AQUATIC SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

VOLUME: 35 ISSUE: 2 2020 E-ISSN 2602-473X

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Publisher

İSTANBUL UNIVERSITY PRESS

İstanbul University Press Journal Adress: İstanbul University Faculty Aquatic Sciences, Ordu Caddesi No:8 34134 Laleli Fatih/İstanbul Turkey E-mail: ase@istanbul.edu.tr

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Editor in Chief: Prof. Devrim MEMİŞ
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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(2): 31-35 • DOI: https://doi.org/10.26650/ASE2020679538

Research Article

The Levels of Plastic-associated Heterotrophic Bacteria on Three Different Types of Plastics

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Cite this article as: Çiftçi Türetken, PS., Altuğ, G., Öksüzoğlu, T. (2020). The levels of plastic-associated heterotrophic bacteria on three different types of plastics. *Aquatic Sciences and Engineering, 35*(2), 31–35.

ABSTRACT

Plastic pollution in marine ecosystems is one of the most important study topics in recent years. The toxicity, mobility and long-term persistence characteristics of plastics create risk in ecosystems, biota and human health. In this study, the levels of heterotrophic bacteria attached to the surfaces of commonly used plastic types; polyvinylchloride (PVC), polyethylene (PE), and polypropylene (PP) were tested in a mechanical experimental system prepared with seawater under controlled conditions in laboratory. The seawater, which was used in the experimental system, was taken under aseptic conditions from the Golden Horn Estuary, located in the Istanbul region of Turkey. Three different types of plastic (PVC, PE and PP), in two different (glass slide (76x26 cm) and virgin micro pellets (5mm diameter) size, were placed in the experiment setup filled with seawater and incubated for 28 days at ambient temperature. At the end of 28 days, the counts of heterotrophic bacteria were tested using the spread plate technique on Marine Agar (Difco), in both plastic surfaces and surrounding seawater. The levels of heterotrophic bacteria were recorded to be lower in the seawater surrounding the micropellets and lam-size plastic samples. The seawater sample bacterial levels were recorded as $12x10^9$ CFU/ml, at the start of the experiment. At the end of the 28th days, it was recorded to be $83x10^9$ CFU/ml. The highest levels of heterotrophic bacteria were recorded as $41x10^{10}$ CFU /cm^{-2} and $61x10^{10}$ CFU /cm^{-2} on the lam-size surfaces and the micropellet surface of the polypropylene samples, respectively. In the experiments, the PP plastic type has been recorded as a more preferred plastic derivative by heterotrophic bacteria according to the PVC and PE plastic types, but there has been no significant difference in the bacterial adhesion rates on the surfaces. The study contributed increasing knowledge on the bacterial approach to microplastics types. However, there is a need for long term studies related to the mechanism of bacteria attached to microplastics.

Keywords: Microplastic, heterotrophic bacteria, polyvinylchloride, polyethylene, polypropylene

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Submitted:
21.06.2019

Revision Requested:
25.11.2019

Accepted:
14.01.2020

Online published:
24.01.2020

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INTRODUCTION

Plastics are synthetic polymers with high insulation against electricity and heat, light, easy to operate structure, and are used in many areas in our daily lives. With the development of synthetic organic polymers in the mid-twentieth century, plastic production in 1950, which was 1.5 million tons per year, exceeded 300 million tons over the years. It is expected to reach 540 million tons in 2020, with the increase in production and consumption (Haward, 2018). Most polymer species are highly persistent in the marine ecosystem. Only when exposed to ultra-violet radiation, they undergo photo catalysis and slowly dissolve. Plastic waste can be of any size, from visible large particles to invisible small particles.

On a global scale, it is estimated that 1.5 million tons of primary plastic are dumped into the

oceans (0.8 million tons/year in the optimist scenario, 2.5 million tons/year in the pessimistic scenario). 98% of the primary microplastic reaching the sea constitutes terrestrial activities and 2% from the activities carried out at sea (Boucher & Friot, 2017). In terrestrial activities, the ratio of those arriving by road transport is 49%, the ratio of those involved by waste water treatment plants is 25%, and the ratio of those transported by wind is 7%.

It is known that microplastics can absorb contaminants in water, toxic compounds such as polychlorobifenil (PCB), and harmful compounds such as nonylphenol (International Pellet Watch, 2018). The release of microplastics with these contaminants in the aquatic environment causes serious effects on the transport of harmful compounds (Mato et al., 2001; Endo et al., 2005).

In addition to the planktonic form, it has been determined that bacteria, known to survive in water as individuals, have developed a different structure in the form of a community in order to continue their lives, as seen in the studies and observations carried out over time (Çiftçi, 2005). The demands of bacteria to hold onto any surface around the sea are intended to evaluate organic matter. The particles of the bacterium called "attachment bacteria" have been identified more intensively than the bacteria that live freely in surface water (Costa, 2011). It is known that microplastics in the marine ecosystem form a suitable environment for bacteria to hold on.

Plastisphere is a term used to describe the habitats on plastics in aquatic ecosystems. The initiation of this mechanism, which is formed by microorganisms, which develops in all kinds of materials, and which is defined as "biofouling", is the attachment of gram negative bacteria to the environment, and then diatoms, protozoa and invertabrates are added to this structure. The long-term permanence of plastics in the marine ecosystem and the transport of bacteria and other microorganisms due to currents causes them to reach different marine areas as invasive species (Zettler et al., 2013; Pauli et a., 2017; Gündoğdu et al., 2017; Hodgson et al., 2018; Rech et al., 2018). These structures formed on plastics increase the weight of micro-macroplastics, and cause them to be transported from surface to bottom in aquatic ecosystems (Kaiser et al 2017; Kooi et al., 2017). For this reasons, the determination of bacterial levels on microplastic surfaces is very important.

The main reason why plastic is widely talked about today and in every aspect of human life is the fact that plastics are not easily degraded and are resistant in nature (Shah et al., 2008; Shimao, 2001). It is known that microorganisms, especially bacteria, are adaptable to ecological niches and natural habitats. The rapid adaptation of microorganisms is also applied to exist in the artificial habitat called Plastic in the oceans. Microbial colonization on the plastic particle in the ocean was reported for the first time in 1972 (Carpenter et al., 1972; Carpenter et al., 1972). Floating plastic parts in the ocean provide an environment for microorganisms to attach (Chen and He, 2015).

The attachment of bacteria to cling to any surface in order to evaluate the nutrients in the environment is more than their tendency to live freely. These tendencies settle on different surfaces in the marine environment and prepare the ground for the collection of bacterial communities. The localization of other microorganisms is a process related to the intensity of the initial bacteria (Hall-Stoodley et al., 2004). There are studies evaluating the binding rates of bacteria depending on the materials. The studies on this subject indicate that the different media characteristics will also be different (Donlan, 2002; Davey and O'toole, 2000).

In this study, the aim is to obtain preliminary data related to the levels of free living and plastic-associated bacteria, using the macro (glass slide size) and micro dimensions of three types of plastics (polyvinylchloride, polyethylene, polypropylene) which are used frequently in Turkey.

MATERIALS AND METHODS

The surface (0-30 cm) seawater sample was taken from Karaköy Port Town in February 2018, under aseptic conditions. The sampling point is shown in Figure 1. The sample was brought to the Istanbul University, Faculty of Aquatic Sciences, with the cold chain on the same day.

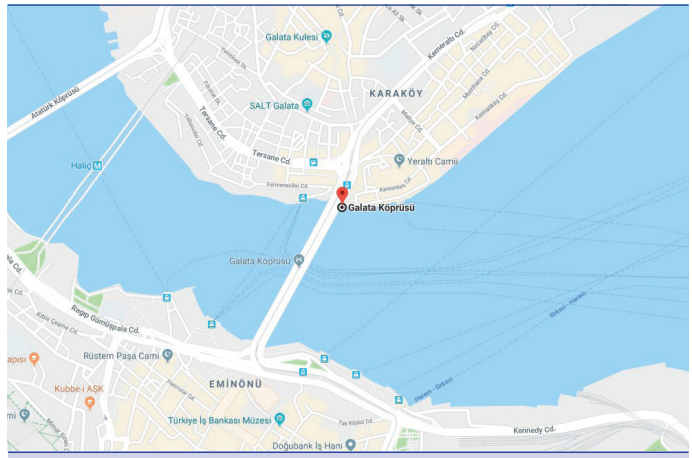

Figure 1. Sampling Point.

Preparation of experimental setup

The experimental devices were prepared in two different ways. The most common used three plastic types were chosen, and they were purchased from a plastic factory. The plastics size tested were arranged considering the experimental setup measurement as glass slide size and 5 mm diameter of micropellet. Similar size materials were used to calculate and compare the area where the bacteria attached. All sizes of polyvinylchloride (PVC), Polyethylene (PE) and Polypropylene (PP) materials were prepared for analysis according to Palanichamy (2002). A 15-liter sea water sample was put into the special design closed circuit system. PVC, PE, PP materials were cut to glass slide (76 x 26 mm) size, and they were sterilized by washing with sterile distilled water. They were placed in the closed circuit system under aseptic conditions to avoid airborne bacterial contamination, and the water circulation rate was set to 13 Hz (Hi-RUN / N100) (Figure 2).

In addition, the 100 ml of seawater samples were introduced into the sterile Erlenmeyer (250 ml) under aseptic conditions. All the micropellet size samples (5 mm in diameter) were weighed as 1g un-

der aseptic conditions, and they were placed in the Erlenmeyer containing seawater. The samples were placed in a shaking incubator, and were incubated at ambient temperature for 28 days (Figure 3).

Bacteriological analysis

The method of spreading plaque to Marine Agar (Difco) was used to determine the heterotrophic bacteria levels in plastic surfaces and the seawater. The Petri dishes were incubated at least 48-72 hours at 22±1 °C. The colony count was carried out and recorded at the end of incubation. All bacteriological analyses were performed in triplicate (APHA, 1998). The first day of bacterial level results were recorded as the bacterial level of the control group, and this data were used to compare the results obtained from the plastic surfaces after the system was shut down (Bordalo et al., 2002).

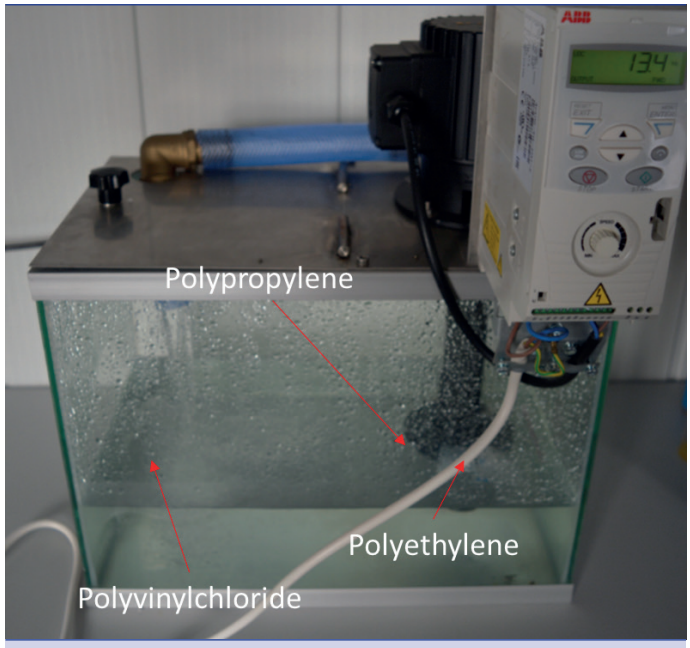

Figure 2. The special design closed circuit system.

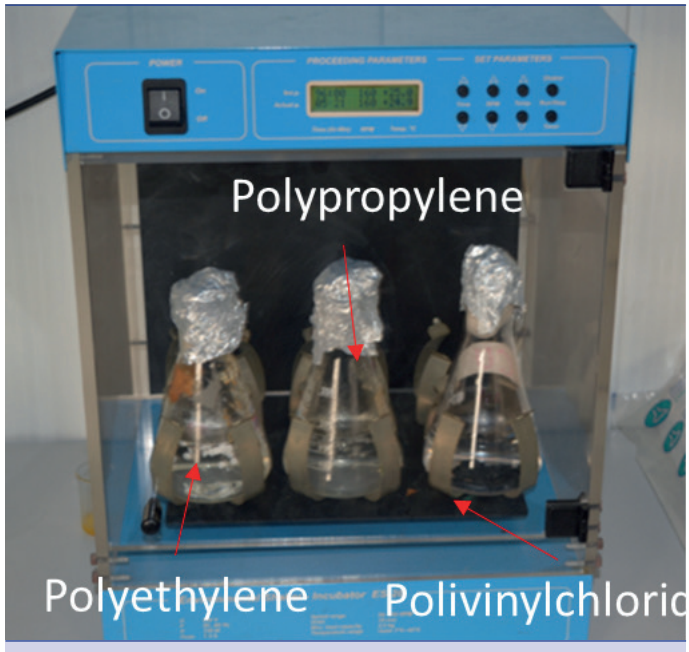

Figure 3. The shaking incubator used in the experiment.

Statistical analysis

Plastic types and bacterial level were compared by one-way ANOVA, followed by Tukey's post-hoc tests. The plastic size and bacterial level tested with one-way ANOVA. Statistical analysis was performed with the SPSS Statistics 21 program.

RESULTS AND DISCUSSION

The results of the first day and last day heterotrophic bacteria levels obtained from the plastic (glass slide size [76 x 26 mm] and micropellet-size [5 mm in diameter]) samples were summarized in Table 1 and Figure 4.

The results of the first day and last day heterotrophic bacteria levels obtained from the sea water samples were summarized in Table 2 and Figure 5.

