba. Limnology and Oceanography,* 484, 1685-1700. DOI: 10.4319/lo.2003.48.4.1685

Şen Özdemir, N., Parrish, C.C., Parzanini, C.C. & Mercier, A. (2019). Neutral and polar lipid fatty acids in five families of demersal and pelagic fish from the deep Northwest Atlantic. ICES *Journal of Marine Science*, 76 (6), 1807–1815. DOI:10.1093/icesjms/fsz054

Şen Özdemir, N., Feyzioglu, A.M., Caf, F. & Yildiz, I. (2020). Can the early stages copepoda (copepodites and naupliies) abundances effect the fatty acid composition of *Sagitta setosa* (chaetognatha) in the southeastern Black Sea? *Ege Journal of Fisheries and Aquatic Sciences,* 37 (4),335-342. DOI: 10.12714/egejfas.37.4.03

Viso, A. C. & Marty, J. C. (1993). Fatty acids from 28 marine microalgae. *Phytochemistry,* 34, 1521–1533. DOI: 10.1016/S0031-9422(00)90839-2

Yip, S. Y. (1984). The Feeding of *Pleurabranchia pileus* Müller (Ctenephora) from Galbay Bay. *Proceedings of the Royal Irish Academy*, 84 (B), 109-122.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Parasitic isopods on some marine fishes caught from the coasts of Sinop in the Black Sea, Turkey

Karadeniz'in Sinop kıyılarından yakalanan bazı deniz balıklarında parazitik isopodlar

Sevilay Okkay[1]* ● Ahmet Özer[2]

[1] Kocaeli University, Faculty of Agriculture, 41285 Kocaeli, Turkey
[2] Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000 Sinop, Turkey

https://orcid.org/0000-0003-4440-3525
https://orcid.org/0000-0002-2890-6766

*Corresponding author: sevilayokkay@gmail.com

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Abstract: Marine fish species such as grey wrasse *Symphodus cinereus*, common sole *Solea solea*, knout goby *Mesogobius batrachocephalus* and rusty blenny *Parablennius sanguinolentus* were studied for their parasitic isopods on the Sinop coast of the Black Sea. Two cymothoid species (*Nerocila bivittata* and *Nerocila orbignyi*) were identified. *Nerocila bivittata* was found on the body surface and fins of grey wrasse, common sole, knout goby, and *N. orbignyi* on the dorsal fin of rusty blenny. The prevalence and mean intensity values of each parasite species on fishes were calculated. Fish species mentioned above were all new hosts for *N. bivittata* and *N. orbignyi* in the Turkish coast of the Black sea.

Keywords: Nerocila, Cymothoidae, Black Sea, Isopoda, parasitism

Öz: Karadeniz'in Sinop kıyılarında yaşayan Çırçır balığı *Symphodus cinereus*, Dil balığı *Solea solea*, Kaya balığı *Mesogobius batrachocephalus* and Horozbina balığı *Parablennius sanguinolentus* deniz balıkları izopod parazitlerinin varlığı yönünden incelendi. İncelenen balıklarda iki cymothoid tür tanımlandı. *Nerocila bivittata* paraziti çırçır balığı, dil balığı ve kaya balığının yüzgeç ve vücut yüzeylerinde, *Nerocila orbignyi* paraziti ise horozbina balığının sırt yüzgecinde tespit edildi. Enfeste balık başına ortalama parazit sayısı ve enfestasyon oranı her bir parazit türü için hesaplandı. Karadeniz'in Türkiye kıyılarında gerçekleştirilen bu çalışmada incelenen tüm balık türleri *N. bivittata* ve *N. orbignyi* türü parazitler için yeni konaklardır.

Anahtar kelimeler: Nerocila, Cymothoidae, Karadeniz, Isopoda, parazitizm

INTRODUCTION

Cymothoidae, a family of Isopoda, commonly infect marine, freshwater or brackish-water teleost fishes (Lester, 2005). The genus *Nerocila* Leach, 1818 is one of the largest of this family with at least 65 species reported from the skin, fin and mouth of their host fishes (Kayış and Er, 2016; Nagler et al., 2016). Members of this genus has been mostly reported from fishes belonging to Labridae and to a lesser extent from other families such as Scorpaenidae, Cottidae, Sparidae, Mugilidae, Centracanthidae, Merluccidae, Monacanthidae, Sciaenidae, Mullidae, Gobiidae, Serranidae, Triglidae, and Platycephalidae (Alas et al., 2008; Nagler and Haug, 2016). Until today, three species namely *Nerocila bivittata*, *N. orbignyi* and *N. acuminata* have been reported on marine fishes in Turkey (Horton and Okamura, 2001; Öktener and Trilles, 2004; Oğuz and Öktener, 2007; Kırkım et al., 2008; Öktener et al., 2009; Kayış and Ceylan, 2011; Kayış and Er, 2012; 2016; Akmirza, 2014; Er and Kayış, 2015; Özcan et al, 2015) (Table 1).

Studies of cymothoid parasites in wild fish provide an opportunity to obtain significant information on the effects of these parasites on their hosts (Horton and Okamura, 2001). Fish culture expanding significantly in the recent decades are under threat of some pathogenic isopod parasites which have a decreasing impact on its economic value (Horton and Okamura, 2001; Rameshkumar and Ravichandran, 2012; Nagler and Haug 2016). So, such studies will also make further contribution to our current knowledge as well as their interactions with culture fish populations. The Black Sea has an increasing interest for fish culture in the recent years and wild fish surrounding culture cages may have a potential to spread isopod parasites by parasite spillover process (Horton and Okamura, 2001; Balta et al., 2008; Kayış et al., 2009).

In the present study, we aimed to determine isopod parasites present on some marine fishes located in the Sinop coasts of the Black Sea and their prevalence and intensity values of infection. Moreover, this study also aimed to identify parasitic isopods in wild fish that pose a high risk for aquaculture as was previously reported from other culture facilities in Turkey.

MATERIAL AND METHODS

The grey wrasse *Symphodus cinereus* (Bonnaterre, 1788) (n=6), common sole *Solea solea* L., 1758 (n=55), knout goby *Mesogobius batrachocephalus* (Pallas, 1814) (n=35) and

rusty blenny *Parablennius sanguinolentus* (Pallas, 1814) (n=48) were collected by angling and trammel nets on the Sinop coast of the Black Sea (N 42°05'68" E 35°10'55") during the period from September 2015 to August 2016. The body surface, fins, mouth and the gill arches of all fish individuals were investigated for isopod parasites at the Faculty of Fisheries and Aquatic Sciences in Sinop, Turkey. Parasite species were identified using an Olympus light microscope (BX53) equipped with a digital camera (DP50) according to the definitions of Bruce (1987), Trilles et al. (1989). Calculation of infestation prevalence (%) and mean intensity follow the definition indicated by Bush et al. (1997).

RESULTS AND DISCUSSION

Two cymothoid isopod species were identified; *Nerocila bivittata* (Risso, 1816) (Figure 1a, b) from grey wrasse (*Symphodus cinereus*), common sole (*Solea solea*) and knout goby (*Mesogobius batrachocephalus*), and *Nerocila orbignyi* (Gluerin-Meneville, 1832) (Figure 1c, d) from rusty blenny (*Parablennius sanguinolentus*). Their infestation sites, prevalence (%) and mean intensity values are provided in Table 1. Briefly, *N. bivittata* was found to be attached on the ventral body surfaces, near by the pectoral fin, dorsal fin and caudal fins of its host fishes with the prevalence 16.6% in grey wrasse, 9.1% in common sole and 8.6% knout goby. Loss of scales, extensive skin erosions and haemorrhages were observed in the infested host fishes (Figure 1e, f). On the other hand, *N. orbignyi* was determined on the dorsal fins of its host fish individuals with the prevalence of 2.1% on the rusty blenny. Hemorrhage or loss of scales was observed on infested host fishes.

Figure 1. Dorsal and ventral views of *N. bivittata* (a, b) and *N. orbignyi* (c, d). The macroscopic appearances were observed in infected fishes: Hemorrhage, loss of scales and extensive skin erosions (e, f)

The present study provides new information on the host lists of both *Nerocila* species. *Symphodus cinereus*, *Solea solea* and *Mesogobius batrachocephalus* are new hosts for *Nerocila bivittata* and *Parablennius sanguinolentus* for *N. orbignyi*. Table 1 summarises current host-parasite list for both isopod species in Turkey.

Table 1. List of host fishes for *Nerocila bivittata* and *N. orbignyi* on the coasts of Turkey, and their infestation prevalence (%) and mean intensity values

Parasite species	Host	Locality	Prevalence (%)	Mean Intensity ± S.E.	References
Nerocila bivittata	*Symphodus cinereus*	Black Sea	16.6	1.0±0.0	This study
	Solea solea	Black Sea	9.1	1.4±0.2	This study
	Mesogobius batrachocephalus	Black Sea	8.6	2.3±0.8	This study
	Syngnathus sp.	Black Sea	-	-	Kayış and Er, 2012
	Hippocampus guttulatus		-	-	
	Trachinus draco	Black Sea	-	-	Kayış and Er, 2016
	Symphodus tinca	Black Sea	-	-	Oğuz and Öktener, 2007
	Parablennius sanguinolentus	Black Sea	11.76	1.0	Er and Kayış, 2015
	Platichthys flesus	Black Sea	3.33	3.5	Er and Kayış, 2015
	Scophthalmus maximus	Black Sea	2.19	3.5	Er and Kayış, 2015
	Hippocampus guttulatus	Black Sea	0.72	1.0	Er and Kayış, 2015
	Dicentrarchus labrax	Black Sea	5.55	1.0	Er and Kayış, 2015
	Belone belone	Black Sea	0.71	1.0	Er and Kayış, 2015
	*Pegusa nasuta**	Black Sea	26.94	2.74	Er and Kayış, 2015
	Symphodus spp.*	Black Sea	16.02	1.54	Er and Kayış, 2015
	*Gobius niger**	Black Sea	5.21	2.18	Er and Kayış, 2015
	*Neogobius melanostomus**	Black Sea			Er and Kayış, 2015
	*Syngnathus spp.**	Black Sea	3.44	3.87	Er and Kayış, 2015
	*Uranoscopus scaber**	Black Sea	2.73	1.35	Er and Kayış, 2015
	*Scorpaena porcus**	Black Sea	1.73	1.33	Er and Kayış, 2015
	Parablennius sanguinolentus	Black Sea	7.4	-	Alaş et al., 2008
	Pagellus erythrinus	Mediterranean Sea	-	-	Monod, 1931 (from Öktener and Trilles, 2004)
	Pagellus sp.	Marmara Sea	-	-	Demir, 1952
	Pagellus sp.	Aegean Sea	-	-	Geldiay and Kocataş, 1972
	Sparus auratus	Aegean Sea	-	-	Kırkım, 1998
	Gobius niger	Aegean Sea	-	-	Kırkım, 1998
	Sciaena umbra	Aegean Sea	-	-	Kırkım, 1998
	Scorpaena scrofa	Aegean Sea	8.33	-	Öktener et al., 2009
	Sciaena umbra	Aegean Sea	-	-	Kırkım et al., 2008
	Labrus merula	Aegean Sea	-	-	Kırkım et al., 2008
	Dentex macrophthalmus	Aegean Sea	-	-	Kırkım et al., 2008
	Symphodus tinca	Aegean Sea	-	-	Kırkım et al., 2008
	Gobius niger	Aegean Sea	-	-	Kırkım et al., 2008
Nerocila orbignyi	*Parablennius sanguinolentus*	Black Sea	2.1	1.0±0.0	This study
	Liza aurata	Black Sea	-	-	Öktener and Trilles, 2004
	Serranus cabrilla	Mediterranean Sea	-	-	Özcan et al., 2015
	Solea solea	Black Sea	-	-	Kayış and Ceylan, 2011
	Dicentrarchus labrax	Aegean Sea	-	-	Horton and Okamura, 2001

**N. bivittata*, *N. acuminata* and *Nerocila* spp. total data of the species was used by the authors

When comparing the prevalence (%) and mean intensity of infestations for both parasite species with previous reports in Turkey, the infestation prevalence (%) values found for *N. bivittata* in the present study are higher than those reported by Er and Kayış (2015) and Alaş et al. (2008) (Table 1). This difference may be due to the differences in the number of fishes examined. On the other hand, previous reports for *N. orbignyi* in Turkish marine fishes did not provide any infestation data to make any comparison. It is clear from current data provided in Table 1 that *N. bivittata* infests more fish species than *N. orbignyi* in Turkish coastal areas.

In conclusion, the present data provided new host records for *N. bivittata* and *N. orbignyi* as well as their infestation indices in fishes collected from of the Black Sea coast of Turkey. We believe that these parasites might cause some potential negative impacts on cultured fish species due to their dispersal around culture cages.

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REFERENCES

Alas, A., Öktener, A., Iscimen, A. & Trilles, J.P. (2008). New host record, *Parablennius sanguinolentus* (Teleostei, Perciformes, Blenniidae) for *Nerocila bivittata* (Crustacea, Isopoda, Cymothoidae). *Parasitology Research*, 102, 645–646. DOI: 10.1007/s00436-007-0805-3

Akmirza, A. (2014). Metazoan parasites of brown meagre (*Sciaena umbra* L. 1758) caught near Gökçeada, Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 38, 299-303. DOI: 10.3906/vet-1209-35

Balta, F., Kayis, S. & Altinok, I. (2008). External protozoan parasites in three trout species in the Eastern Black Sea region of the Turkey: intensity, seasonality, and their treatments. *Bulletin of the European Association of Fish Pathologists*, 28 (4), 157-162.

Bruce, N.L. (1987). Australian species of Nerocila Leach, 1818 and *Creniola* n.gen. (Isopoda: Cymothoidae), crustacean parasites of marine fishes. Records of the Australian Museum, 39, 355-412. DOI: 10.3853/j.0067-1975.39.1987.174

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. Revisited. *Journal of Parasitology*, 84, 575 – 583. DOI: 10.2307/3284227

Demir, M. (1952). The bentic Invertebrates of the Bosphorus and Islands Coasts. IU Science Faculty Hydrobiology 3(A), 362-363.

Er, A. & Kayış, Ş. (2015). Intensity and prevalence of some crustacean fish parasites in Turkey and their molecular identification. *Turkish Journal of Zoology*, 39, 1142-1150. DOI: 10.3906/zoo-1409-35

Geldiay, R. & Kocataş, A. (1972). Isopods Collected in Izmır Bay, Aegean Sea. Crustaceana, 3, 19-30.

Horton, T. & Okamura, B. (2001). Cymothoid isopod parasites in aquaculture: a review and case study of a Turkish sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) farm. Diseases of Aquatic Organisms, 46, 181-188. DOI: 10.3354/dao046181

Kayış, S., Ozcelep, T., Capkın, E. & Altınok, I. (2009). Protozoan and Metazoan Parasites of Cultured Fish in Turkey and their Applied Treatments. *The Israeli Journal of Aquaculture – Bamidgeh*, 61(2), 93-102.

Kayış, Ş. & Ceylan Y. (2011). First report of *Nerocila orbigyni* (Crustacea, Isopoda, Cymothoidae) on *Solea solea (Teleostei, Soleidae) from Turkish Sea. Turkish Journal of Fisheries and Aquatic Sciences*, 11, 167-169. DOI: 10.4194/trjfas.2011.0123

Kayış, Ş. & Er, A. (2012). *Nerocila bivittata* (Cymothidae, Isopoda) infestation on syngnathid fishes in the Eastern Black Sea. *Bulletin of the European Association of Fish Pathologists*, 32(4), 135-139.

Kayış, Ş. & Er, A. (2016). First record of *Nerocila bivittata* (Cymothidae, Isopoda) on Greater Weever (*Trachinus draco*) in the Worldwide. *Yunus Research Bulletin* 4, 335-339. DOI: 10.17693/yunusae.v16i26717.281033

Kırkım, F. (1998). Investigations on the Systematics and Ecology of the Aegean Sea Isopoda Fauna. PhD Thesis, Ege University, 238 pp. (*in Turkish*).

Kırkım, F., Kocataş, A., Katağan, T. & Sezgin, M. (2008). A report on parasitic isopods (Crustacea) from marine fishes and decapods collected from the Aegean Sea (Turkey). *Türkiye Parazitoloji Dergisi*, 32, 382-385.

Lester, R.J.G. (2005). Isopoda (isopods), p. 138-144. In C. Rohde [Ed.], Marine Parasitology. CBI Publishing.

Nagler, C. & Haug T.J. (2016). Functional morphology of parasitic isopods: understanding morphological adaptations of attachment and feeding structures in *Nerocila* as a pre-requisite for reconstructing the evolution of Cymothoidae. *Peer Journal*, e2188. DOI: 10.7717/peerj.2188

Nagler, C., Haug, C., Resch, U., Kriwet, J. & Haug, J.T. (2016). 150 million years old isopods on fishes: a possible case of palaeo-parasitism. *Bulletin of Geosciences*, 91(1), 1–12. DOI: 10.3140/bull.geosci.1586

Rameshkumar, G. & Ravichandran, S. (2012). Problems caused by isopod parasites in commercial fishes. *Journal of Parasite Disease*, 38(1), 138-141. DOI: 10.1007/s12639-012-0210-4

Oğuz, M.C. & Öktener, A. (2007). Four parasitic crustacean species from marine fishes of Turkey. *Türkiye Parazitoloji Dergisi*, 31(1), 79-83.

Öktener, A. & Trilles, J.P. (2004). Report on Cymothoids (Crustacea, Isopoda) collected from marine fishes in Turkey. *Acta Adriatica*, 45, 145-154.

Öktener, A., Trilles, J.P., Alaş, A. & Solak, K. (2009). Cymothoid (Crustacea, Isopoda) records on marine fishes (Teleostei and Chondrichthyes) from Turkey. *Bulletin of the European Association of Fish Pathologists*, 29(2), 51-57.

Özcan, T., Kırkım, F. & Sakallı, U. (2015). *Serranus cabrilla* (Linnaeus, 1758) (Perciformes, Serranidae) a new host record for *Nerocila orbignyi* (Guérin-Méneville, 1832) (Isopoda, Cymothoidae). *Iranian Journal of Fisheries Sciences*, 14(4), 1083-1088.

Trilles, J.P., Radujković B.M. & Romestand B. (1989). Parasites des poissons marins du Monténégro: Isopodes. *Acta Adriatica*, 30(1/2), 279-306. DOI: 10.13140/RG.2.1.3662.5366

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Farklı alıştırma sıcaklıklarında kılıçkuyruk (*Xiphophorus helleri*) ve plati balıklarının (*X. maculatus*) termal tolerans parametrelerinin belirlenmesi

Determination of thermal tolerance parameters of swordtail (*Xiphophorus helleri*) and platy fish (*X. maculatus*) acclimated to different temperature levels

Mahmut Yanar[1]* ● Ali Özdeş[2] ● Erhan Erdoğan[3] ● Ece Evliyaoğlu[4]

[1] Çukurova Üniversitesi, Su Ürünleri Fakültesi, 01330, Adana/Türkiye https://orcid.org/0000-0002-4445-0228
[2] Çukurova Üniversitesi, Su Ürünleri Fakültesi, 01330, Adana/Türkiye https://orcid.org/0000-0002-0271-2445
[3] Munzur Üniversitesi, Su Ürünleri Fakültesi, 62000, Tunceli/Türkiye https://orcid.org/0000-0002-3013-3045
[4] Çukurova Üniversitesi, Su Ürünleri Fakültesi, 01330, Adana/Türkiye https://orcid.org/0000-0003-3578-7336

*Corresponding author: myanar@cu.edu.tr

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Öz: Çalışmada üç alıştırma sıcaklığında kılıçkuyruk (*Xiphophorus helleri*) ve platinin (*X. maculatus*) termal tolerans parametreleri belirlenmiştir. 20, 24 ve 28°C alıştırma sıcaklıklarında platinin kritik termal minimum (CTMin) değerleri sırasıyla 9,41, 10,42 ve 11,95°C iken, kılıçkuyruğun 9,38, 11,53 ve 13,23°C platinin kritik termal maksimum (CTMax) değerleri 37,41, 39,19 ve 40,52°C iken, kılıçkuyruğun 36,94, 38,89 ve 40,07°C olarak gerçekleşmiştir. Buna göre alıştırma sıcaklıkları balıkların alt ve üst sıcaklık toleranslarını 3-4 °C etkilemiştir. CTMin alıştırma tepki oranı (ARR) değerleri alıştırma sıcaklıklarına bağlı olarak kılıçkuyrukta 0,42-0,42, platide 0,20-0,34 arasında değişirken, CTMax ARR değerleri kılıçkuyrukta 0,29-0,48, platide ise 0,33-0,44 arasında değişmiştir. Platinin termal tolerans poligon alanı (232°C^2) kılıçkuyruktan (217,3°C^2) biraz daha geniş bulunmuştur. İki balık türünün alt sıcaklık toleranslarının düşük olması, kışın su sıcaklığının 10°C'ye düştüğü subtropikal iklimlerde bu balıkların coğrafik dağılımlarını ve yetiştiriciliklerini sınırlar.

Anahtar kelimeler: Sıcaklık toleransı, Poeciliidae, TTPA, CTM, ARR

Abstract: Thermal tolerance parameters of swordtail (*Xiphophorus maculatus*) and platy (*X. helleri*) at three acclimation temperatures were determined in the study. The CTMin values at 20, 24 and 28°C acclimation temperature were 9.41,10.42 and 11.95°C respectively for platy and 9.38,11.5 and 13.23°C for swordtail, while CTMax values were 37.41, 39.19 and 40.52°C for platy and 36.94, 38.89 and 40.07°C for swordtail. Accordingly, acclimation temperature affected the lower and upper temperature tolerances of fish by 3-4 °C. The CTMin ARR values varied between 0.42-0.42 in swordtail and 0.20-0.34 in platy, while CTMax ARR ranged between 0.29-0.48 in swordtail and 0.33-0.44 in platy depending on acclimation temperature. Thermal tolerance polygon area of platy (232°C^2) was slightly higher than that of swordtail (217.3°C). The fact that both fish species have lower temperature tolerances limits their geographic distribution and aquaculture in subtropical climates where the water temperature drops to 10°C in winter.

Keywords: Thermal tolerance, Poeciliidae, TTPA, CTM, ARR

GİRİŞ

Akvaryum balıkçılığı dünyada en popüler hobilerden biri olmasının yanı sıra, yan sektörleriyle birlikte küresel çapta 15 milyar USD $'ın üzerinde ticaret hacmine sahip önemli bir sektördür (Penning vd., 2009; Hensen vd., 2010; Rhyne vd., 2012; Raghavan vd., 2013). Tropik ve yarı tropik bölgelerde bulunan Singapur, Tayland, Tayvan, Malezya, Endonezya ve Sri Lanka gibi ülkelerin ekonomik gelişmelerine önemli katkılar sağlamaktadır (Lovell, 2000; Gouveia vd., 2003). Akvaryum balıklarının küresel ekonomiye katkılarına karşın, ülkeler arası yoğun ticareti nedeniyle egzotik tür potansiyelleri yüksektir. Sucul canlıların coğrafik yayılışlarında en önemli ekolojik bariyer su sıcaklığı ve tuzluluktur. Bazı türler termal yetenekleri dolayısıyla bu bariyeri aşarak yeni habitatlara girebilmekte ve mevcut ekosisteme ciddi zararlar verebilmektedir. Örneğin, ABD iç sularında %41'i tropik olmak üzere 53-54 egzotik balık türü girmiş (Lever, 1996; USGS, 2004) ve burada yaşayan pek çok yerli balık türünün yok olmasına neden olmuşlardır (Crossman, 1991; Ross, 1991). Dolayısıyla sucul hayvanların sıcaklık tolerans parametrelerinin belirlenmesi, balıkların yayılışlarında maruz kaldıkları ekstrem sıcaklıklara karşı gösterecekleri yaşam stratejilerinin anlaşılmasını (Bennet ve Beitinger, 1997) ve yabancı türlerin yeni habitatlara adaptasyonları konusunda sağlıklı bir değerlendirme yapılmasını sağlar. Diğer yandan sıcaklık, su ürünleri yetiştiriciliğinde tür tercihinde göz önüne alınan en önemli kriterlerden biridir. Subtropikal iklimlerde tropik balık yetiştiriciliğinde karşılaşılan en önemli sorunlardan birisi düşük su sıcaklığıdır. Balık havuzlarında su sıcaklığı kışın 8-10°C'ye kadar düşer. Diğer yandan tropik ve subtropik bölgelerde sığ ve su değişkenliğinin az olduğu havuzlarda

yazın su sıcaklığı 35°C'ye kadar yükselir. Bu sıcaklık seviyeleri çoğu balık türleri için kritiktir ve her tür için bunların ayrıntılı bilinmesi gerekir.

Sucul canlıların alt ve üst sıcaklık toleranslarının belirlenmesinde yaygın kullanılan yöntemlerden biri, kritik termal metodolojidir (CTM). İlk olarak Cowles ve Bogert (1944) tarafından çöl reptillerinin sıcaklık toleranslarını belirlemek için tanımlanan bu metot, daha sonra çeşitli araştırmacılar (Cox, 1974; Spotila vd., 1979; Lutterschmidt ve Hutchison, 1997; Beitinger vd., 2000) tarafından geliştirilerek balıklara ve diğer akuatik hayvanlara uygulanmıştır. CTM verileri, balıkların ekstrem sıcaklıklara toleransları konusunda mutlak bilgiler vermekten ziyade, göreceli bilgiler verir, türler arasında karşılaştırma yapılmasını sağlar. CTMin ve CTMax verileri kullanılarak hesaplanan termal tolerans poligonu, balığın yaşayabileceği sıcaklık aralığının genişliği konusunda bilgi verir (Bennet ve Beitinger, 1997). Diğer yandan, balıkların sıcaklık değişimine karşı gösterdikleri tepkinin matematiksel bir ifadesi olan alıştırma tepki oranı da sıcaklık toleransında kullanılan diğer bir parametredir (Claussen, 1977). Bu nedenle gerek ekoloji, gerekse balık yetiştiriciliği bakımından balık türlerinin sıcaklık toleransı ile ilgili veri tabanına ihtiyaç vardır ve bu konuda çalışmalar hızlı bir şekilde artmaktadır.

CTM değerleri balığın alıştırma sıcaklığına bağlı olarak değişmektedir. Tarafımızdan daha önce 13 akvaryum balık türünün üç farklı alıştırma sıcaklığında CTMin ve CTMax değerleri ayrıntılı olarak çalışılmıştır (Yanar vd., 2019). Tuckett vd. (2016) kılıçkuyruk balığının düşük sıcaklığa töleransını incelemiş ancak literatürde, bu türün ve plati balıklarının termal toleransının belirlenmesi eksik kalmıştır. Bu çalışmada, dünyada ve ülkemizde oldukça talep gören doğuran (ovovivipar) akvaryum balıklarından (Poecilidae) kılıçkuyruk (*Xiphophorus helleri*) ve platinin (*X. maculatus*) üç farklı alıştırma sıcaklığında (20, 24 ve 28 °C) alt ve üst sıcaklık toleransı (sırasıyla CTMin ve CTMax), termal tolerans poligon alanı (TTPA) ve alıştırma tepki oranı (ARR) belirlenmiştir.

MATERYAL VE METOT

Balıklar yerel üreticilerden sağlanmış olup, kılıçkuyruk ve platinin kırmızı renkli variyetleri kullanılmıştır. Laboratuvara getirilen balıklar 20, 24 ve 28°C alıştırma sıcaklığına sahip 120 L'lik akvaryumlara kademeleri olarak adapte edilmiş ve burada 35 gün yetiştirilmişlerdir. Bu süreç sonunda platinin standart boyu 3,71±0,19 cm, kılıçkuyruğun ise 4,11±0,21 cm olarak ölçülmüştür. Deneme boyunca su sıcaklıkları planlanan sıcaklıklarda kontrol edilmiş, akvaryumlar merkezi bir hava motoruyla havalandırılarak sular oksijenlendirilmiştir. Yem artıkları ve dışkılar her gün sifonlanarak temizlenmiş, yerine havalandırılmış ve dinlendirilmiş taze su eklenmiştir. Bu süreçte 12 saat aydınlık/karanlık ışık periyodu uygulanmıştır. Alıştırma süresinde suların oksijen seviyesi ≥6,5 mg/L, pH 7,5-7,8, sertlik ise 305 mg/L $CaCO_3$ olarak ölçülmüştür. Akvaryum sularının sıcaklık seviyelerinin düzenlenmesi, termostatlı su ısıtıcısı (Xilong AT-700) ve su soğutucu (Resun 650-CL) ile sağlanmıştır.

Alıştırma akvaryumlarında tutulan balıklar 1 gün aç bırakıldıktan sonra CTMin ve CTMax denemeleri için aynı sıcaklıklara sahip 10 L'lik cam akvaryuma aktarılmıştır. Her bir deneme 3 tekerrürlü olup, her bir tekerrürde 5 balık kullanılmıştır. Böylece çalışmada her bir balık türünden CTMin için 45, CTMax için 45 olmak üzere toplam 90 adet birey kullanılmıştır. CTM denemeleri süresince akvaryumlar bir hava motoruyla sürekli havalandırılarak oksijen miktarı uygun düzeylerde tutulmuş, ayrıca sıcaklığın akvaryumun her alanında eşit olması sağlanmıştır.

Balıkların CTMin ve CTMax değerleri 20, 24 ve 28°C su sıcaklıklarında ayrı ayrı belirlenmiştir. Ayrıca elde edilen CTM verileri kullanılarak ARR ve TTPA değerleri hesaplanmıştır. Balıkların sıcaklık tolerans limitlerinin belirlenmesinde Kritik Termal Metodoloji (CTM) yöntemi (Cowles ve Bogert, 1944) kullanılmıştır. Akvaryum balıkları küçük olduğu için, sıcaklığın arttırılması veya düşürülmesinde Becker ve Genewoy (1979) tarafından küçük balıklar için önerilen 0,3°C/d su değişim oranı tercih edilmiştir. Su sıcaklığı balığın motor aktivitesi ve koordinasyonunu yitirdiği ve sonuçta denge kaybının oluştuğu (loss of equilibrium; LOE) ana kadar ısıtılmış (CTMax) veya soğutulmuştur (CTMin). LOE için, balıkların 1 dakikadan fazla dorsoventral oryantasyonu sürdürememesi esas alınmıştır (Bennett ve Beitinger, 1997). Balıkların bireysel olarak kaydedilen CTM değerlerinin aritmetik ortalaması, grupların CTM değeri olarak not edilmiştir. ARR değeri ΔCTM/Δ*T* formülü ile hesaplanmıştır (Claussen, 1977). ΔCTM, alıştırma su sıcaklığının azaltılması veya arttırılması sonucunda balıkların denge kaybına başladıkları ilk ve son sıcaklık noktaları arasındaki farkı, Δ*T* ise alıştırma su sıcaklıkları arasındaki farkı tanımlamaktadır. Çeşitli araştırmacılar (Bennett ve Beitinger, 1997; Eme ve Bennett, 2009) tarafından tanımlanan TTPA, Yanar vd. (2019)'nin önermiş olduğu, koordinat sisteminde horizontal (alıştırma su sıcaklığı) ve vertikal (CTM) aksisler arasında kalan yamuk alanı formülünden [(a+c) x h/2] yararlanılarak hesaplanmıştır. Ardışık her alıştırma sıcaklık aralığı için TTPA = $(CTMax1-CTMin1)^a+(CTMax2-CTMin2)^c$ x $(\Delta AT)^h/2$ formulü kullanılmıştır. Burada "a" ve "c" yamuğun paralel kenarlarını, "h" yamuğun yüksekliğini, "ΔAT" ardışık iki alıştırma sıcaklık arasındaki farkı sembolize etmektedir. Ardışık AT aralıkları arasında kalan alanların toplamı ise, toplam TTPA değerini vermektedir.

CTMin ve CTMax denemeleri süresince balıkların sıcaklık değişimine karşı gösterdikleri hareketlilik, denge kaybı, salgı üretme ve dışkılama gibi tepkiler gözlenerek not edilmiştir. CTM denemelerinden sonra dengesini yitirip bayılan balıklar bulundukları akvaryumlardan alınıp sıcaklığı 24°C olan aklimasyon akvaryumlarına kademeli olarak alıştırılmıştır. Balıklar burada 4 gün gözlemlenerek deneme sonrası yaşama oranları kaydedilmiştir.

Her bir balık türünün alıştırma su sıcaklıklarında CTM değerleri arasındaki farklılıklar, SPSS (versiyon 20,0) programında tek yönlü varyans analizi (ANOVA) ile tespit edilmiş ve gruplar arasındaki farklılıklar Tukey testi ile %5 önem seviyesinde belirlenmiştir. Metindeki veriler ortalama ± standart sapma (SD) olarak kaydedilmiştir.

BULGULAR

Balıkların CTM değerleri alıştırma sıcaklığından (AT) önemli düzeyde etkilenmiştir (P<0,05). AT arttıkça CTMin ve CTMax değerleri artmıştır. Kılıçkuyrukta 20, 24 ve 28°C AT'de CTMin değerleri sırasıyla 9,83, 11,53 ve 13,23°C, CTMax değerleri ise 36,94, 38,89 ve 40,07°C olarak gerçekleşmiştir. Buna göre, 20 ve 28°C AT, kılıçkuyruğun CTMin değerlerinde 3,40°C, CTMax değerinde ise 3,13°C bir fark yaratmıştır. Plati için de benzer durum söz konusudur. 20, 24 ve 28°C AT'de CTMin değerleri sırasıyla 8,82, 10,22 ve 11,03°C, CTMax değerleri ise 37,41, 39,19 ve 40,52°C olarak gerçekleşmiştir. 20 ve 28°C AT baz alınırsa, bu türde CTMin değerlerinde 2,21°C, CTMax değerlerinde ise 3,11°C bir sıcaklık farkı olmuştur (Tablo 1).

