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Thermal Tolerance of Turkish Crayfish (*Astacus leptodactylus*) Acclimated to Three Different Temperatures

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ABSTRACT

Critical thermal maxima (*CTMax*) and minima (*CTMin*) were determined for Turkish crayfish (*Astacus leptodactylus)* acclimated to 15, 20 and 25°C. *CTMin* and *CTMax* were 1.3, 1.1 and 2.0°C, and 37.4, 37.5 and 38.7°C, respectively. Thermal tolerance tests showed that acclimation temperatures (15, 20 and 25°C) had significant effects on *CTMin* values of *A. leptodactylus* (P≤0.05). The area of thermal tolerance assessed using the *CTMin* and *CTMax* boundaries were calculated as 364°C^2. The overall *ARR* values were calculated as 0.07 for *CTMin* and 0.13 for *CTMax* values between 15 and 25 °C acclimation tempera-tures. All the crayfish crumpled at 0.5°C and showed overall spasm at 32.0 – 33.0°C. Farming *A. leptodactylus* in the southeastern part of the Mediterranean region may be practiced in terms of temperature tolerance.

Keywords: *A. leptodactylus*, CTMin, CTMax, thermal polygon, the acclimation response ratio

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Üç Farklı Sıcaklığa Alıştırılan Türk Kereviti (*Astacus leptodactylus*)'nin Sıcaklık Toleransı

Öz: 15, 20 ve 25 °C'ye alıştırılan Türk kereviti için kritik termal maksima (*CTMax*) ve minima (*CTMin*) değerleri belirlenmiştir. *CTMin* ve *CTMax* değerleri sırasıyla 1,3, 1,1 ve 2,0 ile 37,4, 37,5 ve 38,7 °C'dir. Sıcaklık tolerans testleri alıştırma sıcaklıklarının (15, 20 ve 25 °C) *A. leptodactylus CTMin* değerlerine önemli etkilerde bulunduklarını göstermiştir (P≤0,05). Sıcaklık tolerans alanı *CTMin* ve *CTMax* sınırları ile değerlendirilerek 364°C^2 olarak hesaplanmıştır. Genel olarak 15 ile 25 °C arası alıştırma sıcaklıklarında, alıştırma tepki oranı (*ARR*) 0,07 ile 0,13 olarak hesaplananmıştır. Kerevitlerin tamamı 0,5 °C'de kıvrılma ve geneli 32,0- 33,0°C'de kasılma göstermiştir. Akdeniz bölgesinin güneydoğu kesiminde *A. leptodactylus* yetiştiriciliği sıcaklık toleransı açısından uygun olabilir.

Anahtar kelimeler: *A. leptodactylus*, CTMin, CTMax, termal poligon, alıştırma tepki oranı

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Introduction

Survival, reproduction, and growth of poikilothermal aquatic organisms are intimately influenced by temperature fluctuations in their environments. Ectothermic animals use a combination of behavioral and physiological mechanisms to maintain their body temperatures within a narrow range, even under varied environmental conditions (Salas et al. 2014). The thermal preference of a species, therefore, corresponds closely with temperatures that maximize growth and other physiological processes (Huey and Stevenson 1979; Diaz et al. 2011; Tepler et al. 2011). The thermal limits tolerated by an organism are set genetically and are adaptive in determining the geographical distribution of a species (Simčič et al. 2014).

