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Benthic Macro-invertebrate Community Diversity of Orhuwhorun River in Udu Wetlands

Gloria Akohame OSUINDE [1*], John Ovie OLOMUKORO [1]

[1] Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin, Benin City, PMB 1154, Nigeria

ABSTRACT

Benthic macro-invertebrate studies were done on Orhuwhorun River in Udu wetlands in Delta State, Southern Nigeria from March to December, 2011 in three selected stations. Sampling was done using a hand modified Eckman grab for sand and silt, the kick sampling technique and floatation method. They were sorted and identified using identification keys. A total of 2466 individuals were recorded in 66 taxa species belonging to thirteen (13) groups. Crustacean was the dominant group (36.29%) closely followed by gastropoda (35.60%) and diptera (21.04%). Significant similarity in fauna composition was observed. Station 3 had the highest population density with a relative abundance of 58.19% followed by station 2 (32.03%) and station 1 (9.77%). Diptera had the highest species diversity while nematode and lepidoptera had the least amongst the groups. Station 1 had the highest species richness (d) followed by station 3 and least in station 2. Species diversity showed no significant difference between the stations. Values for pollution tolerance index ranged between 13 and 15 at the stations. The highest value for pollution tolerance (PTI) was recorded in station 2 and the least in station 1. Positive significant correlations existed between most benthic organisms. The water quality is described as "fair".

Keywords: Benthic macro-invertebrates, Orhuwhorun River, Diversity, Pollution Tolerance Index (PTI).

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**** CORRESPONDING AUTHOR***

gloriaonde@gmail.com
Phone : +234 703 817 29 68

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Introduction

Nigeria has a coastline of about 853 km inundated with different types of aquatic systems which are majorly estuarine in nature (Uwadiae 2013). One of them is wetlands. Wetlands are transitional lands between terrestrial and deep water habitats where the water table is usually at or near the land surface and usually flooded (Egborge et al. 2003). They include "areas of marsh, fen, peat-land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed 6 m" (Ramsar Convention Secretariat 2007). Wetlands contribute significantly to world biodiversity (Keddy 2010). They are one of the most important ecosystems in the world performing essential ecosystem services including preservation of biodiversity and providing habitat for many endangered species amongst others (Asibor 2009; Hu et al. 2017; Ogbeigbu and Ohiorobo 2020).

The Udu wetlands cover part of an area that harbours the oil and gas industries and its allied industries and is usually susceptible to degradation and fauna loss in the water bodies due to water pollution either directly or indirectly. The loss of biodiversity and its effect seems to be greater for aquatic ecosystems than for terrestrial ecosystens because of its sensitive chemical nature.

The benthic macro-invertebrate community occupies an important trophic level in wetland ecosystems and can also be found in a variety of habitats (Stenert and Maltchik 2007). They mix up of soils by their activities which include burrowing, ingestion and defecation of sediment grains (Aller and Cochran 2019). Nutrient transport across sediment is a major role played by these bottom

dwelling organisms. They also serve as a food source for other aquatic organism in the food chain (Silva et al. 2006) which when stressed such other organisms on the food chain is affected. Diaz et al. (2004) described benthic infauna as opportunistic species that can adapt to any habitat circumstance of possible benefits such as a dynamic salinity regime and variable physical conditions. Their composition, abundance, biomass and distribution patterns are to a large extent determined by a number of interacting variables of physical and chemical parameters like temperatue, dissolved oxygen concentrarion, salinity and biochemical oxygen demand (Ikomi et al. 2005; Hepp et al. 2013).

Benthic invertebrates are useful as bioindicators of environmental degradation in the aquatic ecosystems, ecological monitoring and assessing pollution status (Olomukoro and Osuinde 2015; Arimoro et al. 2015; Anyanwu et al. 2019). Biological monitoring is the systematic use of living organisms (benthic invertebrates) and their responses to their environment in the determination of water quality (Barbour and Paul 2010; Muralidharan 2010).

Aquatic insects give a more accurate interpretation of changing aquatic conditions (Ikomi et al. 2005; Xu et al. 2014) and a more reliable assessment of pollution status in a waterway than other organisms like fish, due to the greater variety of insect species present in a water body representing an entire range of water quality tolerance. Some insects are only found in clean waters while some are facultative that is show no preference for either polluted or clean waters. The poorer the quality of the water body, the fewer the number and types of organisms that can live in it. Some species are more sensitive to chemical and physical changes than other species. If sensitive species are more available then the waterway is described as clean and of good quality. The objective of this paper is to determine the composition and diversity of benthic fauna in Orhuwhorun River and to determine the water quality of the river using the PTI key.

Materials and Methods

Study Area

Orhuwhorun River in Udu wetlands is located between latitude 05°47'-05°52'E and longitude 05°28'-05°33'N (Figure 1). It is a tributary flowing into Warri River and tidal influenced with a 12 hourly cycle. The river is about 7 m above sea level and 10 km long with a sloppy and undulating terrain. The catchment's area is surrounded by homesteads, houses, fish ponds, and shopping complexes. The bottom is more than 50% clay and the depth of the river is about 2.5 m. The study area consists primarily of freshwater swamp, mangrove swamp and tropical rainforest characterized by dense vegetation. It is dark and turbid flanked by red mangrove (*Rhizophora racemosa*), oil palm trees (*Elaeis guinensis*), mahogamy trees, raffia palm, *Hevea brasilensis, Rahia hookeria, Alstonia sp., Ficus sp, Kigelia africana, Aestotrophyllum secundifloum, Clamitus sp., Lemna sp.* and water hyacinth (*Eichhornia crassipes*). The shrubs around the water body include *Alchoma laxiflora, Nephrolepsis biseraa, Amarathus sp* and *Anacandium occidentale* etc. Farming, fishing and felling of trees are the major human activities alongside domestic activities such as bathing, laundry and defecation. The rainy season lasts from March to early November, while the dry season spans from November to February of the following year.

Sampling Stations

Station I

Station I (N 05° 30' 42.6", E 05° 50' 4.5") is located in Orhuwhorun village, 5 m above sea level. It is about 2.8 km upstream of station II; the water is dark and turbid. The water drains primarily through a thick freshwater swamp forest with an average velocity of 0.02 m/s.

Station II

Station II (N 05° 30' 49", E 05° 49' 0.30") is located 2.5 km upstream from station III and is at the center of Igbogidi village axis, 2 m above sea level. The substratum is mainly sand and silt. Human activities are fishing, bathing and washing. The flow rate was faster than station I with an average current velocity was 0.05 m/s.

Station III

Station III (N 05° 30' 52.5", E 05° 48' 18.0") is located at Ekete waterside, 7 m above sea level. The substratum is sandy silt (laterite). Human activities include timber felling, fishing, shrimping and laundry. The water is fast flowing with an average velocity of 0.18 m/s.

Sampling of Macro-benthic Invertebrates

Benthic macro-invertebrates were sampled randomly every two weeks using three methods. First, a 6-inch metal container (modified grab) was used to sample the substratum of the river. It was operated by hand in the shallow waters forced to a depth of 15-20 cm. The contents were sieved and put in sampling containers holding 10% formalin solution. Secondly is the kick sampling method (Victor and Ogbeibu 1985). Here, the water close to the bankroots and macrophytes is kicked with the leg to disturb the organisms and divert the flow of water to another direction where the sieve is placed to collect the detached and floating invertebrates and placed in sampling containers holding 10% formalin

solution. Thirdly, floating aquatic plants-water hyacinth (*Eicchornia crassipes*) was collected midstream and put in plastic buckets containing 10% formalin and left for 3-5 minutes. Then it is shaken vigorously within the bucket and transferred to another bucket of water. It is dusted again to shake off completely any organism still attached to its root (Olomukoro and Osuinde 2015). Then the contents of the buckets are filtered through a set of sieves. All samples were preserved in sampling containers holding 10% buffered formalin solution.

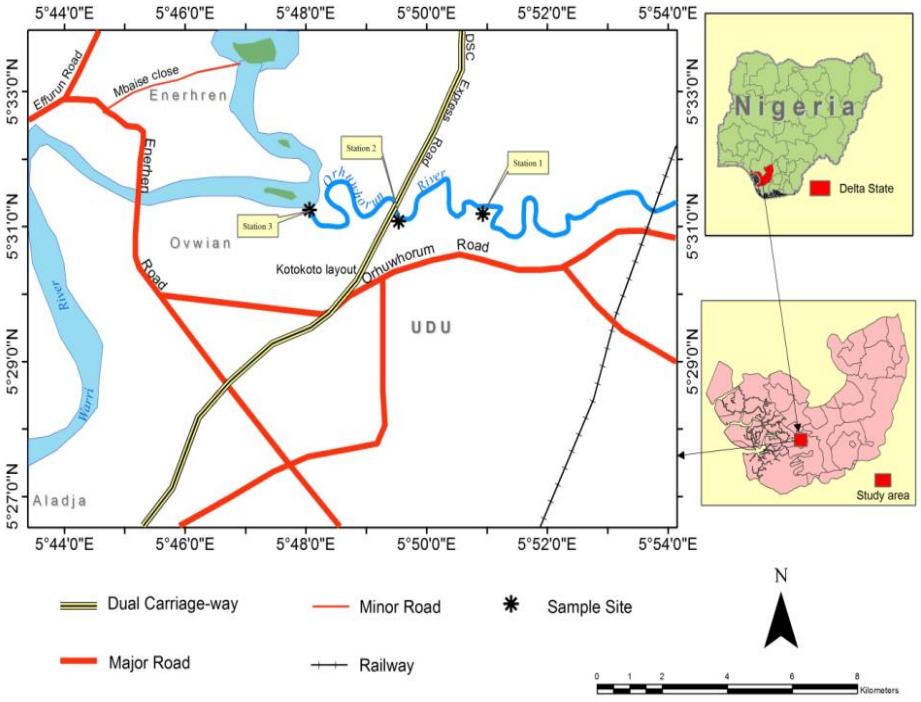

Figure 1. Map of study area showing sampled locations

Sorting, Identification and Counting

Sorting was done using an American optical dissecting microscope; model 570 with a magnification of 25- 40x, preserved in 4% formalin and stored in labeled specimen bottles. Identification and counting was done using a binocular Olympus microscope; model WF 10x. The methods of Olomukoro (1996) and Olomukoro and Ezemonye (2007) were employed for the identification of macroinvertebrate organisms and further confirmed with keys by Needham and Needham (1962), Mellanby (1963), Pennak (1978) and Olomukoro (1983). Some organisms were identified to generic level due to the poor knowledge of taxonomy in Nigerian fresh and brackish water.

Statistical Analysis

Statistical analysis was computed using the SPSS statistical package and micro-soft excel. Biotic indices to ascertain the diversity such as Margalef's index (d), Shanon-Weinner index (H), Evenness index (S) and Dominance index (C) were analyzed using computer software known as PAST (Paleontological Statistics) (Hammer et al. 2001). Comparism between the stations was done using the single analysis of variance (ANOVA) and the significant difference pointed out using the Duncan multiple range tests, (DMR). Correlation between the macro invertebrates were analyzed to show the relationship between the parameters across the stations.

Pollution Tolerance Index

The health status of the water body was assessed using the method described by Klemm et al. (1990) under three groups. The first-pollution sensitive group of Epemeroptera, Trichoptera, and Coleoptera was multiplied by 3. The second-somewhat sensitive group of Decapoda, Zygoptera, Anisoptera and Diptera was multiplied by 2 and the third –pollution tolerant group of Oligochaetes, and gastropods was multiplied by 1. The total was summed up to get a value which was compared with PTI index categories in Table 1 for each station.

Table 1: Pollution tolerance index categories and its description

PTI index	Description
23 and above	Excellent
17-22	Good
11-16	Fair
10 or less	Poor

Results

A total of 2466 individuals belonging to 66 taxa were collected in this study. Station 1 recorded 37 taxa species, station 2 recorded 45 taxa species and station 3 recorded 40 taxa species respectively while the number of individuals in each station were 241, 790 and 1435 respectively (Table 2). Throughout the study period, among the dominant groups, dipteran population occurred more in the rainy season in station 1 except in May than in other stations. While station 3 recorded low numbers of dipteran population throughout the study period (Figure 3).

Table 2. General composition and diversity and abundance in the study stations

Stations Taxa	1	2	3
Nematoda			
Diplogaster sp.	1	-	-
Annelida (Oligochaete)			
Nais simplex	-	-	1
Nais osborni	-	1	-
Nais sp.	1	-	-
Lumbricus sp.	12	1	-
Polychaeta			
Lycastoides alticola	-	-	1
Lycastopsi sp	-	-	1
Namanereis hawaiiensis	2	-	2
Crustacean			
Caridina gabonensis	-	-	1
Potamalpheops monody	-	280	614
Arachnida			
Sesarma alberti	-	-	28
Agyronecta aquatic	-	4	1
Megapus sp	-	1	-
Ephemeroptera			
Baetis bicaudatus	3	-	-
Ephemerella ignita	-	1	-
Cleon simplex	3	1	-
Cleon bellum	8	9	2
Centroptilum sp.	1	4	-
Unidentified ephemeroptera	-	-	1
Odonata (Anisoptera)			
Libellula sp.	1	4	1
Orthemis sp	-	-	1
Gomphid sp	2	-	-
Zygoptera			
Hesperagrion heterodoxum	-	3	-
Enallagma sp	1	2	-
Coenagrion scitulum	-	1	1
Lestes sp.	-	1	-
Hemiptera			
Pelocoris femoratus	1	-	2
Unidentified hemiptera	-	-	1
Lepidoptera			
Lepidoptera larva	6	4	7
Coleoptera			
Berosus sp.	-	1	-
Hydrophilus sp.	1	8	9
Hydroptillid larva	-	3	5
Hydroporus larva	-	-	1
Dysticus marginalis	-	7	1
Phylidrous larva	4	1	-
Unidentified dysticus larva	1	1	2
Unidentified coleopteran	-	1	-
Diptera			
Stilobezzia antenalis	1	-	-
Probezzia sp.	1	-	-
Palpomyia sp.	44	2	1
Forcipomyia sp.	27	1	1
Allaudomyia needhami	5	5	1
Pentaneura sp.	10	40	26
Polypedilum sp.	12	45	62
Tanytarsus sp.	3	15	16

Table 2. Continued

Stations Taxa	1	2	3
Diptera			
Chironomus fractilobus	15	14	17
Chironomus travailensis	7	1	-
Chironomid sp.	5	-	-
Procladius sp.	6	22	6
Unidentified dipteran larva	-	1	-
Unidentified diptera pupa	-	1	-
Cricotopus sp.	2	44	15
Pseudochironomus sp.	3	6	1
Culex sp.	7	6	4
Tanypus sp.	10	6	2
Gastropoda			
Neritina glabrata	-	109	288
Nerita senegalensis	-	33	60
Hydrobia sp.	4	7	4
Hydrobia guyenoti	1	24	70
Potamopyrgus sp.	-	10	21
Potamopyrgus ciliates	-	54	110
T. fuscatus radula	-	3	-
T. fuscatus fuscatus	-	-	48
Planorbis complanatus	1	-	-
Limnea glabra	13	1	-
Limnea auricularia	16	1	-
Total	**241**	**790**	**1435**

The benthic fauna was dominated by Crustaceans (36.29%) closely followed by Gastropods (35.60%) and Diptera (21.04%) (Figure 2). The others fell into the category of rare groups having a population density <5% (Slack et al. 1977). They include Coleoptera (1.82%), Ephemeroptera (1.37%), Arachnida (1.37%), Lepidoptera(0.68%), Oligochaete (0.65%), Anisoptera (0.36%), Zygoptera (0.36%), Polychaeta (0.24%), Hemiptera (0.16%), and Nematoda (0.04%).

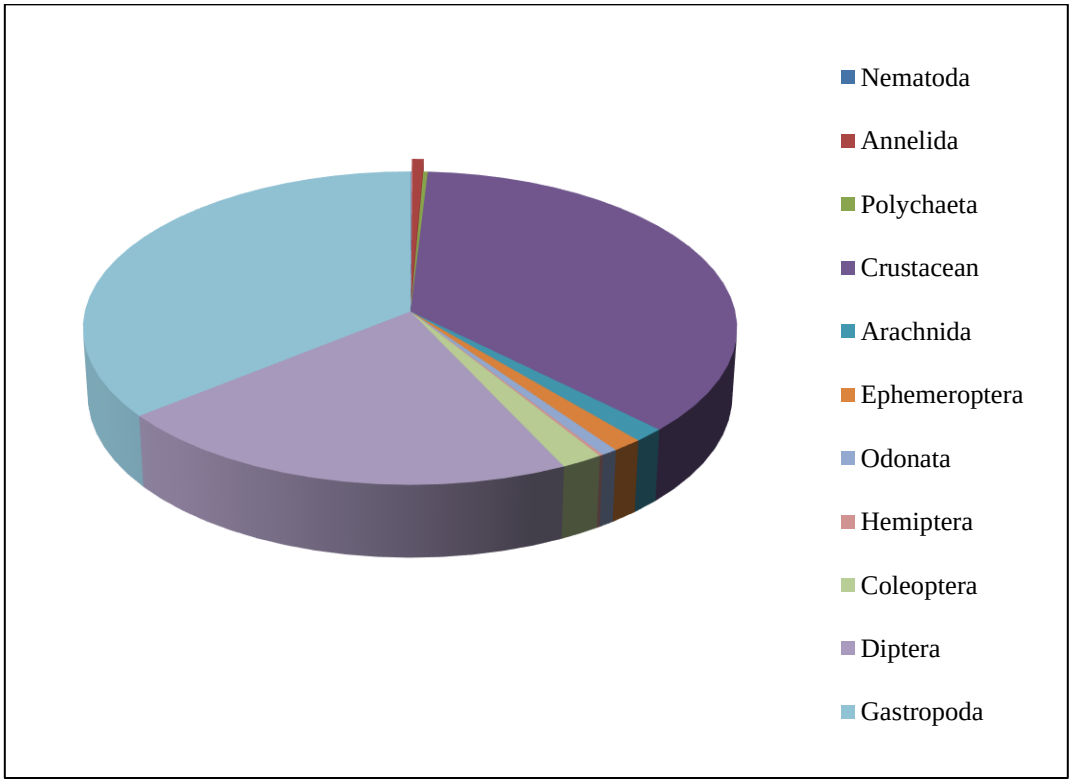

Figure 2: Overall composition of benthic macrofauna in the stations

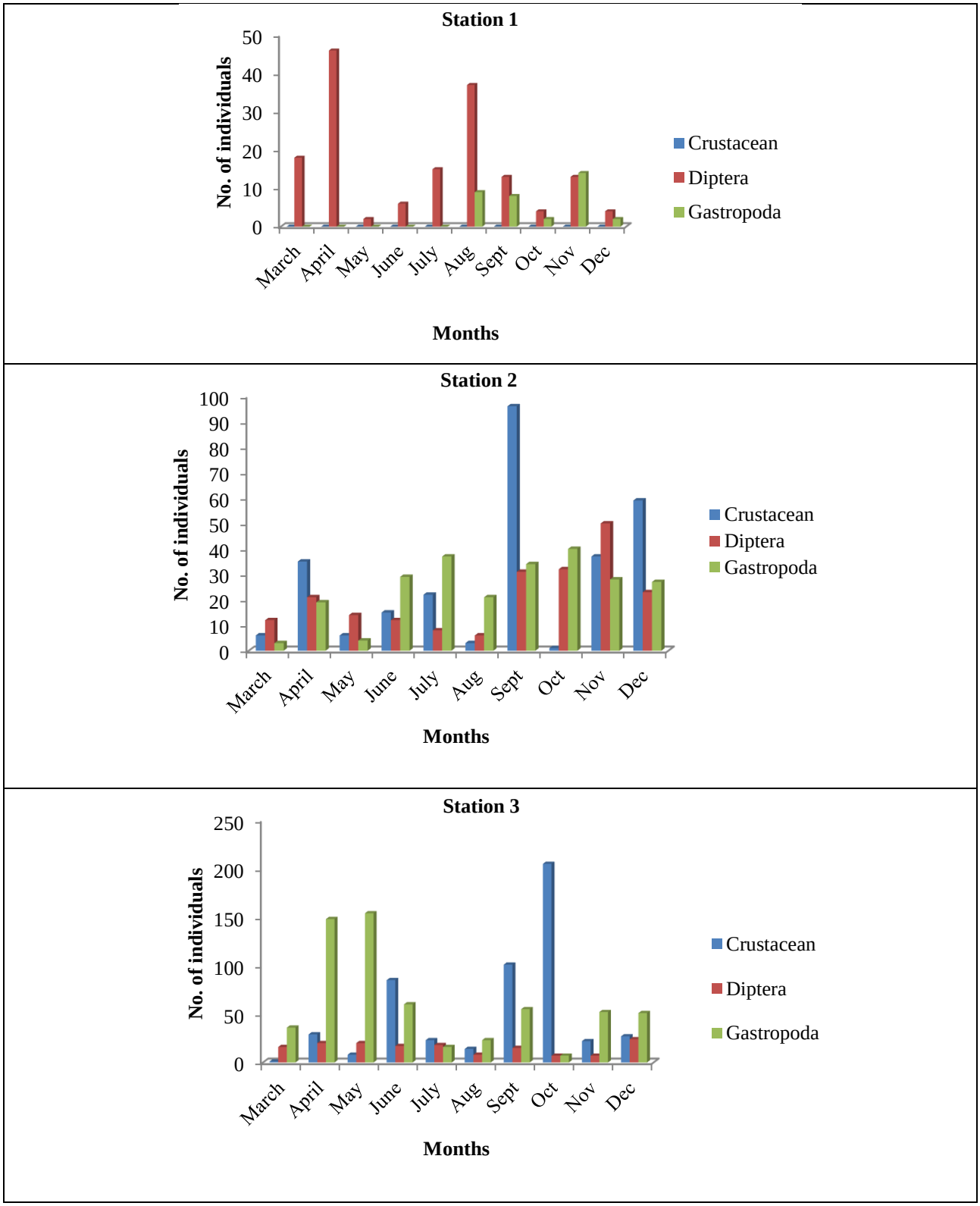

Figure 3: Seasonal variation of the dominant macrobenthic fauna in the three stations

The community structure include Nematode (1 species), Oligochaete (4 species), Polychaete (3 species), Crustaean (2 species), Arachnida (3 species), Ephemeroptera (6 species), Odonata (7 species), Hemiptera (2 species), Lepidoptera (1 species), Coleoptera (8 species), Diptera (18 species) and Gastropods (11 species) (table 2).

Species evenness (E) was highest in station 2 and lowest in station 3. Taxa richness (d) was highest in station 1 and lowest in station 2. Station 2 was highest for general diversity (D) while station 3 had the lowest diversity (table 3). There exist no significant differences in diversity between the stations. Comparism between stations using Sorenson's

quotient showed strong similarity in species composition between the stations. Similarity was highest between stations 2 and 3 (62.7) and least between stations 1 and 3 (54.5).

Table 3: Diversity indices of macrobenthic fauna of the study stations in Orhuwhorun River

INDICES	STN 1	STN 2	STN 3
No. of taxa	37	45	40
Percentage taxa (%)	56.06	68.18	60.60
Number of individuals	241	790	1435
Relative abundance (%)	9.77	32.03	58.19
Margalef's index (d)	1.823	1.349	1.513
Shanon-Weinner's index (H)	1.234	1.398	1.183
Dominance index (D)	0.4592	0.2908	0.3709
Simpson's index (1-D)	0.5408	0.7092	0.6291
Evenness index (S)	0.3122	0.4048	0.2719
Menhinick' s index	0.7086	0.3558	0.3168
PIE	0.5523	0.8237	0.6308

On the pollution tolerance index scale, it gave close values of 13, 15 and 14 at stations 1, 2 and 3 respectively. Across the sampling months, it was highest in September and December (14) and lowest in April (3) (Figure 4).

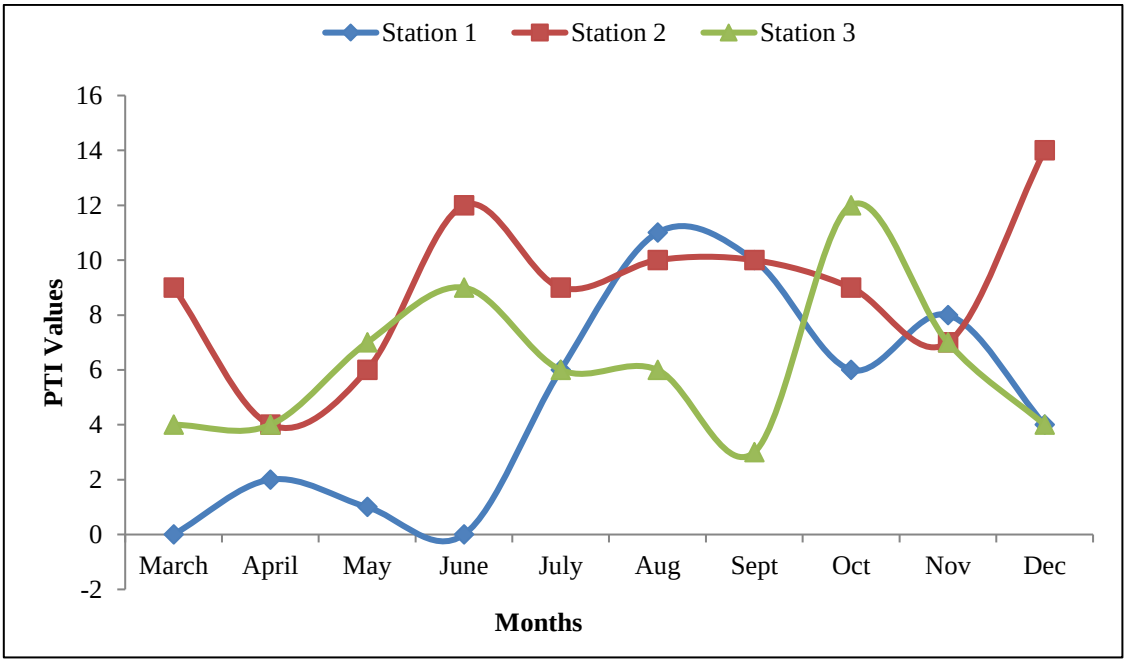

Figure 4. Spatial and temporal variation in pollution levels in the stations

Discussion

It is known that tropical streams record higher numbers of benthic fauna than temperate waters (Bishop 1973). This study recorded 66 taxa in the study period. The high taxa number recorded corroborates Olomukoro (1996) and Ajao and Fagade (2002). Previous works on Udu-ghievwen wetlands recorded 40 taxa (Olomukoro and Dirisu, 2014). Also, Agbede wetlands recorded 42 taxa (Olomukoro et al. 2013) despite its disturbance with lindane. However, It is important to note that the numbers was quite low when compared with other tropical water bodies such as Olomokoro and Egborge (2003) (138 taxa species), Olomukoro (2007) (112 taxa species). Such differences may be as a result of variation in sampling methods, pollution and increased environmental and anthropogenic activities.