Table 1. Heterotrophic Bacterial Levels data obtained from plastic samples

		Incubation Time	
		1st day	28th day
Size	Type	CFU/cm^{-2}	
Glass Slide	PVC	-	$45x10^{10}$
	PP	-	$65x10^{10}$
	PE	-	$60x10^{10}$
Micropellet	PVC	-	$48x10^{10}$
	PP	-	$97x10^{10}$
	PE	-	$87x10^{10}$

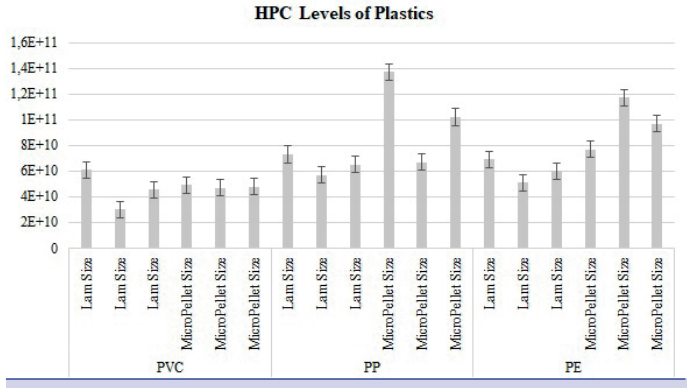

Figure 4. Heterotrophic Bacterial Levels data obtained from plastic samples. *HPC: Heterotrophic Bacterial Count*

Table 2. Heterotrophic Bacterial Levels obtained from the sea water samples

		Incubation Time	
		1st day	28th day
		CFU/ml	
Glass Slide Size	Sea Water	$12x10^9$	$83x10^9$
Micropellet Size	Sea Water (PVC)	$12x10^9$	$20x10^9$
	Sea Water (PE)	$12x10^9$	$86x10^9$
	Sea Water (PP)	$12x10^9$	$42x10^9$

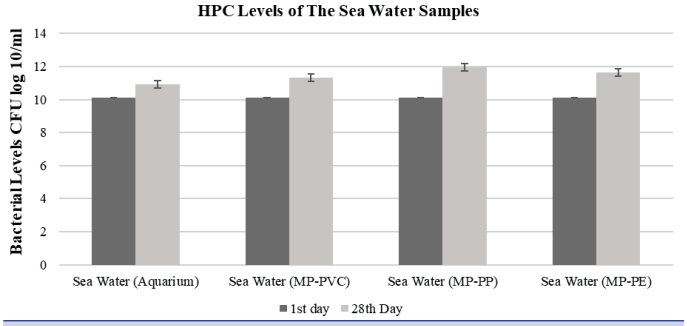

Figure 5. The levels of heterotrophic bacteria obtained from the seawater samples at the first and the end of the experiments. *HPC: Heterotrophic Bacterial Count*

The bacteria levels were found as $45x10^{10}$±2.8 CFU/cm^2, $65x10^{10}$±4.9 CFU/cm^2 and $60x10^{10}$±5.4 CFU/cm^2, respectively, in the glass slide size samples of PVC, PP and PE surfaces. The highest bacterial adhesion rate to glass slide size was detected on the polypropylene (PP) surface. The mean levels of bacteria detected in the PVC, PP and PE micro-pellets was $48x10^{10}$±3.2 CFU/ cm^2, $97x10^{10}$±4.7 CFU/cm^2, and $87x10^{10}$±5.5 CFU/cm^2, respectively, as a result of the 28-day incubation in a shaking incubator. The mean bacterial levels of the seawater including PVC, PP, PE micro pellet were determined as $20x10^9$±8.2 CFU/ml, $86x10^9$±8.2 CFU/ml and $42x10^9$±8.2 CFU/ml, respectively.

The highest total mesophilic aerobic bacteria level was detected on the micro and macro sized polypropylene (PP) plastic surfaces. This finding has shown that the polypropylene plastic variety for the adhesion of bacteria has the appropriate surface and, it was found that there was a statistically significant relationship between the plastic type and the levels of bacteria ($p<0.05$).

Microplastic pellets have higher levels of bacteria than the glass slide size samples ($p<0.05$). This situation is related to the fact that the surface area is larger in the pellet samples. Similarly, Carson et al. (2013) compared microbial colonization on polypropylene, polystyrene and polyethylene in the North Pacific using scanning electron microscopy, and recorded the highest microbial abundance on large surface areas of polystyrene foam. Researches on microbial biofilms on plastic wastes in water have begun to be the subject of more studies in recent years (Davey and O'toole, 2000; McKenney et al., 1998; Muthukumar & Veerappapilla, 2015; Oberbeckmann et al., 2018; Zettler et al., 2013).

Lobelle and Cunliffe (2011) reported that biofilm formation was observed in sinking plastics with early stage biofilm formation on marine microplastic wastes. In the first week of the study, while the number of cultivable heterotrophic bacteria on the plastic surface was $1.4x10^4$ cm^{-2}, it reached $1.2x10^5$ cm^{-2} at the end of the third week. They also did not encounter a species that degrades plastic in early stage biofilm formation (Lobelle and Cunliffe, 2011). The average level of heterotrophic bacteria was reported in the range of 6.4-7.9 CFU/cm^2 in the study conducted on sheet metal, aluminum, stainless steel, glass, galvanized, wood, cotton and rope (Altuğ et al., 2007). In this study, the heterotrophic bacterial levels on PP, PVC and PE surfaces were determined at an average of 11 CFU/cm^2. Zettler et al. (2013) reported that the microbial community on polypropylene and polyethylene particles differed from the community in seawater. In our study, we can sort the plastic varieties according to the bacterial levels they have on them as PP>PE>PVC. The research conducted by Oberbeckmann et al. (2018) examined the microbial biofilm structure and variety on marine microplastics in North European waters in different seasons.

The sector produced 3.5 million tons of plastic raw materials in 2017, and produced around 1.8 million tons in the first 6 months of 2018 all over the world. In Turkey, the total plastic raw materials production was realized at the following rates: low-density polyethylene (31%), high-density polyethylene (9%), PVC (15%), PP (%12), PS (10%), and Polyethylene terephthalate (23%) (AT&T, 2017).

The part of the plastic that is produced in the scope of recycling is not clearly predictable. Plastics such as disposable bags, PET bottles and packaging are known to play a major role as contaminants for our aquatic resources (Browne et al., 2011; Yurtsever, 2015).

CONCLUSION

This research confirms that the plastic surfaces, like all particles in marine ecosystems, might have a potential to carry microbial agents. The domestic and industrial wastes should be prevented from reaching the sea, permanent pollutant sources should be identified and the monitoring studies should be carried out in marine ecosystems. Alternative organic materials should be preferred instead of disposable plastic materials. This study contributes scientific knowledge related for the first time to the levels of heterotrophic bacteria, attached to the plastic surfaces, in our country. Detailed and long-term studies are needed to determine the bacterial profiles and metabolical peculiarities of dominant bacterial species that hold onto plastic surfaces.

Acknowledgment: The authors would like to thank Meryem ÖZTAŞ (MSc), and S. Dilara KARAMAN for their laboratory assistance.

Ethics committee approval: Ethics committee approval is not required.

Conflict of Interests: There are no conflicts of interest to declare.

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(2): 36-42 • DOI: https://doi.org/10.26650/ASE2020596672

Research Article

Age, Growth and Length-weight Relations of Common Sole (*Solea solea* Linnaeus, 1758) from Southern Aegean Sea

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Cite this article as: Cerim, H., Ateş, C. Age, growth and length-weight relations of common sole (*Solea solea* Linnaeus, 1758) from Southern Aegean Sea. *Aquatic Sciences and Engineering, 35*(2), 36–42.

ABSTRACT

In this study, age and sex compositions, length distributions, growth parameters and length-weight relationships of common sole populations were determined in Güllük Bay, southern Aegean Sea, Turkey. Trammel nets and beach seines which have different full mesh sizes were used to obtain samples. Sex ratio (female:male) was found to be 1.14:1. Growth parameters of the common sole in Güllük Bay was described as; L_{∞}=33.95, K=0.208 y^{-1}, t_0=-0.032, L_{∞}=31.98, K=0.236 y^{-1}, t_0=-0.037 y and L_{∞}=29.11, K=0.324 y^{-1}, t_0=-0.030 y, for sexes combined, females and males, respectively. Length-weight relationships for combined sexes, females and males were $W=0.0079L^{3.064}$, $W=0.0072L^{3.101}$ and $W=0.0088L^{3.024}$, respectively. Combined individuals and females showed positive allometric growth and males showed isometric growth. Ages ranged between 0-9 years. Study results could be useful for further common sole fishery management strategies.

Keywords: Common sole, Aegean Sea, trammel net, small-scale fishery, flatfish

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Submitted:
25.07.2019

Revision Requested:
06.12.2019

Last Revision Received:
07.01.2020

Accepted:
14.01.2020

Online published:
28.01.2020

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INTRODUCTION

Flatfishes are a highly diverse fish group. According to taxonomists, 1820 species have been identified, however, 1073 are valid and the Soleidae family includes approximately 281 species (181 valid species) (Eschmeyer & Fong, 2017). 56 of these flatfish species have commercial importance all over the world and 10 in the Mediterranean (Ulutürk, 2012). In Turkey, a few turbots, sole and flounder species are commercial (TUİK, 2016). Common sole (*Solea solea*) is one of the highest commercial flatfish species in Turkey (Türkmen, 2003) as in some other parts of the world (Teixeira, 2007). Therefore, it is a targeted species by fishermen in some periods.

Güllük Bay is an important fishery area for both small-scale and industrial fisheries. Beside trawling and purse-seining, small-scale fishery (especially trammel net fishery) is common in this area. Species-specific fishing gear (such as red mullet nets, shrimp nets, common dentex nets etc.) are densely used. One of these nets is the common sole trammel net and is used in certain periods in Güllük Bay. Especially, the period which is from the middle of September to the middle of February, is locally named as *common sole period*.

Due to having high commercial importance, the common sole stocks need proper management strategies. Therefore, there have been many studies conducted on common sole including growth (Deniel, 1990; Enberg et al., 2008), stock assessment (Mehanna & Salem, 2012), and population parameters (Türkmen, 2003).

Common sole studies are both discontinuous and insufficient in Turkey. The objective of the present study was to determine age and sex compositions, length distributions, growth parameters and length-weight relationships of the common sole population in Güllük Bay for further fishery management strategies.

MATERIALS AND METHODS

Fishing operations were conducted between October 2013 – November 2015 in Güllük Bay and Boğaziçi lagoon, which are in the Southern Aegean Sea, Turkey (Figure 1).

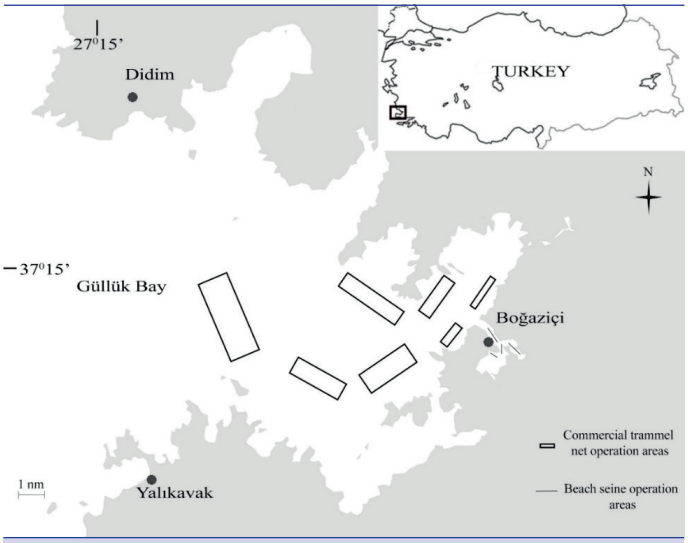

Figure 1. Study area (Cerim, 2017).

Samplings consisted of two parts; first was just total length measurement on board due to difficulties of taking weights of the specimens by a digital laboratory scale, second was laboratory examinations for length, weight and age determinations.

Data were obtained from commercial small-scale fishermen who use 80-90 mm full mesh size trammel nets. Additionally, beach seine and 52-56-64 mm full mesh size PA trammel nets were used to obtain various length classes in the lagoon area. Sampling depths varied between 0.5m and 70m. Samples were stored in ice and were brought to the laboratory.

Total lengths were measured to the nearest 0.1 cm and weighed individually to the nearest 0.01 g. Sagittal otoliths were removed, cleaned and stored in Eppendorf tubes. Otoliths were embedded in polyester, and two thin sections (0.1 mm) were cut along a transverse plane through the focus of the otolith by a Buehler Isomet Lowspeed Saw. The ages were read under a light microscope by three independent experts. Contradictory readings were discarded from age estimations. The theoretical birthday was considered as 1 January (Froglia & Giannetti, 1985). Exact ages were calculated according to sampling month (1 month=0.083 year).

Length weight relationships were calculated according to formula $W=a*L^b$ (Ricker, 1973). Where; L is the total length, W is the total weight, *a* is the intercept of the regression curve and *b* is the regression coefficient. If *b* value is 3, <3 or >3, it means isometry, negative allometry and positive allometry, respectively. Significant difference of *b* values from 3 were tested with the *t*-test (Pauly, 1993).

Growth coefficient (K), age at zero length (t_0) and asymptotic length (L_∞) were estimated with the least square method (Legendre, 1805).

The Chi-square (χ^2) test was used to examine significant differences ($p<0.05$) between sexes.

Growth performance was estimated with the phi-prime test (Ø) (Pauly and Munro, 1984);

$Ø = \log K + 2\log L_\infty$.

RESULT AND DISCUSSION

On board measurements and laboratory examinations

In total, 2165 individuals were evaluated in the study. The total length of 1029 individuals were recorded on board. The total lengths varied between 19.1 and 42.1 cm (Figure 2). The maximum total length, 42.1 cm, was measured on board. It was not possible to determine the sexes of the individuals on board.

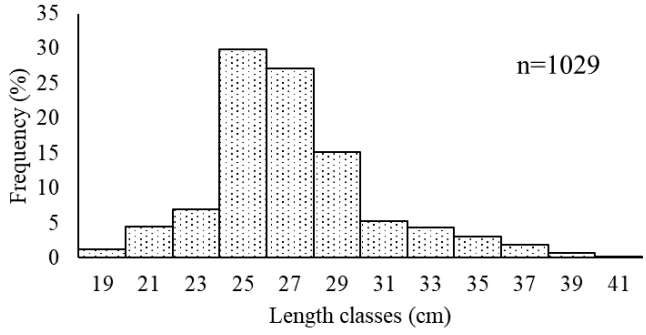

Figure 2. Length-frequency of on board measurements.

According to laboratory examinations, 1136 individuals were evaluated and 607 of the individuals were female and 529 individuals were male. Sex ratio was found as 1.14:1, female to male, respectively. According to the chi square test, a significant difference was found between female and male ($p<0.05$, $\chi^2=5.35$).

Female total lengths ranged between 7.1-31.1 cm and male total lengths ranged between 3.9-28.7 cm (Figure 3).

Total lengths and weights of 1136 individuals were evaluated to determine length and weight relations. Total lengths ranged

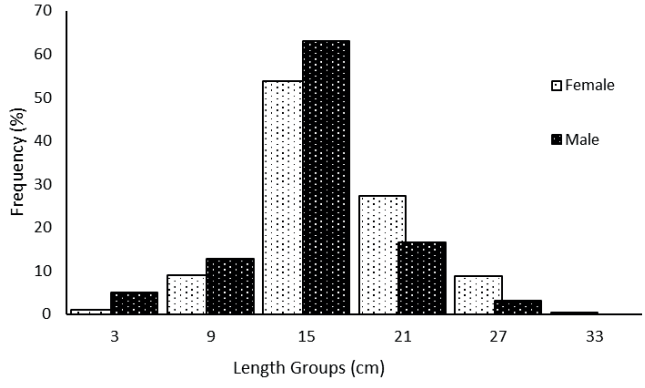

Figure 3. Total length-frequency of common sole.