Tablo 1. Balıkların CTM değerleri
Table 1. CTM values of fish

	Türler	Alıştırma Sıcaklıkları (°C)		
		20	24	28
CTMin	Kılıçkuyruk	9,83±0,40[a]	11,53±0,32[b]	13,23±0,60[c]
	Plati	8,82±0,43[a]	10,22±0,28[b]	11,03±0,47[c]
CTMax	Kılıçkuyruk	36,94±0,32[a]	38,89±0,24[b]	40,07±0,29[c]
	Plati	37,41±0,46[a]	39,19±0,40[b]	40,52±0,24[c]

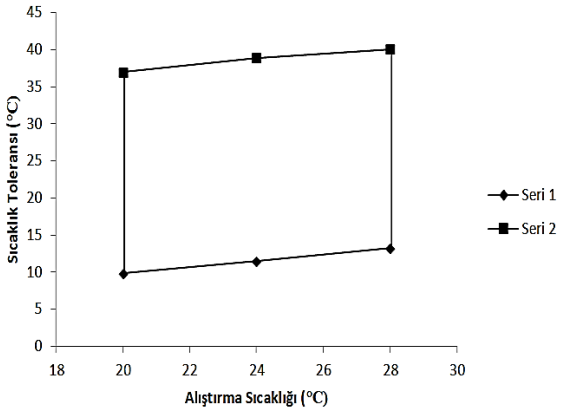
Sonuçlar ortalama±standart sapma (n=15) şeklinde verilmiştir. Her satırda farklı harflerle işaretlenen ortalamalar istatistiki olarak birbirinden farklıdır (P<0,05)

CTMin ARR değerleri alıştırma sıcaklıklarına bağlı olarak kılıçkuyrukta 0,42-0,42, platide 0,20-0,34 arasında değişirken, CTMax ARR değerleri kılıçkuyrukta 0,29-0,48, platide ise 0,33-0,44 arasında değişmiştir (Tablo 2).

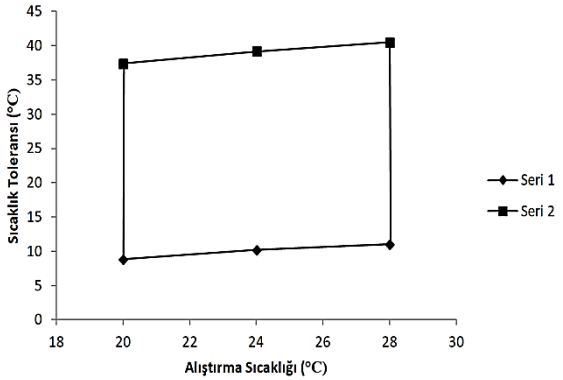
Tablo 2. Balıkların ARR değerleri
Table 2. ARR values of fish

	Türler	Alıştırma sıcaklıkları (°C)		
		20-24	24-28	20-28
CTMin	Kılıçkuyruk	0,42	0,42	0,42
	Plati	0,34	0,20	0,27
CTMax	Kılıçkuyruk	0,48	0,29	0,39
	Plati	0,44	0,33	0,38

Balıkların TTPA değerleri kılıçkuyrukta 217,3°C² (Şekil 1) platide ise biraz daha yüksek olup 232°C² (Şekil 2) olarak hesaplanmıştır.

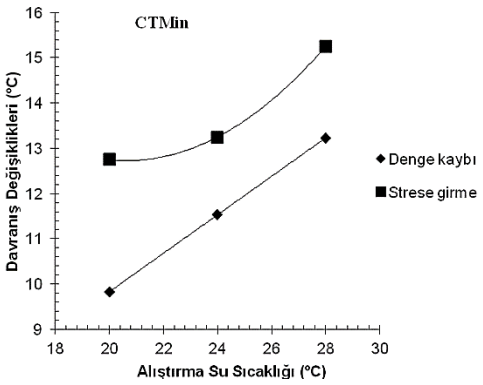


Şekil 1. Kılıçkuyruğun (*X. helleri)* termal tolerans poligon alanı
Figure1. Thermal tolerance polygon area of swordtail (*X. helleri)*



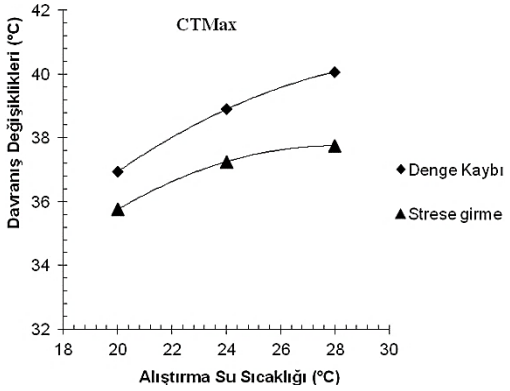
Şekil 2. Platinin (*X. maculatus*) termal tolerans poligon alanı
Figure 2. Thermal tolerance polygon area of platy (*X. maculatus*)

Balıklar kritik sıcaklıklara yaklaşırken denge kaybından (LOE) önce bazı davranış değişiklikleri göstermişlerdir. Bu değişiklikler alt ve üst kritik sıcaklığa göre değişmiştir. Her iki balık türünde CTMin uygulamasında kritik düşük sıcaklığa yaklaşırken denge kaybından önce balıkların hareketlerinde giderek artan bir yavaşlama/durağanlaşma göze çarparken, CTMax uygulamasında kritik yüksek sıcaklığa yaklaşırken denge kaybından önce giderek artan bir hiperaktivite, ani sıçrama ve amaçsız yüzme, ayrıca mukus salınımı ve dışkılama gözlenmiştir. Alıştırma sıcaklığına bağlı olarak gözlenen bu stres davranışları kılıçkuyrukta CTMin uygulamasında su sıcaklığı kritik düşük seviyeye yaklaşırken 15,4-12,8°C'de başlamış, denge kayıpları ise 13,23-9,83°C arasında gerçekleşmiştir (Şekil 3). CTMax uygulamasında ise aynı türde su sıcaklığı kritik yüksek seviyeye yaklaşırken stres davranışları 35,6-37,6°C'de başlamış, denge kayıpları ise 36,94-40,07°C arasında gerçekleşmiştir (Şekil 4). Diğer yandan platide CTMin uygulamasında su sıcaklığı kritik düşük seviyeye yaklaşırken stres davranışları 14,4-11,5°C'de başlamış, denge kayıpları ise 11,03-8,82°C arasında gerçekleşmiştir (Şekil 5). CTMax uygulamasında ise su sıcaklığı kritik yüksek seviyeye yaklaşırken stres davranışları 35,2-37,6°C'de başlamış, denge kayıpları ise 37,41-40,52°C arasında gerçekleşmiştir (Şekil 6).



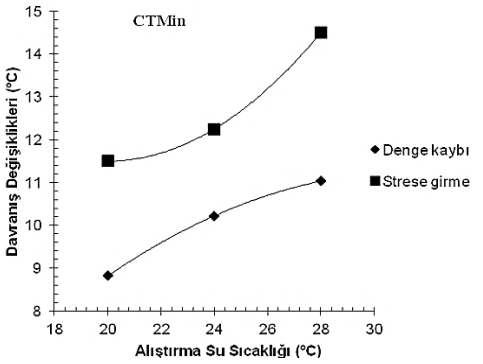
Şekil 3. Kılıçkuyruğun (*X. helleri)* CTMin uygulamasında kritik düşük sıcaklığa tepkisi

Figure 3. The response of swordtail (*X. helleri*) to critical low temperature in CTMin application



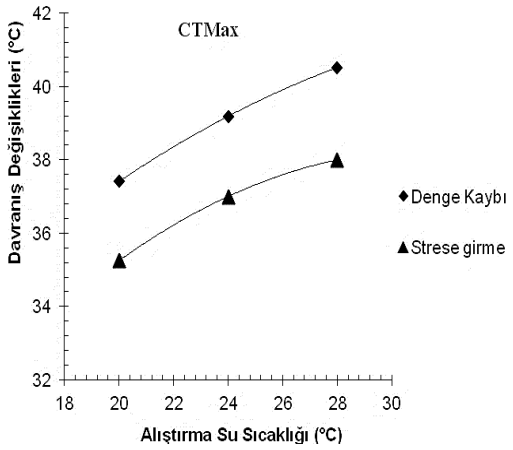
Şekil 4. Kılıçkuyruğun (*X. helleri)* CTMax uygulamasında kritik yüksek sıcaklığa tepkisi

Figure 4. The response of swordtail (*X. helleri)* to critical high temperature in CTMax application



Şekil 5. Platinin (*X. maculatus*) CTMin uygulamasında kritik düşük sıcaklığa tepkisi

Figure 5. The response of platy (*X. maculatus*) to critical low temperature in CTMin application



Şekil. 6. Platinin (*X. maculatus*) CTMax uygulamasında kritik yüksek sıcaklığa tepkisi

Figure 6. The response of platy (*X. maculatus*) to critical high temperature in CTMax application

TARTIŞMA VE SONUÇ

Alıştırma su sıcaklıkları (AT) her iki balık türünün CTM değerlerini önemli düzeyde etkilemiştir. AT'nin Kılıçkuyruğun CTMin ve CTMax değerlerinde sırasıyla 3,40 ve 3,13; platide ise 2,21 ve 3,11°C bir sıcaklık farkı yaratması, AT'nin balıkların sıcaklık tolerans yeteneğini önemli ölçüde etkilediğini göstermektedir. AT'nin bu etkisi Prodocimo ve Freire (2001) tarafından da gösterilmiştir. Balıkların ortam sıcaklığından etkilenmelerinin nedeni, memeli ve kuşlardan farklı olarak, vücut sıcaklıklarının değişken olmalarına (poikloterm), dolayısıyla su sıcaklığına çok daha fazla bağımlı olmalarına bağlanmıştır. Poecilidler genelde tropik balıklardır ve bu aileye ait olan kılıçkuyruk ve platinin alt ve üst sıcaklık limitleri de buna uygun çıkmıştır. İki türün CTMax değerleri birbirlerine yakın olmakla birlikte, platinin CTMin değeri kılıçkuyruğa göre nispeten daha düşük bulunmuştur. Bu iki türün hem düşük ve hem yüksek sıcaklık toleransları ailenin diğer üyelerine göre nispeten düşük gözükmektedir. Örneğin Hernández ve Bückle (2002) moli (*Poecilia sphenops*) için CTMin ve CTMax değerlerini sırasıyla 7,5-12,5°C ve 38,8-43°C; Yanar vd. (2019), yelken kuyruk moli (*P. latipinna*) için 6,80-8,63 °C ve 38,73-41,83°C; lepistes (*P. reticulata*) için 9,41-11,95 ve 39,71- 41,80°C; Bierbach vd. (2010), *P. latipinna* için 7,6 ve 41,0°C olarak bildirmişlerdir. Diğer yandan *P.mexicana'nın* CTMin değerleri 9,1-11,3°C, *P. formasa' nın* ise 9,7°C olarak rapor etmiştir (Beirbach vd., 2010).

CTMin ARR değerleri alıştırma sıcaklıklarına bağlı olarak kılıçkuyrukta 0,42-0,42, platide 0,20-0,34 arasında değişirken, CTMax ARR değerleri kılıçkuyrukta 0,29-0,48, platide ise 0,33-0,44 arasında değişmiştir. Kılıçkuyruk ve platinin ARR verileri ilk olarak bu çalışmada ortaya konmuştur. Aynı aileden

molinin CTMin ve CTMax ARR değerleri sırasıyla 0,22 ve 0,38, lepistesin ise 0,31 ve 0,26 olarak bildirilmiştir (Yanar vd., 2019). Alıştırma sıcaklıklarının CTM değerleri üzerindeki değişiminin matematiksel ifadesi ARR balıkların sıcaklık dalgalanmalarına karşı dayanıklılığını ifade eder (Claussen, 1977). Bir balığın ARR değerinin yüksek olması, sıcaklık dalgalanmalarına karşı toleransının yüksek olduğunu gösterir. Subtropikal iklimlerde mevsimsel geçişlerde sıcaklık dalgalanmaları fazla olduğu için, bu bölgelerde yaşayan balıkların ARR değerlerinin daha yüksek, tropik bölgelerde ise daha düşük olması beklenir (Herrera vd., 1998; Re vd., 2005). Kılıçkuyruk ve plati tropik türler olduğu için, beklenildiği gibi tropik balıkların ARR değerlerini yansıtmaktadır. Yanar vd., (2019) 10 tropik akvaryum balık türünün CTMin ARR değerlerini 0,18 (vatoz, *Hypostomus plecostomus*) ve 0,63 (zebra balığı, *Brachydanio rerio*) aralığında; CTMax ARR değerlerini ise 0,25 (sarı prenses, *Labidochromis caeruleus*) ve 0,67 (tetrazon, *Puntius tetrazona*) aralığında bulmuşlardır. Kılıçkuyruk ve platinin ARR değerleri bu balıkların içinde orta sıralarda gözükmektedir.

Kılıçkuyrukta 217,3°C^2, plati balığında 232°C^2 olarak saptanan TTPA değerleri beklenildiği gibi tropikal balıkların değerlerine oldukça yakındır. Aynı alıştırma sıcaklıklarında, plati ve kılıçkuyrukla aynı aileye ait türlerde yapılan bir çalışmada (Yanar vd., 2019) TTPA değerleri siyah molide 259°C^2, lepisteste 242°C^2 olarak bildirilmiştir. Bu verilere göre, kılıçkuyruk ve platinin TTPA değerleri aynı aileden olan siyah moli ve lepistese göre nispeten daha düşüktür. Diğer bir anlatımla sıcaklık varyansları daha dardır. *Cyprini*d veya *Cyprinodontid* gibi subtropik türlerde ise TTPA değerleri *Carassius auratus* için 1,429°C (Ford ve Beitinger, 2005), *Cyprinidon variegatus* için 1,470°C (Bennett ve Beitinger, 1997) gibi oldukça yüksek değerlerde bildirilmiştir. Dolayısıyla kılıçkuyruk ve platinin TTPA değerlerinin düşük olması, bu balıkların subtropikal iklimlerde coğrafik dağılımları ve yetiştiriciliklerini sınırlayacaktır.

Su sıcaklığı düşerken denge kaybından önce balıkların hareketlerinde giderek artan bir yavaşlama; sıcaklık yükselirken ise artan oranda bir hiperaktivite, ani sıçrama ve amaçsız yüzme, ayrıca mukus salınımı ve dışkılama davranışları, sıcaklığa bağlı stres olarak değerlendirilmiştir. Gerek CTMin gerekse CTMax uygulamasında bu stres davranışları, denge kaybının gerçekleştiği sıcaklıktan yaklaşık 3-4°C önceki sıcaklıklarda başlamıştır. Denge kaybından önce sıcaklık değişimine bağlı bu stres belirtileri, sıcaklığın henüz kritik eşik seviyeye gelmeden önce önlem alınması açısından önemlidir. Yetiştiriciler su sıcaklığının henüz kritik eşik seviyeye gelmeden önce balıkların sıcaklığa bağlı bu tepkilerini bilirlerse, önlem almaları için fırsatları olacaktır. Balıkların kritik sıcaklık seviyesine yaklaşırken gösterdikleri bu stres davranışları, benzer şekilde, Yanar vd., (2019) tarafından 13 akvaryum balık türü üzerinde de gösterilmiştir.

CTM denemeleri sonlandırıldıktan sonra balıklar gözlem akvaryumlarına (24°C) aktarıldıklarından sonra, iki balık türünde yaşama oranı CTMin uygulananlarda %100, CTMax uygulananlarda ise %95-96 dolayında gerçekleşmiştir. Dolayısıyla kritik yüksek sıcaklık, kritik düşük sıcaklığa göre daha öldürücü bulunmuştur. Benzer sonuçlar Yanar vd., (2019) tarafından pek çok balık türünde rapor edilmiştir. Dolayısıyla kısa süreli de olsa, balıkları özellikle yüksek sıcaklık şokundan korunması gerekir.

TEŞEKKÜR

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KAYNAKÇA

Becker, C.D. & Genoway, R.G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes*, 4(3), 245–256. DOI :10.1007/BF00005481

Beitinger, T.L., Bennett, W.A. & McCauley, R.W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental biology of fishes*, 58(3), 237-275. DOI:10.1023/A:1007676325825

Bennett, W.A. & Beitinger, T.L. (1997). Temperature Tolerance of the Sheepshead Minnow, *Cyprinodon variegatus*. *Copeia*, 77-87. DOI: 10.2307/1447842

Bierbach, D., Schleucher, E., Hildenbrand, P., Köhler, A., Rodriguez, L.A., Riesch R. & Plath, M. (2010). Thermal tolerances in mollies (*Poecilia* spp.): reduced physiological flexibility in stable environments? *Bulletin of Fish Biology*, 12(1/2), 83-89.

Claussen, D.L. (1977). Thermal acclimation in ambystomatid salamenders. *Comparative Biochemistry and Physiology* - Part A: 58, 333–340. DOI: 10.1016/0300-9629(77)90150-5

Cowles, R.B. & Bogert, C.M. (1944). A preliminary study of the thermal requirements a desert reptiles. *Iguana*, 83, 265-296.

Cox, D.K. (1974). Effects of three heating rates on the critical thermal maximum of bluegill. *Thermal ecology*, 158-163.

Crossman, E.J. (1991). Introduced freshwater fishes: a review of the North American perspective with emphasis on Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 48(S1), 46-57. DOI:10.1139/f91-303

Eme, J. & Bennett, W.A. (2009). Critical Thermal Tolerance Polygons of Tropical Marine Fishes from Sulawesi, Indonesia. *Journal of Thermal Biology*, 34, 220-225. DOI:10.1016/j.jtherbio.2009.02.005

Ford, T. & Beitinger, T.L. (2005). Temperature tolerance in the goldfish, *Carassius auratus*. *Journal of Thermal Biology*, 30(2), 147-152. DOI:10.1016/j.jtherbio.2004.09.004

Gouveia, L., Rema, P., Pereira, O. & Empis, J. (2003). Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. *Aquaculture Nutrition*, 9(2), 123-129. DOI:10.1046/j.1365-2095.2003.00233.x

Hensen, R.R., Ploeg, A. & Fossa, S.A. (2010). Standard Names for Freshwater Fishes in the Ornamental Aquatic Industry. The Netherlands: OFI Education Publication.

Hernández, R.M. & Bückle R, L.F. (2002). Temperature tolerance polygon of *Poecilia sphenops* Valenciennes (Pisces: Poeciliidae). *Journal of Thermal Biology*, 27(1), 1-5. DOI: 10.1016/S0306-4565(01)00008-0

Herrera, D.F., Uribe, S.E., Ramirez, B.L.F. & Mora, G.A. (1998). Critical thermal maxima and minima of *Macrobrachium rosenbergii* (Decapoda: Palemonidae). *Journal of Thermal Biology,* 23(6), 381-385. DOI: 10.1016/S0306-4565(98)00029-1

Lever, C. (1996). Naturalized fishes of the world. New York: Academic Press.

Lovell, R.T. (2000). Nutrition of ornamental fish. In: Bonagura, J. (Ed.), Kirk's Current Veterinary Therapy XIII-Small Animal Practice (pp. 1191–1196). Philadelphia: W.B. Saunder.

Lutterschmidt, W.I. & Hutchison, V.H. (1997). The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, 75(10), 1561-1574. DOI: 10.1139/z97-783

Penning, M., Reid, G. McG., Koldewey, H., Dick, G., Andrews, B., Arai, K., Garrat, P., Gendson, S., Lange, J., Tanner, K., Tonge, S., Van den Sande, P., Warmolts, D. & Gibson, C. (2009). Turning the tide: A global aquarium strategy for conservation and sustainability. World Association of Zoos and Aquariums, Bern, Switzerland.

Prodocimo, V. & Freire, C.A. (2001). Critical thermal maxima and minima of the platyfish *Xiphophorus maculatus* Günther (Poecillidae, Cyprinodontiformes)-A tropical species of ornamental freshwater fish. *Revista Brasileira de Zoologia,* 18(1), 97-106. DOI: 10.1590/S0101-81752001000500007

Raghavan, R., Dahanukar, N., Tlusty, M., Rhyne, A., Kumar, K., Molur, S. & Rosser, A. (2013). Uncovering an obscure trade: Threatened freshwater fishes and the aquarium pet markets. *Biological Conservation*, 164, 158-169. DOI: 10.1016/j.biocon.2013.04.019

Re, A.D., Diaz, F., Sierra, E., Rodriguez, J. & Perez, E. (2005). Effect of salinity and temperature on thermal tolerance of brown shrimp *Farfantepenaeus aztecus* (Ives) (Crustacea, Penaeidae). *Journal of Thermal Biology*, 30(8), 618-622. DOI: 10.1016/j.jtherbio.2005.09.004

Rhyne, A.L., Tlusty, M.F., Schofield, P.J., Kaufman, L. & Morris Jr, J.A. (2012). Revealing the appetite of the marine aquarium fish trade: the volume and biodiversity of fish imported into the United States. *PLoS One*, 7(5), e35808. DOI: 10.1371/journal.pone.0035808

Ross, S.T. (1991). Mechanisms structuring stream fish assemblages: are there lessons from introduced species? *Environmental Biology of Fishes*, 30(4), 359-368. DOI: 10.1007/BF02027979

Spotila, J.R., Terpin, K.M., Koons, R.R. & Bonati, R.L. (1979). Temperature requirements of fishes from eastern Lake Erie and the upper Niagara River: a review of the literature. *Environmental Biology of Fishes*, 4(3), 281-307. DOI: 10.1007/BF00005485

Tuckett, Q.M., Ritch, J.L., Lawson, K. M., Lawson, L. L. & Hill, J. E. (2016). Variation in cold tolerance in escaped and farmed non-native green swordtails (*Xiphophorus helleri*) revealed by laboratory trials and field introductions. *Biological invasions,* 18(1), 45-56. DOI: 10.1007/s10530-015-0988-y

USGS (U. S. Geological Survey), (2004). Nonindigenous aquatic species database, Gainesville, Florida. Alıntılanma adresi: http://nas.er.usgs.gov (14.09.2020).

Yanar, M., Erdogan, E. & Kumlu, M. (2019). Thermal tolerance of thirteen popular ornamental fish species. *Aquaculture*, 501, 382-386. DOI: 10.1016/j.aquaculture.2018.11.041

http://www.egejfas.org
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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Güneydoğu Karadeniz'de dağılım gösteren kahküllü horozbina balığı *Parablennius tentacularis* (Brünnich, 1768)'de yaş tahmini ve en uygun büyüme modelinin seçimi

Age estimation and the best growth model selection of the tentacled blenny *Parablennius tentacularis* (Brünnich, 1768) in the southeastern Black Sea

Ayşe Van[1]* ● Aysun Gümüş[2] ● Melek Özpiçak[3] ● Serdar Süer[4]

[1] Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Samsun, Türkiye https://orcid.org/0000-0002-8100-4462
[2] Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Samsun, Türkiye https://orcid.org/0000-0002-0217-6494
[3] Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Samsun, Türkiye https://orcid.org/0000-0003-3506-4242
[4] Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Samsun, Türkiye https://orcid.org/0000-0002-4254-4845

*Corresponding author: van_55.1986@hotmail.com
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Öz: Bu çalışmada kapsamında, Güneydoğu Karadeniz kıyıları boyunca dağılım gösteren kahküllü horozbina balığı (*Parablennius tentacularis* (Brünnich, 1768))'na ait 522 birey, Mayıs 2010-Mart 2012 tarihleri arasında dip trolü çalışmaları (ticari balıkçılık ve deniz saha araştırmaları) ile yakalanmıştır. Örneklemin boy dağılım aralığı 4,8-10,8 cm arasında değişmektedir. Eşeylerin boy (K-S test, Z=3,729, P=0,000) ve ağırlık frekans dağılımları (K-S test, Z=3,605, P=0,000) arasındaki fark istatistiksel olarak önemli bulunmuştur. Boy-ağırlık ilişki modeli erkek bireylerde $W= 0{,}009L^{3{,}034}$ ile izometrik ve dişi bireylerde $W= 0{,}006L^{3{,}226}$ ile pozitif allometrik olarak tanımlanmıştır. Otolit ve omur örnekleri yaşın belirlenmesinde kullanılabilecek en doğru sert yapının seçimi için karşılaştırılmış ve otolit en uygun sert yapı olarak seçilmiştir. Mevcut veri seti, en iyi büyüme modelinin tahmini için kullanılmıştır. Bu amaçla, yaygın olarak kullanılan von Bertalanffy, Gompertz ve Lojistik büyüme fonksiyonları ile büyüme parametreleri tahmin edilmiştir. Bu fonksiyonlar aracılığıyla kurulan büyüme modellerinden en doğrusunu seçmek için Akaike'nin Bilgi Kriteri (AIC), $L_{mak.}/L_{\infty}$ oranı ve R^2 ölçütleri kullanılmıştır. Çoklu model çıkarımı ile model ortalamalı parametreler hesaplanmıştır: L'_{∞}=15,091 cm, S.E.(L'_{∞})=3,966, K'=0,232 yıl^{-1}, S.E.(K')= 0,122.

Anahtar kelimeler: *Parablennius tentacularis,* kahküllü horozbina balığı, yaş, büyüme, Akaike'nin bilgi kriteri, çoklu model çıkarımı

Abstract: By the study's coverage, 522 individuals of tentacled blenny (*Parablennius tentacularis* (Brünnich, 1768)), were caught with the bottom trawl operations (commercial fisheries and scientific field surveys) between May 2010 and March 2012 from the southeastern Black Sea. The size distribution range of the sample varied between 4.8-10.8 cm. The difference between sex length (K-S test, Z=3.729, P=0.000) and weight frequency distributions (K-S test, Z=3.605, P=0.000) was found to be statistically significant. The length-weight relationship models were defined as isometric with $W = 0.009L^{3.034}$ in male individuals and positive allometric with $W = 0.006L^{3.226}$ in female individuals. Otolith and vertebra samples were compared for the selection of the most accurate hard structure that can be used to determine the age. Otolith was chosen as the most suitable hard structure. The current data set was used to predict the best growth model. For this purpose, the growth parameters were estimated with the widely used von Bertalanffy, Gompertz and Logistic growth functions. Akaike's Information Criterion (AIC), $L_{mak.}/L_{\infty}$ ratio, and R^2 criteria were used to select the most accurate growth models established through these functions. Model averaged parameters were calculated with multi-model inference (MMI): L'_{∞} = 15.091 cm, S.E. (L'_{∞}) = 3.966, K'= 0.232 year^{-1}, S.E. (K') = 0.122.

Keywords: *Parablennius tentacularis,* tentacled blenny, age, growth, Akaike's information criterion, multi model inference

GİRİŞ

Horozbina türleri (Blenniidae ailesinde) littoral alanlarda yaşayan küçük bentik balıklardır. Vücut boyutu, renk, büyümüş dorsal yüzgeçler, farklı tipteki tentaküller ya da orta yüzgeç üzerinde bulunan özelleşmiş bezler ve sefalik süsler gibi eşeysel dimorfik özelliklere sahip familyalardan biridir (Giacomello ve Rasotto, 2005). Bu familyanın erkeklerinde diğerlerinden farklı olarak testis ve sperma kanalı ile ilişkili testikular beze ve kör keseler gibi yardımcı organlar mevcuttur (Richtarski ve Patzner, 2000). Yumurtalar, demersaldir ve yuvanın iç yüzeyine tek katman halinde bırakılır. Erkeklerde yuva bakımı vardır ve yumurtalar açılıncaya kadar yumurtalara bekçilik yapar.

Blenniidae familyasının bir üyesi olan *Parablennius tentacularis* bireylerinde, siyahımtırak, kırmızı, siyah veya esmer çizgili, küçük lekeli ve bariz 7-8 tane enine siyah çizgi ve çene üzerinde ise ekseriya zikzak şeklinde olan siyahımtırak esmer renkte 3 çizgi bulunmaktadır. Tuzlu ve acı su habitatlarına uyum sağlayabilen Atlanto Mediterranean kökenli demersal bir türdür. Karadeniz, Marmara, Ege ve Akdeniz'de dağılım gösteren tür (Mater vd., 2002) Karadeniz'in özellikle kumlu ve kayalıklı littoral alanlarında bulunmaktadır (Bat vd., 2005). *Parablennius tentacularis* üzerine yapılan çalışmalar özellikle türün eşeysel dimorfizm özellikleri, üremeye yardımcı organ yapıları, üreme taktikleri ve başarıları hakkındadır (Richtarski ve Patzner, 2000; Giacomello ve Rasotta, 2005; Giacomello vd., 2006; Giacomello vd., 2008; Pizzolon vd., 2010).

Balık büyümesi populasyon dinamiklerini belirleyen en önemli süreçlerden biridir. Balığın bireysel büyümesi birbirine ters olan iki yaklaşımın (anabolizma ve katabolizma) sonucudur (von Bertalanffy, 1938). Populasyon analizi için balığın ortalama bireysel büyümesinin açıklanması matematiksel bir ifadeye ihtiyaç duyar ki bu ifade yaşa karşı türlerin boyutu ile bağlantılıdır (Katsanevakis, 2006). Büyüme oranı ve yaş ya da boy arasındaki ilişkiler her biri teorik hesaplamalar ya da ampirik gözlemler vasıtasıyla türetilen formüller ile hesaplanabilir. Bu parametreler arasındaki çeşitli ilişkiler için birkaç model önerilmiştir. Von Bertalanffy balıklarda büyümeyi tanımlayan en popüler modeldir. Bununla birlikte alternatif modeller hesaba katılmaksızın bu modelin kullanılması, modelleme içine kaynağı belli olmayan hatalar ekleyebilir ve bu belirsizlik sonraki stok değerlendirmesini etkileyebilir. Ayrıca, görmezden gelinen alternatif modeller eksik değerlendirilmiş standart hata gibi katsayı tahminlerini ortaya çıkarabilir (Lin ve Tzeng, 2009). Bu modeller genellikle diferansiyel eşitliklerle temsil edilir ve çözümler balık büyümesi modellemesinde kullanılır (Katsanevakis, 2006; Katsanevakis ve Maravelias, 2008; Lin ve Tzeng, 2009). Büyüme çalışmalarında en güçlü ya da en doğru modeli seçmek için veri setine birden fazla model uygulanabilmektedir. Model ise bir kritere bağlı olarak seçilebilmektedir. MSE (the mean squares errors), R^2 (the coefficient of determination), AIC (Akaike's Information Criterion), APE (the least average prediction error), X^2 (chi-square) lnL values (the loglikelihood) gibi katsayılar model seçiminde tercih edilebilmektedir (Lin ve Tzeng 2009; Zhu vd., 2009).

Bilgi teorisine dayanan model seçimi, biyolojik bilimlerde göreceli yeni bir paradigmadır ve sıfır hipotezine dayanan genel metotlardan oldukça farklıdır (Johnson ve Omland, 2004; Katsanevakis, 2006; Lin ve Tzeng, 2009). Son zamanlarda omurgalı ve omurgasız hayvanların büyümesini tanımlayan en iyi modelin seçimi için kullanılmaktadır. Seçim süreç verilerin toplanması, doğru veri/örnek büyüklüğünün oluşturulması ile başlar. Modelin yapısının doğru kurulup kurulmadığının sınanmasında araştırmacıya yardımcı olacak teknikler ise model yapı testleridir. Bu aşamada, diğer kriterlerin aksine güçlü bir teorik alt yapıya sahip olan, Kullback-Leibler (Kullback ve Leibler, 1951) uyumsuzluğuna ve bilgi teorisine dayanan Akaike'nin Bilgi Kriteri (AIC) (Akaike, 1973; Katsanevakis, 2006) geleneksel yaklaşımlardan daha uygun ve daha güçlü bir alternatif sunmaktadır (Akaike, 1981; Burnham ve Anderson 2002) ve model karşılaştırma çalışmalarında da sıklıkla tercih edilmektedir (Katsanevakis ve Maravelias, 2008; Burnham vd., 2011; D'Alberto vd., 2017). Ortalama büyüme modelinin yanı sıra, veriyi en iyi modele uydurmak için bilgi kriterlerini kullanan çoklu model çıkarımı [Multi-Model Inference (MMI)] önerilir çünkü bu çıkarım model seçimindeki belirsizliği giderebilme yeteneğine sahiptir.

Çalışma, model seçimi ve MMI 'ye dayalı olarak balık büyüme çalışmalarında bir fenomen olan von Bertalanffy modelinin, seçilen tür için uygun olup olmadığını göstermeyi amaçlamaktadır.

MATERYAL VE METOT

Bu çalışmada araştırma materyalini oluşturan *P. tentacularis* türüne ait 522 örnek, Kızılırmak – Yeşilırmak ve Melet Irmağı şelf sahalarından Mayıs 2010-Mart 2012 tarihleri arasında, ticari trol balıkçı tekneleri ve gerçekleştirilen deneysel saha sörveyleri aracılığıyla 0 ila 60 m derinlikten dip trolü ile yakalanmıştır.

Laboratuvara getirilen örneklerin toplam boyları (TL, cm; ±0,1 mm) ve ağırlıkları (W, ±0,01 g) ölçülmüş, eşeyleri kaydedilmiştir. Yaş tayininde kullanılacak iki sert yapı (otolit ve omur) bireylerden çıkarılarak, prosedürlere uygun olarak okuma için hazır hale getirilmiştir. Her iki yapıda binoküler mikroskop ile üstten aydınlatma altında, sırasıyla X40 ve X20'lik büyütmede incelenmiştir. Yaş verileri iki okuyucunun üç tekrarlı okumaları neticesinde elde edilmiştir. İki okuyucunun tekrarlı okumaları ve güvenilir yapının seçimi için hesaplanan katsayı CV (değişim katsayısı) ve uygulanan istatistiki testler APE (ortalama yüzde hata) sert yapıların değerlendirilmesinde kullanılmıştır.

Erkek ve dişi bireylerde gözlenen morfolojik farklılıklar nedeniyle yapılan işlemler eşeyler için ayrı ayrı hesaplanmıştır. Eşeylerin boy ve ağırlık ortalamaları bağımsız iki örneklem t-testi (α=0,05), boy, ağırlık ve yaş frekans dağılımları Kolmogorov-Smirnov Z testi (α=0,05) (K-S test) ve eşeylerin yaş ortalamaları Mann-Whitney U testi ile karşılaştırılmıştır.

