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Short Comminication

First Record of *Contracaecum rudolphii* Hartwich, 1964 in *Carassius gibelio* (Bloch, 1782) From Turkey

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ABSTRACT

Thirty-eight individuals of Prussian carp, *Carassius gibelio* (Bloch, 1782) were collected from Karataş Lake, Burdur-Turkey and analyzed for parasite fauna. We found the nematode larvae of *Contracaecum rudolphii* Hartwich, 1964 in one sample (prevalence 2.63%, mean intensity of infestation 27 parasites per fish). The individual parasite was found around the pancreas, fibrous connective tissue and its mesentery. To our knowledge, this is the first record of anisakid nematode, *Contracaecum rudolphii* in Turkey. Therefore, a new locality has been added to the geographical distribution of the parasite species. Furthermore, slight to severe inflammatory cells were seen on the infected tissue. Granulomatous reaction characterized by mononuclear cells and fibrous tissue proliferations were also seen around the parasite located areas.

Keywords: Nematoda, Anisakidae, first record, histopathology

INTRODUCTION

The nematodes of the genus *Contracaecum* belonging to the family Anisakidae are able to infect both terrestrial and aquatic animals (Shamsi, 2019). The cosmopolitan *Contracaecum rudolphii* Hartwich, 1964 has a complex life cycle, involving invertebrates such as copepods, ostracods or gammarids as first intermediate hosts, teleost fish as second intermediate or paratenic host and piscivorous birds e.g. cormorants as final hosts (Bartlett, 1996; Dziekońska-Rynko, Rokicki, Mierzejewska, Wziątek, & Bielecki, 2013).

As far as we know, the first-stage larva formed in the embryonated eggs released with the faeces of definitive host moult to the second-stage, a variety of organisms including mollusks and crustaceans being able to eat eggs or larvae of *Contracaecum* parasites (Shamsi, 2019). Fish play the role of common hosts for the third larval stage after ingesting the already infested invertebrates (Dziekońska-Rynko & Rokicki, 2007). Infections of cyprinids under laboratory conditions have been proved by various authors that found nematode three-stage nematode larvae encysted in the intestinal wall or located in the internal organs of the fish (Moravec, 2009; Dziekońska-Rynko et al., 2013).

At the adult stage, *C. rudolphii sensu lato (s.l.)* is a parasite common in fish-eating birds such as great cormorant, *Phalacrocorax carbo* (Szostakowska & Fagerholm, 2007; Al-Moussawi, 2017). Dziekońska-Rynko, Rokicki, & Wziątek B (2008) suggested that nematodes prefer to develop in warm water bodies when cormorants become infected during their wintering season.

In Turkey, the first case of intense infection with adult *Contracaecum* sp. in a piscivorous bird was reported by Girişgin, Alasonyalilar-Demirer, & Girişgin (2012) during the necropsy of the Dalmatian pelican, *Pelecanus crispus*. So far, larval forms of *Contracaecum* sp. were confirmed in the helminthofauna of several fish species from Turkey: *Alburnus alburnus*, *Barbus* *lacerta*, *B. plebejus escherichi*, *Carassius auratus*, *C. carassius*, *Capoeta tinca*, *Scardinius erythrophthalmus*, *Rutilus rutilus* and *Vimba vimba* (Koyun & Altunel, 2007; Selver, Aydogdu, & Cirak 2009; Koyun, Ulupınar, & Gül, 2015). Moreover, larvae of *Contracaecum* sp. were mentioned for the first time in 2016 in the intestine of *Carassius gibelio* from Marmara Lake, western Turkey (Demir & Karakişi, 2016). Nevertheless, there is still no information upon larvae of *Contracaecum rudolphii* parasitizing fish species in Turkey. This study describes the first case of identified *C. rudolphii* Hartwich, 1964 in a freshwater fish host in Turkey.

MATERIALS AND METHODS

Overall, 38 Prussian carp (Figure 1) were caught using nets by local commercial fishermen in November 2018 in Karataş Lake, Burdur. Fish speciemens were identified to species level according to Kottelat and Freyhof (2007). The individuals were transported alive to the research laboratory of Biology, Burdur Mehmet Akif Ersoy University.

Figure 1. *Carassius gibelio* from Karataş Lake.

The total body length (cm) and weight (g) of each *Carassius gibelio* individuals were recorded. After all fish were sacrificed, their skin, fins, gills, oesophagus, liver, gall-bladder, stomach and intestine of the samples were dissected out and placed in petri dishes with a physiological solution. To determine the presence of parasite specimens, all parts were thoroughly examined under a binocular microscope. Parasite individuals found in the host fishes were removed using a preparation needle. The parasites were fixed in formaldehyde stained with Mayer's haematoxylin and identified using the reference keys (Pritchard & Kruse, 1982; Anderson, 1992). The percentage of hosts infected with the parasites (prevalence, %) and intensity were computed according to Bush, Lafferty, Lotz, & Shostak (1997).

The gut and mesenteries samples collected from the fish during necropsy were fixed in 10% neutral formalin. The samples were then routinely prepared by automatic tissue processor equipment (Leica ASP300S, Wetzlar, Germany) and embedded in paraffin wax. Tissue sections were cut into 5-µm-thickness by a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Then, samples were stained with hematoxylin-eosin (HE), placed on a coverslip with mounting media, and examined under a light microscope.

RESULTS AND DISCUSSION

The total length of Prussian carp individuals varied between 20 and 33 cm, while the weight values ranged between 153 and 560 grams. Among the total number of 38 specimens of *Carassius gibelio* examined for parasite presence, only one individual fish was infected with nematode *Contracaecum rudolphii* (prevalence 2.63%, mean intensity of infestation 27 parasites per fish) (Figure 2).

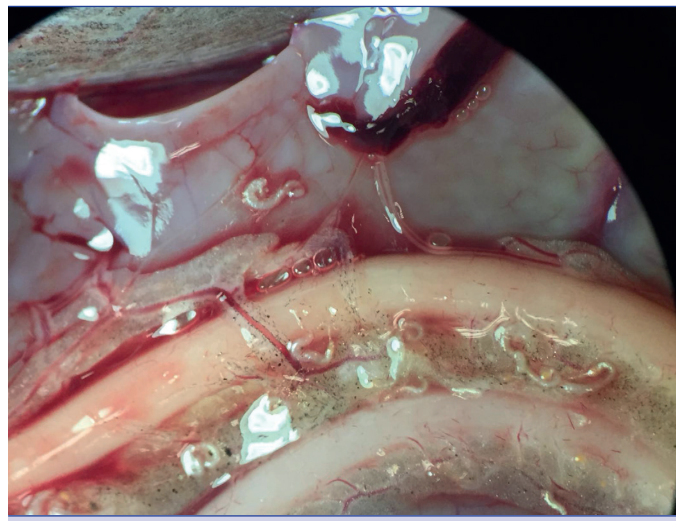

Figure 2. *Contracaecum rudolphii* in Carassius gibelio.

During the study, at the gross examination, hyperemias at the mesenteric vessels of infected host samples were observed. Parasites were found around the pancreas, fibrous connective tissue and its mesentery. Slight to severe inflammatory cells were seen on the infected tissue. Granulomatous reaction characterized by mononuclear cells and fibrous tissue proliferations were also seen around the located parasite areas. Some granulomas contained more than one parasite. Some granulomatous became necrotic when effected with the parasite. Numerous melano macrophages were found around the necrotic granulomas (Figure 3).

Based on genetic data evidence, Li et al. (2005) identified the existence of two-strains of the parasite, *C. rudolphii*-A and *C. rudolphii*-B. From these strains, *C. rudolphii*-B was determined as parasite of freshwater fishes (Szostakowska & Fagerholm, 2007; Moravec, 2009). In the present study, the larval parasite species, *C. rudolphii* was identified using morphologic and anatomic features detailed by Anderson (1992), in *Carassius gibelio* from Karataş Lake. According to the above knowledge, the larval parasite species *C. rudolphii* determined in the present study might belong to *C.rudolphii*-B. In further research, DNA sequencing is obviously required in order to establish that the taxon identified in this study is *C.rudolphii*-B.

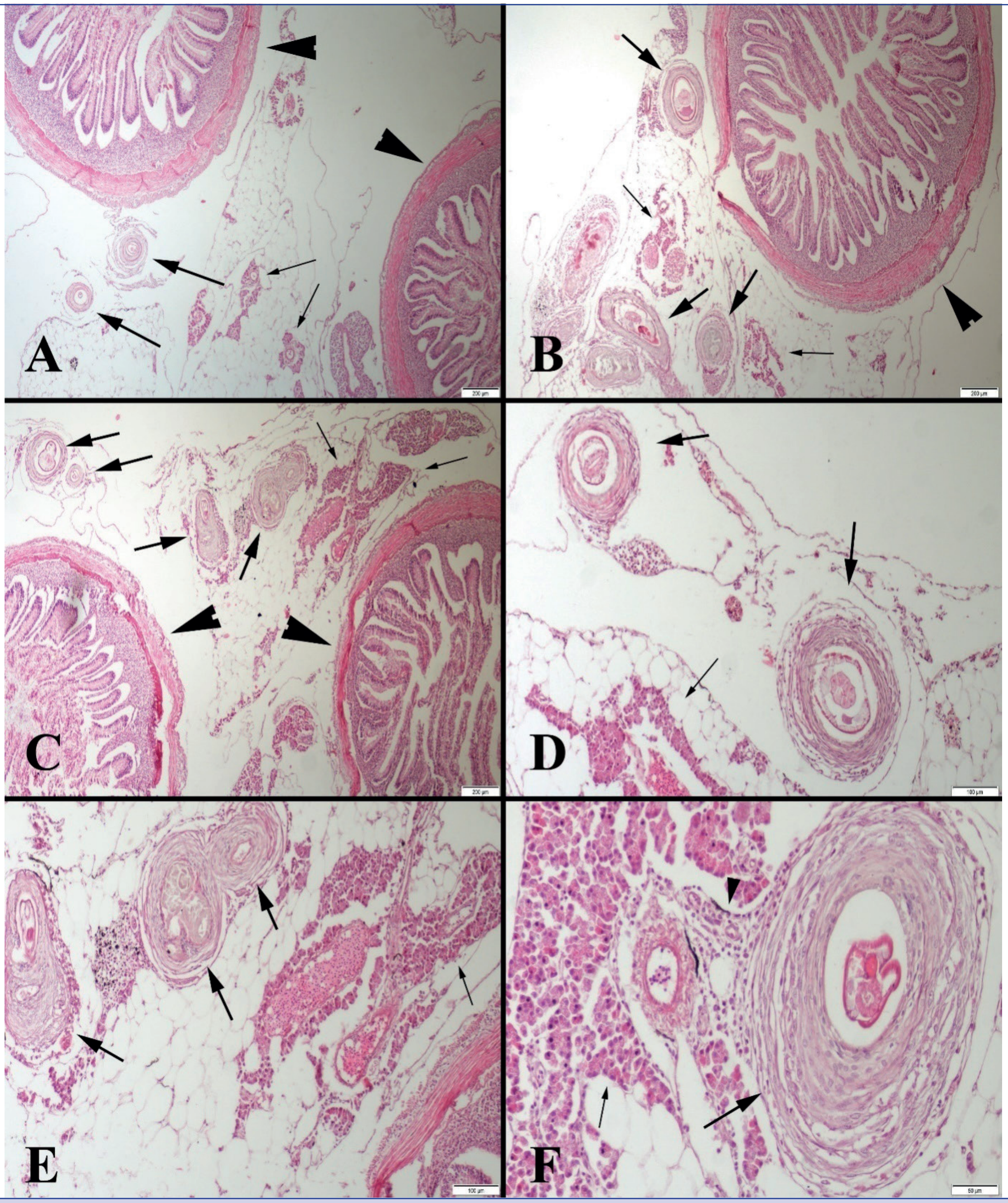

Figure 3. (A) Numerous parasites (thick arrows) localized at mesentery of the intestine (arrow heads) near the pancreas (thin arrows), HE, Bar= 200µm. (B) Another area and numerous parasite (thick arrows) around the intestine (arrow heads) and the pancreas (thin arrows), HE, Bar= 200µm. (C) Parasites (thick arrows), near the gut (arrow heads) and pancreas (thin arrows), HE, Bar= 200µm. (D-E) Higher magnification of the parasites (thick arrows), near the pancreas (thin arrows), HE, Bar= 100µm. (F) Fibrous connective tissue (arrow) around the parasite and inflammatory cell infiltrations (arrow head), HE, Bar= 50µm.

To date, another taxon, *Contracaecum microcephalum* was recorded for the Prussian carp from Srebarna Nature Reserve, Bulgaria (Shukerova, 2005). Different pervalence values of *Contracaecum* infection were reported from fish species (Stoyanov, Mutafchiev, Pankov, & Georgiev, 2017; Oztürk & Yesil, 2018; Dziekońska-Rynko, Mierzejewska, Kubiak, Rydzewska, & Hliwa, 2018; Chunchukova & Kirin, 2018; Sokolova et al., 2018). Low values of prevalence for *Contracaecum* infection in fish host in the present research are consistent with other studies (Mancini et al., 2008; Roumbedakis et al., 2013; Stoyanov et al., 2017). This might be explained by reduced feeding activity of infested fish by copepods at low temperatures in the cold season (Barson, 2004).