The overall diversity of a water body is a product of all dynamic spatial and temporal changes affecting the communities and a reflection of the extent to which a biotope is disturbed by human activity (May 1981). According to the intermediate disturbance hypothesis of diversity, disturbance either by predation or by physical process prevents species from reaching densities where competition begins and thus allows more species to co-exist than where there is no distubance (Gray 1985). This was observed in station 2 where species diversity is high (1.4) that is having more species co-existing together and at the same time having reduced taxa richness

(1.35). While in station 1 where there was relative calmness and much reduced disturbance by physical processes, the species diversity was low (1.23) and the taxa richness high (1.82). Olomukoro et al. (2013) was also of the view that human activities can significantly alter the eco-balance of any aquatic system.

Also observed in this study is that a high abundance of benthic invertebrates in aquatic ecosystem does not simultaneously mean greater species diversity in the system as seen in the upstream and midstream stations. This is seen in station 3 having the highest abundance of benthic invertebrates (58.19%) and the least in species diversity (1.18) while station 2 which had a reduced abundance of species (32.03%) recorded the highest diversity (1.4).

The nature of the substratum or particle size distribution was a vital factor that influenced the occurrence and distribution of the benthic fauna. The dominant fauna were Diptera, Gastropoda and Crustaceans (P>15%). The dominance of diptera in tropic freshwater bodies has been acknowledged (Ogbeibu 2001; Olomukoro and Ezemonye 2007). The clayey sediment at the river bottom may also have supported the ubiquitous dipteran and gastropod population during exposure periods due to its ability to retain much water as the organisms burrow into the soil to find shelter. Other factors which support dipteral population include current (velocity, temperature, season, total suspended solids and vegetation).

An important factor in shell formation is bicarbonate and acidity. These major factors affect gastropod distribution Thus they survive well in nuetral to slightly alkaline waters which favors the toughness and rigidity of their shells (Olomukoro and Azubuike 2009). This was the prevailing condition in this study. A study conducted by Spyra (2017) showed that a more diverse gastropod fauna was found in neutral ponds, whereas the lowest degree of diversity was found in ponds with the lowest pH. Gastropods were more downstream due to favorable conditions such as the presence of leaf litter which serve as food for them and extensive canopy cover provided by the trees and mangrove vegetation against predators (Lajtner et al. 2022). Economically important gastropod species such as *Tympanotonus sp.* and crustaceans are eaten by the locals in the area and by fish. Thus they play a vital role in the aquatic food web. They are detritus feeders and largely influenced by desiccation (Stickle et al. 2017).

Eutrophication was an observed phenomenon in station 1 as a result of increased nutrients from flood, sewage discharge and runoff from dumpsites into the river at this point. Also, some portions of the river bank are used as a dumpsite releasing nutrient leak into the watercourse. Similar observation by Mandal et al. (2012) reported nutrient (phosphate) contamination from sewage discharge, use of detergents in water and runoffs laden with fertilizers. Eutrophication alters habitat structure for benthic organisms, reduces water clarity and affects oxygen levels in the water and increased heat. This may have affected the crustacean population as they were very much reduced at station 1 when compared to the other two stations. Studies have shown that crustaceans are susceptible to oxygen depletion and increased heat (Verberk et al. 2018). Generally, demand for oxygen in aquatic organisms increases abnormally with every 10°C increase in temperature which directly affects their physiological activities (Halim et al. 2018).

A wide variety of benthic organisms usually indicate clean water conditions. This is drawn from the fact that high quality water provides an optimum environment for the existence of a large number of species. Polluted water on the other hand imposes one or more limiting factors on the benthic community and restricts the variety of species that can survive in such conditions. Sensitive species of Ephemeroptera, Odonata, and Coleoptera occurred in low numbers at all three stations. They are usually abundant in waters unpolluted with organic waste and lots of dissolved oxygen (Ezemonye et al. 2004). A low number of these sensitive species clearly indicates a compromised state in the water body. The close values of 13, 15 and 14 at stations 1, 2 and 3 (Figure 4) respectively from the PTI scale fell in category of 11-16 which describes the water quality as “fair” (Klemm et al. 1990). Olomukoro et al 2015 also reported PTI values in this category in Ekpan creek. Unlike the report given by Olomukoro and Dirisu (2013) who recorded water quality with values less than 10 in most stations. Olomukoro and Anani (2019) reported fair in some rivers, poor in others and excellent in a few rivers.

The water quality status of the study area depicts susceptibility to pollution and contamination as anthropogenic activities increases along the river course. A strategic management plan is thereby recommended to preserve its diversity and maintain natural conditions in the wetland area.

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Morphometric Characteristics of Freshwater Crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823) Caught in Sapanca Lake, Türkiye

Muhammed Ali BALTACI [1], Hamdi AYDIN [2*], Nadide SEYHUN [3], Tomris DENİZ [4]

[1]Sapanca Inland Waters Fish Culture Research and Application Unit, Istanbul University, İstanbul, Türkiye

[2]Faculty of Arts and Sciences, Biology Department, Kocaeli University, Kocaeli, Türkiye

[3]Food Technology Department, Kocaeli University, Kocaeli, Türkiye

[4]Faculty of Aquatic Sciences, İstanbul University, İstanbul, Türkiye

ABSTRACT

This is the first study providing information on some morphometric parameters for *Pontastacus leptodactylus* from Sapanca Lake (Sakarya), Türkiye. This study was conducted from June - 2016 to October - 2017 and a total of 264 crayfish were caught, being 146 females and 118 males. The results revealed that the average total length for the population was 91.89±15.68 mm (*TL*±SD) (94.75±15.91 mm for females, 88.36±14.72 mm for males), and the average total weight for the total population was 27.27±16.84 g (*TW*±SD) (29.02±17.54 g for females, 25.09±15.74g for males). The sex ratio was computed as 1.24:1 (female to male). A strong positive statistical relationship was determined between the total length and total weight of females, males, and both sexes (r^2: 0.90 – 0.95). This statistically strong relationship was also valid between other body parts, such as carapace length, width, and weight. The regression analyses for total length-total weight relationships also revealed that the whole population exhibited positive allometric growth (b=3.186) regardless of sex, and the condition factor for all sex groups was computed to be over 3 (*K*=3.23±0.60 SE).

Keywords: *Pontastacus leptodactylus*, morphometric characteristics, Sapanca Lake

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*** CORRESPONDING AUTHOR**

aydin@kocaeli.edu.tr
Phone : +90 532 315 39 03
Fax : +90 262 303 20 03

Sapanca Gölü'nde (Türkiye) Yakalanan Tatlısu Kerevitlerinin (*Pontastacus leptodactylus* Eschscholtz, 1823) Morfometrik Özellikleri

Öz: Bu çalışma, Sapanca Gölü (Sakarya, Türkiye) tatlısu kerevitlerinin *(Pontastacus leptodactylus*) bazı morfometrik parametreleri hakkında bilgi sağlayan ilk çalışmadır. Çalışma Haziran 2016-Ekim 2017 tarihleri arasında gerçekleştirilmiş, 146 dişi ve 118 erkek olmak üzere toplam 264 kerevit yakalanmıştır. Sonuçlar, popülasyonun ortalama toplam uzunluğunun (*TL*±SD) 91,89±15,68 mm (dişiler için 94,75±15,91 mm, erkekler için 88,36±14,72 mm) ve ortalama ağırlığının (*TW*±SD) 27,27±16,84 g (dişiler için 29,02±17,54 g ve erkekler için 25,09±15,74 g) olduğunu ortaya koymuştur. Cinsiyet oranı 1.24:1 (dişi:erkek) olarak bulunmuştur. Dişi, erkek kerevitler ve her iki cinsiyetin total boy ve total ağırlığı arasında istatistiksel olarak güçlü bir pozitif ilişki belirlenmiştir (r^2: 0.90 – 0.95, P<0.05). Bu istatistiksel güçlü ilişkinin, diğer morfometrik parametreler, özellikle kabuk uzunluğu, genişliği ve ağırlığı arasında da geçerli olduğu tespit edilmiştir. Toplam boy-toplam ağırlık ilişkileri için yapılan regresyon analizleri de cinsiyetten bağımsız olarak tüm popülasyonun pozitif allometrik büyüme gösterdiğini (b=3.186) ve tüm cinsiyet grupları için kondisyon faktörünün 3'ün üzerinde (*K*=3.23±0.60 SE) olduğunu ortaya koymuştur

Anahtar kelimeler: *Pontastacus leptodactylus*, morfometrik özellikler, Sapanca Gölü

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Introduction

Crayfish are distributed in tropical and subtropical latitudes and are habituated in rivers, streams and lakes, dam lakes, marshes areas, freshwater caves and springs, and terrestrial burrows (Richman et al. 2015). More than 640 freshwater crayfish species are distributed around the globe in various water resources, except Indian and Antarctica

continental (Crandall and Buhay 2008; Reynolds 2011). Although the number of species is high, the number of crayfish species of commercial importance does not exceed seven species (FAO 2022). *Pontastacus leptodactylus* is the indigenous freshwater crayfish species that are widely spread in Türkiye's inland waters and it is also a commercially important inland water species nowadays. Although Türkiye's crayfish fishing started in 1961 from Sapanca Lake at first (Bolat 2001), official catch data was not recorded until 1977. It was the most important inland water product, especially between 1977 and 1985. However, there was a dramatic decrease in its population due to the crayfish plague, which was recorded in Türkiye in 1984 (Furst 1988; Baran and Soylu 1989; Rahe and Soylu 1989; Aydın et al. 2015). After 1985, despite increased crayfish stocks, the amount of harvested crayfish has not reached the levels of previous years in Türkiye. In Türkiye, the average amount of year of crayfish harvested between 2008 and 2017 was 650 tons (TÜİK 2022). According to the data of the Turkish Statistical Institute, 60 tons of crayfish were caught in 1977, 50 tons in 1982, 56 tons in 1985, 7 tons in 1986, 6.5 tons in 1987 and 17 tons in 1988 in Sakarya province (TÜİK 1977,1982,1985,1986,1987,1988). From these data for the years 1977 to 1988, commercial crayfish fishing in Sakarya province was carried out only in Sapanca Lake, and it was assumed that the amount of crayfish caught belonged only to this lake. The adverse effects of the crayfish plague have been severe and long-lasting in some water sources, while in some water sources, it has been lighter and shorter in Türkiye. Sapanca Lake has been one of the water sources where crayfish disease has a heavy impact in Türkiye. Sapanca Lake was one of the important water sources in Türkiye for crayfish harvesting until 1980, but after 1984 the crayfish stock in the lake collapsed considerably due to crayfish plague (*Aphanomyces astaci*).

For the protection of crayfish populations, the size and the characteristics of the population must be known. There is no study about the crayfish population inhabiting Sapanca Lake. Therefore, this study was the first report on the morphometric structure, growth characteristics, and the status of crayfish (*P. leptodactylus* Eschscholtz, 1823) population of the Sapanca Lake.

Materials and Methods

Study Area

Sapanca Lake is located in the eastern region of Marmara, Türkiye. Half of the lake is located in the province of Sakarya and the other half is located within the boundaries of Kocaeli. It's surface area varies between 46 and 60 km^2 depending on the amount of water entering the basin of the lake. The length of the lake is 16 km and the widest part is 6 km and the north and south are surrounded by mountains. The average depth of the lake is 29 m and its deepest point is 52 - 54 m (Uzunay and Soylu 2006).

Field Sampling and Data Collection

The crayfish samples were collected monthly from Sapanca Lake between June 2016 and October 2017. In order to catch crayfish samples, a total of 80 nets of single-entrance fyke nets (each with a 34 mm mesh size) were used. Before starting the research, 4 different areas of the lake (front of the Eşme Beach, Seka Camp area, Arifiye Gölbaşı and Kurtköy Creek) 3 times were used fyke nets in May 2016 and June 2016. As a result of these preliminary searches, the front of Eşme Beach has been selected as the sampling area because the highest number of crayfish is harvested in the coastal region. Fyke nets were located in water along 50 m of shore and harvested 2 days later. Fishing trials have been performed 14 times monthly between June 2016 and October 2017. Caught live crayfish were put into styrofoam boxes and carried the same day to Istanbul University Sapanca Inland Water Research and Application Unit. All crayfish were counted and separated by sex. The sex of the crayfish was determined by morphologically, checking the presets of the copulatory swimmerets. After this, each crayfish was weighed with a digital scale (0.001 g sensitivity). Total length (TL), carapace length (CL) (from the tip of the rostrum to the posterior median edge of the cephalothorax), carapace width (CW), abdomen length (AL), rostrum length (RL), head length (HL), and areola length (ARL) were measured with a digital calliper (to the nearest 0.01 mm). For measuring the body parts, the methods of Rhodes and Holdich (1984) were used. During the study, in the sampling area, the water surface temperature, oxygen and pH values were measured with a multiparameter (YSI, USA).

Data Analysis

Freshwater crayfish, as in fish, has a nonlinear relationship between length and weight that can be expressed as $W = aL^b$ (Froese 2006), where W = weight of the samples in g, L = length of the samples in cm, *a* and *b* are constant parameters of the regression equation; b is the slope value of the line in the regression equation giving information about the body shape of fish, and a is the intersection point of the regression equation giving information on the food capacity of the environment. To determine whether the weight increase of the freshwater crayfish population analyzed was isometric or

allometric, the length and weight values underwent regression analysis, and coefficients a and b were calculated by the least-squares method (Ricker 1975; Pauly 1984). The condition factor (K) was calculated from the equation, $K = 100W/TL^3$, where W is observed total body weight (g) and TL (cm) is body total length (Ricker 1975).

The Catch per unit effort (CPUE) was calculated as follows for each harvest: $CPUE= \Sigma N_c / \Sigma N_{fn}$ where ΣN_c is the sum of a number of crayfish in harvest and ΣN_{fn} is the sum of fyke-net set during the study (Bolat et al. 2011; GFCM 2018).

Sex ratios were calculated for caught *P. leptodactylus* each month. The chi-square test (χ^2) was used to test for differences in the male-female ratio, with a significance level of p=0.05. The t-test was used to determine if there were potential and meaningful differences between the acquired *b* values and cubic growth. For this process, the standard error of the *b* values was first calculated, and its relationship with the value in the t distribution table of 95% confidence interval was analyzed (Sokal and Rohlf 1987). The calculated r (correlation value) shows the relationship between the independent variable (e.g. length) and the dependent variable (e.g. weight) (Romaire et al. 1977; Harlıoğlu 1999). Differences in length class distributions of crayfish among males and females were assessed by the Kolmogorov-Smirnov two-sample test. In order to test any statistically significant differences for slopes and elevation between both sexes analysis of covariance (ANCOVA) was performed at the significance level of P<0.05.

Based on sexes for harvested crayfish, the CPUE values were calculated both for below (<100 mm TL) and above (≥100 mm TL) minimum landing size (MLS) according to months. We compared mean catch per unit effort (CPUE; number of crayfish/trap/hour) of legal and sub-legal *P. leptodactylus* among months using one-way analysis of variance (ANOVA; a p 0.05) or with the Kruskal–Wallis H-test when the ANOVA assumption could not be satisfied. All the analyses were performed using the statistical programs Microsoft Excel 2007 and SSPS 16 for Windows. In statistically evaluating the data acquired from the research, the significance tests were done based on the p=0.05 confidence limits (Ricker 1973).

Results

The results estimated in the current study aimed to demonstrate the length-weight relationships, condition factor and CPUE for 34 mm mesh size on commercial fyke net fishery for *P. leptodactylus* first time in the Sapanca Lake. A total of 264 crayfish were caught of 146 females (55.30%) and 118 males (44.70%) from Sapanca Lake during the study. The crayfish total length (*TL*) caught ranged in size from 63.70 to 133.43 mm and in total weight (*TW*) from 8.00 to 87.10 g. Total body length ranged from 63.70 -133.43 mm for males and from 68.81 - 132.13 mm for females, with a total weight between 8.00-87.10 g for males and 9.80 to 73.60 g for females. The average total length for the population was estimated as 91.89±15.68 mm (*TL*±SD) (94.75±15.91 mm (*TL*±SD) for females, 88.36±14.72 mm (*TL*±SD) for males), and the average total weight for the population was estimated as 27.27±16.84 g (*TW*±SD) (29.02±17.54 g (*TW*±SD) for females, 25.09±15.74 g (*TW*±SD) for males) (Table 1). The dominant total length classes were 75 - 90 mm for both sexes (Figure 1). While female individuals were bigger than male individuals in terms of average total length and total weight, the mean total length and the mean total weight were not significantly different in the sexes (t=3.402, p=0.06; t:1.894, p=0.059, respectively).

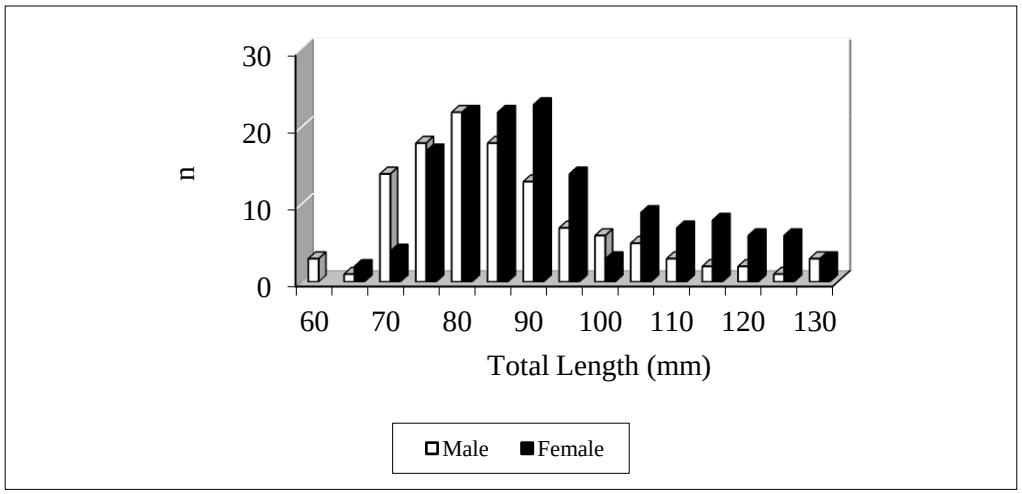

Figure 1. Length frequency distribution by sex for *P. leptodactylus* in the Sapanca Lake

Table 1. Descriptive statistics of various morphometric measurements of *P. leptodactylus*. (*TL*: total length (mm); *CL*: carapace length (mm); *CW*: carapace width (mm); *AL*: abdomen length (mm); *RL*: rostrum length (mm); *ARL*: areola length (mm); *HL*: head length (mm); *AW*: abdomen width (mm); *TW*: total weight (g))

	Sex	Min-Max	Mean±Sx	t-student test
TL	F	68.81-132.13	94.75±15.91	
	M	63.70-133.43	88.36±14.72	t:0.000
	M + F	63.70-133.43	91.89±15.68	P>0.05
CL	F	31.96-67.85	47.20±8.36	
	M	30.76-69.19	44.83±7.86	t:0.018
	M + F	30.76-69.16	46.14±8.20	P>0.05
CW	F	15.23-33.70	22.82±4.40	
	M	14.50-36.83	22.16±4.69	t:0.185
	M + F	14.50-36.83	22.53±4.54	P>0.05
AL	F	28.96-69.42	48.44±8.59	
	M	29.08-65.85	43.63±7.26	t:0.000
	Total	28.96-69.42	46.31±8.36	P>0.05
RL	F	8.75-21.50	14.07±2.54	
	M	9.64-19.14	13.38±2.12	t:0.011
	M + F	8.75-21.50	13.76±2.38	P>0.05
ARL	F	10.26-21.64	14.83±2.71	
	M	10.18-22.24	14.53±2.51	t:0.251
	M + F	10.18-22.24	14.70±2.62	P>0.05
HL	F	18.84-47.42	31.90±5.72	
	M	21.62-47.72	30.57±5.29	t:0.046
	M + F	18.84-47.72	31.31±5.56	P>0.05
TW	F	9.80-73.60	29.02±17.54	
	M	8.00-87.10	25.09±15.74	t:0.056
	M + F	8.00-87.10	27.27±16.84	P>0.05

F: female; M: male

Although size-frequency distributions show a size predominance over females, in length-frequency distributions, females and males show almost the same size, with mean female size exceeding that of males, and the *K-S* test revealed the existence of significant differences in size distributions of total length and abdomen length (Kolmogorov-Smirnov two-sample test: nm=118, nf=146, D=0.185; D=0.253; D=0.185; P<0.05, respectively), and there is no difference between male and female individuals in other measured length distributions (P>0.05).

Even though the sex ratio for the whole population was 1.24:1 in favor of the females, the $\chi 2$ analysis showed significant differences from the ratio of 1:1 ($\chi 2$ = 30.254, P = 0.0043). According to months, sex rates have changed and the number of caught crayfish was high in winter but decreased in summer. Male individuals were caught at most (30 individuals) in February and at least (1 individual) in November. Female individuals were caught at most in March 2016 (26 individuals) and at least (1 individual) in August and November 2016 (Table 2).

Table 2. Sex ratios of *P. leptodactylus* caught by months in the Sapanca Lake

	Female	Male	Sex ratio (F:M)	Chi-square	p-value
June 2016	16	2	8:1	9.3596*	0.0022
July 2016	4	3	1.33:1	0.1335	0.7148
August 2016	1	2	0.5:1	0.3237	0.5694
September 2016	5	4	1.25:1	0.1019	0.7495
November 2016	1	1	1:1	0.0000	1.000
January 2017	9	15	0.6:1	1.2125	0.2708
February 2017	12	30	0.4:1	5.5213*	0.0188
March 2017	26	11	2.36:1	4.4926*	0.0340
April 2017	25	14	1.79:1	2.2461	0.1339

May 20117	19	19	1:1	0.0000	1.000

Table 2. Continue

June 2017	7	5	1.4:1	0.2977	0.5853
July 2017	14	7	2:1	1.9349	0.1642
August 2017	3	2	1.5:1	0.1905	0.6625
October 2017	4	3	1.33:1	0.1335	0.7148
Total			**1.24:1**	**0.8207**	**0.3649**
< 100 mm	**104**	**96**	**1.08:1**	**0.1067**	**0.7439**
≥ 100 mm	**42**	**22**	**1.91:1**	**3.8685***	**0.0492**

∗Significant

The highest level of female: male ratio was determined at 8:1 in June 2016. The lowest level of female:male ratio was observed at 0.4:1 in February 2017. There were statistically significant differences in the number of the catch between sexes individuals in the months of sampling in June 2016 and February 2017, respectively (χ^2 = 8.214; X^2 = 12.141; P<0.05, respectively). There was no significant difference between the sexes in other months of sampling (P>0.05). In the study period, water temperature varied between 7.7 and 27.1 °C.

The descriptive statistics and estimated parameters of the length-weight relationship parameters are given in Figure 2, Figure 3, Figure 4, Table 3 and Table 4.