Boy- ağırlık ilişkisinin hesabında $W = a \times L^b$ eşitliği kullanılmıştır (Bagenal ve Tesch, 1978). b değerinin 3'ten farklılığı t-testi ile karşılaştırılmıştır. Eşeyler için hesaplanan *a* ve *b* sabitleri arasındaki farklılık ise ANCOVA (α = 0.05) ile test edilmiştir.

Otolitler Leica marka stereo mikroskoba bağlı kamera-monitör ve Leica IM50 görüntü analiz programı kullanılarak görüntülenmiştir. Balık boyutu – yaş, otolit boyutu (genişlik ve boy)- yaş arasındaki ilişkiler doğrusal ve eğrisel regresyon tekniği ile açıklanmıştır.

Von Bertalanffy, Gompertz ve Lojistik büyüme fonksiyonları yaş-boy verisine uygulanmıştır. Parametreler, tüm modellere doğrusal olmayan regresyon için kalıntı kareler toplamının (RSS) bir kriter olduğu Levenberg-Marguardt algoritması uygulanmasıyla hesaplanmıştır. Bu üç büyüme modelinin fonksiyonu aşağıdaki gibidir;

Von Bertalanffy Model $L_t = L_\infty(1 - e^{-k(t-t_0)})$,
Gompertz Model $L_t = L_\infty e^{-e^{-k\,(t-t_0)}}$,
Lojistik Model $L_t = \frac{L_\infty}{(1+e^{-k(t-t_0)})}$

Formüllerde, L_t yaşa karşı hesaplanan ortalama boyu, L_∞ asimptotik boyu, K büyüme katsayısını, t_0 sıfır boyundaki balığın yaşını ifade eder. Munro'nun büyüme performans indeksi; Φ=Log(K)+2Log(L_∞) eşitliğinden faydalanılarak hesaplanmıştır (Pauly ve Munro, 1984).

Doğru ya da veriyi en iyi sekilde yansıtan modelin seçimi için AIC (Akaike, 1973), R^2 kriteri (Cameron ve Frank, 1995) ve $L_{mak.}/L_{\infty}$ oranı kullanılmıştır. Akaike bilgi kriterinin formülü aşağıda verilmiştir;

$\mathrm{AIC} = n\ln(\hat{\sigma}^2) + 2k,\ \hat{\sigma}^2 = \mathrm{RSS}/n$, RSS kalıntı kareler toplamı, n toplam örnek sayısı, k parametre sayısıdır. Her bir model için bir AIC değeri hesaplanmıştır. Kural olarak en düşük AIC değerine sahip olan model seçilmelidir. Ancak AIC göreceli bir ölçümdür ve AIC değeri için atanan sınır keyfi olabilir, bu nedenle problemi ortadan kaldırmak ve temelde modellerin olasılık miktarını belirlemek için her bir modelin "Ağırlıklı Akaike" (w_i) (Burnham ve Anderson, 2002) değeri hesaplanmıştır. Formül aşağıdaki gibidir;

$$w_i = \frac{(\exp(-0.5\Delta_i))}{\sum_{k=1}^{R}\exp(-0.5\Delta_k)}$$

burada w_i, Ağırlıklı Akaike değerini, $\Delta_i = AIC_i - \min AIC$ (AIC_i, model için hesaplanan AIC değeri, min AIC, tüm modeller için düşük AIC değeri) ifade eder. R test edilen modellerin sayıdır. Eğer $\Delta_i > 10$ ise model karşılaştırma için kullanılmaz, red edilir. $\Delta_i < 2$ ve $4 < \Delta_i < 7$ arasında ise modelin desteğe ihtiyacı vardır, karşılaştırma için kullanılabilir. Bu aşamadan sonra doğru modelin seçilebilmesi için ağırlıklı akaike değerinin 0,9 'ün üzerinde olması gerekmektedir. Eğer bu kabulü sağlayan model yoksa Çoklu Model Çıkarımı (MMI) (Burnham ve Anderson, 2002) yapılmalıdır. Ağırlıklı Akaike'ye dayalı ortalanmış model olan çoklu model çıkarımı, güçlü parametre tahminleri yapmak için önerilmektedir. Bu, veri birden daha çok modelle desteklenmesi gerektiğini ifade eder. MMI model ortalamalarıyla sağlam bir büyüme eğrisi çıkarmaktadır. Bu terim, yalnızca en iyi modelle parametre tahmininin yerine, parametrenin tahmini tüm modeller hesaba katıldığında yapılabilirliğinin ifadesidir. MMI formülü şöyledir;

$$TL_s\,(A) = \sum_{k=1}^{R} w_i \times TL_i(A)$$

burada, TL_s (A) yaş üzerinden balığın toplam boyunu veren yapay fonksiyonu, k MMI ye dahil güvenilir model(ler)in sayısını ve TL_i (A) tek bir modele göre hesaplanan yaşa karşı toplam boy değerini ifade eder.

MMI yaklaşımdan sonra, model-ortalamalı asimptotik boy L'_{∞} ve büyüme katsayısı K' her bir modelin hesaplanan w_i değeri ile üç model kullanılarak ağırlıklı bir ortalamadan tahmin edilmiştir. Model-ortalamalı asimptotik boy aşağıdaki gibi formüle edilir:

$\grave{L_{\infty}} = \sum_{i=1}^{R} w_i\, L_{\infty,i}$ L'_{∞} 'un koşulsuz standart hatası şu formülle tahmin edilir (Burnham ve Anderson, 2002); $S.E.\left(\grave{L}_{\infty}\right) = \sum_{i=1}^{R} w_i\ \left(var\left(L_{(\infty,\, i|i)}\right) + \left(L_{\infty,i} - \grave{L_{\infty}}\right)^2\right)^{1/2}$

burada var(L_{∞},i|i) i. modele göre hesaplanmış asimptotik boyun varyansını ifade eder. $\left(L_{\infty,i} - \grave{L}_{\infty}\right)^2$ model seçim varyansıdır. Koşulsuz standart hatayı hesaplamak için, model seçim varyansı koşullu varyansa eklenir. Bu toplamın karekökü Ağırlıklı Akaike' ye göre ağırlıklı hale getirilir ve toplanır. Büyüme katsayının standart hatası da aynı formülden yola çıkarak hesaplanmıştır.

BULGULAR

Bu çalışmada, örnekleme sahaları içinde 0-60 m derinlik konturlarından dip trolü ile 522 adet *Parablennius tentacularis* elde edilmiştir. Çalışma sırasında türün eşeyleri arasında morfolojik farklılaşma vardır. Üreme dönemi olan yaz mevsimde erkek bireylerin renklerinde koyulaşma olduğu saptanmıştır. Ocak ayında örneklenen erkek bireylerin yaz mevsimindeki bireylere göre daha açık kahve renkli olduğu kaydedilmiştir. Türde var olan supraorbital tentaküller dişi bireylerde üreme döneminde oldukça incelmiş ve kısalmıştır. Ancak Ocak ayı örneklerinde, tentaküllerin oldukça uzamış ve kalınlaşmış olduğu görülmüştür. Aynı zamanda, türün dişi bireylerinde çene desenlemesi dikkat çekmiştir ve dişiler beyaz bantların varlığı ile erkek bireylerden ayırt edilebilmektedir. Bu bantlar üreme döneminde daha belirgin iken, diğer dönemlerde varlığını korumakla birlikte belirginliği yitirmektedir. Erkeklerde anal yüzgecin önünde, anüs açıklığının arkasında 'anal bez' adı verilen bir yapı bulunmaktadır. İlave olarak her ne kadar türün üreme dönemi erken yaz (Mayıs-Temmuz) olarak nitelendirilse de sonbahar döneminde (Ekim) de olgun safhadaki (safha IV) bireylere rastlanılmıştır.

Örneklemde 306 dişi birey ve 146 erkek birey belirlenmiştir. Dişi: erkek oranı 1:0,47 hesaplanmıştır ve bu orana ki-kare analizi yapılmış ve eşey oranında fark anlamlı bulunmuştur (χ^2 test, 518,509, sd=2, P=0,000). Örneklemin boy aralığı 4,8-10,8 (7,439±0,043) cm arasında değişmektedir. Ortalama total boy dişilerde 7,21 cm ve erkeklerde 8,11 cm'dir. Dişi bireylerde ortalama ağırlık 3,84 g ve erkek bireylerde 5,70 g'dır. Eşeyler arasında ortalama boy (t=10,290, sd=450, P=0,000) ve ağırlık (t=10,523, sd=450, P=0,000) arasında anlamlı farklılık bulunmuştur. Eşeylerin boy ve ağırlık frekans değerlerini karşılaştırmak için iki örneklem Kolmogorov-Smirnov Z testi uygulanmıştır. Eşeylerin boy (K-S test, Z=3,729, P=0,000) ve ağırlık (K-S test, Z=3,605, P=0,000) frekans dağılımları arasındaki fark istatistiksel olarak önemli bulunmuştur.

Boy-ağırlık ilişkisi, dişiler için $W=0{,}006\ L^{3{,}226}$ (*b için %95 G.A. 3,108-3,345*), erkekler için $W=0{,}009\ L^{3{,}034}$ (*b için %95 G.A. 2,879-3,171*) ve toplam örneklem için $W=0{,}007\ L^{3{,}180}$ (*b için %95 G.A. 3,102-3,258*) şeklinde tanımlanmıştır. LWR modeli eşeyler arasında farklı bulunmuştur (ANCOVA, $F=41{,}314_{2,\,490}$, $R^2=0{,}929$, P=0,000).

Çalışmada, yaş tayininde güvenilir yapı seçiminde kullanılan yapılar (otolit ve omur) iki okuyucu tarafından üç tekrarlı olarak okunmuştur. Her bir okuyucu için yapılara ait ortalama yaş, yüzde uyum, ortalama yüzde hata ve değişim katsayısı hesaplanmıştır. Omurda yaş okumaları sonucu 6 yaş grubu (0-V yaş), otolitte ise 5 yaş grubu (I-V yaş) tespit edilmiştir. Tablo 1'de iki okuyucunun okumaları sonucu elde edilen yaş verilerinin karşılaştırması yer almaktadır. Öncelikle değişim katsayısı değerlerine daha sonra ise değişim katsayısı ile paralel sonuçların bulunduğu yüzde uyum ve ortalama yüzde hata değerlerine bakılarak en güvenilir sert yapının otolit olduğuna karar verilmiştir.

Tablo 1. İki okuyucunun otolit ve omur yaş verilerinin karşılaştırılması
Table 1. Comparison of age data of two readers on otolith and vertebra

		Ortalama Yaş		Uyum (%)	Ortalama Yüzde Hata (%)		Değişim Katsayısı (%)	
Sert Yapılar	N	Okuyucu 1.	Okuyucu 2	1-2 Okuyucu	Okuyucu 1.	Okuyucu 2.	Okuyucu 1.	Okuyucu 2.
Otolit	**100**	2,61	2,49	89	10,07	14,73	5,90	8,97
Omur	**100**	2,33	2,08	78	15,58	14,65	14,07	13,15

397 bireyde otolit ile yaş tahmini yapılmıştır. Eşeylere göre yaş dağılım yüzdeleri şöyledir: dişi bireylerde I-V yaş gruplarına dağılım sırasıyla %3,4, %60,3, %34,2, %1,7 ve %0,4'tür. Erkek bireyler I yaş grubu tespit edilememekle birlikte II-V yaş gruplarının dağılımı %38,3, %43,9, %11,2 ve %6,5 'dir. Dişi bireylerde II yaş grubu baskın iken erkek bireylerde III yaş grubu baskındır. Eşeylerin yaş ortalamaları arasında bir farkın olup olmadığı Mann-Whitney U testi ile sınanmıştır ve aralarında istatistikî olarak anlamlı bir fark bulunmuştur (Mann-Whitney U test, Z=-5,292, P= 0,000).

Tablo 2'de otolit boyutları ile yaş arasındaki regresyon analizi ile ilişki parametreleri (keşişim noktası (a), eğim (b)), iki değişkenin birbirleriyle uyum derecesi hakkında bilgi veren korelasyon katsayısı (r) ve değişkenler arasındaki önemlilik düzeyini belirleyen P değeri hesaplanmıştır. Doğrusal ve doğrusal olmayan regresyon teknikleri uygulandığında otolit boyutu ve yaş arasında eğrisel bir ilişki olduğu sonucuna varılmıştır. Dolayısıyla, yaş artışıyla birlikte otolit boyutları artışında bir azalma söz konusudur.

Tablo 2. *P. tentacularis* örnekleminde otolit boyutu ile yaş arasındaki ilişki parametreleri
Table 2. Relationship parameters between otolith length-age in *P. tentacularis*

	Değişken			Denklem Parametreleri					
	Bağımlı	Bağımsız	İlişki Türü	N	a	b	b_{SE}	R^2	P
Genel	OB	Yaş	Eğrisel	522	1,708	0,283	0,015	0,750	0,000
	OG	Yaş	Eğrisel	522	1,076	0,233	0,011	0,771	0,000

Büyüme parametreleri von Bertalanffy, Gompertz ve Lojistik büyüme eşitlikleri kullanılarak hesaplanmıştır(Tablo 3). Hesaplamalar sonucu her bir model için elde edilen RSS değeri AIC hesabında kullanılmıştır. Bunun yanı sıra, R^2 kriteri ve $L_{mak.}/L_\infty$ oranı göz önüne alınarak tür için en uygun büyüme modelinin seçiminde kullanılmıştır.

Tablo 3. *P. tentacularis* için farklı modeller yardımıyla hesaplanan büyüme parametreleri
Table 3. Estimated growth parameters of *P. tentacularis* with different models

	Büyüme Parametreleri			
Modeller	L_∞ (cm)	K ($yıl^{-1}$)	t_0 (yıl)	Φ
Von Bertalanffy	17,470	0,119	-2,252	1,560
Gompertz	13,819	0,261	1,163	1,698
Lojistik	12,511	0,399	1,462	1,796

Bu çalışmada, veriyi en doğru temsil eden modelin belirlenmesinde kullanılan kriterler belli kabullere dayanmaktadır. Şöyle ki, en düşük AIC, $L_{mak.}/L_\infty$ oranı ve en yüksek R^2 değerine sahip model seçilmedir. Modellerin AIC değeri, en düşük von Bertalanffy büyüme fonksiyonu için elde edilmiştir. Sırasıyla gompertz ve lojistik fonksiyonlar gelmektedir. En uygun modelin seçimde ölçüt olarak kullanılan diğer parametrelerde (R^2 ve $L_{mak.}/L_\infty$) AIC değerini destekler niteliktedir. Çalışma burada bırakıldığında bu tür için, model seçim belirsizliği önemsenmediğinde en uygun modelin von Bertalanffy büyüme fonksiyonu olduğu kararına varılır. Ancak AIC değeri için verilen karar keyfi bir atamadır ve 'en iyi' model olma olasılığının miktarının belirlemek için her bir modelin w_i değerleri hesaplanmıştır. Elde edilen w_i değerleri değerlendirilmeden önce formülde yer alan Δ_i (=AIC_i-minAIC) parametresi üzerinden modeller hakkında karar verilmesi gerekmektedir. Üç modelde de Δ_i >10 olmadığından tüm modeller seçim işlemine tabi tutulmuştur. Modellerin sahip olduğu w_i değerleri >0,9 olmadığından uygun modele karar verme işlemi bir basamak daha ileri gitmiştir (Tablo 4). Zira, modellerden biri için hesaplanan bu değer 0,9'un üzerinde olsaydı, model seçimi tamamlanmış olacaktı. Buradan çıkan sonuç, modeller tek başına türün büyümesinin tanımlanmasında yeterli değildir. Bu aşamadan sonra uygulanan MMI ile her bir yaş grubuna karşı bir TL_s değeri hesaplanmış ve ortalama bir büyüme modeli elde edilmiştir. Çalışma sonunda, veri von Bertalanffy, Gompertz ve Lojistik büyüme fonksiyonlarının herhangi biriyle değil tamamının hesaba katılmasıyla sağlam bir modelle açıklanmıştır. Von Bertalanffy model büyümenin tanımlanması için güçlü bir eğrinin üretimine %43,3; Gompertz model %32,9 ve Lojistik model ise %23,8'lik bir katkı sağlamıştır.

Çoklu model çıkarımı ile elde edilen ortalama modelin model ortalamalı asimptotik boy değeri 15,091 ve standart hatası 3,966, model ortalamalı büyüme katsayısı 0,232 ve standart hatası 0,122 olarak hesaplanmıştır. Ortalama modelin asimptotik boy değeri çalışmada kullanılan üç kritere göre (model seçim belirsizliği önemsenmediğinde) en iyi model olarak nitelendirilebilen von Bertalanffy modelinden küçük olduğu görülmektedir. Von Bertalanffy modeline, model seçim belirsizliği dahil edildiğinden normalin üstünde ya da altında tahminler yapabilir. Bu model, çıkarım için kullanıldığı zaman hatalı nokta tahminine ve uyumun yanlış değerlendirilmesine neden olabilir.

Tablo 4. *P. tentacularis'* de yaş-boy veri setine uygulanan 3 modelin karşılaştırılması. (Var. (L'∞), model ortalamalı asimptotik boy varyansı; MSV, model seçim varyansı; S.E.(L'∞), model ortalamalı asimptotik boy koşulsuz standart hatası; Var.(K'), model ortalamalı büyüme katsayı varyansı; S.E.(K'), model ortalamalı büyüme katsayı koşulsuz standart hatası)

Table 4. Comparison of 3 models applied to age-length data set in *P. tentacularis*. (Var. (L'∞), model- averaged unconditional variance of L∞; MSV, model selection variance; S.E.(L'∞), model- averaged unconditional standard error of L∞; Var.(K'), model averaged variance of K; S.E.(K'), model-averaged unconditional standard error of K)

	MODEL			
	Von Bertalanffy	Gompertz	Lojistik	
k	3	3	3	
R^2	0,646	0,645	0,644	
L_{mak}/L_∞	0,618	0,781	0,863	
RSS	153,072	153,289	153,528	
AIC	-370,400	-369,842	-369,222	
Δ_i	0,000	0,558	1,178	
wi	0,433	0,329	0,238	
S.E.(L∞)	5,270	1,989	1,288	
Var. (L'∞)	27,771	3,961	1,664	
MSV (L'∞)	5,673	1,611	6,641	
S.E.(L'∞)	2,504	0,777	0,686	
S.E.(K)	0,065	0,067	0,068	
Var.(K')	0,004	0,004	0,005	
MSV(K')	0,013	0,001	0,028	
S.E.(K')	0,056	0,023	0,043	
Model-ortalamalı parametreler	L'∞	S.E.(L'∞)	K'	S.E.(K')
	15,091	3,966	0,232	0,122

TARTIŞMA

Genel bilgiler türün 1 ila 15 m derinlikler arasında dağılım gösterdiğini ifade etmektedir (Mater vd., 2002). Kuzey Adriyatik Denizi ve İtalya'da yapılan çalışmalarda 2 ila 6 m derinlikte kayalık alanlarda türe rastlanılmıştır (Giacomello ve Rasotto, 2005; Giacomello vd., 2008). Akdeniz sularında *D. annularis, Gobius sp., Mullus surmuletus, Sarpa salpa, Sciaena umbra, S. porcus, Serranus cabrilla, S. ocellatus, S. rostratus, S. tinca, Synodus saurus, Posidonia oceanica* gibi türlerle birlikte 18-38 m derinlikten deniz çayırı alanlarından yakalandığı bildirilmiştir. Bayhan vd.(2008)'nin Gediz nehir ağzı lagünlerinde (İzmir Körfezi) balık faunası çeşitliğini araştırdıkları çalışmalarında, çamurlu habitatlardan 10-15 m derinliklerden yakaladıkları türler arasında bulunan *P.tentacularis* 'in, Mugilidae, Gobiidae, Sparidae, Labridae gibi familyalara ait bazı türler ile bir arada bulunduğu görülmektedir. Karadeniz balık kontrol listelerinde demersal-bentik habitatta yaşayan türün korunma durumu Romanya'da yok olma riski değerlendirmesini yapmak için yeterli veri olmadığı, Türkiye'de ise hassas yani yüksek tehlike riski altında olduğu bildirilmiştir (Yankova vd., 2010). Bu çalışma, *P. tentacularis* türünün, örnekleme sahası içinde 0-60 m derinlikler arasında dağılım gösterebildiğini ve bentopelejik bir tür olduğunu ortaya koymuştur. Türün dağılımı ve bolluğunda mevsimsel bir farklılaşma olduğu belirtilerek, bu durumun yuva yapma davranışının bir sonucu olabileceği vurgulanmıştır (Deudero vd., 2008).

Çalışma sırasında erkek ve dişi bireylerin morfolojik bazı özelliklerinin farklılığı dikkat çekmiştir. Zira, Blenniidae familyasına ait türlerin eşeyleri arasında morfolojik farlılıkları istatistiki testlerle ortaya koyan çalışmalar mevcuttur (Richtarski ve Patzner, 2000; Giacomello ve Rasotto, 2005; Giacomello vd., 2006; Giacomello vd., 2008). Vücut büyüklüğü açından bakıldığında, erkek bireylerin dişilerden daha büyük olduğu tespit edilmiştir ki, eşeylerin boy ve ağırlık ortalamaları arasında anlamlı bir farkın olması bu kanıyı doğrulamaktadır. Eşeyler arasında subraorbital tentakül boyutu ve mevsimsel (üreme dönemi boyunca) farklılaşma olduğu yönünde saptamalarımız mevcuttur. Erkeklerde tentaküllerin daha gelişmiş ve üreme periyodu (erken yaz dönemi) süresinde her iki eşeyde de uzamaya doğru bir eğilim olduğu rapor edilmiştir (Giacomello ve Rasotto, 2005; Giacomello vd., 2006). Giacomello ve Rasotto (2005) erkeklerin dişi bireylerden daha büyük, eşeyler arasında vücut boyutu açından önemli eşeysel dimorfizm olduğu istatistiki testlerle de ifade etmişlerdir. Familyanın üyeleri üzerine yapılan çalışmalarda sıklıkla üzerinde durulan konu, 'anal bez, bulb bez ya da testucular bez' olarak adlandırılan üremeye yardımcı organlar ile ilgilidir. Çalışmamız sırasında mikroskop altında incelenen erkek bireylerde anal yüzgeç üzerindeki beze benzeri yapıların varlığı dikkat çekmiştir. Erkek anal bezler antimikrobial özelliğe sahip bir salgı üretmektedir ve bu salgıdan yoksun kalan yumurtaların hayatta kalma ihtimali çok düşüktür. Dişiler daha fazla salgı üretebilme yeteneğinde olan büyük bezlere sahip olan erkekleri tercih eder. Bunun en önemli nedeni antimikrobial üretimden doğrudan fayda sağlanmak istenmesidir (Pizzolon vd., 2010). Anal bez boyutlarında mevsimsel bir farklılaşma olduğu tespit edilmiştir (Giacomello ve Rasotto, 2005). Bu mevsimsel varyasyon hormonal kontrol altında olabileceği ve androjen ile muamele edilen anal bezler büyüme eğilimi göstermiştir (Oliveira vd., 2001). Son olarak, bir çok araştırmacı, vücut ya da supraorbital tentakül boyutu, anal bez varlığı gibi özellikler erkek bireylerin çiftleşme başarısını etkilediğini ifade etmişlerdir (Oliveira vd., 1999; Giacomello ve Rasotto, 2005; Giacomello vd., 2006).

Bu çalışmada, *P. tentacularis* eşeylerinde boy-ağırlık ilişkisi modeli farklılık göstermiştir. Dişilerde model pozitif allometrik iken erkeklerde izometriktir. Batı Karadeniz'de, boy aralığı 5,5 ile 11,0 cm'dir ve boy ağırlık ilişkisi pozitif allometrik olarak tanımlanmıştır (Yıldız vd., 2018). Marmara Denizi'nde 3,5-10,0 cm boy aralığı verilmiştir ve boy ağırlık ilişkisi izometrik olarak tanımlanmıştır (Keskin ve Gaygusuz, 2010). Kara vd. (2016) doğu Ege Denizi'nde boy aralığını 10,4-12,5

cm bildirmişler ve pozitif allometrik boy ağırlık ilişkisi tespit etmişlerdir. Bu çalışmalarda eşey ayrımı yapılmaksızın tanımlamalar yapılmıştır. Çalışmamızda genel örneklem için ilişki pozitif allometriktir.

Sert yapıların değerlendirilmesi aşamasında iki okuyucunun tekrarlı okumaları ve güvenilir yapının seçimi için hesaplanan katsayı ve uygulanan istatistiki testlerin birinci amacı ortalama yaşa bakarak şayet varsa normalin altında veya üstünde hesaplamaları tespit etmektir. Nitekim omur normalin altında değerler vermiştir. Okuyucular arasındaki yüzde uyum ise, yaş yapılarının değerlendirilmesi esnasında, okuyucuların kriterlerinin benzerliğini ölçer ki, bu çalışmada en yüksek uyum otolitten elde edilmiştir. Yüksek uyum değerini ortalama yüzde hata ve değişim katsayısı da desteklemiştir. Eşeylerin ortalama yaşları arasında herhangi bir farkın olup olmadığı sınanmış ve fark anlamlı bulunmuştur. Öyleyse, örneklem içinde dişi bireyler daha küçük yaş gruplarında yoğunlaşmakta ve ileri yaşlara erişen birey sayılarının erkeklere oranla daha az olduğu görülmektedir. Familyanın bazı diğer üyelerinde yaş-büyüme, otolit yapısı, populasyon parametrelerinin tahmini gibi çalışmalar mevcuttur. Santos vd. (1995), Azores'ten örnekledikleri *Parablennius sanguinolentus parvicornis* 'in yaş ve populasyon yapısını çalışmışlardır. Yaş okumalarını otolit üzerinden yapmışlar ve büyümenin tanımlanmasında von Bertalanffy büyüme fonksiyonunu kullanmışlardır. Aynı lokalite de Azevedo ve Homem (2002)' nin *Parablennius ruber* üzerine yaptıkları çalışmada, aylık boy-frekans verisinden modal analizi yaparak boya karşı yaş verisi elde etmişler ve von Bertalanffy büyüme modelini uygulayarak parametre tahmini yapmışlardır. Kuzey-doğu İspanya'da bulunan Matarranya Nehri'nden örneklenen *Salaria fluviatilis* 'te yaş tayini operkulum kemikleri kullanılarak yapılmıştır ve Bhattacharya metodu ile yaş sınıflarını temsil eden boy kategorileri tanımlanmış sonrakinde ise güçlü yıl sınıfının izlenmesi analizi (Modal Progression Analysis) uygulanmıştır (Vinyoles ve De Sostoa, 2007).

Çalışmamız balık büyümesini modellenen alanlarda bilgi teorisine dayanan yaklaşımların nasıl uygulandığı ve model seçiminde tahmin edilen büyüme parametrelerinin nasıl hesaba katılacağını göstermiştir. Üç aday model veri setine uygulanmıştır ki, bu modeller bu alanda yapılan çalışmalarda da yaygın olarak kullanılan alternatiflerdir. En iyi model Akaike bilgi kriteri (AIC)'den biçimlenen hata-düzeltme katsayına göre seçilmiştir. Her bir modelin en iyi model olma olasılığı 'Ağırlıklı Akaike' w_i ile hesaplanmıştır. w_i' ye dayanan ortalama model her bir durum için sınanmıştır. Bu aşamadan sonra, üç model kullanılarak, her bir modelin w_i' ye göre ağırlıklı tahmini ve yaşa karşı ortalama boy değerleri ile Çoklu Model Çıkarımı (MMI) yaklaşımı uygulanmıştır.

Model seçimi özellikle markalama-yeniden yakalama ve filogenetik çalışmalarda yaygın olarak kullanılmaktadır. Uyum derece testleri ve model ortalaması da markalama-yeniden yakalama çalışmalarında sıklıkla kullanılmaktadır. Son zamanlardaki eğilim biyolojik süreçleri anlamak için çok sayıda model kullanarak ilgili parametreleri tahmin etmeye doğrudur (Johnson ve Omland 2004).

Model seçim kriterleri hem uygulanabilirliği hem de karmaşıklığı dikkate alır ve çok sayıdaki modeli eş zamanlı karşılaştırma imkânı sağlar (Burnham ve Anderson, 2002; Johnson ve Omland 2004). Diğer bir ifade ile model seçimi, gözlemlere göre desteklenen en iyi hipotezin tanımlamasını yapmak için kullanılır (Katsanevakis, 2006). Model seçimi sıklıkla sadece tek bir 'best' modeli seçen bir metot olarak düşünülmektedir ve daha sonraki çıkarsama ve parametre tahmini bu model koşuluna bağlıdır. Bu yaklaşım alternatiflerin hiçbir şekilde katkısı olmaksızın tek bir modelin keyfi toplanmasından daha iyidir, ancak hala bilgi teorisinden tam olarak yararlanılmayan basit bir yaklaşım vardır. Model seçimi sadece tek bir 'en iyi' modeli arama olarak görülmemeli; bunun yerine, daha güvenilir çıkarımlar aday modeller setine dayandırılmalıdır (Katsanevakis, 2006).

Von Bertalanffy büyüme fonksiyonu en çok çalışılan ve tüm boy-yaş modelleri arasında yaygın olarak uygulanan modeldir. Çalışmamızda bu modelle birlikte iki alternatif fonksiyon kullanılmıştır: Gompertz ve Lojistik. Büyümenin ifade edilmesi ya da verinin en doğru temsili için tek bir model kullanılması birtakım sorunları da beraberinde getirmektedir. En iyi model olmaksızın, model çıkarsama için kullanıldığında hatalı parametre tahmini ve yanlış bir değerlendirme yapacaktır. Bu durumda, sürecin en başında tek bir modelin en iyi olduğu kabulü yapılır ve daha sonra karşılaşılacak olan hatalar göz ardı edilebilir. Bu anlayışından yola çıkarak birçok araştırmacı farklı konularda çok sayıda modeli denemiştir. Modelin yapısının doğru kurulup kurulmadığının sınanmasında, iyi olarak tanımlanmış bir tahmini ölçmek ve analiz etmek için kullanılan birkaç kriterden biri olan AIC değeri kullanılmıştır (Katsanevakis, 2006; Katsanevakis vd., 2007; Lin ve Tzeng, 2009; Alp vd., 2011; Mercier vd., 2011; Ohnishi vd., 2012). Çoğu çalışmada büyüme parametreleri seçime gerek duyulmaksızın tek model gibi varsayılarak von Bertalanffy büyüme modeli kullanılmaktadır. Model seçimi yapılan birçok çalışmada von Bertalanffy ya aday model içine dahil edilmemiştir ya da en güçlü model olarak seçilmemiştir (Cerdenares-Ladro´n de Guevara vd., 2011; Wang ve Ma, 2016) Bununla birlikte; Katsanevakis ve Maravelias (2008)'ın literatürden topladıkları 133 yaş-boy verisinde von Bertalanffy büyüme modeli durumlar içinde %34.6 oranında en iyi model olarak seçilmiştir ve bu modeli %30.1 ile power model izlemiştir. Çalışmalarda ağırlıklı akaike değerlerine göre von Bertalanffy büyüme modeli ya en güçlü olarak (0,99 ya da 1) ya da en zayıf (<0,01) şekilde desteklenmiştir (Ahmed vd., 2012; Alp vd., 2011).

Çalışmalarda parametre tahminlerinde sıklıkla von Bertalanffy büyüme modelinin tercih edilmemesi, VBGM den farklı modellerin tür veri setlerine uygulanması gerekmektedir.

Genellikle, eğrinin kurulması ve şekillenmesi tek bir modele ve parametre tahminleri ve yorumları yalnızca kullanılan modele dayandırılmamalıdır. Von Bertalanffy büyüme modelinin balık büyüme çalışmalarında her zaman öncelikli ve tek model olması anlayışı terk edilmelidir. Böylece, büyümenin ve yaşam döngüsü parametrelerinin daha gerçekçi olarak tanımlanması sağlanabilir.

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KAYNAKÇA

Ahmed, Z.F., Hossain, M.Y. & Ohtomi, J. (2012). Modelling the growth of silver hatchet chela *Chela cachius* (Cyprinidae) from the Old Brahmaputra River in Bangladesh using multiple functions. *Zoological Studies*, 51(3), 336-344.

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In 2nd international symposium on information theory (B. N. Petrov, and F. Csaki, eds.), p. 267–281. Akademiai Kiado, Budapest.

Akaike, H. (1981). Likelihood of a model and information criteria. *Journal of Econometrics*, *16*(1), 3-14.DOI: 10.1016/0304-4076(81)90071-3

Alp, A., Kara, C., Üç kardeş, F., Carol, J. & Garcı´a-Berthou, E. (2011). Age and growth of the European catfish (*Silurus glanis*) in a Turkish Reservoir and comparison with introduced populations. *Reviews in Fish Biology and Fisheries*, 21,283–294. DOI: 10.1007/s11160-010-9168-4

Azevedo, J.M.N. & Homem, N. (2002). Age and growth, reproduction and diet of the red blenny *Parablennius ruber* (Blenniidae). *Cybium*, 26(2), 129-133.

Bagenal, T.B. & Tesch, F.W. (1978). Age and Growth. In: Methods for Assessment of Fish Production in Freshwaters, (Bagenal, T.B., Ed), Blackwell Science Publication, pp. 101-136, Oxford, UK.

Bat, L., Erdem, Y., Ustaoğlu, S., Yardım, Ö. & Satılmış, H.H. (2005). Türkiye'nin Orta Karadeniz Kıyısı Balıkları Üzerine Bir Araştırma. *Journal of the Black Sea / Mediterranean Environment*, 11, 281 –296.