Table 1. Total length-weight parameters

Sex	n	*a*	*b*	C.I. of *b*	S.E. of *b*	R^2	Growth Type
Combined	1136	0.0079	3.064	3.046-3.080	0.03617	0.9915	A+
Female	607	0.0072	3.101	3.085-3.139	0.03535	0.9866	A+
Male	529	0.0088	3.024	2.997-3.042	0.03586	0.9925	I

from 3.9 to 31.1 cm and weights ranged from 0.24 to 458.67 g. Total lengths and weight regressions were calculated for combined sexes, females and males, separately. *b* values of combined, female and male individuals were compared with isometric growth. While combined individuals and females showed positive allometric growth ($p>0.05$), males showed isometric growth ($p<0.05$) (Table 1).

Length-frequency distributions provide a vision (Khan & Khan, 2014) to help understand when the fishing pressure starts and ends. Length range (2.9-42.1cm) shows us the significance of the area in terms of maintaining common sole's generations. According to Turkish fishery communiques, the minimum landing size of common sole is 20cm. However, an individual with only 9.1 cm total length was caught in a commercial 52mm trammel net. Capture of small individuals by commercial nets means fishing pressure is at the beginning of the common sole lifespan. Cerim & Ateş (2016) found optimum catch sizes to be 23.20 and 27.02 cm for 80 mm and 90 mm full mesh sized trammel nets, respectively. These findings are suitable in terms of length-based fishery management. However, the 16-22 cm length range (the second most observed length range in length-frequency distribution) originated from lagoon samplings (52, 56 and 64 mm full mesh size trammel nets). According to length-frequency distributions, as a great majority of small individuals are captured in the lagoon area, Boğaziçi lagoon area could be characterized as a spawning or nursery area. Therefore, lagoon fisheries should be managed by taking into consideration this feature. On the other hand, if the fishing pressure exceeds the optimal population growth, common sole stocks may collapse in the future.

Age determinations

The otoliths of 235 individuals were used for age determination. Fishes were chosen randomly to obtain various length classes without considering their sexes. Therefore, the number of females and males varies.

Ages were between 0-9 years. Ages distributed between 1-8 year for females (148 individuals) and 1-7 years for males (75 individuals) (Figure 4). For some fish, sex determinations were impossible due to having too small or transparent gonads and no internal organs (occasionally, internal organs are eaten by an Isopod species after the fish is entangled in the net). However, these individuals (i.e. 12 unsexed individuals) were incorporated into age determination (some of 0, 8 and 9 years old individuals belong to this unsexed group). Length related ages of combined females and males are shown in Tables 2, 3 and 4.

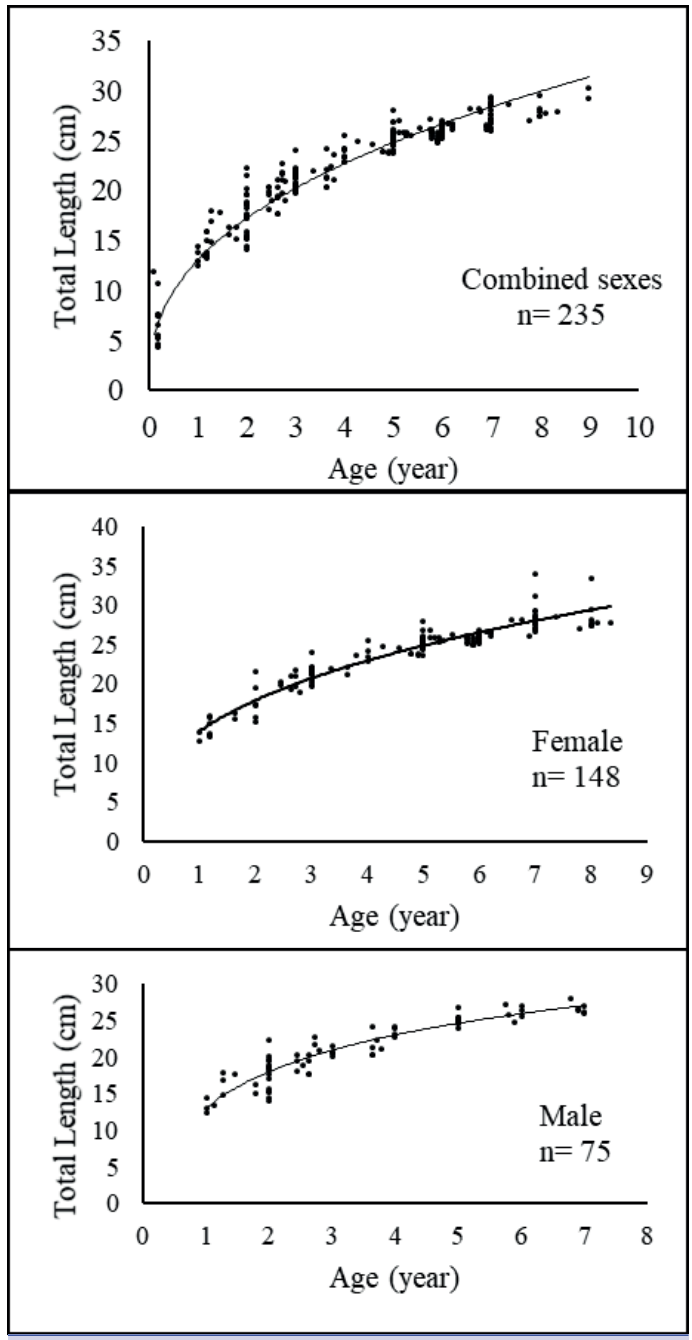

Figure 4. Age-Length relationship of common sole.

Length at ages are given in Table 5. Methods of the previous studies' age determinations were similar. However, some of the total lengths which were related to their ages in other previous studies, are larger or smaller than the present study results. Firstly, the difference in size-compositions could be an effect on these variations. On the other hand, these variations could have originated due to some environmental factors such as pollution (Authman et al, 2015), fishing and temperature (Tu et al., 2018) and especially food availability (Ujjania et al., 2012; Gupta & Banerjee, 2015). Furthermore, size variation may be affected by genet-

Table 2. Age-length key for the common sole in Güllük Bay based on otolith readings

	Age (years)										N
	0	1	2	3	4	5	6	7	8	9	
2-4.9	3										3
5-7.9	7										9
8-10.9		1									1
11-13.9		5	5								10
14-16.9		3	16								19
17-19.9			13	12							25
20-22.9			3	25	10						38
23-25.9				1	8	35	25	7			76
26-28.9						3	14	25	6		48
29-31.9								3	3	2	8
Total	10	9	37	38	18	38	39	35	9	2	
Mean (cm)	5.7	12.8	16.8	20.5	22.6	24.9	25.8	26.8	28.0	30.3	235
S.D.	1.2	1.2	2.3	1.4	1.4	0.7	0.5	0.6	0.5	1.5	

Table 3. Age-length key for female common sole in Güllük Bay based on otolith readings

	Age (years)									N
	0	1	2	3	4	5	6	7	8	
2-4.9										
5-7.9										
8-10.9										
11-13.9		2	2							4
14-16.9		1	9							10
17-19.9			4	6						10
20-22.9			2	19	4					25
23-25.9				1	7	25	16	4		53
26-28.9						2	13	20	6	41
29-31.9								2	3	5
Total		3	17	26	11	27	29	26	9	
Mean (cm)		13.7	16.5	21.1	23.4	24.8	25.8	28.2	28.5	148
S.D.		2.0	2.2	1.1	1.3	0.9	0.5	1.7	1.9	

Table 4. Age-length key for male common sole in Güllük Bay based on otolith readings

	Age (years)								N
	0	1	2	3	4	5	6	7	
2-4.9									
5-7.9									
8-10.9		1							1
11-13.9		3	3						6
14-16.9		2	7						9
17-19.9			9	6					15
20-22.9			1	6	6				13
23-25.9					1	10	9	3	23
26-28.9						1	1	5	7
29-31.9								1	1
Total		6	20	12	7	11	10	9	
Mean (cm)		12.4	17.0	20.1	22.3	25.1	26.1	26.6	75
S.D.		1.4	2.1	1.5	1.5	0.7	0.8	0.6	

Table 5. Age-length distribution of common sole from Güllük Bay and other locations

	Sex	Age (year)										Locations
		0	I	II	III	IV	V	VI	VII	VIII	IX	
Ghirardelli (1959)	F+M	-	16.8	21.4	23.9	25.6	33.1	-	-	-	-	Adriatic
Hoşsucu et al. (1999)	F	-	16.80	21.30	24.52	26.98	29.36	31.90	-	-	-	İzmir Bay
	M	-	15.30	20.06	22.75	25.08	27.04	-	-	-	-	
Oral (1996)	F+M	-	16.42	21.52	25.32	27.65	29.90	31.88	33.20	-	-	Sea of Marmara
	F	-	16.57	22.24	25.70	27.90	29.90	31.88	33.20	-	-	
	M	-	16.83	21.18	23.99	25.68	-	-	-	-	-	
Gonzales & Carillo (1985)	F	-	11.3	17.0	22.9	26.6	32.0	-	-	-	-	Atlantic
	M	-	13.0	16.9	20.3	23.1	26.7	-	-	-	-	
Ramos (1982)	F	-	17.1	22.4	26.5	30.3	33.7	36.3	38.4	-	-	Western Medit.
	M	-	17.5	21.1	24.8	27.4	30.4	33.4	36.0	-	-	
Papaconstantinou et al. (1990)	F+M	-	18.09	24.16	26.61	28.39	25.99	33.0	-	-	-	Aegean Sea
Froglia & Gianetti (1985)	F+M	-	18.0	25.63	30.94	32.5	36.25	-	-	-	-	Adriatic
Gurbet (2000)	F+M	-	-	22.5	25.8	-	-	-	-	-	-	İzmir Bay
	F+M	-	-	20.5	25.1	30.1	-	-	-	-	-	Aliağa-Çandarlı Bay.
	F+M	-	-	21.5	26.0	36.0	-	-	-	-	-	Edremit Bay
Piccinetti & Giovanardi (1984)	F+M	-	18-20	21-30	-	-	-	-	-	-	-	Adriatic
This study	F+M	5.7	12.8	16.8	20.5	22.6	24.9	25.8	26.8	28.0	30.3	
	F	-	13.7	16.5	21.1	23.4	24.8	25.8	28.2	28.5	-	Güllük Bay
	M	-	12.4	17.0	20.1	22.3	25.1	26.1	26.6		-	

Table 6. Some growth parameters of common sole from Güllük Bay and other study locations

	Sex	n	K (yr^{-1})	t_0 (yr)	L_∞ (cm)	Ø*	Locations
Ramos (1982)	F	179	0.220	-0.749	46.40	2.68	Western Mediterranean
	M	151	0.240	-1.085	38.80	2.56	
Froglia & Gianetti (1985)	F+M	671	0.041	-3.574	38.25	1.78	Adriatic
Vianet et al. (1989)	F	287	0.270	-0.410	51.56	2.86	
	M	274	1.030	-0.070	26.38	5.86	Gulf of Lion
	F+M	561	0.240	-0.770	48.83	2.76	
Papaconstantinou (1990)	F+M		0.380	-0.410	34.88	2.66	Amvrakikos Gulf
Deniel (1990)	F	558	0.329	0.075	48.20	2.88	France
	M	351	0.397	0.093	42.40	2.85	
Oral (1996)	F+M	523	0.273	-1.166	37.12	2.58	
	F	218	0.729	-1.065	35.79	2.97	Sea of Marmara
	M	206	0.629	-0.911	28.63	2.71	
Stergiou et al. (1997)			0.380	-0.410	34.90	2.67	Aegean Sea
Hoşsucu et al. (1999)	F+M	340	0.280	-1.109	34.75	2.53	
	F	184	0.170	-1.956	42.45	2.49	İzmir Bay
	M	156	0.330	-1.043	31.14	2.51	
Türkmen (2003)	F	553	0.181	-1.550	29.95	2.21	İskenderun Bay
	M	550	0.221	-1.310	26.03	2.18	
Mehanna & Salem (2012)		2179	0.330	-0.450	44.36	2.81	Egypt
Mehanna et al. (2015)	F+M		0.580	-0.003	35.81	2.87	
	F		0.620	-0.009	36.24	2.91	Egypt
	M		0.550	-0.060	34.77	2.82	
This Study	F+M	1136	0.208	-0.032	33.95	2.38	
	F	607	0.236	-0.037	31.98	2.38	Güllük Bay
	M	529	0.324	-0.030	29.11	2.44	

* Ø estimated by the present author.

ic factors (Exadactylos et al, 2013). Phi-prime values of previous studies showed no differences with the present study ($p>0.05$). Therefore, the growth of common sole could not be correlated with just food availability and other environmental factors could be responsible for length at age variation.

L_∞ values are different from other studies (Table 6). This variation could emanate from different sampling gears and maximum catch lengths. Moreover, the L_∞ of combined sexes was higher than the L_∞ of males and females. This situation was due to the incorporation of unsexed individuals into the age estimation.

Possible effects on growth

Growth of fishes is different from other animals. After maturation, although growth slows down due to the transferring of resources to reproductive parts of body, it continues (Enberg et al., 2008). Flatfishes also have similar lifecycles to other fishes and this similarity is likely to reflect temperature, food availability and energetics (Nash & Geffen, 2015).

Growth of common sole were revealed by different observations and considerations. According to some researchers, growth of common sole does not depend on food limitation (van der Veer et al., 2001; Pihl, 1989). Exadactylos et al. (2013) mentioned a potential genetic effect on growth and size variability in cultured common sole and turbot. Nash & Geffen (2015) stated that many flatfish species show an increased growth rate within increasing exploitation levels due to a decrease in population size and an increase in food availability under these circumstances. On the other hand, during the first 2 to 3 years, juveniles are found in nurseries before migrating to deeper waters (ICES, 2012) and also food availability and temperature effects the growth on the nursery grounds (Nash & Geffen, 2015).

Growth may be affected by interspecific food competitions. Vinagre (2007) found that priority of cohort colonization of both *S. senegalensis* and *S. solea* in estuaries effects growth rate and *S. senegalensis* has a higher growth rate than *S. solea*. In early colonization, low competition and high food availability may affect the growth rate. Besides, Molinero et al. (1991) determined that diets of *S. solea* and *S. senegalensis* are very similar in the western Mediterranean and *S. senegalensis* is now extending its range to the west Mediterranean Sea and is thought to be competing with *S. solea*, at least in the north-west part of the basin (Tous et al., 2015). Similarly, *S. solea* may have food competition with other flatfishes (e.g. *Microchirus ocellatus, Monochirus hispidus, Citharus liguatula, Arnoglossus spp.* etc.).

Except competitions, water pollution may be another reason that effects fish growth. Bhatnagar & Devi (2013) stated that good water quality is important for survival and growth of fish. Güllük Bay sampling area is the most important aquaculture center in Turkey. Yıldız et al. (2002) conducted a study in Güllük Bay about marine pollution sources and they mentioned the Sarıçay river (sewage from the Milas district discharges into the Sarıçay river), Güllük Harbor and marine traffic, tourism, aquaculture systems, domestic wastes and atmospheric pollutants may have a pressure on Güllük Bay. Fish growth may be negatively affected by the existence of the aforementioned pollution types.