Similar changes were noticed by Dezfuli et al. (2016) in intestinal walls of *Anguilla anguilla*. In gastrointestinal tract of great cormorant *Phalacrocorax carbo*, Rokicki, Sołtysiak, Dziekońska-Rynko, & Borucińska (2011) reported *C. rudolphii* causing lesions consisted of severe or diffuse gastritis. Larval stages of nematodes forming ulcerative eosinophilic granulomas have been found by Amato, Monteiro, & Amato (2006) in the proventriculus of *Phalacrocorax brasilianus*.

Li et al. (2005) have postulated that *C. rudolphii*-A and *C. rudolphii*-B were not related with human anisakidosis, although these two sibling species have shown a zoonotic potential due to larval development in numerous fishes.

In conclusion, *Contracaecum rudolphii* is recorded for the first time in *Carassius gibelio* from Turkey. Accordingly, a new locality has been added to the geographical distribution of the parasite species. Also, hyperemia and necrotic granulomas caused by the parasite species were determined.

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Research Article

Determination of Trace/Toxic Mineral Risk Levels for Different Aged Consumers of Three Fish Species Caught in the Marmara Sea

Nuray Erkan[1], Muammer Kaplan[2], Özkan Özden[1]

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ABSTRACT

The Marmara Sea is a semi closed fishing area in Turkey and has valuable fish species such as the common sole, black scorpionfish and horse mackerel. Domestic waste, generated by the population of over 25 million living in the coastal zone as well as industrial facilities in the region are the main causes of pollution in the Marmara Sea. In the current study, the levels of toxic metals (arsenic, cadmium, mercury, and lead) in samples of three fish tissue were analysed over a year-long period and compared with international results. The health risks for different age groups caused by the consumption of these fish were estimated. The results revealed that regular weekly consumption of sole and horse mackerel caught from the Marmara Sea posed health risks for child and youth populations. These fish species should be consumed more carefully due to potential arsenic-related hazards and carcinogenic risks for vulnerable consumer groups.

Keywords: Trace toxic mineral, HI, THQ, Health risk, The Marmara Sea, Common sole, Black scorpionfish, Horse mackerel

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INTRODUCTION

Metals are one of the basic components of nature, rapid industrialization, urbanization, mining, related changes in land use and associated enhanced terrestrial runoff (to seas) with anthropogenic activities which can significantly increase their content (Bat et al. 2012; Saha et al. 2016). Biological material from aquatic sources are most easily contaminated by environmental pollution, especially due to their location in the nutritional chain. Therefore, contaminated seafoods is an important contributory factor in the transfer of environmental pollutants to consumers. Because of this reason consumption of fisheries' products is one of the main steps in the transfer of environmental pollutants to consumers. A lot of nutrient components and elements found in seafood are crucial for sustaining human life, but metals such as mercury, cadmium and lead do not play a known role in biological systems, and dietary intakes of these metals - even at very low concentrations - may cause toxic effects (Storelli et al. 2012). The trace toxic minerals from sea pollutants accumulating in fish tissues are the major risk factor and therefore need to be monitored for the protection of human health. The metal toxicity associated with fish consumption depends mainly on the metal content, how often it is consumed, and on the age of the consumers (Safahieh et al. 2011). Consumption of trace toxic minerals/ contaminated fishes may result in a weakening of the endocrine system in children and adults, and these compounds have neurotoxic and nephrotoxic effects (Herreros et al. 2008).

Another important hazard rooted in environmental pollution is arsenic and its presence in seafood is an important source of arsenic exposure in humans, and according to the European Commission Scientific Cooperation Project

(SCOOP) this ratio is may be 50% higher than other foods. Fish and shellfish are usually contaminated with arsenobetain, while arseno-lipids are found in fatty marine fish. Moreover, the inorganic form of arsenic is more toxic than organic arsenic. These compounds are genotoxic and generally have a neurotoxic effect, in the central nervous system of developing foetuses, infants and growing children (Mania et al. 2015; Koesmawatia et al. 2015).

Common sole (*Solea solea*) and black scorpionfish (*Scorpaena porcus*) are demersal fish species which do not leave their habitats, whereas horse mackerel (*Trachurus trachurus*) is a seasonal migratory pelagic fish in the Marmara sea. In 2017, the total production quantity of Turkey's sea fish catch was reported as 269676.4 metric tons (Turkish Statistical Institute (TUIK), 2018). The total catch was 486.4 tons for common sole, 306 tons for black scorpionfish, and 8065.6 tons for horse mackerel in Turkey. These fish species mainly live in the Marmara Sea where pollution is one of the major challenges. The domestic waste of densely populated cities (Istanbul and İzmit with a total population of nineteen million people) in the northeastern region of the Marmara Sea, maritime traffic and the waste of heavy industry plants in the İzmit Gulf are the main causes of pollution.

Fish are good indicators for the long term monitoring of metal accumulation in the marine environment. The trace toxic minerals in fish produce toxic effects at high concentrations (Keskin et al. 2007; Tepe et al. 2008; Türkmen and Ciminli, 2007). The possible health risks to consumers may vary depending on the age groups and the amount of fish consumed. In recent years, the target hazard ratio (THQ) and/or total target hazard area (TTHQ) have been used to assess health risks (Copat et al. 2013).

The data on trace toxic minerals levels in common sole, black scorpionfish and horse mackerel from Turkey are very limited and little information is available in the literature to quantify the health risks related with the consumption of contaminated fish from the Marmara Sea.

Therefore the main aims of the study are twofold: firstly, to determine the levels of arsenic cadmium, mercury and lead in selected fish species samples over a one-year period, and to compare the results with the limits set by International Organisations. Secondly, to assess the health risks of trace toxic minerals to consumers of different age groups.

MATERIALS AND METHODS

Samples

The common sole (*Solea solea*), black scorpionfish (*Scorpaena porcus*) and horse mackerel (*Trachurus trachurus*) samples (caught from the Marmara Sea) were purchased from a local fish market in Istanbul. Fish samples were collected on a monthly basis throughout the fishing season. The maximum, minimum and average of all values were presented and evaluated. Each species (10 samples for common sole, 20 samples for black scorpionfish and 100 samples for horse mackerel in each sampling) was gutted, filleted and muscle tissue was minced for analysis. The minced samples were then placed in individual polythene bags and kept frozen prior to analysis. Determination of arsenic (As), cadmium (Cd), total mercury (Hg), and lead (Pb) amounts in each fish species were analysed according to the Method 3051A (US Environmental protection Agency (US EPA) 2007) method.

Toxic metals analysis

Fish samples were accurately weighed (approximately 0.3 g) and digested in a closed-vessel microwave digestion system (ETHOS 1, Milestone, Italy) using 6 mL nitric acid (65% v/v) and 4 mL hydrogen peroxide (30% v/v) (Merck, Darmstadt, Germany) at 200°C for 20 min. After cooling to room temperature (20-23°C), the solution was quantitatively transferred into a 25 mL volumetric flask and diluted to volume with ultra-pure water obtained from the Milli-Q system (Millipore, Bedford, MA, USA) and stored at 4°C prior to analysis.

The toxic metal contents of the fishes (three sub-samples of each material) were analysed using a Thermo Scientific iCAP Qc ICP-MS instrument (Thermo Fisher Scientific GmbH, Germany) equipped with an Elemental Scientific SC-2DX auto sampler (Omaha, NE, USA). The ICP-MS sample introduction system consists of a PFA concentric nebulizer coupled with a peltier-cooled cyclonic spray chamber. The iCAP Q interface consists of a pair of standard Ni sample and skimmer cones. The ICP-MS was operated in kinetic energy discrimination mode, using helium as the collision cell gas with a flow rate of 4.5mL min^{-1}. The following operating parameters were also set: RF power, 1,550 W; plasma gas flow rate, 14 L min^{-1}; auxiliary gas flow rate, 0.89 L min^{-1}; carrier gas flow rate, 0.91 L min^{-1}; spray chamber temperature, 2.70°C.

Arsenic, ^{75}As; cadmium, ^{111}Cd; mercury, ^{202}Hg; and lead, ^{208}Pb were the isotopes monitored. Quantitative analysis of the samples was performed using a five-point calibration curve (0.5, 1.0, 2.5, 5.0, 10.0 µg/L) constructed for each isotope. To cover the mass range of isotopes a mixture of internal standard elements (^{6}Li, ^{45}Sc, ^{73}Ge, ^{103}Rh, ^{111}In, ^{159}Tb, ^{175}Lu and ^{209}Bi) at 2 µg/L concentration level were used. The response factors of both higher and lower mass internal standards were used to correct the concentration of each isotope monitored.

The toxic metal content results were given as µg/g in wet weight (w.w.) of fish samples and the accuracy of the analytical method was monitored by analysing certified reference materials from mussel tissue (Catalogue No. ERM-CE278k).

Estimation of potential public health risks

Nine different age-categories (US Environmental protection Agency (US EPA) 2011) were used for estimating health risks: **A1** 1-3 years children, **A2** 4-6 years children, **A3** 7-10 years children, **A4** 11-14 years adolescents, **A5** 15-19 years adolescents, **A6** 20-24 years adults, **A7** 25-54 years adults, **A8** 55–64 years adults and **A9** >65 years seniors. Body weights (kg) were 14 kg for A1, 21 kg for A2, 32 kg for A3, 51 kg for A4, 67 kg for A5, 72 kg for A6, 77 kg for A7, 77 kg for A8 and 72 kg for A9. The weekly fish consumption value was obtained by measuring one portion as 150 g/week/person for A4, A5, A6, A7, A8, A9 and 50 g/week/person for A1, A2, A3. The risk assessments of the fish samples for the different groups were made according to the methods and calculations given by Erkan and Özden (2017) (Table 1).

Table 1. Risk Assessments calculation methods

Risk Assessment Name	Formula	Explanation
Estimated Weekly Intake (EWI)	$\frac{WFC \times C}{BW}$	**WFC:** Weekly Consumption Values (g/week/person) **C:** Concentration of contaminant (µg/g) **BW:** Body Weight (kg)
Hazard Index (HI)	$\frac{CWICC}{PTWI}$	**CWICC:** Calculated Weekly Intake for Certain Contaminant **PTWI:** Provisional tolerable weekly intake (µg/week/kg bw) in European Food Safety Authority (EFSA) (2009a) for As, in EFSA (2009b) for Cd, in EFSA (2019) for MeHg and in EFSA (2010) for Pb
Target Hazard Quotient (THQ)	$\left(\frac{EF \times ED \times FIR \times C}{RFD \times BW \times TA}\right) \times 0.001$	**EF**: Exposure Frequency (365 days/year) **ED**: Exposure Duration (years) for different age groups. **FIR**: Food Ingestion Rate (/person/day) for children and adult age groups **C**: Concentration of contaminant (µg/g) **RFD**: Oral Reference Dose (µg/g/day for As, Cd, Hg, Pb) (Qin et al., 2015) **BW**: Body Weight (kg) **TA**: Averaging exposure time for non -carcinogens (365 days/ year x ED)

Table 2. The concentration of trace toxic minerals in three fish species of the Marmara Sea.

Fish/ Season	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Sole -Spring	1.103	<0.001	0.038	0.061
Sole -Summer	5.244	<0.001	0.122	0.046
Sole-Autumn	3.796	<0.001	0.060	0.042
Sole-Winter	20.450	<0.001	0.055	0.020
Season average	**7,648 ±7.539**	**<0.001**	**0.069 ±0.032**	**0.042 ±0.015**
European Commission (EC)'s upper limits (mg/kg)	**-***	**-***	**-***	**-***
Horse mackerel -Spring	13.30	<0.001	0.170	0.052
Horse mackerel -Summer	2.147	0.004	0.021	0.056
Horse mackerel -Autumn	1.217	0.004	0.010	0.037
Horse mackerel -Winter	2.740	0.005	0.024	0.047
Season average	**4.852 ±4.909**	**0.004 ±0.000**	**0.056 ±0.066**	**0.048 ±0.007**
EC's upper limits (mg/kg)	**-***	**0.10**	**0.50**	**0.30**
Scorpion fish -Spring	4.301	<0.001	0.025	0.071
Scorpion fish -Summer	1.153	<0.001	0.094	0.029
Scorpion fish -Autumn	3.314	<0.001	0.100	0.033
Scorpion fish -Winter	2.319	<0.001	0.138	0.099
Season average	**2.772 ±1.168**	**<0.001**	**0.089 ±0.041**	**0.058 ±0.029**
EC's upper limits (mg/kg)	**-***	**0.50**	**0.50**	**0.30**

• No limits in EC

Total THQ (TTHQ) of heavy metals for seafood is the sum of the following composition: **TTHQ (individual seafood) = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant n)**.

The EWI, HI and THQ calculations of this study were conducted using 100% Hg (EFSA, 2012) and 10% As (Qin et al., 2015).