Bayhan, B., Sever, T.M. & Kaya, M. (2008). Diversity of fish fauna in Gediz Estuary Lagoons (Izmir Bay/Aegean Sea). *Jornal of Animal and Veterinary Advances*, 7(9),1146-1150, ISSN: 1680-5593.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: A practical information-theoretic approach. Springer, New York, USA, 488 p.

Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology,* 65(1),23-35. DOI: 10.1007/s00265-010-1029-6

Cameron, A.C. & Frank, A.G. (1995). An R-squared measure of goodness of fit for some common nonlinear regression models, Windmeijer.

Cerdenares-Ladro`n De Guevara, G., Morales-Bojórquez, E. & Rodríguez-Sánchez, R. (2011). Age and growth of the sailfish *Istiophorus platypterus* (Istiophoridae) in the Gulf of Tehuantepec, Mexico. *Marine Biology Research*, 7(5),488-499. DOI: 10.1080/17451000.2010.528201

D'Alberto, B.M., Chin, A., Smart, J.J., Baje, L. & White, W.T. (2017). Age, growth and maturity of oceanic whitetip shark (*Carcharhinus longimanus*) from Papua New Guinea. *Marine and Freshwater Research,* 68(6), 1118-1129. DOI: 10.1071/MF16165

Deudero, S., Morey, G., Frau, A., Moranta, J. & Moreno, I. (2008).Temporal trends of littoral fishes at deep *Posidonia oceanica* seagrass meadows in a temperate coastal zone. *Journal of Marine Systems*, 70,182–195. DOI: 10.1016/j.jmarsys.2007.05.001

Giacomello, E. & Rasotto, M.B. (2005). Sexual dimorphism and male mating success in the tentacled blenny, *Parablennius tentacularis* (Teleostei: Blenniidae). *Marine Biology*, 147, 1221–1228. DOI: 10.1007/s00227-005-0023-4

Giacomello, E., Marchini, D. & Rasotto, M.B. (2006). A male sexually dimorphic trait provides antimicrobials to eggs in blenny fish. *Biology Letters*, 2,330–333. DOI: 10.1098/rsbl.2006.0492

Giacomello, E., Neat, F.C. & Rasotto, M.B. (2008). Mechanisms enabling sperm economy in blenniid fishes. *Behavioral Ecology and Sociobiology*, 62,671–680. DOI: 10.1007/s00265-007-0491-2

Johnson, J.B. & Omland, K.S. (2004). Model selection in ecology and Evolution. *TRENDS in Ecology and Evolution,* 19,2. DOI: 10.1016/j.tree.2003.10.013

Kara, A., Sağlam, C. & Acarlı, D. (2016). Length-weight relationships of fish captured by wire pots in Izmir Bay (eastern Aegean Sea, Turkey). In: *2nd International Congress on Applied Ichthyology & Aquatic Environment* (pp. 10-12).

Katsanevakis, S. (2006). Modelling fish growth: model selection, multi-model inference and model selection uncertainty. *Fisheries Research,* 81, 229-235. DOI: 10.1016/j.fishres.2006.07.002

Katsanevakis, S., Thessalou-Legaki, M., Karlou-Riga, C., Lefkaditou, E., Dimitriou, E. & Verriopoulos, G. (2007). Information-theory approach to allometric growth of marine organisms. *Marine Biology*, 151(3), 949-959. DOI: 10.1007/s00227-006-0529-4

Katsanevakis, S. & Maravelias, C.D. (2008). Modelling fish growth: multi-model inference as a better alternative to a priori using von Bertalanffy equation. *Fish and Fisheries,* 9, 178-187. DOI: 10.1111/j.1467-2979.2008.00279.x

Keskin, Ç. & Gaygusuz, Ö. (2010). Length-weight relationships of fishes in shallow waters of Erdek Bay (Sea of Marmara, Turkey). *IUFS Journal of Biology*, 69(2), 87-94.

Kullback, S. & Leibler, R.A. (1951). On information and sufficiency. *The Annals of Mathematical Statistics*, 22, 79–86. DOI: 10.1214/aoms/1177729694

Lin, Y.J. & Tzeng, W.N. (2009). Modelling the growth of Japanese eel *Anguilla japonica* in the lower reach of the Kao-Ping River, southern Taiwan: an information theory approach. *Journal of Fish Biology*,75, 100–112. DOI: 10.1111/j.1095-8649.2009.02268.x

Mater, S., Kaya, M. & Bilecenoğlu, M. (2002). Türkiye deniz balıkları atlası. Ege Üniversitesi Basımevi. Ege Üniversitesi, Su Ürünleri Fakültesi Yayınları, No:68,169 s.

Mercier, L., Panfili, J., Paillon, C., N'diaye, A., Mouillot, D. & Darnaude, A.M. (2011). Otolith reading and multi-model inference for improved estimation of age and growth in the gilthead seabream *Sparus aurata* (L.). *Estuarine, Coastal and Shelf Science,* 92, 534-545. DOI: 10.1016/j.ecss.2011.02.001

Ohnishi, S., Yamakawa, T., Okamura, H. & Akamine, T. (2012). A note on the von Bertalanffy growth function concerning the allocation of surplus energy to reproduction. *Fishery Bulletin*, 110,223–229.

Oliveira, R.F., Almada, V.C., Forsgren, E. & Goncalves, E.J. (1999). Temporal variation in male traits, nest aggregations and mating success in the peacock blenny, *Salaria pavo*. *Journal of Fish Biology,* 54,499–512. DOI: 10.1111/j.1095-8649.1999.tb00631.x

Oliveira, R.F., Canario, A.V.M. & Grober, M.S. (2001). Male sexual polymorphism, alternative reproductive tactics, and androgens in combtooth blennies (Pisces: Blenniidae). *Hormones and Behavior*, 40,266–275. DOI: 10.1006/hbeh.2001.1683

Pauly, D. & Munro J.L. (1984). Once more on growth comparison in fish and invertebrates, ICLARM. *Fishbyte,* 2, 21.

Pizzolon, M., Giacomello, E., Marri, L., Marchini, D., Pascoli, F., Mazzoldi, C. & Rasotto, M. B. (2010). When fathers make the difference: efficacy of male sexually selected antimicrobial glands in enhancing fish hatching success. *Functional Ecology*, 24, 141–148. DOI: 10.1111/j.1365 2435.2009.01608.x

Richtarski, U. & Patzner, R. A. (2000). Comparative morphology of male reproductive systems in Mediterranean blennies (Blenniidae). *Journal of Fish Biology*, 56, 22–36. DOI: 10.1111/j.1095-8649.2000.tb02084.x

Santos, R.S., Nash, R.D.M. & Hawkins, S.J. (1995). Age, growth and sex ratio of the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis. Journal of the Marine Biological Association of the United Kingdom,* 75, 751-754. DOI: 10.1017/S0025315400039175

Vinyoles, D. & De Sostoa, A. (2007). Life-history traits of the endangered river blenny *Salaria fluviatilis* (Asso) and their implications for conservation. *Journal of Fish Biology*, 70, 1088–1108. DOI: 10.1111/j.1095-8649.2007.01371.x

Von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth lows. II). *Human Biology*, 10, 181–213.

Wang, X.A. & Ma, A. (2016). Comparison of four nonlinear growth models for effective exploration of growth characteristics of turbot *Scophthalmus maximus* fish strain. *African Journal of Biotechnology*, 15(40), 2251-2258. DOI: 10.5897/AJB2016.15490

Yankova, M., Raykov, V., Ivanova, P., Mgeladze, M., Diasamidze, R., Agapov, S., Grinchenko, M., Öztürk, B., Oral, M., Bat, L., Düzgüneş, E., Shlyakhov, V., Boltachev, A. & Karpova, E. (2010). Black Sea Fish Check List. Black Sea Commission Publication, 58p.

Yıldız, T., Zengin, M., Uzer, U., Akpınar, I.O. & Karakulak, F.S. (2018). Length-weight relationships for 24 fish species collected from the western Black Sea (Turkey). *Cahiers de Biologie Marine*, 59, 159-165.

Zhu, L., Li, L. & Liang, Z. (2009). Comparison of six statistical approaches in the selection of appropriate fish growth models. *Chinese Journal of Oceanology and Limnology,* 27(3), 45. DOI: 10.1007/s00343-009-9236-6

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of total replacement of dietary fish oil by vegetable oils on growth performance, nutritional quality and fatty acid profiles of rainbow trout (*Oncorhynchus mykiss*) at optimum and high temperature conditions

Yemdeki balık yağının bitkisel yağlarla tamamen değiştirilmesinin optimum ve yüksek su sıcaklığında beslenen gökkuşağı alabalığının (*Oncorhynchus mykiss*) büyüme performansı, besin kalitesi ve yağ asidi profili üzerine etkileri

Seval Dernekbaşı[1]* ● Ayşe Parlak Akyüz[2] ● İsmihan Karayücel[3]

[1] Sinop Üniversitesi, Su Ürünleri Fakültesi, Yetiştiricilik Anabilim Dalı, Akliman, Sinop
[2] Kuzey Su Ürünleri San. ve Tic. Ltd. Şti. Bafra, Samsun
[3] Sinop Üniversitesi, Su Ürünleri Fakültesi, Yetiştiricilik Anabilim Dalı, Akliman, Sinop

https://orcid.org/0000-0001-5735-2486
https://orcid.org/0000-0001-9099-4368
https://orcid.org/0000-0003-2520-7545

*Corresponding author: sevalyaman@hotmail.com

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Abstract: The present study investigated the effects of total replacement of dietary fish oil by different vegetable oils on growth performance, nutritional quality and fatty acid profiles of rainbow trout (*Oncorhynchus mykiss*) at optimum and high temperature conditions. Five *iso*-nitrogenous and *iso*-lipidic diets including 100% fish oil and vegetable oils were prepared for rainbow trout fingerlings with an average weight of 11.27±0.01 g. After the fish were fed experimental diets for 9 weeks at the optimum temperature (17.74±0.01°C), all groups were fed an FO diet containing only fish oil for 4-weeks at the upper optimum temperature conditions (19.28±0.11°C). In both feeding trials, experimental fish were hand-fed *ad libitium* twice a day. Results showed that growth performance and feeding efficiency were significantly better ($p<0.05$) in groups fed by VO-based diets compared to groups fed by FO based diet at optimum temperature. Survival was 100% in CANO, SFO, CO PNO groups and 94.12±3.39% in FO (control) group at the end of the 9 weeks. Growth, feed consumption and survival of fish fed the upper-optimum temperature were significantly differed ($p<0.05$). In particular, while the survival rate of the groups fed with vegetable oil-based diets at optimum temperature and then fed only fish oil remained 100%, this rate decreased to 54.17±1.39% in the control group. Eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and n-3 PUFA of fish fed by VO based diets were low. When all groups were fed only FO diet for 4 weeks at upper-optimum temperatures, EPA, DHA and n-3 PUFA ratios increased. In addition, after the upper-optimum temperature trial, increases in the nutritional quality indices of fish meat were also detected.

Keywords: Salmonids, feed formulation, growth metrics, compensatory baiting

Öz: Bu çalışmada, balık yağının farklı bitkisel yağlarla toplam ikamesinin, optimum ve yüksek sıcaklık koşullarında gökkuşağı alabalığının (*Oncorhynchus mykiss*) büyüme performansı, beslenme kalitesi ve yağ asidi profilleri üzerindeki etkileri araştırılmıştır. Ortalama 11,27±0,01 g ağırlıktaki gökkuşağı alabalığı yavruları için %100 balık yağı ve bitkisel yağlardan oluşan beş izo-nitrojenli ve izo-lipidik diyet hazırlanmıştır. Balıklar optimum sıcaklıkta (17,74±0,01°C) 9 hafta deneysel diyetlerle beslendikten sonra, tüm gruplara optimum üstü sıcaklık koşullarında (19,28±0,11°C) 4 hafta boyunca sadece balık yağı içeren bir FO (kontrol) diyeti verilmiştir. Her iki besleme denemesinde de deneysel balıklar günde iki kez elle doyuncaya kadar beslenmiştir. Sonuçlar, optimum sıcaklıkta FO bazlı diyetle beslenen gruplara kıyasla, VO bazlı diyetlerle beslenen gruplarda büyüme performansı ve yemleme veriminin önemli ölçüde daha iyi ($p<0,05$) olduğunu göstermiştir. Yaşama oranı, 9 hafta sonunda CANO, SFO, CO PNO gruplarında %100 ve FO (kontrol) grubunda %94,12±3,39 olarak tespit edilmiştir. Optimum sıcaklıkta beslenen balıkların büyümesi, yem tüketimi ve hayatta kalması önemli ölçüde farklılık göstermiştir ($p<0,05$). Özellikle bitkisel yağ bazlı diyetlerle optimum sıcaklıkta beslenen ve daha sonra sadece balık yağı ile beslenen grupların hayatta kalma oranı %100 kalırken, kontrol grubunda bu oran %54,17±1,39'a gerilemiştir. VO bazlı diyetlerle beslenen balıkların eikosapentaenoik asit (EPA, 20:5n-3), dokosaheksaenoik asit (DHA, 22:6n-3) ve n-3 PUFA düşük olduğu tespit edilmiştir. Tüm gruplara optimum sıcaklıklarda 4 hafta boyunca sadece FO diyeti verildiğinde EPA, DHA ve n-3 PUFA oranları artmıştır. Ayrıca optimum üst sıcaklık denemesinin ardından balık etinin besin kalitesi endekslerinde de artışlar tespit edilmiştir.

Anahtar kelimeler: Alabalıklar, yem formülasyonu, büyüme ölçümleri, telafi yemlemesi

INTRODUCTION

In the fish feed industry, fish oil costs increased due to the decline in fish stocks and rising demand (Izquierdo et al., 2005; Şahan et al., 2017), and this increase in fish oil prices was reflected in feed costs. In fish feeds, it is very important to prepare a balanced ration to meet the needs of fish. Balanced feed rations play an important role in implementing most of the physiological functions for fish. Creating an economical and balanced ration for feed, finding alternative fish oil sources that can meet the need for the similar fish fatty acids without causing metabolic deterioration and health impairment will reduce the cost of the market sector and utility the economy (Sahan et al., 2016).

Due to the fact that the production of vegetable oils is easy and inexpensive, it can be used as an alternative to fish oil in different proportions and it is generally accepted by the researchers that there is no negative effect on growth of fish (Torstensen et al., 2000; Caballero et al., 2002; Yildiz et al., 2015; Yıldız et al., 2018). These rates can be up to 100% in freshwater fish while it was determined as 60% in marine fish (Izquierdo et al., 2005). Most vegetable oils are rich in unsaturated 18C fatty acids such as oleic (18:1n-9), linoleic (18:2n-6) and alpha-linolenic acids (α-18:3n-3) but are poor sources for n-3 HUFAs, so the flesh of farmed fish given feeds containing high concentrations of vegetable oils may contain a limited amount of n-3 HUFAs (Sonu, 2018). This dilemma has led to the development of using only a fish oil diet to restore n-3 PUFA levels in fish after a growth period on vegetable oil (VO) based diet. Feeding fish only fish oil after grow-out period based on vegetable oil diet is one strategy to restore eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels (Codabaccus et al., 2013).

Rainbow trout is defined as a species of cold water fish, and especially in trout farming, an increase in water temperature can lead to a decrease in feed intake, growth rate and survival rate and an increase in disease incidence. In recent years, researchers have accelerated the studies on the use of vegetable oils showing antioxidant features as an alternative antioxidant in order to prevent diseases, increase growth performance and strengthen the immune system. Antioxidation is important for nutrition because of reducing physiological stress in organs and cells (Bayaz, 2014). Studies showed that these substances have quite beneficial effects and their main advantage is that they are of natural origin and do not pose any threat to human, fish health or the environment. (Gabor et al., 2012).

For this reason, the current study, after feeding rainbow trout with vegetable oil-based and fish-oil diets at optimum temperature, in all groups, fed by diets containing only fish oil at upper-optimum temperature for a certain period of time, in particular, it was tried to determine how it would affect survival and growth. In addition, it is aimed to regain the nutritional quality indexes that decreased as a result of feeding with n-3 PUFA and vegetable oils in a certain period after the end of the growth period by using fish oil diets.

MATERIAL AND METHODS

Experimental diets and feeding

Five experimental diet groups were formulated to be iso-nitrogenous (48%) and iso-lipidic (17%), containing different lipid sources (Table 1).

Fish oil and feed ingredients were obtained from a local fish feed manufacturer (Sibal A.Ş. Black Sea Feed, Sinop, Turkey). Canola and peanut oils were purchased from commercial companies (Tokul Tarım A.Ş., İzmir/Turkey and Başpınar Toprak Mah. ve Nak. Ltd. Şti., Osmaniye, Turkey, respectively). Sunflower and corn oil were purchased from a supermarket (ŞOK Marketler T.A.Ş., Sinop, Turkey). Diets were prepared by a laboratory pelleting machine after 35- 40 g of distilled water was added into 1000 g mixture of ingredients and the pellets were dried at 40°C for 12 h. Diets were crushed to the approximate size of 1-2 mm and kept at -20°C.

Table 1. Ingredients (g/kg) and proximate composition (%) of the experimental diets containing the different vegetable oils and fish oil

	Experimental diets				
	FO (Control)	CANO	CO	SFO	PNO
Ingredients (g kg^{-1})					
Fish meal	350	350	350	350	350
Extracted soybean meal	250	250	250	250	250
Wheat flour	130	130	130	130	130
Corn protein	146	146	146	146	146
Fish oil	120	0	0	0	0
Canola oil	0	120	0	0	0
Corn oil	0	0	120	0	0
Sunflower oil	0	0	0	120	0
Peanut oil	0	0	0	0	120
Vitamin premix(*)	2	2	2	2	2
Mineral premix(*)	2	2	2	2	2
Proximate Composition (%)					
Moisture	4.08	4.34	3.69	3.63	4.81
Protein	47.28	47.74	47.69	47.63	47.24
Lipid	17.71	17.23	17.69	17.59	17.29
Ash	9.74	9.55	9.22	9.39	9.28
NFE+Crude fiber	21.19	21.14	21.71	21.79	21.38
Gross energy (kJg^{-1})	21.75	21.76	21.95	21.82	21.58

* Vitamin-mineral premix (mg/kg premix): vitamin A, 210000 IU; Vitamin D_3, 35000 IU; vitamin E, 7000 mg; vitamin K_3, 322 mg; vitamin B_1, 588 mg; vitamin B_2, 252 mg; vitamin B_6, 294 mg; vitamin B_{12}, 826 mcg; niacin, 1400 mg; biotin, 7583 mcg; 182 mg folic acid, pantothenic acid, 1722 mg; inositol, 17220 mg; vitamin C, 933.31 mg; Ca, 1414mg. NFE+Crude fiber=100-(%protein+ %lipid+ %ash+ %moisture)

The experiment was conducted in triplicates in randomly assigned tanks. During 9 weeks of optimum temperature and 4 weeks of the upper-optimum temperature, fish in all groups were hand fed twice a day (at 09:00 am and 15:00 pm) to apparent satiety under a natural light regime.

Fish facilities and sampling

Rainbow trout fingerlings were obtained from a commercial trout farm (Kuzey Su Ürünleri Inc., Samsun, Turkey) and acclimated in the Research and Application Center of Sinop University, Fisheries Faculty (Sinop, Turkey) for one week before the start of the experiment. Fish were stocked in centrally drained three 4000 l rectangle fiberglass tanks in a flow-through water system in an indoor facility during acclimatization. After acclimatization, fish (mean weight of 11.27±0.01 g) were fasted for a day, weighted and

randomly distributed among fifteen fiberglass circular tanks (approximately, the water volume of 300-L; 60 cm in high; 80 cm in diameter) at a density of 17 fish per tank. Water inflow was adjusted to 4 l/min and supplemental aeration was provided via airstone diffusers. The fish were individually weighted at the beginning and end of the experiment with a 0.01 g sensitive electronic balance. The water quality parameters were monitored on a weekly basis and the following parameters were recorded. In this study was measured as average temperature of 17.74±0.01°C, dissolved oxygen of 6.06±0.04 mg^{-l} and pH 8.21. At the beginning of the experiment, twenty fish were sacrificed, weighed and fish fillets were homogenized for analysis. At the end of the optimum temperature trial and the upper-optimum temperature trial, five fish from each tank were sampled and fish fillets were homogenized. Homogenized fish fillet samples were stored at -80°C until used to proximate and fatty acid analysis.

Upper-optimum temperature

Fish used in the optimum temperature experiment were used in the experiment. The fish remaining after the optimum temperature trial were weighed. A new trial was created to be fed only the control diet for 4 weeks at the upper-optimum temperature. After this point, all fish were fed by a control diet (Table 1), which had the same composition as the FO diet used in the optimum temperature trial. In the upper-optimum temperature trial, the average water temperature was 19.28±0.11°C during 4 weeks. At the end of the trial, fish were individually weighed and five fish from each tank were randomly selected, euthanized by excess anesthetic (clove oil) and stored at -80°C for future analysis.

Proximate and fatty acid analysis

The analyses of proximate composition on feed and fillet were performed according to the standard methods of AOAC (1995): dry matter after drying in an oven at 105°C until constant weight, crude protein (Nx6.25) by Kjeldhal method after acid digestion, ash content by incineration in a muffle furnace at 550°C for 12 h, crude lipid after extraction with petroleum ether by the Soxhlet method.

Total lipid was determined by modified Bligh and Dyer Method (Hanson and Olley, 1963). 0.25 g of extracted oil from fish fillets and diets was dissolved by adding 4 ml of heptane and 0.4 ml of 2N KOH was added. This mixture was stirred in vortex for 2 minutes, then centrifuged at 5000 rpm for 5 minutes. After centrifugation, 1.5-2 ml of the heptane phase was collected and transferred to glass tubes for GC/MS analysis. The injection of samples into the device was performed with the autosampler AI 1310. Samples were analyzed by Thermo Scientific ISQ LT model GC/MS gas chromatography by spectrometer. For this analysis, with 0.25µm film thickness was used a Trace Gold TG-WaxMS capillary column (Thermo Scientific code: 26088-1540) in 0.25µm inner diameter and 60µm length. The injection block temperature was adjusted to 240°C and the column temperature program to be increased from 100°C to 240°C. Helium gas (1 ml/min) was used as a carrier gas and 1:20 split ratio was applied. The MS unit (ISQ LT) was used in electron ionization mode. Fatty acids are defined by comparing the standard FAME mixture of 37 components [Chem-Lab Fame mix (37C) standard solution; Art. Nr. CL40. 13093; Lot Nr. 221.561.102.100] with respect to their arrival time.

The nutritional quality indexes of the fillet lipids

In the study, the nutritional quality indexes of fillet lipids were calculated according to the formulas below (Dagtekin et al., 2017; Yu et al., 2018; Kocatepe et al., 2019).

Index of Atherogenicity (AI) = [C12:0 + (4*C14:0) + C16:0] / (ΣMUFA + n-6 PUFA + n-3 PUFA)

Index of Thrombogenicity (TI) = (C14:0 + C16:0 + C18:0) / (0.5*ΣMUFA + 0.5*n-6 PUFA + 3*n-3 PUFA) + (n-3 PUFA/n-6 PUFA)

Flesh Lipid Quality Index (FLQ) = (C20:5 n3 + C22:6 n3 / Σtotal FA)*100

Hypocholesterolemic / hypercholesterolemic (h/H) = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0)

Statistical analysis

Anderson-Darling and Levene's tests were used for homogeneity of variances and equality of variance of groups, respectively. The effects of dietary oils on muscle fatty acid compositions and the significance of differences in growth between control and treated groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's method for multiple comparisons. Arcsine square root transformations of percentage data were conducted for homogeneity of variances before statistical analysis. Differences were considered significant when $p<0.05$. Analyses were performed using Minitab 17 software for Windows.

RESULTS

Fatty acid composition of the diets

Fatty acid (FA) composition of fillets reflected the fatty acid composition of diets, although the differences in fatty acid composition of fish in the groups was less than the differences in fatty acid composition among their respective diets. The fatty acid composition of the experimental diets was shown in Table 2.

Table 2. Fatty acid composition of the experimental diets (total fatty acids %)

	Experimental Diets				
	FO (Control)	**CANO**	**SFO**	**CO**	**PNO**
C14:0	5.67±0.04^{a}	1.28±0.00^{d}	1.50±0.02^{a}	1.30±0.02^{d}	1.42±0.00^{c}
C15:0	1.09±0.04^{a}	0.17±0.00^{b}	0.21±0.00^{b}	0.19±0.00^{b}	0.19±0.00^{b}
C16:0	13.51±0.14^{b}	12.58±0.16^{c}	12.32±0.13^{c}	10.87±0.18^{d}	14.96±0.11^{a}
C17:0	1.35±0.08^{a}	0.25±0.05^{b}	0.23±0.00^{b}	0.23±0.02^{b}	0.23±0.00^{b}
C18:0	6.27±0.02^{c}	7.29±0.07^{b}	8.35±0.09^{a}	4.65±0.01^{d}	6.36±0.06^{c}
C20:0	0.37±0.08^{a}	0.05±0.00^{b}	0.05±0.00^{b}	0.13±0.01^{b}	0.05±0.00^{b}
C22:0	1.54±0.08^{c}	4.86±0.00^{a}	1.96±0.01^{b}	1.05±0.01^{d}	0.62±0.01^{e}
C24:0	0.49±0.02^{a}	0.05±0.00^{b}	0.04±0.00^{b}	0.05±0.01^{b}	0.05±0.00^{b}
ΣSFA	**30.27±010^{a}**	**26.52±0.04^{b}**	**24.65±0.01^{c}**	**18.45±0.19^{e}**	**23.87±0.05^{d}**
C14:1	0.38±0.02^{a}	0.05±0.00^{b}	0.06±0.00^{b}	0.06±0.01^{b}	0.06±0.00^{b}
C15:1	0.19±0.01^{a}	0.03±0.00^{b}	0.03±0.00^{b}	0.03±0.00^{b}	0.03±0.00^{b}
C16:1	6.72±0.04^{a}	1.86±0.04^{c}	1.86±0.04^{c}	1.74±0.02^{d}	1.96±0.03^{b}
C17:1	0.58±0.04^{a}	0.16±0.01^{b}	0.14±0.00^{b}	0.16±0.0^{b}	0.15±0.00^{b}
C18:1 n-9c	11.81±0.53^{d}	32.92±0.30^{a}	22.13±0.02^{c}	31.84±0.56^{b}	21.50±0.14^{c}
C18:1 n-9t	3.33±0.11^{b}	2.06±0.55^{c}	1.49±0.05^{cd}	4.32±0.11^{a}	1.37±0.01^{d}
C20:1	3.05±0.06^{b}	3.09±0.00^{b}	1.73±0.04^{d}	3.40±0.04^{a}	1.93±0.01^{c}
C20:1 n-9	2.90±0.06^{a}	1.65±0.03^{c}	1.90±0.03^{b}	1.61±0.01^{c}	1.86±0.01^{b}
C24:1	1.23±0.05^{a}	0.35±0.00^{c}	0.39±0.00^{c}	0.64±0.03^{b}	0.39±0.00^{c}
ΣMUFA	**30.25±0.36^{c}**	**42.15±0.34^{b}**	**29.72±0.09^{c}**	**43.79±0.72^{a}**	**29.23±0.16^{c}**
C18:2 n-6c	11.26±0.30^{c}	20.92±0.32^{b}	37.88±0.19^{a}	22.08±1.10^{b}	37.55±0.12^{a}
C18:2 n-6t	0.51±0.02^{a}	0.08±0.01^{b}	0.06±0.00^{b}	0.08±0.01^{b}	0.06±0.00^{b}
C18:3 n-3	1.62±0.08^{b}	3.20±0.05^{a}	1.09±0.01^{d}	1.66±0.02^{b}	1.46±0.02^{c}
C18:3 n-6	4.15±0.02^{c}	3.58±0.01^{d}	3.27±0.03^{e}	10.74±0.21^{a}	4.45±0.02^{b}
C20:2	0.65±0.04^{a}	0.14±0.01^{c}	0.13±0.01^{c}	0.22±0.00^{b}	0.16±0.01^{c}
C20:3 n-6	0.44±0.02	nd	nd	nd	nd
C20:4 n-6	1.54±0.08^{a}	0.22±0.01^{b}	0.22±0.00^{b}	0.18±0.00^{b}	0.22±0.00^{b}
C20:5n-3	8.01±0.19^{a}	1.49±0.03^{b}	1.28±0.03^{bc}	1.19±0.01^{c}	1.31±0.01^{bc}
C22:2	0.18±0.01^{a}	0.15±0.00^{b}	0.13±0.00^{c}	0.09±0.01^{e}	0.10±0.00^{d}
C22:6 n-3	10.83±0.13^{a}	1.37±0.01^{b}	1.38±0.02^{b}	1.28±0.01^{b}	1.41±0.02^{b}
ΣPUFA	**39.16±0.25^{c}**	**31.11±0.30^{e}**	**45.42±0.09^{b}**	**37.49±0.87^{d}**	**46.71±0.12^{a}**
Σn-3 PUFA	**20.45±0.23^{a}**	**6.05±0.02^{b}**	**3.74±0.06^{d}**	**4.12±0.02^{c}**	**4.18±0.00^{c}**
Σn-6 PUFA	**17.88±0.43^{d}**	**24.79±0.30^{c}**	**41.43±0.16^{a}**	**33.07±0.89^{b}**	**42.28±0.11^{a}**
n-3/n-6	**1.14±0.04^{a}**	**0.24±0.00^{b}**	**0.09±0.00^{c}**	**0.12±0.00^{c}**	**0.10±0.00^{c}**
EPA/DHA	**0.74±0.01^{b}**	**1.09±001^{a}**	**0.93±0.00^{a}**	**0.93±0.00^{a}**	**0.93±0.01^{a}**

nd: not detected, FO: Fish oil, CANO: Canola oil, CO: Corn oil, SFO: Sunflower oil, PNO: Peanut oil. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, Values are means of three determinations.

Growth performance, feeding efficiency and survival

In the optimum temperature, significant differences were detected in both the growth performance and feeding efficiencies between FO and VO based diet trials (Table 3). The weight of all fish increased by approximately 30 to 43 fold and specific growth rate (SGR) varied between 2.27 and 2.69% day^{-1} while the weight gains (WG) were between 30.73 and 42.38 g. Feed conversion ratios (FCR), hepatosomatic index (HSI) were not significantly different among the groups and ranged from 1.29–1.61 and 1.39–2.08%, respectively. The survival was high among the groups fed by VO based diets (100 %), but it was 94.12±3.39% in the groups fed by FO based diet. No significant differences were detected among the trial groups in terms of vicerosomatic index (VSI) values (p>0.05).

In the upper-optimum temperature, significant differences in the survival, growth performance, FCR and other body parameters were detected among all fish after fed by FO (control) diet for four weeks (Table 3; p>0.05). At the end of the trial, fish in FO group had significant lower final body weight (FBW), weight gain (WG), FCR and SGR (43.40 g, 1.73 g, 2.05 and 0.14, respectively) compared to CANO (55.39 g, 4.52 g and 0.30), SFO (74.30 g, 23.43 g and 1.33), CO (67.12 g, 16.58 g and 1.01) and PNO groups (77.76 g, 27.09 g and 1.52). The lowest HSI value was detected in the SFO group, similarly among in CANO, CO and PNO groups, while it was 1.45 % in FO group. The VSI value was significantly higher in SFO and CO groups than the FO, CANO and PNO groups (p>0.05). The survival rate was significantly lower in the FO group, while it was 100% in the other groups (p>0.05).

Table 3. Growth performance, feed efficiency and other body parameters of rainbow trout fingerlings fed optimum and upper-optimum temperature

	Optimum Temperature (17ºC)					Upper-Optimum Temperature (19ºC)				
	FO	CANO	SFO	CO	PNO	FO	CANO	SFO	CO	PNO
IBW (g)	11.26±0.01	11.27±0.02	11.26±0.01	11.27±0.05	11.29±0.04	41.67±0.80	50.86±4.54	50.87±2.42	50.54±0.46	50.67±1.01
FBW (g)	42.17±0.59^{a}	52.70±6.68^{b}	51.12±1.66^{b}	53.65±1.68^{b}	51.88±1.59^{b}	43.40±1.69^{a}	55.39±2.95^{b}	74.30±5.94^{d}	67.12±2.67^{c}	77.76±3.23^{e}
WG (g)[1]	30.73±0.67^{a}	41.42±6.66^{b}	39.85±1.66^{b}	42.38±1.72^{b}	40.57±1.63^{b}	1.73±2.47^{a}	4.52±3.92^{b}	23.43±5.99^{d}	16.58±2.46^{c}	27.09±2.34^{e}
SGR(%)[2]	2.27±0.03^{a}	2.64±0.22^{b}	2.60±0.06^{b}	2.69±0.06^{b}	2.63±0.06^{b}	0.14±0.20^{a}	0.30±0.28^{b}	1.33±0.31^{d}	1.01±0.12^{c}	1.52±0.09^{e}
FCR[3]	1.61±0.04^{a}	1.35±0.11^{b}	1.40±0.08^{b}	1.29±0.05^{b}	1.35±0.02^{b}	2.05±0.45^{d}	1.75±0.36^{c}	1.08±0.69^{a}	1.18±0.56^{b}	1.02±0.57^{a}
HSI (%)[4]	2.08±0.08^{a}	1.47±0.01^{b}	1.39±0.08^{b}	1.42±0.01^{b}	1.56±0.02^{b}	1.45±0.05^{b}	1.55±0.11^{c}	1.14±0.07^{a}	1.54±0.17^{c}	1.51±0.09^{c}
VSI (%)[5]	12.15±1.22^{a}	12.94±0.49^{a}	12.68±1.41^{a}	13.16±0.15^{a}	12.87±0.08^{a}	11.22±0.63^{a}	11.29±0.72^{a}	13.53±0.99^{b}	13.07±0.06^{b}	11.15±0.93^{a}
Survival (%)	94.12±3.39^{a}	100^{b}	100^{b}	100^{b}	100^{b}	54.17±1.39^{a}	100^{b}	100^{b}	100^{b}	100^{b}

Data are the mean values of three replicate ± SD. Means with different superscript letter in a row are significantly different (p>0.05).
[1]Weight gain (WG, g)= Final body weight - initial body weight. [2]Specific growth rate (SGR)= [(ln final wet weight - ln initial wet weight)/days] x 100. [3]Feed conversion rate (FCR)= total feed intake/weight gain. [4]Hepatosomatic index (HSI) = (liver weight / body weight) x 100. [5]Viserosomatic index (VSI)= (viscera weight/body weight) x 100

Proximate composition of fillets

Fish fillet proximate compositions were significantly influenced by dietary groups at optimum temperature (Table 4, p<0.05). However, except for ash, moisture, crude protein and lipid were not different in the experimental groups compared to the initial samples. In addition, the moisture content in the groups fed by FO based diet was higher than in the other groups (p<0.05).