One of the approaches to quantify lower and upper-temperature tolerance of aquatic animals in the laboratory is known as the critical thermal methodology (*CTM*) (Cowles and Bogert, 1944). Thermal tolerance is quantified through the determination of the critical thermal maxima (*CTMax*) where the temperature is increased gradually until a critical point (lost of equilibrium) is reached (Kumlu et al. 2010a; González et al. 2010). The acclimation response ratio (*ARR*) is an index of the magnitude of the thermal acclimation of an organism (Claussen 1977; Herrera et al. 1998; Díaz

et al. 2002; Pérez et al. 2003; Díaz et al. 2004; Re et al. 2005). It is suggested that crustacean species that inhabit cold and temperate regions have low values of *ARR*, in contrast to those from subtropical and tropical regions, which present high ARR values; this indicates that the *ARR* values are dependent on the geographic gradient where the organisms are distributed. Attributes of thermal tolerance polygons provide important insights into fish ecology and distribution and have been used to identify temperature-related survival tactics (Bennett and Beitinger 1997). The usefulness of thermal tolerance polygons lies inheritability to impart markedly more information than tolerance endpoints alone. Overall polygon area (reported as $°C^2$) provides a convenient and useful comparative index of eurythermicity between species (Eme and Bennett 2009).

Turkish crayfish (*A. leptodactylus*) is accepted as a cold-water species with high economic value (Holdich 1993; Wickins and O'C Lee 2003; Harlioğlu 2004). The knowledge of the critical thermal minima and maxima provides a relevant ecological index since the Turkish crayfish in nature can find such spatial temperatures temporarily, as acute fluctuations outside of their limit of tolerance. The *CTM* provides a relevant and ecological index when it is applied to Turkish crayfish (*A. leptodactylus)* in its habitat. Paladino et al. (1980) mentioned that, in aquaculture, it is highly important to know *CTMax* and *CTM* values as they are indicators of the thermal resistance of organisms. Firkins (1993) reported thermal tolerance values for the *A. leptodactylus* originating from England; however, basic *CTM* studies are necessary to implement at different acclimation temperatures for *A. leptodactylus* originating from Turkey. Therefore, to detect geographic influences on thermal tolerance values in crayfish, research is needed that allows for direct comparison.

The aim of this study was to determine the critical thermal maximum and minimum, acclimation response ratio (*ARR*) and the zone of thermal preference of Turkish crayfish acclimated to three temperatures in order to evaluate the possibility of culturing the species in the south eastern region of the Mediterranean of Turkey.

Materials and Methods

Critical Thermal Tolerance

Sub-adult crayfish were caught in the Keban Dam Lake and transported in styrofoam boxes with wet towels to the laboratory of Çukurova University, Faculty of Fisheries (Adana – Turkey). The organisms were placed in a 1000-L tank, provided with a biological filter at 20°C for 1-week for adaptation to indoor culture conditions. Each group was then acclimated to 15, 20 and 25°C in thermostatically controlled tanks at a rate of 1°C increase or decrease per day. The animals were cultured at the respective temperatures for a period of 35 days prior to the onset of the experiment in round fibreglass tanks (300-L) in order to stabilize their physiology and allow metabolic compensation in flow through rearing systems. Each tank was fitted with 25 plastic pipes (5 cm in diameter and 10 cm length), which serves as hiding places for the animals. Crayfish (20-25 g) were fed twice a day (09.00, 17.00) by 2% of their biomass with artificial pelleted feed containing 35% protein. Water temperature was regularly checked twice a day with a thermometer. The food remainder, feces and moults were extracted daily from the reservoirs by siphoning. The oxygen content and pH of rearing tank was measured by an oxygen meter (OxiGuard®, Birkerød, Denmark) and a WTW pH-meter (Germany). Each temperature group was then divided into two groups to determine the *CTMin* and *CTMax* in separate trials. *CTMin* and *CTMax* trials were conducted in rectangular plastic containers (0.8 m x 0.35 m x 0.5 m) containing thermostatically controlled water baths. Three containers were allocated for each acclimation group and 5 crayfish were randomly allocated to each container (n=15). During the experiment, the crayfish were not fed. Continuous aeration was provided for all containers to maintain dissolved oxygen above 5 ppm using an air blower. The photoperiod was maintained in 12 h light/12 h dark. The trials were separately conducted at cooling or heating rates of 0.3 °C min^{-1}. *CTMin* or *CTMax* was determined as the sub lethal thermal point at which locomotory movements became disorganized and crayfish lost the ability to escape from conditions, which ultimately lead to death. The *CTMin* and *CTMax* values were calculated as the arithmetic mean of the collective endpoint of individuals of a random sample of crayfish. The *ARR* was calculated by dividing the tolerance change by the total change in acclimation temperature according to Claussen (1977).