RESULTS AND DISCUSION

The concentration of toxic metals determined in the fish tissues are presented in Table 2. Arsenic concentration was found to be the highest of all the toxic metals measured in the three fish species studied. Arsenic toxicity depends on the form of arsenic: inorganic arsenic in drinking water is much more toxic than organic arsenic in seafood. The trivalent form of inorganic arsenic types is also more toxic. Arsenobetaine and arsenosugars is the major form of arsenic in marine fish and most other seafood. Arsenolipid is the form of arsenic present in fish oils and fatty fish tissue likely to be present in other seafood. According to the Turkish Food Codex (2011) there is no reported limit value for arsenic in fish and fish products. Similarly, Falcó et al. (2006) and Martorell et al. (2011) have reported high arsenic concentrations of 4.55 µg/g and 11.614 µg/g of fresh weight in muscle tissues of sole fish from the Catalonia Region of Spain. Unlike our results, Erkan et al. (2009) and Özden et al. (2010) reported lower amounts of arsenic content in scorpion fish (0.189-0.244 µg/g) and sole

Table 3. Trace toxic minerals levels in literature for the three fish species.

Sample area	Fish	As (µg/g)	Cd (µg/g)	Hg (µg/g)	Pb (µg/g)	Literature
Trabzon (Turkey) local fish market	*Trachurus trachurus*	0.63	3.58	-	<LOD	Aydın and Tokalıoğlu, 2015
Coastal Waters of the Black Sea,	*Trachurus trachurus*	-	0.043-0.048	-	0.17-0.23	Bat et al. 2012
	Solea solea	-	0.020-0.023	-	0.03-0.08	
Iskenderun Bay	*Solea lascaris*	-	0.04	NE	0.39	Renieri et al. 2014
Aegean Sea	*Scorpaena porcus*	-	0.8	NE	0.66	
Marmara Sea	*Scorpaena porcus*	0.189	0.001	0.672	0.007	Erkan et al. 2009
	Scorpaena scrofa	0.244	0.011	0.405	0.032	
Varna (Bulgarian) local fish market	*Trachurus mediterraneus ponticus)*	-	0.045	-	0.166	Stoyanova et al. 2015
Black Sea	*Trachurus trachurus*	-	0.045-0.052	-	0.363-0.638	Yaman et al. 2013
Iskenderun Bay	*Trachurus mediterraneus*	-	-	-	1.037	Yilmaz, 2003
Istanbul (Turkey) local fish market	*Solea solea*	0.34	0.05	0.56	0.39	Özden and Erkan, 2016
	Trachurus trachurus	0.25	0.04	0.29	0.17	
Istanbul (Turkey) local fish market	*Trachurus trachurus*	0,026-0.644	0.001-0.016	0.37-1.282	0.015-0.807	Özden, 2010
Marmara Sea	*Trachurus mediterraneus*		0.011	0.035	0.063	Keskin et al. 2007
	Solea solea		0.022	0.329	0.133	
Granada (Spain) local fish market	*Trachurus trachurus*	0.032	0.253	0.146	0.672	Rivas et al. 2014
	Trachurus mediterraneus	0.043	0.141	0.204	0.814	
Marmara Sea (Yalova)	*Solea solea*	-	0.02	-	0.17	Türkmen, 2011
North Eagean Sea (Çanakkale)		-	0.07		0.25	
Eagean Sea (İzmir		-	0.26	-	0.48	
Meditreanean (Mersin Bay)		-	0.38	-	0.37	
Bulgarian Black Sea coast	Thrachurus mediterraneus ponticus	0.73	0.008	0.16	0.06	Makedonski et al. 2017
Istanbul (Turkey) local fish market	*Solea solea*	0.153-0.820	<0.001-0.233	0.135-1.858	0.006- 0.912	Özden et al. 2010

NE: not estimated.

(0.153-0.820 µg/g). Other researchers reported lower arsenic levels of 1.73 µg/g (Falcó et al. 2006), 3.141 µg/g (Martorell et al. 2011), 0.026-0.644 µg/g (Özden, 2010), 0.032-0.043 µg/g (Rivas et al. 2014), 0.63 µg/g (Aydın and Tokalıoğlu 2015) and 0.73 µg/g (Makadonski et al. 2017) in horse mackerel.

The mean concentration of Cd detected in sole and scorpion fish was <0.001 µg/g and higher mean values of 0.004 µg/g wet wt. was found in horse mackerel. The Cd content of sole, ranging from <0.001-0.233 µg/g and of horse mackerel ranging from 0.141 to 0.253 µg/g, was reported by Özden et al. (2010) and Rivas et al. (2014). However, this value was higher (for horse mackerel 3.58 µg/g, for scorpion fish 0.8 µg/g, for sole 0.38 µg/g than that reported by other authors (Aydın and Tokalıoğlu, 2015; Renieri et al. 2014; Türkmen, 2011).

The average amounts of mercury in the tissues of the investigated species were 0.069±0.032 µg/g w/w. (ranging from 0.038–0.122), 0.056±0.066 µg/g (0.010-0.170) and 0.089±0.041 µg/g (0.025–0.138) for sole, horse mackerel and scorpion fish, respectively.

The results obtained in Hg content analysis of the tissue samples are similar to the results of Keskin et al. (2007) in common sole (0.329 µg/g); Erkan et al. (2009) in scorpion fish (0.405-0.672 µg/g); Rivas et al. (2014) in horse mackerel (0.146-0.204 µg/g).

The average lead content was found to be 0.042, 0.048 and 0.58 µg/g for common sole, horse mackerel and scorpionfish respectively. In this study, Scorpionfish contained the highest lead concentration.

The lead content of sole was reported by Guérin et al. (2011) as 0.011 µg/g. Bat et al. (2012) and Türkmen (2011) reported higher lead cocentrations of 0.03-0.08 µg/g and 0.17-0.48 µg/g in sole, respectively. In another study, even higher lead concentrations of 0.672-0.814 µg/g were determined in pink salmon (Rivas et al. 2014).

The trace metal limits for the European Communities are for Hg 0.5 mg/kg (µg/g) in fishery products, for Pb 0.3 mg/kg (µg/g) in fish, for Cd 0.05 mg/kg (µg/g) in lean fish, 0.1 mg/kg (µg/g) in wedge sole, eel, horse mackerel, sardine, and anchovy (European Commission (EC) No 1881/2006). Unlike mercury, lead and

Table 4. HI and THQ risk factors for people of different ages and kilograms in three fish species from Marmara Sea.

		As		Cd		Hg		Pb		S	
		HI	THQ	HI	THQ	HI	THQ	HI	THQ	HI	THQ
Sole	A_1	0.364	1.300	<0.001	<0.001	0.379	0.352	0.012	0.014	0.757	1.666
	A_2	0.243	0.867	<0.001	<0.001	0.253	0.235	0.008	0.010	0.504	1.110
	A_3	0.159	0.569	<0.001	<0.001	0.166	0.154	0.005	0.006	0.331	0.728
	A_4	0.098	1.051	<0.001	<0.001	0.102	0.284	0.003	0.012	0.203	1.346
	A_5	0.076	0.815	<0.001	<0.001	0.079	0.221	0.003	0.010	0.157	1.044
	A_6	0.071	0.759	<0.001	<0.001	0.074	0.205	0.002	0.008	0.146	0.972
	A_7	0.066	0.710	<0.001	<0.001	0.069	0.192	0.002	0.008	0.137	0.908
	A_8	0.066	0.710	<0.001	<0.001	0.069	0.192	0.002	0.008	0.137	0.909
	A_9	0.071	0.759	<0.001	<0.001	0.074	0.205	0.002	0.008	0.146	0.972
Horse mackerel	A_1	0.231	0.825	0.011	0.002	0.308	0.286	0.014	0.016	0.564	1.129
	A_2	0.154	0.550	0.008	0.001	0.205	0.190	0.009	0.011	0.376	0.753
	A_3	0.101	0.361	0.005	0.001	0.135	0.125	0.006	0.007	0.247	0.494
	A_4	0.062	0.667	0.003	0.002	0.083	0.231	0.004	0.013	0.152	0.912
	A_5	0.048	0.517	0.002	0.001	0.064	0.179	0.003	0.010	0.118	0.708
	A_6	0.045	0.481	0.002	0.001	0.060	0.167	0.003	0.010	0.110	0.659
	A_7	0.042	0.450	0.002	0.000	0.056	0.156	0.002	0.009	0.103	0.615
	A_8	0.042	0.450	0.002	0.001	0.056	0.156	0.002	0.009	0.103	0.616
	A_9	0.045	0.481	0.002	0.001	0.060	0.167	0.003	0.010	0.110	0.659
Scorpion fish	A_1	0.132	0.471	<0.001	<0.001	0.489	0.454	0.017	0.020	0.639	0.944
	A_2	0.088	0.314	<0.001	<0.001	0.326	0.303	0.011	0.013	0.426	0.629
	A_3	0.058	0.206	<0.001	<0.001	0.214	0.199	0.007	0.009	0.279	0.413
	A_4	0.036	0.381	<0.001	<0.001	0.132	0.367	0.004	0.016	0.171	0.763
	A_5	0.028	0.296	<0.001	<0.001	0.102	0.285	0.003	0.012	0.133	0.592
	A_6	0.026	0.275	<0.001	<0.001	0.095	0.265	0.003	0.012	0.124	0.551
	A_7	0.024	0.257	<0.001	<0.001	0.089	0.248	0.003	0.011	0.115	0.515
	A_8	0.024	0.257	<0.001	<0.001	0.089	0.248	0.003	0.011	0.115	0.515
	A_9	0.026	0.275	<0.001	<0.001	0.095	0.265	0.003	0.012	0.124	0.551

HI ≥ 1, unacceptable, THQ ≥ 1, unacceptable cadmium levels were considerably below the legal limits for all three fish species studied.

The hazard index, the ratio of the weekly intake value to the PTWI value for As, Cd, Hg, and Pb calculated for different aged categories of people, was found to be well below the value of 1 (HI<1) in all three fish species (Table 4). According to the hazard index value, consumption of these fish species for the nine different age groups does not pose any health risk. Concentrations of the contaminants in food, may cause risks to the consumer in long-term consumption even if they do not exceed legal limit values. In this process, the body weight of the consumer group and the amount of food consumed are effective in the formation of health risk. Therefore, any health risk has been estimated by making THQ calculations in recent years. The THQ value being equal to or higher than 1 indicates that there is a potential health risk. In this study, the THQ values calculated for the heavy metals in selected fish species showed that arsenic-derived health risks could occur for certain groups of individuals. The total THQ values from consumption of sole have been found above 1 for the following age groups; A1 (1-3 years old children), A2 (4-6 years old children), A4 (11-14 years adolescents) and A5 (15-19 years adolescents) (Table 4). Also, the total THQ was calculated higher than 1 for the first group consuming horse mackerel. Similar to our findings, Vieira et al. 2011 calculated the THQ value for different age groups in sardine, chub and horse mackerel collected from Portuguese waters (Northeast and Eastern Central Atlantic Ocean) during one year. They reported metal concentrations below the tolerable limits adopted by the European Commission Regulation and the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO). However, their recommendation is that the fish investigated should be consumed more carefully due to possible hazard and carcinogenic risks from arsenic. In another study, Martorell et al. (2011) reported no risk for As, Cd, Hg, and Pb for individuals of different ages and kilograms in seafood products in Catalonia.

CONCLUSIONS

The THQ results calculated for different age groups has revealed that, due to their high arsenic content, regular weekly consumption of sole and horse mackerel from the Marmara Sea poses a health risk for the young age groups - 4-19 years old. Although there is no health risk calculated for Scorpion fish and the toxic metals studied, it is important to establish local research and toxic metal monitoring policies in the Marmara Sea fishing areas. As a result, it is recommended that periodic monitoring studies be conducted to evaluate the relationship between exposure to these toxic metals and fish and seafood consumption. It is also important that the results should be shared with the community and necessary warnings should be made in a timely manner.

Conflict of interests: The authors declare that they have no conflict of interest.

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Research Article

Spawning Period and Size at Maturity of Scaldback, *Arnoglossus kessleri* Schmidt, 1915 (Pleuronectiformes: Bothidae), Caught by Beam Trawl in The Black Sea, Turkey

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ABSTRACT

The scaldback, *Arnoglossus kessleri*, is a small fish that belongs to the Bothidae family. This species is a demersal fish inhabiting sandy and muddy bottoms where it lives, generally buried in the sand. Even in Turkey, the biology of *A. kessleri* is unknown, and there are very few complete studies of the biology of this fish in the Mediterranean Sea, including the Aegean Sea and the Sea of Marmara. To describe the spawning period, size at maturity (L_m), and length at maximum yield per recruit (L_{opt}) of *A. kessleri*, and to assess the differences in these parameters from other populations, a total of 12 months samplings were conducted between December 2012 and November 2013 in the Black Sea. The monthly fluctuation of the *GSI* values showed that the spawning period was between June and September in the study area. The L_m was estimated as 5.76 cm for females and 6.03 cm for males. The L_{opt} was calculated from the empirical relationships between the L_{opt} and L_m, and it was determined as smaller than L_m for both sexes (L_{opt} = 5.55 cm for female and L_{opt} = 5.82 cm for male) and also the reproductive load, L_m/L_{max}, of females was estimated as a bit (1.4%) larger than males. The results of this study were offered as biological input parameters regarded as a reference for the management of the Black Sea stocks of the scaldback species.