Except for crude protein, differences in moisture, ash and crude lipid contents were determined in fish fed by FO diet at upper-optimum temperature (Table 4, p>0.05). The lowest moisture and fat were detected in the SFO group. The highest protein and fat and the lowest ash were in the CO group. High moisture and low crude lipid were determined in the FO group.

Table 4. Chemical composition of fillet of rainbow trout fingerlings for different phases of the experiment

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Optimum Temperature(17ºC)				
Initial	81.65±0.44^{a}	15.81±0.18^{a}	2.66±0.01^{a}	2.64±0.09^{a}
FO(Control)	76.50±0.07^{b}	18.54±0.13^{b}	5.73±0.68^{b}	2.58±0.05^{a}
CANO	75.49±0.10^{c}	19.26±0.02^{b}	6.70±0.23^{b}	2.22±0.13^{a}
SFO	75.31±0.05^{c}	19.14±0.14^{b}	5.61±0.37^{b}	2.27±0.19^{a}
CO	74.72±0.03^{c}	18.76±0.22^{b}	5.59±0.84^{b}	2.16±0.15^{a}
PNO	75.33±0.05^{c}	18.74±0.15^{b}	5.96±0.70^{b}	2.18±0.14^{a}
Upper-Optimum Temperature (19ºC)				
FO(Control)	77.28±0.06^{d}	20.56±0.13^{a}	3.86±0.47^{a}	2.35±0.41^{b}
CANO	76.82±0.02^{c}	20.53±0.48^{a}	4.76±0.23^{bc}	3.19±0.44^{a}
SFO	74.59±0.03^{a}	20.74±0.39^{a}	4.47±0.25^{b}	2.83±0.06^{a}
CO	76.60±0.04^{c}	21.10±0.30^{a}	5.47±0.03^{c}	2.33±0.91^{b}
PNO	75.45±0.06^{b}	20.32±0.26^{a}	4.87±0.11^{bc}	2.59±0.31^{b}

Data are mean ± SD. Means with different superscript letter in a column are significantly different (p>0.05).

Fatty acid composition of the fillets

Total lipid and fatty acid profiles showed the effects of the respective dietary treatments (Table 5). The levels of SFA were significantly increased ($p<0.05$) in fish fed by VO based diets, whereas the groups fed by FO based diet had the lowest content (28.88%) at optimum temperature. Among the FA classes, fillet contents of monoenes were reduced in the groups fed by CANO, SFO and CO based diets, except groups fed by PNO based diet. Although the PNO based diet was the lowest MUFA content, the groups fed by PNO based diet had the highest fillet MUFA content. The highest LA increase was observed in the groups fed by SFO based diet having the lowest value of 20.92% ($p<0.05$). Likewise, the inclusion of vegetable oils in diet led to increased ALA content ($p<0.05$) in all groups fed by VO based diets, apart from groups fed by SFO based diet. While EPA and DHA were significantly high ($p<0.05$) in the groups fed by FO based diet in comparison to the other groups, ARA was increased in the groups fed by CO, SFO, PNO and CANO based diets, respectively. The groups fed by FO based diet had the highest ($p<0.05$) n-3 PUFA content, EPA/DHA and n-3/n-6 ratios.

LA content of the SO group decreased significantly after 4 weeks from the beginning of the upper-optimum temperature trial and reached almost the same level of the FO group at the end of the trial (Table 5). The same situation was also observed for OA content. EPA, DHA and ARA contents of fish in the growth out period were increased significantly after 4 weeks of FOFD trial. At the end of the growth-out trial period, although fish in the FO group had the highest ($p<0.05$) n-3 PUFA content, EPA/DHA and n-3/n-6 ratios, after 4 weeks of FOFD trial, n-6 PUFA ratios decreased and n-3 ratios increased. In all groups, EPA/DHA was equal.

Table 5. Fatty acid compositions of fillet of rainbow trout fingerlings fed experimental diets (% of total fatty acids) at optimum and upper-optimum temperature

	Optimum Temperature (17ºC)					Upper-Optimum Temperature (19ºC)				
	FO	**CANO**	**SFO**	**CO**	**PNO**	**FO**	**CANO**	**SFO**	**CO**	**PNO**
C14:0	4.04±0.00^{a}	2.93±0.91^{b}	1.51±0.00^{c}	1.53±0.02^{c}	1.63±0.00^{c}	3.54±0.05^{a}	2.49±0.01cd	2.42±0.00d	2.53±0.04c	2.66±0.03b
C15:0	1.20±0.00^{a}	0.74±0.38^{b}	0.27±0.00^{c}	0.27±0.01^{c}	0.30±0.01^{bc}	1.04±0.01^{a}	0.62±0.01c	0.60±0.01c	0.66±0.02b	0.68±0.02b
C16:0	12.18±0.06^{b}	12.84±0.20^{a}	10.10±0.00^{c}	12.06±0.21^{b}	11.90±0.07^{b}	13.14±0.23^{a}	11.38±0.01d	11.58±0.00cd	11.94±0.15bd	12.26±0.18b
C17:0	1.38±0.00^{a}	0.79±0.47^{b}	0.22±0.01^{c}	0.23±0.00^{bc}	0.28±0.00^{bc}	1.20±0.03a	0.68±0.01c	0.58±0.00d	0.73±0.02b	0.71±0.01bc
C18:0	6.46±0.03^{b}	7.85±0.77^{a}	7.80±0.03^{a}	7.74±0.15^{a}	7.97±0.04^{a}	7.36±0.13b	7.11±0.05c	7.78±0.02a	7.11±0.13bc	7.92±0.09a
C20:0	0.92±0.01^{c}	1.81±0.94^{bc}	3.58±0.01^{a}	3.41±0.10^{a}	2.61±0.02^{ab}	0.84±0.02e	1.75±0.01d	2.89±0.00a	2.79±0.06b	2.00±0.03c
C22:0	2.05±0.01^{c}	2.45±0.51^{bc}	3.26±0.00^{a}	3.29±0.05^{a}	2.98±0.02^{ab}	2.20±0.03d	2.94±0.02b	3.16±0.01a	3.19±0.06a	2.80±0.04c
C24:0	0.66±0.01^{a}	0.36±0.26^{ab}	0.07±0.01^{b}	0.08±0.05^{b}	0.08±0.01^{b}	0.54±0.01a	0.28±0.01c	0.26±0.01c	0.31±0.02b	0.32±0.01b
ΣSFA	**28.88±0.11^{b}**	**29.75±0.41^{a}**	**26.79±0.004^{d}**	**28.59±0.01^{b}**	**27.73±0.14^{c}**	**29.85±0.51a**	**27.23±0.09b**	**29.25±0.04a**	**29.25±0.49a**	**29.32±0.39a**
C14:1	0.42±0.01^{a}	0.24±0.15^{ab}	0.07±0.00^{b}	0.07±0.54^{b}	0.08±0.00^{b}	0.35±0.00a	0.19±0.01d	0.17±0.00e	0.20±0.00c	0.22±0.01b
C15:1	0.27±0.04^{a}	0.12±0.07^{b}	0.04±0.00^{b}	0.04±0.00^{b}	0.04±0.00^{b}	0.21±0.04a	0.12±0.02c	0.13±0.01bc	0.14±0.02bc	0.18±0.00ab
C16:1	5.58±0.02^{a}	4.29±0.96^{b}	2.31±0.00^{c}	2.49±0.00^{c}	2.74±0.00^{c}	5.15±0.10a	3.71±0.02c	3.52±0.00d	3.73±0.04c	4.02±0.06b
C17:1	0.85±0.00^{a}	0.55±0.21^{b}	0.21±0.00^{c}	0.24±0.04^{c}	0.29±0.01^{c}	0.74±0.01a	0.46±0.01c	0.41±0.00d	0.48±0.02c	0.53±0.01b
C18:1 n-9c	13.35±0.04^{c}	10.46±2.30^{d}	18.32±0.04^{b}	18.57±0.00^{b}	22.91±0.10^{a}	11.54±1.43c	17.88±0.04a	15.32±0.08b	14.25±0.20b	18.60±1.35a
C18:1n-9t	2.68±0.06^{ab}	3.23±0.61^{a}	1.85±0.01^{c}	1.84±0.32^{c}	2.25±.015^{bc}	2.61±0.08ab	2.72±0.17a	2.53±0.00ab	2.42±0.00ab	2.26±0.25b
C20:1	2.67±0.00^{abc}	3.43±0.90^{ab}	2.24±0.01^{c}	2.56±0.04^{bc}	3.75±0.00^{a}	2.48±0.03c	2.96±0.00b	2.38±0.00d	2.22±0.03e	3.24±0.04a
C20:1 n-9	2.67±0.01^{a}	2.12±0.39^{b}	1.12±0.01^{c}	1.17±0.04^{c}	1.20±0.01^{c}	2.21±0.02a	1.81±0.01b	1.65±0.01d	1.74±0.04c	1.75±0.03bc
C24:1	1.48±0.01^{c}	1.73±0.29^{c}	2.99±0.01^{ab}	3.02±0.01^{a}	2.63±0.03^{b}	1.52±0.02d	1.81±0.01c	2.67±0.00a	2.61±0.04a	2.15±0.03b
ΣMUFA	**29.96±0.02^{b}**	**26.16±2.27^{c}**	**29.14±0.02^{b}**	**29.99±0.05^{b}**	**35.89±0.20^{a}**	**26.77±1.13c**	**31.64±0.09a**	**28.75±0.06b**	**27.76±0.37bc**	**32.93±0.94a**
C18:2 n-6c	11.50±0.01^{c}	13.82±2.82^{c}	24.90±0.03^{a}	17.80±0.49^{b}	14.63±0.01^{bc}	10.70±0.17b	12.12±0.05b	15.47±0.14a	15.87±1.30a	11.89±0.23b
C18:2 n-6t	0.65±0.00^{a}	0.15±0.04^{b}	0.11±0.00^{b}	0.12±0.00^{b}	0.15±0.00^{b}	0.10±0.00e	0.31±0.01c	0.29±0.00d	0.33±0.01b	0.35±0.00a
C18:3 n-3	1.08±0.01^{bc}	1.17±0.17^{b}	0.67±0.00^{d}	0.93±0.01^{c}	1.53±0.01^{a}	0.96±0.01c	1.04±0.02b	0.83±0.00e	0.88±0.03d	1.31±0.02a
C18:3 n-6	3.88±0.01^{ab}	5.08±1.41^{a}	1.87±0.00^{c}	2.46±0.02^{bc}	2.25±0.01^{bc}	3.51±0.05b	4.27±0.00a	2.49±0.01d	2.71±0.02c	2.63±0.04c
C20:2	2.28±0.01^{c}	2.72±0.57^{bc}	3.31±0.00^{ab}	3.73±0.05^{a}	3.05±0.00^{ab}	1.84±0.02e	2.33±0.02d	3.11±0.00a	2.80±0.06b	2.51±0.03c
C20:3 n-6	1.16±0.01^{c}	2.11±1.03^{bc}	3.71±0.00^{a}	3.80±0.06^{a}	3.16±0.00^{ab}	1.02±0.02e	2.13±0.02d	2.88±0.01a	2.60±0.05b	2.32±0.03c
C20:4 n-6	2.05±0.01^{b}	2.40±0.46^{b}	3.26±0.00^{a}	3.29±0.05^{a}	2.98±0.02^{a}	2.20±0.03d	2.94±0.02b	3.16±0.01a	3.19±0.06a	2.80±0.04c
C20:5n-3	5.97±0.17^{a}	3.98±1.65^{b}	0.98±0.01^{c}	1.08±0.02^{c}	1.42±0.02^{c}	5.71±0.09a	3.91±0.01b	3.35±0.01d	3.53±0.10c	3.57±0.04c
C22:2	0.19±0.00^{a}	0.15±0.00^{b}	0.12±0.01^{c}	0.12±0.01^{c}	0.11±0.00^{c}	0.14±0.02ab	0.13±0.01b	0.15±0.00a	0.14±0.00ab	0.14±0.00ab
C22:6 n-3	11.95±0.01^{a}	12.17±3.07^{a}	4.77±0.02^{b}	5.95±0.08^{b}	6.68±0.02^{b}	16.86±0.21a	11.61±0.01b	9.89±0.02d	10.54±0.12c	9.86±0.12d
ΣPUFA	**40.70±0.13^{b}**	**43.73±1.79^{a}**	**43.68±0.05^{a}**	**39.25±0.65^{b}**	**35.94±0.04^{c}**	**43.02±0.61a**	**40.76±0.00b**	**41.61±0.11ab**	**42.56±0.86a**	**37.35±0.55c**
Σn-3PUFA	**19.00±0.17^{a}**	**17.31±5.54^{a}**	**6.41±0.01^{b}**	**7.95±0.10^{b}**	**9.62±0.02^{b}**	**23.53±0.31a**	**16.55±0.01b**	**14.07±0.03d**	**14.94±0.24c**	**15.33±0.42c**
Σn-6PUFA	**19.23±0.03^{c}**	**23.56±5.76^{bc}**	**33.84±0.04^{a}**	**27.46±0.48^{ab}**	**23.16±0.02^{bc}**	**19.23±0.03^{c}**	**23.56±5.76^{bc}**	**33.84±0.04^{a}**	**27.46±0.48^{ab}**	**23.16±0.02^{bc}**
n-3/n-6	**0.99±0.01^{a}**	**0.83±0.40^{ab}**	**0.19±0.00^{c}**	**0.29±0.00^{c}**	**0.42±0.00^{bc}**	**0.99±0.01^{a}**	**0.83±0.40^{ab}**	**0.19±0.00^{c}**	**0.29±0.00^{c}**	**0.42±0.00^{bc}**
EPA/DHA	**0.50±0.01^{a}**	**0.31±0.06^{b}**	**0.20±0.00^{c}**	**0.18±0.00^{c}**	**0.21±0.00^{c}**	**0.50±0.01^{a}**	**0.31±0.06^{b}**	**0.20±0.00^{c}**	**0.18±0.00^{c}**	**0.21±0.00^{c}**

Data are reported as mean ± standard errors of three replicate (3). Means with different superscript letter in a row are significantly different ($p>0.05$). SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid

The nutritional quality indices of the fillet lipids for different phases

At the end of the optimum temperature trial, the IA, IT, FLQ and h/H values decreased in the groups fed with vegetable oil diets compared to the control group. Differences were determined between groups ($p<0.05$). At the end of the upper-optimum temperature trial, it was determined that the nutritional quality indexes increased in the groups fed with vegetable oil diets (Table 6).

Table 6. The nutritional quality indices of the fillet lipids of rainbow trout fingerlings for different phases of the experiment

	IA	IT	FLQ	h/H
Optimum Temperature (17ºC)				
FO(Control)	0.42±0.00^{a}	0.27±0.00^{c}	18.00±0.08^{a}	2.83±0.00^{d}
CANO	0.37±0.03^{a}	0.31±0.03^{bc}	16.20±2.72^{a}	2.79±0.06^{d}
SFO	0.23±0.00^{b}	0.38±0.00^{a}	5.76±0.01^{b}	4.56±0.00^{a}
CO	0.28±0.00^{b}	0.40±0.00^{a}	7.18±0.01^{b}	3.50±0.00^{c}
PNO	0.27±0.00^{b}	0.37±0.00^{ab}	8.13±0.02^{b}	3.71±0.01^{b}
Upper-optimum Temperature (19ºC)				
FO(Control)	0.40±0.00^{a}	0.26±0.00^{c}	22.65±0.17^{a}	2.88±0.06^{c}
CANO	0.31±0.00^{d}	0.27±0.00^{b}	15.57±0.02^{b}	3.57±0.00^{a}
SFO	0.32±0.00^{cd}	0.31±0.00^{a}	13.29±0.02^{d}	3.43±0.00^{ab}
CO	0.33±0.00^{bc}	0.30±0.00^{a}	14.13±0.13^{c}	3.34±0.06^{b}
PNO	0.34±0.01^{b}	0.31±0.00^{a}	13.48±0.09^{d}	3.22±0.06^{b}

Data are mean ±SD. Means with different superscript letter in a column are significantly different ($p>0.05$)

DISCUSSION

Fish oil has been widely used as the main lipid source of the n-3 polyunsaturated fatty acids (n-3 PUFA) in farmed fish feed. Fish oil production has decreased in recent years due to the decrease in fish stocks. These increases feed costs considerably. Thus, the current trend is finding suitable alternatives to fish oils in aquafeeds, without compromising overall growth performance and final nutritional quality of farmed fish. In this case, the best alternative could be vegetable oil. However, the alternative vegetable oil is poor sources for n-3 HUFAs. One way to improve the nutritional quality of farmed fish fed by alternative vegetable oil may be to use finishing feeds containing fish oil before harvesting. Firstly, the aim of this study was to determine the effect of feeding with vegetable oil-based diets for 9 weeks at optimum temperature on the growth, feed utilization and fatty acid profile of rainbow trout. Secondly, by continuing the study for another 4 weeks, all groups at upper-optimum temperature were re-fed with only fish oil-based diet (control), and at the end of this period, it was aimed to determine the gradual recovery of fatty acid profiles and nutritional quality of rainbow trout and especially its effect on survival rate.

A number of earlier studies have utilized vegetable oil in feed formulations for salmonids at a replacement level of up to 100%, and no negative effect in growth rates and feed conversion have been observed (Torstensen et al. 2000; Caballero et al. 2002; Fonseca-Madrigal et al., 2005; Yildiz et al. 2015; Yıldız et al. 2018; Yu et al. 2018). Similar results were determined in the present study. However, after feding the fish by VO based diets in the growth-out period, fish weights in groups fed by FO based diet was less than the other experimental groups, despite final fish weights of the all trial groups increased in 9 weeks at the top optimal temperature. In parallel with this situation, the lowest WG, FCR, SGR, survival and highest HSI were detected in the groups fed by FO based diet. Similar results were also reported by Babalola and Apata (2012). In their study using different vegetable oil (soybean, palm, coconut, groundnut and melon seed oil) in *Heterobranchus longifilis* fingerling diets, although survival, feed intake and HSI were similar for all experimental groups, WG, SGR and FCR of fish fed by soybean oil based diet decreased significantly. As a result, it was reported that the usage of different vegetable oils in the diets were created no negative effect on ther growth composition of *Heterobranchus longifilis* fingerlings. VSI values of the present study were equal for both groups fed by FO and VO based diets in the growth-out period. Yildiz et al. (2015) reported that the VSI value of rainbow trout fed by diet including sunflower oil was lower than the those of fish fed by diets including other vegetable oil (sesame and linseed oil).

In the optimum temperature trial, while the survival rate decreased in the groups fed by FO based diet, it was not changed in the other groups. After previously fed by VO based diets, HSI, VSI and growth performance of fish fed by FO diet for four weeks at upper-optimum temperature were different among the all trial. The decrease in the survival rate determined in the optimum temperature trial continued in the upper-optimum temperature trial. While the survival rates of all groups (CANO, SFO, CO, PNO groups) in the upper-optimum temperature trial were 100%, this rate decreased to 54.17±1.39% in the groups fed by FO based diet. In the present study, feeding with vegetable oil diets at top and upper optimal temperatures was most effective at the survival rate. This case could be originated from the antioxidant property of the vegetable oils. It was proved that vitamin E, one of the strongest natural antioxidants (Altıner et al., 2017), has an immune-enhancing effect compared to a synthetic antioxidant (McDowell et al. 1996). Canola, sunflower, corn and peanut oils used in the present study are also rich in vitamin E (İşler, 2018). Also, It was reported that the presence of low vitamin E in the fish diets decreased the resistance to stress of the fish under acute and chronic stress and increased mortality (Montero et al., 2001).

After the optimum temperature trial period, fatty acid profiles of diets containing VO were reflected the FA profiles of fish fillet, except for groups fed by CANO based diet which was characterized by high LA and OA and low n-3 PUFA

contents. In the present study, OA, LA and n-3 PUFA contents in the groups fed by CANO based diet were almost same as those in the groups fed by FO based diet. Several studies showed that salmonids respond differently to distinct VO, making it difficult to make direct comparison among studies. Bell et al., (2003) reported that fillet fatty acid profile showed significant increases in OA and LA as well as ALA with the addition of rapeseed oil in the Atlantic salmon (*Salmo salar*) diets. Bell et al. (2001) reported that the inclusion of RO in levels exceeding of 50% may result in significant reductions in (n-3)/(n-6) PUFA ratio and the EPA and DHA concentrations, but, this does not prevent higher rapeseed oil levels from being used in dietary formulations for Atlantic salmon, such diets could be brought back to "normal" values of 18: 2 (n-6), EPA and DHA concentrations by feeding with fish oil diets at a convenient time before marketing. Yildiz et al. (2015) reported that 100% replacement of fish oil with vegetable oil (sunflower, sesame and linseed oil) resulted in decrease of total n-3 PUFA levels and increased levels of n-6 PUFA in both fillet and liver, except for linseed oil groups in rainbow trout. They also indicated increased levels of n-6 and LA with inclusion of sunflower oil whereas ALA increased with inclusion of linseed oil. The reduced accumulation of dietary monoenes and LA coupled with good growth, survival and feed utilization in their study were capable of meeting essential fatty acid (EFA) requirements of rainbow trout by the use of monoenes and LA. This stiuation was also reported that residual fish oil in the fishmeal in the diets containing VO could be enough to provide essential HUFAs for normal growth and development of rainbow trout (Yıldız et al., 2018).

In the present study, DHA and ARA concentrations in fish fillet were higher than the dietary concentrations. This case was also detected by Caballero et al. (2002) for rainbow trout. The simlar results were also reported for DHA levels in European sea bass (Montero et al., 2005; Mourente and Bell, 2006), gilthead sea bream (*Sparus aurata* L.) (Menoya et al., 2004) and turbot (*Psetta maxima*) (Regost et al., 2003). Specific fatty acids were selectively retained in the fish fillet; especially DHA and ARA concentrations were higher than the dietary concentrations (Fountoulaki et al., 2009).

At the end of the upper-optimum temperature trial, the fatty acid composition of groups fed by CANO based diet was closer to the the fatty acid composition of the groups fed by FO based diet. The fatty acid composition of the other groups increased almost to the doubles. Similar results declared by Yıldız et al. (2018) and Codabaccus et al. (2013) for rainbow trout and by Ng et al.,o (2013) for red hybrid tilapia (*Oreochromis sp.*), Fountoulaki et al. (2009) for gilthead sea bream and Bell et al. (2003) for Atlantic salmon.

Total substitution of dietary FO with vegetable oils (CANO, SFO, CO and PNO) caused a decline in the fillet PUFAs (EPA and DHA) in the present study. This is compatibles previous studies (Caballero et al., 2002; Bell et al., 2004; Thanuthong et al., 2011; Codabaccus et al., 2013; Yıldız et al., 2018). At the end of the growth-out trial, DHA and EPA were significantly lower. PUFA retention in the present study followed the pattern; DHA>EPA. DHA showed higher retention than EPA in fish fed by VO based diets. At the end of the trial, an increase in DHA and EPA in the fillets was detected in all groups. The present study showed that 4 weeks at upper-optimum temperature for the restoration of ARA, EPA and DHA was sufficient, especially in the groups fed by CANO based diet. Returning fish previously fed by diet including 100% vegetable oil to fish oil diet for a period before harvest allowed restoration of flesh (n-3) HUFA concentrations up to 80% comparing with fish fed by FO throughout the seawater phase, although 18:2(n-6) remained significantly higher (Sonu, 2018). It was declared that the fatty acid composition of the fish fillets was restored applying a FOFD strategy for 16 weeks after feeding with vegetable oil diets (canola and linseed oils) for 25 weeks in murray cod (*Maccullochella peelii peelii*, Mitchell) (Turchini at al., 2006). However, studies with marine fish emphasized that ARA and DHA but not EPA could be restored by longer FOFD trials (trials ≥ 5 months) (Yıldız et al., 2018). It was reported that EPA levels were not restored even after 104 days of FOFD application in gilthead sea bream (Izquierdo et al., 2005), 150 days of FOFD application in European sea bass (Montero et al., 2005), 56 days of FOFD application in turbot (Regost et al., 2003). However, Fountoulaki et al. (2009) concluded that re-feeding gilthead sea bream previously fed by VO based diets with a FOFD for 120 days was not adequate for restoration of DHA, ARA and EPA. This stuation suggests that rainbow trout has the ability to convert fatty acids more quickly.

Several indicators have been described to determine the nutritional quality of food lipids in human consumption. In the present study, to identify the nutritional quality of fillet lipids, the IA, IT, FLQ and h/H levels were measured in fillets. In accordance with nutritional recommendations, it was reported that lipids with lower indices of atherogenicity (IA) and thrombogenicity (IT) can hinder the aggregation of platelets and reduce the levels of esterified FA, cholesterol and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases (Turan et al., 2007). That's why IA and IT indexes should be low to prevent cardiovascular diseases related to lipid intake (Ulbricht and Southgate, 1991). However, the high value of h/H ratio represents high-quality lipids. In this study, IA and IT indices were measured to be lower than 1.0 but high in h/H values in all groups. Ouraji et al. (2009) and Stancheva et al. (2014) determined that higher values of IA and IT (>1.0) are detrimental to human health. Monge-Ortiz et al. (2018) reported that lower levels of IA and IT values are beneficial to human health. Flesh Lipid Quality Indice (FLQ) indicates the global dietetic quality of lipids and their potential effects on the development of coronary disease (Senso et al., 2007). Lipid quality of the flesh is directly related to EPA and DHA ratios (Dagtekin et al., 2017). A relatively high FLQ value was obtained in this study. It was reported that omega-3 fatty acids, which are beneficial for both healthy and people suffering from

cardiovascular diseases, are lower the risk of arrhythmias, which can lead to sudden death (URL 2, 2016). Therefore, Yu et al. (2018) reported that consuming both the IA and IT indices together with high FLQ with low efficacy is an indication that it may help prevent the development of coronary heart disease, which is more favorable in terms of lipid quality for human consumption. In this context, it can be said that the European seabass obtained after the present study have a good nutritional quality for human consumption.

As a result, this study was conducted to investigate the growth effectiveness of rainbow trout fed by VO based diets at optimum temperature and then fed by FO diet at upper-optimum temperature. Optimum temperature trial results of current study suggest that it is possible to replace FO totally with VO in rainbow trout diets without any significant effect on growth and feed utilization. FA profile of fish fed by VO based diets reflected dietary FA composition characterized by reduced contents of ARA, EPA and DHA. Feeding by diets including different VO during nine weeks of optimum temperature period followed by a FO diet for four weeks at upper-optimum temperature was sufficient for a large restoration % values of EPA and DHA in rainbow trout fillet. Although a large restoration of EPA and DHA was achieved for fish previously fed by VO based diets at both optimum and upper-optimum temperatures, the n-3/n-6 ratios were not fully restored in the all groups of upper-optimum temperature trial. In spite of this, implementing such a feeding strategy to rainbow trout culture will probably offer the best balance between feed cost-effectiveness and fillet quality.

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REFERENCES

Altıner, A., Atalay, H. & Bilal, T. (2017). Bir antioksidan olarak E vitamini. *Balıkesir Sağlık Bilimleri Dergisi*, 6(3),149-157. DOI: 10.5505/bsbd.2017.47450

AOAC, (1995). *Animal Feed*. W. Horwitz (Ed.). Official Methods of Analysis of the Association of Analytical Chemists, 13th Edition 7, 125. USA

Babalola, T.O. & Apata, D.F. (2012). Effects of dietary lipid source on growth, digestibility and tissue fatty acid composition of *Heterobranchus longifilis* fingerlings. *Journal of Agriculture and Rural Development in the Tropics and Subtropics,* 113(1), 1–11

Bayaz, M. (2014). Esansiyel yağlar: Antimikrobiyal, antioksidan ve antimutajenik aktiviteleri. *Akademik Gıda*, 12(3), 45-53

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar)* affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr.,* 131, 1535-1543. DOI: 10.1093/jn/131.5.1535

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R. & Cramptom, V.O. (2003). Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J Nutr.,* 133, 2793–2801. DOI: 10.1093/jn/133.9.2793

Bell, J.G., Henderson, R.J., Tocher, D.R. & Sargent, J.R. (2004). Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids,* 39(3), 223–232. DOI: 10.1007/s11745-004-1223-5

Caballero, M.J., Obach, A., Roselund, G., Montero, D., Gisvold, M. & Izquierdo, M.S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 214, 253–271. DOI: 10.1016/S0044-8486(01)00852-3

Codabaccus, M.B., Ng, W.-K., Nichols, P.D. & Carter, C.G. (2013). Restoration of EPA and DHA in rainbow trout (*Oncorhynchus mykiss*) using a finishing fish oil diet at two different water temperatures. *Food Chemistry,* 141, 236-244. DOI: 10.1016/j.foodchem.2013.02.032

Dagtekin, B.B.G., Misir, G.B., Kutlu, S. & Basturk, O. (2017). Comparison of biochemical, fatty acids and lipid quality indexes of Prussian carp (*Carassius gibelio*) caught from Lake Çıldır on different seasons. *Mediterranean Fisheries and Aquaculture Research*, 1(1), 2-14.

Fonseca-Madrigal, J., Karalazos, V., Campbell, P.J., Bell, J.G. & Tocher, D.R. (2005). Influence of dietary palm oil on growth, tissue fatty acid composition, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.,* 11, 241-250. DOI: 10.1111/j.1365-2095.2005.00346.x

Gabor, E.F., Ichim, O. & Şuteu, M. (2012). Phyto-additives in rainbow trout (*Oncorhynchus mykiss*) nutrition. *Biharean Biologist*, 6(2), 134-139

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B. & Alexis, M.N. (2009). Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile: Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture*, 289, 317-326. DOI: 10.1016/j.aquaculture.2009.01.023

Hanson, S.W.F. & Olley, J. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochem J.,* 89, 101-102

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G. & Ginés, R. (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture*, 250, 431–444. DOI: 10.1016/j.aquaculture.2004.12.001

İşler, N. (2018). *Kolza (Kanola) tarımı*. M.K.Ü. Ziraat Fakültesi Tarla Bitkileri Bölümü. http://www.mku.edu.tr/files/898-0e9fe1a8-1d54-4c38-8ac4-cdbdccae03ad.pdf

Kocatepe, D., Erdem, M.E., Keskin, I., Köstekli, B. & Kaya Y. (2019). Differences on lipid quality index and amino acid profiles of European Anchovy caught from different area in Turkey. *Ukrainian Journal of Food Science,* 7(1), *6-15.* DOI: 10.24263/2310-1008-2019-7-13

McDowell, L.R., Williams, S.N., Hidiroglou, N., Njeru, C.A., Hill, G.M., Ochoa, L. & Wilkinson, N.S. (1996). Vitamin E supplementation for the ruminant. *Animal Feed Science Technology*, 60, 273-296. DOI: 10.1016/0377-8401(96)00982-0

Menoyo, D., Izquierdo, M.S., Robaina, L., Cinés, R., Lopez-Bote, C.J. & Bautista, J.M. (2004). Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *Br. J. Nutr.,* 92, 41–52. DOI: 10.1079/BJN20041165

Monge-Ortiz, R., Tomas-Vidal, A., Rodriguez-Barreto, D., Martínez-Llorens, S., Perez, J., Jover Cerda, M., Lorenzo, A. (2018). Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles: effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. Aquaculture Nutrition, 24(1),605-615. DOI: 10.1111/anu.12595

Montero, D., Tort, L., Robaina, L.,Vergara, J.M. & Izquierdo, M.S. (2001). Low vitamin E in diet reduces stress resistance of gilthead seabream (*Sparus aurata*) juveniles. *Fish and Shellfish Immunology*, 11, 473-490. DOI: 10.1006/fsim.2000.0324

Montero, D., Robaina, L., Caballero, M.J., Gines, R. & Izquierdo, M.S. (2005). Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time course study on the effect of a re-feeding period with 100% fish oil diet. *Aquaculture*, 248, 121–134. DOI: 10.1016/j.aquaculture.2005.03.003

Mourente, G. & Bell, J.G. (2006). Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European seabass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comparative Biochemistry and Physiology Part B,* 145, 389-399. DOI: 10.1016/j.cbpb.2006.08.012

Ng, W-K., Chong, C-Y., Wang, Y. & Romano, N. (2013). Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. *Aquaculture*, 372-375, 97-110. DOI: 10.1016/j.aquaculture.2012.10.030

Ouraji H., Shabanpur B., Abediankenari A., Shabani A., Nezami A., Sudagar M. & Faghani S. (2009). Total lipid. fatty acid composition and lipid oxidation of Indian White shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. *Journal of the Science of Food and Agriculture*, 89(6), 993–997. DOI: 10.1002/jsfa.3545

Regost, C., Arzel, J., Robin, J., Roselund, G. & Kaushik, S.J. (2003). Total replacement of fish oil by soybean or oil with return to fish oil in turbot (*Psetta maxima*) 1. Growth performance, flesh fatty acid profile and lipid metabolism. *Aquaculture*, 217, 465–482. DOI: 10.1016/S0044-8486(02)00259-4

Sahan, A., Eroldogan, O.T., Kurutas, E.B., Yilmaz, H.A. & Demirkale, I. (2016). The effects of cottonseed oil as alternative to fish oil on oxidative stress biomarkers and some hematological parameters in European sea bass (*Dicentrarchus labrax* L.). *Fresenius Environmental Bulletin,* 25(7), 2639-2645

Senso L., Suarrez M.D., Ruiz-Cara T. & Garcira-Gallego M. (2007). On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*), *Food Chemistry*, 101, 298-307. DOI: 10.1016/j.foodchem.2006.01.036

Sonu, B. (2018). Fish-oil finishing diets: A strategy to recover long-chain polyunsaturated fatty acids in fish previously fed alternative dietary lipids. *International Journal of Life Sciences,* 6(2), 466-472

Stancheva M., Merdzhanova A., Dobreva D.A. & Makedonski L. (2014). Common carp (*Cyprinus carpio*) and European catfish (*Silurus glanis*) from Danube River as sources of fat soluble vitamins and fatty acids, *Czech Journal of Food Sciences*, 32, 16–24. DOI: 10.17221/31/2013-CJFS

Şahan, A., Yilmaz, H.A. & Eroldogan, O.T. (2017). Determination of hematological status as health indicator of European sea bass (*Dicentrarchus labrax* L.) fed different dietary fatty acids. *Turkish Journal of Agriculture - Food Science and Technology,* 5(4), 445-450. DOI: 10.24925/turjaf.v5i4.445-1106

Thanuthong, T., Francis, D.S., Senadheera, S.P.S.D., Jones, P.L. & Turchini, G.M. (2011). Fish oil replacement in rainbow trout diets and total dietary PUFA content: I) effects on feed efficiency, fat deposition and the efficiency of a finishing strategy. *Aquaculture*, 320(1), 82–90. DOI :10.1016/j.aquaculture.2011.08.007

Torstensen, B.E., Li, Ø. & Frøyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)-effects of capelin-, palm- and oleic acid enriched sunflower oil as dietary lipid sources. *Lipids,* 35, 653–664. DOI: 10.1007/s11745-000-0570-6

Turan, H., Sönmez, G. & Kaya, Y. (2007). Fatty acid profile and proximate composition of the thornback ray (*Raja clavata*, L. 1758) from the Sinop coast in the Black Sea. *Journal of Fisheries Sciences,* 1(2), 97–103. DOI: 10.3153/jfscom.2007012

Turchini, G.M., Francis, D.S. & De Silva, S.S. (2006). Modification of tissue fatty acid composition in Murray cod (*Maccullochella peelii peelii*, Mitchell) resulting froma shift from vegetable oil diets to a fish oil diet. *Aquaculture Research*, 37, 570-585. DOI: 10.1111/j.1365-2109.2006.01465.x

Ulbricht, T. & Southgate, D. (1991). Coronary heart disease: seven dietary factors, *The Lancet,* 338, 985–992. DOI: 10.1016/0140-6736(91)91846-M

URL2, (2016). HealthyLiving, HealthyEating, Healthy Diet Goals, Fish and Omega-3 Fatty Acids. Retrivied from http://www.heart.org/HEARTORG/HealthyLiving/HealthyEating/HealthyDietGoals/Fish-and-Omega-3-FattyAcids_UCM_303248_Article.jsp#. jtw499l-M8, In: Dagtekin, B.B.G., Misir, G.B., Kutlu, S. & Basturk, O., (2017). *Mediterranean Fisheries and Aquaculture Research,* 1(1), 2-14.