Statistical Analysis

Following determination of the normality and homoscedasticity of the data, a one-way ANOVA was used to compare *CTM* tolerance of the species (*SPSS* 20.1). Duncan's multiple range test (*DMRT*) was carried out for post hoc mean comparisons. Regression analysis was also carried out to evaluate the relationship between acclimation temperature and *CTMin* and *CTMax* ($p \leq 0.05$).

Results

Water quality parameters in the rearing tanks at three acclimation temperatures were maintained at

levels appropriate for the species (Table 1). Thermal tolerance tests showed that acclimation temperatures (15, 20 and 25°C) had significant effects on *CTMin* values of *A. leptodactylus* ($P \leq 0.05$). At the heating/cooling rate of 0.3°C min^{-1} the *CTMin* values ranged between 1.1 and 2.0°C, while *CTMax* values ranged between 37.4 and 38.7°C (Table 2). During the *CTMin* trials, the crayfish, regardless of the acclimation temperatures, started to lose their balance and laid down laterally at temperatures of 3.0–5.0 °C, and all the crayfish crumpled at 0.5°C. In the *CTMax* trials, the crayfish started to show overall spasms at 32.0 – 33.0 °C secreting mucus like fluids at between 33.0 and 37.0 °C. The thermal tolerance polygon area was calculated as 364°C^2 between 15 and 25°C acclimation temperatures used in the experiments (Figure 1). The overall *ARR* values were calculated as 0.07 for *CTMin* and 0.13 for *CTMax* values between 15 and 25 °C acclimation temperatures (Table 3).

Table 1. Water quality parameters in the rearing tanks.

Water temperature (°C)	Dissolved oxygen (ppm)	pH	NH_3 (mg/L)
15.0±1.0 20.0±1.0 24.9±1.0	5.0-6.0	6.60-7.40	0.0

Table 2. CTMin and CTMax values of *Astacus leptodactylus* acclimated to 15, 20 and 25° C. Each value is a mean ± standart deviation (n = 15).

	Acclimation Temperature		
	15 °C	20 °C	25 °C
CTMin	1.3 ± 0.8^{ab}	1.1 ± 0.7^{b}	2.0 ± 0.8^{a}
CTMax	37.4 ± 1.4^{a}	37.5 ± 0.9^{a}	38.7 ± 1.6^{a}

Table 3. The calculated acclimation response ratios (*ARR*) for *Astacus leptodactylus* acclimated to 15, 20 and 25° C.

		Acclimation Temperature		
		15-20°C	20-25°C	15-25°C
ΔCTmin	CTMin	-0.2	0.9	0.7
ΔT	CTMin	5	5	10
ARR	CTMin	-0.04	0.18	0.07
ΔCTmax	CTMax	0.1	1.2	1.3
ΔT	CTMax	5	5	10
ARR	CTMax	0.02	0.24	0.13

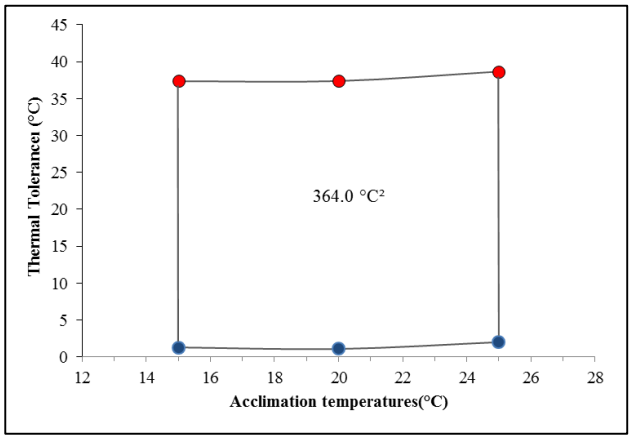

Figure 1. Thermal tolerance polygon of freshwater crayfish *A. leptodacylus* over three acclimation temperatures (15, 20 and 25°C) using *CTM* values.