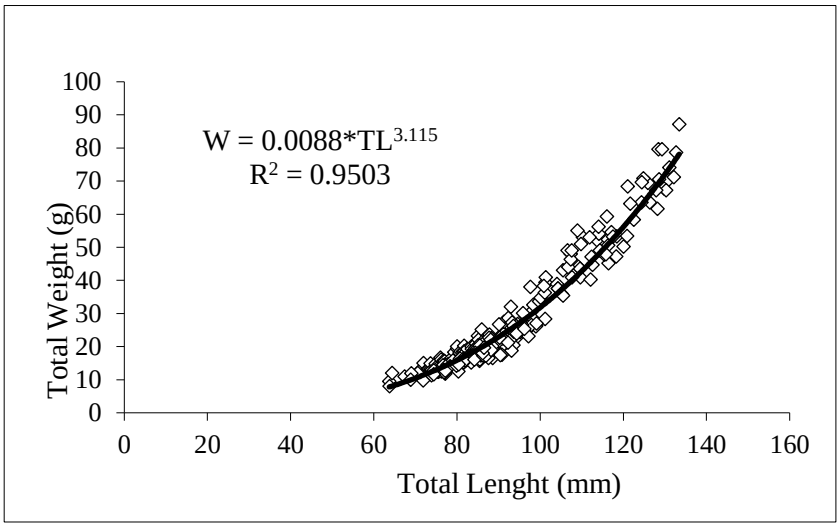

Figure 2. Total length-total weight relationships of crayfish population in Sapanca Lake

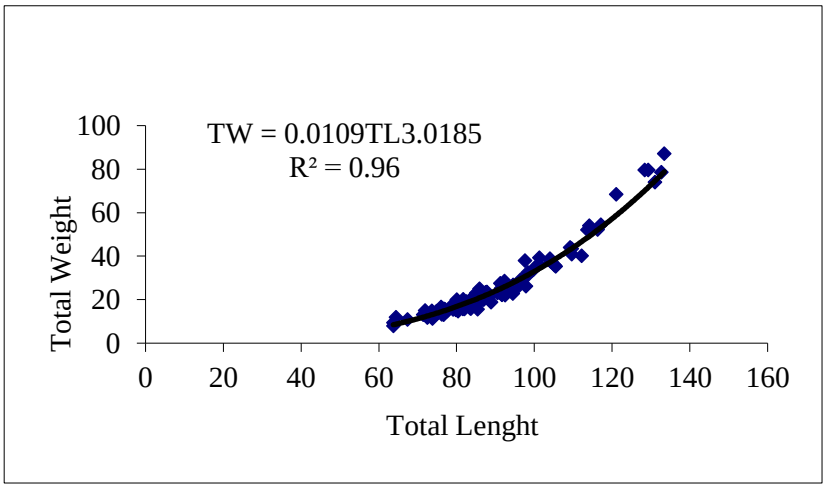

Figure 3. Total length-total weight relationships of male crayfish in Sapanca Lake

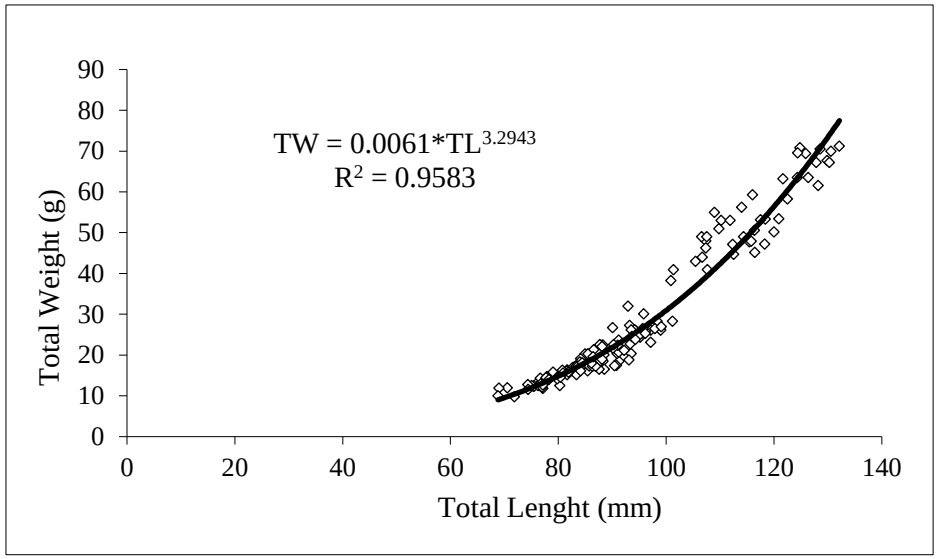

Figure 4. Total length-total weight relationships of female crayfish in Sapanca Lake

Table 3. Descriptive statistics and length-weight relationship parameters for *P. leptodactylus* in the Sapanca Lake (*TW*, total weight (g); *TL*, total length (mm); *CL*, carapace length (mm); *SE*(b)., standard error of the slope b; *CL*(b), 95% confidence limits of slope b; r^2, is the coefficient of determination; *A*-, negative allometry; *A*+, positive allometry; *I*, isometry growth)

Relation	Sex	W = a Lb	SE(b)	CL(b)	r^2	Type of growth	Ancova	F (DF)	P
TL - TW	M	$TW = 0.0109\ TL^{3.018}$	0.057	2.961-3.076	0.960	I			
	F	$TW=0.006108\ TL^{3,294}$	0.057	3.237-3.352	0.958	A+	Slope	1.573	0.211
	M + F	$TW = 0.00002\ TL^{3.115}$	0.044	3.071-3.159	0.950	A+	Intercept	21.44	0.000
CL - TW	M	$TW = 0.0002\ CL^{3.021}$	0.069	2.952-3.090	0.943	I			
	F	$TW = 0.0001\ CL^{3.134}$	0.066	3.069-3.200	0.940	A+	Slope	4.391	0.037
	M + F	$TW = 0.0002\ CL^{3.062}$	0.048	3.014-3.111	0.939	I	Intercept	640.7	0.000

Table 4. Morphometric relationships between length and lengths for *P. leptodactylus* caught in the Sapanca Lake (*TL*, total length (mm); *CL*, carapace length (mm); *CW*, carapace width (mm); *AL*, abdomen length; *RL*, rostrums length; *HL*, head length and *ARL*, areola length; *SE*(*b*)., standard error of the slope b; *CL(b)*, 95% confidence limits of slope b; r^2, is the coefficient of determination; *I*, isometry growth)

	Sex	**Log *W* = Log a +b Log *L***	**SE(b)**	**CL(b)**	**r^2**	**Type of growth**	**Ancova**	**F (DF)**	**P**
TL - CL	M	LogCL= - 0.4868 + 0.5123 LogTL	0.010	0.503-0.522	0.960	I			
	F	LogCL= - 0.4867 + 0.5012 LogTL	0.009	0.492-0.510	0.956	I	Slope	0.509	0.476
	M + F	LogCL = 0.1135 + 0.4994 LogTL	0.007	0.493-0.506	0.954	I	Intercept	19.569	0.000
TL - CW	M	LogCW = -3.837 + 0.291 LogTL	0.006	0.285-0.297	0.949	I			
	F	LogCW = -2.8718 +0.2725 LogTL	0.006	0.267-0.278	0.942	I	Slope	0.390	0.533
	M + F	LogCW =- 2.8708+ 0.2758 LogTL	0.004	0.271-0.280	0.938	I	Intercept	258.4	0.000
TL - AL	M	LogAL = -1.6607+ 0.5149 LogTL	0.010	0.505-0.525	0.958	I			
	F	LogAL = -5.301+ 0.5767 LogTL	0.011	0.565-0.588	0.948	I	Slope	0.700	0.193
	M + F	LogAL = - 4.906+ 0.5635 LogTL	0.008	0.555-0.572	0.942	I	Intercept	41.208	0.000
TL - ARL	M	LogARL =- 0.1630+ 0.167 LogTL	0.004	0.163-0.171	0.926	I			
	F	LogARL = -0.8222+0.1673LogTL	0.004	0.164-0.171	0.937	I	Slope	0.002	0.961
	M + F	LogARL = - 0.1601-0.1632LogTL	0.003	0.159-0.166	0.918	I	Intercept	53.719	0.000
TL - HL	M	LogHL = -0.3977 + 0.3525LogTL	0.007	0.345-0.359	0.951	I			
	F	LogHL = 1.065 + 0.3305 LogTL	0006	0.324-0.336	0.955	I	Slope	5. 424	0.021
	M + F	LogHL = 0.736 + 0.336 LogTL	0005	0.331-0.341	0.951	I	Intercept	13.520	0.000
TL - RL	M	LogRL = 0.3905 + 0.1528 LogTL	0.005	0.148-0.158	0.888	I			
	F	LogRL = 0.5708 + 0.1438LogTL	0.004	0.139-0.149	0.852	I	Slope	1.534	0.217
	M + F	LogRL = 0.8694 + 0.1435 LogTL	0.004	0.139-0.147	0.851	I	Intercept	32.800	0.000

F: female; M: male

Table 5. A comparison of sex distribution and growth characteristics of *P. leptodactylus* populations in some water resources in Turkey (*M*= Male, *F*= Female, Growth type: *I* = isometric growth, *+A* = positive allometric growth, *-A* = negative allometric growth)

Water resources	Sex rate (F : M)	Sex	N	Growth type
Aktaş Lake, CL-W (Aksu and Kurt Kaya, 2017)	0.94 : 1	M	81	A^+
		F	76	A^-
		M + F	157	A^+
Eğirdir Lake, CL-W (Bolat and Kaya, 2016)	0.75 : 1	M	1289	A^-
		F	1719	A^-
		M + F	3008	A^-
Gaga Lake, CL-W (Yılmaz et al., 2011)	0.98 : 1	M	131	A^-
		F	129	A^-
		M + F	260	
Trakya District Lakes (Deniz et al., 2010)	0.56 : 1	M	1558	A^+
		F	880	A^-
		M + F	2438	A^+
Apolyont Lake, CL-W (Berber and Balık, 2009)	0.68 : 1	M	843	I
		F	573	A^-
		M + F	1416	I
Manyas Lake, CL-W (Berber and Balık, 2006)	0.53 : 1	M	731	I
		F	387	A^-
		M + F	1118	A^-
This study (Sapanca Lake)	1.24:1	M	118	I
		F	146	A^+
		M + F	264	A^+

While the TL-TW and CL-TW relationships were observed with positive allometry growth for females and also for pooled data for TL-TW (b>3, t-test, P>0.05), and all remaining length-weight relationships were shown isometry growth for females, males and pooled data (b<3, t-test, P<0.05). Though the student's t-test showed no significant statistical difference between the *b* values of male and female individuals, the all length-weight and length-length relationships were calculated separately for females, males, and all samples (Tables 3 and 4). The slope and intersection of length-weight and length-width relationships were compared among sexes by ANCOVA to test for equality of slope and intersection in data. There was no significant difference between the slope and intersection of male and female groups (ANCOVA; P<0.05).

The condition factors (K) for studied crayfish ranged from 2.52 to 5.16 for males, 2.12 to 5.95 for females and mean condition factors were calculated at 3.33±0.38 for males and 3.15± 0.73 for females.

The catch data from 14 fishing operations was standardized with CPUE by number and biomass. A total of 10107.22 g of crayfish was caught on the 48-trapping/hour during the study period. The mean CPUE for all traps was determined as 0.3929 n/fyke-net/hour and 15.2478 g/fyke-net/hour at the end of the study. The lowest and highest mean CPUE were established 0.0417 n/fyke-net/hour in November 2016 and 0.8750 n/fyke-net/hour in February 2017, respectively (Figure 5). The lowest and highest average CPUE was 1.4021 g/fyke-net/hour in November 2016 and 42.529 g/fyke-net/hour in April 2017 by weight (Figure 6). The mean CPUE values below MLS, according to the highest and lowest number and weight of harvested crayfish were calculated as 0.2976 n/fyke-net/hour and 9.7927 g/fyke-net/hour respectively. The mean CPUE values above MLS, according to the highest and lowest number and weights of harvested crayfish were calculated as 0.0952 n/fyke-net/hour and 5.2478 g/fyke-net/hour, respectively. Although there is a statistically significant difference in terms of individual CPUE values in number when compared to below MLS and above MLS (Hn=3.9213, p=0.4768), there is no statistical difference found according to their weights (Hw=0.0423, p=0.837). There is also no statistical difference observed in terms of CPUE value below MLS and above MLS according to sexes (Hn=0.0007, p=0.97948; Hw=0.3201, p=0.57154; Hn=0.5772, 267 p=0.44741; Hw=0.1746, p=0.67605).

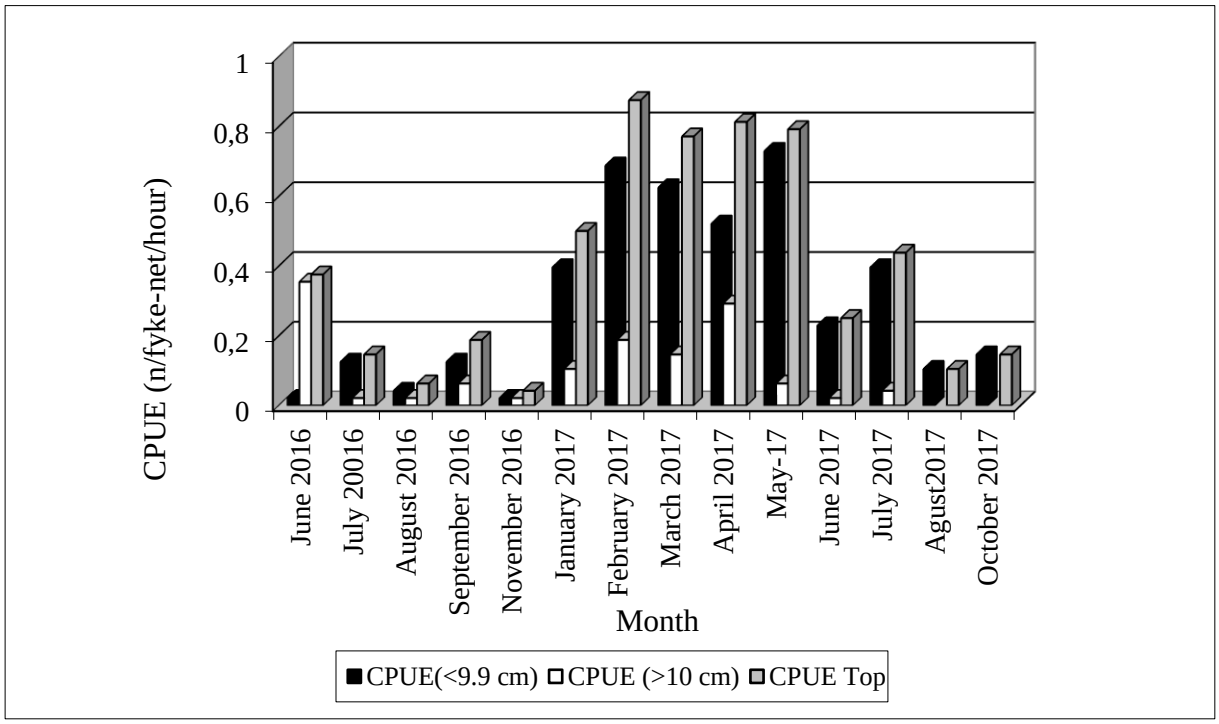

Figure 5. Comparison of catch per unit effort (CPUE) between below-MLS and above-MLS by the number of individuals

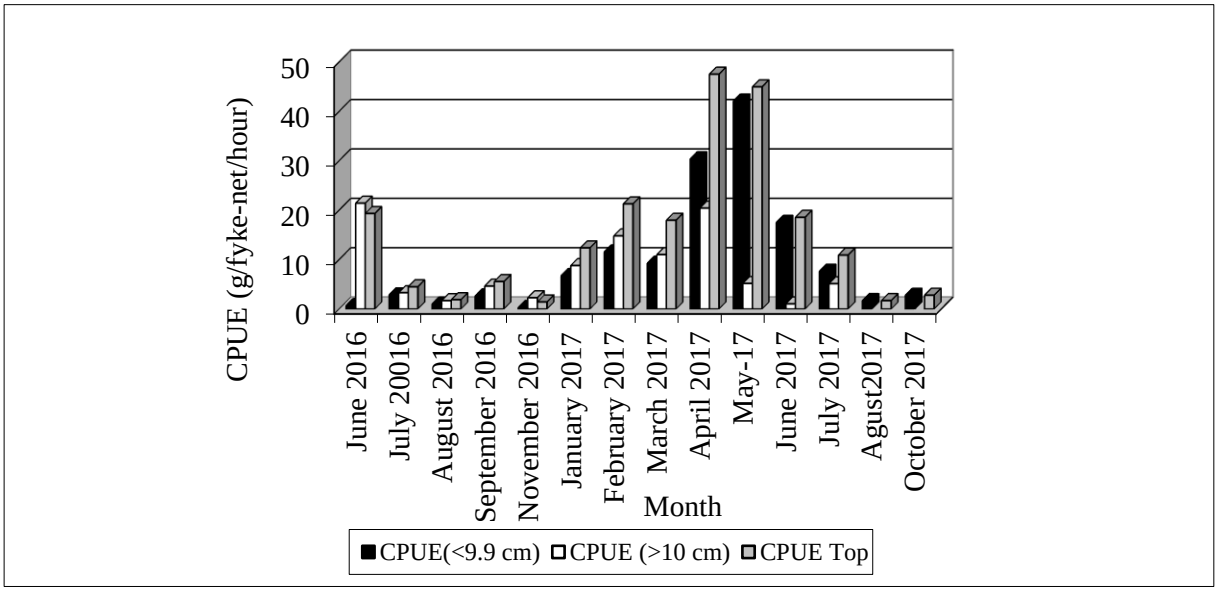

Figure 6. Comparison of catch per unit effort (CPUE) between below-MLS and above-MLS by weight

Discussion

The study is the first scientific report on some morphometric characteristics of freshwater crayfish (*P.leptodactylus*) in Sapanca Lake in Türkiye. The sex ratio, length and weight compositions of crayfish populations were evaluated. Besides, length-weight and length-length relationships, condition factors, and CPUE were calculated for the crayfish population in Sapanca Lake. Although many studies have been performed on the biological and morphological characteristics of *P. leptodactylus* since 1977, there has been no knowledge of this species in Sapanca Lake. Regarding all this, our study reveals important first findings.

Male, female and combined sexes of *P. leptodactylus* were measured and descriptive statistics are given related to them in Table 1. In general, the total weight of the male individuals of crayfish is heavier and longer than females (Romaire et al. 1977; Rhodes and Holdich 1979; Harlıoğlu and Güner 2006). It has been found in most of the studies on *P. leptodactylus* in Türkiye that due to the size of their chela lengths and their weight, male individuals have a greater average length and weight when compared to female individuals (Balık et al. 2005; Büyükçapar et al. 2006; Güner 2008; Aydın et al. 2015). Unlike other water sources in Türkiye, Sapanca Lake female crayfish weight and length is greater than the average male crayfish. It was determined that the mean TL, CL, AL, RL, HL and TW of the female individuals were slightly longer and heavier than those of males. In this study, the average body length and body weight of female crayfish were found to be greater than male individuals. The difference between these morphological characters is due to the predominance of environmental and food sources.

In the research period, 14 times sampling was made and a total of 264 specimens consisting of 146 females (55.3%) and 118 males (44.7%) with the sex ratio of 1.24:1 (female to male) were analyzed. But it was found that a sex ratio change occurs in *P. leptodactylus* caught in different months in the Sapanca Lake. Chi-squared test analysis showed significant differences from the ratio of 1:1. It has been reported that in a natural population, the female to male ratio will vary between 1:1 and 1:1.3 (Avşar 1998). The pooled data values acquire for our study were in the reasonable range expected for a natural population. Some researchers have also reported similar results in their studies in different inland waters in Türkiye (Erdemli 1982; Karabatak and Tüzün 1989, Köksal 1980, Kuşat and Bolat 1995). All these studies taken into consideration, it can be said that the sex ratio is also an important factor in stock evaluations since the reproductive equilibrium and the process of renewal of species are directly affected by the sex ratio in the ecosystem.

In the present results, the males, females, and both sexes of length-weight relationships showed the ideal value and little more than that of 3. While the Student t-test showed isometric growth and the slope of males, females, and both sexes did not exhibit significant interaction, the b value of the TL-W and CL-W relationships were significantly different for females and pooled data (TL-W) (Table 3). In this study, an isometric growth for CL-W of males and

pooled data and positive allometry of females was detected for crayfish, which is in apparent contradiction with some studies in other geographical areas (Table 5). The overall results indicate that *P. leptodactylus* showed an isometric pattern of growth in the studied habitat and the present conditions that exist in the collection site are conducible for the feeding and optimum growth of crayfish.

The determination coefficients (r^2) ranged from 0.851 to 0.960. Similar results were found for *P. leptodactylus* caught from Iznik Lake and Manyas Lake (Aydın et al. 2015; Berber and Mazlum 2009), Keban Dam Lake (Yüksel and Duman 2012), and Terkos Lake (Güner 2006).

The length-weight relationship and the condition factor are considered to be valid not only for fish but also for the evaluation of shellfish. The difference in growth types and condition factors of crayfish among locations may be a reflection of a number of factors, including population density, food abundance, photoperiod, water level fluctuations, and water quality (Weya et al. 2017). The mean condition factor of the crayfish population of Sapanca Lake was 3.23±0.60 (a minimum of 2.12 - a maximum of 5.95). Unlike other water sources in Türkiye, Sapanca Lake female crayfish's weight and length is greater than the average male crayfish, and female and pooled data crayfish are both showing positive allometric growth and condition factor greater than 3. These differences are thought to be due to factors such as the water characteristics, nutrition, and low crayfish density in the Sapanca Lake. The rate of disease symptoms in crayfish caught from Lake İznik was determined as 5.27% (Aydin et al. 2015). İn this research, however, crayfish plaque signs were observed in approximately 11% of the total crayfish caught. According to these results, it was observed that the effect of the plague disease still continues in Sapanca Lake and it has a negative effect on the development of the crayfish stock.

As seen in Figures 5 and Figure 6, CPUE values below MLS were the lowest in November and the highest in May 2017 according to the individual number and weight of the crayfish harvested by months. Depending on the crayfish harvested by months, while the number of individuals, CPUE values above MLS were the lowest in November 2016 and by weight in June 2017. However, the highest CPUE values in June 2016 were determined both by number and weight. Variation of individual numbers of crayfish monthly, CPUE indicated that catches increased considerably from February to May, while it was seen to increase significantly from April to June in terms of weight. Previously, Balık et al. (2002) determined the average CPUE value as 1.10 crayfish/fykenet/night for the lengths above MLS and reported that the CPUE of the caught crayfish in June and July was quite low compared to other months and increased gradually from June to December in Iznik Lake. Additionally, Demirol and Yüksel (2014) calculated the CPUE value per fyke net as 5.74 g/day for the lengths above MLS for 2012 in the Keban Dam Lake. In our case, there is a gradual increase observed in CPUE values for crayfish caught above MLS from January 2017 until April 2017. It was determined that the CPUE values of the crayfish caught in Sapanca Lake were very low compared to these lakes. CPUE value and size distributions of catch crayfish can be affected by the timing of trapping and associated environmental variables such as temperature, aquatic plants, etc. In addition, it is influenced by the density of fyke nets or traps.

Based on these findings in our presented study, it can be concluded the fact that the sampled crayfish are mostly caught below the MLS. The observed highest catch amount in winter and spring can be explained that the place where the traps are set and the catch is made, is covered with high water plants, as well as the high temperature of the summer months so the crayfish do not enter the traps as a result of hiding among the plants due to molting. Taking into consideration the above mentioned, calculating CPUE is very important to evaluate stock and provide information management engagements for the future and studies on sustainable developments.

Although numerous studies have been carried out on biological and morphological traits of *P. leptodactylus* since 1980 in Türkiye water bodies, there was no study of this species in the Sapanca Lake as yet. Considering all this, our study brings out important initial findings for the literature.

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Relationships of the *Cyprideis torosa* (Ostracoda, Crustacea) with Seasonal Occurrences, Carapace Type and Physicochemical Variables in Kocaçay Delta (Türkiye)

Okan KÜLKÖYLÜOĞLU[1*], Mehmet YAVUZATMACA[1], Enis AKAY[2, 3], Nurhayat DALKIRAN[2]

[1] Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, Bolu, Türkiye
[2] Department of Biology, Faculty of Arts and Science, Bursa Uludağ University, Nilüfer, Bursa, Türkiye
[3] Medical Laboratory Techniques, Department of Medical Services and Techniques, Istanbul Vocational School of Health and Social Sciences, Fatih, İstanbul, Türkiye

ABSTRACT

To understand the spatial and temporal continuing occurrence patterns and relationships of *Cyprideis torosa* to several physicochemical variables, monthly samples from seven stations were collected from Kocaçay Delta (Bursa, Türkiye) between 2018 and 2019. Four (*Cyprideis torosa, Koencypris ornata, Candona* cf. *lindneri, Candona meerfeldiana*) of 14 ostracod taxa are new records for the ostracod fauna of Bursa province, where the total numbers of recent species increased to 33. *Cyprideis torosa* was the only dominant species found in almost all samples throughout the sampling. Based on the carapace type of the species, three groups can be divided as i) Type-1 (smooth carapaces), ii) Type-2 (noded carapaces), and iii) Type-3 (carapaces noded on one valve and smooth/rare on the other). Different occurrence patterns of the species with overlapping ecological ranges were observed among the stations. Except for station 1, all the types were encountered from other stations. Beginning from December 2018, nearly all populations had mostly Type-1 individuals until March. During April-May, individuals with Types-2 and 3 appeared to increase until October, while individuals in the Type-2 group were solely found from three stations (2, 3, and 5) in March and May 2018. There was a significant difference in salinity, magnesium, and calcium values among the stations ($P<0.05$) but only total nitrogen, temperature, and calcium showed a medium correlation to carapace type. In all cases, populations with noded individuals were found in narrower ecological ranges for those variables than other populations with smooth individuals.

Keywords: Coding, seasonality, ecological tolerances, subfossils, recent distribution

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*** CORRESPONDING AUTHOR**

kulkoyluoglu_o@ibu.edu.tr
Phone : +90 374 254 1222

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Introduction

The genus *Cyprideis* (Cytherideidae) includes about 31 species (Meisch et al. 2019). Of which, *Cyprideis torosa* with wider geographic distribution than other species (Karanovic 2012) has been found primarily on highly saline (euryhaline), brackish or marine habitats (so-called "halophilic species" (Bronstein 1947)) and less frequently in freshwater habitats (Hartmann 1964). This is because the species shows broad tolerance ranges to salinity (Aladin 1993) and temperature values in several different aquatic ecosystems such as springs (Gülen 1985) and small water bodies and irrigation canals (Akdemir and Külköylüoğlu 2021). Also, live individuals of *C. torosa* have been recorded from streams (Külköylüoğlu et al. 2020) and a lake (Lake Bafa) in Turkey (Akdemir et al. 2020) where electrical conductivity values were below 400 µS/cm, referring to freshwater conditions. *Cyprideis torosa* was also reported from several other inland water types (e.g., springs, ditches, pits, ponds and brooks) in Germany, where electrical conductivities ranged in 750 to

77400 µS/cm (Scharf et al. 2017). Wouters (2017) - pointed out that species can be found from coastal zones of fresh, brackish, and marine habitats with high salinity values where the species was reported from 0.2 to 80‰ of salinity ranges in waters (e.g., see Bardawil inner lagoon) (Rosenfeld and Vesper 1977). Moreover, *C. torosa* can be active in waters with broad temperature ranges from 1.0°C (Ustaoğlu et al. 2012) to ca. 34.5°C (Bodergat et al. 2014). Dykan (2016) underlined that the species is highly abundant in density in a range of 0-25 ºC temperature between 3 and 5 m depth at Northern Black Sea coasts. Additionally, laboratory experiments (see details in Jahn et al. 1996) also showed that at least 50% of *C. torosa* tolerated hypoxic (reduced oxygen of 70% air saturation) conditions along with very high (1 mM and 1.8 mM) hydrogen sulfide concentrations for three weeks. Hence, it is clearly seen that *C. torosa* is one of the most tolerant species to several environmental variables, which can explain its wide geographic distribution ranging from fresh to euryhaline coastal waters.