Yildiz, M., Köse, I., Issa, G. & Kahraman, T. (2015). Effect of different plant oils on growth performance, fatty acid composition and flesh quality of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, 46, 2885–2896. DOI: 10.1111/are.12441

Yıldız, M., Eroldoğan, T.O., Ofori-Mensah, S., Engin, K. & Baltacı, M.A. (2018). The effects of fish oil replacement by vegetable oils on growth performance and fatty acid profile of rainbow trout: Re-feeding with fish oil finishing diet improved the fatty acid composition. *Aquaculture*, 488, 123-133. DOI: 10.1016/j.aquaculture.2017.12.030

Yu, J., Chang, J., Niu, H., Hu, Z. & Han, Y. (2018). Effect of variation in the dietary ratio of linseed oil to fish oil on growth, body composition, tissues fatty acid composition, flesh nutritional value and immune indices in Manchurian trout, *Brachymystax lenok. Aquaculture Nutrition*, 1-11. DOI: 10.1111/anu.12863

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Expression of cytochrome P450 aromatase isoforms in female *Alburnus tarichi* (Guldenstaedtii, 1814)

Dişi Van balığı (*Alburnus tarichi*, Guldenstaedtii, 1814)'nda sitokrom P450 aromataz izoformlarının ekspresyonu

Guler Unal[1*] ● Emily Marquez[2] ● Mara H. O'brien[3] ● Pericles Stavropoulos[4] ● Ian P. Callard[5]

[1] Adnan Menderes University, Faculty of Health Sciences, Aydın, Turkey
[2] Boston University, Faculty of Science, Department of Biology, Boston, USA
[3] Boston University, Faculty of Science, Department of Biology, Boston, USA
[4] Missouri University, Department of Science and Technology, Missouri, USA.
[5] Boston University, Faculty of Science, Department of Biology, Boston, USA

https://orcid.org/0000-0003-1035-4809
https://orcid.org/0000-0003-1391-4664
https://orcid.org/0000-0002-5646-2541
https://orcid.org/0000-0003-0985-6203

*Corresponding author: histoloji35@gmail.com

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Abstract: In this study, we aimed to clone brain-derived *Cyp19b* and ovary-derived Cyp19a the P450 aromatase gene isoforms and to indicate the expression levels of these genes in the hypothalamus and ovary tissues from reproductively arrested ovarian development (RA) and non-arrested ovarian development (RN) *Alburnus tarichi* from Lake Van, Turkey. The expression levels of *Cyp19b* and *Cyp19a* genes were predominant in the hypothalamus and ovary, respectively. The level of *Cyp19b* mRNA was significantly lower in the hypothalamus and ovary from RA fish than in the hypothalamus and ovary from RN fish (*P<0.05*). The expression level of *Cyp19a* was significantly lower in the ovary of RA fish (*P<0.05*) than RN fish while no difference was found in the hypothalamus of both RA and RN fish (*P<0.05*). According to these findings, we suggest that the RA fish represent a segment of the population and these fish may be more sensitive to endocrine disruption compound/s than others.

Keywords: P450 aromatase, gene expression, *Alburnus tarichi,* Lake Van

Öz: Bu çalışmanın amacı, Van Gölü'nden yakalanan dişi *Alburnus tarichi*'de P450 aromataz izoformları olan beyin kaynaklı *Cyp19b* ve ovaryum kaynaklı *Cyp19a* genlerini klonlanmak ve üreme bakımından ovaryum gelişimi engellenmiş (RA) ve ovaryum gelişimi normal olan (RN) balıklardan alınan hypothalamus ve ovaryum örneklerinde bu genlerin ekspresyon seviyelerini belirlemektir. Cyp19b gen ekspersyonu beyinde, Cyp19a gen ekspresyonu ise ovaryumda daha fazlaydı. Cyp19b mRNA seviyesi, RA balıkların ovaryum ve hipotalamusunda, RN balıkların ovaryum ve hipotalamusundan belirgin olarak düşüktü (*P<0,05*). CYP19a ekspresyon seviyesi, hem RN ve hem de RA balıkların hipotalamusunda fark bulunmazken RA balıkların ovaryumunda, RN balıkların ovaryumundan belirgin olarak düşüktü (*P<0,05*). Bu sonuçlara bize, RA balıkların populasyonun bir parçası olduğunu ve bu balıkların endokrin bozucu bileşik veya bileşiklere diğer balıklardan daha çok duyarlı olabileceğini düşündürmektedir.

Anahtar kelimeler: P450 aromatase, gen ekspresyonu, *Alburnus tarichi,* Van Gölü

INTRODUCTION

Increasing P450 aromatase is a catalyze enzyme which converts testosterone to estrogens. In most vertebrates, it is encoded on a single *Cyp19* gene, two structurally and functionally different *Cyp19* genes are found in many teleost, (Kishida and Callard, 2001; Chang et al., (2005); Greytak et al., 2005; Barney et al., 2008; Lange et al., 2008). These genes are expressed mainly in the ovary and brain and they encode *Cyp19a/Cyp191a* and *Cyp19b/Cyp191a2*, respectively. The *Cyp19b* gene is expressed earlier than *Cyp19a* during early embryonic development and has an important role in gonadal sex differentiation (Callard et al., 2001; Chiang et al., 2001; Barney et al., 2001). However, in adult fish, brain aromatase activity is high, approximately 100- to 1000-fold greater than that detected in the similar brain regions of mammals (Pasmanik and Callard, 1985).

The roles of *Cyp19a* and *Cyp19b* genes in developmental programming and estrogen regulation are different (Kishida and Callard, 2001; Tchoudakova et al., 2001). In unfertilized zebrafish eggs, aromatase isoforms are derived maternally but *Cyp19b* expression starts 5hpf much earlier than *Cyp19a* (48 hpf) post-fertilization. In addition, the expression of *Cyp19b* but not *Cyp19a* mRNA is up-regulated by 17β-estradiol (E_2) (Sawyer et al., 2006). *Cyp1919b* is a potential target of endocrine disrupting chemicals EDCs). In some studies, *Cyp19b* mRNA is strongly up-regulated by E_2 and E_2 mimics EDCs such as nonylphenol(NP) and 17alpha-ethinyl estradiol (EE_2) whereas *Cyp19a* was largely unaffected (Kazeto et al., 2003; Fenske and Segner, 2004; Cheshenko et al., 2006). Similarly, the levels *Cyp19a* mRNA in ovary, testis and brain did not change after *in vitro* treatment with E_2, testosterone and 17,20β,21-trihydroxy-4-pregnen-3-one for six hours (Nunez and Applebaum, 2006). The *A. tarichi*, the vitellogenesis starts about in October and continues to March (Unal et al., 1999). The sampling fish from Van Edremit Region (VER) of Lake Van

(Figure 1) have been previously described as reproductively non-arrested (RN) and reproductively arrested (RA) fish (Ünal et al., 2007). In that study, RA fish have the following characteristics assessed at collection and sacrifice: lower gonadosomatic index, lower plasma E_2 levels, and reduced ovaries with oocytes that are developmentally blocked before the vitellogenic stage. We previously indicated di-(2-ethlhexyl) phthalate (DEHP) in the sediment from VER of Lake Van (Unal et al., 2014). At the same study, low estrogen receptor alfa (ERα) and vitellogenin mRNA levels were measured in the liver of RA fish. DEHP is widely used as a plasticizer in flexible vinyl products. Plastics may contain from 1 to 40% DEHP by weight and are used in many consumer products. DEHP is the most comment pollutant chemical of our general environment, and it have a potential to accumulate in soil, sediment, underground water and also air because of its low soluble and vaporization abilities (EPA, 2001). Domestic and industrial wastewater treatment plants (Marttinen et al., 2003) are the major source of DEHP contaminant to fresh water such as river and lake. There is also a small factory between university campus and waste treatment plant, in Van (Figure.1).

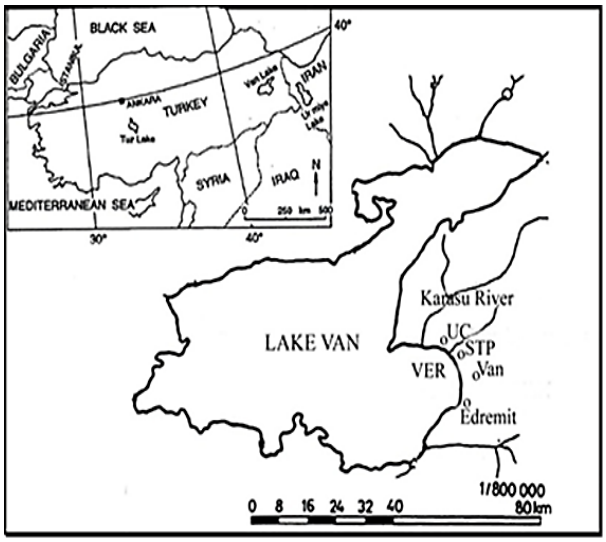

Figure 1. Fish sampling sites at lake Van. Ver, Van Edremit region; stp, sewage treatment plant; uc, university campus

The purpose of this study was to determine the cDNA sequences of P450 aromatese subtypes (*Cyp19b* and *Cyp19a*), and to measure the mRNA expressions in the hypothalamus and ovary of RN (vitellogenic) and RA female *A. tarichi* from VER in Lake Van, a site with known contamination by household waste and potentially contaminated by other sources of pollution. *A. tarichi* is a cyprinid fish endemic to Lake Van basin, in Turkey, and it has been an economical important for this region.

MATERIALS AND METHODS

Fish collection and tissue sampling

RN female *A. tarichi* were caught by netting from VER of Lake Van, Turkey (Figure 1) in April 2010 and 2013. They were transferred to the laboratory in aired water tank and they killed by decapitation. After opened the abdomen of fish, RA and RN fish were separated according to ovaries and testis structure (Ünal et al., 2007). The ovaries, liver and brain were removed after dissection of 4-5 years old fish (about 20-21 cm fork length). For this 10 RN and 10 RA female fish were used. Tissues were treated with RNA-later (Sigma), frozen at -80°C and shipped to Prof.Dr. Ian P. Callard's laboratory on dry ice. They were stored at -80ºC until RNA extraction.

RNA extraction, cloning and gene expression analysis

Total RNA was extracted from frozen ovary and hypothalamic tissues using Trizol (Sigma Aldrich St. Lous, MO) following the manufacturer's instructions. The hypothalamus was removed with the brain and trimmed from the sides and the area containing the hypothalamus was used for RNA extraction. RNAs were run on a 1% agarose gel to assess quality, and total RNA quantified using a Nanodrop (Thermo Fisher Scientific). 5 µg of total RNA from each tissue was reverse transcribed using SuperScript II transcriptase and oligo $(dT)_{20}$ primer according to the manufacturer's instructions (Invitrogen). PCR was performed in a 50 µl final volume using Sigma Readymix (Sigma). PCR conditions and primers used to clone partial cDNAs of CYP19B and CYP19A were taken from Tchoudakova and Callard (1998) and Lange et al. (2008), respectively (Table 1). The amplification products were excised from the gel, and extracted using the MinElute gel extraction kit (Qiagen). The PCR fragment were ligated into pGEM T-easy plasmid (Promega) and transformed into competent *E. coli* cells (Bioline). After amplification, the DNA fragments were extracted using the Wizard Miniprep kit (Invitrogen) and sequenced (MWG/Eurofins Operon, Huntsville, AL, USA).

The real-time quantitative PCR (qPCR) primers were designed using PrimerExpress 2.0 (Applied Biosystems, Foster City, CA, USA) from deduced cDNA sequences (Table 1). An amplification efficiency value was obtained for each primer by using serial dilutions of cDNA of each tissue. The reverse transcribed mRNA was measured by qPCR using target-specific assays. qPCR was performed on an ABI Prism 7900HT sequence detection system (Applied Biosystems) with SYBR green fluorescent label. *β-actin*, the reference gene, also was cloned from hypothalamus, ovary and liver tissues of RN fish and measured the expression level of its. It was used as an internal control to normalize mRNA expression values.

Table 1. Primers for CYP19B and CYP19B Cloning and Quantitative PCR in *A. tarichi*. Primer direction is noted. F, forward; R, reverse; qPCR, quantitative PCR. *β-actin* was used to normalize the qPCR data from the *A. tarichi*

Primer No	Oligo/ direction	Sequence (5' to 3')	Position	Reference
CYP19B				
1	CYP19B-F	AGGTWCCAKCCNGTBGTSGACTTC	1173-1197	Tchoudakova and Callard (1998)
2	CYP19B-R	CACCATNGCDATRWRYTTNCC	1395-1416	Tchoudakova and Callard (1998)
3	CYP19B-F	RGTBTGGATCWVYGGAGARGA	338-359	Tchoudakova and Callard (1998)
4	CYP19B-R	GTAACGACTGGGAACGCTGT	167-186	From *Alburnus tarichi*
5	qCYP19B-F	GCACAAGTCCGAGTTCTTCA	943-962	
6	qCYP19B-R	CCGAACGGCTGGAAGTAA	1015-1032	
CYP19A				
1	CYP19A-F	GGNYTNCARTGYATHGGNATG		Lange et al., (2008)
2	CYP19A-R	GTRTCNGGNGCNGCDAT		Lange et al., (2008)
3	qCYP19A-F	CTGCACAAGAAGCACAAGAGAGA	358-378	
4	qCYP19A-R	TCGAGTTTTTCTGCATGTGTCA	458-479	

Data analysis

Data deduced by qPCR were first analyzed using the Applied Biosystems Sequence Detection System 2.2.1. Analyses were conducted with qGene to normalize the data obtained (Simon, 2003). Relative quantification was performed by a modified comparative critical threshold method that corrects for different PCR amplification efficiencies among primer pairs (Simon, 2003). Normalized gene expression is given as mean normalized expression (MNE)= ($E_{PPI}^{meanCTPP1}$)/$E_{ERα}^{meanCTERα}$) where E=PCR efficiency ($E=10^{-(1/slope)}$) and mean threshold cycle (CT) is the average CT from the three replicates (Pfaffl, 2001). Data were rejected if the %SEM was greater than 20%. The average MNE was determined for each set of replicates obtained from an individual animal, and standard deviation calculated for each SE of MNE. The MNEs were then averaged for each group analyzed. Statistical analyses were performed using the PROG GLM in the SAS 9.3 package. Student's t tests were used to compare expression of *Cyp19b* and *Cyp19a* genes. Significance was set at $P<0.05$.

RESULTS

We isolated a partial of *Cyp19b* and *Cyp19a* cDNA from vitellogenic *A. tarichi* hypothalamus and ovary, respectively. For the isolation of *Cyp19b* and *Cyp19a* genes, they were amplified by PCR using degenerate and designed primers (Table 1). Isolated sequences of *Cyp19b* (**GenBank accession no. JF2975565.1**) and *Cyp19a*genes (**GenBank Accession no. JF297564.1**) were deposited in GenBank. Expression of *Cyp19b* and *Cyp19a* genes was observed in the hypothalamus and ovary of fish (Figure 2). The expression of *Cyp19b* and *Cyp19a* was dominant in the hypothalamus and ovary, respectively. The level of Cyp19b mRNA in the hypothalamus was measured 992.3-fold higher than ovary while Cyp19a mRNA in the ovary was 14.3-fold higher than hypothalamus.

The *Cyp19b* and *Cyp19a* expression levels were measured in the hypothalamus and ovary of both RN and RA *A. tarichi*. The levels of *Cyp19b* mRNA were significantly lower in the hypothalamus (Figure 3A) and ovary (Figure 3B) of RA fish ($P<0.05$). The expression of *Cyp19a* was significantly lower in the ovary of RA fish than RN fish ($P<0.05$) while no significant difference was apparent in *Cyp19a* expression in the hypothalamus of RN and RA fish ($P>0.05$; Figure 4).

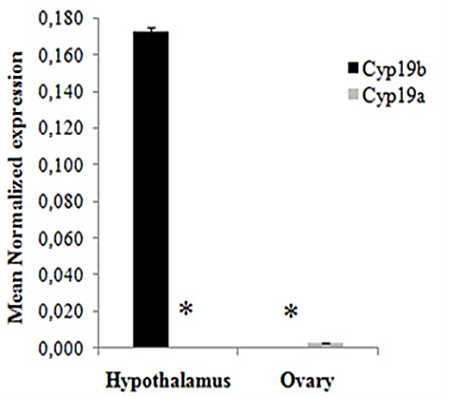

Figure 2. Tissue Distribution of *A.tarichi* Aromatase Isomers. mRNA levels of *Cyp19b* and *Cyp19a* in the hypothalamus and ovary from vitellogenic fish. Letters indicate significant differences

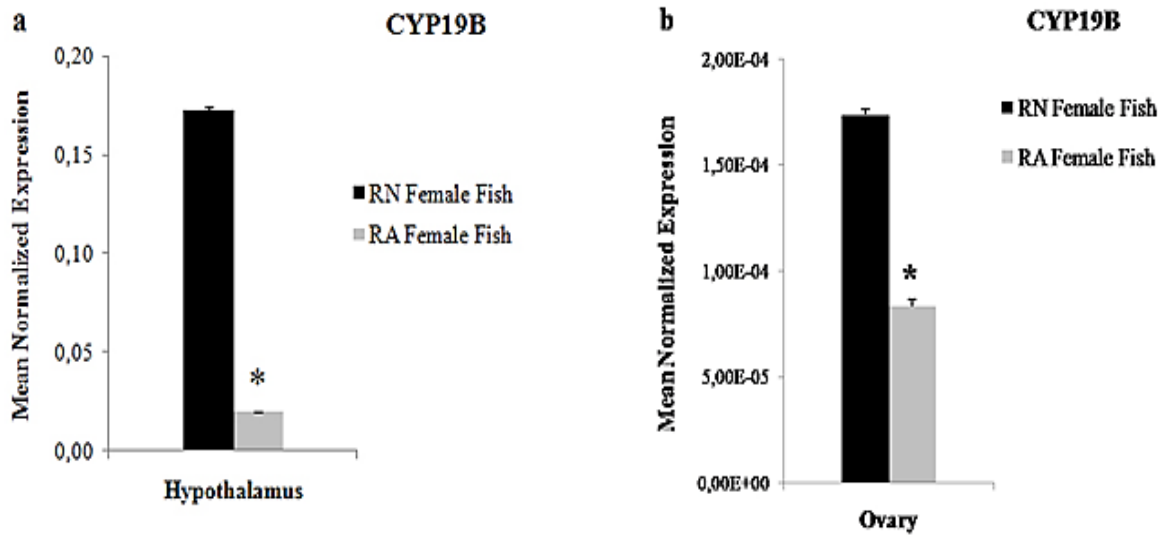

Figure 3. *Cyp19b* mRNA levels in the hypothalamus (a) and ovary (b) of reproductively non-arrested (RN) and reproductively arrested (RA) female *A. tarichi* sampled from Van Edremit Region, Lake Van. Eachbar shows mRNA levels normalized with *β-actin*. Asterisks indicate significant differences (*P*<0.05)

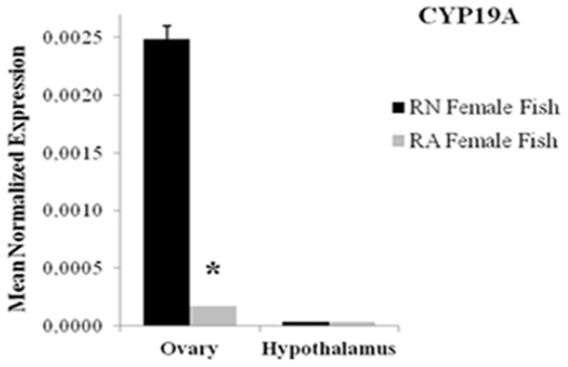

Figure 4. *Cyp19a* mRNA Levels in Ovary and Hypothalamus of Reproductively Non-arrested (RN) and Reproductively Arrested (RA) Female *A. tarichi* Sampled from Van Edremit Region, Lake Van. Each bar shows mRNA levels normalized with *β-actin*. Asterisks indicate significant differences (*P*<0.05)

DISCUSSION

In this study, we measured the expression levels of *Cyp19b* and *Cyp19a* genes in the hypothalamus and ovary of RN (vitellogenic stage) and RA *A. tarichi* from VER of Lake Van. Previous studies of fish determined that two *Cyp19* loci have been isolated, brain-derived *Cyp1919b* and ovary-derived *Cyp19a*, and they have different functional programs (Callard et al., 2001; Cheshenko et al., 2008; Kishida and Callard, 2001; Tchoudakova and Callard, 1998; Tchoudakova et al., 2001). *Cyp19b* and *Cyp19a* mRNA were both detected in the hypothalamus and ovary of RN *A. tarichi*, and*Cyp19b* mRNA abundance was ~993-fold higher in the hypothalamus than in ovary whereas *Cyp19a* mRNA was ~71-fold higher in the ovary than in the hypothalamus. In teleost fish, it is well known that the brain aromatase activity (10-100-fold) and expression are higher than ovary (Greytak et al., 2005; Pasmanik and Callard, 1985; Villeneuve, 2006).

The level of *Cyp19b* transcript was found to be significantly lower in RA fish tissues than in RN fish. Several studies indicate that P450 aromataseB (not the P450 aromataseA) transcripts are up-regulated by E_2 (Barney et al., 2008; Kishida and Callard, 2001; Kishida et al., 2001; Sawyer et al., 2006; Tchoudakova et al., 2001) due to the fact that the 5'-flanking region of the *Cyp19b* gene includes two estrogen response element (EREs), and an ERE half-site (ERE1/2) (Callard et al., 2001). In accordance with these results, the low *Cyp19b* expression in the hypothalamus and ovary of RA fish correlates with low plasma E_2 level and GSI in RA fish (Ünal et al., 2007).

In fish, it is well known that the physiological functions of brain aromatase are implicated in development of central the nervous system, regeneration and sex differentiation (Forlano et al., 2001) while ovarian aromatase is involved in ovarian differentiation (Kwon et al., 2001; Matsuoka et al., 2006) and gametogenesis (Kazeto et al., 2004). In adult fish studied, *Cyp19b* expression varies seasonally, depending on reproductive cycle. For instance, in goldfish (Gelinas et al., 1998) and channel catfish (Kazeto et al., 2003; Kazeto et al., 2005; Rasheeda et al., 2010), it was reported that*Cyp19b* transcript level begins to increase during ovarian recrudescence (from preparatory phase to regressed phase), increases further in the pre-spawning phase, and is followed by a steep decline. In accordance with these reports, the low *Cyp19b* mRNA level which is found in the hypothalamus and ovary of RA *A. tarichi* were anticipated because the ovaries of these fish include only cortical alveoli oocytes during the vitellogenic stage (Ünal et al., 2007). This low *Cyp19b* mRNA

level in the hypothalamus and ovary of RA fish from VER suggest that *A. tarichi* from VER may have been exposed to EDC/s which down-regulates the brain aromatase expression in some fish.

The level of *A. tarichi Cyp19a* mRNA was significantly lower in the ovary of RA fish than in RN fish tissue, while no differences were found in the brain of both RA and RN fish. Ovarian aromatase expression begins after brain aromatase expression during early embryonic development and peaks during gonad development (Chiang et al., 2001; Kishida et al., 2001; Matsuoka et al., 2006). These results suggest that the high expression of *Cyp19a* may be necessary in trigger gonadal differentiation. Also in adult fish, high expression of *Cyp19a* is necessary for vitellogenesis. In the adult fathead minnow (*Pimephales promelas*) ovarian aromatase activity and *Cyp19a*transcript were higher in reproductively active fish (vitellogenic oocyte) than non-reproductive (maturation stage) and juvenile fish (Villeneuve et al., 2006). Similarly, in the rainbow trout, *Oncorhynchus mykiss* (Nakamura et al., 2005) and Atlantic croaker, (*Micropogonias undulates*) (Nunez and Applebaum, 2006), P450aromatase transcript levels were higher in the middle vitellogenic stage than in post vitellogenic and post-ovulated follicles. *In situ* hybridization studies have shown that *Cyp19a* mRNA expression was localized in the follicle cell layer in the pre-vitellogenic and vitellogenic stages of growth while no signal was seen in the primary growth and maturation stage (Dong and Willett, 2008; Kazeto et al., 2004). According to our results, the low *Cyp19a* mRNA expression in the ovary from RA fish which included cortical alveoli but not pre-vitellogenic and vitellogenic oocytes from RA *A. tarichi* (Ünal et al., 2007) would be expected result. These low *Cyp19a* mRNA levels in the hypothalamus and ovary of RA fish from VER suggest that *A. tarichi* in VER may have been exposed to EDC which is unknown factor, such as down-regulates the ovarian aromatase expression in a subset fish (RA).

In conclusion, we suggest that the RA fish represent a segment of the population which may be more sensitive to EDC exposure. Further studies are required to determine the primary sites and the causes of these reproductive abnormalities in *A. tarichi* in VER of Lake Van.

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REFERENCES

Barney, M.L., Patil, J. G., Gunasekera, R. M. & Carter, C. G. (2008). Distinct cytochrome P450 aromatase isoforms in the common carp (*Cyprinus carpio*): Sexual dimorphism and onset of ontogenic expression. *General and Comparative Endocrinology*, *156*(3), 499-508. DOI:10.1016/j.ygcen.2008.03.013

Callard, G. V., Tchoudakova, A. V., Kishida, M. & Wood, E. (2001). Differential tissue distribution, developmental programming, estrogen regulation and promoter characteristics of cyp19 genes in teleost fish. *The Journal of Steroid Biochemistry and Molecular Biology*, *79*(1-5), 305-314. DOI:10.1016/S0960-0760(01)00147-9

Chang, X., Kobayashi, T., Senthilkumaran, B., Kobayashi-Kajura, H., Sudhakumari, C. C. & Nagahama, Y. (2005). Two types of aromatase with different encoding genes, tissue distribution and developmental expression in Nile tilapia (*Oreochromis niloticus*). *General and Comparative Endocrinology*, *141*(2), 101-115. DOI:10.1016/j.ygcen.2004.11.020

Cheshenko, K., Brion, F., Le Page, Y., Hinfray, N., Pakdel, F., Kah, O., … Eggen, R. I. (2006). Expression of zebra fish Aromatase cyp19a and cyp19b genes in response to the ligands of estrogen receptor and aryl hydrocarbon receptor. *Toxicological Sciences*, *96*(2), 255-267. DOI:10.1093/toxsci/kfm003

Cheshenko, K., Pakdel, F., Segner, H., Kah, O. & Eggen, R. I. (2008). Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. *General and Comparative Endocrinology*, *155*(1), 31-62. DOI:10.1016/j.ygcen.2007.03.005

Chiang, E. F., Yan, Y., Guiguen, Y., Postlethwait, J. & Chung, B. (2001). Two Cyp19 (P450 Aromatase) genes on duplicated Zebrafish chromosomes are expressed in ovary or brain. *Molecular Biology and Evolution*, *18*(4), 542-550. DOI:10.1093/oxfordjournals.molbev.a003833

Dong, W. & Willett, K. L. (2008). Local expression of CYP19A1 and CYP19A2 in developing and adult killifish (*Fundulus heteroclitus*). *General and Comparative Endocrinology*, *155*(2), 307-317. DOI:10.1016/j.ygcen.2007.05.018

EPA (2001). Sampling procedures for the 2001 National Sewage Sludge Survey. Office of Science and Technology Washington, D.C. www.epa.gov.