Keywords: Scaldback, *Arnoglossus kessleri*, spawning period, size at maturity, fisheries management, Black Sea

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INTRODUCTION

The Bothidae family of sinistral flatfishes (left-eye flounder) contains about 20 genera and 164 species (Froese & Pauly, 2019), and many bothid genera have wide geographic distributions with representative species widely distributed throughout temperate and tropical seas (Gibson, Nash, Geffen, & van der Veer, 2015). *Arnoglossus* is a speciose genus with members distributed from off the Atlantic coast of Europe and Africa, in the Mediterranean and Black seas, throughout the Indo-West and South Central Pacific to the Nazca Submarine Ridge in the southeastern Pacific (Gibson et al., 2015). There have been reports of six marine fish species distributed along the Turkish coasts belonging to the Bothidae family: *Arnoglossus imperialis* (Rafinesque, 1810), *Arnoglossus kessleri* (Schmidt, 1915), *Arnoglossus laterna* (Walbaum, 1792), *Arnoglossus rueppelii* (Cocco, 1844), *Arnoglossus thori* (Kyle, 1913) and *Bothus podas* (Delaroche, 1809), but only three fish species belonging to this family: *A. kessleri*, *A. laterna* and *A. thori* have been reported from the Black Sea (Bilecenoğlu, Kaya, Cihangir, & Çiçek, 2014).

According to FishBase (Froesa & Pauly, 2019; https://www.fishbase.org) and the IUCN red list of threatened species (Golani, Kada, Nouar, Quignard & Cuttelod, 2011; de Sola, Nielsen, Monroe, Costa & Herrera, 2014); the scaldback, *A. kessleri* (Pleuronectiformes: Bothidae), is endemic to the Mediterranean and Black seas, and presents all through the Mediterranean Sea coast, except for Morocco and Algeria. It is found on the upper part of the continental shelf, and feeds on small fish and invertebrates. It is an uncommon species, but it is unknown if it is rare due to fishing activities. Therefore, *A. kessleri* is listed as a data deficient (DD) species in the IUCN red list of threatened species (Golani et al., 2011; de Sola et al., 2014).

The life history parameters, including only weight-length parameters (WLRs) and conditions for *A. kessleri,* were previously reported from different geographic regions, such as the Aegean Sea (İlkyaz, Metin, Soykan & Kinacigil, 2008; Altın, Ayyıldız, Kale & Alver, 2015; Bayhan, Sever & Taşkavak, 2008; Türker-Çakır, Koç, Basusta, & Basusta, 2008), the Marmara Sea (Ozen, Ayyildiz, Oztekin & Altin, 2009; Türker-Çakır, Akalın, Ünlüoğlu, Bayhan & Hoşsucu, 2003; Keskin & Gaygusuz, 2010), the Mediterranean (Ergüden, Altun & Ergüden, 2018) and also the Black Sea (Ak, Kutlu & Aydin, 2009). Moreover, previous research for *A. kessleri* has been rather fragmentary in the Black Sea. Only one study has been conducted on the WLRs parameters in the Black Sea coast of Turkey so far (Ak et al., 2009). Furthermore, reproduction biology parameters such as spawning season, size at maturity and reproductive load, etc. are key population input parameters in the assessment and management of fish stocks (Tsikliras, Antonopoulou & Stergiou, 2010; Tsikliras & Stergiou, 2014). But, these reproduction biology parameters of *A. kessleri* have not previously reported from different geographic regions and also from the Black Sea. The objective of the present study was to contribute first information on the spawning period, to provide new findings on length at maximum yield per recruit (L_{opt}) and reproductive load (L_m/L_{max}) of this species, and also to present a first estimation on the size at maturity (L_m), necessary for the introduction of suitable management plans for *A. kessleri* in the Black Sea.

MATERIALS AND METHODS

Study area and sampling

Samples of the scaldback (*A. kessleri*) were collected using an experimental purposes beam trawl with 15 mm cod-end stretched mesh size up to 30 m water depths between December 2012 and November 2013 on the Rize province coasts of the southeastern Black Sea, Turkey (Fig. 1). Seasons were grouped as winter (December - February), spring (March - May), summer (June - August) and autumn (September - November). Although the beam trawl fishery is banned in the Rize province coasts of the southeastern Black Sea during the year, sampling surveys were conducted with a special permit to determine crab population dynamics project samples. The total length (*TL*) of *A. kessleri* was measured after blot drying with a piece of clean towel. All specimens were measured to the nearest 1 mm, and weighed to the nearest 0.01 g. Total wet weight (*W*) and gonad weight (W_g) were recorded to the nearest 0.1 g.

Maturity and spawning period

The sex and the stage of maturity were recorded by macroscopic and/or microscopic examination of the gonads. The developmental stages of the gonads were classified for *A. kessleri*, taking

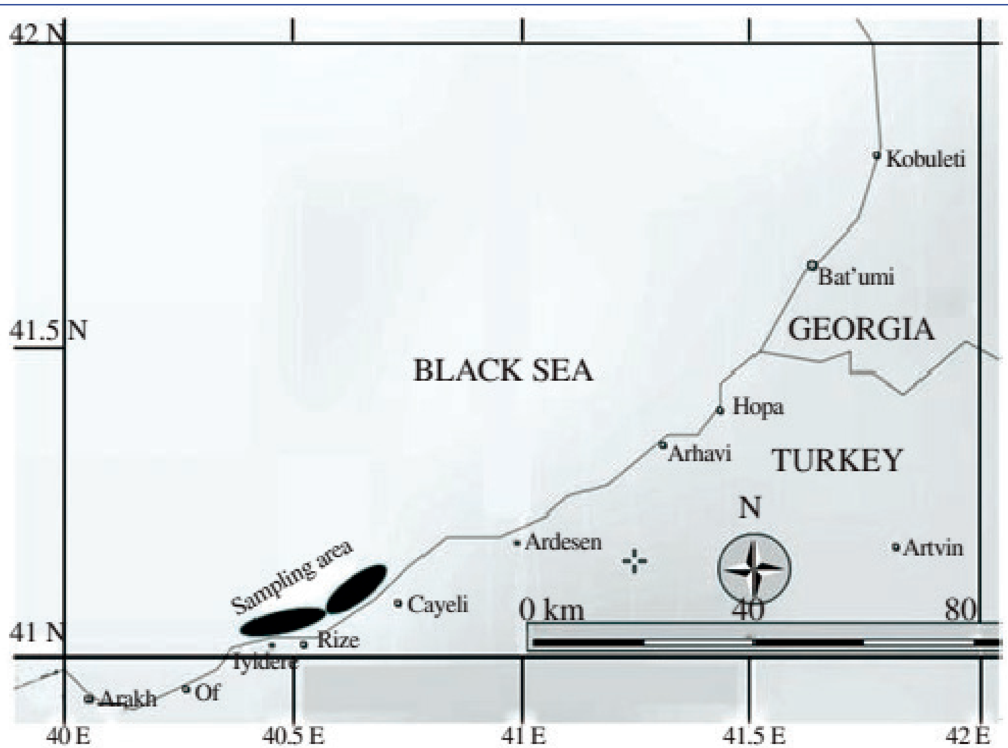

Figure 1. Beam trawl fishing operations sampling area on the Rize coasts in the southeastern Black Sea, Turkey.

into account the criteria proposed for Actinopterygii species by Holden & Raitt (1975) and for the greenback flounder, *Rhombosolea tapirina* by Barnett (1998). So, the maturity stages of the examined gonads were determined within four categories, based on the stages of the morphological characteristics: (stage I) immature virgin; (stage II) developing virgin and/or recovering; (stage III) ripe and/or spawner; (stage IV) resting or spent.

The spawning period was graphically determined for both sexes by the monthly variation of the mean values of the gonadosomatic index (*GSI*) as:

$$GSI = \frac{Wg}{W} = 100,$$

where, W_g is gonad weight (g), W is total scaldback weight (g).

Size at sexual maturity (L_m)

Size at sexual maturity (defined here as the length at which 50% of a population become sexually mature for the first time, L_m) was determined for females and males by calculating the proportion of mature females and males in the 0.5 cm size classes in the spawning period. Individuals with stage 2 and 3 in the gonad development stage were considered to be mature (Holden & Raitt, 1975). The proportion of mature females and males by size were fitted to the logistic equation:

$$P = \frac{1}{1 + e^{a+bL}},$$

where, P is the proportion of mature females or males, a and b are the coefficients of the equation, and L is the total length. Size at sexual maturity (L_m), corresponding to 50% sexually mature for females and males, was calculated from - (a/b).

Length at maximum yield per recruit (L_{opt})

Froese and Binohlan (2000) suggested that if an estimate of length at first maturity is available, the length at maximum yield per recruit, L_{opt} can be estimated. So, the L_{opt} for both sexes of scaldback was calculated from the following empirical equation suggested by Froese & Binohlan (2000).

$$\log L_{opt} = 1.053 \times \log(L_m) - 0.0565,$$

where, L_m is the size at sexual maturity (or the length at which 50% of a population become sexually mature for the first time) of scaldback.

Reproductive load (L_m/L_{max})

L_m/L_{max} ratio can be used to compare potential trends in maturation and energetic investment in reproduction, and/or growth for fish species (Tsikliras & Stergious, 2014), and the ratio also expresses the proportion of the potential growth span of the species that is covered before maturation (Beverton, 1963), so the L_m/L_{max} ratio (%) was calculated to express the reproductive load for both males and females of the scaldback.

RESULT AND DISCUSSION

A total of 1548 *A. kessleri* individuals were sampled during the study period, and it was determined that 44.1% of the samples were female (n = 682) and 55.9% male (n = 866). The sex ratio (female/male) was calculated as 0.79 and the χ^2 analysis showed that there was a statistically significant difference between the number of males and females (χ^2, $P < 0.05$).

Spawning period

A total of 1548 scaldback (682 females and 866 males) were sampled during the study. But only a total of 878 scaldback gonads (483 female and 395 male) were examined and used for gonadosomatic index (*GSI*) estimation. Looking at the monthly changes in the *GSI* values variation (Fig. 2), the peak of the *GSI* values was clearly exhibited in June 2013 and then, the *GSI* values decreased until August 2013, and reached the lowest level in September 2013 for both female and male. This monthly fluctuation

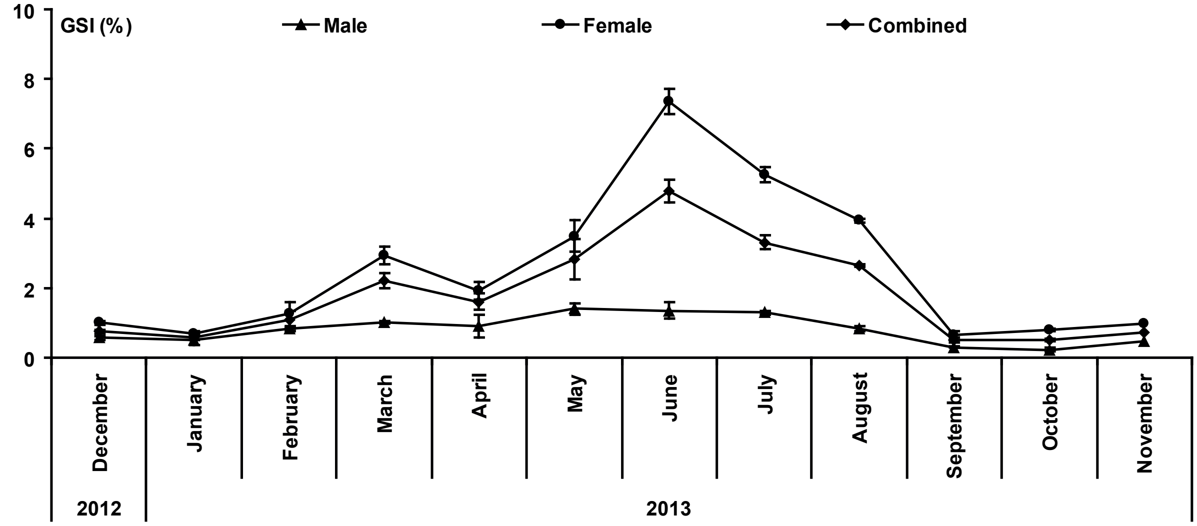

Figure 2. Plot of the mean gonadosomatic index (GSI) by month and sex for Scaldback, *Arnoglossus kessleri* collected between December 2012 and November 2013 in the southeastern Black Sea, Turkey. Vertical lines indicate standard errors of the mean.

of the *GSI* values showed that the spawning period of scaldback was between June and September in the study area.