Discussion

The importance of acclimation temperature on *CTMin* and *CTMax* values has been well documented for various freshwater crustacean species (Layne et al. 1987; Espina et al. 1993; Firkins 1993; Díaz et al. 2004; Manush et al. 2004). Similar to the above studies, acclimation temperature was also found to significantly influence the *CTMin* values of *A. leptodactylus* in the present study.

At 0.3°C min^{-1} cooling/heating rate, the *CTMin* ranged from 1.1 to 2.0 °C, and the *CTMax* ranged from 37.4 to 38.7 °C for crayfish reared in three acclimation temperatures from 15 to 30°C. The *CTMax* values obtained for *A. leptodactylus* were similar to those (ranged from 34.2 to 39.8 °C) reported by Díaz et al. (2002) for *Macrobrachium acanthurus*.

A species living in the heterothermic environment must be tolerant to physiological temperature changes. The *CTMax* values in *A. leptodactylus* corresponded to total disorientation response and hampered locomotive activity resulting finally in death. Similar results were also reported by Hernández-Rodriguez et al. (1996) for *Macrobrachium tenellum*, Kumlu et al. (2010a) and Kumlu et al. (2010b) for *Litopenaeus vannamei*. During the *CTMin* trials, the crayfish, regardless of

the acclimation temperatures, started to lose their balance and laid down laterally at temperatures of 5.0–3.0 °C. After the *CTMin* tests, almost 100% of the animals recovered when transferred to pre-trial temperatures (Beitinger et al. 2000; Manush et al. 2004). However, at *CTMax* tests, the crayfish started to secrete mucus like fluids from their body at temperatures of 33.0-37.0 °C.

Thermal tolerance values varied within species with multiple estimates (Westhoff and Rosenberger 2016). For example, the thermal tolerance range for *A. leptodactylus* which was reported at 4-32 °C by Köksal (1998) and from 9 to 36 °C by Firkins and Holdich (1993). Moreover, it is known that thermal tolerance zone is dependent on acclimation temperature, strain or population differences as well as the size of the animals used. In the current study, thermal tolerance polygon for *A. leptodactylus* at three acclimation temperatures (15, 20 and 25 °C) was calculated as 364°C^2 (Figure. 1), but Firkins (1993) found the thermal tolerance area for *A. leptodactylus* originating from England at four acclimation temperatures (5, 10, 20 and 25 °C) as 927°C^2. Thermal tolerance traits could be attributed to geographic and climatic differences among ancestral source populations in this species. On the other hand, Manush et al. (2004) calculated thermal tolerance polygon for *Macrobrachium rosenbergii* at three acclimation temperatures (25, 30 and 35 °C), and found a value of 255 °C^2, much lower than that of Herrera et al. (1998), who calculated this value as >800 °C^2 for the same species. Díaz et al. (2002) reported the polygon area of 644°C^2 for *M. acanthurus* at wider acclimation temperatures (20, 23, 26, 29 and 32 °C).