Cyprideis torosa is a polymorphic species with different populations, including individuals with noded or unnoded (smooth) carapace. Historically speaking, these two types were considered two other species and forms due to the presence (forma *torosa*) or absence (forma *littoralis*) of nodes on the carapace (see Sars 1928; Klie 1938; Elofson 1941). However, it was later pointed out that such difference was probably related to changes in environmental conditions (e.g., salinity) (Van Morkhoven 1962; Keyser 2005; Frenzel et al. 2012; Frogley and Whittaker 2016; Berndt et al. 2019). Moreover, finding both forms from the same sites has already been reported recently (e.g., Triebel 1941; Külköylüoğlu et al. 1993; 1995; Scharf et al. 2017; this study) and fossil forms (Nazik et al. 2008; Witt 2010; Tuncer 2020), supporting that the two forms belonging to a single species as *Cyprideis torosa* (Meisch 2000). Besides the presence of some morphological characters (e.g., length of carapace, posteroventral spine on the right valve, the shape of hemipenis and clasping organs, length of terminal claw and medial seta of the fourth segment of the first antenna) (Van Harten 1975; Wouters 2017), presence/absence of nodes has been commonly used for species identification both in fossil and live (recent) samples. There are plenty of studies (e.g., see Sandberg 1964; Kilenyi 1972; Vesper 1972a, b; Heip 1976a, b; Keyser and Aladin 2004; Keyser 2005; Wouters 2017) on the occurrence of the nodes. It was earlier considered that node formation might be a genetic response. However, as shown by the studies of Keyser and Aladin (2004) and Keyser (2005), the appearance of the nodes corresponds to decreasing salinity levels during the molting stages due to osmoregulation. Therefore, nodes are most likely environmentally induced. This implies that individuals with nodes are more likely to be found in oligohaline habitats (<5‰ of salinity). Indeed, a few well-known long-term studies based on monthly samples (Vesper 1972a, b; Heip 1976a, b) and studies with seasonal samplings (e.g., Külköylüoğlu et al. 1993; 1995) provided supportive evidence for the previous works. These studies underlined that noding might be related to the monthly occurrence of the species because of the monthly or seasonal influence of air temperature on the aquatic ecosystems.

As stated above, *C. torosa* has a wide geographic distribution (Sandberg 1964). According to Wouters (2002, 2003, 2017 and references in there), the species has been known from Africa, Asia, Europe and above the Arctic Circle (see Schornikov 2011). Hence, its occurrence may be questionable in Australia (De Deckker and Lord 2017). Furthermore, King and Kornicker (1970) and Heip (1976a) had already reported the species from several sites in North America, but Wouters (2017) indicated that the species reported there were synonyms, and *C. torosa* was not known from the Americas. Besides, Sandberg (1964), in his detailed work on the species, provided a list of *Cyprideis* species from the Americas but not *C. torosa*. However, Sandberg (1964, see p. 93) did not collect specimens from North America but used several materials obtained from the A. G. Davis Collection (British Natural History Museum) and juvenile valves from Kijkduin (Holland). In contrast, recently, Pint and Frenzel (2017) reported the species from Nevada (North America) and Chile (South America) while it was also reported from Texas (Külköylüoğlu et al. 2021a). Thus, considering all the studies above (and several others cited in there) and doubts about its occurrence in Australia suggest that the species almost exhibit cosmopolitan distribution in all continents except the poles.

Although a quantity of studies on the species' ecology, biology and distribution exists in the literature, there is no extensive and comparative study on its monthly occurrence patterns along with carapace type and distribution of fossil and recent populations of the species in Turkey. However, an important issue that the presence of the species with or without nodes can aid to (i) understand salinity and temperature changes and/or fluctuations in aquatic bodies, (ii) estimate and possibly reconstruct the past aquatic conditions sought in paleontological studies, (iii) compare levels of changes between the past and present water quality measurements, and (iv) create proxy models and scenarios for future aquatic conditions which can elaborate our understanding

about the possible impact of climatic changes. Therefore, the aims of the present study focus on three points to (1) accomplish the first monthly sampling of *C. torosa* along with its correlation to some environmental variables (e.g., salinity and temperature) in the Kocaçay Delta, (2) search relationship between occurrence patterns and carapace type, and (3) compare species distribution both in subfossil and recent populations in Turkey.

Materials and Methods

The sampling area (Figure 1) is located in Kocaçay Delta Floodplain Forest (ca. 42.000 hectares of surface area), which is known to be one of the essential floodplains in Turkey. Kocaçay stream (aka Susurluk Stream) reaches the Marmara Sea after it receives water from several different aquatic bodies, such as Susurluk Stream and Lake Uluabat in the east of Karacabey District (Bursa province). As a result, it develops a large floodplain delta including three lakes (Poyraz, Arapçiftliği and Dalyan lakes, on the East and West of the stream, respectively), swamps, sand dunes, and floodplain (longoz) forest (Keçeli and Ursavaş 2019) where it emerges to the Marmara Sea.

Monthly samples were taken from seven stations (Figure 1) from the Kocaçay Delta (Bursa, Turkey) between 30 March 2018 and 04 April 2019. Water samples for chemical analyses were collected from each station in plastic bottles (1 lt). Chemical analyses were done after APHA (1998) methods.

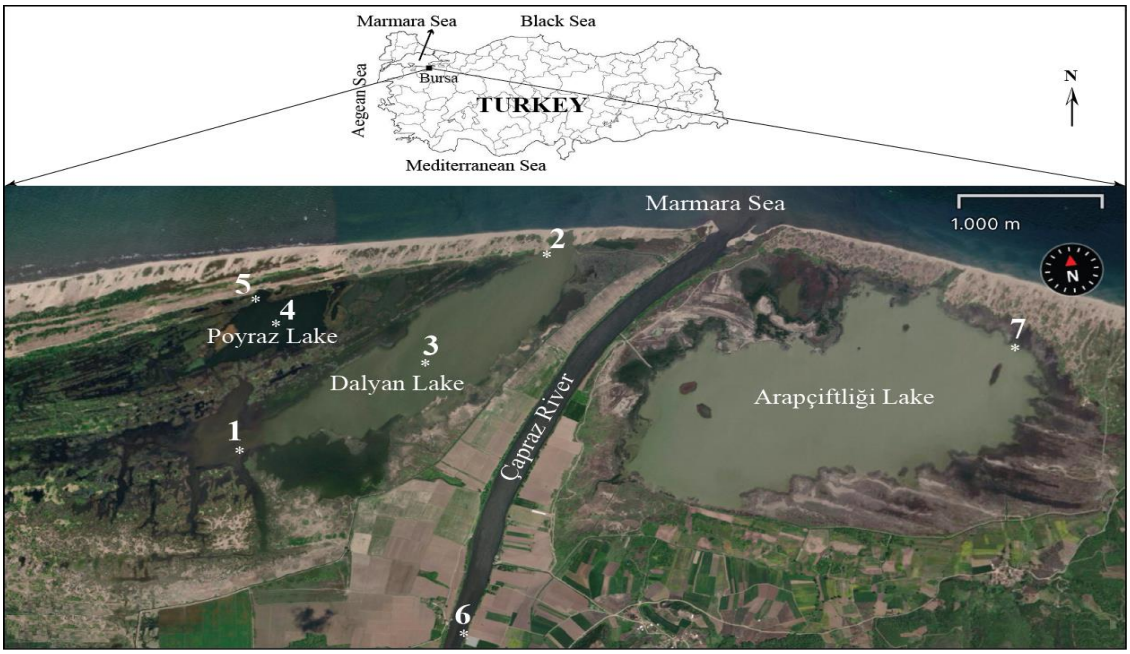

Figure 1. The seven sampling stations in Kocaçay Delta (Bursa, Turkey).

Lovibond Senso multiprobe was used to measure water temperature (°C), pH, dissolved oxygen (mg/L) and electrical conductivity (mS/cm) in situ. Ostracod samples taken from the littoral zones (stations 1, 2, 5, 6, 7) were collected from the shores (ca. 1 m^2 area with a maximum of 1 m depth) with a hand net (0.5 mm mesh size) and fixed with 70% alcohol in 200 ml plastic bottles. Other samples taken from the pelagic sites (stations 3 and 4) of the lakes were collected with Ekman bottom grab (152x152x152 mm in size) (ENVCO). This includes the samples collected from the uppermost sediment layer. These samples were filtered through a Retsch brand stainless sieve and separated from the sediment as much as possible. Then, samples were fixed in plastic bottles with 70% alcohol in situ. In the laboratory, all individual samples were separately washed under the tap water through three standard seized sieves (0.5, 1.0, 2.0 mm of mesh size) and fixed with 70% ethanol for future studies. We used a stereomicroscope (Olympus SZ-STLA) to sort specimens from the sediment and dissect them in lactophenol solution. Individual samples were examined with fine needles (no: 000) and covered with a cover slide while related information (gender, dimensions, sampling date, site name etc.) was noted on each of them. Whenever possible, taxonomic identification at the species level was made under a light microscope (Olympus BX-51). A taxon is left as "sp." if lacking undamaged and adult individuals. The carapace and valves of dissected species were kept on micropaleontological slides. We followed the taxonomic keys of Meisch (2000) during identification. All samples were placed in the Limnology Laboratory, Department of Biology, Bolu Abant İzzet Baysal University, Bolu, Turkey. The measured values were compared among

the stations with the non-parametric t-test with equal variances (significant if $P < 0.05$). To comprehend possible relationships among carapace type (Type-1 (smooth carapaces), Type-2 (both carapaces noded), and Type-3 (both carapaces noded on one valve and smooth on the other), abundance values (numbers of live adult individuals) and measured physicochemical variables explained above, we used Spearman correlation analysis (0-0.33 low, 0.33-0.66 medium, >0.66 strong correlation) with binary data and ternary plots obtained from the PAST 4.03 program (Hammer et al. 2001).

Results

A total of 10 recent (extant, living) and four subfossils (dead valves and carapaces) ostracod taxa (*Neglecandona angulata, C.* cf. *Lindneri, C. meerfeldina, Candona* sp., *Cypria* sp., *Cyprideistorosa, Cypridopsis* sp., *Eucypris* sp., *Heterocypris salina, Ilyocypris* sp., *Koencypris ornata, Limnocythere* sp., *Plesiocypridopsis* sp. and *Potamocypris* sp.) were reported from the present study. Four reported taxa (*C. torosa, K. ornata, C.* cf. *lindneri,* and *C. meerfeldiana*) increased the number of documented recent species up to 33 in the Bursa province. *Cyprideis torosa*, the most frequently occurring dominant species, was observed in almost all samples throughout the sampling period (but see a few exceptions) (Figure 1, Table 1). Based on the carapace type of the species, three groups can be separated as i) Type-1 with smooth carapaces, ii) Type-2 with noded carapaces, and iii) Type-3 with carapaces noded on one side and smooth on the other or nodes are smaller on the left valve than right one. Different occurrence patterns were observed among the stations (Table 1). While one live female and three subfossils (carapace and valves) of *C. torosa* with all smooth carapaces were found at station 1, populations with all three types of carapaces were randomly encountered from other stations. Live individuals were found in wide ranges of salinity (0.21-28.89 mS/cm) and water temperature (6.03-34°C), corresponding to the known ranges (Table 2). There was a significant difference in the values of salinity, magnesium (Mg), and calcium (Ca) among some stations ($P<0.05$), while no significant difference was found for other variables. These differences were especially apparent between two stations (1st and 6th stations) located far from the Marmara Sea. Spearman correlation analyses exhibited medium but insignificant correlations for water temperature, Ca and total nitrogen with carapace type. At the same time, none of the variables examined here revealed a significant correlation with the abundance values (Table 3). Carapace type tends to show variations among the seasons from November until April, and almost all populations had mostly smooth carapaces. During April-May, individuals with Types-2 and three carapaces appeared to increase until the end of October. In all cases, populations with noded individuals were found in narrower ranges for those variables than those with smooth, smooth, and noded individuals. At the same time, the first rank was changed between the smooth and smooth-noded populations at different sites and sampling times (Table 1). A comparison of the fossil and recent populations showed that the distribution and reports of fossil forms were wider than current *C. torosa* populations in Turkey (Figure 2, Appendix). Overall, electrical conductivity (referring to salinity) seems more effective on the species occurrence and abundance than Mg and Ca alone (Figure 3). Results suggest that the occurrence of nodes on the carapace can be affected by both temporal and spatial conditions.

Discussion

Co-occurrence and salinity

With the additional reports, the number of non-marine ostracods in Bursa province increased to 33 species. This number is more than many other provinces of similar size in Turkey (Külköylüoğlu et al. 2021b). However, it is still considered an underestimation since previous reports are based on random or primarily one-time sampling efforts, and there is no extensive study on the ostracod fauna of the province. Meanwhile, looking at the abundance of individual species, it is seen that *C. torosa* is the only dominant species encountered, along with nine other recent taxa (Table 1). Of which, four candonids (*N. angulata*, *C. meerfeldiana*, *C.* cf. *Lindneri*, *Candona* sp.) were the most accompanying taxa, with *C. torosa* followed by others (*Cypria* sp., *Heterocypris salina*, *Eucypris* sp., *Potamocypris* sp., *Plesiocypridopsis* sp.). It is already known that some of these taxa can be found in fresh to saline habitats together with *C. torosa* (Meisch 2000; Scharf et al. 2017; Pint and Frenzel 2017; McCormack et al. 2019). However, a comparison of the abundance values amid taxa portrayed that *C. torosa* was generally overnumbered (>98%) during our study. Plotnikov et al. (2021) reported that *C. torosa* was

Figure 2. Distribution of fossil (*) and recent (▲) *Cyprideis torosa* in Turkey (see Appendix for the references).

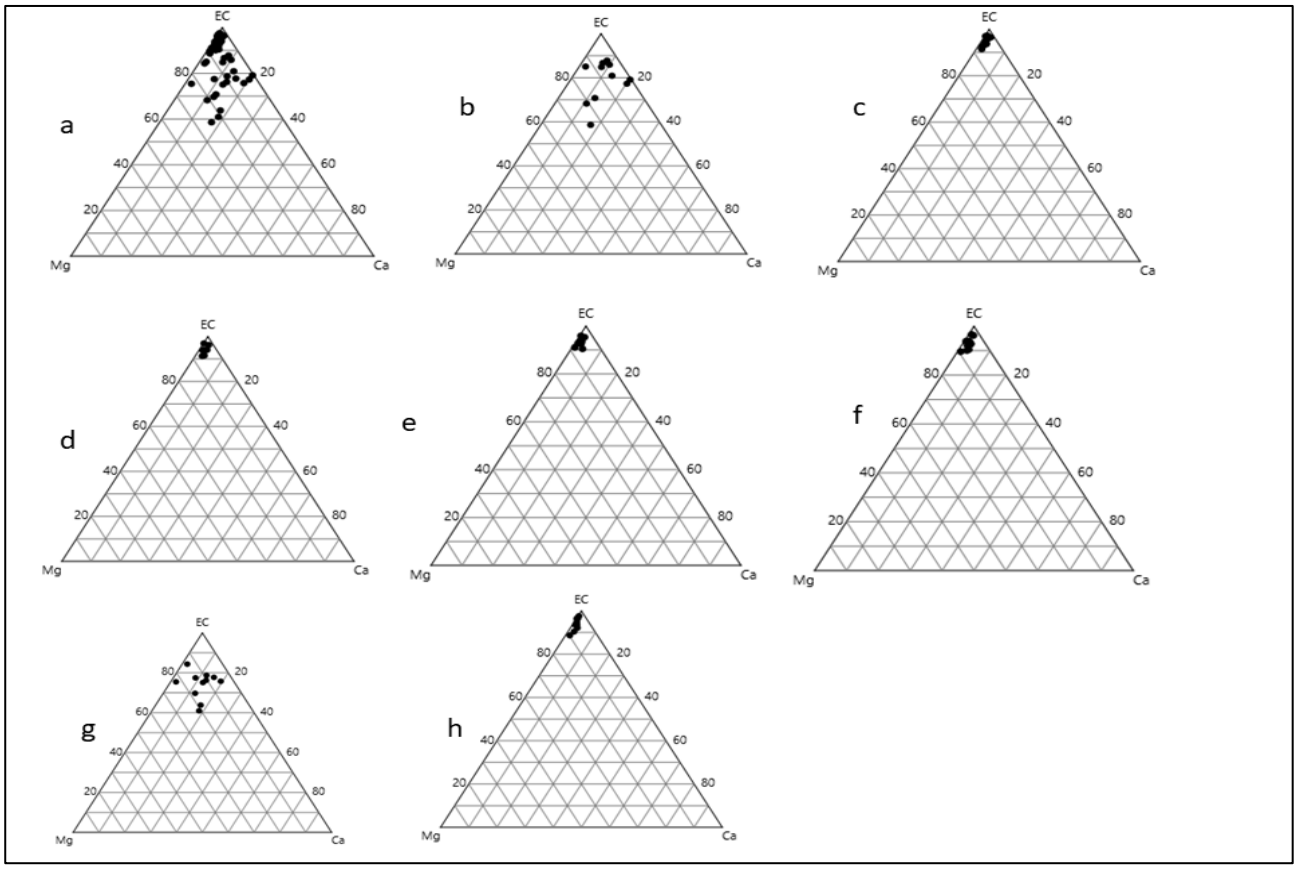

Figure 3. Ternary plots with percentage values show relationtionships among Ca, Mg and EC for all data (a) and stations 1-7 (b-h).

Table 1. Monthly distribution of *Cyprideis torosa* among seven stations (St No). Abbreviations: m (adult male), f (adult female), S (smooth individuals), N (noded individuals), NS (noded + smooth individuals) carapaces. Recent (extend, live individuals), Subfossil (surface sediment samples) (adult carapace or valves), Rec Juv (live juveniles) and Fos Juv (subfossil juveniles/only carapace or valves).

Date	St No	Carapace	Recent	Subfossil	Rec Juv	Fos Juv
30.03.2018	1					
30.03.2018	2	S	1			
30.03.2018	3	S	4f			
30.03.2018	4	S	1m1f	8		
30.03.2018	5	N	1f			
30.03.2018	6					
30.03.2018	7					
04.05.2018	1					
04.05.2018	2	N	2m16f			
04.05.2018	3	N	6m1f	1		
04.05.2018	4	NS	>100	>100		
04.05.2018	5	NS	3m9f	>100		
04.05.2018	6	NS		2		
04.05.2018	7	NS	7m18f	1		
28.05.2018	1	S				
28.05.2018	2	S		2		
28.05.2018	3	S	1m			
28.05.2018	4	S	1m3f		2	
28.05.2018	5					
28.05.2018	6					
28.05.2018	7	S		1		
02.08.2018	1					
02.08.2018	2	S	2			2
02.08.2018	3	S	1m4f		1	
02.08.2018	4	S	3f	1		
02.08.2018	5	NS	>100	>100	>100	>100
02.08.2018	6	NS		1	1	
02.08.2018	7	NS	>100	>100	>100	>100
05.09.2018	1					
05.09.2018	2	NS	10m35f	1	1	
05.09.2018	3	NS	8m11f	1	1	
05.09.2018	4	S	3f	20	1	
05.09.2018	5	NS	>100	>100	>100	>100
05.09.2018	6	NS	1f	1	2	
05.09.2018	7	NS	>100	>100	>100	>100
29.09.2018	1					
29.09.2018	2	NS	8m28f	1	5	
29.09.2018	3	NS	3m2f	1	4	
29.09.2018	4	NS	8m49f	>100	>100	

Table 1. Continued.

Date	St No	Carapace	Recent	Subfossil	Rec Juv	Fos Juv
29.09.2018	5	NS	>100	>100	>100	>100
29.09.2018	7	NS	>100	>100	>100	>100
24.10.2018	1	S	1f	2		
24.10.2018	2	S	7m9f	1	1	
24.10.2018	3	S	17m53f	>100	>100	
24.10.2018	4	NS	2m6f	>100	>100	
24.10.2018	5	NS	31m40f	>100		>100
24.10.2018	6	S		1		
24.10.2018	7	S	27m42f	>100	>100	>100
20.12.2018	1	S		1		
20.12.2018	2					
20.12.2018	3	S	2m3f	5		
20.12.2018	4	S	2m8f	>100	>100	
20.12.2018	5	S		2		
20.12.2018	6	S		1		
20.12.2018	7					
17.01.2019	1					
17.01.2019	2	S		3		
17.01.2019	3	S	2m38f	2		
17.01.2019	4	S	7m4f	>100	>100	
17.01.2019	5	S	4m3f	9		
17.01.2019	6	S		1		
17.01.2019	7					
20.02.2019	1					
20.02.2019	2	S	5m5f	1	1	
21.02.2019	3	NS	10m40f	1	>100	
22.02.2019	4	S	>100	>100		
23.02.2019	5	S	2f	2		
24.02.2019	6					
25.02.2019	7	S		11		
04.04.2019	1					
04.04.2019	2					
04.04.2019	3	S	2m9f	1		
04.04.2019	4	S	2m1f	2		
04.04.2019	5	N		1		
04.04.2019	6					
04.04.2019	7	S	4m2f	2		

Table 2. Mean, maximum (MAX) and minimum (MIN) values (n = 77) of 13 variables for *Cyprideis torosa* with noded, nodes + smooth, and smooth carapaces. Abbreviations: DO (dissolved oxygen, mg/L), EC (electrical conductivity, mS/cm), water temperature (T°C), salinity (Sal, ppt), phosphate (PO_4-P), total phosphate (TP), nitrite (NO_2-N), nitrate (NO_3-N), total nitrogen (TN), and suspended solid matters (SSM) (all units are in mg/L unless otherwise indicated).

	DO	**pH**	**EC**	**TºC**	**Sal**	**Mg**	**Ca**	**PO_4-P**	**TP**	**NO_2-N**	**NO_3-N**	**TN**	**SSM**	***C. torosa***
MEAN	7.13	8.18	12.82	17.08	7.44	694.33	254.59	0.055	0.138	0.0140	0.167	3.531	35.35	Noded
MAX	9.80	8.51	18.06	19.60	10.67	875.52	384.77	0.171	0.209	0.0490	0.403	4.827	46.70	
MIN	5.10	7.84	3.89	15.18	2.05	228.69	24.05	0.010	0.077	0.0010	0.073	2.197	22.30	
MEAN	7.06	8.42	16.47	24.18	9.92	937.83	342.58	0.102	0.326	0.0260	0.229	5.425	70.33	Noded+ Smooth
MAX	11.90	8.79	44.70	34.00	28.89	3112.96	801.60	0.369	1.253	0.2010	1.099	16.370	175.30	
MIN	3.40	7.93	0.64	13.20	0.31	82.69	68.14	0.006	0.054	0.0001	0.027	1.595	13.40	
MEAN	7.45	8.25	10.41	15.76	6.26	555.23	197.85	0.071	0.203	0.0090	0.263	3.220	54.67	Smooth
MAX	10.80	8.83	28.30	30.17	17.42	2315.24	673.34	0.277	0.587	0.1350	1.738	5.893	215.30	
MIN	1.90	7.37	0.442	6.03	0.21	2.43	12.02	0.003	0.050	0.0006	0.038	1.925	11.20	

the most common species with long-term tolerance to salinity increase in the Aral Sea. These authors found different occurrence frequencies of the species between Large Aral (salinity range 8-13 ppt) and Small Aral (salinity range 0-3 ppt). The species was the last survivor during the salinity increase in the lake. However, they argued that increasing salinity could even cause the extinction of *C. torosa* in Large Aral but the species present in Small Aral, implying that even *C. torosa* has some upper limits of salinity tolerance.

During the present study, there are, however, variations of the species occurrence pattern among the stations. For example, one possible explanation for such an occurrence may be that station 1 receives a water discharge from a small creek (Figure 1). Besides, it is also faced with a seasonal sea water intrusion when the sea water level rises over the narrow coastal barrier into the lake area (Dalkıran N., pers. comm). Thus, one may argue that this water back up from the creek may reduce salinity (also referring to electrical conductivity) at that sampling point lower than *C. torosa* prefers. However, the species can tolerate wide salinity (and temperature) values. In a microcosm study, Frenzel et al. (2012) showed highest numbers of reproductive rates at the salinities ranged from 3 to 8 psu (Practical Salinity Unit = ppt) while noded valves being most abundant below 2 psu were also found up to 7 psu.

Furthermore, the authors reported smooth valves above the limit 7 psu. Accordingly, their study showed a similar trend for both males and females. In our case, however, salinity ranges between station 1 (0.16 and 0.69 ppt) and station 6 (0.21 and 0.47 ppt) overlap. Therefore, an insignificant level of salinity range does not explain why *C. torosa* was relatively abundant in station 6 but station 1.

Pint and Frenzel (2017) recently proposed a flowchart for paleoenvironmental interpretationbased on species dominance. Hence, if the dominancy of the species is more than 90%, the habitat can be characterized as hypersaline or with oxygen deficiency. In contrast, dominancy with less than 90% refers to fresh to brackish waters. Although their application is suggested to use fossil occurrences of the species, it can also be used to determine habitats with present conditions. We collected *C. torosa* from the stations (but cf. station 1) with more than 90% of dominancy almost all year round. This finding suggests that the delta is of hypersaline conditions, but this does not support oxygen deficiency due to a relatively high mean oxygen value (ca. 7.16 mg/L).

Table 3. Results of Spearman correlation analyses between the numbers of living adult *C. torosa* (NumInd) and carapace type (Car. Type) with 12 variables. Bold numbers show medium but not significant correlations for T°C, Ca and TN. See the text and Table 2 for the units. "NumInd" represents the calculated values after abundances.