Spawning season, size at maturity and reproductive load of *A. kessleri* have not previously reported from different geographic regions and neither from the Black Sea. So, this study provides the first information on the spawning season and first maturity length. Based on the monthly fluctuation of the *GSI* values, the reproduction of *A. kessleri* was between June and September in the Black Sea. Similarly, the breeding period of *A. kessleri* was reported as between May and August by FishBase (Froese & Pauly, 2019), based on Nielsen's (1986) report. But, this information is not based on field study. On the one hand, the spawning period of other Mediterranean scaldfish, *A. laterna* was reported from late June to August in the west coast of Scotland (northeast Atlantic) (Gibson & Ezzi, 1980). Furthermore, the spawnin period of *A. laterna* was reported as throughout almost the year, but with the maximum spawning in August, and continuing until October in the east-central Aegean Sea, in Turkey by İlkyaz, Metin, Soykan & Kinacigil (2017), and from February to June in the Yumurtalık Bight, in Turkey (western Mediterranean) by Özütok & Avşar (2004). Our findings also show reproduction in the similar time periods indicated by some of the previous studies. Although there is evidence that the duration of the spawning period may vary both annually and geographically, it was suggested that length of daylight is the main factor controlling the ovarian cycle, and that temperature controls the rate of egg laying (Holden, 1975). Moreover, all the studies' findings indicate a different time interval for the spawning period of *Arnoglossus sp,* and these results show that the spawning period has a close relation to the ecological characteristics of the water system in which the *Arnoglossus* species lives (İlkyaz et al., 2017).

Length at maximum yield per recruit (L_{opt})

The L_{opt} was estimated from the empirical relationships between length at maximum yield per recruit and size at maturity. Thus, L_{opt} was calculated as 5.55 cm for females and 5.82 cm for males, and also, L_{opt} was determined as smaller than L_m for both sexes of scaldback.

The L_{opt} is an important fisheries management parameter, because it can be a useful tool in defining routine fisheries management measures, such as MLS, closed seasons, etc. (Holt, 1958; Gulland, 1983; Frose & Binohland, 2000). But, estimation of L_{opt} requires knowledge of basic population parameters such as natural mortality (*M*) and the von Bertalanffy growth function parameter, *K*. Those two parameters are not easily obtained. Therefore, an empirical relationship ($\log L_{opt} = 1.053*\log L_m - 0.0565$) was reported between L_{opt} and L_m to provide an estimation of this parameter (Frose & Binohland, 2000). To calculate L_{opt} values for *A. kessleri*, we used Frose & Binohland's (2000) empirical equation and L_{opt} , calculated as 5.6 cm for females and 5.8 cm for males. L_{opt} values for females (5.6 cm) was smaller than for males (5.8), and L_{opt} values < L_m values for both sexes (male L_m = 6.0 cm, female L_m = 5.8 cm) of *A. kessleri*. It was reported that when using the recommended empirical formula for the calculation of L_{opt} from L_m, there are differences between short-lived small fish and long-lived large fish species. Namely, if L_{opt} estimates from L_m, the L_{opt} value is generally smaller than at lower L_m values, and it is generally longer than at higher L_m values. E.g. for small L_m values (e.g. L_m = 10.5 cm), L_{opt} is calculated at a lower (10.4 cm) than L_m ,and also for higher L_m values (e.g. L_m = 90 cm), L_{opt} is calculated at a higher (100.3 cm) than L_m. Since the *Arnoglossus* species, such as *A. laterna,* is a short-lived small fish species (Özütok & Avşar 2002; İlkyaz et al., 2017), it may be considered normal that L_{opt} values < L_m values for both sexes of *A. kessleri* stock in the Black Sea.

Reproductive load (L_m/L_{max})

The L_m/L_{max} ratio (%) of the scaldback calculated as 74.4 for males and 75.8 for females, and also L_m/L_{max} ratio of female was estimated as a bit (1.4%) larger than male. The L_m/L_{max} ratio of different species, such as *A. laterna* and *A. thori* belonging to Bothidae family, ranged between 0.59 and 0.70 for females and between 0.59 and 0.65 for males in the Mediterranean and Aegean Sea, and also the L_m/L_{max} ratio of female was generally larger than male (Özütok & Avşar 2004; İlkyaz et al., 2017). In the present study, similar results were found for both sexes of *A. kessleri* stocks in the Black Sea. The L_m/L_{max} ratio for fish species may vary within and between different species due to latitude variation, depending on water temperature and nutritional quality or availability of food, energy output, etc. (Abookire & Macewicz, 2003; Trip et al., 2014; Tsikliras and Stergious, 2014).

Size at maturity (L_m)

Total length of mature females ranged between 4.6 and 7.6 cm (mean: 41.6±0.61 cm; n = 166) and between 4.9 and 7.1 cm (mean: 6.1±0.21 cm; n = 84) for males.

Size at sexual maturity for females and males is shown in Fig. 3. The relationship between total length and the proportion of mature males was:

$$P = \frac{1}{1+e^{11.403-1.892*TL}}$$

and for female it was

$$P = \frac{1}{1+e^{7.853-1.364*TL}},$$

from this, the estimated size for 50% sexual maturity (TL_{50}) was 6.03 cm for males and 5.76 cm for females (Fig. 3).

We found that the sexual maturity size for males (L_m = 6.03 cm) was longer than for females (L_m = 5.76 cm) in the Black Sea. These L_m results for both sexes are the first reported values in the literature for *A. kessleri*. However, there are reported L_m results for other species, such as *A. laterna* belonging to *Arnoglossus* genus. Namely, the L_m values (total length) of *A. laterna* were reported as 11.9 cm for females and 11.4 cm for males in the east-central Aegean Sea, Turkey (İlkyaz et al., 2017), as 6.6 cm for males and 6.7 cm females in the Yumurtalık Bight (western Mediterranean, Turkey) by Özütok & Avşar (2004), as 6.8 cm for combined sex in the Adriatic Sea, Italy (Giovanardi & Piccinetti, 1984), and as 6-7 cm (standard length) for combined sex in the west coast of Scotland (northeast Atlantic) (Gibson & Ezzi, 1980). These differences between the studies conducted in different seas are most probably due to species difference, different length composition used to size at maturity calculation, different sampling methods, different environmental conditions such as

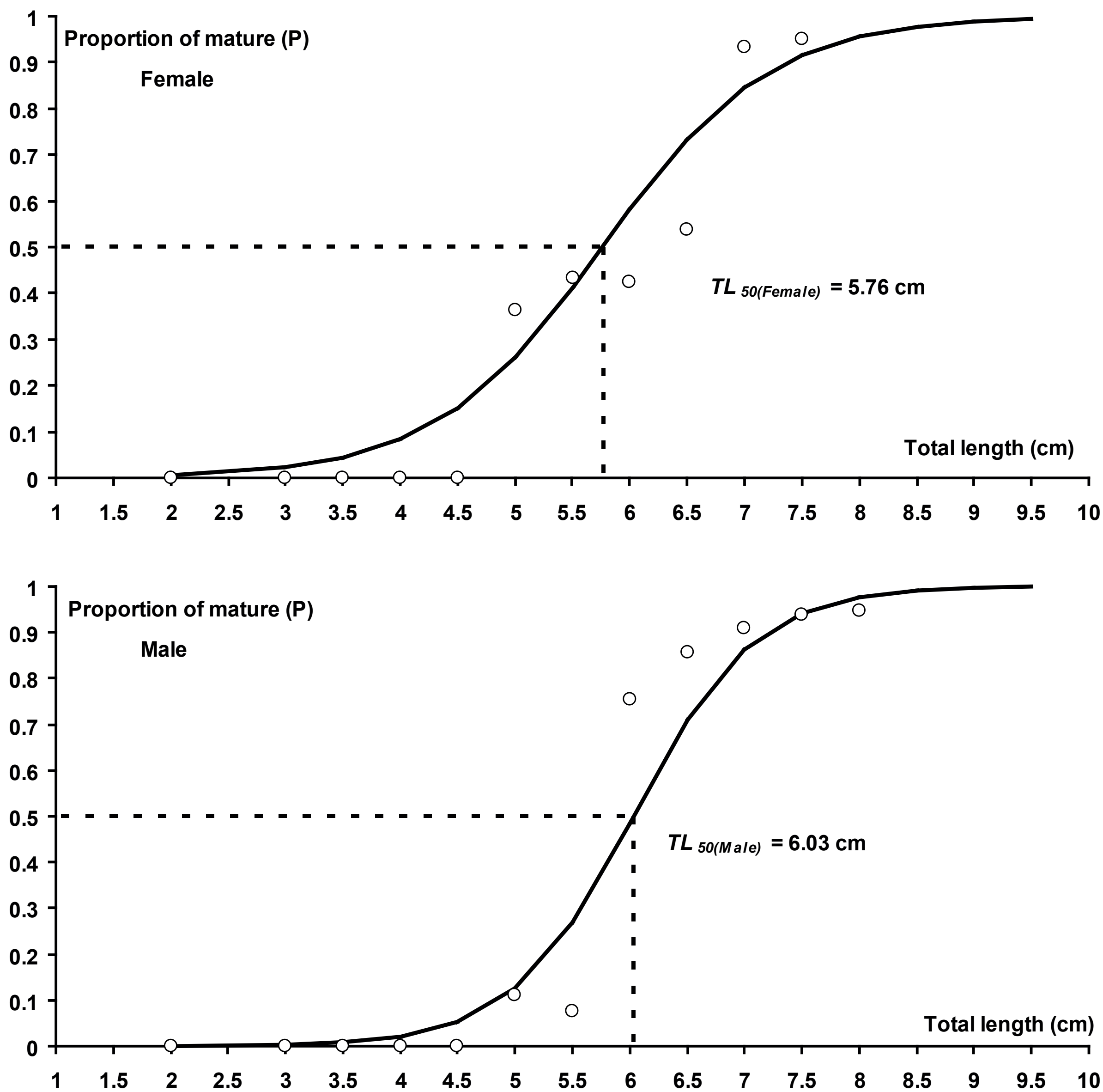

Figure 3. Plot of the size at sexual maturity by sex for Scaldback, *Arnoglossus kessleri* collected between December 2012 and November 2013 in the southeastern Black Sea, Turkey.

temperature, and different fishing pressure levels among the research areas and years, and also differences in the criteria for determining maturity. Furthermore, this variability may be due to environmental differences such as phenotypic variability (Tsikliras & Stergiou, 2014), differences of biotic and abiotic factors such as temperature, salinity, competition, etc. Size at maturity is an important fisheries management parameter, because it is the basis for setting the minimum landing size (MLS) of fish stocks, i.e. the MLS under which fish should not be caught (Tsikliras & Stergiou, 2014). Moreover, in the Black Sea (eastern Black Sea of Turkey), *A. kessleri* are not caught as a target fish species by fishermen, and also it is not amongst commercially important fish species, so no MLS limits are defined for the catch in the Black Sea and also other areas. The results of the present study could be used as biological input parameters regarded as a reference (e.g., the MLS: 6 cm total length) for the management of the Black Sea stocks of this species.

CONCLUSION

The presently reported study provides the first information on the spawning period and size at maturity for the scaldback, *A. kessleri* for the Black Sea. The results of this study were offered as biological input parameters regarded as a reference for the management of the Black Sea stocks of the scaldback species.

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

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

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Research Article

Research into the Epipelic Diatoms of the Meriç and Tunca Rivers and the Application of the Biological Diatom Index in Water Quality Assessment

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ABSTRACT

The Meriç River is one of the longest rivers of the Balkans and the Tunca River is the most significant tributary of the Meriç River. In the present study, the epipelic diatoms of the Meriç and Tunca Rivers were investigated and the water quality was evaluated from a physicochemical and biological perspective. Epipelic (EPP) diatoms were collected from the middlestream of the Meriç River (Edirne Province of Turkey) and from the downstream of the Tunca River (before emptying into the Meriç River) and certain physicochemical parameters including dissolved oxygen (DO), oxygen saturation (OS), pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity, nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), sulphate (SO_4), chemical oxygen demand (COD) and oxidation-reduction potential (ORP) were measured during the field studies. The Biological Diatom Index (IBD) was used to determine the trophic status of the Meriç and Tunca Rivers in terms of EPP diatoms, and Cluster Analysis (CA) was applied to the detected biological data in order to classify the identified diatom taxa in terms of their dominance in the system. According to the results of the physicochemical analysis, the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, NO_3, NH_4, SO_4 and COD parameters; and have III. – IV. Class water quality in terms of NO_2 and PO_4 parameters. 24 diatom species were recorded in the Meriç River by counting a total of 403 valves and a total of 19 diatom species were identified by counting a total of 409 diatom valves in the Tunca River. *Cyclotella atomus* Hustedt, *Navicula gregaria* Donkin, *Nitzschia palea* (Kützing) W.Smith and *Nitzschia subacicularis* Hustedt, were determined as the most dominant species in the Meriç River and *Navicula erifuga* Lange-Bertalot, *Navicula gregaria* Donkin and *Navicula rostellata* Kützing were recorded as the most dominant taxa in the Tunca River. According to the result of the IBD, the investigated rivers were found to be in a meso-eutrophic state and according to the results of CA, three statistical clusters were formed for both rivers, and were named as "dominant taxa", "frequent taxa" and "rare taxa".