CONCLUSION

Monitoring of biological parameters constitutes the main data for fisheries. Therefore, fishery management should be structured on biological data to understand the status and to manage fish stocks. More studies should be conducted in different fishing areas to gain more information about wild stocks for managing commercial flatfish fisheries and aquaculture trials.

Conflicts of interest: The authors have no conflicts of interest to declare.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

Financial Disclosure: This study was funded by Muğla Sıtkı Koçman University, Scientific research Project Office with Phd thesis project number BAP 13/119.

Acknowledgement: We would like to thank Boğaziçi and Göltürkbükü sole fishermen for their valuable support and to Burak YABA and Cihan BULUT for checking grammar and spelling.

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İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(2): 43-51 • DOI: https://doi.org/10.26650/ASE2020646725

Research Article

Water Quality Assessment by Means of Bio-Indication: A Case Study of Ergene River Using Biological Diatom Index

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Cite this article as: Tokatlı, C., Solak, C. N., Yılmaz, E. (2020). Water quality assessment by means of bio-indication: A case study of ergene river using biological diatom index. *Aquatic Sciences and Engineering, 35*(2), 43–51.

ABSTRACT

The Ergene River is the most significant fluvial ecosystem located in the Thrace Region of Turkey. But it is being exposed to an intensive organic – inorganic pollution by means of agricultural – industrial applications conducted around its basin. In this research, the epipelic (EPP) diatoms of the Ergene River were investigated and the water quality was evaluated using the determined physical, chemical and biological data. EPP diatom samples were collected from upstream, middlestream and downstream of the Ergene River and certain physicochemical parameters (dissolved oxygen, oxygen saturation, pH, electrical conductivity, total dissolved solids, salinity, turbidity, nitrate, nitrite, ammonium, phosphate, sulphate, fluoride, chemical oxygen demand, biological oxygen demand and oxidation-reduction potential) were measured during the field – laboratory studies. Also, the Biological Diatom Index (BDI) was used to determine the trophic status of the Ergene River in terms of EPP diatoms. According to the results of the physicochemical analysis, upstream of the Ergene River has Class I – II water quality and middle – downstream of the Ergene River have Class III – IV water quality in general. According to the results of the biological analysis, 24 diatom species were recorded in the upstream samples, 4 diatom species were recorded in the middlestream samples. and 7 diatom species were recorded in the downstream samples. *Cymbopleura amphicephala* (Nägeli) Krammer, *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot and *Nitzschia capitellata* Hustedt were determined as the most dominant species in the up – middle – downstream of Ergene River respectively. According to the result of the BDI, the upstream of Ergene River was found to be in an oligotrophic state – has high water quality and the middle – downstream of Ergene River were found to be in a eutrophic state – have poor water quality.

Keywords: Ergene River, Bentic diatoms, Biological Diatom Index, Water quality

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Submitted:
19.10.2019

Revision Requested:
04.01.2020

Accepted:
11.01.2020

Online published:
10.02.2020

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INTRODUCTION

Diatoms are algae that are the only organism on the planet with cell walls composed of transparent, opaline silica. Diatom cell walls are also ornamented by intricate and striking patterns of silica. Diatoms have chlorophylls a and c that are light-absorbing molecules. They collect energy from the sun and turn it into chemical energy by means of photosynthesis. Diatoms remove carbon dioxide from the atmosphere and convert it to organic carbon, and release the oxygen. Therefore, they are of vital importance for all organisms living in both aquatic and terrestrial environments. Diatoms are particular about the quality of water. For example, species have distinct ranges of pH and salinity where they will grow. They also have ranges and tolerances for other environmental factors, including nitrogenous or phosphorus concentration, flow regime, elevation, and organic or inorganic toxicants. Therefore, diatoms are also vital for assessment and monitoring of the envi-

ronmental condition of water ecosystems (Round et al., 1990; Compton, 2011).

Bioindicator organisms have been widely used in the scientific community for an effective water quality assessment research (Martin et al., 2010; Solak and Acs, 2011; Tokatlı and Dayıoğlu, 2011; Delgado et al., 2012; Atıcı and Udoh, 2016). Diatoms, which are one of the most important aquatic producer groups, can be found in all surface waters all the time. They are accepted as an important part of bioindicator organisms because of having quick reactions to environmental changes. Therefore, diatoms have been used to evaluate environmental conditions in many countries as indicators of water pollution (Ács et al., 2004; Goma et al., 2004; Atıcı and Obalı, 2006; Solak et al., 2007; Kalyoncu et al., 2009; Atıcı and Obalı, 2010; Tokatlı, 2013; Aydın and Büyükışık, 2014; Tan et al., 2017; Tokatlı et al., 2020). Diatom indices are one of the most widely used water quality assessment techniques and the Biological Diatom Index (BDI) is one of the most convenient indexes for evaluating water quality by using diatom communities (Coste et al., 2009).

The Ergene River is the most significant lotic ecosystem for the Thrace Region of Turkey and it is well documented that this system is being exposed to intensive anthropogenic pressure by means of agricultural and industrial applications conducted around the watersheds (Erkmen and Kolankaya, 2006; Tokatlı and Başlatlı, 2016; Tokatlı, 2017). The aim of the present research was to determine the epipelic diatoms of the Ergene River and to evaluate its water quality by using certain physicochemical parameters and the BDI.

MATERIAL AND METHOD

Study area

Water samples and epipelic (EPP) diatoms were collected from 3 selected stations on the Ergene River in autumn (rainy season) of 2018. A map of the Ergene River Basin and the selected stations is shown in Figure 1.

Physical and chemical parameters

The dissolved oxygen, oxygen saturation, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity and ORP parameters were determined using a Hach Lange branded "HQ40D Multiparameter" device during the field studies; the turbidity parameter was determined using a Hach Lange branded "2100Q Portable Turbiditymeter" device during the field studies; the nitrate, nitrite, ammonium, phosphate, sulphate, fluorine and COD parameters were determined using a Hach Lange branded "DR3900 Spectrophotometer" device during the laboratory studies; the BOD parameter was determined using a Hach Lange branded "BOD Trak II Biological Oxygen Demand" device during the laboratory studies.

Epipelic (EPP) diatoms

A glass pipe with a diameter of 0.8 cm and a length of 100 – 150 cm was used for capturing EPP diatom samples. Then the diatom samples collected from the field were cleaned with acid (98% H_2SO_4 and 35% HNO_3) and mounted on a microscope for observation at a magnification of 1000X. Slides were prepared and approximately 400 valves were enumerated on each slide to determine the relation and abundance of each taxa (Sladecova, 1962; Round, 1993). Diatoms were identified according to Cox (1996) and Krammer and Lange-Bertalot (1986; 1988; 1991a; 1991b).

The Biological Diatom Index (BDI)

The Biological Diatom Index (IBD) values of the up – middle – downstreams of the Ergene River were automatically calculated using the "Calculate IBD with Excel" program. The trophic statuses and quality classes of freshwater according to BDI values are given in Table 1 (Lenoir and Coste, 1996).

Table 1. Scale of BDI

Index Value	Quality Class	Trophic Status
> 17	High Quality	Oligotrophic
15 – 17	Good Quality	Oligo – Mesotrophic
12 – 15	Moderate Quality	Mesotrophic
9 – 12	Low Quality	Meso – Eutrophic
< 9	Poor Quality	Eutrophic

RESULTS AND DISCUSSION

Physical and chemical data

The results of the physicochemical data detected in the Ergene River and some national – international limit values are given in Table 2. According to the criteria of the Water Pollution Control Regulation in Turkey, upstream of the Ergene River has Class I – II water quality and middle – downstream of the Ergene River have Class III – IV water quality in general (Uslu and Türkman, 1987; Turkish Regulations, 2015).

It is known that the use of organic and inorganic fertilizers, and municipal and industrial wastewater discharges are the most important factors in increasing the amount of nitrogenous and phosphorus in water (Wetzel, 2001; Manahan, 2011). In a study performed in the Meriç, Tunca and Ergene Rivers, water qualities were investigated. As a result of this research and similar to the present study, water quality of the Meriç, Tunca and Ergene Rivers were reported as Class III – IV in terms of nitrite, ammonium and phosphate accumulations (Tokatlı, 2015). In another study performed in the same river basin, the Meriç – Ergene River ecosystem was found to have Class I – II water quality in terms of temperature, DO, COD, pH, TDS, nitrate, ammonium and sulphate parameters; Class II water quality in terms of nitrite parameters; and Class III – IV water quality in terms of phosphate, BOD and fecal coliform parameters in general (Tokatlı, 2019). According to the DSI observation reports, nitrogen and phosphorus are the main concerns affecting the water quality of Meric – Ergene River Basin (Kendirli et al., 2005). Similar to the data reported by the DSI, the nitrite and phosphate concentrations in the water of middle – downstream of the Ergene River were detected in quite high levels and they have Class III – IV water quality in terms of these parameters.

Biological data

During the present investigation, a total of 31 diatom species were identified from the epipelic (EPP) habitat of the Ergene River by counting a total of 497 valves in the upstream samples, 67

valves in the middlestream and 62 valves in the downstream. A list of identified diatom taxa with the frequency values of the investigated stations is given in Table 3. Also, the microscopy pictures of identified diatoms are given in Figure 2 and the relative abundance values of the detected EPP diatoms (higher than 1%) are given in Figure 3. *Cymbopleura amphicephala* (Nägeli) Krammer, *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot and *Nitzschia capitellata* Hustedt, nom. inval. were determined as the most dominant species in the up – middle – downstream of the Ergene River respectively. *Cymbopleura amphicephala*, which was recorded as the most dominant taxon (relative abundance of 41%) for the upstream samples, is known as a cosmopolitan species found in oligo – mesotrophic waters with a low to moderate electrolyte content. *Nitzschia umbonata*, which was recorded as the most dominant taxon (relative abundance of 79%) for the middlestream, is a common species in eutrophic electrolyte rich waters and tolerating extremely polluted conditions. *Nitzschia capitellata*, which was recorded as the most dominant taxon (relative abundance of 37%) for the downstream, is a widespread species occurring in electrolyte rich and brackish waters and tolerating extremely polluted conditions (Taylor et al., 2007).

The biological diatom index (IBD)

The Biological Diatom Index (BDI), the formula of which was developed by Zelinka and Marvan (1961), is a standardized biological water quality assessment method. It is based on a total of 209 diatom taxa and provides significant information about the tro-

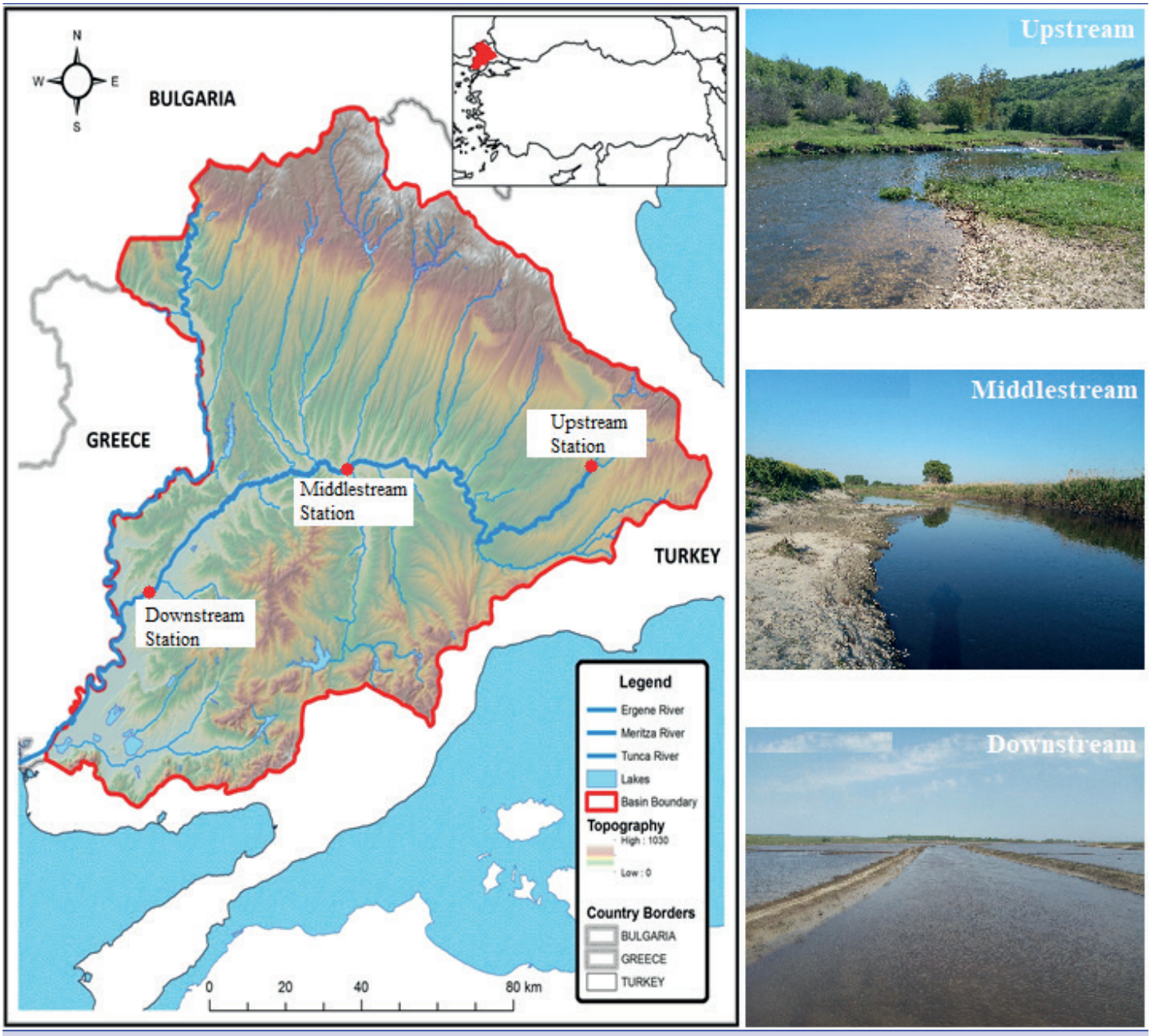

Figure 1. The Ergene River Basin and the selected stations.