Variable	**NumInd**	**Car. Type**
DO	-0.045	-0.150
pH	0.190	0.158
TºC	0.119	**0.389**
Salinity	0.237	0.234
Mg	0.235	0.291
Ca	0.223	**0.342**
PO_4-P	0.092	0.067
TP	0.147	0.189
NO_2-N	0.135	0.033
NO_3-N	0.061	-0.102
TN	0.258	**0.429**
SSM	0.249	0.075

Mg, Ca and noding

This explanation above may have a value since salinity, Mg and Ca measurements were significantly (t-test, $P<0.05$) different between stations (1st and 6th) and others (2-5, 7th) where the species exhibited seasonal occurrence patterns with high abundance. No significant difference ($P>0.05$) was found for other variables. Station 6 is located on the Çapraz River, which flows continuously through the Marmara Sea, but intrusion from the sea occurs seasonally. Thus, its water is mixed all the time, where both smooth and smooth-noded individuals were collected during the study. Both elements are necessary for the carapace formation, while Ca is generally higher than Mg in the carapace. However, with a few exceptions, the Mg values of the stations were found to be almost always much higher than Ca during the present study. These differences were apparent between two stations (1 and 6) which were the furthest in the distance to the Marmara Sea. Indeed, we found *C. torosa* from the known ranges of these variables obtained in the literature. What is, however, the imperative is to associate species' frequent occurrences amid the stations with or without (or both) noded carapaces. As mentioned, carapace morphology seems to be related to salinity (and temperature) changes in waters that noded individuals tend to be found more commonly in freshwaters than brackish or saline waters. In addition to these variables, however, previous studies (Keyser 2005; Frenzel et al. 2012) pointed out that node formation might also be correlated to deficiency of Ca level, suggesting that the numbers of nodes can be increased in the waters with low Ca. Although the correlation was medium and not significant (Table 3), our results support the opposite of this view that the mean Ca level (197.85 mg/L) was the lowest among other groups where there were only individuals with smooth carapaces (Table 2). While working on another species (*Limnocythere inopinata*) in Lake Van (Turkey) known with Ca limitation (0.105 to 0.087 mmol/L) (Reimer et al. 2009), a similar finding was outlined by McCormack et al. (2019) that node formation may be influenced by several other factors that Mg may be one of them. Our values are much higher than these and may seem good enough to build a carapace structure. However, this does not explain the absence (except one female) of *C. torosa* at station 1, although its chemical composition is similar to station 6, where the species was relatively higher in numbers and occurrences.

Several studies (Meisch 2000) showed that some species and/or genera could be associated with lower salinity ranges. For example, finding members of the genus *Candona* from station 1 may support this view due to their freshwater habitat preferences with low salinities (Neale 1988; Karanovic 2012), but we still need to find out why *C. torosa* was not found and/or was not common there. This question is essential because some taxa reported here (e.g., *Heterocypris salina*, *Eucypris* sp., *Plesiocypridopsis* sp., *Cypridopsis* sp.) are already known to survive in wide ranges of salinity, temperature and/or pH values (Delorme 1991). As indicated in their excellent review, Dettman and Dwyer (2012) underlined that several other factors can influence carapace chemistry and structure. Hence, there is no single explanation for the relationships between the formation of nodes on the carapace and Mg, Ca and/or Mg/Ca in waters. On the other hand, Figure 3 suggests that its electrical conductivity is more closely related to species occurrence/abundance than Mg and Ca alone. Moreover, our results with Mg and node formation support a similar explanation for Ca, where individuals without nodes were solely found below the mean (555 mg/L) of Mg level.

Temperature, seasonality and noding

Herman et al. (1983) showed that *C. torosa* has one generation, and several factors can affect its life cycle and occurrences effectively. For example, salinity increase can be directly intimated with temperature. This is the case for *C. torosa*. Heip (1976a, b), after more than four years of continuous work, illustrated that the adults' abundance and occurrence were triggered and closely related to water temperature above 15°C. Our results support this approach with a few exceptions in some months (see Table 1) where adults are high in numbers below this proposed temperature level. For example, there were more adults at station 3 during January and February 2019, when the water temperature was 6.03 and 13.2°C, respectively. In contrast, a medium correlation between water temperature and species abundance was not significant. Nevertheless, this does not change the general view proposed by Heip (1976a) that the numbers of adults increase with increasing temperature (and salinity), but this should be investigated in detailed studies.

On the other hand, relating the temperature to monthly occurrences of the noding, it is valuable to indicate that adults without nodes mostly begin at the end of the fall season (November) until the spring season (April). Similarly, the individuals without nodes (but with a few exceptions) were reported all year round from a eutrophic lake, Lake Küçükçekmece (Turkey) (Külköylüoğlu et al. 1993). In another monthly study, however, Külköylüoğlu et al. (1995) reported a similar pattern of the noded and smooth individuals of *C. torosa* from a brackish water lake (Lake Büyükçekmece) (now the lake is freshwater characteristics due to separation from the

Marmara Sea in 1985) in summer (June) and winter (December) seasons. In both studies, authors failed to measure the salinity values of the lakes. Külköylüoğlu et al. (1995) underlined that node formations might be a critical issue for the species because it probably helps the species movement on the sediment in freshwater conditions while the species may not need the nodes in saline waters due to lifting force. Unfortunately, these authors did not ask a specific question about the study's correlation between noding and salinity.

Additionally, these explanations may not represent the true nature of the correlation between node formations and water chemistry. However, they help to deduce an understanding of it. Nevertheless, as shown in previous studies (see above), node formations are possibly a response to environmental factors.

pH, alkalinity and noding

Alkalinity was suggested as an influential factor in the carapace structure and formation of nodes (Van Harten 2000; McCormack et al. 2019); for instance, De Deckker and Lord (2017, p.4) stated that "…*It is unfortunate that neither Vesper nor Heip measured alkalinity of the waters during their long investigations of the life cycles of torosa, and this needs to be examined in the future to understand ostracod shell composition better. Alkalinity, combined with ionic analysis of the ambient waters, will lead to identifying the calcite saturation nature of the waters in which ostracods moult and grow*." We did not measure alkalinity during the present study, but pH values were calculated. Moreover, we know that water pH and alkalinity are not the same, but they are closely related (Boyd et al. 2017). This relationship implies that increasing pH values (>7, referring to alkaline waters) means high alkalinity. In a very comprehensive work by Boyd et al. (2017), this relationship in waters is provided as pH = 6.6, alkalinity = 1 mg/L; pH = 7.3, alkalinity = 5 mg/L; pH = 7.6, alkalinity = 10 mg /L; pH = 8.3, alkalinity = 50 mg/L. This information may be applied to the studies; for example, *C. torosa* was reported in the waters of Terschelling Island, where pH values were measured between 7.5 and 8.5 (Scharf and Hollwedel 2010). The implication is that alkalinity was at least ten or more in the island's waters. During the present study, we have a total of 77 pH measurements. There are only 16 of 77 cases where pH values were below 8.0. Of these, there are only three cases (pH = 7.84, 7.92, and 7.96) where we identified live *C. torosa* (the first two with noded individuals and the last with smooth individuals, respectively). In comparison, we found no ostracods or only valves/carapaces in six and seven cases (mostly smooth and noded-smooth individuals but no single population with solely noded individuals found), respectively. The remaining cases (61 of 77) include pH values ≥8.0. Adapting the equations of Boyd et al. (2017), we may link the pH values (now referring to alkalinity values above) to the noding on carapaces. The mean pH values (8.14-8.47) among the stations did not show a significant difference, but it can be inferred that the species may prefer waters with alkalinities above 10 mg/L or even 50 mg/L. This can be helpful to information provided here for the first time that such a view may be used in fossil forms for understanding past environmental conditions in paleontological studies.

Fossil vs Recent (live) forms

In Turkey, *C. torosa* was reported from Early Miocene (Ilgar and Nemec 2005) corresponding to the previous records (cf. Van Harten 2000; Witt 2010; Wouters 2017). When we compare dispersion of the fossil and live species reported so far (Figure 2), the numbers of fossil records from about 24 provinces (aka cities) are higher than living specimens in 20 provinces. With a few exceptions (Figure 2), living forms have been mostly coupled with fossil records reported from nearby the west and northwest coastal zones (around the Marmara Sea) of Turkey. Although there are extensive studies in some provinces (e.g., Sinop, Çankırı, Eskisehir, Elazığ, Konya), which include about 1000 water samples, there are only surface sediment samples of (subfossils) *C. torosa* populations reported from them. The last four of these cities (and more others, see Figure 2) are far away from the seas and are located within Anatolia, where fossils were found in several different water bodies. Two other similar proxies can be worth discussing: First, *C. torosa* with smooth and noded individuals were reported from Holocene samples of Lake Sevan (Armenia) (Wilkinson et al. 2005). The lake is at 1900 m asl and has no connection to the seas. The authors pinpointed those smooth forms were encountered in a Holocene sequence more than noded forms, implying that the lake salinity had increased during at least the last 5000 years or late Holocene. Second, similarly, in Germany, Scharf et al. (2017) reported Quaternary fossils of *C. torosa* from 32 of 45 inland sites far away (more than 200 km) from the Baltic and the North seas. The opposite situation is also true for live populations with a few cases. There can be at least three possible ways to delineate this situation (1) lack of studies, (2) unsuitable habitats for the species, and (3) no time for the species migration yet. On the other hand, we believe that such a map showing overlapping ranges of fossil and live forms can help

us understand species distribution since the Early Miocene in Turkey.

Overall, in conclusion, as stated above, alkalinity was not directly measured in situ, so we cannot explain its correlation with the noding of the carapace. However, combining salinity and/or alkalinity with other environmental and biotic variables may be a better way to apply them in future studies. Indeed, total nitrogen (and phosphorous) portrayed a medium correlation (P>0.05) to the species abundance among the stations. Consulting Figure 1 and the site description above, one can recognize agricultural activities or so-called "human activities" around the study area. According to Chen et al. (2015), the global distribution of TN and TP values in lakes can be found between 0.526 mg/L and 0.014 mg/L. Our mean values are all higher than these (cf. Table 2). This implies possible sources of nitrogen and phosphate and their compounds reaching the sampling sites due to human activities. *C. torosa* can overcome all these artificial inputs due to its high tolerance ranges. As indicated by Frenzel et al. (2012), *C. torosa* can be used as a suitable indicator species because the populations inhabit or prefer a wide range of salinities. For example, individuals of the athalassic populations from stable water bodies can be used to describe continuous and detailed water bodies.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection was performed by Enis Akay and Nurhayat Dalkıran. Ostracod samples preparation and analyses were provided by Okan Külköylüoğlu and Mehmet Yavuzatmaca. The first draft of the manuscript was written by Okan Külköylüoğlu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix

List of provinces in Turkey where the fossil and recent forms of *Cyprideis torosa* were reported. Also, references used in these lists are provided below.

Fossil forms

Adana (Nazik and Gökçen 1995; Şafak 2003; Avşar et al. 2006; Darbaş et al. 2008; Darbaş and Nazik 2010), Antalya (Alçiçek 2007), Aydın (İlhan and Öner 2019; İlhan 2020), Balıkesir (Leroy et al. 2002; Nikolaou et al. 2016; Parlak and Nazik 2016; Darbaş and Demircan 2017; Vardar and Öner 2017), Çanakkale (Atay and Tunoğlu 2002; Atabey et al. 2004; Çağatay et al. 2006; Meriç et al. 2019), Çankırı (Yavuz et al. 2017), Denizli (Şafak 2010; Karayiğit et al. 2015; Tuncer et al. 2016; Rausch et al. 2019; İlhan et al. 2020;

Rausch et al. 2020), Edirne (Sakınç et al. 2000), Tekirdağ (Sakınç et al. 2000), Elazığ (Koç-Taşgın et al. 2012), Eskişehir (Bassiouni 1979; Tunoğlu et al. 1995), İzmit (Gülen et al. 1995; Matzke-Karasz and Witt 2005; Kırcı-Elmas et al. 2021), Hatay (Boulton et al. 2007; Meriç et al. 2012a; Tekin et al. 2019), İstanbul (Şafak et al. 1999; Meriç et al. 2000; Çevik Üner and Özkar Öngen 2009; Witt 2010; Şekeryapan 2011; Meriç et al. 2013; Şafak 2016; Doğan et al. 2020), İzmir (Meriç et al. 2012b; Berndt 2014; Yümün et al. 2016; Stock et al. 2019; Stock et al. 2020), Kahramanmaraş (Şekeryapan et al. 2016), Karaman (Ilgar and Nemec 2005), Konya (Karakaş Kadir 1998; Beker et al. 2008), Malatya (Nazik et al. 2008), Mersin (Melis et al. 2015; Şafak and Nurlu 2018; Şafak 2019), Muğla (Gül et al. 2019), Sakarya (Kerey et al. 2004), Sinop (Şekeryapan 2011), Sivas (Koçyiğit 1989) and Yalova (Matzke-Karasz and Witt 2005; Rückert-Ülkümen et al. 2006).

Recent forms

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Determination of Amino Acid Profile and Some Characteristics of Collagen Extracted from Skin and Bone of Mangar (*Luciobarbus esocinus* Heckel, 1843)

Mustafa GÖÇER [1*] Yasemen YANAR [2*] Muhsin AYDIN [3*]

[1] Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adiyaman University, Adiyaman, Türkiye

[2] Faculty of Fisheries, Department of Seafood Processing Technology, Cukurova University, Adana, Türkiye

[3] Department of Biology, Faculty of Science and Letters, Adiyaman University, Adiyaman, Türkiye

ABSTRACT

Acid soluble collagens from bone (ASC-B) and skin (ASC-S) of mangar (*Luciobarbus esocinus* Heckel, 1843) were extracted, characterized, and their amino acid profiles were determined. To best of our knowledge, the current study is the first research that used this species as a source of collagen. Both ASC-S and ASC-B from mangar skin and bone contained glycine as the major amino acid and high amounts of proline, hydroxyproline, alanine, and glutamic acid. On the basis of dry weight, yields of ASC-S and ASC-B were 9.38 and 3.71%, respectively. Furthermore, fourier transform infrared spectroscopy proved that both collagens were integrated and native. Additionally, the results of X-Ray Diffraction (XRD) demonstrated that both of the collagens reserved their helical structures. The screened collagens had prominent absorptions at 230 nm by UV-Vis spectra. Additionally, the SEM studies have shown that both ACS-S and ASC-B have porous and fibrous nature. According to the UV–Vis and Fourier Transform Infrared Spectrophotometer (FTIR) results, extracted collagens were characterized as type I collagen based on their amino acid composition. According to the obtained results, the collagen isolated from mangar can potentially be an alternative source of vertebrate collagens for use in the diet and other industries such as medical and pharmaceutical industries.

Keywords: Mangar, ASC, FTIR, SEM, type I collagen

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*** CORRESPONDING AUTHOR**

m.aydin@adiyaman.edu.tr
Phone: +90 416 223 38 00 hy/ Ext. 4212

Ferkenin (*Luciobarbus esocinus* Heckel, 1843) Deri ve Kemiklerinden Ekstrakte Edilen Kollajenin Amino Asit Profili ve Bazı Özelliklerinin Belirlenmesi

Öz: Ferkenin (*Luciobarbus esocinus* Heckel, 1843) kemiğinden ve derisinden asitte çözünür kolajenler (ASC) ekstrakte edildi, karakterize edildi ve amino asit profilleri belirlendi. Bildiğimiz kadarıyla, yapılan bu çalışma bahsi geçen türü kolajen kaynağı olarak kullanan ilk araştırma olma niteliği taşımaktadır. Ferkenin derisi ve kemiğinden elde edilen ASC'lerin içerisinde ana amino asit olarak glisin ve yüksek miktarlarda prolin, hidroksiprolin, alanin ve glutamik asit içerdiği belirlendi. Kuru ağırlık, ASC-deri ve ASC-kemik verimleri sırasıyla %9,38 ve %3,71 olarak belirlendi. Ayrıca, fourier dönüşümü kızılötesi spektroskopisi (FTIR) ile her iki kolajenin de entegre ve doğal olduğu kanıtlandı. Ek olarak, X-Işını Kırınımı (XRD) sonuçları şunu gösterdi: her iki kolajen sarmal yapılarını korumuştur. Araştırılan kolajenler UV-Vis spektrumları tarafından 230 nm'de belirgin absorpsiyonlara sahiptir. Ayrıca, SEM çalışmaları hem ACS-deri hem de ASC-kemiğin gözenekli ve lifli yapıya sahip olduğunu göstermiştir. UV–Vis ve FTIR sonuçlarına ve ekstrakte edilen kolajenler, amino asit bileşimlerine göre tip I kolajen olarak karakterize edildi. Bu çalışma ile, ferkeden izole edilen kolajenin, diyet, tıp ve ilaç endüstrileri gibi diğer endüstrilerde kullanım için omurgalı kolajenine göre potansiyel olarak alternatif bir kaynak olabileceği belirlenmiştir.

Anahtar kelimeler: Ferke, ASC, FTIR, SEM, tip I kolajen

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Introduction

The *Luciobarbus esocinus* (mangar, ferke in Turkish) is a species of ray-finned fish in the genus *Luciobarbus*, native to the Tigris–Euphrates River system in the middle eastern countries that include Iran, Iraq, Syria, and Turkey (Freyhof 2014). This species was described as a vulnerable species by The International Union for Conservation of Nature's (IUCN) red list of threatened species. It is among the most important endemic species found in the Euphrates and Tigris rivers (Ozgur 2016). Mangar is usually hunted by locals as a source of food. It can reach about 2.3 m and weigh up to 140 kg (Fricke et al. 2007) and among the most preferred freshwater fish by local people.

As the main component of connective tissue, collagen is the major fibrous glycoprotein found mainly in the skin, bone, cartilage, tendon, and connective tissues of mammals and fish (Huo and Zhao 2009; Aberoumand 2012; Salvatore et al. 2021). Collagen makes up from 25% to 35% of the whole-body protein content and up to 85% of the skin of mammals. However, it makes up from 19% to 38% of the whole-body protein content and up to 96% of the skin of fish (Dave et al. 2019). A wide range of collagen products are available commercially, but not all collagens are from the same source, nor are they suitable for certain types of dietary requirements (Noorzai and Verbeek 2020). Many of these collagens are sourced from livestock production in different farms that include cattle, pig, and chicken, while others come from aquatic sources including fish, shellfish, jellyfish, and crustaceans. Cattle are more widely used in collagen production in compare to porcine and fish sources because of its lower price and the abundance of its skin and bones. However, there are increased concerns about the transmission of diseases such as bovine spongiform encephalopathy (BSE) to humans due to consumption of bovine-based products (Nathanson et al. 1997; EFSA Panel on Biological Hazards (BIOHAZ) et al. 2020).

Due to its resistance to stretching and its fibrous structure, collagen provides strength and elasticity of the skin as well as playing an important role in strengthening blood vessels and tissue development (Sherratt 2009). Collagen's low immunogenicity and high biocompatibility make it a preferred biomaterial in biomedical applications. Beside its industrial uses, there is a great interest in collagen's anti-aging effects in many medical fields such as plastic surgery, burn surgery, and even weight management (Borumand and Sibilla 2014; Sionkowska et al. 2020). For this reason, many studies have been done to find alternative sources of collagen. Recently there has been an increase in the trend towards collagen derived from aquatic sources. Fish-derived collagen has a higher level of bioavailability since fish-derived collagen peptides have been proven to be easily digested absorbed and distributed throughout the body as much as 1.5 time in compare to collagen derived from bovine or porcine sources (Sadowska et al. 2003). In many studies, it has been reported that even fish waste constitutes a high potential source of collagen (Kiew and Mat Don 2012; Wang et al. 2013; Mahboob et al. 2015). Therefore, the isolated collagen has been known that it can be used as a supplement to the diet.

In the current study, mangar (*Luciobarbus esocinus* (Heckel, 1843)) was investigated for its collagen resources and their amino acid profiles. *L. esocinus* (Heckel, 1843) is found in some Middle Eastern countries such as Iran, Turkey, Syria, and Iraq. It is one the most important endemic species found in the Euphrates and Tigris River system, which is located within the Middle East (Ozgur 2016). Since mangar is one of the most consumed fresh water fish in the region and to best of our knowledge there has been no previous study involving collagen extraction from its skin and bones, with this study we determined that whether the skin and bones of mangar can be used as an alternative source of collagen.

Materials and Methods

Materials

Two mangar fish (*L. esocinus* Heckel,1843), each weighed about 6 kg and approximate length was 88 cm, were purchased from the fish market in May 2019, then brought to the laboratory and cleaned, its skin and bones were separated from the body and they were frozen at -20 °C until reuse. Fish skin and bones were thawed in the refrigerator at +4 °C and later was brought to room temperature before application of the extraction procedure.

Methods

Sample preparation

The preparation of the collagen samples was performed with minor modifications of the method that was previously described by Nagai and Suzuki (2000) (Nagai and Suzuki 2000).

All sample preparation procedures were carried out at not exceeding +4 °C. Each sample was studied as duplicate in all examinations.

Characterization of collagens

Collagen yields

Collagen yield was calculated using the dry weight of the material as specified in the following formula.

$$\text{Collagen yield (g/100g)} = \frac{\text{Weight of lyophilised Collogen}}{\text{Initial weight of lyophilised fish skin}} \times 100$$

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis of collagen samples (one repeat) was performed as previously described by Kittiphattanabawon and colleagues (Kittiphattanabawon et al. 2005). Lyophilized collagen samples were gelled with 0.05 M acetic acid at a solid/liquid ratio of 1:40 (w/v) and then stored at +4 °C for two days. Measurements were done by using Mettler Toledo, Model DSC 3, (Schwerzenbach, Switzerland). In an aluminum pan, the gelled samples (5-10 mg) were weighed. The screening was carried out at a temperature of 10 °C, increasing at a rate of 1 °C/minute. As a cooling medium, liquid nitrogen was used. An indium thermogram was used to calibrate the temperature against an empty aluminum container. The DSC thermogram was used to calculate the maximum transition temperature (Tm) and total denaturation enthalpy (ΔH).

X-Ray diffraction analysis

Crystal structures of lyophilized collagen samples were determined using an X-ray diffraction (XRD; PANalytical X'Pert High Score Empyrean, 45kV, 40mA) with CuKa (λ =1.54) radiation in the scanning range of 5 °C to 45 °C at 0.5 °C/min scan speed and 0.02 °C step interval.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of collagen (one repeat was performed) were obtained from 2 mg collagen in about 100 mg of potassium bromide (KBr) under dry conditions. All spectra were performed using a JASCO ATR Pro One Model 6700 FT/IR spectrometer (JASCO International Co. Ltd., Hachioji, Tokyo, Japan) at a data acquisition rate of 4 cm^{-1} from 4000 to 600 cm^{-1}. Analysis of spectral data was performed using Spectra Manager TM II cross-platform software program (JASCO).

UV-Vis measurement

A Cary 100 UV-Vis Spectrophotometer was used to obtain UV spectra of collagen (one repeat) samples (Agilent Technologies). The samples were dissolved in 0.5 M acetic acid at 0.2 mg mL^{-1} concentration. Readings were taken in the 200-400 nm wavelength range against 0.5 M acetic acid (negative control).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed (one repeat) using the Quanta 650 model, FEI®, (Columbus, Ohio, USA). The surface of the samples was made conductive by coating them with Gold-Palladium (Au/Pd) (approximately 2Å/second). Samples were observed using 30 kV, and EDS technique was used to determine the major compounds of the samples surface regions.

Amino acid composition

Collagen samples were hydrolyzed in 6 N HCl for 24 hours at 110 °C under vacuum. HPLC was used to analyze amino acids (Shimadzu model Nexera-X2 device). Two microliters of the derivatized sample were injected into the Shimadzu XR-ODS II shim-pack column. The temperature of the column oven was set to 40 °C. KH_2PO_4 solution (A) (1 mM K_2HPO_4 in water) and (B) Acetonitrile/Methanol/Water (45/40/15) were used as mobile phases. The retention times and peak areas of the standards were used to define and calculate amino acids. All of this method was performed as duplicates from the initial stage.

Statistical Analysis

In the current study, the results are expressed as mean ± standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences SPSS (Pallant 2020). One Way ANOVA was used to determine the differences between groups. The T-test was also used for pair comparisons. Any *p* value below 0.05 ($P<0.05$) were considered as statistically significant.

Results

Collagen Yield

Based on the wet weight, the yields of acid-soluble collagens extracted from mangar skin (ASC-S) and bones (ASC-B) were 9.38% and 3.71%, respectively. Collagen yield obtained from the mangar skin was found to be higher than its bone ($P<0.05$).

Thermal Stability of Collagen by Differential Scanning Calorimeter (DSC)

The maximum transition temperatures (Tmax) of acid-soluble collagen extracted from template skin and bones dissolved in 0.5 M acetic acid are shown in Figure 1. Tmax and enthalpy (ΔH) value of ASC-S was found as 32.62 °C, 0.230 J/g. However, there were three different Tmax and enthalpy (ΔH) values for ASC-B and they were found as 1st 32.66 °C, 0.253 J/g, 2nd 38.12 °C, 0.452 J/g, and 3rd 41.37 °C, 0.320 J/g. The amino acid composition of collagen, particularly the imino acid composition, influences its thermal stability. While proline and hydroxyproline provide the spatial structure of collagen with pyrrolidine rings, hydroxyproline improves collagen's thermal stability by forming inter-chain hydrogen bonds that stabilize the triple helical structure of collagen (Gelse et al. 2003). Therefore, the Tmax value has a positive relationship with the imino acid content. In fact, the reason why the Tmax value of cattle skin collagen is higher than the value we obtained at 40.8 °C is the higher amount of imino acid it contains (Komsa-Penkova et al. 2000).