Keywords: Meriç River, Bentic diatoms, Biological Diatom Index, Cluster Analysis

INTRODUCTION

Rapid world population growth, a lack of environmental awareness in society and developments in industry have been the cause of significant environmental problems - especially so in the last century. One of the biosphere components affected most by this pollution is undoubtedly freshwater ecosystems. Therefore monitoring water quality is a necessity for the sustainability and protection of water ecosystem health (Çiçek et al., 2013; Köse et al., 2014; Tokatlı et al., 2016). Using only physical and chemical water quality monitoring methods may be inadequate, and especially in recent years, biological monitoring methods and bio-indicator organisms have been widely used in the scientific community for effective research (Martin et al., 2010; Solak and Acs, 2011; Tokatlı and Dayıoğlu, 2011; Delgado et al., 2012; Atıcı and Udoh, 2016).

Diatoms, which are known to be a large part of the benthos (often 90 – 95%), can be found in all surface waters all the time. They are also one of the most important aquatic producer groups and have quick reactions to the changes in environmental variables. Therefore, diatoms, which are accepted as an important part of bio-indicator organisms, have been used to evaluate environmental conditions in many countries as indicators of water pollution (Ács et al., 2004; Goma et al., 2004; Atıcı and Obalı, 2006; Solak et al., 2007; Kalyoncu et al., 2009; Atıcı and Obalı, 2010; Tokatlı, 2013; Aydın and Büyükışık, 2014; Tan et al., 2017). Diatom indices developed for different conditions in different habitats are one of the most widely used water quality assessment techniques and the Biological Diatom Index (IBD) is one of the most convenient indexes for evaluating the water quality by using diatom communities (Coste et al., 2009).

The Meriç and Tunca Rivers are two of the most significant lotic ecosystems for the Balkans and it is well documented that they are being exposed to intensive anthropogenic pressure - especially from agricultural and domestic applications conducted on their watersheds (Erkmen and Kolankaya, 2006; Tokatlı and Baştatlı, 2016; Tokatlı, 2017). The aim of this study was to determine the epipelic diatoms of selected stations on the Meriç and Tunca Rivers and to evaluate/compare the water quality by using certain limnologic parameters and the Biological Diatom Index (IBD).

MATERIAL AND METHOD

Study Area

Water and epipelic (EPP) diatom samples were collected from 2 selected stations on the Meriç and Tunca Rivers in autumn (November) 2018. A map of the study area and the selected stations is shown in Figure 1.

Physical and Chemical Parameters

The dissolved oxygen, oxygen saturation, pH, EC, TDS, salinity and oxidation - reduction potential (ORP) parameters were determined using a Hach Lange branded "HQ40D Multiparameter" device during the field studies; the turbidity parameter was determined using a Hach Lange branded "2100Q Portable Turbiditymeter" device during the field studies; the nitrate, nitrite, ammonium, phosphate, sulphate and chemical oxygen demand (COD) parameters were determined using a Hach Lange branded "DR3900 Spectrophotometer" device during the laboratory studies.

Epipelic (EPP) Diatoms

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

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

The Biological Diatom Index (IBD)

The Biological Diatom Index (IBD) values of the Meriç and Tunca Rivers were automatically calculated using the "Calculate IBD with Excel" program. The trophic statuses and quality classes of freshwater according to IBD values are given in Table 1 (Lenoir and Coste, 1996).

Table 1. Scale of IBD

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

Statistical Data

Cluster Analysis (CA) according to Bray Curtis was applied to the results using the "Past" package program in order to classify the diatom species in terms of their relative abundances.

RESULTS AND DISCUSSION

Physical and Chemical Data

The results of the physicochemical data detected in the 2 stations selected on the Meriç and Tunca Rivers and some national–international limit values are given in Table 2. According to the criteria of the Water Pollution Control Regulations in Turkey, the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, nitrate, ammonium, sulphate and COD parameters; and have III. – IV. Class water quality in terms of nitrite and phosphate parameters (Uslu and Türkman, 1987; Turkish Regulations, 2015).

Nitrite is known as an intermediate product in the biological oxidation process reaching from ammonium to nitrate. It may reach high concentrations in low–oxygen and in especially organically contaminated water. It is also known that organic and inorganic fertilizers, municipal and industrial wastewater discharges are the most important factors in increasing the amount of ammonia and phosphate in water (Wetzel, 2001; Manahan, 2011). Özkan and Elipek (2006) investigated the water quality of the Meriç River using certain physicochemical parameters. As a result of this research, the Meriç River was reported as having II. Class water quality level in terms of phosphate, II – III. Class water quality level in terms of nitrate, and III – IV. Class water quality level in terms of nitrite. In a study performed in the same basin, and similar to the present study, the Meriç, Tunca and Ergene Rivers were reported as III. – IV. Class in terms of nitrite, ammonium and phosphate accumulations in water (Tokatlı, 2015). In another study performed in the Meriç-Ergene River Basin, the water and sediment qualities of the basin components were investigated. According to the results of this research, and similar to the present study, the Meriç River Basin was found to have I. – II. Class water quality in terms of temperature, DO, COD, pH, TDS, nitrate, ammonium and sulphate parameters; II. Class water quality in terms of nitrite parameters; and III. – IV. Class water quality in terms of phosphate, BOD and fecal coliform parameters in general (Tokatlı, 2019). According to the DSI observation reports, nitrogen and phosphorus are the main concerns affecting the water quality of Meric River (Kendirli et al., 2005). Similar to the data reported by the DSI, the nitrite and phosphate concentrations in the water from the Meriç and Tunca Rivers were detected in quite high levels and they have III. – IV. Class water quality in terms of these parameters.

Biological Data

During the present study, a total of 36 diatom species were identified from the epipelic (EPP) habitats of the Meriç and Tunca rivers by counting a total of 403 valves in the Meriç and 409 valves in the Tunca. A list of identified diatom taxa with the frequency values of the investigated stations is given in Table 3 and the rel-

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

Limit Values and the Results		Parameters													
		DO (mg/L)	OS (%)	pH	EC (mS/cm)	TDS (mg/L)	Sal (%$_0$)	Tur (NTU)	NO_3 (mg/L)	NO_2 (mg/L)	NH_4 (mg/L)	*PO_4 (mg/L)	SO_4 (mg/L)	COD (mg/L)	ORP (mV)
•Water Quality Classes (SKKY, 2015)	I. Class	8	90	6.5-8.5	400	500	-	-	5	0.002	0.2	0.02	200	25	-
	II. Class	6	70	6.5-8.5	1000	1500	-	-	10	0.01	1	0.16	200	50	-
	III. Class	3	40	6.0-9.0	3000	5000	-	-	20	0.05	2	0.65	400	70	-
	IV. Class	<3	<40	Out of 6.0-9.0	>3000	>5000	-	-	>20	>0.05	>2	>0.65	>400	>70	-
Drinking Water	TS266 (2005)	-	-	6.5-9.5	2500	-	-	5	50	0.5	0.5	-	250	-	-
	EC (2007)	-	-	6.5-9.5	2500	-	-	-	50	0.5	0.3	-	250	-	-
	WHO (2011)	-	-	-	-	-	-	-	50	0.2	-	-	-	-	-
♦Fish Health (EC, 2006)	Cyprinid Species	4	-	6-9	-	-	-	-	-	0.03	0.2	-	-	-	-
	Salmonid Species	6	-	6-9	-	-	-	-	-	0.01	0.04	-	-	-	-
Present Study	**S1** (Meriç)	9.51 I. Class	101.9 I. Class	8.31 I. Class	327 I. Class	176 I. Class	0.17	0.46	1.480 I. Class	**0.017** III. Class	0.016 I. Class	**0.161** III. Class	60.7 I. Class	7.8 I. Class	200.1
	S2 (Tunca)	8.98 I. Class	96.9 I. Class	8.07 I. Class	777 II. Class	426 I. Class	0.43	2.46	2.230 I. Class	**0.072** IV. Class	0.249 II. Class	**1.960** IV. Class	69.6 I. Class	39.1 II. Class	196.4

*: According to another water quality classification specified by Uslu and Türkman (1987)
•: Bold data mean III. – IV. Class water quality
♦: Underlined data are not suitable for fish health
DO – Dissolved oxygen, OS – Oxygen saturation, Sal – Salinity, Tur – Turbidity
TS266 – Turkish Standards Institute, EC – European Communities, WHO – World Health Organization

Table 3. Identified diatom taxa

Diatom Taxa	Tunca River	Meriç River
Amphora pediculus (Kützing) Grunow	+	+
Bacillaria paxillifera (O.F.Müller) T.Marsson	+	-
Cocconeis pediculus Ehrenberg	+	-
Craticula subminuscula (Manguin) Wetzel & Ector	-	+
Cyclotella atomus Hustedt	-	+
Cyclotella meneghiniana Kützing	-	+
Cymbella tumida (Brébisson) Van Heurck	+	-
Diatoma vulgaris Bory	-	+
Encyonema minutum (Hilse) D.G.Mann	-	+
Frustulia vulgaris (Thwaites) De Toni	+	+
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin	-	+
Gyrosigma kuetzingii (Grunow) Cleve	+	-
Melosira varians C.Agardh	+	+
Navicula amphiceropsis Lange-Bertalot & U.Rumrich	-	+
Navicula capitatoradiata H.Germain ex Gasse	-	+
Navicula erifuga Lange-Bertalot	+	+
Navicula germainii J.H.Wallace	+	-
Navicula gregaria Donkin	+	+
Navicula perminuta Grunow	-	+
Navicula radiosa Kützing	-	+
Navicula recens (Lange-Bertalot) Lange-Bertalot	+	-
Navicula rostellata Kützing	+	+
Navicula simulata Manguin	+	
Nitzschia amphibia Grunow	-	+
Nitzschia clausii Hantzsch	+	-
Nitzschia dissipata (Kützing) Rabenhorst	-	+
Nitzschia dubia W.Smith	+	-
Nitzschia inconspicua Grunow	+	-
Nitzschia linearis W.Smith	-	+
Nitzschia palea (Kützing) W.Smith	+	+
Nitzschia sociabilis Hustedt	-	+
Nitzschia subacicularis Hustedt. nom. inval.	-	+
Pantocsekiella ocellata (Pantocsek) K.T.Kiss & Ács	-	+
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	-	+
Stephanodiscus hantzschii Grunow	+	-
Tryblionella calida (Grunow) D.G.Mann	+	-

ative abundance values of the detected EPP diatom species, which have relative abundance values higher than 1% for the Meriç and Tunca Rivers, are given in Figure 2. *Cyclotella atomus* Hustedt, *Navicula gregaria* Donkin, *Nitzschia palea* (Kützing) W. Smith and *Nitzschia subacicularis* Hustedt, nom. inval. were found as the most dominant species in the Meriç River and *Navicula erifuga* Lange-Bertalot, *Navicula gregaria* Donkin and *Navicula rostellata* Kützing were found as the most dominant taxa in the Tunca River.

Navicula rostellata, which is found as the most dominant taxon (relative abundance of 50%) for the Tunca River, is a cosmopolitan eutrophic species. *Navicula erifuga*, which is found as the second most dominant taxon (relative abundance of 25%) for the Tunca River, is a cosmopolitan species found in eutrophic, brackish waters or those with very high electrolyte content. Both species with very high abundance values in the Tunca River are tolerant to critical levels of pollution (Taylor et al., 2007). *Navicula gregaria*, which is found as the most dominant taxon (relative abundance of 52%) in the Meriç River, is a cosmopolitan species. It is very common in eutrophic to hypertrophic freshwaters with a moderate to high electrolyte content. *N. gregaria* tolerant of strongly polluted conditions may also be found in brackish waters. It is also known as a good indicator species for these conditions. *Cyclotella atomus*, which is found as the second most dominant taxon (relative abundance of 12%) in the Meriç River, occurs in the electrolyte rich waters (Taylor et al., 2007).

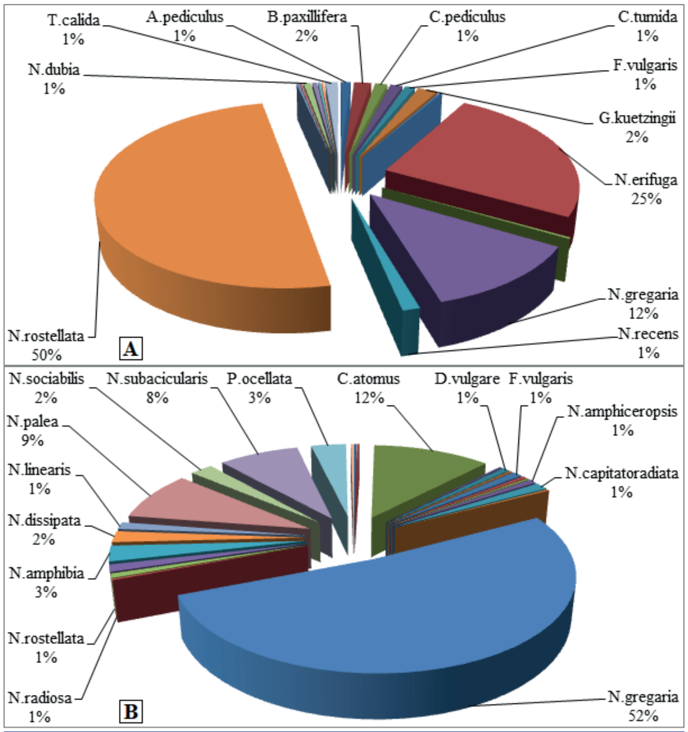

Figure 2. Relative abundance of diatoms in Tunca (A) and Meriç (B) rivers.