Depending on the acclimation temperatures, the ARR values ranged from -0.04 to 0.24 for Turkish crayfish at the heating or cooling rate of 0.3 °C min^{-1}. The overall ARR values were calculated as 0.13–0.24, which are similar to those reported for some other crayfish species i.e. *Astacus pallipes* (0.12-0.18) by Spoor (1955) and Bowler (1963), *Orconectes rusticus* (0.23-0.25) by Claussen (1980) and Layne et al. (1987), *O. virilis* (0.15) by Claussen (1980). For a tropical crustacean, the giant freshwater prawn (*M. rosenbergii*), Herrera et al. (1998) reported the *ARR* values as 0.44–0.58, while for a subtropical crayfish, the red claw (*Cherax quadricarinatus*), Díaz et al. (2004) reported the *ARR* as 0.33–0.66. Hence, when we compare our results with those of the above, it is clear that *A. leptodactylus* is more resistant to low temperatures rather than the upper range and is a cold-temperature species. Culturing of *A. leptodactylus* in the southeastern region of the Mediterranean may be practiced in geographic zones where the environmental temperatures are near the final preferendum and do not exceed *CTMax*.

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Ontogenetic Diet Shift of Invasive Gibel Carp (*Carassius gibelio*, Bloch 1782) in Karamenderes River (Turkey)

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ABSTRACT

The ontogenetic diet shift of invasive *Carassius gibelio* (Bloch, 1782) was investigated in Karamenderes River, Turkey. The fieldwork was performed during summer 2012, autumn 2012 and spring 2013. The fishes were caught by electrofishing and using gill nets. Nine fork length groups were used in order to assess the ontogenetic diet shift. The gut contents were assessed by the index of relative importance that was calculated from the frequency of occurrence, numerical abundance, and volumetric analyses. The most abundant length groups of *C. gibelio* were 18-20 cm, 6-8 cm, and 27-29 cm length groups during summer 2012, autumn 2012 and spring 2013, respectively. The feeding intensity was the lowest in the length groups of 15-17 cm during summer 2012, in 3-5 cm length group in autumn 2012 and in 24-26 cm length group in spring. Seasonal variations were observed in the ontogenetic diet shift of *C. gibelio*. Large specimens consumed more animal materials during summer and more algae in autumn. There was not any significant niche overlap recorded between small and large specimens except summer. Any niche overlap between small and large specimens might be advantageous for the establishment success of invasive Gibel carp in Karamenderes River.

Keywords: Gut contents, feeding intensity, diet overlap, IRI

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Karamenderes Çayı'nda (Türkiye) İstilacı Gümüşi Havuz Balığının (*Carassius gibelio*, Bloch 1782) Beslenmesindeki Ontogenetik Değişim

Öz: Bu çalışmada Karamenderes Çayı'nda bulunan istilacı *Carassius gibelio* (Bloch, 1782) türünün beslenmesindeki ontogenetik değişimin belirlenmesi amaçlanmıştır. Arazi çalışmaları Yaz 2012, Sonbahar 2012 ve İlkbahar 2013 mevsimlerinde gerçekleştirilmiştir. Balıkların yakalanmasında elektroşoker ile çeşitli ağlar kullanılmıştır. Beslenmedeki ontogenetik değişimi belirlemek için, balıklar çatal boylarına göre dokuz gruba ayrılmıştır. Balıkların sindirim kanalı içerikleri besin bulunma sıklığı, sayısal bolluk ve hacimsel oran kullanılarak nisbi önem indeksi ile hesaplanmıştır. *C. gibelio* bireylerinin mevsimlere göre bol olan boy grupları sırasıyla Yaz 2012 (18-20 cm), Sonbahar 2012 (6-8 cm) ve İlkbahar 2013 (27-29 cm) şeklindedir. Beslenme şiddeti Yaz 2012'de 15-17 cm, Sonbahar 2012'de 3-5 cm ve İlkbahar 2013'de 24-26 cm boy gruplarında en az olduğu belirlenmiştir. *C. gibelio* bireylerinin ontogenetik beslenme alışkanlığında zamansal ve mekânsal olarak farklılıklar gözlenmiştir. Büyük bireyler yaz mevsiminde daha çok hayvansal besin ve sonbahar mevsiminde alglerle beslendiği ve küçük bireylerle büyük bireylerin besinleri arasında yaz mevsimi haricinde önemli bir çakışma olmadığı belirlendi. Küçük ve büyük bireylerin besinleri arasında herhangi bir çakışmanın olmaması, gümüşi havuz balığının Karamenderes'de yerleşme başarısı için bir avantajı olabilir.