Fenske, M. & Segner, H. (2004). Aromatase modulation alters gonadal differentiation in developing zebrafish (*Danio rerio*). *Aquatic Toxicology*, *67*(2), 105-126. DOI: 10.1016/j.aquatox.2003.10.008

Forlano, P. M., Deitcher, D. L., Myers, D. A. & Bass, A. H. (2001). Anatomical distribution and cellular basis for high levels of Aromatase activity in the brain of teleost fish: Aromatase enzyme and mRNA expression identify glia as source. *The Journal of Neuroscience*, *21*(22), 8943-8955. DOI:10.1523/JNEUROSCI.21-22-08943.2001

Gelinas, D., A. Pitoc, G. & V. Callard, G. (1998). Isolation of a goldfish brain cytochrome P450 aromatase cDNA:. *Molecular and Cellular Endocrinology*, *138*(1-2), 81-93. DOI: 10.1016/S0303-7207(98)00015-X

Greytak, S. R., Champlin, D. & Callard, G. V. (2005). Isolation and characterization of two cytochrome P450 aromatase forms in killifish (*Fundulus heteroclitus*): Differential expression in fish from polluted and unpolluted environments. *Aquatic Toxicology*, *71*(4), 371-389. DOI:10.1016/j.aquatox.2004.12.007

Kazeto, Y., Goto-Kazeto, R., Place, A. & Trant, J. (2003). Aromatase expression in zebrafish and channel catfish brains: Changes in transcript abundance associated with the reproductive cycle and exposure to endocrine disrupting chemicals. *Fish Physiology and Biochemistry*, *28*(1-4), 29-32. DOI:10.1023/B:FISH.0000030466.23085.94

Kazeto, Y., Place, A. R. & Trant, J. M. (2004). Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. *Aquatic Toxicology*, *69*(1), 25-34. DOI: 10.1016/j.aquatox.2004.04.008

Kazeto, Y. & Trant, J. M. (2005). Molecular biology of channel catfish brain cytochrome P450 aromatase (CYP19A2): Cloning, preovulatory induction of gene expression, hormonal gene regulation and analysis of promoter region. *Journal of Molecular Endocrinology*, *35*(3), 571-583. DOI:10.1677/jme.1.01805

Kishida, M. & Callard, G. V. (2001). Distinct cytochrome p450 Aromatase Isoforms in Zebrafish (*Danio rerio*) brain and ovary are differentially Programmedand estrogen regulated during early Development. *Endocrinology*, *142*(2), 740-750. doi.org/10.1210/endo.142.2.7928

Kishida, M., McLellan, M., Miranda, J. A. & Callard, G. V. (2001). Estrogen and xenoestrogens upregulate the brain aromatase isoform (P450aromB) and perturb markers of early development in zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *129*(2-3), 261-268. DOI:10.1016/S1096-4959(01)00319-0

Kwon, J. Y., McAndrew, B. J. & Penman, D. J. (2001). Cloning of brain aromatase gene and expression of brain and ovarian aromatase genes during sexual differentiation in genetic male and female Nile tilapia *Oreochromis niloticus*. *Molecular Reproduction and Development*, *59*(4), 359-370. DOI:10.1002/mrd.1042

Lange, A., Katsu, Y., Ichikawa, R., Paull, G. C., Chidgey, L. L., Coe, T. S. & Tyler, C. R. (2008). Altered sexual development in roach (*Rutilus rutilus*) exposed to environmental concentrations of the pharmaceutical 17α-Ethinylestradiol and associated expression dynamics of Aromatases and estrogen receptors. *Toxicological Sciences*, *106*(1), 113-123. DOI: 10.1093/toxsci/kfn151

Marttinen, S. K., Kettunen, R. H., Sormunen, K. M. & Rintala, J. A. (2003). Removal of bis(2-ethylhexyl) phthalate at a sewage treatment plant. *Water Research*, *37*(6), 1385-1393. DOI: 10.1016/S0043-1354(02)00486-4

Matsuoka, M. P., Van Nes, S., Andersen, Ø., Benfey, T. J. & Reith, M. (2006). Real-time PCR analysis of ovary- and brain-type aromatase gene expression during Atlantic halibut (*Hippoglossus hippoglossus*) development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *144*(1), 128-135. DOI: 10.1016/j.cbpb.2006.02.008

Nakamura, I., Evans, J. C., Kusakabe, M., Nagahama, Y. & Young, G. (2005). Changes in steroidogenic enzyme and steroidogenic acute regulatory protein Messenger RNAs in ovarian follicles during ovarian development of rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, *144*(3), 224-231. DOI:10.1016/j.ygcen.2005.06.004

Nunez, B. S. & Applebaum, S. L. (2006). Tissue- and sex-specific regulation of CYP19A1 expression in the Atlantic croaker (*Micropogonias undulatus*). *General and Comparative Endocrinology*, *149*(2), 205-216. DOI:10.1016/j.ygcen.2006.06.005

Pasmanik, M. & Callard, G. V. (1985). Aromatase and 5α-reductase in the teleost brain, spinal cord, and pituitary gland. *General and Comparative Endocrinology*, *60*(2), 244-251. DOI:10.1016/0016-6480(85)90320-X

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), 45e-45. DOI:10.1093/nar/29.9.e45

Rasheeda, M., Sridevi, P. & Senthilkumaran, B. (2010). Cytochrome P450 aromatases: Impact on gonadal development, recrudescence and effect of hCG in the catfish, *Clarias gariepinus*. *General and Comparative Endocrinology*, *167*(2), 234-245. DOI:10.1016/j.ygcen.2010.03.009

Sawyer, S. J., Gerstner, K. A. & Callard, G. V. (2006). Real-time PCR analysis of cytochrome P450 aromatase expression in zebrafish: Gene specific tissue distribution, sex differences, developmental programming, and estrogen regulation. *General and Comparative Endocrinology*, *147*(2), 108-117. DOI:10.1016/j.ygcen.2005.12.010

Simon, P. (2003). Q-gene: Processing quantitative real-time RT-PCR data. *Bioinformatics*, *19*(11), 1439-1440. DOI:10.1093/bioinformatics/btg157

Tchoudakova, A. & Callard, G. V. (1998). Identification of multiple CYP19 genes encoding different cytochrome P450 Aromatase Isozymes in brain and ovary*. *Endocrinology*, *139*(4), 2179-2189. DOI:10.1210/endo.139.4.5899

Tchoudakova, A., Kishida, M., Wood, E. & Callard, G. V. (2001). Promoter characteristics of two cyp19 genes differentially expressed in the brain and ovary of teleost fish. *The Journal of Steroid Biochemistry and Molecular Biology*, *78*(5), 427-439. DOI:10.1016/S0960-0760(01)00120-0

Unal, G., Çetinkaya, O. & Elp, M. (1999). Histological Investigation of Gonad Development of *Chalcalburnus tarichi* (P. 1811). *Turkish Zoology*, *23,* 329–338. DOI:10.1016/j.ygcen.2006.06.005

Unal, G., Marquez, E. C., Feld, M., Stavropoulos, P. & Callard, I. P. (2014). Isolation of estrogen receptor subtypes and vitellogenin genes: Expression in female *Chalcalburnus tarichi*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *172-173*, 67-73. DOI:10.1016/j.cbpb.2014.04.002

Ünal, G., Türkoğlu, V., Oğuz, A. R. & Kaptaner, B. (2007). Gonadal histology and some biochemical characteristics of *Chalcalburnus tarichi* (Pallas, 1811) having abnormal gonads. *Fish Physiology and Biochemistry*, *33*(2), 153-165. DOI:10.1007/s10695-006-9126-1

Villeneuve, D.L., Knoebl, I., Kahl, M. D., Jensen, K. M., Hammermeister, D. E., Greene, K. J. & Ankley, G. T. (2006). Relationship between brain and ovary aromatase activity and isoform-specific aromatase mRNA expression in the fathead minnow (*Pimephales promelas*). *Aquatic Toxicology*, *76*(3-4), 353-368. DOI:10.1016/j.aquatox.2005.10.016

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REVIEW DERLEME

Seafood associated human pathogenic non-enveloped viruses

Su ürünleri kaynaklı insan patojenik zarfsız virüsleri

Bahar Tokur[1*] ● Koray Korkmaz[2]

[1] Ordu University Fatsa Faculty of Marine Sciences Department of Fishery Technology Engineering 52400 Fatsa/ORDU https://orcid.org/0000-0002-7087-5801

[2] Ordu University Fatsa Faculty of Marine Sciences Department of Fishery Technology Engineering 52400 Fatsa/ORDU https://orcid.org/0000-0003-2940-6592

*Corresponding author: baharorhun@gmail.com

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Abstract: Non-enveloped human pathogenic viruses, highly stable in the environment, can be transmitted by different routes, such as contaminated food and water. The waterborne transmission of non-enveloped viruses to humans causes illnesses when individuals are exposed to contaminated water resources such as agricultural water, drainage, outdoor water, field or subsurface water and even drinking water. In addition to waterborne transmission, viral foodborne transmission may consist because of contagious seafood, through infected food handlers due to inadequate hygienic activities, aerosol containing viruses produced by infected people. Most hazardous non enveloped enteric viruses assocated with water and seafood cause a significant and emerging food safety and public health problem and threat. In this review, norovirus (NoVs), hepatitis E virus (HEV) and hepatitis A (HAV), human adenovirus, rotavirus A and sapovirus are evaluated as seafood associated human pathogenic non-envoleped viruses.

Keywords: Waterborne transmission, seafood borne transmission, pathogenic non-enveloped viruses

Öz: Çevresel ortamda oldukça kararlı olan zarfsız insan patojenik virüsleri, kontamine olmuş gıda ve su gibi farklı yollarla bulaşabilirler. Zarfsız virüslerin insanlara su yoluyla bulaşması, genellikle tarımsal su, drenaj, evsel atık suları, tarım veya yer altı suyu ve hatta içme suyu gibi kirli su kaynaklarına maruz kaldıklarında, hastalıklara neden olur. Su yoluyla bulaşmaya ek olarak, gıda kaynaklı viral bulaşma, virüsleri taşıyan deniz ürünleri, yetersiz hijyenik faaliyetler nedeniyle enfekte olmuş gıda çalışanları ve enfekte kişiler tarafından üretilen aerosol içeren virüslerden kaynaklanabilir. Su ve su ürünleri ile ilişkili zararlı zarfsız enterik virüslerin çoğu, önemli ve ciddi gıda güvenliği ve halk sağlığı sorununa ve tehdidine neden olur. Bu derlemede, su ürünleri ile ilişkili insan patojenik zarfsız virüsler olarak norovirüs (NoVs), hepatit E virüsü (HEV) ve hepatit A (HAV), insan adenovirüs, rotavirüs A ve sapovirüs değerlendirilmiştir.

Anahtar kelimeler: Su kaynaklı bulaş, su ürünleri kaynaklı bulaş, zarfsız patojenik virüsler

INTRODUCTION

Viruses are a submicroscopic intracellular parasites that, by nature, made up of RNA or DNA genome encapsulated protein shell, varying in size from 15 to 400 nm and involving just a small number of particles are required to spread disease. Such infections don't happen randomly: each virus group has its own characteristic host diversity and cell selection (Eterpi et al. 2010; Koopmans and Duizer, 2004). A complete sample of the virus is called a virion. The virion 's primary purpose is to transmit the RNA or DNA genome when they infect a host cell. The viral genome, frequently with related basic proteins is packaged into the virion, a symmetric protein capsid. The nucleic acid-associated protein together with the genome are known as nucleoprotein, forms by the association of the nucleocapsid (Gelderblom, 1996).

Among different types of natural water applications, such as agricultural water, drainage, outdoor water, field or subsurface water and drinking water, human pathogenic viruses implicated among water transmission are often observed. (Grabow, 2007; Pinon and Vialette, 2018). It can spread more than 140 forms of pathogenic viruses from the aquatic world (Schwartzbrod and WHO, 1995). All identified waterborne pathogenic viruses present a major threat to public safety in the marine ecosystem which are spread mainly through fecal-oral routes, which is regularly released into coastal and estuarine environments by the runoff of processed and untreated wastewater (Rao et al., 1986; Griffin et al., 2003; Kovać et al., 2009) and urine, and respiratory secretions from the infected host which enter into sewage water (Wang et al., 2018). The average viral concentration in wastewater changes between 10^2 and 10^3 PFU 1^{-1}, and it can be estimated at between 10^1 and 10^2 PFU 1^{-1} in treated wastewater and 10^3 PFU Kg^{-1} in treated sludge, depending on the type of treatment and its yield. According to the intensity of faecal pollution, the concentration in the surface water changes, but can be determined at 10^1 PFU 1^{-1} in river water and between 1 and 10 PFU 1^{-1} in contaminated sea water (Chang et al., 1995). Humans can be infected the pathogenic virus infected in the fecal contaminated water through production in watercourses by collecting, handling, preparation, processing, carrying and storage chain (Blumenthal et al., 2000; Elbashir et al., 2018; Lee and

Rangdale, 2008; Schwartzbrod and WHO, 1995). These polluted ecosystems will end up with unhealthy seafood.

Worldwide an estimated 8 billion per year gastrointestinal disease occur annually (Chan et al., 2019). Viruses associated with acute gastroenteritis, include diarrhea, fever, headache, vomiting, abdominal cramps, and myalgia, may occur as a result of contamination of the marine environment with feces (Adelodun et al., 2020; Koopman et al., 1982). Viral pathogens from water environment have also been reported which include norovirus (NoVs), hepatitis E virus (HEV) and hepatitis A (HAV), human adenovirus, rotavirus A and sapovirus (Bosch et al., 2005; Joshi et al., 2019; WHO, 2006). This work will focus on different human pathogenic non-enveloped viruses associated with seafood and their characteristics.

Human pathogenic non-enveloped viruses

Noroviruses

Norovirus is a small organized RNA virus, and a class known as human pathogens belong to the family Caliciviridae. Virus particles are 27–37 nm in diameter and unwrapped, which cause their high tenacity and disinfection resistance (Bachofen, 2018). The NoV genome of approximately 7.5 kb is positive-sense single-stranded RNA with three free read frames encoding both structural and non-structural proteins (Campos and Lee, 2014). The viral protein 1, the capsid protein, is the viral capsid's most significant component, while the viral protein 2 is inserted within the capsid at low copy numbers (Figure 1).

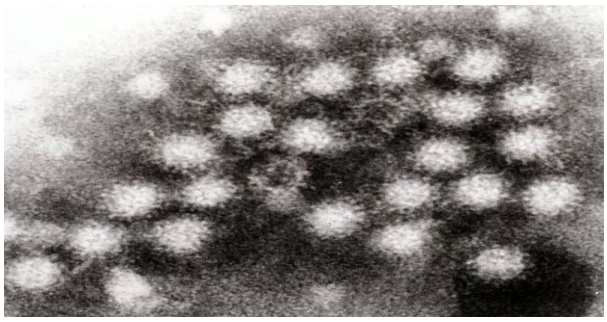

Figure 1. Transmission electron micrograph of Norovirus particles in feces by Graham Beards at English Wikipedia (CC BY 3.0. https://commons.wikimedia.org/w/index.php?curid=5736176

Norovirus (NoV) is well-known to be the primary causative agent of seafood transmitted illness (Bachofen, 2018; Grabow,2007; Li et al., 2014; Terregino and Arcangeli, 2017) and the emergence in viral gastroenteritis outbreaks is a global safety issue which is also blamed for economic losses (Kobayashi et al., 2019; Pavoni et al., 2013). Several geographic areas worldwide have been confirmed to have numerous seafood associated outbreaks of various norovirus strains (Stals et al., 2013; Vidal et al., 2005). The spread of NoV is primarily due to its low infectious dose is sufficient to cause infection and gastroenteritis needed for the infection (Khora, 2018; Koopmans et al., 2002; Teunis et al., 2008). The long-term survival and longevity of NoV on infected surfaces used in food processing areas often lead significantly to transmission of the disease (Moorman, 2017).

The main route for norovirus transmission is harvesting or catching of seafood from faecally contaminated waters (Butt et al., 2004; Campos and Lee, 2014; Terregino and Arcangeli, 2017), NoV-infected food handling (Grabow 2007; Li et al. 2014; Widdowson et al., 2000), contaminated surfaces and utensils used in food preparation areas because of the long-term stability and persistence of NoV (Cheesbrough et al., 2000; Evans et al., 2002; Lamhoujeb et al., 2009; Mattison et al., 2007; Sharp et al., 2012; Tuladhar et al., 2013). Hardstaff et al. (2018) stated personnel working as food handler (mostly in the kitchen) were tested for NoV in 44 of 51 (86%) outbreaks and food handlers, the median proportion of positive samples, showed 46% (interquartile range of 25–76%). In fact, inadequate preparation, such as steaming clams, has led to disease and outbreaks even before they expand rather than to higher temperatures that destroy noroviruses (Centers for Disease Control and Prevention, 2010; DuPont, 1986; Le Guyader and Atmar, 2007; Morse et al., 1986). Consumption of contaminated raw/undercooked oysters are at risk for secondary infection of NoV in household contacts (Guix et al., 2019). Cooking shellfish (e.g. by steaming) can not inactivate the virus, so after ingestion of cooked shellfish, incidents of infection have occurred (Le Guyader and Atmar, 2007; Li et al., 2014; McDonnell et al., 1997). In comparison, NoV is often immune to certain commercial food storage practices and can withstand boiling, freezing, acidification, decreased water movement and changed packaging environment (Baert et al. 2009).

Shellfish, in particular moulds, clams and oysters, crabs, prawns, finfish, shirmps are usually implicated in seafood-borne norovirus outbreaks because of their feeding patterns as filter feeders and their capacity to absorb the virus from polluted water (Das et al., 2020; Woods et al., 2016). Throughout this filter feeding, bacteria and viruses may be stored and accumulated in their bodies due to land-based waste outflow or harvester runoff disposal (Bellou et al., 2013; Kohn et al., 1995; Le Guyader et al., 2006). Oysters are capable of great flesh and intestine bioaccumulation at amounts up to 99 times greater than the local waters during the autumn / winter season (Burkhardt and Calci, 2000) and remain contagious well after depuration (McLeod et al., 2009). NoV, which can be insufficiently removed by standard decontamination procedures (McLeod et al., 2017; Muniain-Mujika et al., 2002) the result is gastroenteritis outbreaks after consumption of shellfish (Le Guyader et al., 2006; Le Guyader et al., 2008; Webby et al., 2007). Le Guyader et al. (2008) and Doré et al. (2010) both stated that NoV was still measureable in some samples after three to four weeks of purification in open seawater. This may suggest that low levels of NoV in oysters purified for three to four weeks in clean open seawater is small risk to consumers. However,

Doré et al. (1998) stated that a the low levels of NoV particles may be able to inducing infection of NoV in oysters after four weeks of purification (McLeod et al., 2017).

Hepatitis A virus

Hepatitis A virus (HAV) has an assRNA (+) genome of 7.5 kb size and a Picornaviridae family member belonging to the Hepatovirus class (2). Particles of the virus come in two versions: naked, unenveloped 27 nm diameter icosahedral virions with a protein capsid surrounding them (Butt et al., 2004; Feng et al., 2013). Electron micrograph of "Hepatovirus A" virions is shown in Figure 2

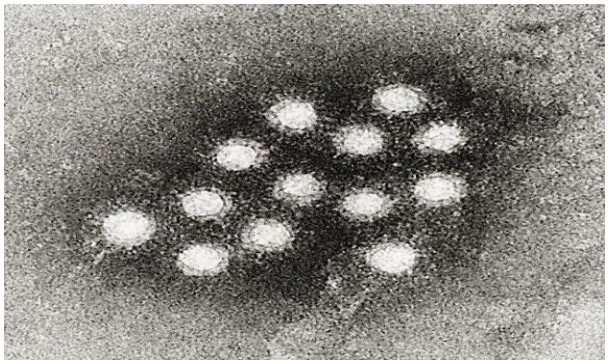

Figure.2. Electron micrograph of "Hepatovirus A" virions. This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #2739 (https://commons.wikimedia.org/wiki/File:Hepatitis_A_virus_02.jpg#/media/File:Hepatitis_A_virus_02.jpg)

Hepatitis A is the most severe virus infection related to the consumption of seafood (Grabow, 2007; 8; Khora, 2018; Iwamato et al., 2010). The WHO (2015) reports that 14 million foodborne infections and 27,731 fatalities in 2010 were triggered by hepatitis A virus (HAV) (Kirk et al., 2015). Incidence of contamination ranges across areas of the world, with the largest incidence in developed nations where there could be inadequate water management and hygiene procedures (Rodriguez-Lazaro et al., 2012). In 1955, when 629 cases were connected with oyster ingestion, the first HAV-linked seafood-borne epidemic involving oysters was reported in Sweden (La Rosa et al., 2012; Lindberg-Braman, 1956). 1988 witnessed the biggest outbreak in Shanghai, China, in which more than 288,000 people were poisoned after consuming fresh or poorly cooked clams (Butt et al., 2004). Between 1986 and 2012, 46 HAV outbreaks were reported and linked to seafood vehicles worldwide, such as oysters, clams, moulds and cockles (Bellou et al., 2013).

Bivalve molluscs, such as soft clams, hard clams, moulds and oysters, have been infected with HAV outbreaks and pollution most often happens when shellfish growing areas become polluted with human sewage (Khora, 2018). Through successful filtration, shellfish accumulate the virus many times in their tissue. The infectious dosage is small, maybe 10–100 virus particles, which is undoubtedly the explanation why shellfish collected from areas were feasibly infected (Butt et al., 2004). In fact, shellfish are consumed with their digestive tracts in place, unlike many other seafoods. Shellfish, mostly consumed raw or lightly cooked, may also protect viruses by adequate cooking, unlike other foods (Patwardhan, 2019).

Hepatitis A virus transmission is spread by consumption of fecal polluted water and/or seafood, inadequate ventilation, bad personal hygiene and close contact with an infected person (including asymptomatic carriage) (Bosch 1998; Butt et al., 2004; Tallon et al., 2008; Richards, 2013; WHO, 2016). In the primary instances, the most significant risk factor was the intake of raw seafood, during interaction with individuals (Germinario et al., 2000).

The HAV has environmental stability, which makes it viable in water or on fomites for many weeks and including freezing, heat, chemicals, and desiccation (Khora, 2018). For instance, the virus can keep living for a long time in tap water (up to 60 days), in river water (over 6 weeks), in groundwater (over 8 weeks) and in sea water (up to 30 weeks) (Crance et al., 1998; Enriquez et al., 1995; Sobsey, 1989; Springthorpe et al., 1993). In fact, hepatitis A virus is heat-resistant and can tolerate steaming, so proper cooking of seafood diminishes the risk of ingestion of live hepatitis A. Since shellfish is widely prepared in ways that are inadequate to inactivate the virus, several preventive techniques are aimed to manage the contamination before the food is processed (Iwamato et al., 2010).

Hepatitis E virus

Hepatitis E virus (HEV) as is a a tiny (32–34 nm) single-stranded, positive-sense RNA virus coated with protein classified in the family Hepeviridae, RNA molecule of approximately 7.2 kb in size (Khora, 2018) and comprising of four known Genotypes (1–4), at least two new putative mammalian HEV genotypes and one floating genus of HEV avian (Yugo and Meng, 2013; Van der Poel, 2014). Electron micrograph of Hepatitis E viruses (HEV) is given in Figure 3.

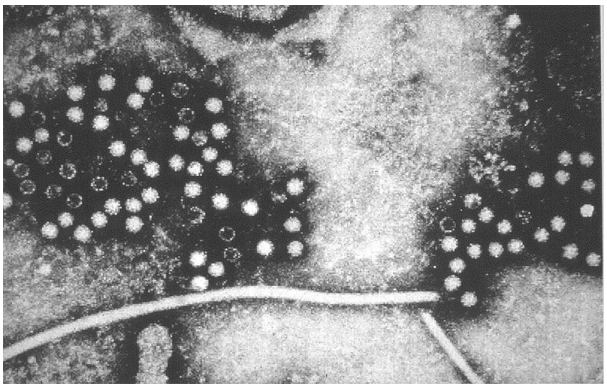

Figure3. Electron micrograph of Hepatitis E viruses (HEV). This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #5605, https://commons.wikimedia.org/w/index.php?curid=1882568

Hepatitis E virus (HEV) is a virus that causes acute hepatitis outbreaks and endemics in humans, which can be transmitted mainly to people and other animals from fecal-oral routes and animals (Sooryanarain and Meng, 2019). Unfortunately, this ensures that some species may act as reservoirs for HEV strains that infect humans (Grabow, 2007). Insufficient management and storage of wastewater, the usage of contaminated river water for coomon activities and contamineted of drinking and irrigation water contribute to numerous epidemics in developed countries (Ceylan et al., 2003; Fenaux et al., 2019).

HEV can easily pollute the surface water, entering through food production chains, especially via shellfish cultivation areas and irrigation waters function as a threat to public health (Di Cola et al. 2020). The surface water's quality directly affects people using the source as drinking water, and intense agricultural activities result in high levels of viruses in these sources (Yugo and Meng, 2013).

Coastal waters can also be polluted with HEV, contributing to the aggregation of the virus in shellfish 's digestive tissues, which presents a possibility of human infection by ingestion (Yugo and Meng, 2013; Van der Poel, 2014). Very commonly, moulds, cockles and oysters are consumed raw or partially fried, and HEV is stable at pH 2 to 9 (Wolff et al., 2020), frozen for more than 10 years (Emerson et al., 2005) and fresh, undercooked or partially steamed infected seafood will pass HEV to consumers (Crossan et al., 2012; Namsai et al., 2011). People who travel to hyperendemic and endemic areas of the world are at elevated risk of contamination with HEV from polluted water and seafood, in which developed countries are not excluded (Yugo and Meng, 2013; Zuckerman, 2003). Said et al. (2009) reported severe HEV infections in 33 participants on a 2008 world cruise caused by shellfish (crabs, prawns, moulds, lobsters, and scallops) and mixed seafood (a combination of shrimp, mussels, salmon, hake, cod, and squid). Recent studies have been showed that HEV infections may be associated with shellfish consumption (Rivadulla et al., 2019; Webb et al., 2020; Zhang et al., 2017).

Rotavirus A

Rotaviruses (RVs), a member of the genus Rotavirus of the Reoviridae family, consisting of a rectangular segmented double-stranded RNA genome in a non-enveloped icosahedral capsid 60–80 nm in diameter (Gerba et al., 1996; Grabow, 2007; Estes, 2001). The genome of 16–27 kb is bound by a triple layer of capsid covered by a double protein coat (Sattar et al., 1994). The capsid has a unique double layer with spikes between the layers giving it the wheel-like outlook (Latin "rota") hence the name rotavirus is derived from the Latin meaning rota (wheel) (Gerba et al., 1996; Grabow, 2007; Khora, 2018). Electron Micrographs of Rotaviruses is shown in Figure 4.

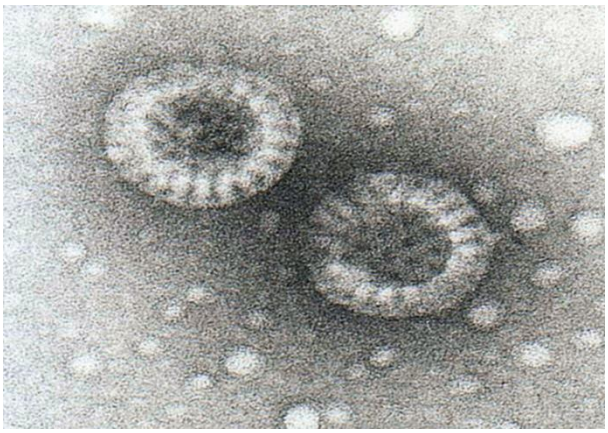

Figure 4. Electron Micrographs of Rotaviruses by Dr Graham Beards at en.wikipedia, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=16014758

Given the global adoption of rotavirus vaccines, RVA is the main agent of acute gastroenteritis in infants worldwide, with many deaths / year cases (> 200,000), especially in low-income and undeveloped countries (Crawford et al., 2017). In 2003, 114 million cases of rotavirus infection were recorded worldwide in children who were less than 5 years of age, 24 million of which needed outpatient visits and 2,3 million of which needed to be hospitalized. (Parashar et al., 2003). Rotaviruses are released from the feces of infected people in exceptionally high numbers (up to 1010 g–1) and can remain in the atmosphere for long periods of time (Carter, 2005) contributing to the potential for pollution of recreational and drinking water. (Gerba et al., 1996). The infectious dose is estimated at between 100 and 1000 particles of the virus (Ward et al., 1986). The viral particles are extremely robust and dangerously contagious to harsh ambient conditions. Despite exceptionally high levels of faecal excretion and confirmation of the possibility of waterborne transmission, the predominant route of transmission is via the fecal-oral route under poor hygienic conditions. (Khora, 2018; Magana-Arachch and Wanigatunge, 2020). Several waterborne rotavirus outbreaks which have caused illnesses in both adults and children (Chia et al., 2018; Tozan et al., 2016; Sattar, 2001).

RoV has been frequently detected in found in both freshwater and marine water sources and can also be concentrated by shellfish with higher prevalences and levels (Cook et al. 2004; Lees, 2000; Lodder and de Roda Husman, 2005; Prevost et al., 2015; Souza et al., 2018). Keller et al. (2013), found genomes of Rotavirus in Brazil, with a range of 76 and 88% of water samples and 100% of mussel samples. Quiroz-Santiago et al. (2014) detected that shellfish grown in China is infected by Rotavirus with a 7 % ratio identified. Shellfish bioaccumulate viruses in their gills, digestive glands, and other tissues during filter feeding (Asahina et al., 2009; Schwab et al., 1998; Wang et al., 2008).

Rotavirus also occurs in infected and non-polluted fresh water (Hurst and Gerba, 1980). Amoroso et al. (2020) examined a two-phase kinetic virus elimination with a high reduction in the first 24 h of depuration and rotavirus were completely removed after 5 days in experimentally contaminated Mytilus galloprovincialis.

Human Adenovirus

Adenoviruses are double-stranded, non-enveloped, icosahedral DNA viruses (Bosch, 2007). Human adenoviruses (HAds) belong to the genus Mastadenovirus in the Adenoviridae family consisting of a double-stranded DNA genome in a non-enveloped icosahedral capsid with a diameter varying from 80 to 110 nm (Figure 5).

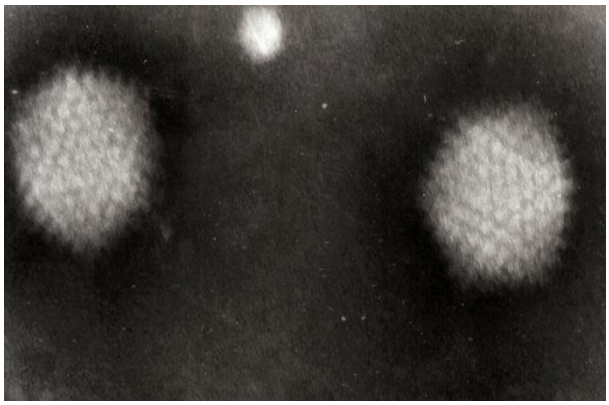

Figure 5. Transmission electron micrograph of two Adenovirus particles By GrahamColm at English Wikipedia, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=3921907

Adenoviruses are linked to a number of human diseases and were responsible for waterborne outbreaks (Crabtree et al., 1997). There have been many cases of waterborne recreational outbreaks attributed to adenovirus within years (Bonadonna and La Rosa, 2019; Koopmans et al., 2017; Zhang et al., 2016). These viruses have been commonly seen in riversides, coast waters, swimming pool waters, and drinking water supplies all over the world where they may survive until four months (Jiang et al., 2001; Jiang, 2006). Adenovirus prevents environmental deterioration and water treatment rather than other measures of pollution (De Moraes Tavares et al., 2005; Griffin et al., 2008; Luz et al., 2015; Jiang et al., 2007).

Adenovirus can be transmitted either directly from person to person or via the fecal-oral route that can result from the ingestion of polluted water during outdoor practices or from the processing of shellfish obtained from polluted waters and contaminated food and liquid intake (Benabbes et al., 2021; Gyawali and Hewitt, 2020).

Formiga-Cruz et al. (2002) reported that, with respect to the existence of human enteric viruses, Adenovirus was the most wide spreading virus group found in oyster (*C. gigas* and *O. edulis*) and mould (*M. edulis* and *M. galloprovincialis*) samples across Europe. Choo and Kim (2006) reported that 50.9% of oysters harvested from the wholesale fishing industry in Noryangjin were infected with contagious adenovirus. Ghalyoun and Alçay (2018) detected adenovirus before the fishing season ended in 46.15% of the musk sample from three separate locations in Istanbul. Luz et al. (2015) reported that the findings of the analysis revealed substantial bioaccumulation of adenoviruses in shrimp, demonstrating the magnitude of fecal pollution 's effect on aquatic environments. In 18.6% of shellfish tests from the Norwegian coast, Myrmel et al. (2004) have found Adenovirus with more accurate tests in the winter season.

Sapovirus

It is a small (27–40 nm) nonenveloped RNA virus which belongs to the Caliciviridae family (like NoV) and forms its own genus. Similar to NoV, only observed sapoviruses (SaV) in 1977 was extremely immune to negative environmental factors (Grabow, 2007), usually infecting humans (HuCVs), as well as two other genera synonymous with animal diseases, including primates, birds, reptiles and insects (Figure 6)

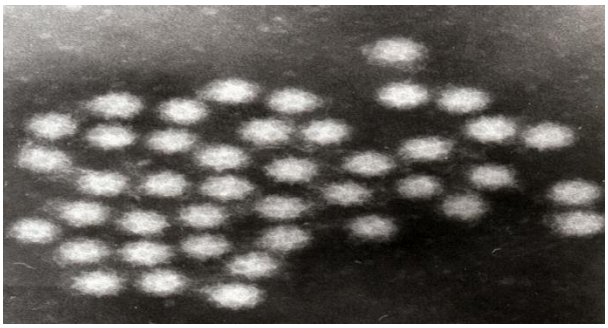

Figure 6. Transmission electron micrograph of Sapporo viruses By Graham Beards at English Wikipedia, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=56088018

SaV is spread via the fecal–oral path discharge into environmental waters and collected by shellfish (i.e., or oysters clams) (Hansman et al., 2007; Khora, 2018; Nakagawa-Okamoto et al., 2009; Oka et al., 2015), as well as in contaminated food related with the intake of shellfish (Nakagawa-Okamoto et al., 2009), water, materials, and human interactions. Lizuka et al. (2010) reported that SaV was found in fecal specimens of 17 individuals who consumed restaurant food and one asymptomatic food handler, as well as in stripped shellfish and residual liquids in shellfish containers, which triggered a gastroenteritis spread in a restaurant, June 2008. Ueki et al. (2010) show that SaV can be accumulated in oysters which are grown in an estuary in Japan that receives treated sewage. During a three-year period (2015–2017), Fusco et al. (2019) detected sapovirus (SaV; 18.8%) in bivalve mollusc samples from three littoral zones of the Campania region in South West Italy.

CONCLUSION

Seafoodborne and waterborne viruses may exist in any form of untreated water due to pollution induced by faecal content of human or animal origin and directly infected through interaction with body secretions and fluids containing contagious viral particles or indirectly by aerosols or other polluted fomites. Thus, the human pathogenic viruses can infect seafood and its products anytime from water and food environments. Non-enveloped viruses are commonly known to have a greater tolerance to desiccation and are therefore harder to transmit than viruses with an envelope tag, which often correlates to their mode of transmission. For this cause, a significant danger is the intake of food that is cooked only minimally before consumption or eaten with fresh vegetables, shellfish or other conventional meat specialities. When the food can't be accurately decontaminated during processing, it is essential to prepare the food such as cooking.

REFERENCES

Adelodun, B., Ajibade, F.O., Ighalo, J.O., Odey, G., Ibrahim, R.G., Kareem, K.Y. & Adeniran, K.A. (2020). Assessment of socioeconomic inequality based on virus-contaminated water usage in developing countries: a review. *Environmental Research*, 110309. DOI: 10.1016/j.envres.2020.110309

Amoroso, M.G., Langellotti, A.L., Russo, V., Martello, A., Monini, M., Di Bartolo, I. & Fusco, G. (2020). Accumulation and Depuration Kinetics of Rotavirus in Mussels Experimentally Contaminated. *Food and Environmental Virology*, 12(1), 48-57. DOI: 10.1007/s12560-019-09413-0

Asahina, A.Y., Lu, Y., Wu, C., Fujioka, R.S. & Loh, P.C. (2009). Potential biosentinels of human waste in marine coastal waters: Bioaccumulation of human noroviruses and enteroviruses from sewage-polluted waters by indigenous mollusks. *Journal of Virological Methods*, 158(1-2), 46-50. DOI: 10.1016/j.jviromet.2009.01.013

Bachofen, C. (2018). Selected viruses detected on and in our food. *Current Clinical Microbiology Reports*, 5(2), 143-153. DOI: 10.1007/s40588-018-0087-9

Baert, L., Debevere, J. & Uyttendaele, M. (2009). The efficacy of preservation methods to inactivate foodborne viruses. *International journal of food microbiology*, *131*(2-3), 83-94. DOI: 10.1016/j.ijfoodmicro.2009.03.007

Bellou, M., Kokkinos, P. & Vantarakis, A. (2013). Shellfish-borne viral outbreaks: a systematic review. *Food and Environmental Virology*, 5(1), 13-23. DOI: 10.1007/s12560-012-9097-6

Benabbes, L., Anga, L., Faouzi, A., Rhaissi, H. & Nourlil, J. (2021). Detection of human enterovirus and adenovirus in shellfish collected in Morocco Mediterranean Coast. *Journal of Microbiology, Biotechnology and Food Sciences*, *2020*, 97-100.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G. & Stott, R. (2000). Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bulletin of the World Health Organization*, 78, 1104-1116.