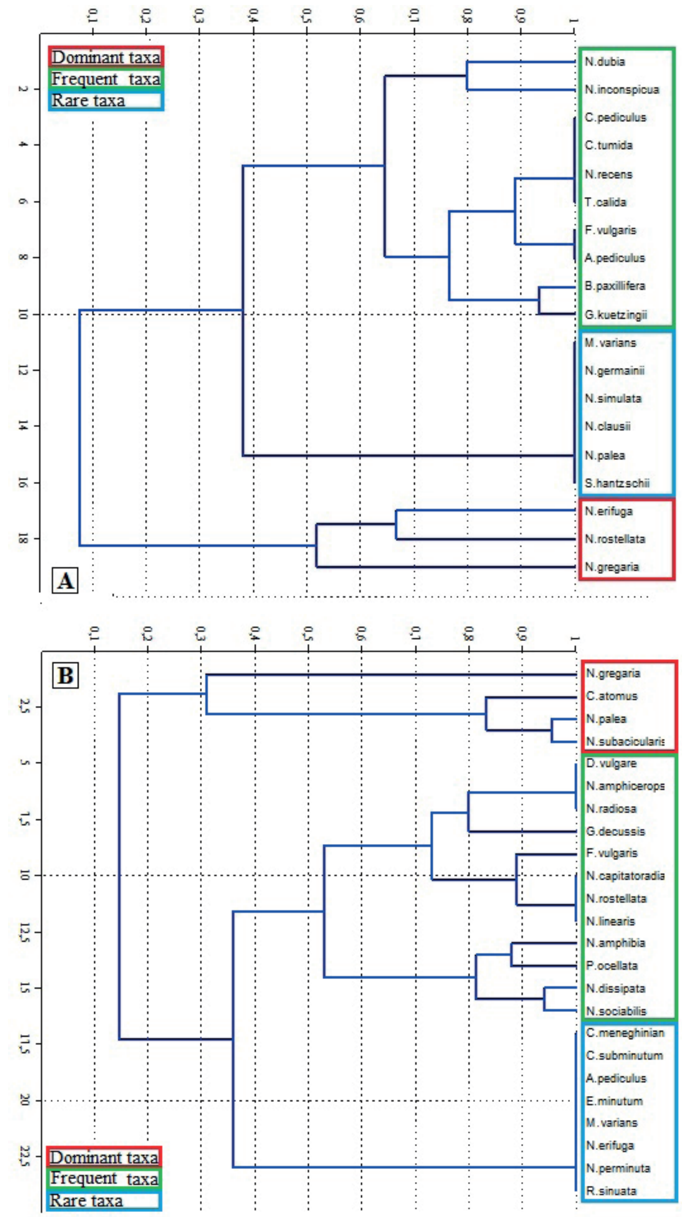

Figure 3. Tree dendrograms of CA for Tunca (A) and Meriç (B) Rivers.

Cluster Analysis (CA)

Cluster Analysis (CA), which classifies the objects, is one of widely used multivariate statistical techniques and hierarchical agglomerative clustering is the most common approach in CA applications (Shrestha and Kazama, 2007; Kazi et al., 2009; Tokatlı et al., 2014). In the present study, CA was applied to the results in order to classify the EPP diatoms of the Meriç and Tunca Rivers according to their relative abundance values. According to the results of CA, three statistically significant clusters were formed both for the diatoms of the Meriç and Tunca Rivers and these detected clusters were named as "dominant taxa", "frequent taxa" and "rare taxa" (Figure 3).

It is known that the Meriç River, which is one of the largest rivers flowing on Europa territory, is a transboundary fluvial ecosystem. Therefore the quality of its waters is of substantial importance for irrigation, industrial, recreation and domestic use for Bulgaria, Greece and Turkey and the control-management of the Meriç catchment is of mutual interest for the neighboring countries. In a study performed in the upstream of the Meriç River Basin, Cluster Analysis (CA) was applied for a model assessment of the water quality of the Meriç River on Bulgarian territory using long-term monitoring data from 21 sampling sites characterized by 8 surface water quality indicators. The application of CA to the indicators resulted in 3 significant clusters showing the impact of biological, anthropogenic and eutrophication sources and the results identified the dominant role of the industrial wastes and agricultural activities in water pollution (Papazova and Simeonova, 2013).

The Biological Diatom Index (IBD)

The Biological Diatom Index (BDI) is a standardized biologically water quality assessment method. The BDI, the formula of which was developed by Zelinka and Marvan (1961), is based on a total of 209 diatom taxa and provides information about trophic levels of the investigated aquatic ecosystems (Coste et al., 2009). In the present study, a total of 36 diatom taxa were identified and 34 of them were used to calculate the Biological Diatom Index (BDI) scores of the Meriç and Tunca Rivers in order to determine the trophic statuses. The BDI index values of the investigated rivers are given in Figure 4. According to the calculated BDI values for the EPP habitats of the investigated aquatic ecosystems, the Meriç and Tunca Rivers were in "meso – eutrophic state" and had "low water quality" (score range of 9 – 12) in general.

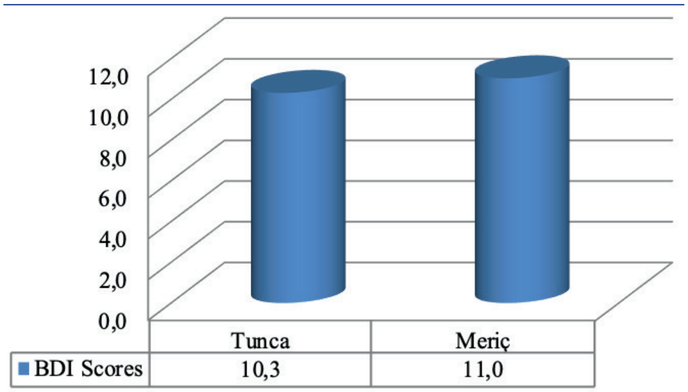

Figure 4. BDI scores of Tunca and Meriç Rivers.

The physicochemical parameters used to assess the water quality may only indicate the current status of aquatic habitat. But the diatoms, which should be used in monitoring programs of rivers ecological assessment according to the Water Framework Directive (WFD), may indicate the long term changes in aquatic ecosystems. Therefore they have been widely used for the bio-assessment of lotic and lentic ecosystems for a long time in almost all the countries of Europe due to their broad distribution and their ability to integrate changes occurring in water composition (Acs et al., 2004; Torissi and Dell'Uomo, 2006; Solak and Acs, 2011).

Diatom indices are widely used worldwide to determine the quality and trophic levels of aquatic systems. In a study performed in Portugal, the water qualities of the Caiman and Mau River Basins were evaluated using the Specific Pollution Index (SPI) and the Biological Diatom Index (BDI). According to the results of this research, the water and diatom data showed a medium to good water quality. However, samples collected near the mining areas (Coval da Mó) revealed a low abundance and diversity of diatom taxa indicating a stressed environment (Nunes et al., 2003). In another study performed in Poland, the Biological Diatom Index (BDI) was used for the estimation of water quality in the central section of the Pilica River, located in Łódź province. As a result of this study, the ecological state of the Pilica River changed from good (oligo – mesotrophic) to moderate (mesotrophic), which corresponded with the physicochemical analysis of the water (Szulc and Szulc, 2013). Pham (2017) used the Biological Diatom Index (BDI) to evaluate the water quality of the Dong Nai River (Vietnam). As a result of this study, similar to the present study, the water quality of the investigated river varied from good (oligo – mesotrophic), moderate (mesotrophic), to low (meso – eutrophic) levels, based on the BDI values. Although water quality assessment using diatom indices has been used worldwide for many years, several studies have been also been carried out in Turkey especially in the last 15 – 20 years. Gürbüz and Kıvrak (2002) were applied saprobity index (SI), trophic diatom index (TDI) and the percentage pollution tolerant valves in order to assess the water quality of Karasu River and they found that the Karasu River were eutrophicated and organically polluted. Kalyoncu et al. (2009) investigated the Darıören Stream by ecological methodologies to evaluate the impact of the pollution on epilithic diatom assemblages. SLA, EPI-D, TDI and DESCY indices were used by Solak (2011) in Upper Porsuk River (Kütahya) and according to the results of this study, the water quality levels of the investigated stations were found in different trophic conditions.

In this study, the Biological Diatom Index (IBD) was used to assess the water quality of the Meriç and Tunca Rivers and this data was compared with the limnological parameters data. Similar to the resulting limnological data, the Meriç and Tunca Rivers were found to be in a "meso–eutrophic state" and had "low water quality" in terms of the calculated IBD values. In a number of studies performed in the Gürleyik, Ankara and Seydisuyu Streams (Central Anatolian Region of Turkey), the IBD was used to evaluate the water quality of these lotic ecosystems. According to the results of these studies, and in line with the investigated physicochemical data, the Gürleyik, Ankara and Seydisuyu Streams were found to be in a mesotrophic state (Tokatlı, 2012, Atici ve Ahiska, 2005; Atıcı et al., 2018). In the present study, the detected similarities in water quality status between the results of the IBD scores and the limnological parameters indicate that the IBD may be used to reflect changes in ecological conditions of the basin – potentially after making some revisions in the index.

CONCLUSION

Biological water quality assessment is much more effective (especially in the long term) in reflecting any effects on water ecosystems than investigated psychochemical data. Therefore any limnological data has to be supported by biological data to make a much better assessment. In this study, the epipelic diatoms of the Meriç and Tunca Rivers were investigated and the water quality of these fluvial ecosystems were evaluated using the Biological Diatom Index. As a result of this study, it was determined that the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, NO_3, NH_4, SO_4 and COD parameters; and have III. – IV. Class water quality in terms of NO_2 and PO_4 parameters and the water of these rivers is in a meso–eutrophic state according to the results of the IBD. It was also determined that the biologically determined water qualities of the rivers showed a high similarity with the physico–chemically determined water qualities - especially in terms of nitrogen and phosphorous compounds. The results of this study also showed the benefits of using biotic and abiotic factors together in water quality assessment studies and show that minor changes in environmental conditions may cause major effects in the diatom communities. While the sampling frequency is perhaps not sufficient and more research is needed for the assessment of quality status of the investigated water ecosystems, the results of the present study do have the characteristics of a preliminary research with the aim of providing resources for any future bio-indication studies in the region.

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Research Article

Relationships between Body Size, Weight and Fecundity of the Endangered Fish *Alburnus carinatus* Battalgil, 1941 in the Manyas Lake (Turkey)

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ABSTRACT

Alburnus carinatus Battalgil, 1941 is an endangered fish species, which has a narrow distribution range in two shallow lake basins in northwest Anatolia. The present study aimed to describe the reproductive potential of this endemic fish with the relationships between body size, weight, and fecundity. A total of 101 female *A. carinatus* were captured from the Manyas Lake between January and June 2019. The standard length and body weight of these samples varied between 8.1–15.0 cm and 9.38–65.45 g, respectively. The length-weight relationship of the female *A. carinatus* was calculated as $W = 0.020 \times SL^{2.959}$ ($r^2 = 0.866$) with isometric growth. Absolute fecundity ranged from 1512 eggs (8.1 cm SL) to 3203 eggs (10.3 cm SL) with a mean of 2281 eggs (SD = 415.2). Fecundity–length relationship was $F = 5.86 \times SL^{2.67}$ ($r^2 = 0.702$) and the fecundity–length relationship was $F = 476.6 + 116.5 \times L$ ($r^2 = 0.688$). Mean relative fecundity was calculated as 244 eggs.cm^{-1} (SD = 34.1) (ranged from 179.8 to 310.9 eggs.cm^{-1}) and 148 eggs.g^{-1} (SD = 14.9) (ranged from 115.1 to 171.8 eggs.g^{-1}).

Keywords: Endemism, shallow lake, length-weight relationship, spawning potential, Anatolia

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INTRODUCTION

Until recently, most of the Turkish shemayas (*Alburnus* spp.) have been described as subspecies of *Chalcalburnus chalcoides*, however recent detailed studies on the morphological and molecular features of this genus have revealed that descriptions of some species were incorrect and that the species diversity for Turkish shemayas was high (Özuluğ & Freyhof, 2007a). Turkey - particularly Anatolia - is the center of biodiversity and endemism of the genus *Alburnus* and a total of 27 species were recognized with recent studies (Krupp, 1992; Kottelat & Freyhof, 2007; Özuluğ & Freyhof, 2007a; Özuluğ & Freyhof, 2007b; Elp, Şen, & Özuluğ, 2015; Özuluğ, Geiger, & Freyhof, 2018; Freyhof & Turan, 2019; Fricke, Eschmeyer, & Van der Laan, 2019). One of these newly described species, *Alburnus carinatus* Battalgil, 1941 is a fish endemic to northwest Anatolia living in the Manyas and Uluabat lake basins. According to the IUCN Red List (2019), this species is classified as Endangered due to habitat restriction. Additionally, the Manyas and Uluabat lakes, where this endemic fish live, are under heavy environmental pressure from pollution and water extraction (Özuluğ & Freyhof, 2007a; Dorak, Köker, Sağlam, Akçaalan, & Albay, 2017). Invasive *Carassius gibelio* (Bloch, 1782) reported from the Manyas Lake in the 2000s (Emiroğlu, Arslan, Malkoç, Koç, & Çiçek, 2008) can be considered to be another potential threat for *A. carinatus* in the lake.