Anahtar kelimeler: Sindirim kanalı içeriği, beslenme şiddeti, diyet çakışması, IRI

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Introduction

Carassius gibelio (Bloch, 1782) is one of the major invasive species which was introduced to Trace region first in the 1980s (Özuluğ et al. 2004; Ilhan et al. 2005) and spreaded over many freshwater systems rapidly throughout Turkey (Aydın et al. 2011; Ekmekçi et al. 2013). In general, this species is known as a generalist, it has opportunistic omnivorous feeding strategy and feeds on different foods in different environments (Sakai et al. 2001; Gaygusuz et al. 2006; Ekmekçi et al. 2013). It is obvious that high variety in food resources of this

invasive species will affect many other indigenous species living in the same habitat (Goodell et al. 2000) and make it more advantageous among competitors. In addition to having advantages of this species in the interspecific relationship, the high variety of food resources may change during ontogeny and the intraspecific resource partitioning may another advantage of this species in the introduced ecosystems. There are many studies about ecological traits (Lockwood et al. 2013; Ekmekçi et al. 2013; Tarkan 2013), gut contents (Specziár et al. 1997; Rybczyk 2006; Yılmaz et al. 2008; Rogozin et al. 2011; Partal 2014; Partal and Yalçın Özdilek 2017) and feeding characteristics (Specziár et al. 1997; Rybczyk 2006; Yılmaz et al. 2008; Rogozin et al. 2011; Partal 2014; Yalçın Özdilek and Jones 2014; Partal and Yalçın Özdilek 2017) of this species. *C. gibelio* has been first recorded in Karamenderes river which is on the Northwestern part of Turkey in 2007 (Yalçın Özdilek 2008). There are some records on the feeding habits of *C. gibelio* from Karamenderes river (Yalçın Özdilek and Jones 2014; Partal and Yalçın Özdilek 2017), however, there is a gap in the knowledge about ontogenetic diet shift of this species. There is a limited study on the ontogenetic diet shift of *C. gibelio* and the data on this subject with the ontogenetic niche overlap and trophic position will serve to understand the establishment success of this species.

The morphological, physiological and behavioral changes during the developmental stage may result in ontogenetic diet shift (Wilbur 1980; Miller and Rudolf 2011; De Roos and Persson 2013; Nakazawa 2015). In addition, changes in foraging ability depending on the growth may cause the ontogenetic diet shift in fish (Bergman and Greenberg 1994; Jeppesen et al. 2003; Alcaraz and García-Berthou 2007; Nakazawa 2015). Shifting in feeding pattern is common with a function of age and length in many animal species (Wilbur 1980; Miller and Rudolf 2011; De Roos and Persson 2013; Nakazawa 2015). Data on the ontogenetic diet shift is very important for evaluating the ecological role of a species (Werner and Gilliam 1984; Post 2003). Intraspecific competition reduces the population growth particularly in the limited resource condition (Bolnick et al. 2011). The data on the ontogenetic diet shift of invasive *C. gibelio* may serve to take some measures related to mitigating the adverse effects of this invasive species. We aimed to reveal the food diversity in different length groups and the ontogenetic diet shift of *C. gibelio* quantitatively in this study. This study will serve to understand if this species has such advantage make it successive in establish and spread.

Materials and Methods

Study area and sampling

Karamenderes River, about 109 km in length, originates from the Kaz and Ağı Mountains and directs to West and North and flows into Çanakkale strait after watering Kumkale plate in Biga Peninsula, Çanakkale. There are two reservoirs along the river. One is in the Bayramiç province, which is about 86.5 cubic hectometer water capacity, and the other is in Pınarbaşı village, which is smaller than the other. These reservoirs are used for irrigation purposes (Figure 1).