Bonadonna, L. & La Rosa, G. (2019). A review and update on waterborne viral diseases associated with swimming pools. *International journal of environmental research and public health*, *16*(2), 166. DOI: 10.3390/ijerph16020166

Bosch, A., (1998). Human enteric viruses in the water environment. A minireview. *Int. Microbiol*. 1, 191–196.

Bosch, A., Abad, F.X. & Pintó, R.M. (2005). Human pathogenic viruses in the marine environment. *In oceans and health: pathogens in the marine environment* (pp. 109-131). Springer, Boston, MA. DOI: 10.1007/0-387-23709-7_5

Bosch, A., (2007). Human viruses in water. Elsevier Academic Press, Amsterdam, The Netherlands

Burkhardt, W. & Calci, K.R. (2000). Selective accumulation may account for shellfish-associated viral illness. *Applied and Environmental Microbiology*, 66(4), 1375-1378. DOI: 10.1128/AEM.66.4.1375-1378.2000

Butt, A.A., Aldridge, K. E. & Sanders, C. V. (2004). Infections related to the ingestion of seafood Part I: viral and bacterial infections. *The Lancet infectious diseases*, *4*(4), 201-212. DOI: 10.1016/S1473-3099(04)00969-7

Campos, C.J. & Lees, D.N. (2014). Environmental transmission of human noroviruses in shellfish waters. *Applied and Environmental Microbiology*, 80(12), 3552-3561. DOI: 10.1128/AEM.04188-13

Carter, M.J., (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology*, 98, 1354-1380. DOI: 10.1111/j.1365-2672.2005.02635.x

Centers for Disease Control and Prevention (2010) Norovirus: Technical fact sheet. http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm. Accessed 01 March 2011

Ceylan, A., Ertem, M., Ilcin, E. & Ozekinci, T. (2003). A special risk group for hepatitis E infection: Turkish agricultural workers who use untreated waste water for irrigation. *Epidemiology & Infection,* 131(1), 753-756. DOI: 10.1017/S0950268803008719

Chan, J.S.H., Chao, A.C.W., Cheung, V.C.H., Wong, S.S.K., Tang, W., Wu, J.C.Y. & Ng, S.C. (2019). Gastrointestinal disease burden and mortality: A public hospital-based study from 2005 to 2014. *Journal of gastroenterology and hepatology*, *34*(1), 124-131. DOI: 10.1111/jgh.14377

Chang, A.C., Page, A.L. & Asano, T. (1995). *Developing human health-related chemical guidelines for reclaimed wastewater and sewage sludge applications in agriculture* (No. WHO/EOS/95.20). World Health Organization.

Cheesbrough, J.S., Green, J., Gallimore, C.I., Wright, P.A. & Brown, D.W.G. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiology & Infection*, *125*(1), 93-98. DOI: 10.1017/S095026889900432X

Chia, G., Ho, H.J., Ng, C.G., Neo, F. J.X., Win, M.K., Cui, L. & Chow, A. (2018). An unusual outbreak of rotavirus G8P [8] gastroenteritis in adults in an urban community, Singapore, 2016. *Journal of Clinical Virology*, *105*, 57-63. DOI: 10.1016/j.jcv.2018.06.004

Choo, Y.J. & Kim, S.J. (2006). Detection of human adenoviruses and enteroviruses in Korean oysters using cell culture, integrated cell culture-PCR, and direct PCR. *The Journal of Microbiology*, *44*(2), 162-170.

Cook, N., Bridger, J., Kendall, K., Gomara, M.I., El-Attar, L. & Gray, J. (2004). The zoonotic potential of rotavirus. *Journal of Infection*, *48*(4), 289-302. DOI: 10.1016/j.jinf.2004.01.018

Crabtree, K.D., Gerba, C.P., Rose, J.B. & Haas, C.N. (1997). Waterborne adenovirus: a risk assessment. *Water Science and Technology*, 35(11-12), 1-6. DOI: 10.1016/S0273-1223(97)00225-4

Crance, J.M., Gantzer, C., Schwartzbrod, L. & Deloince, R. (1998). Effect of temperature on the survival of hepatitis A virus and its capsidal antigen in synthetic seawater. Environmental Toxicology and Water Quality: *An International Journal*, 13(1), 89-92. DOI: 10.1002/(SICI)1098-2256(1998)13:1<89::AID-TOX7>3.0.CO;2-3

Crawford, S.E., Ramani, S., Tate, J.E., Parashar, U.D., Svensson, L. & Hagbom, M. (2017). Rotavirus infection. *Nature Reviews Disease Primers*, 3(1), 1-16. DOI: 10.1038/nrdp.2017.83

Crossan, C., Baker, P.J., Craft, J., Takeuchi, Y., Dalton, H.R. & Scobie, L. (2012). Hepatitis E virus genotype 3 in shellfish, United Kingdom. *Emerging Infectious Diseases*, 18(12), 2085. DOI: 10.3201/eid1812.120924

Das, O., Lekshmi, M., Kumar, S. & Nayak, B.B. (2020). Incidence of norovirus in tropical seafood harbouring fecal indicator bacteria. *Marine Pollution Bulletin*, *150*, 110777. DOI: 10.1016/j.marpolbul.2019.110777

De Moraes Tavares, T., De Paula Cardoso, D.D.D. & De Brito, W M.E.D. (2005). Vírus Entéricos Veiculados Por Água: Aspectos Microbiológicos E Decontrole De Qualidade Da Água. *Revista De Patologia Tropical/Journal of Tropical Pathology*, 34(2), 85-104

Di Cola, G., Fantilli, A.C., Pisano, M.B. & Ré, V.E. (2020). Foodborne transmission of hepatitis A and hepatitis E viruses: a literature review. *International Journal of Food Microbiology*, 108986. DOI: 10.1016/j.ijfoodmicro.2020.108986

Doré, W.., Henshilwood, K. & Lees, D.N. (1998). The development of management strategies for control of virological quality in oysters. *Water Science and Technology*, 38(12), 29-35. DOI: 10.1016/S0273-1223(98)00796-3

Doré, B., Keaveney, S., Flannery, J. & Rajko-Nenow, P. (2010). Management of health risks associated with oysters harvested from a norovirus contaminated area, Ireland, February–March 2010. *Eurosurveillance*, 15(19), 19567.

DuPont, H.L. (1986). Consumption of raw shellfish—is the risk now unacceptable?.The *New England Journal of Medicine, 314, 707-708.* DOI: 10.1056/NEJM198603133141109

Elbashir, S., Parveen, S., Schwarz, J., Rippen, T., Jahncke, M. & DePaola, A. (2018). Seafood pathogens and information on antimicrobial resistance: A review. *Food Microbiology*, 70, 85-93. DOI: 10.1016/j.fm.2017.09.011

Emerson, S.U., Arankalle, V.A. & Purcell, R.H. (2005). Thermal stability of hepatitis E virus. *The Journal of Infectious Diseases*, 192(5), 930-933. DOI: 10.1086/432488

Enriquez, C.E., Hurst, C.J. & Gerba, C.P. (1995). Survival of the enteric adenoviruses 40 and 41 in tap, sea, and waste water. *Water Research*, 29(11), 2548-2553. DOI: 10.1016/0043-1354(95)00070-2

Estes, M.K. (2001). Rotaviruses and their replication. *Fields Virology*. 4edn, pp. 1747–1785. (Eds) Knipe D M, Howley P M, Grifûn D E, Lamb R A, Martin M A, Roizman B.. Philadelphia

Eterpi, M., McDonnell, G. & Thomas, V. (2010). Virucidal activity of disinfectants against parvoviruses and reference viruses. *Applied Biosafety*, 15(4), 165-171. DOI: 10.1177/153567601001500402

Evans, M.R., Meldrum, R., Lane, W., Gardner, D., Ribeiro, C.D., Gallimore, C.I. & Westmoreland, D. (2002). An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiology and Infection, 129(2), 355-360. DOI:10.1017/S0950268802007446

Fenaux, H., Chassaing, M., Berger, S., Gantzer, C., Bertrand, I. & Schvoerer, E. (2019). Transmission of hepatitis E virus by water: An issue still pending in industrialized countries. *Water research*, *151*, 144-157. DOI: 10.1016/j.watres.2018.12.014

Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L., ... & Lemon, S.M. (2013). A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature*, *496*(7445), 367-371. DOI: 10.1038/nature12029

Formiga-Cruz, M., Tofino-Quesada, G., Bofill-Mas, S., Lees, D.N., Henshilwood, K. & Allard, A.K. (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden, and the United Kingdom. *Applied and Environmental Microbiology,* 68(12), 5990-5998. DOI: 10.1128/AEM.68.12.5990-5998.2002

Fusco, G., Anastasio, A., Kingsley, D.H., Amoroso, M.G., Pepe, T. & Fratamico, P.M. (2019). Detection of hepatitis a virus and other enteric viruses in shellfish collected in the gulf of Naples, Italy. *International Journal of Environmental Research and Public Health*, 16(14), 2588-. DOI: 10.3390/ijerph16142588

Gelderblom, H.R. (1996). Structure and classification of viruses. *In Medical Microbiology*. 4th edition. University of Texas Medical Branch at Galveston.

Gerba, C.P., Rose, J.B. & Haas, C.N. (1996). Sensitive populations: who is at the greatest risk?. *International Journal of Food Microbiology*, 30(1-2), 113-123.DOI: 10.1016/0168-1605(96)00996-8

Germinario, C., Lopalco, P.L., Chicanna, M. & Da Villa, G. (2000). From hepatitis B to hepatitis A and B prevention: the Puglia (Italy) experience. *Vaccine*, 18, S83-S85.DOI: 10.1016/S0264-410X(99)00473-9

Ghalyoun, F. & Alçay, A.Ü. (2018). Investigation of Rotavirus, Adenovirus and Astrovirus in Mussels and Shrimps Using Multiplex Real-time PCR. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 24(5), 761-767. DOI: 10.9775/kvfd.2018.19997

Grabow, W.O. (2007). Overview of health-related water virology. *Perspectives in Medical Virology*, 17, 1-25. DOI: 10.1016/S0168-7069(07)17001-4

Griffin, D.W., Donaldson, K.A., Paul, J.H. & Rose, J.B. (2003). Pathogenic human viruses in coastal waters. *Clinical Microbiology Reviews*, 16(1), 129-143. DOI**:** 10.1128/CMR.16.1.129-143.2003

Griffin, J.S., Plummer, J.D. & Long, S.C. (2008). Torque teno virus: an improved indicator for viral pathogens in drinking waters. *Virology Journal*, 5(1), 1-6. DOI: 10.1186/1743-422X-5-112

Guix, S., Pintó, R. M. & Bosch, A. (2019). Final consumer options to control and prevent foodborne Norovirus infections. *Viruses*, *11*(4), 333. DOI: 10.3390/v11040333

Gyawali, P. & Hewitt, J. (2020). Faecal contamination in bivalve molluscan shellfish: can the application of the microbial source tracking method minimise public health risks?. *Current Opinion in Environmental Science & Health*, *16*, 14-21. DOI: 10.1016/j.coesh.2020.02.005

Hansman, G.S., Oka, T., Okamoto, R., Nishida, T., Toda, S. & Noda, M. (2007). Human sapovirus in clams, Japan. *Emerging Infectious Diseases*, 13(4), 620. DOI: 10.3201/eid1304.061390

Hardstaff, J.L., Clough, H.E., Lutje, V., McIntyre, K.M., Harris, J.P. & Garner, P. (2018). Foodborne and food-handler norovirus outbreaks: a systematic review. *Foodborne Pathogens and Disease,* 15(10), 589-597. DOI: 10.1089/fpd.2018.2452

Hurst, C.J. & Gerba, C.P. (1980). Stability of simian rotavirus in fresh and estuarine water. *Applied and Environmental Microbiology*, 39(1), 1-5. DOI: 10.1128/AEM.39.1.1-5.1980.

Iwamoto, M., Ayers, T., Mahon, B.E. & Swerdlow, D.L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical microbiology reviews*, *23*(2), 399-411. DOI**:** 10.1128/CMR.00059-09

Jiang, S., Noble, R. & Chu, W. (2001). Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. *Applied and Environmental Microbiology*, 67(1), 179-184. DOI: 10.1128/AEM.67.1.179-184.2001

Jiang, S.C. (2006). Human adenoviruses in water: occurrence and health implications: a critical review. *Environmental Science and Technology*, 40(23), 7132-7140. DOI: 10.1021/es060892o

Jiang, S.C., Chu, W. & He, J.W. (2007). Seasonal detection of human viruses and coliphage in Newport Bay, California. *Applied and Environmental Microbiology*, 73(20), 6468-6474. DOI: 10.1128/AEM.01370-07

Joshi, M.S., Lole, K.S., Barve, U.S., Salve, D. S., Ganorkar, N.N., Chavan, N.A. & Gopalkrishna, V. (2019). Investigation of a large waterborne acute gastroenteritis outbreak caused by group B rotavirus in Maharashtra state, India. *Journal of medical virology*, *91*(10), 1877-1881. DOI: 10.1002/jmv.25523

Keller, R., Justino, J.F. & Cassini, S.T. (2013). Assessment of water and seafood microbiology quality in a mangrove region in Vitória, Brazil. Journal of water and health, 11(3), 573-580. DOI: 10.2166/wh.2013.245

Khora, S.S. (2018). Risk From Viral Pathogens in Seafood. *In Diet, Microbiome and Health* (pp. 439-481). Academic Press. DOI: 10.1016/B978-0-12-811440-7.00015-6.

Kirk, M.D., Pires, S.M., Black, R.E., Caipo, M., Crump, J.A. & Devleesschauwer, B. (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS Medicine, 12(12), e1001921. DOI: 10.1371/journal.pmed.1001923

Kobayashi, Y., Shimazu, T., Murata, K., Itou, T. & Suzuki, Y. (2019). An endogenous adeno-associated virus element in elephants. *Virus Research,* 262, 10-14. DOI: 10.1016/j.virusres.2018.04.015

Kohn, M.A., Farley, T.A., Ando, T., Curtis, M., Wilson, S.A. & Jin, Q. (1995). An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters: implications for maintaining safe oyster beds. *JAMA*, 273(6), 466-471. DOI:10.1001/jama.1995.03520300040034

Koopman, J.S., Eckert, E.A., Greenberg, H.B., Strohm, B.C., Isaacson, R. E. & Monto, A.S. (1982). Norwalk virus enteric illness acquired by swimming exposure. *American Journal of Epidemiology*, 115(2), 173-177. DOI: 10.1093/oxfordjournals.aje.a113288

Koopmans, M., von Bonsdorff, C.H., Vinjé, J., de Medici, D. & Monroe, S. (2002). Foodborne viruses. *FEMS Microbiology Reviews*, 26(2), 187-205. DOI: 10.1111/j.1574-6976.2002.tb00610.x

Koopmans, M. & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90(1), 23-41. DOI: 10.1016/S0168-1605(03)00169-7

Koopmans, M., Bosch, A. & Le Guyader, S. (2017). Viruses. In *Foodborne Diseases* (pp. 289-303). Academic Press. DOI: 10.1016/B978-0-12-385007-2.00014-0

Kovač, K., Gutiérrez-Aguirre, I., Banjac, M., Peterka, M., Poljšak-Prijatelj, M., Ravnikar, M., ... & Raspor, P. (2009). A novel method for concentrating hepatitis A virus and caliciviruses from bottled water. Journal of Virological Methods, 162(1-2), 272-275. DOI: 10.1016/j.jviromet.2009.07.013

La Rosa, G., Fratini, M., della Libera, S., Iaconelli, M. & Muscillo, M. (2012). Emerging and potentially emerging viruses in water environments. *Annali Dell'istituto Superiore Di Sanità*, 48, 397-406. DOI: 10.4415/ANN_12_04_07

Lamhoujeb, S.F.I., Ngazoa, S.E. & Jean, J. (2009) Molecular study of the persistence of infectious human norovirus on foodcontact surfaces. *Food* and *Environmental Virology*, 1, 51–56. DOI: 10.1007/s12560-009-9010-0

Le Guyader, F.S., Bon, F., DeMedici, D., Parnaudeau, S., Bertone, A. & Crudeli, S. (2006). Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. *Journal of Clinical Microbiology*, 44(11), 3878-3882. DOI: 10.1128/JCM.01327-06

Le Guyader, F.S. & Atmar, R.L. (2007). Viruses in shellfish. *Perspectives in medical virology*, *17*, 205-226. DOI: 10.1016/S0168-7069(07)17010-5

Le Guyader, F.S., Le Saux, J.C., Ambert-Balay, K., Krol, J., Serais, O., Parnaudeau, S. & Atmar, R.L. (2008). Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology*, 46(12), 4011-4017. DOI: 10.1128/JCM.01044-08

Lee, R. J. & Rangdale, R.E. (2008). *Bacterial Pathogens in Seafood*, Edited by T. Borresen.DOI: 10.1533/9781845694586.3.247

Lees, D., (2000). Viruses and bivalve shellfish. *International Journal* of *Food Microbiology***,** 59, 81–116. DOI: 10.1016/s0168-1605(00)00248-8

Li, D., Stals, A., Tang, Q.J. & Uyttendaele, M. (2014). Detection of noroviruses in shellfish and semiprocessed fishery products from a Belgian seafood company. Journal of Food Protection, 77(8), 1342-1347. DOI: 10.4315/0362-028X.JFP-14-016

Lindberg-Braman, A.M. (1956). Clinical observations on the socalled oyster hepatitis. *American Journal of Public Health* 53, 1003–1011.

Lizuka, S., Oka, T., Tabara, K., Omura, T., Katayama, K., Takeda, N. & Noda, M. (2010). Detection of sapoviruses and noroviruses in an outbreak of gastroenteritis linked genetically to shellfish. *Journal of Medical Virology,* 82(7), 1247-1254. DOI: 10.1002/jmv.21791

Lodder, W.J. & de Roda Husman, A.M. (2005). Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. *Applied and Environmental Microbiology*, 71(3), 1453-1461. DOI: 10.1128/AEM.71.3.1453-1461.2005

Luz, R.B., Staggemeier, R., Fabres, R.B., Soliman, M.C., Souza, F.G. & Gonçalves, R. (2015). Bioaccumulation of animal adenoviruses in the pink shrimp. *Brazilian Journal of Microbiology*, 46(3), 715-723. DOI: 10.1590/S1517-838246320140323

Magana-Arachchi, D.N. & Wanigatunge, R.P. (2020). Ubiquitous waterborne pathogens. In *Waterborne Pathogens* (pp. 15-42). Butterworth-Heinemann. DOI: 10.1016/B978-0-12-818783-8.00002-5

Mattison, K., Karthikeyan, K., Abebe, M., Malik, N., Sattar, S.A., Farber, J.M. & Bidawid, S. (2007). Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *Journal of Food Protection*, 70: 500–503. DOI: 10.4315/0362-028X-70.2.500

McDonnell, S., Kirkland, K.B., Hlady, W.G., Aristeguieta, C., Hopkins, R.S., Monroe, S.S. & Glass, R.I. (1997). Failure of cooking to prevent shellfish-associated viral gastroenteritis. *Archives of Internal Medicine,* 157(1), 111-116. DOI: 10.1001/archinte.1997.00440220117015

McLeod, C., Hay B., Grant C., Greening G., & Day D., (2009). Localization of norovirus and poliovirus in Pacific oysters. *Journal of Applied Microbiology*, 106: 1220-1230. DOI: 10.1111/j.1365-2672.2008.04091.x

McLeod, C., Polo, D., Le Saux, J C. & Le Guyader, F.S. (2017). Depuration and relaying: a review on potential removal of norovirus from oysters. *Comprehensive Reviews in Food Science and Food Safety*, 16(4), 692-706. DOI: 10.1111/1541-4337.12271

Moorman, E.A. (2017). Alternative Chemical Disinfection Technologies for Inactivation of Human Norovirus on Surfaces. Msc Thesis, 80p.

Morse, D.L., Guzewich, J.J., Hanrahan, J.P., Stricof, R., Shayegani, M. & Deibel, R. (1986). Widespread outbreaks of clam-and oyster-associated gastroenteritis. *New England Journal of Medicine*, 314(11), 678-681. DOI: 10.1056/NEJM198603133141103

Muniain-Mujika, I., Girones, R., Tofiño-Quesada, G., Calvo, M. & Lucena, F. (2002). Depuration dynamics of viruses in shellfish. *International Journal of Food Microbiology*, 77(1-2), 125-133. DOI: 10.1016/S0168-1605(02)00324-0

Myrmel, M., Berg, E.M.M., Rimstad, E. & Grinde, B. (2004). Detection of enteric viruses in shellfish from the Norwegian coast. *Applied and Environmental Microbiology*, 70(5), 2678-2684. DOI: 10.1128/AEM.70.5.2678-2684.2004

Nakagawa-Okamoto, R., Arita-Nishida, T., Toda, S., Kato, H., Iwata, H. & Akiyama, M.(2009). Detection of multiple sapovirus genotypes and genogroups in oyster-associated outbreaks. *Japanese Journal of Infectious Diseases*, 62(1), 63-6.

Namsai, A., Louisirirotchanakul, S., Wongchinda, N., Siripanyaphinyo, U., Virulhakul, P. & Puthavathana, P. (2011). Surveillance of hepatitis A and E viruses contamination in shellfish in Thailand. *Letters in Applied Microbiology*, 53(6), 608-613. DOI: 10.1111/j.1472-765X.2011.03152.x

Oka, T., Wang, Q., Katayama, K. & Saif, L.J. (2015). Comprehensive review of human sapoviruses. *Clinical Microbiology Reviews*, 28(1), 32-53. DOI: 10.1128/CMR.00011-14

Parashar, U.D., Hummelman, E.G., Bresee, J.S., Miller, M.A. & Glass, R.I. (2003). Global illness and deaths caused by rotavirus disease in children. *Emerging Infectious Diseases*, 9(5), 565. DOI: 10.3201/eid0905.020562

Patwardhan, M.H. (2019). Heat sensitization of human enteric viruses by plant extracts and validation of their thermal inactivation by bacterial surrogates. Master's Thesis, University of Tennessee. https://trace.tennessee.edu/utk_gradthes/5454

Pavoni, E., Consoli, M., Suffredini, E., Arcangeli, G., Serracca, L. & Battistini, R. (2013). Noroviruses in seafood: a 9-year monitoring in Italy. *Foodborne Pathogens and Disease*, 10(6), 533-539. DOI: 10.1089/fpd.2012.1399

Pinon, A. & Vialette, M. (2018). Survival of viruses in water. *Intervirology*, 61(5), 214-222. DOI: 10.1159/000484899

Prevost, B., Lucas, F.S., Goncalves, A., Richard, F., Moulin, L. & Wurtzer, S. (2015). Large scale survey of enteric viruses in river and waste water underlines the health status of the local population. *Environment International*, 79, 42-50. DOI: 10.1016/j.envint.2015.03.004

Quiroz-Santiago, C., Vázquez-Salinas, C., Natividad-Bonifacio, I., Barrón-Romero, B.L. & Quiñones-Ramírez, E.I. (2014). Rotavirus G2P [4] detection in fresh vegetables and oysters in Mexico City. *Journal of Food Protection*, 77(11), 1953-1959. DOI: 10.4315/0362-028X.JFP-13-426

Rao, V.C., Metcalf, T.G. & Melnick, J.L. (1986). Development of a method for concentration of rotavirus and its application to recovery of rotaviruses from estuarine waters. *Applied and Environmental Microbiology,* 52(3), 484-488. DOI: 10.1128/AEM.52.3.484-488.1986

Richards, G.P. (2013). Foodborne and waterborne enteric viruses. In: Fratamico, P.M., Bhunia, A.K., Smith, J.L. (Eds.), *Foodborne Pathogens Microbiology and Molecular Biology*, Chapter: Foodborne and Waterborne Enteric Viruses. Caister Academic Press, pp. 121-143.

Rivadulla, E., Varela, M.F., Mesquita, J.R., Nascimento, M.S. & Romalde, J.L. (2019). Detection of hepatitis E virus in shellfish harvesting areas from Galicia (Northwestern Spain). *Viruses*, *11*(7), 618. DOI: 10.3390/v11070618

Rodriguez-Lazaro, D., Cook, N., Ruggeri, F.M., Sellwood, J., Nasser, A. & Nascimento, M.S.J. (2012). Virus hazards from food, water and other contaminatedenvironments. *FEMS Microbiology Reviews*, 36, 786–814. DOI :10.1111/j.1574-6976.2011.00306.x

Said, B., Ijaz, S., Kafatos, G., Booth, L., Thomas, H.L. & Walsh, A. (2009). Hepatitis E outbreak on cruise ship. *Emerging Infectious Diseases*, 15, 1738-1744. DOI: 10.3201/eid1511.091094

Sattar, S.A., Springthorpe, V.S. & Ansari, S.A., (1994). Rotavirus. In: Hui, Y.H., Gorham, J.R., Murrell, K.D., Cliver, D.O. (Eds.), *Foodborne Disease Handbook: Diseases Caused by Viruses, Parasites, and Fungi*, vol. 2. Marcel Dekker, New York, NY, pp. 81–111.

Sattar, S.A. (2001). Survival of microorganisms on animate and inanimate surfaces and their disinfection. In: Rutala, W.A. (Ed.), *Disinfection, Sterilization and Antisepsis: Principles and Practices in Healthcare Dacilities.* Association for Professionals in Infection Control and Epidemiology, Inc, Washington, DC, pp. 195–205. Schwab, K. J., Neill, F. H., Estes, M. K., Metcalf, T. G., & Atmar, R. L. (1998). Distribution of Norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR. *Journal of Food Protection*, 61(12), 1674-1680. DOI: 10.4315/0362-028X-61.12.1674

Schwartzbrod, L. (1995). *Effect of human viruses on public health associated with the use of wastewater and sewage sludge in agriculture and aquaculture* (No. WHO/EOS/95.19). World Health Organization.

Sharp, T.M., Crawford, S.E., Ajami, N.J., Neill, F.H., Atmar, R.L. & Katayama, K. (2012). Secretory pathway antagonism by calicivirus homologues of Norwalk virus nonstructural protein p22 is restricted to noroviruses. *Virology Journal*, 9(1), 1-6. DOI: 10.1186/1743-422X-9-181

Sobsey, M.D. (1989). Inactivation of health-related microorganisms in water by disinfection processes. *Water Science and Technology*, 21(3), 179-195.DOI: 10.2166/wst.1989.0098

Sooryanarain, H. & Meng, X.J. (2019). Hepatitis E virus: reasons for emergence in humans. *Current opinion in virology*, *34*, 10-17. DOI: 10.1016/j.coviro.2018.11.006

Souza, D.S.M., Dominot, A.F.Á., Moresco, V. & Barardi, C.R.M. (2018). Presence of enteric viruses, bioaccumulation and stability in Anomalocardia brasiliana clams (Gmelin, 1791). *International Journal of Food Microbiology*, 266, 363-371. DOI: 10.1016/j.ijfoodmicro.2017.08.004

Springthorpe, S.V., Loh, C.L., Robertson, W.J. & Sattar, S.A. (1993). In situ survival of indicator bacteria, MS-2 phage and human pathogenic viruses in river water. *Water Science and Technology*, 27(3-4), 413-420. DOI: 10.2166/wst.1993.0383

Stals, A., Uyttendaele, M., Baert, L, & Van Coillie, E. (2013). Norovirus transfer between foods and food contact materials. *Journal of Food Protection*, 76(7), 1202-1209. DOI: 10.4315/0362-028X.JFP-12-392

Tallon, L.A., Love, D.C., Moore, Z.S. & Sobsey, M.D. (2008). Recovery and sequence analysis of hepatitis a virus from springwater implicated in an outbreak of acute viral hepatitis. *Applied and Environmental Microbiology*, 74(19), 6158-6160. DOI: 10.1128/AEM.02872-07

Terregino, C. & Arcangeli, G. (2017). Shellfish and berries: the ready-to-eat food most involved in human norovirus outbreaks. In *The Norovirus* (pp. 31-47). Academic Press. DOI: 10.1016/B978-0-12-804177-2.00003-8

Teunis, P.F., Moe, C.L., Liu, P., E. Miller, S., Lindesmith, L. & Baric, R.S. (2008). Norwalk virus: how infectious is it?. *Journal of Medical Virology*, 80(8), 1468-1476.DOI: 10.1002/jmv.21237

Tozan, E., Duman, P., Elbasan, H.D., Aktepe, Ö., Sezen, F., Temel, F. & Sucaklı, M.B. (2016). A gastroenteritis outbreak investigation, NIGDE city center, March 2014. *Türkiye Halk Sağlığı Dergisi*, *14*(1), 1-12. DOI: 10.20518/thsd.83892

Tuladhar, E., Hazeleger, W.C., Koopmans, M., Zwietering, M.H., Duizer, E. & Beumer, R.R. (2013). Transfer of noroviruses between fingers and fomites and food products. *International Journal of Food Microbiology*, 167(3), 346-352. DOI: 10.1016/j.ijfoodmicro.2013.09.018

Ueki, Y., Shoji, M., Okimura, Y., Miyota, Y., Masago, Y. & Oka, T.(2010). Detection of sapovirus in oysters. *Microbiology and Immunology*, 54(8), 483-486. DOI: 10.1111/j.1348-0421.2010.00239.x

Van der Poel, W. H. (2014). Food and environmental routes of Hepatitis E virus transmission. *Current Opinion in Virology*, 4, 91-96. DOI: 10.1016/j.coviro.2014.01.006

Vidal, R., Solari, V., Mamani, N., Jiang, X., Vollaire, J. & Roessler, P.(2005). Caliciviruses and foodborne gastroenteritis, Chile. *Emerging Infectious Diseases*, 11(7), 1134. DOI: 10.3201/eid1107.041062

Wang, D., Wu, Q., Yao, L., Wei, M., Kou, X. & Zhang, J. (2008). New target tissue for food-borne virus detection in oysters. *Letters in Applied Microbiology*, 47(5), 405-409. DOI: 10.1111/j.1472-765X.2008.02445.x

Wang, H., Sikora, P., Rutgersson, C., Lindh, M., Brodin, T. & Björlenius, B., (2018). Differential removal of human pathogenic viruses from sewage by conventional and ozone treatments. *International Journal of Hygiene And Environmental Health*, 221(3), 479-488. DOI: 10.1016/j.ijheh.2018.01.012

Ward, R.L., Knowlton, D.R. & Winston, P.E. (1986). Mechanism of inactivation of enteric viruses in fresh water. *Applied and Environmental Microbiology*, 52(3), 450-459. DOI: 10.1128/AEM.52.3.450-459.1986

Webb, G.W., Kelly, S. & Dalton, H.R. (2020). Hepatitis A and Hepatitis E: Clinical and Epidemiological Features, Diagnosis, Treatment, and Prevention. *Clinical Microbiology Newsletter*, *42*(21), 171-179. DOI: 10.1016/j.clinmicnews.2020.10.001

Webby, R.J., Carville, K.S., Kirk, M.D., Greening, G., Ratcliff, R.M. & Crerar, S.K.(2007). Internationally distributed frozen oyster meat causing multiple outbreaks of norovirus infection in Australia. *Clinical Infectious Diseases*, 44(8), 1026-1031. DOI: 10.1086/512807

WHO (2006) The world health report 2006: working together for health. World Health Organization. Guidelines for the safe use of wastewater, excreta and greywater. Volume 2: *Wastewater use in agriculture.* World Health Organisation, Geneva, Switzerland.

WHO (2015). WHO estimates of the global burden of foodborne diseases. *Foodborne Disease Burden Epidemiology Reference Group*, 2007–2015.

WHO (2016). Hepatitis A. Available at: http://www.who.int/mediacentre/factsheets/fs328/en/ (Accessed 17 October 2016).

Widdowson, M.A., van Doornum, G. ., van der Poel, W. ., de Boer, A., Mahdi, U.,& Koopmans, M. (2000). Emerging group-A rotavirus and a nosocomial outbreak of diarrhoea. The Lancet, 356(9236), 1161-1162. DOI: 10.1016/S0140-6736(00)02760-4

Wolff, A., Günther, T., Albert, T., Schilling-Loeffler, K., Gadicherla, A.K. & Johne, R. (2020). Stability of hepatitis E virus at different pH values. *International journal of food microbiology*, *325*, 108625. DOI: 10.1016/j.ijfoodmicro.2020.108625

Woods, J.W. Calci, K.R., Marchant-Tambone, J.G. & Burkhardt III, W. (2016). Detection and molecular characterization of norovirus from oysters implicated in outbreaks in the US. *Food Microbiology*, 59, 76-84. DOI: 10.1016/j.fm.2016.05.009

Yugo, D.M. & Meng, X.J. (2013). Hepatitis E virus: foodborne, waterborne and zoonotic transmission. *International Journal of Environmental Research and Public Health*, 10(10), 4507-4533. DOI: 10.3390/ijerph10104507

Zhang, L., Zhao, N., Sha, J., Wang, C., Jin, X., Amer, S. & Liu, S. (2016). Virology and epidemiology analyses of global adenovirus-associated conjunctivitis outbreaks, 1953–2013. *Epidemiology & Infection*, *144*(8), 1661-1672. DOI:10.1017/S0950268815003246

Zhang, L., Li, K., Huang, S., Liu, D., Rehman, M.U., Lan, Y. & Mehmood, K. (2017). Seroprevalence and risk factors associated with hepatitis E virus infections among people and pigs in Tibet, China. *Acta Tropica*, *172*, 102-106. DOI: 10.1016/j.actatropica.2017.04.033

Zuckerman, J. N. (2003). Hepatitis E and the traveller. *Travel Medicine and Infectious Disease*, 1, 73- 76. DOI: 10.1016/S1477-8939(03)00039-5