Bagenal & Tesch (1978) have defined the fecundity as the seasonal spawning potential or as the number of eggs ripening during the spawning period of a female. Fecundity is generally calculated for commercial fish species due to their importance in assessing their economic potential. In terms of ecosystem management, however, the fecundity of non-commercial fish species is highly critical for the assessment of the ecological roles of these species. The survival of endemic *A. carinatus* in the Manyas Lake, where environmental pressures are intense, is critical. The present study aims to reveal the reproductive potential of this endangered fish with the relationships between body size, weight, and fecundity. Additionally, this study will contribute to the gap in knowledge of the length-weight relationship and some reproductive characteristics of this fish species.

MATERIAL And METHOD

Study area

The Manyas Lake located in the western part of Anatolia is a shallow (mean depth = 1.7 m, maximum depth = 3.4 m) and a turbid lake (Albay & Akçaalan, 2003; Dorak et al., 2017). The main water sources that feed the lake are the Sığırcı, Köydere, Kocaçay, Karadere and Mürvetler streams as well as annual rainfall (Figure 1). The lake was designated as a Ramsar Site in 1994 in terms of being on the migration routes of some bird species and used by these birds as a nesting and shelter area (Yeniyurt & Hemmani, 2011).

Sampling and data analysis

Fish samples were captured by a fisherman in the Manyas Lake between January and June 2019. For fishing, cast nets (10 mm mesh size) were used in the northeast shorelines of the lake. The captured samples were immediately transferred to the Laboratory of Istanbul University Faculty of Aquatic Sciences Department Freshwater Resources and Management with cold conditions and then stored at -18°C until the investigations. The fish samples were measured for standard length (SL), fork length (FL) and total length (TL) to the nearest 0.1 cm and weighed for total body weight (W) on a digital balance with a 0.01 g accuracy. Length-length transformations (FL vs. TL; SL vs. TL; SL vs. FL) in the fish were calculated using linear regression analysis to be useful in comparing the results of different studies, which will use different length measures.

The length-weight relationship (LWR) was calculated using the equation: $W = aL^b$, where W is the total weight (g), L is the standard length (cm), *a* and *b* are regression parameters (Le Cren 1951; Froese, 2006). The equation ($W = aL^b$) was converted into the natural logarithmic form ($ln W = ln a + b \times ln L$), and parameters *a* (regression intercept) and *b* (slope) were calculated by the regression analysis (King, 2007). To determine the growth type (isometry or allometry) of the species, ninety-five percent confidence limits (95% CI) of parameter *b* was estimated by the equation: $95\% \text{ CI} = x \pm (t_{0.05} \times SE)$ (x: *b*; t: table value of *t* (t-test at 95% confidence)); SE: standard error value of *b*) (King, 2007).

The gonads were removed and weighed to the nearest 0.0001 g (Precisa XB 220A). For the estimation of absolute fecundity, the gravimetric method was used: sub-samples of approximately 1 g were taken from 3 different parts of the ovaries (the anterior, medial and posterior parts) and the number of eggs in the sub-samples was multiplied by the weight of the ovary using the following equation; F = number of eggs in the sub-samples × gonad weight / sub-sample weight (Le Cren, 1951). Relative fecundity (RF) was calculated as the ratio between absolute fecundity (F) and body length and weight (SL and W). The relationships between fecundity and fish length were estimated by exponential regression analysis ($F = aL^b$) and the relationships between fecundity and fish weight were estimated by linear regression analysis ($F = a + b \times L$; where F is fecundity, L is the standard length (cm), *a* is the regression constant, and *b* is the regression coefficient).

RESULT AND DISCUSSION

A total of 109 *A. carinatus* specimens (101 females and 8 males) were captured from the Manyas Lake. The standard length and body weight of the specimens varied between 8.1–15.0 cm and 9.38–65.45 g for females and 8.0–11.8 cm and 9.80–32.03 g for males, respectively. As the individual numbers of the males were quite low, they were not used in the following calculations. The length-length relationships of the female fish specimens were

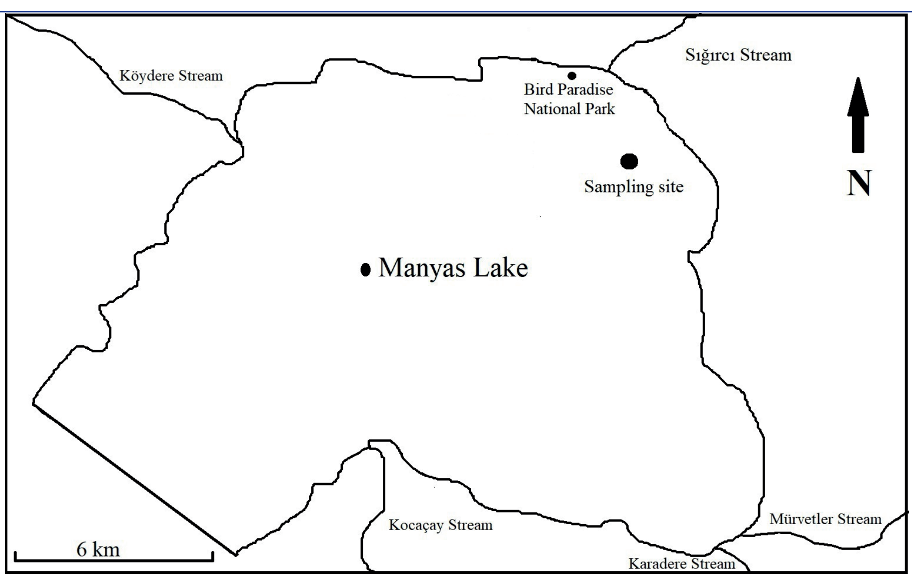

Figure 1. Sampling site in the Manyas Lake.

calculated as follows; FL=0.900×TL–0.019 (r^2=0.986), SL=0.078×TL–0.010 (r^2=0.977) and SL=0.868×FL+0.038 (r^2=0.985).

The length-weight relationship of female *A. carinatus* was calculated as $W = 0.020 \times SL^{2.959}$ (r^2 = 0.866) (Figure 2). In terms of growth type, 95% CI of parameter *b* was calculated between 2.84 and 3.08 and the results showed that the growth type of the population was isometric (*b* = 3).

Froese (2006) indicated that the range of the parameter *b* usually encountered in fishes was within the expected range of 2.5 – 3.5. In this study, the value of *b* for female *A. carinatus* was within this expected range and therefore, the result can be used as valid data for the FishBase database. Because there is no other study on the length-weight relationship of this endemic fish, a comparison could not be made.

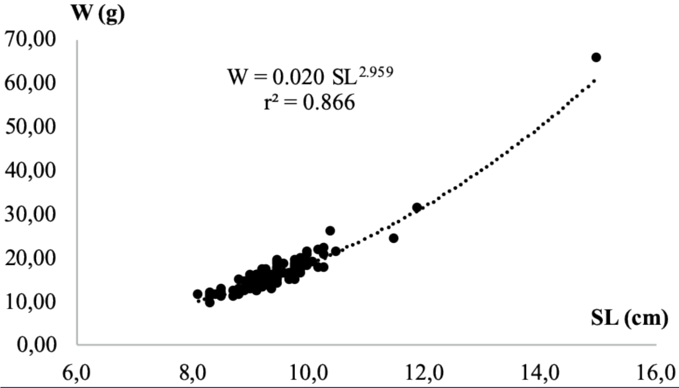

Figure 2. Diagram of the length–weight relationship of *A. carinatus* living in the Manyas Lake.

For estimating the fecundity of the species, a total of 47 individuals captured between April and June (2019) were used. The average absolute fecundity in mature females was 2281 eggs (SD = 415.2), ranging from 1512 eggs (8.1 cm SL) to 3203 eggs (10.3 cm SL). The relationship between body length, weight, and absolute fecundity were positively correlated; the fecundity-length relationship was $F = 5.86 \times SL^{2.67}$ (r^2 = 0.702) and the fecundity-length relationship was $F = 476.6 + 116.5 \times W$ (r^2 = 0.688) (Figure 3, 4).

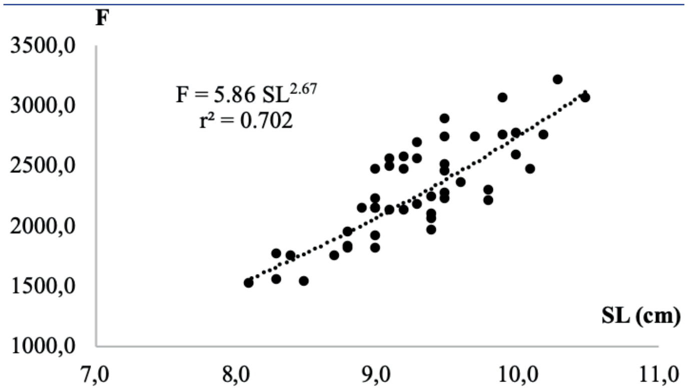

Figure 3. Diagram of the fish length–fecundity relationships of *A. carinatus* living in the Manyas Lake.

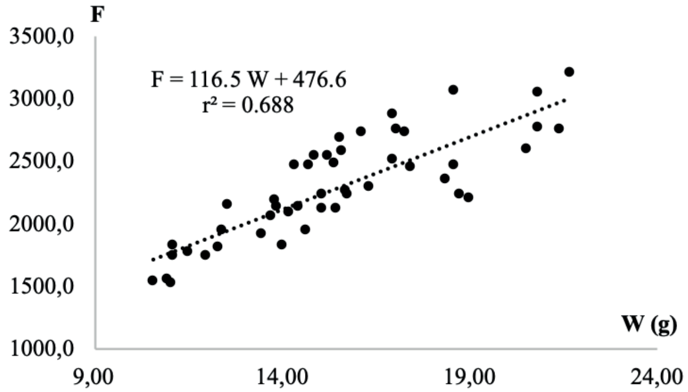

Figure 4. Diagram of the fish weight–fecundity relationships of *A. carinatus* living in the Manyas Lake.

Mean relative fecundity was calculated as 244 eggs.cm^{-1} (SD = 34.1) (ranged from 179.8 to 310.9 eggs.cm^{-1}) and 148 eggs.g^{-1} (SD = 14.9) (ranged from 115.1 to 171.8 eggs.g^{-1}).

Fecundity is affected by several factors such as fish size, weight, age, and life-history traits of fishes as well as the environmental conditions such as food supply, population density, and temperature (Ünlü & Balcı, 1993; Ali & Wootton, 1999). In this study, a positive correlation between fecundity and fish length and fish weight was found: fecundity increased with the increase of fish length and weight and the larger fish produced more eggs. While the coefficient of the determination (r^2) was above 0.7 in the fish length–fecundity relationship (r^2 = 0.702), it was calculated as 6.88 for the fish weight–fecundity relationship. This small difference might be related to the narrow size/weight range of the fish samples.

There are many factors threatening the presence of endemic *A. carinatus* in the Manyas Lake. The agricultural waste of cultivated areas, livestock activities, poultry farms around the lake and the streams are the main pollution sources of the lake and the water quality was identified as eutrophic due to its nutrient concentrations and biological characteristics (Albay & Akçaalan, 2003; Dorak et al., 2017). Invasive *C. gibelio*, which was detected in the 2000s in the lake (Emiroğlu et al., 2018) and is accepted as a sperm parasite (Perdikaris et al., 2012), may threaten *A. carinatus* with food and habitat competition. Carnivorous fish species (*Esox lucius* Linnaeus, 1758 and *Silurus glanis* Linnaeus, 1758) living in the lake, waterfowls, which are permanently or periodically found in the Lake basin, and local fishermen can create prey pressure on this fish. Additionally, the recently constructed Manyas Dam is thought to be another threat for *A. carinatus*, which migrates to the streams for reproduction.

The conservation status of *A. carinatus* has been assessed as Endangered because its area of occupancy (AOO) is estimated to be less than 300 km². However, there is no action plan for the conservation of this special fish. In order to prevent the extinction of this endemic fish and to ensure the continuity of the population in its limited distribution areas (Manyas and Uluabat lake basins), its bio-ecological characteristics and habitat requirements should be identified in detail. It is highly important to avoid threats such as pollution, invasive fish introduction, overfishing, and habitat degradation. In addition, local people and fishermen should be informed about the importance of this endangered species.

CONCLUSIONS

In conclusion, this study has provided some baseline information on the length-weight relationship and fecundity features of endangered *A. carinatus*. Since there is no published information about these features of this endemic fish, the results are expected to be useful for future studies.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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