Table 2. Results of detected parameters and some national – international limit values

Limit Values and the Results		Parameters															
		DO (mg/L)	OS (%)	pH	EC (mS/cm)	TDS (mg/L)	Sal ($‰_0$)	Tur (NTU)	NO_3 (mg/L)	NO_2 (mg/L)	NH_4 (mg/L)	$*PO_4$ (mg/L)	SO_4 (mg/L)	F (mg/L)	COD (mg/L)	BOD (mg/L)	ORP (mV)
Water Quality Classes (SKKY. 2015)	I. Class	8	90	6.5-8.5	400	500	-	-	5	0.002	0.2	0.02	200	1	25	4	-
	II. Class	6	70	6.5-8.5	1000	1500	-	-	10	0.01	1	0.16	200	1.5	50	8	-
	III. Class	3	40	6.0-9.0	3000	5000	-	-	20	0.05	2	0.65	400	2	70	20	-
	IV. Class	<3	<40	Out of 6.0-9.0	>3000	>5000	-	-	>20	>0.05	>2	>0.65	>400	>2	>70	>20	-
Drinking Water	TS266 (2005)	-	-	6.5-9.5	2500	-	-	5	50	0.5	0.5	-	250	1.5	-	-	-
	EC (2007)	-	-	6.5-9.5	2500	-	-	-	50	0.5	0.3	-	250	1.5	-	-	-
	WHO (2011)	-	-	-	-	-	-	-	50	0.2	-	-	-	1.5	-	-	-
Fish Health (EC. 2006)	Cyprinid Species	4	-	6-9	-	-	-	-	-	0.03	0.2	-	-	-	-	-	-
	Salmonid Species	6	-	6-9	-	-	-	-	-	0.01	0.04	-	-	-	-	-	-
Present Study	**Up stream**	9.66 Class I	107.6 Class I	7.96 Class I	503 Class II	262 Class I	0.26	0.95	0.920 Class I	0.009 Class II	0.012 Class I	0.013 Class I	13.1 Class I	0.087 Class I	5.4 Class I	0.4 Class I	191.6
	Middle Stream	**1.86** Class IV	**20.7** Class IV	7.54 Class I	**2940** Class III	**1622** Class III	1.65	32.30	0.725 Class I	**0.124** Class III	**2.210** Class IV	**1.330** Class IV	185.0 Class I	0.466 Class I	**112.0** Class IV	**21.0** Class IV	143.3
	Down Stream	**3.77** Class III	**42.1** Class III	7.46 Class I	**2910** Class III	**1607** Class III	1.63	17.70	0.592 Class I	**0.144** Class III	**2.180** Class IV	**1.320** Class IV	158.0 Class I	0.583 Class I	**83.3** Class IV	**11.0** Class III	130.0

*: According to another water quality classification specified by Uslu and Türkman (1987)

: Bold data means III. – IV. Class water quality

: Underlined data is not suitable for fish health

DO – Dissolved oxygen, OS – Oxygen saturation, Sal – Salinity, Tur – Turbidity; F: Fluoride; ORP: Oxidation – Reduction Potential

TS266 – Turkish Standards Institute, EC – European Communities, WHO – World Health Organization

Table 3. Identified diatom taxa

Species Code	Diatom Taxa	Upstream	Middlestream	Downstream
1	*Amphora pediculus* (Kützing) Grunow	+	-	-
2	*Cocconeis pediculus* Ehrenberg	+	-	-
3	*Craticula subminuscula* (Manguin) C.E.Wetzel & Ector	-	+	-
4	*Cyclotella meneghiniana* Kützing	+	+	-
5	*Cymbella excisa* Kützing	+	-	-
6	*Cymbopleura amphicephala* (Nägeli) Krammer	+	-	-
7	*Diatoma vulgaris* Bory	+	-	-
8	*Diploneis separanda* Lange-Bertalot	+	-	-
9	*Encyonema ventricosum* (C.Agardh) Grunow	+	-	-
10	*Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin	+	-	-
11	*Gomphonema italicum* Kützing	+	-	-
12	*Grunowia sinuata* (Thwaites) Rabenhorst	+	-	-
13	*Melosira varians* C.Agardh	+	-	+
14	*Navicula amphiceropsis* Lange-Bertalot & U.Rumrich	-	-	+
15	*Navicula antonii* Lange-Bertalot	+	-	-
16	*Navicula capitatoradiata* H.Germain ex Gasse	+	-	-
17	*Navicula cryptocephala* Kützing	+	-	-
18	*Navicula cryptotenella* Lange-Bertalot	+	-	-
19	*Navicula gregaria* Donkin	-	-	+
20	*Navicula radiosa* Kützing	+	-	-
21	*Navicula reichardtiana* Lange-Bertalot	+	-	-
22	*Nitzschia amphibia* Grunow	-	-	+
23	Nitzschia capitellata Hustedt, nom. inval.	-	+	+
24	*Nitzschia dissipata* (Kützing) Rabenhorst	+	-	-
25	*Nitzschia linearis* W.Smith	+	-	-
26	*Nitzschia sublinearis* Hustedt	+	-	-
27	*Nitzschia subtilis* (Kützing) Grunow	+	-	-
28	*Nitzschia umbonata* (Ehrenberg) Lange-Bertalot	-	+	+
29	*Tryblionella hungarica* (Grunow) Frenguelli	-	-	+
30	*Ulnaria acus* (Kützing) Aboal	+	-	-
31	*Ulnaria ulna* (Nitzsch) Compère	+	-	-

phic levels of the investigated aquatic habitat (Coste et al., 2009). In this research, a total of 31 diatom taxa were identified and they were used to calculate the Biological Diatom Index (BDI) scores of the up – middle – downstreams of the Ergene River in order to determine the trophic status. The BDI values of the investigated locations are given in Figure 4. According to the calculated BDI values for the EPP habitats, upstream of the Ergene River was found to be in an oligotrophic state – has high water quality (score range of >17) and middle – downstream of the Ergene River were found to be in a eutrophic state – have poor water quality (score range of <9).

The physical and chemical parameters used to assess water quality may only indicate the current status of the investigated aquatic ecosystem. However, the bioindicator organisms like diatoms may indicate the long-term changes in water ecosystems. Therefore, they have been widely used for the bio-assessment of aquatic habitats in almost all the countries of Europe due to their broad distribution and their quick reaction to environmental changes in water (Acs et al., 2004; Torissi and Dell'Uomo, 2006; Solak and Acs, 2011). Many diatom indices have been developed and they are widely used to determine the quality and trophic levels of water ecosystems. In a study performed in Poland, the Biological Diatom Index (BDI) was used for the assessment of water quality in the Pilica River. As a result of this study, the ecological state of the Pilica River changed from good (oligo – mesotrophic) to moderate (mesotrophic) (Szulc and Szulc, 2013). In another research performed in Vietnam, the BDI was used to evaluate the water quality of the Dong Nai River. As a result of this study, the water quality of the investigated river varied from good (oligo – mesotrophic), moderate (mesotrophic), to low (meso – eutrophic) levels (Pham, 2017). Several studies have also been carried out in different aquatic habitats of Turkey. Gürbüz and Kıvrak (2002) applied Saprobity Index (SI) and Trophic Diatom Index (TDI) in order to assess the water quality of Karasu River. According to the results of this investigation, the Karasu River was found to be in a eutrophicated state and organically polluted. Kalyoncu et al. (2009) investigated the Darıören Stream by ecological methodologies to evaluate the impact of pollution on epilithic diatom assemblages. Solak (2011) also used the SLA, EPI-D, TDI and DESCY indices to evaluate the water quality of the Upper Porsuk River (Kütahya). In two studies performed in the Gürleyik and Seydisuyu Streams, the BDI was used to assess the water qualities and the results of these studies showed that in line with the investigated physicochemical data, the Gürleyik and Seydisuyu Streams were found to be in a mesotrophic state (Tokatlı, 2012, Atıcı et al., 2018).

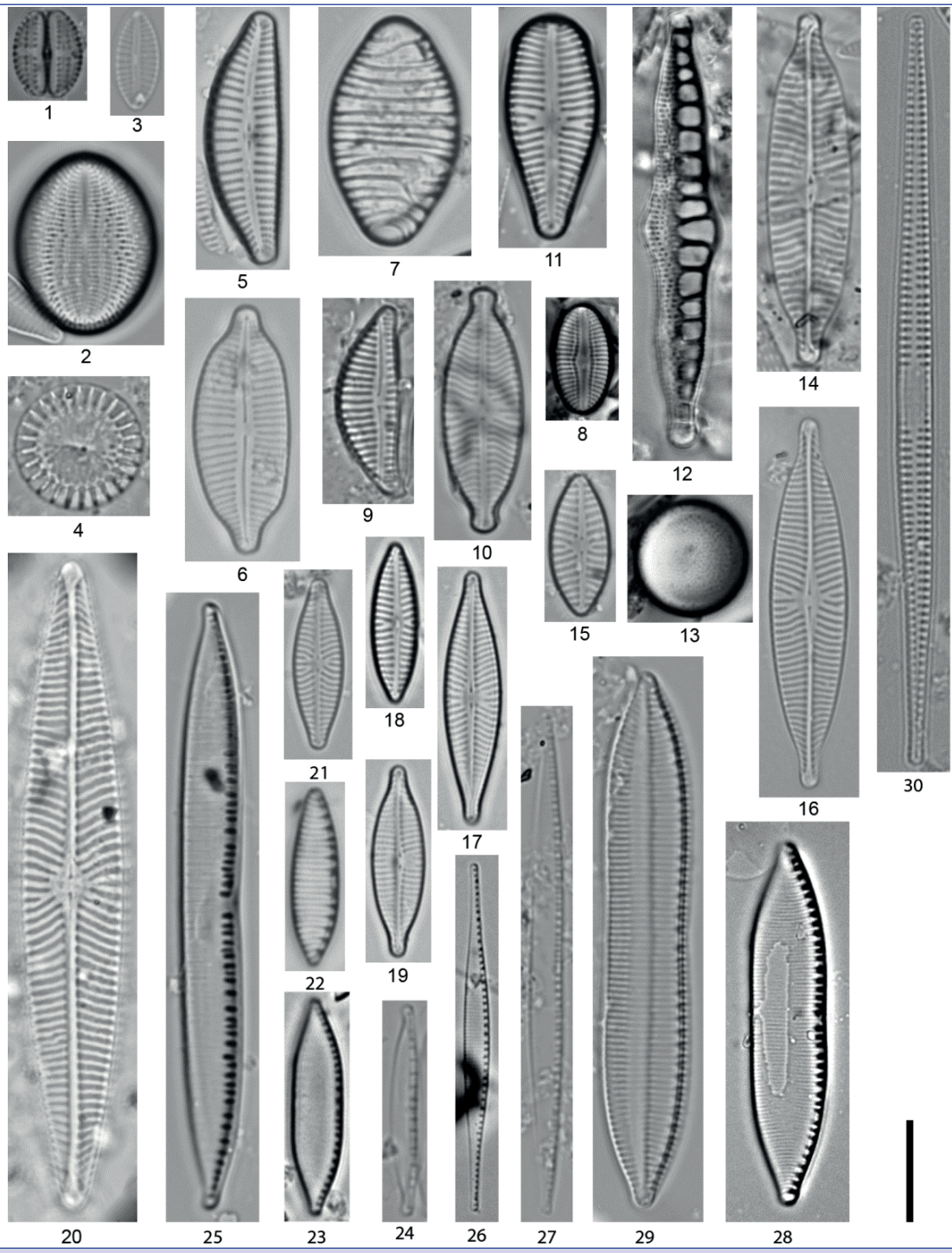

Figure 2. Microscopy pictures of Ergene River diatoms.

In the present investigation, the BDI was used to assess the water quality of the Ergene River and the detected data was compared with the results of limnological data. Similar to the resulting limnological data, upstream of the Ergene River was found to be in an oligotrophic state and has high water quality according to the result of the BDI and has Class I – II water quality according to the results of physicochemical parameters. Middle – downstream of the Ergene River were found to be in a eutrophic state and have poor water quality according to the result of the BDI and have Class III – IV water quality according to the results of physicochemical parameters. The detected similarities in water quality status between the results of the BDI scores and the psychochemical parameters indicate that the BDI may be used to reflect changes in ecological conditions of the Ergene River.

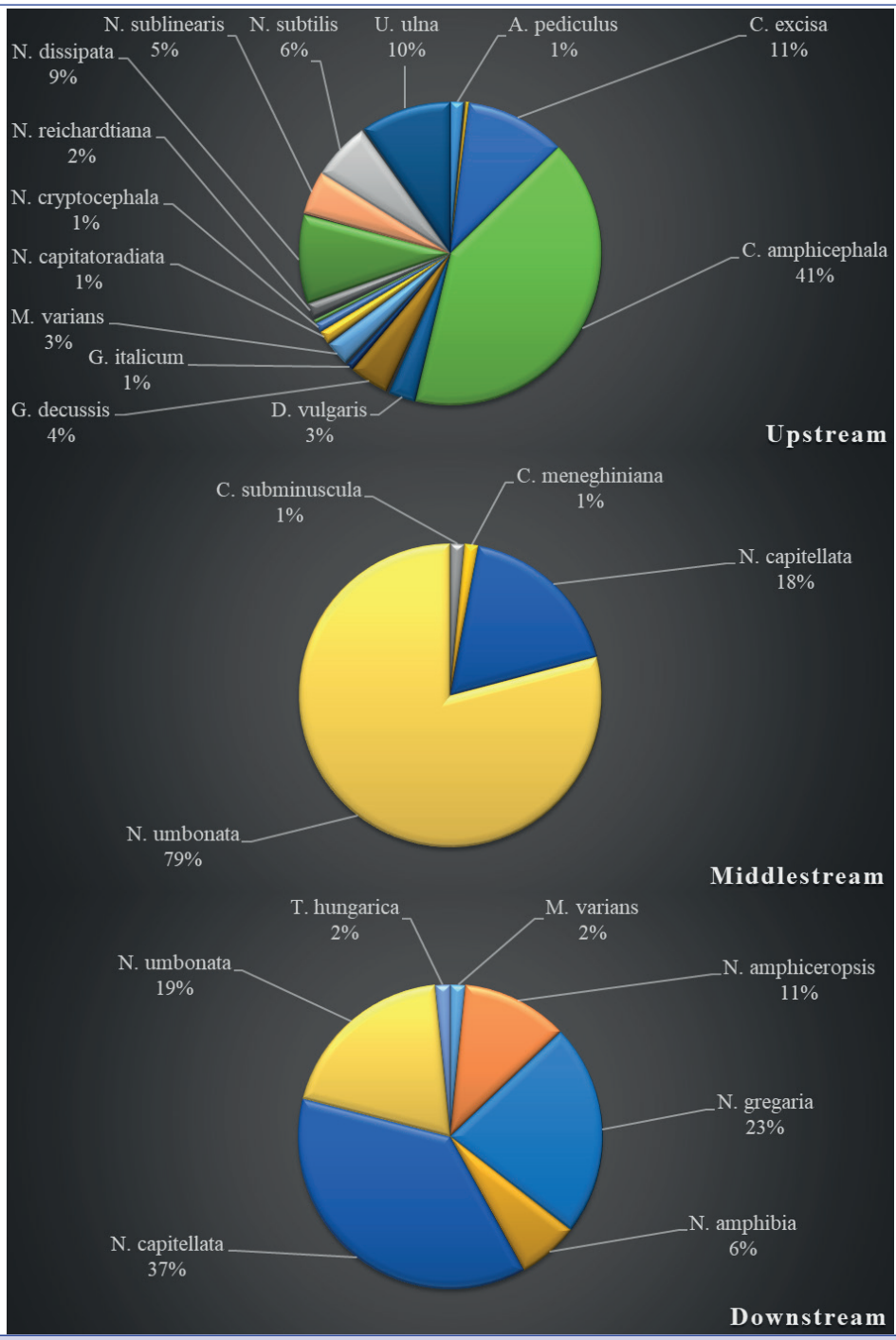

Figure 3. Relative abundance of diatoms in the Ergene River.