C. gibelio is first recorded in Karamenderes at the lower part of the Pınarbaşı village after fish stocking studies on Bayramiç Dams by the activities of Directorate of National Water Affairs (Yalçın Özdilek 2008). The samplings were conducted in three seasons, during summer 2012 (July-August 2012), autumn 2012 (October-November 2012), and spring 2013 (May 2013). Sampling could not be performed in winter because of inconvenient weather conditions for sampling. The fish sampling has performed at 14 stations along the Karamenderes River from the upper parts of the dams to the river mouth. The names of the stations from up to down are Karaköy 1, Karaköy 2, Evciler, Evciler trout farm, Çırpılar, Mollahasanlar, Bayramiç-Çan road, Ahmetçeli, Sarmısaklı, Pınarbaşı, Kalafat, Kumkale bridge (3), Kumkale closed end (2), Kumkale open end (1) (Figure 1).

Different sampling device was used for fishing according to habitat characteristics. Fish were collected by scanning about 20 m lengths of the river during 20 minutes in every station by electrofishing (SAMUS) on the upper sites of Karamenderes. Gill nets in different mesh size (18 mm, 22 mm, 25 mm, and 32 mm knot to knot) and different lengths (160 m-2.5 m, 100 m-2 m, 15 m-2 m, and 30 m-1 m) were used for fishing (average of 24 hours) in the river mouth stations. In addition, fyke net composed of 8-37 sets each has 12 m long and a cast net, which has 10 mm mesh size and its radius 140cm were used for fishing in some stations. All the fish samples were transferred to the laboratory in an icebox and after identification, they preserved in a deep freeze with labeled.

Laboratory procedures

The gut contents of 215 specimens of totally 251 specimens could be examined. Before dissection, the fork length and weight were measured by the standard ruler (± 0.01 mm) and a balance (± 0.1g). After dissection, the total gut length from the

esophagus to the anus was measured using the same ruler. The sex of the specimens was determined under a stereomicroscope. The gut contents were evacuated in a graduated cylinder, which contains 70 % ethanol. The total gut volumes were measured by the replacement of ethanol level.

Diluted gut contents in a Sedgewick- Rafter lam were examined under a stereomicroscope x10 magnitude. The number and sizes (Sun and Liu 2003) of each food category were recorded after the description of the taxon at the possible level.

The percentage of empty guts, vacuity index (*VI* %), were used to assess the feeding intensity (Hureau 1966). The feeding intensity was assessed taking into consideration the length groups, sex, season and stations. Vacuity Index (*VI*) was used for calculating the feeding intensity by using

VI = empty gut number x 100 / total gut number equation (Hureau 1966; Costa and Cabral 1999).

The percentage of the relative index (*IRI* %) was evaluated to assess gut contents data using the frequency of occurrence (*F* %), numerical (*N* %) and volumetric (*V* %) methods (Pinkas et al. 1971; Prince 1975; Hyslop 1980).

IRI = (*N* %+*V* %) x *F* %

F_i %= *i* prey items frequency of occurrence in the gut x 100 / total number of full guts

N_i %= *i* prey items total number x 100 / prey items total number

V_i %= *i* prey items total volume x 100 / prey items total volume

$$H = -\sum p_i ln p_i$$

$$p_i = N_i / N$$

equation. The trophic level was calculated as

$$TL_k = 1 + \left(\sum_{j=1}^{11} P_j \times TL_j \right)$$

in the equation (Cortés 1999). The *IRI* % values of each food category were used to calculate the diversity and trophic position.

Fish were grouped into nine-length class categories and the differences in *IRI* % value of each food category in each length group were tested by nonparametric Kruskal Wallis test.