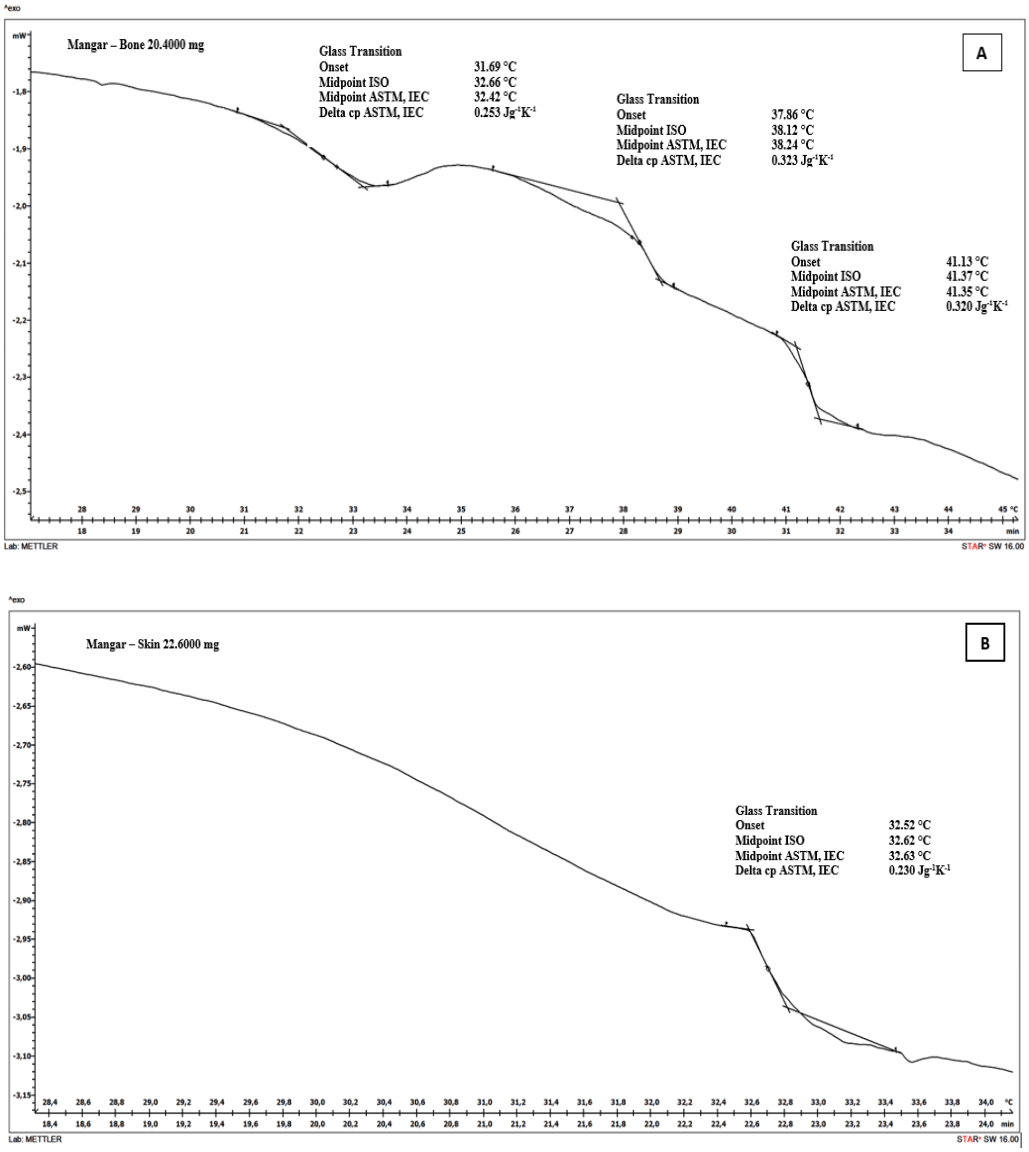

Figure 1. DSC thermogram of ASC-B (a) and ASC-S (b) from the skin and bone of mangar dispersed in 0.05 M acetic acid.

X-Ray Diffraction (XRD)

XRD is frequently used to investigate the crystal structure of polymers. Refraction occurs when an X-ray encounters crystalline particles, and the position and density of the diffraction peak reflect the structural properties of the crystals (Bigi et al. 2001). The XRD curves for both ASC-S and ASC-B have characteristic two break peaks at

diffraction angles (2θ) of approximately 6.93º and 22.78º for ACS-S and 7.68º and 22.17º for ASC-B, as shown in Figure 2. The first sharp peak corresponds to the collagen's triple helix structure, while the second large peak represents the distance between the chains. These findings confirm that both collagens retain their triple helix structure and are not denatured.

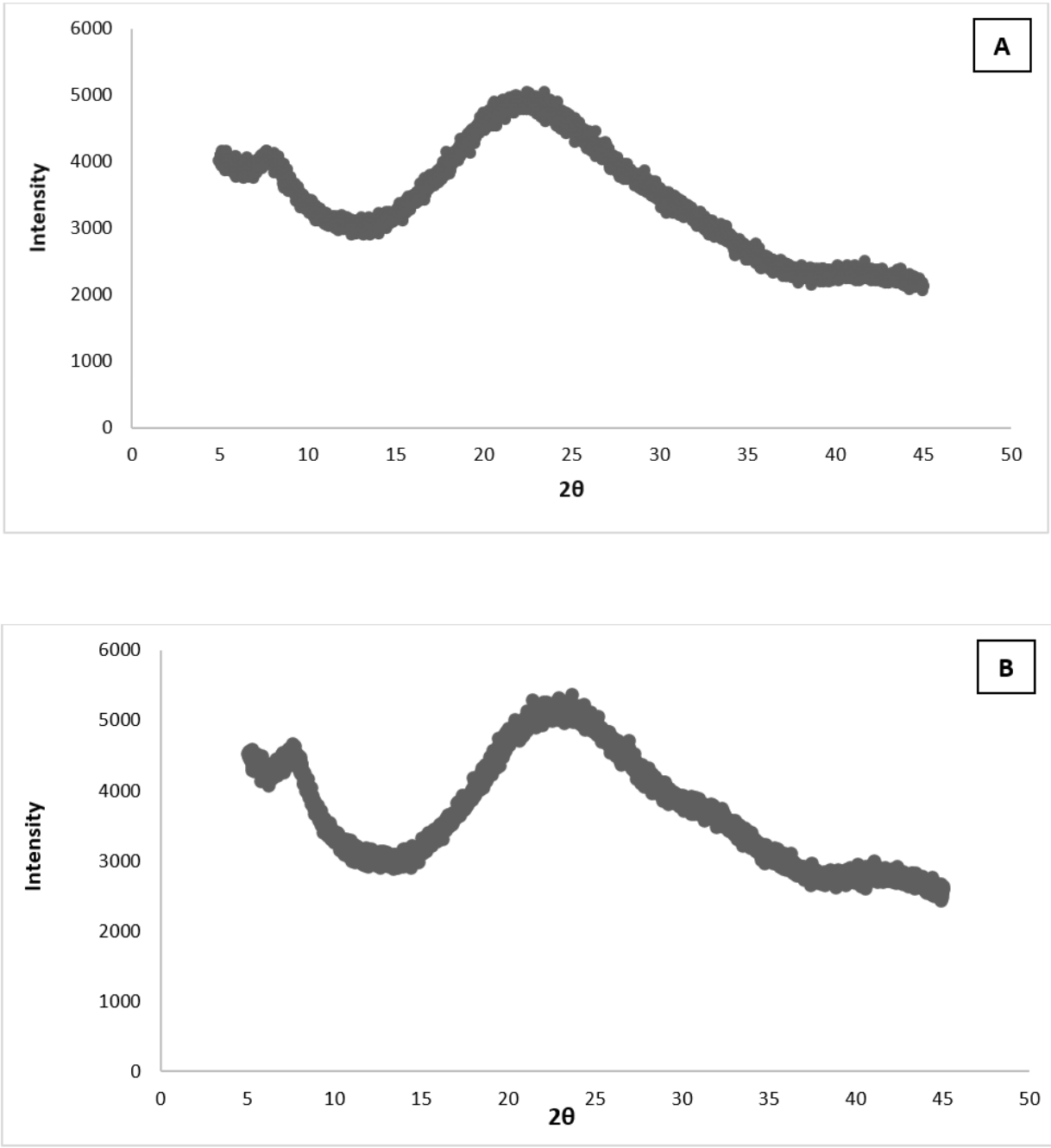

Figure 2. X-ray diffraction spectra of mangar's ASC-S (a) and ASC-B (b).

Fourier Transform Infrared (FTIR)

FTIR spectra of collagen extracted from mangar skin and bones are shown in Figure 3. Collagen exhibited similar spectral properties with five distinct collagen absorption bands (amide A, amide B, and amide I, II, and III), indicating the presence of high proline and hydroxyproline aminoacids in the collagen molecule; these are typical collagen bands and indicate that the collagen obtained was determined to be type I collagen. Amide A absorption peaks of ASC-S and ASC-B were found to be 3219.58 and 3290.93 cm^{-1}, respectively.

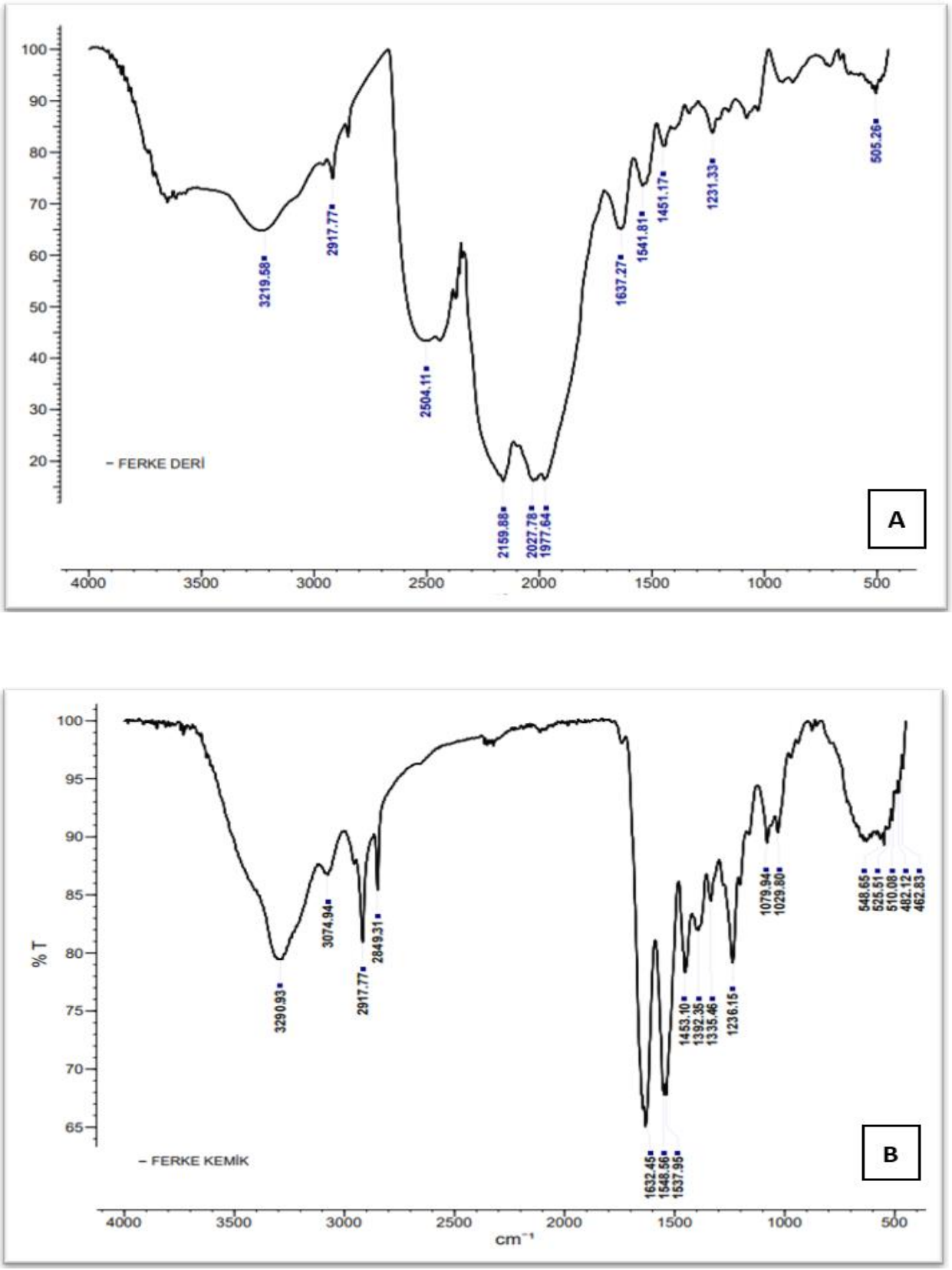

Figure 3. The FTIR spectra of acid-soluble collagens from skin (a) and bone (b) of mangar.

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy Analysis

UV-Vis spectroscopy was used to assess the purity of collagen. ASC-S and ASC-B UV-Vis measurement results are shown in Figure 4. There is a single absorption peak and showed maximum absorbance at 230 and 232 nm, respectively.

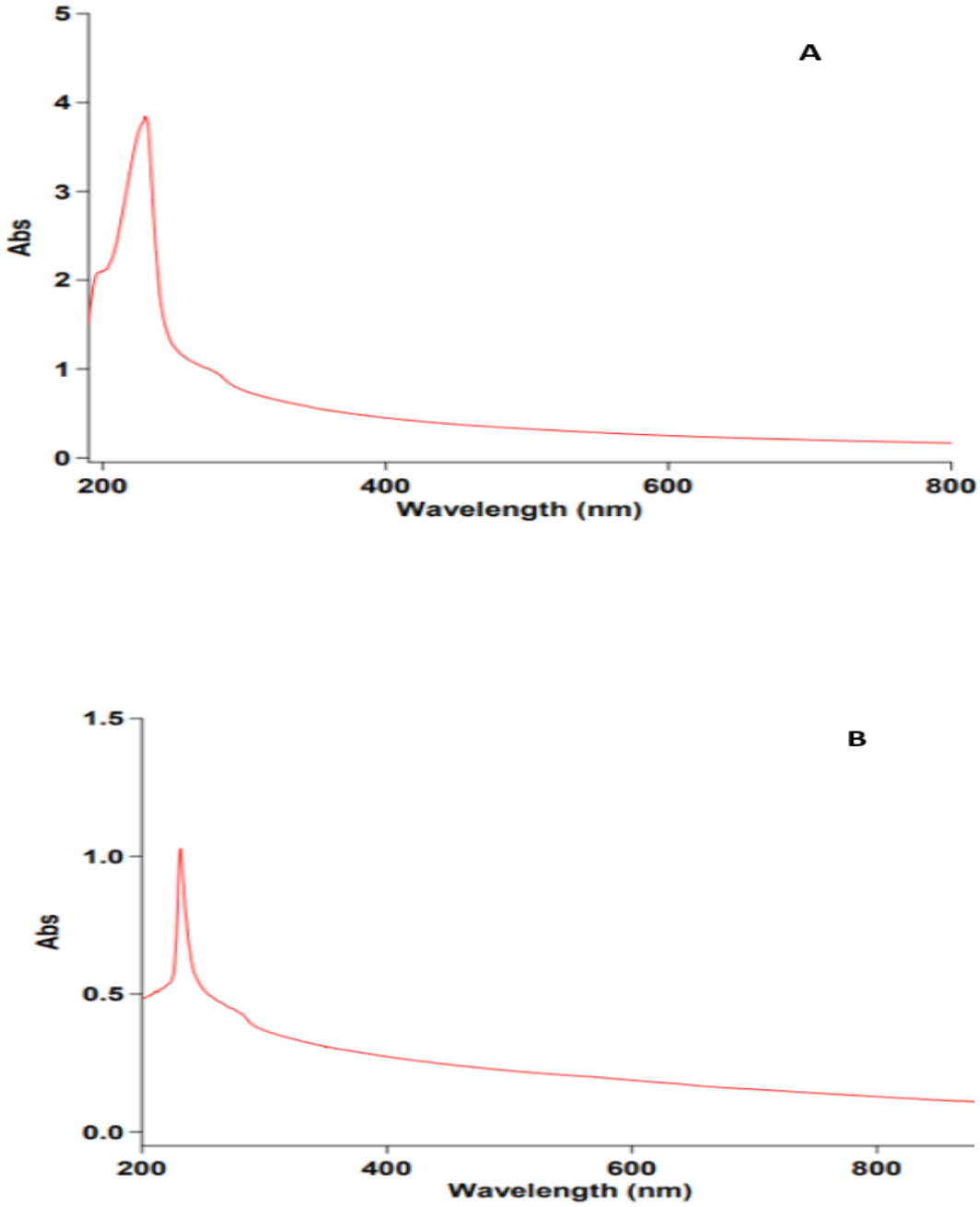

Figure 4. UV- Spectra of acid-soluble collagens from skin (a) and bone (b) of mangar

Scanning Electron Microscopy (SEM)

The morphological structures of the extracted lyophilized (freeze dried) collagens (ASC-S and ASC-B) were visualized by scanning electron microscopy (SEM) under three different magnifications ×200, ×500, ×1000 and ×2,000 (Figure 5 and Figure 6). Both of the lyophilized collagens were seen as soft, white, and spongy with a porous structure when naked eye observations were made. However, SEM analysis revealed that both collagens had a dense, irregular, and partially wrinkled surface image bound by randomly wrapped filaments. This is probably due to dehydration during lyophilization. Likewise, some similar results have been reported by various research such as the observation of collagen obtained from *Arabibarbus gripus* skin and bone (Göçer 2022), *Amur sturgeon* skin (Wang et al. 2014) and *Istiophorus platypterus* skin (Tamilmozhi et al. 2013).

In this study, both of the studied collagens were alike in many ways. They are characterized by poor organization, intersecting fibers, entangled bundles, and some fibrils have intricated meshes in contact with others. Both collagens' fibrils of varying thickness were intertwined throughout the porous matrix. As a result, the collagen SEM images

show that they are type I collagen with a fibrillar structure.

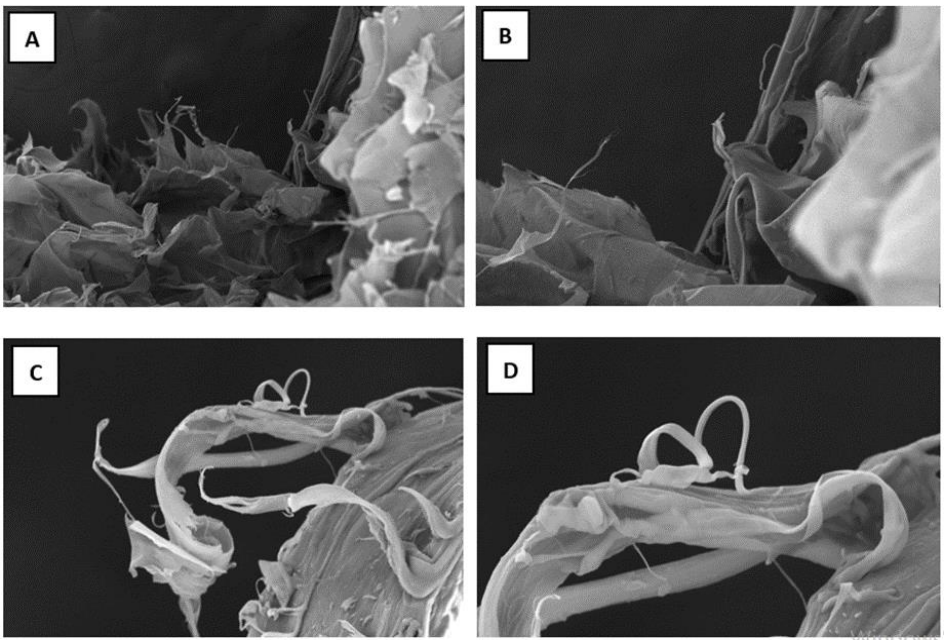

Figure 5. SEM images of acid-soluble collagen from skin of mangar A: ×200, B: ×500, C: ×1.000, D: ×2.000

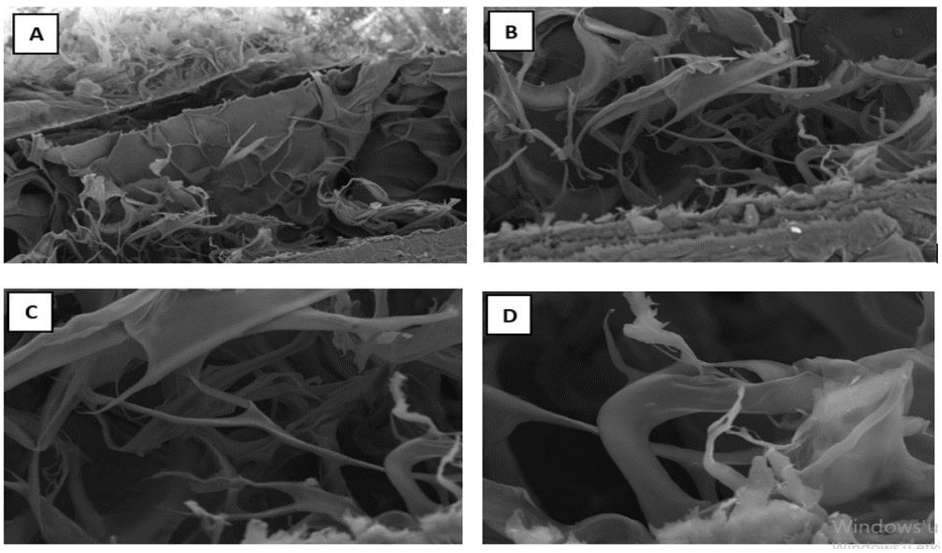

Figure 6. SEM images of acid-soluble collagen from bone of mangar A: ×200, B: ×500, C: ×1.000, D: ×2.000

Amino Acid Composition

Table 1 shows the amino acid compositions of acid-soluble collagen extracted from mangar skin (ASC-S) and bones (ASC-B). Both collagens have amino acid compositions that are similar. Due to the characteristic (Gly-Pro-Hyp) triple-helix repeats of all collagens, the ASC-S and ASC-B samples were high in glycine (Gly), proline (Pro), and hydroxyproline (Hyp). Tryptophan and cystine were not detected. Proline and hydroxyproline found in both ASC-S and ASC-B, which are important imino acids that ensure the structural integrity of collagen. The total amount of imino acid (Pro+Hyp) is 19.32% and 19.17% for ASC-S and ASC-B, respectively, and is statistically similar ($P>0.05$).

Table 1. Amino acid profiles (g/100g protein) of skin (ASC-S) and bone (ASC-B) collagens of mangar

Amino Acid	ASC-S	ASC-B
Aspartic acid	4.91±0.07[b]	5.58±0.06[a]
Glutamic acid	9.46±0.45	9.31±0.17
Serine	3.84±0.07[b]	4.17±0.04[a]
Glycine	29.46±0.56	30.23±0.09
Threonine	3.58±0.33	3.51±0.07
Arginine	7.51±0.26	7.18±0.10
Alanine	9.61±0.18	9.26±0.05
Tyrosine	0.38±0.01	0.36±0.01
Cysteine	0	0
Valine	1.2±0.09	1.06±0.08
Methionine	1.04±0.12	0.97±0.06
Tryptophan	0	0
Phenylalanine	2.17±0.21	1.98±0.03
Leucine	2.39±0.04	2.33±0.08
Lysine	3.25±0.03[a]	2.86±0.11[b]
Hydroxyproline	9.63±0.10	9.48±0.05
Proline	9.69±0.27	9.69±0.22
Total imino acid (Hydroxyproline + proline)	19.33±0.38	19.18±0.24

±, represents standard deviations. The superscript letters [a] and [b] indicate to the statistical differences ($p<0.05$, n=6) between groups within the same line. ND, not determined.

Discussion

Collagen Yield

Similar to the current study, it was found that the yield of collagen obtained from *Priacanthus tayenus* bone (1.6%) to be lower than the yield of collagen obtained from its skin (10.9%) (Kittiphattanabawon et al. 2005). The authors also suggest that their ASC-B yields were lower than the current study, whereas their ASC-S yields were higher. Additionally, the obtained results of this study suggested that the collagen yield of this study is higher than the yield of collagen extracted from carp bone (1.06%) (Duan et al. 2009) by over than 3.5 folds.Unlikely, Doğdu et al. (2019) extracted collagen from silver cheeked pufferfish *Lagocephalus sceleratus* skin and the collagen yield was found to be 50.9%, which is much higher than the current study (Doğdu et al. 2019). A very recent study was accomplished by Jaziri et al. (Jaziri et al. 2022) on skin of lizardfish (*Saurida tumbil* Bloch, 1795) the yields of acetic acid-extracted collagen (AESkC), lactic acid-extracted collagen (LESkC), and citric acid-extracted collagen (CESkC) were 11.73 ± 1.14%, 11.63 ± 1.10%, and 11.39 ± 1.05% (based on wet weight), respectively. That suggests that even if different acids were used in order to extract collagen from skin of *S. tumbil* the collagen yield was around 11%, which is very close to our study results. Reátegui-Pinedo and gis colleagues (Reátegui-Pinedo et al. 2022) isolated collagen from tilapia (*Oreochromis niloticus*) skin the yields were for acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC), 19% and 21%, respectively. In another study, Benjakul et al. (2010) was found the collagen yield (7.7% and 7.1%) extracted from *P. tayenus* and *Priacanthus macracanthus* skin, both of these results represented very close collagen yield to the current study (Benjakul et al. 2010). However, Wei et al. (2019) isolated and characterized collagen from sturgeon fish and reported that the collagen yield was 5.73% (Wei et al. 2019). The current study's results represent about twice as much collagen yield than Wei et al. (2019)'s study. In the current study, the same method which was adopted without any modification or with minor modification of Nagai and Suzuki (2000)

was used as the above-mentioned previous studies. Since all reviewed studies results vary from each other in terms of the collagen yield percentages, all of these studies suggest that the collagen yield could be species specific.

Thermal Stability of Collagen by Differential Scanning Calorimeter (DSC)

In a study conducted by Göçer (2022) Tmax and enthalpy values of ASC-S and ASC-B isolated from *Arabibarbus grypus* were found as 31.59 °C, 0.358 J/g and 32.25 °C, 0.452 J/g, respectively. Whereas, as shown in Figure 1 in this study, Tmax and enthalpy value of ASC-S of was found as 32.62 °C, 0.230 J/g and there were three different Tmax and enthalpy values for ASC-B and they were found as 1st 32.66 °C, 0.253 J/g, 2nd 38.12 °C, 0.452 J/g, and 3rd 41.37 °C, 0.320 J/g. These differences could be due to use of different species for the isolation of ASC-S and ASC-B. The cattle's imino acid content in ASC-S was 19.26%, while the ASC-B was 19.89%. This value was found to be lower than the collagen obtained from many cold climate fish (Ciarlo et al. 1997). This explains why collagen isolated from subtropical and tropical fish have better thermal stability (Hsieh et al. 2016).