CONCLUSION

It is clearly known that biological water quality assessment is much more effective than investigated physicochemical data in terms of reflecting any environmental effects on aquatic ecosystems. Therefore, in order to make a better aquatic ecosystem quality assessment research, physicochemical data should be supported by biological data. In the present study, the epipelic diatoms of the Ergene River were investigated and the water quality of this significant river was evaluated using the Biological Diatom Index (BDI).

As a result of this research, 24 diatom species were recorded in the upstream samples, 4 diatom species were recorded in the middlestream samples and 7 diatom species were recorded in the downstream samples. *Cymbopleura amphicephala* which has a narrow ecological valence and low tolerance, *Nitzschia umbonata* and *Nitzschia capitellata* which have a wide ecological

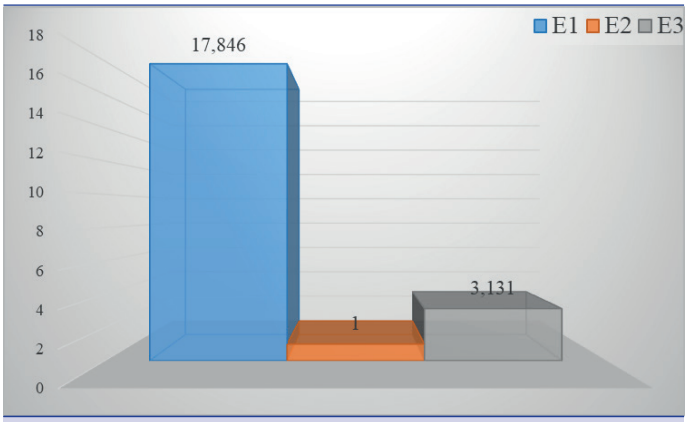

Figure 4. BDI scores of the Ergene River.

valence and high tolerance were determined as the most dominant species in the up – middle – downstreams of the Ergene River respectively. It was also determined that upstream of the Ergene River was found to be in a Class I – II water quality status and middle – downstream of the Ergene River were found to be in a Class III – IV water quality statuses according to the results of limnological parameters. And, similar to the abiotic data, upstream of the Ergeen River was found to be in an oligotrophic state and has high water quality and middle –downstreams of the Ergene River were found to be in a eutrophic state and have poor water quality according to the results of BDI.

The results of this study also revealed the benefits of using biotic and abiotic factors together in water quality assessment studies and showed that minor changes in environmental conditions may cause major effects in the diatom communities. While the sampling frequency is perhaps not sufficient and more research is needed for the assessment of quality status of the investigated water ecosystem, the results of the present research do have the characteristics of a preliminary research with the aim of providing resources for any future bioindication investigation in the region.

Financial Disclosure: The present study was funded by the Commission of Scientific Research Projects at Trakya University (Project No. 2018/154).

Ethics committee approval: Ethics committee approval is not required.

Conflict of Interest: The authors have no conflicts of interest to declare.

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İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(2): 52-56 • DOI: https://doi.org/10.26650/ASE2020652073

Research Article

Bacterial Community Composition of Sapanca Lake During a Cyanobacterial Bloom

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Cite this article as: Özbayram, E. G., Köker, L., Akçaalan, R., İnce, O., Albay, M. (2020). Bacterial community composition of sapanca lake during a cyanobacterial bloom. *Aquatic Sciences and Engineering, 35*(2), 52–56.

ABSTRACT

Microbial community compositions and functions of freshwater ecosystems vary due to the environmental parameters and water chemistry. Transient bloom events play a crucial role on the community profiles. In this study, a specific focus was set to provide a snapshot of the bacterial community composition in Lake Sapanca, associated with cyanobacterial bloom by high throughput sequencing method. For this purpose, a sample was collected in the shore of Lake Sapanca during a cyanobacterial bloom, and the bacterial community profile was examined by 16S rRNA amplicon sequencing using the Illumina MiSeq platform. Cyanobacteria represented 94% of the all reads. The bacterial community was re-calculated to evaluate the bacterial diversity in detail by filtering cyanobacterial sequences. The community was dominated by Proteobacteria (44%) and Bacteroidetes (33%) species which are abundant in freshwater ecosystems having an ability to degrade complex organics. Among the classified genera, *Flavobacterium* and *Rheinheimera* dominated the bacterial community suggesting a strong link between those species and the cyanobacterial bloom. The experimental work presented here provides one of the first investigations of total bacterial communities in Lake Sapanca by the high throughput sequencing method. Further work is needed with more sampling points and time series to fully understand the bacterial diversity and dynamics.

Keywords: Bacterial community, cyanobacterial bloom, illumina miseq, Sapanca lake, 16S rRNA

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Submitted:
28.11.2019

Revision Requested:
17.01.2020

Last Revision Received:
20.01.2020

Accepted:
21.01.2020

Online published:
30.01.2020

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INTRODUCTION

Freshwater habitats have a vital role in global biogeochemical cycles. However, cyanobacterial blooms are getting more widespread in freshwater lakes due to nutrient runoff and climate change, and have become a serious risk on the sustainability of these ecosystems (Cai et al., 2014; Liu et al., 2019) which cause difficulties to secure and maintain ecosystem health (Woodhouse et al., 2016).

Microorganisms drive crucial functions in the freshwater ecosystems and have a role in the degradation of organic materials, energy conversion and nutrient recycling, overall contribute to the ecosystem balance (Su et al., 2017a; Zhu et al., 2019). Microbial community compositions and functions vary due to the water chemistry, nutrient concentrations, hydrodynamic stability and climate (Steffen et al., 2012), and they respond to environmental alterations quickly being critical indicators (Su et al., 2017b). Furthermore, community structures are highly dependent on transient bloom events which affect the abundance and activity of these communities (Eiler & Bertilsson, 2004). Since microbial communities are key players on biogeochemical cycles, there is still limited information on the characterization and function of bacterial communities inhabiting freshwater

ecosystems, especially during bloom events (Eiler & Bertilsson, 2004; Steffen et al., 2012).

Recent developments in advanced molecular genetic technologies enable us to have deep information about microbial diversity and interactions between the community structure and their environment (Nakatsu, Byappanahalli, & Nevers, 2019). Since the 16S rRNA gene is considered as a molecular marker of prokaryotes and used to investigate microbial communities in a wide variety of habitats, it has also been used to asses microbial community dynamics during cyanobacterial blooms (Nakatsu et al., 2019; Zhu et al., 2019).

Lake Sapanca, a freshwater lake, is located in the northeast of the Marmara Region of Turkey. It is a source for drinking water for the cities of Sakarya and Kocaeli, as well as for industrial usage (Akçaalan et al., 2014; Leroy & Albay, 2010), and has been monitored according to the presence of cyanobacteria and cyanotoxin for years to indicate the ecological status of the lake, and it has now a well-established cyanobacteria diversity (Akçaalan et al., 2007; Akçaalan et al., 2014). Moreover, there are some studies in the literature on Lake Sapanca which evaluated the total bacterial counts, pathogenic bacteria and petroleum-resistant bacteria presence by a culture dependent technique (Altuğ et al. 2006; Çiftçi Türetken et al., 2018), and seasonal dynamics of pathogens by the microarray method (Akçaalan et al., 2018). So far, there have been no attempts to examine the total bacterial community structure in Lake Sapanca, and it has remained unclear. This study, therefore, sets out to assess the bacterial community composition in Lake Sapanca during a cyanobacterial bloom by the high throughput sequencing method. The experimental work presented here provides one of the first investigations into the bacterial community profile of Lake Sapanca by a next generation sequencing platform.

MATERIALS AND METHODS

Physico-chemical characterization

A surface cyanobacterial bloom occurred in Lake Sapanca on 8 April, 2019 and the sample was collected during the bloom event in the shore. pH, temperature and dissolved oxygen were measured with a portable multiparameter (6600, YSI, USA) on the sampling date.

DNA extraction and amplicon sequencing

First, 10 mL the sample was filtered with a 0.22 µm filter, and total DNA was extracted from that filter paper using a MoBio PowerWater® DNA Isolation Kit (MoBio Laboratories, Inc., CA, USA) according to the manufacturer's protocol. The DNA quantification was performed by NanoDrop 1000 (Thermo Fisher Scientific, Inc., DE, USA), and the extracted DNA was stored at -20°C for further analysis.

The bacterial community composition of the sample was analyzed with the ZymoBIOMICS™ Service - Targeted Amplicon Sequencing (Zymo Research, Irvine, CA). 16S ribosomal RNA gene targeted sequencing was performed using the Quick-16S™ NGS Library Preparation Kit (Zymo Research, Irvine, CA). Shortly, the 16S primers used amplified the V3-V4 region of the 16S rRNA gene (341f-CCTACGGGNGGCWGCAG and 805r-GACTACHVGGGTATCTAATCC). The PCR products were quantified with qPCR fluorescence readings, and pooled together based on equal molarity. The final pooled library was cleaned up with Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation® and Qubit®. The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with >10% PhiX mix and in paired-end mode.

The Dada2 pipeline was used to infer the amplicon sequences from raw reads (Callahan et al., 2016). The raw sequence reads were trimmed with Trimmomatic-0.33 (Bolger, Lohse, & Usadel, 2014). Whereas, SeqPrep were used to assemble the two paired-end reads to have a complete amplicon sequence with (https://github.com/jstjohn/SeqPrep). Usearch (v. 6.1) was used to check and remove chimeric amplicon sequences (Edgar, 2010) in ref mode against a curated database (http://drive5.com/uchime/rdp_gold.fa). Amplicon sequences smaller than 320 bp were removed. For each sample, up to 40,000 sequences were randomly sampled to reduce the potential bias caused by uneven sampling. These amplicon sequences were compiled, clustered and analyzed with Qiime 1.9.1 (Caporaso et al., 2010). OTUs were picked by the workflow of pick_open_reference_otus.py using the GreenGene database (gg_13_8) as reference database. Singleton OTUs were removed. Taxonomy assignment was performed with Qiime v.1.9.1 (Caporaso et al., 2010). The microbial community structures were shown by Krona graphs (Ondov, Bergman, & Phillippy, 2011; Ozbayram et al., 2017).

RESULTS AND DISCUSSION

The physical properties of Lake Sapanca during the bloom are depicted in Table 1. The water was slightly alkaline and the characteristics of the lake matched those observed in early studies (Akçaalan et al., 2007, 2014) showing a typical O_2 saturation level during the bloom event with high dissolved oxygen.

Table 1. Physical properties of Lake Sapanca during the cyanobacterial bloom

Parameter	Lake Sapanca
Temperature (°C)	15.9
pH	8.51
Conductivity (uS/cm)	215.6
Dissolved Oxygen (mg/L)	13.57
O_2 Saturation (%)	130.3

The number of raw reads and after filtration were 78,735 and 65,748, respectively and the rarefaction curve reached a plateau. The microbial community composition of the bloom sample was presented at multiple taxonomic levels by a Krona chart in Figure 1. The microbial community comprised 7 phyla, however, the microbial community was dominated by Cyanobacteria members, representing 94% of all sequences as it was expected. At the genus level, all of the Cyanobacteria reads belonged to *Planktothrix*. The results are in keeping with previous observational studies,

which showed that the cyanobacterial bloom was mainly caused by *Planktothrix rubescens* in Lake Sapanca (Akçaalan et al., 2007, 2014). Proteobacteria and Bacteroidetes were the following phyla, representing 5% of the microbial community. The results are in agreement with those of previous studies, indicating that the bacterial community was dominated by Proteobacteria, Actinobacteria, and Bacteriodetes during the phytoplankton bloom (Berg et al., 2009; Eiler, Bertilsson, & Centre, 2007; Zhu et al., 2019).

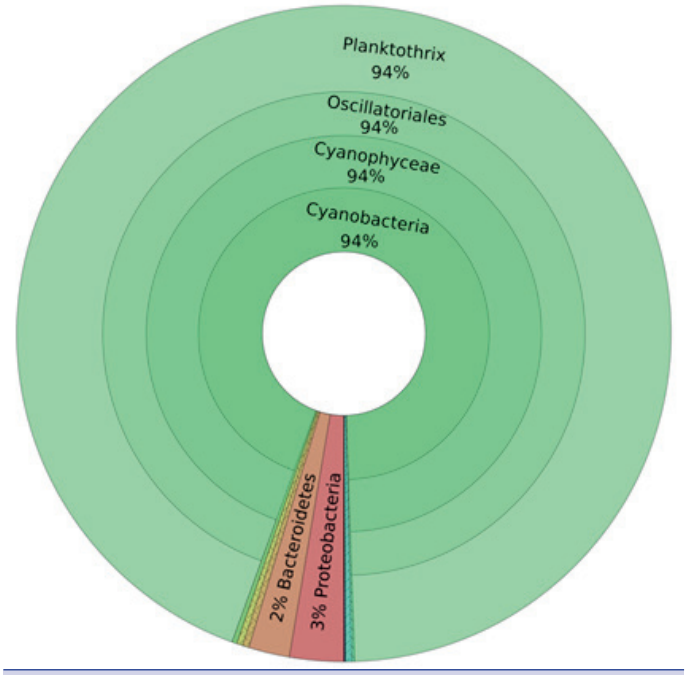

Figure 1. Krona chart illustrating the microbial community composition.

To understand the microbial community diversity better, the bacterial reads were evaluated excluding Cyanobacterial sequences, and re-calculated relative abundances of the bacterial community are depicted in Figure 2. As it is clear from the chart, more than half of the reads were represented by Proteobacteria (44%) and Bacteroidetes (33%). Actinobacteria was the third dominant phylum, representing only 5% of the bacterial community, followed by Planctomycetes (5.3%), Verrucomicrobia (5%) and Gemmatimonadetes. Proteobacteria and Bacteroidetes have the ability to become abundant in the presence of bioavailable organic material, and dominate the community (Eiler et al., 2007). The members are known to be able to decompose complex organic materials, and the peptides of the organic carbon plays a crucial role with their ABC membrane transporters which may support toxin degradation (Lezcano et al., 2017).

Within Proteobacteria, Betaproteobacteria was the dominant class, representing almost half of the total reads in Proteobacteria, followed by Gammaproteobacteria and Alphaproteobacteria. Betaproteobacteria was found as abundant in freshwater ecosystems (Zhu et al., 2019). Flavobacteriia (29%) was the dominant class within Bacteroidetes, and showed a relatively high abundance compared to Sphingobacteriia (2%) and Cytophagia (2%). Most of the Flavobacteriia members are chemoorganotrophs, and are able to use complex organic materials as a carbon source (Parulekar et al., 2017). In terms of family level, 25% of the bacterial community was represented by Flavobacteriaceae (phylum: Bacteroidetes), which was by far the most abundant among all the families. Comamonadaceae (phylum: Proteobacteria) was the second most abundant family, representing 14% of the total reads. Moreover, 9% of the bacterial community was assigned to Chromatiaceae (phylum: Proteobacteria). The families Burkholderiaceae, Sphingomonadaceae, Pseudomonadaceae, Cryomorphaceae and Phycisphaeraceae together represented 18% of the bacterial community.

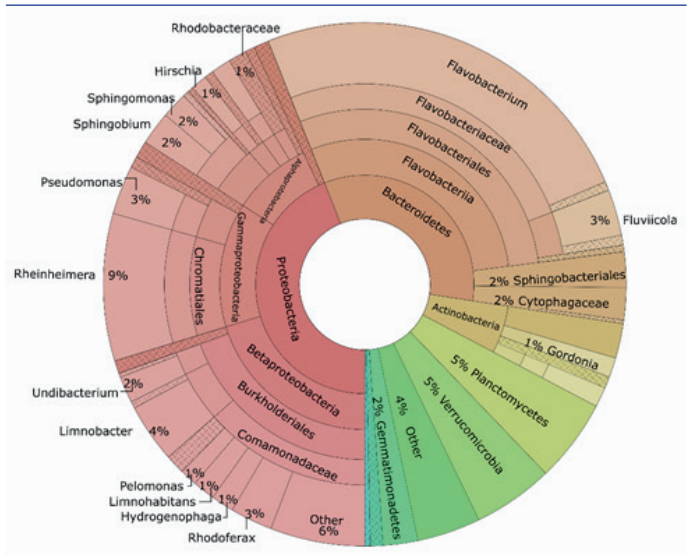

Figure 2. Krona chart illustrating the bacterial community composition (excluding Cyanobacteria).

The overgrowth of Cyanobacterial species causes bacterial community changes with an increasing abundances of the members which have an ability to decompose organic matter and toxic compounds (Lezcano et al., 2017; Su et al., 2017b). Thus, it is expected to observe Bacteroidetes members in high abundance during the phytoplankton blooms (Eiler et al., 2007). Among the classified genera, *Flavobacterium* (phylum: Bacteroidetes) dominated the bacterial community, accounting for 25% of the total sequences (Figure 3). The high abundance suggests that a strong link may exist between the bloom and *Flavobacterium* species which can degrade various biomacromolecules and carbohydrates. The members can react to transient nutrient loads immediately, which is a result of phytoplankton blooms (Buchan, LeCleir, Gulvik, & González, 2014). Whereas some of *Flavobacterium* species have a potential to degrade cyanotoxin, some of them are reported to have a role in denitrification (Parulekar et al., 2017). *Rheinheimera* was the second most dominant genus, representing 9% of the bacterial community. *Rheinheimera* has also higher abundances in the aquatic environments and can hy-

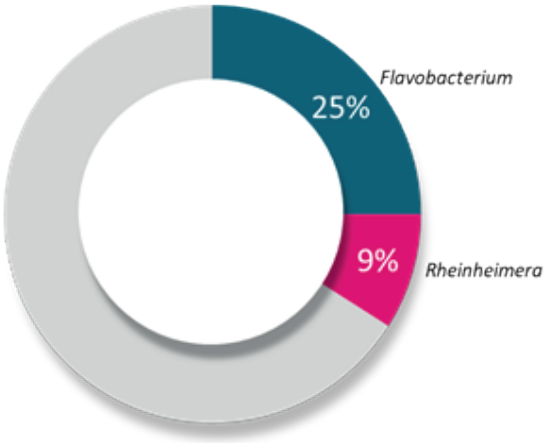

Figure 3. The dominant bacterial genera representing more than 5% of the all sequences are shown in colors. The grey part represented the rest of the bacterial genera which represented <5% of the all sequences.

drolyze organic materials. It is speculated that, *Rheinheimera* species can regulate phosphate exchange in the cyanobacterial mucilage capsule resulting in the enhancement of *Microcystis* growth (Parulekar et al., 2017).

CONCLUSION

The present research explores, for the first time, the bacterial communities associated with cyanobacterial blooms in Lake Sapanca by 16S rRNA targeted amplicon sequencing. This study has shown that the bacterial community was dominated by bloom-associated phyla, Proteobacteria and Bacteroidetes, having the ability to grow on complex organic materials.

These findings provide a snapshot of the bacterial communities in Lake Sapanca during the cyanobacterial bloom. Further work is needed, with more sampling, to fully understand the bacterial diversity and dynamics.

Conflict of Interest: The author has no conflicts of interest to declare.

Ethics Committee Approval: Ethics committee approval is not required.

Financial Disclosure: The authors declared that this study received no financial support.

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İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(2): 57-63 • DOI: https://doi.org/10.26650/ASE2020654619

Research Article

Age and Growth of Red Porgy, *Pagrus pagrus* from the Island of Gökçeada, North Aegean Sea

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Cite this article as: Ayyıldız, H., Altın, A., Kızılkaya, B. Age and growth of red porgy, *Pagrus pagrus* from the Island of Gökçeada, North aegean sea. *Aquatic Sciences and Engineering, 35*(2), 57-63

ABSTRACT

The aim of this study was to describe the age, growth and relationships between otolith morphometry and fish length, weight and age of the red porgy, *Pagrus pagrus*. This study was carried out from the shores of Gizli liman to Kefalos Cape in the north of Gökçeada Island, between March and June 2018. The samples were collected at depths of 40-120 m by long lines. The 66 individuals obtained from red porgy ranged in total length (TL) from 13.5 to 50 cm. The calculated length–weight relationship was determined as $W=0.016TL^{2.9653}$ ($R^2=0.98$). Individual ages were macroscopically determined by counting the annuli of sagittal otoliths. Estimated ages ranged from 1–12 years. The von Bertalanffy growth curve was fitted to the age/total length data as follows: $L\infty=51.48$ cm, K= 0.18 and $t_0=-0.27$. The red porgy sagittal otolith length, width and mass were measured between 4.96–14.72 mm, 3.49–7.85 mm and 0.0199–0.2460 g, respectively. No significant differences in otolith morphometry were found between left and right otoliths. This study provides valuable data for the stock assessment of common pandora in the fishing grounds of Gökçeada Island located in the North Aegean Sea.

Keywords: Age structure, otolith morphometry, *Pagrus pagrus*, Gökçeada

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Submitted:
03.12.2019

Revision Requested:
18.02.2020

Last Revision Received:
19.02.2020

Accepted:
01.03.2020

Online published:
12.03.2020

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INTRODUCTION

Age and growth data provide important information about both individuals and their populations (Michael et al., 2013). Thus, determining the age and growth of the fish species has been and will continue to be one of the most important activities carried out by fisheries biologists (Jackson, 2007). Age and growth information are helpful in describing the present status and past history of fish populations along with the future program of the fishery management (Khan & Khan, 2014). Otoliths are highly useful anatomical structures because they provide the most accurate estimates in determining the age of many fish species (Campana & Thorrold, 2001). Otoliths are used in balance and/or hearing in all bony fishes (Campana, 1999). In the inner ear, there are three pairs of otoliths called lapillus, asteriscus and sagitta (Green et al., 2009; Tuset et al., 2008). These are natural data loggers that record information about growth and environment at different temporal scales into their microstructures and chemistry (Campana, 1999; Kalish, 1989). In addition, otoliths show a complete chronological record of the life of fish (Campana, 1999). Otoliths continue to grow throughout the life of the fish, and this growth occurs by the accumulation of protein and calcium secreted by the macular cells surrounding the surface of the otolith (Campana & Neilson, 1982). The use of otoliths to determine the age of the fish began in 1899 with the observations of Reibisch (Campana, 1999). Age estimates were made by counting the macro bands representing the annual growth rings in the otoliths of the adult individuals of some tropical fish.

The red porgy, *Pagrus pagrus*, is a demersal marine fish associated with a variety of temperate to

subtropical habitats (Labropoulou et al., 1999; Vassilopoulou & Papaconstantinou, 1992). This species is distributed throughout the Atlantic Ocean and Mediterranean Sea at depths of 18 to 280 m (Manooch & Hassler, 1978). Adults of this species inhabit rocky or gravel habitats (Alekseev, 1982; Manooch & Hassler, 1978). This species is a protogynous hermaphrodite that reveals an unbalanced sex ratio in favor of females (Manooch & Hassler, 1978; Vassilopoulou & Papaconstantinou, 1992). Red porgy is a carnivorous fish species that can reach a weight of up to 15-20 kg. It has great economic importance for coastal fisheries in the Turkish waters. According to Türkstat data, commercial landings of red porgy have experienced a serious decline since 2009. Based on these data, it can be said that red porgy stocks are being overexploited.

The red porgy is listed in the IUCN Red List of Threatened Species as a species of least concern, with a recommendation of improved and targeted fisheries regulations and protection for this species (Russell et al., 2014). It is important to study these species, broadly distributed and economically important, in order to implement management policies. The purpose of our study was to determine the age of red porgy accurately in order to develop age-length keys and growth model from Gökçeada Island, Turkey. We also examined the relationship between otolith morphometric measurements (length, width, and mass) and the total length of the fish.

MATERIAL AND METHODS

This study was carried out from Gökçeada Island, Turkey (Figure 1). Samplings were conducted using long lines, from 40-120 m depths between March and June 2018.

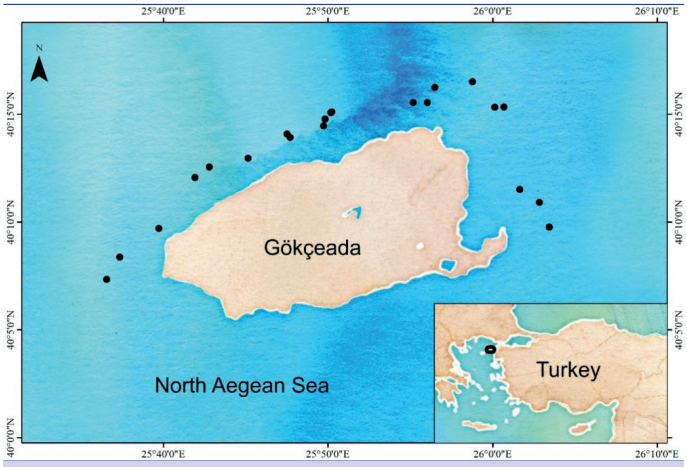

Figure 1. Sampling stations where red porgy, *Pagrus pagrus* were collected with long lines from the island of Gökçeada, Turkey, March – June 2018.

Length-weight relationships

The red porgy was measured for total length (TL) and total weight (W). Sexes were determined using morphological examination in the laboratory. The Mann–Whitney U test was applied to examine the differences between sexes according to the total length. The relationship between the total length and the weight was calculated for each sex separately using a power function:

$$W = aTL^{b}$$

where, b is the regression coefficient and a is the regression constant. The regression parameters a, b and the coefficient of determination (R^2) were estimated for all individuals and for each sex. The allometric index value (b) was compared to the theoretical value of 3 by a t-test (Zar, 1984).

Age and growth

Sagittal otoliths of red porgy were extracted, dried and stored in eppendorf tubes. One otolith was randomly selected and immersed in a plastic vial with glycerine solution for an hour. Sagittal otolith annual rings were counted from the core to the outer edge under a light microscope (Figure 2). Two readers independently counted the annual rings without prior knowledge of fish length. Estimates of the precision of growth ring counts between readers were determined using the average percentage error (APE) of (Beamish & Fournier, 1981) and coefficient of variation (CV) (Chang, 1982).

The von Bertalanffy growth curve was fitted to the length at age data using non-linear least squares parameter estimation (Von Bertalanffy, 1938);

$$TL = L_{\infty}\,[1\text{-}e^{(-K(t-t0))}]$$

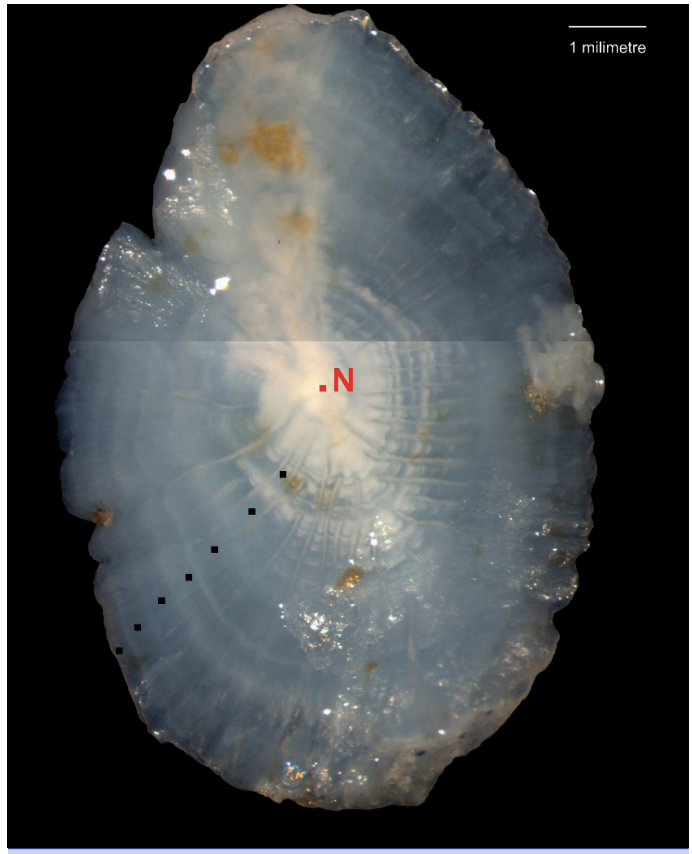

Figure 2. Image of the annual rings seen in a sagittal otolith of red porgy, *Pagrus pagrus* collected from Gökçeada, Turkey. This fish was aged at 7 years and had a total length of 37.5 cm female.

where TL is the fish length at age t, L_∞ the theoretical asymptotic length, K the growth rate coefficient, and t_0 the theoretical age when the fish length is zero.

Otolith morphometry

Otolith length (OL) and width (OW) were measured to the nearest 0.01 mm using Q Capture Imaging Software and weighed (OM) to the nearest 0.00001 g using a Shimadzu electronic balance. OL was defined as the longest axis between the anterior and posterior otolith edge and OW as a distance from the dorsal to the ventral edge (Figure 3). Differences between left and right otoliths were tested by paired t-test. The relationships between the fish length and the otolith morphometry were investigated. Relations between age and otolith weight were calculated using the exponential model.

RESULTS AND DISCUSSION

A total of 66 red porgy, *Pagrus pagrus,* were sampled from Gökçeada, Turkey. The red porgy individuals ranged in size from 13.5 to 50 cm total length (Table 1). Female and male total lengths ranged from 19.9 to 37.5 cm and from 13.5 to 50 cm, respectively (Figure 4). The Mann–Whitney test revealed significant differences between sexes, regarding TL (U=203, Z=2.2117; $p<0.05$). The overall male: female ratio (M:F=1:2) was biased in favor of females. The maximum length and weight reported in this study are the biggest reported values from Turkey. In their studies from Turkey İşmen et al. (2013) and Ozvarol (2014) reported that the larger individuals were 44.5 and 19 cm in total length, respectively.