X-Ray Diffraction (XRD)

As it mentioned above, Figure 2 shows the XRD curve for both ASC-S and ASC-B has characteristic two break peaks at diffraction angles (2θ) of approximately 6.93º and 22.78º for ACS-S and 7.68º and 22.17º for ASC-B. The first sharp peak is related to the triple helix structure of the collagen, while the second large peak indicates the distance between the chains. These results confirm that both of the collagens preserve the triple helix structure and is not denatured. Similar results have been obtained by several studies include carp scale collagen study by Zhang et al.(Zhang et al. 2007), *O. niloticus* skin collagen (Sun et al. 2017), *Gadus macrocephalus* skin collagen (Sun et al. 2017), Atlantic cod and Atlantic salmon skin collagen (Alves et al. 2017), and *A. grypus* skin and bone (Göçer 2022).

Fourier Transform Infrared (FTIR)

FTIR spectra of collagen extracted from mangar skin and bones showed that Amide A absorption peaks of ASC-S and ASC-B were 3219.58 and 3290.93 cm^{-1}, respectively. According to Sai and Babu (2001), Amide A band generally originates from N-H stress vibration and occurs in the wavelength range of 3400-3440 cm^{-1} (Sai and Babu 2001). However, Doyle et al (1975) mentioned that when the NH group of a peptide is involved in the hydrogen bond, the position can shift to a low frequency, usually around 3300 cm^{-1} (Doyle et al. 1975). Therefore, the shift of amide A towards lower wavelengths, as observed in this study, indicates that hydrogen bonded hydroxyl groups are present in both skin and bone collagens. Amide I band of ASC-S and ASC-B were 1637.27 and 1632.45 cm^{-1}, respectively. These results are consistent with the 1625-1690 cm^{-1} range that is the position of the general amide I bands of collagen. Similar results were acquired by Shalaby and colleagues (Shalaby et al. 2020). Amide II band was found to be 1541.81 cm^{-1} for ASC-S and 1548.56 cm^{-1} for ASC-B, amide II band is generally seen at wavelengths of 1550-1600 cm^{-1} (Krimm and Bandekar 1986), its shift to lower wavelengths represents the formation of hydrogen bond. The triple helix structure of collagen can also be presented by the ratio of the density between the absorption peak of amide III and the absorption peak of 1450 cm^{-1}. In our study, the Amide III absorption peaks of ASC-S and ASC-B were 1231.33 and 1236.15 cm^{-1}, respectively. The ratio of the density between the absorption peak of Amide III and the absorption peak of 1450 cm^{-1} was 1.18 (ASC-S/ASC-B=1.17).

Göçer (2022) found that amide A absorption peaks of ASC-S and ASC-B of *A. grypus* were found to be 3265.86 and 3292.86 cm-1, respectively. The researcher indicated that collagen presents similar spectral properties with five characteristic collagen absorption bands including amide A, B, I, II, and III, indicating the presence of high proline and hydroxyproline amino acids in the collagen molecule, which are considered as typical bands for collagen and they mean that the obtained collagen is type I collagen the same as determined in the current study. Matmaroh et al. (2011) stated that a value approaching 1.0 indicates that collagen still has a triple helix structure.

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy Analysis

This spectroscopy is usually used in order to assess the purity of collagen (Kumar and Rani 2017). As presented in Figure 4, there is a single absorption peak and showed maximum absorbance at 230 and 232 nm, respectively. This range is the distinctive absorbance of type I collagen. Generally, the highest protein absorbance is observed at 280 nm; however, our results have

shown maximum absorbance at 230-232 nm due to the absence of tryptophan amino acid and low tyrosine amino acid content in both ASC-S and ASC-B. Similar study was done by Jaziri and his research group (Jaziri et al. 2022) on skin of lizardfish (*Saurida tumbil* Bloch, 1795) and they found that the max absorbances were for AESkC, LESkC, and CESkC at 230.5 nm, 230.0 nm, and 231.5 nm, respectively. In another study, UV absorption spectrum of *O. niloticus* "tilapia" skin collagen samples were between 232 and 234 (Reátegui-Pinedo et al. 2022).

Scanning Electron Microscopy (SEM)

The morphological structures of the extracted and lyophilized ASC-S and ASC-B were observed as soft, white, and spongy with a porous structure when no magnification applied. However, when SEM results were examined, both of the collagens were found to have a dense, irregular, and partially wrinkled surface image bound by randomly wrapped filaments. This could be due to dehydration during lyophilization. Some similar results have been reported by various research groups, the observation results of collagen obtained from *Salmo salar* L. (Mørkøre et al. 2020), *Amur sturgeon* skin (Wang et al. 2014) and *Istiophorus platypterus* skin (Tamilmozhi et al. 2013).

Amino Acid Composition

The amino acid compositions of ASC-S and ASC-B collagen of mangar presented results as expected. As with other collagens, tryptophan and cystine were not detected (Yata et al. 2001; Muyonga et al. 2004; Jongjareonrak et al. 2005). Proline and hydroxyproline found in both ASC-S and ASC-B are important imino acids that ensure the structural integrity of collagen. The total amount of imino acid (Pro + Hyp) is 19.32% and 19.17% for ASC-S and ASC-B, respectively, and is statistically similar ($P>0.05$). This value is similar to the values reported for *O. niloticus* (19.8 - 19.4%) (Potaros et al. 2009) and Carp (19.4%) (Zhang et al. 2011); higher than the values reported for tilapia, (17.75%), grass carp (17.90%) and silver carp (17.78%) (Tang et al. 2015); lower than the value reported for tilapia (25.4%) (Grossman and Bergman 1992). The difference in imino acid content between different species is due to the different habitats of different species, especially to the difference in temperature (Singh et al. 2011).

In conclusion, collagens were successfully extracted and characterized from mangar skin and bone. Both extracted collagens were type I collagen, with a typical amino acid composition. The FTIR and XRD analyses revealed that their triple helical structure was preserved after the extraction processes. Both extracted collagens demonstrated maximum absorption at 230-232 nm and no absorption at 280 nm. Both collagens' SEM images revealed interconnected pores with lace-like fibers. To conclude, the positive characteristics exhibited by the extracted collagens in this study, there is a high potential for use as a valuable collagen alternative in diet, medical and pharmaceutical (can be used extensively in various medical applications). For example, its strength and flexibility may help in the repair and regeneration of skin and nutraceuticals industries.

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First report of leech parasitism in freshwater turtles for Turkish wetlands

Mustafa CEYLAN [1, 2,*], Osman ÇETİNKAYA [1]

[1]Faculty of Eğirdir Fisheries, Isparta University of Applied Sciences, 32260, Isparta, Türkiye.
[2]Medicinal Leech Application and Research Center, Isparta University of Applied Sciences, 32260, Isparta, Türkiye.

ABSTRACT

Research on the diversities of parasites and hosts, as well as their relationships, can provide significant contributions to understanding, utilizing, conserving, and properly managing aquatic ecosystems. The freshwater mud turtle *Emys orbicularis* and Balkan turtle *Mauremys rivulata*, as well as the leech *Placobdella costata*, are distributed in Turkish wetlands; however, the relations between these organisms are not well-known. In this study, the parasitism of Glossiphonid leech *P. costata* on freshwater turtles, *E. orbicularis*, and *M. rivulata* was assessed for the first time through field observations and laboratory examinations in Turkish wetlands. The infection prevalence in turtles was found to be 76%, with an intensity of 5.00 and an abundance of 3.82, all of which were higher in females. Although the infection prevalence was similar for both *E. orbicularis* and *M. rivulata*, infection intensity and abundance were higher in *M. rivulata*. Leeches were mostly sampled from the plastron and at the least from the head. The condition of the parasitic leech was found to be high, and *P. costata* preferred to feed on and parasitize predominantly female individuals of both turtle species.

Keywords: *Placobdella costata*, *Emys orbicularis*, *Mauremys rivulata*, infection prevalence, infection intensity, abundance.

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* CORRESPONDING AUTHOR

mustafaceylan@isparta.edu.tr
Phone : +90 505 947 95 52
Fax : +90 246 214 64 45

Türkiye'deki sulak alanlar için tatlı su kaplumbağalarında sülük parazitliğine ilişkin ilk rapor

Öz: : Parazitlerin ve konakçıların çeşitliliğinin yanı sıra aralarındaki ekolojik ilişkilerin araştırılması, sucul ekosistemlerin anlaşılması, kullanılması, korunması ve uygun şekilde yönetilmesine önemli katkılar sağlayabilmektedir. Benekli kaplumbağa *Emys orbicularis* ve Balkan kaplumbağası *Mauremys rivulata* ile *Placobdella costata* sülük türü Türkiye sulak alanlarında yayılış göstermektedir; ancak bu organizmalar arasındaki ilişkiler tam olarak bilinmemektedir. Bu çalışmada, Glossiphonid sülüklerden *P. costata* türünün tatlı su kaplumbağaları, *E. orbicularis* ve *M. rivulata* üzerindeki parazitliği, saha gözlemleri ve laboratuvar incelemeleri yoluyla Türkiye sulak alanlarında ilk kez incelenmiştir. Kaplumbağalardaki enfeksiyon prevalansı %76, yoğunluğu 5,00 ve bolluğu 3,82 olarak bulunmuştur ve bunların hepsi dişilerde daha yüksek bulunmuştur. Enfeksiyon prevalansı hem *E. orbicularis* hem de *M. rivulata* için benzer olmasına rağmen, enfeksiyon yoğunluğu ve bolluğunun *M. rivulata* türünde daha yüksek olduğu belirlenmiştir. Sülükler en çok plastrondan, buna karşın en az ise kafadan örneklenmiştir. Sülüklerin kondisyonlarının yüksek olduğu ve *P. costata* türünün her iki kaplumbağa türünün ağırlıklı olarak dişi bireylerini parazitlemeyi tercih ettiği dikkat çekmiştir.

Anahtar kelimeler: *Placobdella costata*, *Emys orbicularis*, *Mauremys rivulata*, enfeksiyon prevalansı, enfeksiyon yoğunluğu, bolluk

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Introduction

Parasites play a crucial role in shaping ecosystems by influencing host behavior and fitness, trophic interactions, food webs, competition, biodiversity, and keystone species. This indicates that parasites are vital components that shape the structure of communities and ecosystems (Preston and Johnson 2010; Friesen et al. 2020). The study of parasite diversity is particularly significant due to the potential importance of sympatric diversity in certain parasite taxa and the opportunity to independently test evolutionary hypotheses in the numerous distinct lineages where parasitism has evolved (Poulin and Morand 2020). Parasites are well known for utilizing their hosts as a source of food and habitat (Siddall and Burreson 1996; O'Keeffe et al. 2021).

The diverse functions of leeches, including parasitic species, are of great importance in aquatic ecosystems. They are known for their therapeutic effects, special enzyme production, and use as raw materials for drug and cream production. Additionally, they serve as transmission vectors for numerous pathogens and can be used as wetlands quality indicators and for estimating of aquatic biodiversity (Siddall and Desser 1992; Kazancı et al. 2015; Sig et al. 2017; Williams et al. 2020; Kermanian et al. 2022; Lynggaard et al. 2022).

Freshwater turtle leech, *Placobdella costata*, is widely distributed in Euro-Asia, North Africa, and Turkey, in areas where its hosts are found. It is exclusively an ectoparasite of turtles (Sağlam 2001; Bielecki et al. 2007; Ayaz et al. 2008; Fediras et al. 2017), although it may also have other hosts, such as birds, amphibians, or reptiles. The reproductive period of *P. costata* coincides with its host's migration to breeding grounds, facilitating the dispersal of the parasite (Bielecki et al. 2012).

Turtles play a vital role in maintaining the health of aquatic ecosystems, making them significant organisms in wetland environments (Santori et al. 2020). In Turkey, the European pond turtle (*Emys orbicularis*) is found in freshwaters in Thrace, as well as in western and central Anatolia. Unfortunately, this species has experienced population decline due to the destruction and drying out of their natural habitats (Baran et al. 2005). *E. orbicularis* is also facing challenges in Europe, primarily due to anthropogenic habitat changes (Bielecki et al. 2007). The International Union for Conservation of Nature (IUCN) has listed this species as lower risk/near threatened (NT) globally (Çiçek et al. 2015). The Balkan's striped turtle (*Mauremys rivulata*) inhabits lakes, slow-moving waters, ditches, and wetlands. It is found in Thrace, as well as western and southern Anatolia. This species feeds on small invertebrates, aquatic insects, fish, and frogs (Baran et al. 2005; Ayaz and Budak 2008).

Leeches are a commonly observed parasitic organism affecting freshwater turtles (McCoy et. al. 2007; Readel et al. 2008). Several factors determine leech parasitism on turtles, including the species of both the leech and turtle, microclimate usage, sex, reproductive stage, body size, and environmental characteristics such as the month of capture, turtle abundance, vegetation, turbidity, wetland size, and availability of basking structures. The prevalence of leeches on turtles shows significant variation among turtle species, with the highest incidence observed in bottom-walking and adult turtles, throughout the year. Furthermore, leech intensity is highest in larger turtles and in turbid waters (Readel et al., 2008).

The aims of the study was to investigate occurrence of leech parasitism on freshwater turtles and to evaluate infestation prevalence and densities, abundance, species preferences, sex, attachment sites of body in wetlands around Lake Eğirdir, the second largest freshwater lake of Turkey.

Materials and Methods

Study Area

This research was conducted in the wetlands Aşağı Tırtar (38°14'31.8"N, 30°53'27.3"E), Boyalı (38°04'31.0"N, 30°51'12.4"E), Gelendost (38°02'56.0"N, 30°57'14.6"E), Kayaağzı (38°08'30.3"N, 30°45'12.8"E), and Akbük (38°09'11.9"N, 30°51'11.0"E), which are sublacustrine regions of Lake Eğirdir that are actively connected to the lake. These habitats are characterized as shallow and partially isolated from the lake and are covered by dense macrophytes. The fauna of these wetlands includes various aquatic, amphibian, and terrestrial animal species, such as freshwater turtles, waterfowls, frogs, snails, fish, wild boar, horse, donkey, dogs, wild cats, water vole, leeches, and others (Ceylan 2016).

Turtle and Leech Sampling

The sampling procedures were carried out on a monthly basis in 2013 and seasonally in 2014. The turtles were manually collected and subsequently transported to the laboratory for identification of species, determination of sex, measurement of body size and weight, and examination for leeches. The identification of turtle species was performed in accordance with Baran and Atatür (1998), while the determination of sex was carried out using eye color and plastron shape, as described by Zuffi and Gariboldi (1995), Ayaz et al. (2008), and Kaviani and Rahimibashar (2015).

Once in the laboratory, the shells and soft-tissue extremities of the turtles were carefully examined for leeches. Any leeches found on the turtles were extracted and fixed in 70% ethanol, without contraction, by gradually dripping the solution into a petri dish. Identification of the leeches was carried out under a stereo microscope, following the procedures described by Sawyer (1986) and Nesemann and Neubert (1999). The body length (L, cm) and weight (W, g) of each leech were determined, and the condition factor was calculated using the formula $K=W/L^3 \times 100$, as described by Ceylan et al. (2017). Following the completion of all procedures, the turtles were returned to the habitats from which they were collected.

Indicators of Parasitism

To determine the indicators of parasitism such as infestation prevalence, infestation density, and

abundance, the formulas provided by Laghzaoui et al. (2020) were utilized:

Infestation prevalence = (Number of turtles with leeches / Total number of turtles) x 100

Infestation density = Number of leeches / Number of turtles with leeches

Abundance = Number of leeches / Total number of turtles

Statistical Analyses

Descriptive statistics were computed, and normality of the data was assessed using the Shapiro-Wilk test (for n<30). The Mann-Whitney U test and Kruskal-Wallis H test were utilized to compare turtle species, sex, and the preferred body parts of the leech. Correlations were evaluated using the Spearman Correlation method. Values were reported as the mean ± standard deviation (Mean ± SD). The data were analyzed using IBM SPSS Statistics version 25.0 software package for Windows (IBM Corp., Armonk, NY, USA) with a significance level of alpha set at $\alpha = 0.05$.

Ethical Statement

The research was conducted with the endorsement of the Animal Experiments Local Ethics Committee of the Mediterranean Fisheries Research, Production, and Training Institute (Date: 29.02.2012, ID: 01-227).

Results

Turtles

During the study, a total of 17 turtles, belonging to two different species, were sampled primarily in May (Figure 1 and Table 1). Of the 17 turtles sampled, 13 were *Emys orbicularis* (Linnaeus, 1758) and four were *Mauremys rivulata* (Valenciennes, 1833). Fifteen turtles were in good condition, while two of them were deceased. Means of body weight, carapace length, carapace width and carapace height were 446±333 g, 13.3±3.7 cm, 9.4±2.5 cm, and 5.5±1.6 cm for *E. orbicularis*, and 574±569 g, 15.8±6.6 cm, 10.6±3.0 cm and 6.1±2.4 cm for *M. rivulata*, respectively. Among the *E. orbicularis* turtles, 76.9% were female and 23.1% were male; however, the difference was not statistically significant (Chi Square, P>0.05). In *M. rivulata*, an equal sex ratio was observed.

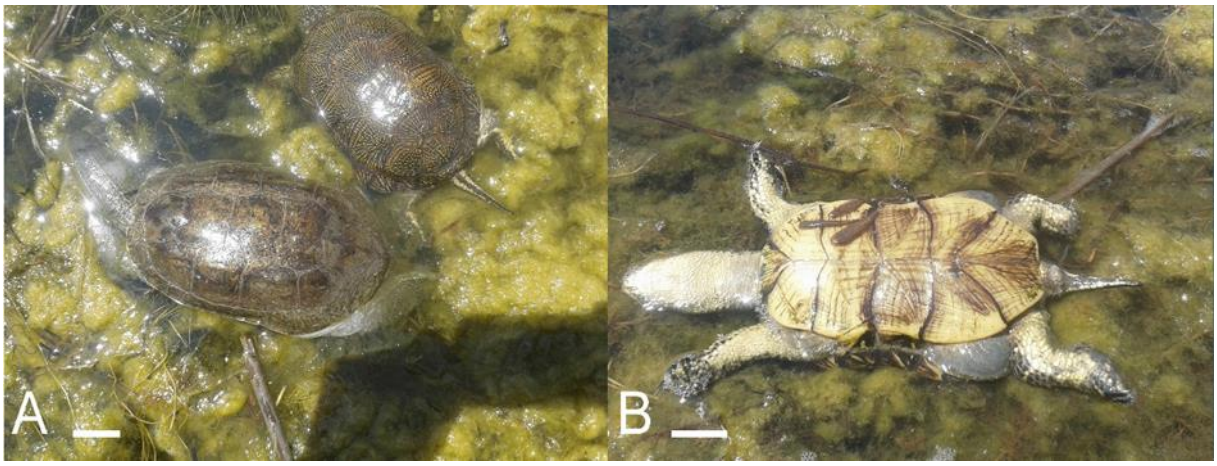

Figure 1. A: Turtles *Emys orbicularis* (top) and *Mauremys rivulata* (bottom) were found dead in the Boyalı wetland of Lake Eğirdir. B: Leeches of *Placobdella costata* in the plastron of *Emys orbicularis*. Scale = 1 cm.

Table 1. Monthly leech parasitism in turtle species*

	Months											
Turtle species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
E. orbicularis				3(7)	4(10)	1(6)	1(2)		1(2)			
M. rivulata							2(6)				1(32)	

* The figures situated outside the parentheses denote the count of turtles that have been parasitized by leeches, whereas the numbers within the parentheses indicate the overall quantity of leeches.

The morphological features of *E. orbicularis* include a carapace that is swollen and round, with a layer of skin in between that is not fully fused with the plastron. The carapace is typically black or brown, with yellowish spots and small stripes, while the plastron can vary from black to yellowish in color. Additionally, there are small yellow spots on the legs, tail, and neck of the species.

The morphological features of *M. rivulata* include a fused carapace and plastron on the sides. The carapace displays varying shades ranging from light to dark olive green, and different shades of

brown. Meanwhile, the plastron may appear either black or dark brown. The head, neck, and feet of *M. rivulata* exhibit black coloration with yellowish stripes present on the head and feet.

Leeches

All of the leeches found on the turtles were identified as *Placobdella costata* (Fr. Müller, 1846) (Figure 2). A total of 65 leeches were detected on the turtles, with 27 found on *E. orbicularis* and 38 on *M. rivulata*. Of the two deceased turtles that were sampled, one (*M. rivulata*) had no leeches, while the other (*E. orbicularis*) had three leeches in the plastron region. The average occurrence of *P. costata* was similar for both turtle species ($p>0.05$), indicating that *P. costata* showed no preference between the two turtle species.

Figure 2. Dorsal (left) and ventral (right) views of *Placobdella costata* sampled from turtles. Scale = 0.5 cm.

According to our findings, leeches were found to be primarily (48%) colonized in the plastron of the turtles, and significantly more frequent compared to other body parts (average of 9 leeches, $p<0.05$). The remaining body parts, namely the hind leg (20%), carapace (17%), foreleg (12%), and head (3%) exhibited no significant differences when compared to the plastron (Table 2). In addition, our results showed that leeches parasitized predominantly female individuals, as 56 out of 65 leeches (86%) were sampled from female turtles.

Table 2. The number of *P. costata* in different body parts of the turtles

		Body parts					
Turtle species	Sex	Plastron	Carapace	Head	Hind leg	Foreleg	Total
E. orbicularis	Female	8	2	2	6	4	22
	Male	2	0	0	3	0	5
Total of species		10	2	2	9	4	27
M. rivulata	Female	17	9	0	4	4	34
	Male	4	0	0	0	0	4
Total of species		21	9	0	4	4	38
Total of turtles		31	11	2	13	8	65

The body weight, length, and condition factor of the leeches were determined to be 0.11±0.07 g, 1.71±0.45 cm, and 2.171±1.102, respectively. A significant positive correlation was found between the body weight and length of the leeches ($r = 0.701$, $p<0.01$), and the length-weight relationship was estimated as $W=0.356*L^{1.962}$ (n=25). Additionally, the growth pattern of the leeches was determined to be negative allometric.

The morphological features of *P. costata* are as follows: the dorsal surface is convex, while the ventral surface is partly concave. The head is slightly broader, but not distinct from the body. The oral cavity is located at the anterior margin of the anterior sucker, and the posterior sucker is small. There are two pairs of eyes, which are often fused. The median segments consist of three rings. The genital pores are separated from each other by two annuli. Live individuals typically have a dark greenish-brown color with a row of white spots along the body margin. On the dorsal side, there are five longitudinal bands with prominent papillae, one of which is located in the center and has four dark spots. The other two pairs of stripes are paramedian and have prominent papillae, as shown in Figure 2.

Indicators of Parasitism

The infection prevalence among all turtles was determined to be 76.4%, with 77% in *E. orbicularis* and 75% in *M. rivulata*. The infection intensity was found to be 2.70 in *E. orbicularis* and 12.67 in *M. rivulata*, while the abundance was 2.08 in *E. orbicularis* and 9.50 in *M. rivulata*. In both species, the infection prevalence, infection intensity, and abundance values were higher among female turtles than males, as shown in Table 3.

Table 3. Infection prevalence, infection intensity and abundance of *P. costata* in turtles

Turtle species	Sex	Infection prevalence (%)	Infection intensity	Abundance
E. orbicularis	Female	80	2.75	2.20
	Male	67	2.50	1.67
General of species		77	2.70	2.08
M. rivulata	Female	100	17.00	17.00
	Male	50	4.00	2.00
General of species		75	12.67	9.50
General for turtles		76	5.00	3.82

Discussion

The distribution of two freshwater turtle species, *E. orbicularis* and *M. rivulata*, and the leech *P. costata*, is well documented in Turkish wetlands (Kazancı et al. 1992; Sağlam 2001; Özbek and Sarı 2007; Ayaz and Budak 2008, Bayrakcı et al. 2017). However, the parasitism of the Glossiphonid leech *P. costata* on these two freshwater turtle species, *E. orbicularis* and *M. rivulata*, has not been previously assessed in Turkish wetlands. Therefore, this study presents the first field observation and laboratory examination of this parasitic relationship.

The morphology of *P. costata* was consistent with previous reports in various habitats (Sawyer 1986; Neubert and Nesemann 1999; Elliott and Dobson 2015). Predominantly, the leeches found on the turtles were attached to the plastron, followed by the legs, carapace, and the head region (head and neck). Different attachment sites have been documented for the same leech by Bielecki et al. (2007), Bashirichelkasari and Yadollahvandmiandoab (2017), and Fediras et al. (2017). No significant correlation was found between parasite density and turtle size. The main peak of parasitism was observed in May and July, likely related to the rise in water temperature, feeding, and reproductive activity (Bielecki et al. 2012). Infection prevalence was found to be higher than in some other aquatic habitats (Bielecki et al. 2012; Fediras et al. 2017).

Aquatic vegetation cover, which serves as both shelter and a food source, is a crucial factor in determining the spatial distribution of turtles (Lebboroni and Chelazzi 1991). The wetlands surrounding Lake Eğirdir, with their abundant and healthy aquatic vegetation cover, as well as an ample supply of prey such as fish and frogs, provide an advantageous habitat for turtles and their parasitic leeches. However, turtle populations are facing significant threats from habitat destruction, over-collection for food and pets, and climate change (Bielecki et al. 2012; Lovich et al. 2018).

Three live *P. costata* were found on the deceased turtle, while the cause of death of the turtles is unclear. Previous studies have reported scavenging feeding characteristics of predatory leech species

(Davies et al. 1997; Pfeiffer et al. 2005; Ceylan et al. 2017). However, this finding needs to be analyzed in more details.

Further investigation, including hematological, histological, and biochemical analyses, as well as examination of host-parasite relationships, is necessary to fully understand the parasitism of *P. costata* in freshwater turtles.