Figure 3. Morphometric measurements of red porgy sagittal otolith.

Length-weight relationships

The parameters of the length–weight relationships are provided for each sex and all individuals in Table 2. Our data suggested that red porgy from Gökçeada showed negative allometric growth. The parameter b of length–weight relationships was significantly different from 3 ($p<0.05$). The allometric exponent b of females was greater than males. Females grew isometrically while males grew allometrically. Most of the previous studies have shown that red porgy showed negative allometric growth (İşmen et al., 2013; Pajuelo & Lorenzo, 1996; Vassilopoulou & Papaconstantinou, 1992).

Age and growth

Ages were determined successfully from 66 otoliths of common pandora that ranged in size from 13.5 to 50 cm TL. Based on the annual growth ring counts of red porgy otoliths, the maximum observed age was 12 years and the minimum observed age was 1 year old (Table 3). Most of the fish, accounting for 78.8% of the total sample, were between 2 and 4 years old. The APE and CV were calculated as 7.4% and 5.2%, respectively. Only males represented age classes of older than 8 years.

The Von Bertalanffy (1957) model was used to describe red porgy growth (Figure 5). The estimated parameters of the equation were; L_∞=51.48; W_∞=1904.7; K=0.1861 and t_0=-0.27.

The calculated asymptotic length for the red porgy of the Gökçeada population shows similarity with other studies especially for the population in the Aegean Sea and the Gulf of Mexico (İşmen et al., 2013; Nelson, 1988; Vassilopoulou & Papaconstantinou, 1992). Lower asymptotic lengths were presented for the populations in Buenos Aires, South Atlantic and in the Southern Aegean Sea (Cotrina & Raimondo, 1997; Harris & McGovern, 1997; Machias et al., 1998). The calculated asymptotic length values given by some authors (Cotrina & Raimondo, 1997; Harris & McGovern, 1997; Machias et al., 1998) are smaller than those of the older individuals obtained from our study. These differences may be due to the size range of the sampled fish and/or the environmental factors where the samples were collected. The findings observed in this study mirror those of previous studies (Hood & Johnson, 2000; İşmen et al., 2013; Potts & Manooch III, 2002) that found a lower k value indicating a slower growth rate.

Otolith morphometry

Otolith length, width and mass ranged between 4.96–14.72 mm, 3.49–7.85 mm and 0.0199–0.2460 g, respectively (Table 4). No significant differences in otolith morphometrics were found be-

Table 1. Summary of the total lengths in centimeters of red porgy collected from Gökçeada. The number of specimens (n) and range, mean, and standard deviation of the mean (SD) for total length are provided.

Capture Date	Female					Male					Undetermined				
	n	Min.	Max.	Mean	SD	n	Min.	Max.	Mean	SD	n	Min.	Max.	Mean	SD
March 18	3	26.3	27.1	26.63	0.42	…	…	…	…	…	…	…	…	…	…
April 18	33	19.9	37.5	26.04	3.26	12	19.9	50	31.88	8.55	10	18.1	22	19.59	1.18
May 18	…	…	…	…	…	6	13.5	37.4	25.65	8.53	1	19.6	19.6	19.6	…
June 18	…	…	…	…	…	…	…	…	…	…	1	22.1	22.1	22.1	…

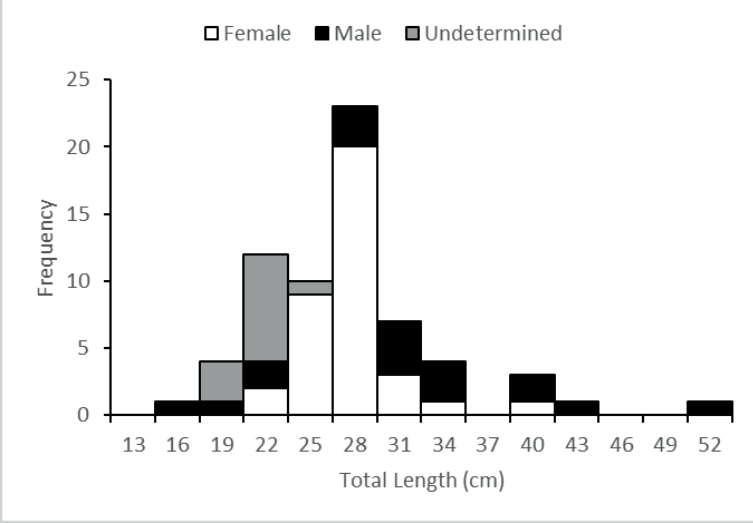

Figure 4. Length–frequency distribution of males and females of red porgy collected from Gökçeada, Turkey.

Table 2. Parameters of the length–weight relationships for males, females, and all individuals of red porgy from Gökçeada, Turkey.

	n	a	b	R^2	p
Female	36	0.0129	3.031	0.964	<0.01
Male	18	0.0210	2.887	0.996	<0.01
Undetermined	12	0.0267	2.784	0.692	<0.01
All	66	0.0160	2.965	0.986	<0.01

Table 3. Age–length key for red porgy from Gökçeada, Turkey.

Total Length (cm)	Age (Year)										
	1	2	3	4	5	6	7	8	9	12	n
13 - 16.9	1										1
17 - 20.9	1	10	3								14
21 - 24.9			12								12
25 - 28.9			11	14							25
29 - 32.9				2	2	1		1			6
33 - 36.9					1	1	1				3
37 - 40.9							2	1			3
41 - 44.9									1		1
45 - 48.9											0
49 - 52.9										1	1
n	**2**	**10**	**26**	**16**	**3**	**2**	**3**	**2**	**1**	**1**	**66**

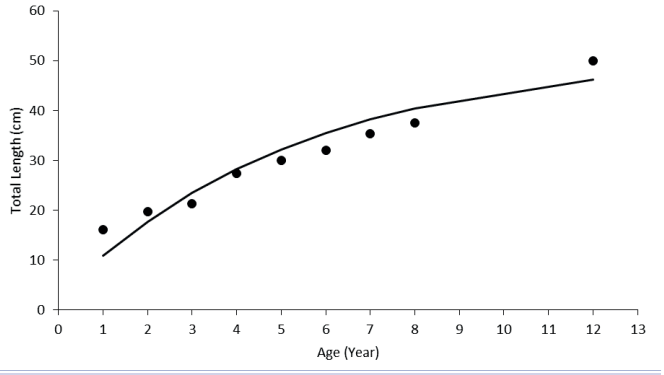

Figure 5. The von Bertalanffy growth curves for red porgy from Gökçeada, Turkey.

tween left and right otoliths (paired t-test, P>0.05). OL, OW and OM showed significant linear relations with the TL (Table 5). The relationship between otolith morphometry and age is shown in Figure 6. An exponential model explains between 80% and 91% of the variation in age.

Otolith morphology has proven to be a powerful tool in species discrimination of many teleost fishes (Rani et al., 2019; Volpedo & Echeverria, 2003). This paper investigates the relationship of fish TL and age with otolith morphometrics (OL and OW) by a power model and OM by linear regression. The results of this study indicate that OL, OW and the OM were linearly correlated to the fish TL. The OM was found to be a better parameter than the others (OL and OW) in estimating fish length and weight. These results are consistent with those of other studies and suggest that fish size and weight could be estimated using the otolith morphometrics (Altin & Ayyildiz, 2018; Ayyildiz & Altin, 2018; Ayyildiz et al., 2014; Yilmaz et al., 2015). In addition, the best model for predicting the fish age of common pandora was found to be OM in this study.

CONCLUSION

In conclusion, the findings of the present study show that total length and weight can be estimated from its otolith morphometric measurements or vice versa. Information about the otolith morphometry could prove to be an important tool for the identification of fish species and determines the prey size that is obtained from the stomach contents of piscivorous predators. In addition, most of the individuals we caught in our study were between 2 and 4 years old. There are no restrictions on fishing for red porgy in Turkish waters nor on the amount that can be caught. However, as this species can reach sexual maturity at the age of 2-4 years when their length corresponds to about 24 cm, the results of this study indicate that it is proper to catch this species in this region when the fish are between 2 and 4 years old. The results provide essential information needed for management policies of red porgy from the North Aegean Sea. Further studies will be required on the age, growth and reproduction of this species to enable effective and sustainable management of red porgy fisheries.

Table 4. Otolith length (OL), width (OW) and mass (OM) measurements according to the age of red porgy from Gökçeada, Turkey.

Age	Left										Right									
	OL				OW			OM			OL				OW			OM		
	Min.	Mak.	Ort.	Std	Min.	Mak.	Ort.	Std	Min.	Mak.	Min.	Mak.	Ort.	Std	Min.	Mak.	Ort.	Std	Min.	Mak.	Ort.	Std
1	5.01	5.01	5.01	---	3.5	3.5	3.5	---	0.02	0.02	0.02	---	4.96	4.96	4.96	---	3.49	3.49	3.49	---	0.02	0.02	0.02	---
2	6.94	7.63	7.28	0.29	4.11	4.74	4.43	0.26	0.04	0.04	0.04	0	7.01	7.42	7.27	0.19	4.17	4.71	4.43	0.23	0.04	0.04	0.04	0
3	6.99	9.33	7.92	0.77	4.54	5.49	4.94	0.31	0.05	0.08	0.06	0.01	7.28	9.36	8.11	0.74	4.45	5.5	4.85	0.38	0.04	0.08	0.06	0.02
4	8.77	9.95	9.34	0.47	5.33	5.85	5.59	0.22	0.08	0.10	0.09	0.01	8.90	9.83	9.39	0.45	5.22	5.7	5.47	0.2	0.08	0.10	0.09	0.01
5	9.815	10.82	10.28	0.51	5.85	6.41	6.1	0.28	0.11	0.11	0.11	0.01	9.98	10.74	10.37	0.38	5.72	6.24	5.96	0.26	0.11	0.11	0.11	0.01
6	10.15	10.83	10.55	0.35	6.26	6.4	6.34	0.07	0.12	0.13	0.13	0.01	10.58	11.03	10.88	0.26	5.87	6.25	6.11	0.21	0.12	0.13	0.12	0.01
7	11.36	12.06	11.62	0.39	6.34	6.97	6.69	0.32	0.14	0.16	0.15	0.01	11.41	12.3	11.74	0.49	6.2	6.80	6.45	0.31	0.13	0.16	0.15	0.01
8	10.73	11.82	11.28	0.77	6.71	7.03	6.87	0.23	0.15	0.15	0.15	---	11.92	11.92	11.92	---	6.74	6.74	6.74	---	0.15	0.16	0.16	0.01
9	13.23	13.23	13.23	---	7.56	7.56	7.56	---	0.19	0.19	0.19	---	13.52	13.52	13.52	---	7.37	7.36	7.37	---	0.19	0.19	0.19	---
10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
11	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
12	14.53	14.53	14.53	---	7.8	7.8	7.8	---	0.25	0.24	0.25	---	14.72	14.72	14.72	---	7.85	7.85	7.85	---	0.24	0.243	0.24	---

Table 5. Parameters of the relationships between the otolith morphometry and the fish total length and weight for red porgy from Gökçeada, Turkey

Otolith–fish relationships	Model	n	a	b	r^2	p
TL - OL	Linear	66	-8.49	3.83	0.96	<0.05
TL - OW	Linear	66	-15.66	7.65	0.96	<0.05
TL - OM	Linear	66	11.97	158	0.99	<0.05
W - OL	Exponential	66	0.058	3.79	0.96	<0.05
W - OW	Exponential	66	0.105	4.56	0.96	<0.05
W - OM	Exponential	66	11415	1.53	0.98	<0.05

n= number of specimens, a= slope of the regression line, b= y-intercept, r^2= coefficient of determination; TL= fish total length, OL= otolith length, OW= otolith width, OM= otolith mass

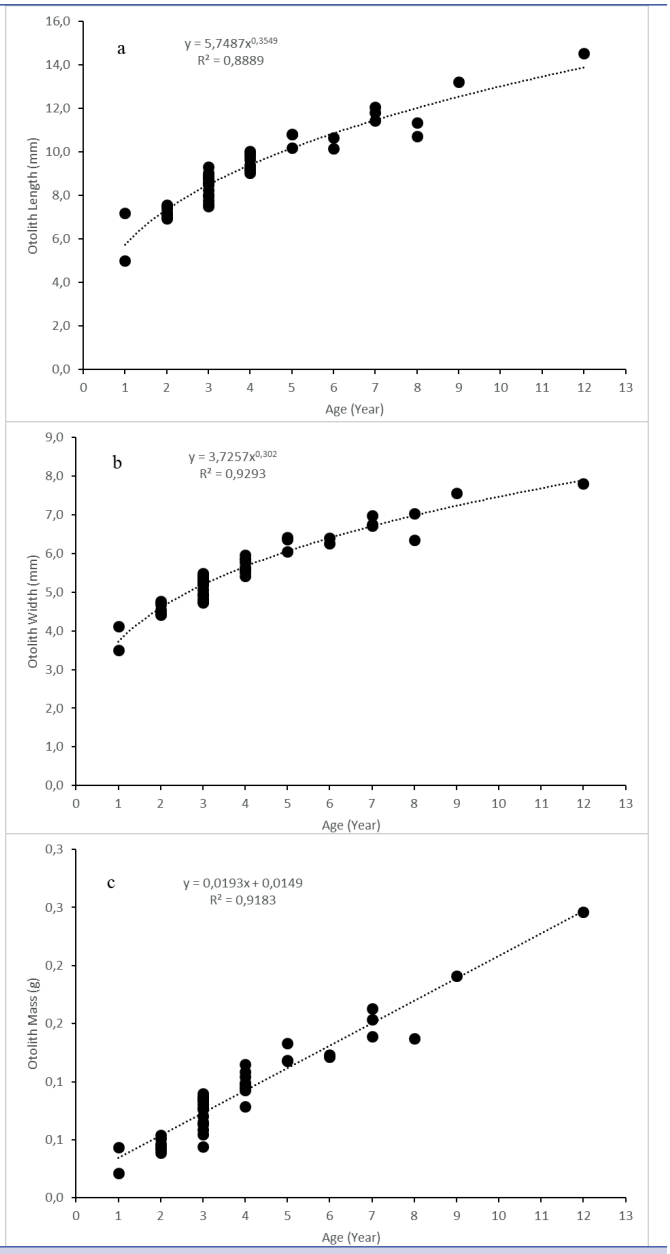

Figure 6. The relationship between otolith morphometry and age for red porgy from Gökçeada, Turkey; Otolith length (a), Otolith width (b), Otolith mass (c).

Ethics Committee Approval: This study was carried out in accordance with animal welfare and trial ethics.

Conflict of Interest: Author has no conflict of interest to report.

Financial Disclosure: This study was funded by Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, project number: FBA-2018-1406.

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