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Effects of Cyanide on Some Histological and Immunohistochemical Parameters of Common Carp (*Cyprinus carpio*)

Mustafa Kavasoğlu[1]* Kazım Uysal[2] Ayşe Nur Değer[3]

[1] Kütahya Dumlupınar University, Gediz Vocational School, Medical Laboratory Techniques Program, Kütahya
[2] Kütahya Dumlupınar University, Faculty of Arts and Sciences, Department of Biology, Kütahya
[3] Kütahya Health Sciences University, Faculty of Medicine, Department of Medical Pathology, Kütahya

ABSTRACT

In this study, Bcl 2 and Caspase 3 expressions and histomorphological changes were investigated in the liver, gill and skin tissues of carp (*Cyprinus carpio*) in which 0,1 mg/L and 0,2 mg/L concentrations of cyanide were added to their environment. It was determined that the lipid accumulation, lymphocyte infiltration, fibrosis and regeneration in the liver tissues; hyperplasia, cell aggregates and goblet cells in the skin epithelium and gill filaments of fish exposed to cyanide. As a result of the study, it was observed that Bcl-2 expressions decreased and caspase-3 expressions increased in all tissues of fish exposed to cyanide at concentrations of 0,1 mg/L and 0,2 mg/L. Changes in Bcl-2 and caspase-3 expression levels result in disruption of the apoptosis mechanism in the liver, gill and skin tissues. At the end of the study, it was concluded that the examined parameters were a good indicator for cyanide intoxication.

Keywords: *Cyprinus carpio*, cyanide, histology.

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*** CORRESPONDING AUTHOR**

mustafa.kavasoglu@dpu.edu.tr
Phone: +90 274 443 55 00

Sazan Balığında (*Cyprinus carpio*) Bazı Histolojik ve İmmunhistokimyasal Parametreleri Üzerine Siyanürün Etkileri

Öz: Bu çalışmada bulundukları ortama 0,1 mg/L ve 0,2 mg/L konsantrasyonda siyanür eklenen sazan balıklarının (*Cyprinus carpio*) karaciğer, solungaç ve deri dokusu Bcl-2 ve kaspaz-3 ekspresyonları ve histomorfolojik açıdan değişimleri araştırılmıştır. Siyanüre maruz kalan balıkların karaciğer dokularında lipid birikimi, lenfosit infiltrasyonu, fibrosis ve rejenerasyon, deri epitelinde ve solungaç filamentlerinde ise hiperplazi, hücre agregatları ve goblet hücreleri tespit edilmiştir. Yapılan çalışma sonunda 0,1 mg/L ve 0,2 mg/L konsantrasyonlarda siyanüre maruz bırakılan sazan balıklarının incelenen tüm dokularında Bcl-2 ekspresyonlarının azaldığı, kaspaz-3 ekspresyonlarının ise arttığı görülmüştür. Bcl-2 ve kaspaz-3 ekspresyon düzeylerindeki değişimler ilgili dokulardaki apoptosis mekanizmasının bozulması sonucunu doğurmaktadır. Çalışma sonunda incelenen parametrelerin siyanür intoksikasyonu için iyi bir indikatör olduğu sonucuna ulaşılmıştır.

Anahtar kelimeler: *Cyprinus carpio,* siyanür, histoloji.

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Introduction

Cyanide is an anion radical formed by the triple bond of a carbon and a nitrogen atom. Cyanide has both organic and inorganic compounds, the organic compounds are named as nitryl and has no toxic potential. But inorganic cyanide salts, like sodium cyanide and potassium cyanide have extremely high toxic potential. The most poisonous forms of cyanide are free cyanide (CN) and hydrogen cyanide (HCN) which is in gas form. Cyanide which is not carcinogen like heavy metals, causes sudden death when exposed to extremely overdose. In doses that do not result in death, it may leave permanent effects on metabolism.

Negative effects are seen on many animal species as a result of cyanide intoxication when industrially produced cyanide is transferred to environment. In literature the cited values are as

follows, on rabbits 30 min. LC_{50} value is 188 ppm, on rats 60 min. LC_{50} value is 143 ppm (Ballantyne 1983); on mouse 5 min. LC_{50} value is 323 ppm (DiPasquale and Davis 1971). Of course, not all concentrations of cyanide are lethal in animals. Generally, 0.2 ppm is considered as the level in which toxic effect starts for animals (Sarı et al. 1999). But this concentration may cause lethal effect for aquatic organisms. It was reported in literature that even 0.01 ppm concentration causes lethal effect on fish (Blaha 1976).

Since cyanide can easily bond with metals, its use in gold and silver mining results in the damage of industrially produced cyanide to the aquatic environment. In aquatic environment, fish is the most affected living species by cyanide pollution. Fish is extremely vulnerable to pollutants since they take place in the last ring of food chain in aquatic environment. In literature, the lethal and toxic effects of cyanide for the fish has been widely studied. It is known that cyanide inhibits many enzyme systems of fish, destroys genetic structure, creates problems for their motive power and food intake (David and Kartheek 2016; David et al. 2008; Prashant 2011). Above all, this study will be the first about the effect of cyanide on histopathologic parameters of carps. From this point of view, in this study, taking into account the dangerous properties of cyanide, it was aimed to determine the toxic potential of cyanide in carp, a species with a wide ecological valence.

Materials and Methods

Test Environment, Fish Nutrition and Anesthesia

In this study sump systems, consisting of 5 aquariums whose dimensions are 30x40x60 cm, were used. The bottom aquarium in the system was used as a cleaning tank and there were no fish in it. The temperature of the aquariums was set to 22°C, and oxygen ratio of the water was never less than 6 mg/L. The fish used in this study were caught using tunnel net from the lake in Kütahya Dumlupınar University and then they were placed in aquarium units in the Kütahya Dumlupınar University Faculty of Arts and Sciences, Biology Department. After 15 days of acclimation period they were measured for their length and weight and placed in the aquarium randomly. 96 fish (48 for the 3-day experiment and 48 for the 15-day experiment) in total were used in the experiment. For each experimental period, 16 fish were determined as the control group and cyanide was not applied to these fish. As a source of cyanide, sodium cyanide (NaCN) was preferred. After adding the cyanide, the experiment continued for 3 days and 15 days. The water quality in the aquariums has been kept at a level that will not adversely affect the health of the fish (Lloyd 1992). No fish died during the experiment. At the end of the experimental period, the fish were taken from the aquariums and transferred to the anesthesia pool. As an anesthetic, clove oil, which has fewer negative effects than other chemical anesthetics, was used at a concentration of 600 mg/L (Han et al. 2016).

Histopathological analyses

Liver, gill and skin tissues dissected from fish were first fixed in 10% neutral formaldehyde solution for 48 hours for microscopic examination. Tissues taken from the formaldehyde solution were passed through increasing degrees of alcohol (%70, %80, %90, %100) for to remove water. Afterwards, it was passed through xylol for transparency and kept in molten paraffin so that it can be cut in the microtome device. The tissues which took the shape of block were cut in a microtome device in 4 microns thick. Staining procedure was applied to prepared samples.

The parts cut and prepared in microtome device were evaluated and their pictures were taken with Leica DCM 4000 (Germany) computer aided imaging system, Leica Q Vin 3 software. Degeneration criteria chart was formed as a result of analysis carried out with Hematoxylin Eosin (H&E) staining method.

H&E staining scoring was formed as below:

0: No change
+1: Slight tissue change
+2: Moderate tissue change
+3: Severe tissue change (Murussi et al. 2016; Poleksic and Mitrovic-Tutundzic 1994).

For immunohistochemical staining (Bcl-2, Caspase-3) Ab-7973 Abcam for Bcl-2 and RB-1197-P0 Thermo Scientific antibodies for Caspase-3 were used. DAB staining was performed as chromogen for to identify positive cells. Hematoxylin was applied for background staining for one minute. The stained sections were passed through the increasing alcohol series and after the water was removed, they were kept in xylol for 5 minutes to make them transparent and covered with entellan. To define expression ratio and apoptotic index, cell count was carried out in five independent different regions with 20x zoom lens.

Statistical analysis

SPSS 22 software was used to interpret and evaluate the gathered data. Graphs were created by calculating the mean and standard errors. To reveal statistical difference among groups One-Way ANOVA was used, and since it was supposed that there was homogeneous distribution among groups, Tukey test was used. To demonstrate the difference between 3-day and 15-day period experiments, Student t-test was

used. Results were analyzed at P<0,05 significance level.

Results

Histomorphological analyzes

When the findings obtained from the study were examined, it was determined that the pathological score level in the liver, gill and skin tissues of the fish exposed to cyanide for three and fifteen days increased significantly (Table 1).

With cyanide exposure in the liver, lipid accumulation in hepatocytes, lymphocyte infiltration, fibrosis, regeneration and loss of hepatic cord structures were observed (Figure 1).

In the gill tissue of cyanide exposed fish, hypertrophic and hyperplastic lamellae were formed, hyperplasia was seen in lamellae and cell aggregate accumulated (Figure 2).

Bcl – 2 expressions

In this study, Bcl-2 expression decreased significantly with the presence ofcyanide in all three tissues whose histopathological analyzes were performed (Table 2). In particular, a statistically significant decrease was observed in all tissues of *C. carpio* exposed to cyanide at a concentration of 0.2 mg/L (P<0,05).

Caspase-3 expressions

It was observed that Caspase-3 expressions in liver, gill and skin tissues of the fish used in this study increased significantly with cyanide exposure (Table 3). At the sametime, there is a direct correlation between increasing cyanide concentration and Caspase-3 expressions.

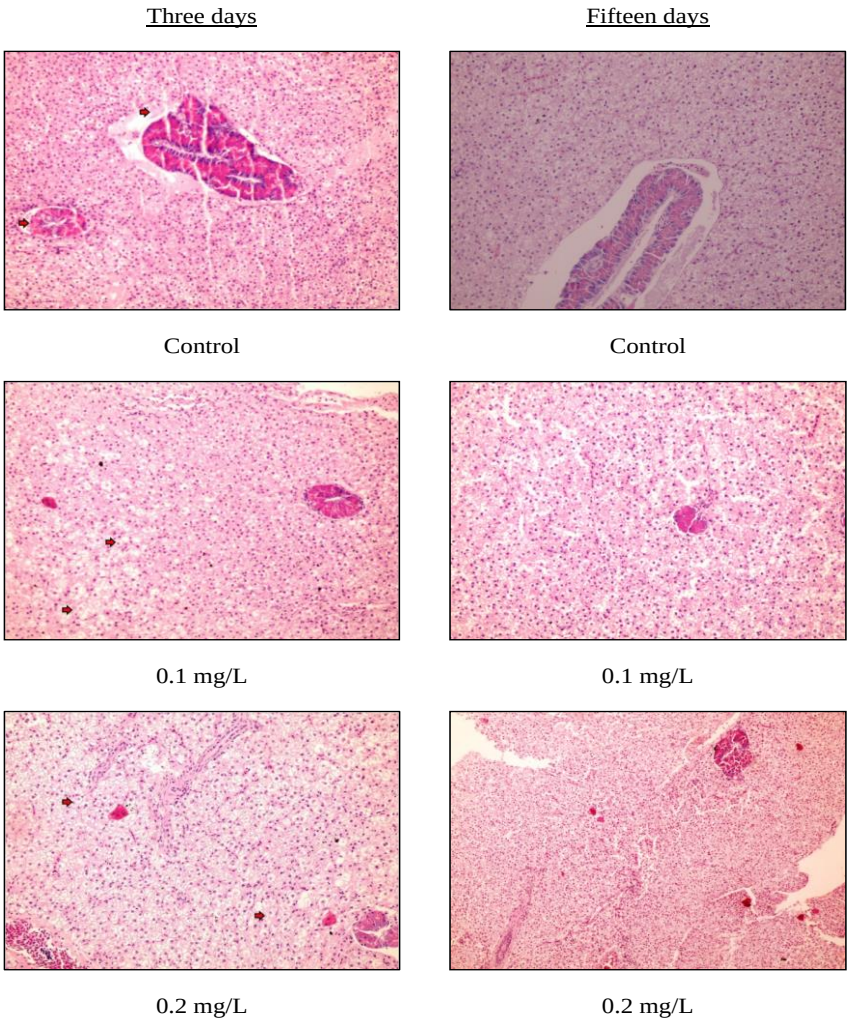

Figure 1. Histomorphological examinations of liver tissue (x200 magnification).

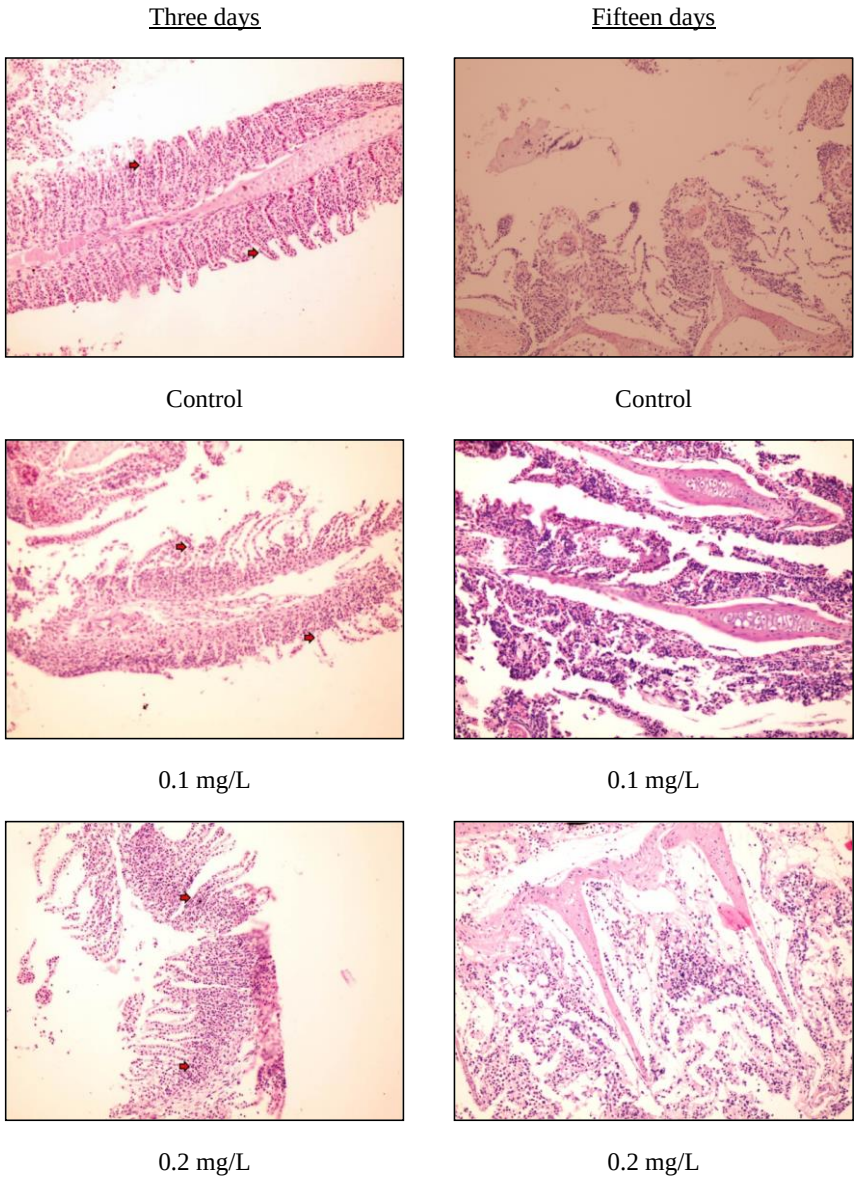

Figure 2. Histomorphological examinations of gill tissue (x200 magnification).

Table 1. H&E scores of fish used in the study

	Hematoxylin & Eosin (score)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Liver	0.40±0.16^{a}	1.00±0.26^{a}	2.60±0.16^{b}	0.60±0.16^{a}	1.20±0.25^{a}	2.20±0.2^{b}
Gill	0^{a}	1.30±0.21^{b}	2.20±0.20^{b}	0.20±0.13^{a}	1.80±0.25^{b}	2.30±0.21^{b}
Skin	0^{a}	0.80±0.62^{b}	2.00±0.47^{b}	0.20±0.13^{a}	1.20±0.29^{b}	1.80±0.29^{b}

(C: Control; Values shown with different letters contain statistical significance)

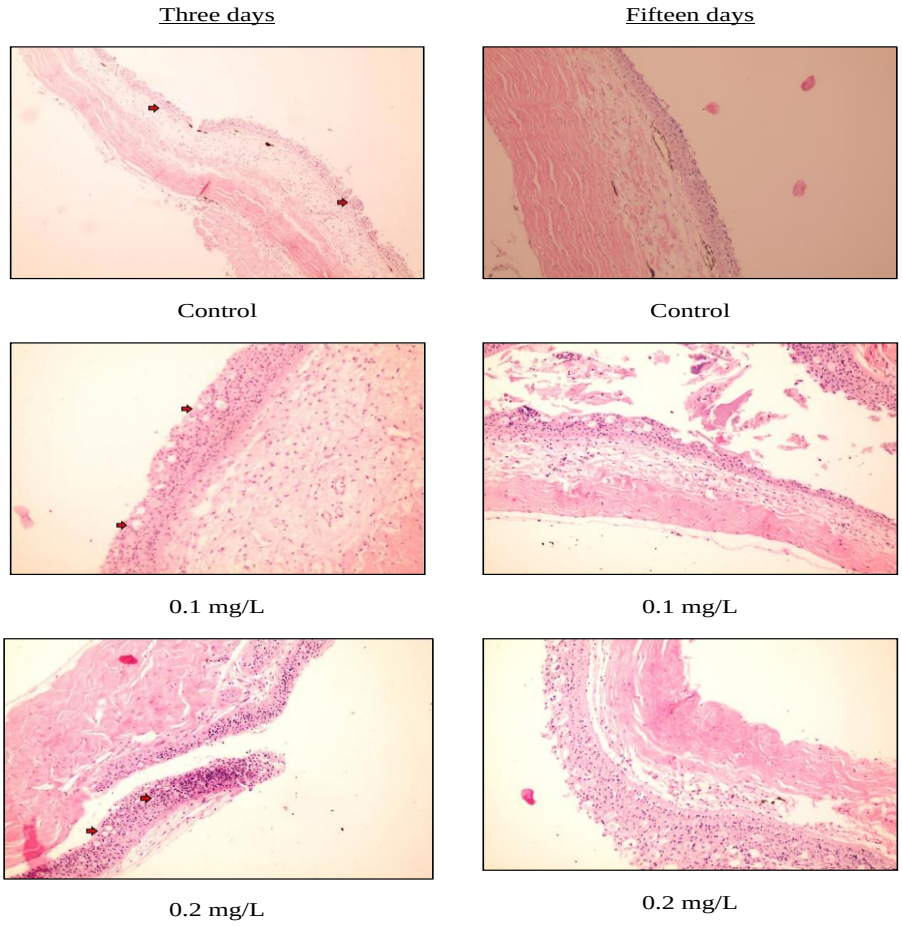

Figure 3. Histomorphological examinations of skin tissue (x200 magnification).

Table 2. Bcl – 2 expression percentages of fish used in the study

	Bcl – 2 (%)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Liver	17.51±1.14^{a}	10.57±1.02^{b}	6.82±0.98^{b}	13.58±1.6^{a}	12.5±1.2^{ab}	5.99±0.66^{b}
Gill	25.95±2.84^{a}	22.74±3.08^{ab}	10.46±1.34^{b}	29.00±1.8^{a}	19.25±1.71^{b}	8.75±1.3^{b}
Skin	27.32±2.82^{a}	15.84±1.27^{b}	9.94±0.71^{b}	32.85±2.91^{a}	26.78±2.78^{ab}	13.44±1.84^{b}

(C: Control; Values shown with different letters contain statistical significance)

Table 3. Caspase - 3 expression percentages of fish used in the study

	Caspase – 3 (%)					
	3 Days			15 Days		
	C	0.1 mg/L	0.2 mg/L	C	0.1 mg/L	0.2 mg/L
Liver	1.44±0.23^{a}	9.80±1.16^{b}	14.97±1.54^{b}	3.84±0.84^{a}	8.15±1.59^{ab}	15.21±1.79^{b}
Gill	8.00±1.01^{a}	11.28±1.68^{ab}	22.10±1.39^{b}	8.10±1.31^{a}	11.45±1.13^{ab}	27.35±1.51^{b}
Skin	7.22±1.4^{a}	14.12±1.73^{b}	19.5±1.4^{c}	8.18±1.23^{a}	18.54±1.15^{b}	22.81±1.47^{b}

(C: Control; Values shown with different letters contain statistical significance)

DISCUSSION

As stated in the results, various changes were detected in all three tissues used in the studycompared to the control group. Hypertrophy is defined as the overgrowth of an organ or tissue, while hyperplasia is defined as the overdevelopment of the tissue by increasing the number of cells. David and Kartheek (2016), exposed *C. carpio* to 0,1 mg/L concentration of NaCN. They found similar pathologic findings as in our study like hyperplasia in liver tissue and

lymphocyte infiltration. Maceda-Veiga et al. (2013), reported that there was excessive metal accumulation in sewage contaminated water supply and this condition resulted negative effects like lymphocytic infiltration and lipid accumulation in liver histopathology of *Squalius laietanus*. Ben Ameur et al. (2012), reported that intense vacuolization, lipid accumulation in hepatocytes and necrosis in some parts of the tissue were detected in the livers of *Mugil cephalus* and *Dicentrarchus labrax*, which they caught from Bizerte Lagoon. Al-Ghanbusi et al. (2012), detected hypertrophy and hyperplasia in the gill tissues of *Aphanius dispar*, which they exposed to deltamethrin at different concentrations. At the same time, they found that some close secondary lamellae in the gills fused to each other with deltamethrin exposure. In an experiment with simazine exposure to *C. carpio*, hyperplasia was detected in the gill tissues. (Oropesa-Jimenez et al. 2005). Again, with the application of azadirachtin at different concentrations to *C. carpio*, an increase and hypertrophy of the epithelial cells of the secondary lamellae in the gill tissues were observed. In addition, hyperplasia and hypertrophy in mucus and chloride cells and aneurysm in the lamellae were detected (Murussi et al. 2016). Pathological alterations occurred in liver and gill tissues exposed to various chemicals are similar to the alterations occurred in gill tissues of fish exposed to cyanide in this study. At the same time, it shows that different chemicals effect on these organs extremely negatively. Pathological conditions such as hypertrophy and hyperplasia can be explained as the tissue overgrowth, creating a gap between the blood and the external environment, and trying to prevent the chemical from entering the metabolism (Cengiz 2006; Poleksic and Mitrovic-Tutundzic 1994). In the literature, it is seen that studies on the effects of cyanide on fish tissues are quite inadequate.

Bcl-2 is a protein found in mitochondrial external membrane which represses apoptosis. Bcl-2 inhibits caspase-mediated apoptosis pathway and necrosis which occurred as a result of oxidants and hypoxia (Green and John 1998; Arockiaraj et al. 2015). It is known that cyanide causes hypoxia and oxidative damage in living things. Therefore, it is important to know the expression of the Bcl-2 protein in the presence of cyanide. Vidal et al. (2008), carried out apoptosis dependent gene characterization of *C. carpio* and for the first time conducted the characterization of Bcl-2 protein. Cao et al. (2013) exposed *C. carpio* to fluoride for ninety days and observed the rate of apoptosis and Bcl-2 expression in the liver. It was reported that there was a positive correlation between fluoride exposure and increased apoptosis rate, and a negative correlation between Bcl-2 expression. (Cao et al. 2013). Yuan et al. (2016), identified and clarified Bcl-2 gene of *Ictalurus punctatus* for the first time and reported that in a condition of bacterial contamination and hypoxia Bcl-2 expression decreased. Studies show that Bcl-2 expression rates decrease in fish metabolism when exposed to any chemical or biological effects. This situation causes the acceleration of apoptosis in the tissue and accelerated apoptosis is a pathological condition. When the literature is examined, it can be said that there is no study on the relationship between cyanide exposure and Bcl-2 expression in fish tissues and this study is the first. Caspase-3 is an important apoptotic protease and enables apoptosis. The active Caspase-3 inhibits deoxyribonuclease enzyme and inactivates it and provides DNA fractionation. At the same time, it inhibits DNA repair and enables apoptosis irreversibly (Elvitigala et al. 2012). Jiang et al. (2015), exposed *C. carpio* var. Jian to copper and reported a significant increase of Caspase-3 expression in muscle tissue. Also reported that copper exposure increased apoptosis. Morcillo et al. (2016) investigated the expression rates of Caspase-3 in kidney and blood leukocytes after the treatment of *Sparus aurata* and *Dicentrarchus labrax* with various metals. As a result of their studies, it was determined that the Caspase-3 ratios of fish exposed to cadmium, mercury and arsenic concentrations at EC_0 and EC_{50} ratios increased significantly. In the study, it was observed that lead also increased the expression of Caspase-3, but this situation was not statistically significant. The study reported increase of Caspase-3 expression resulted in increase of apoptosis (Morcillo et al. 2016). Kumaresan et al. (2016), defined caspase family in the tissues of *Channa striatus* and examined for the expression ratio of caspase family against bacterial and fungal threats. The highest expression of Caspase-3 in the studied species was detected in the spleen, a lymphoid organ. They reported that Caspase-3 expressions were significantly increased in bacterial and fungal threats, especially at 24 hours, compared to control groups (Kumaresan et al. 2016). When the results reported in the literature and obtained from this study are examined, it is concluded that Caspase-3 expressions increase when fish are exposed to a chemical or biological agent, and this may increase apoptosis in cells.

When all the data obtained from the study were examined, the following conclusions can be drawn:

It was determined that 0,1 mg/L and 0,2 mg/L concentrations of cyanide exposure caused various histomorphological differences in liver, gill, skin tissues of *C. carpio*, decreased Bcl-2 expressions and increased Caspase-3 expressions. These finding sheds light on the idea that studies at the cellular level should be done with electron microscopy in order to examine the damage caused by cyanide in more detail. It has been reported in the literature that histopathological parameters are suitable indicators for determining the toxic effects of pollutants on fish. In this study, it was observed that histopathological analyzes gave significant results.

In this study, *C. carpio* were used because they can live in all freshwater ecosystems and are resistant to pollutants. Considering the responses against pollutants may differ among living organisms and more vulnerable species may have negative effects with lower concentrations, so toxic effects of cyanide should also be investigated in different species. There is also a need to study the effects of different components of cyanide at different times.

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