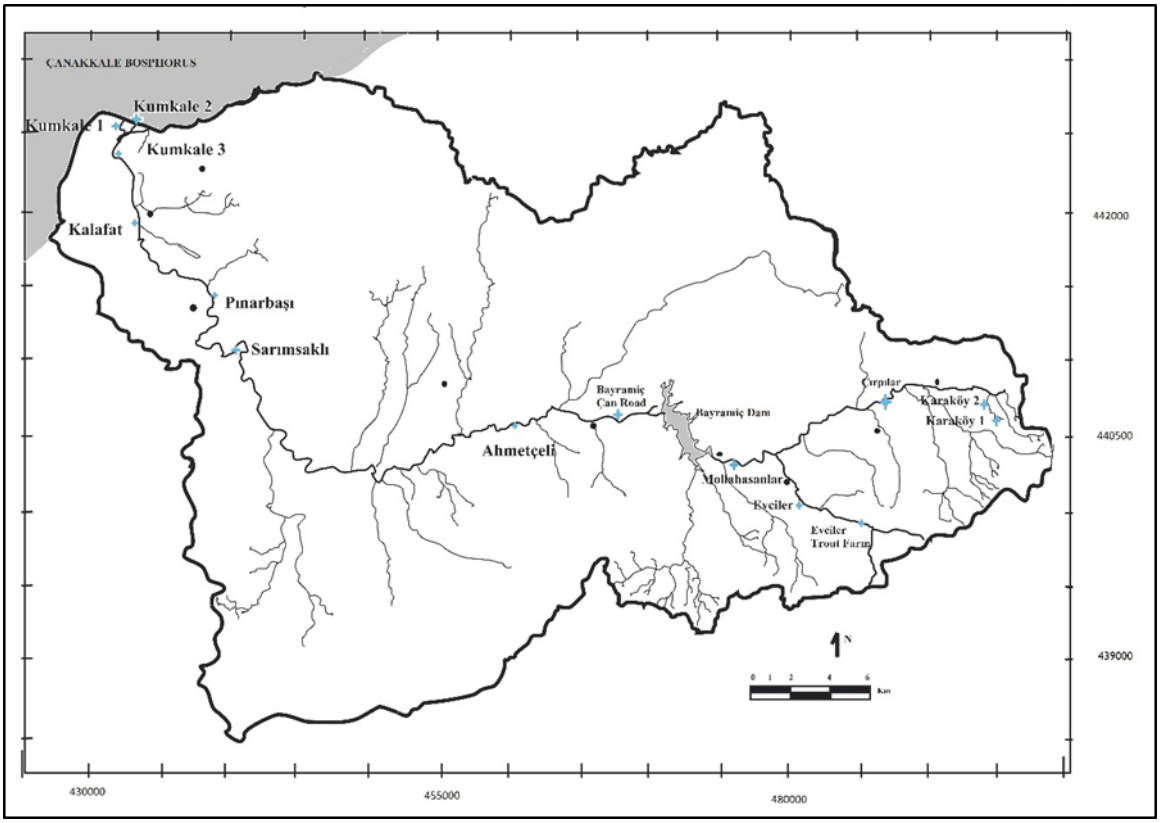


Figure 1. Sampling area (Partal and Yalçın Özdilek 2017 (adapted)).

Results

A total of 215 specimens were all caught at the lower stations of Bayramiç Reservoir. There were no specimens encountered at the upper stations of this dam. 62 %, 27 % and 12 % of the specimens were collected during summer 2012, autumn 2012 and spring 2013, respectively. The spatial and seasonal relative abundance of specimens indicates that

the most abundant specimens were recorded at the Kumkale River mouth station in summer, Ahmetçeli station in autumn and Kumkale bridge (3) station in Spring with the percentages of 60.9, 14.9 and 16.1, respectively (Figure 2). The most abundant length group was 6-8 cm *FL* with the percentage of 16.7 in total. The 18-20 cm, 6-8 cm, and 27-29 cm *FL* groups were the most abundant length groups in summer, autumn and spring seasons, respectively (Figure 3).

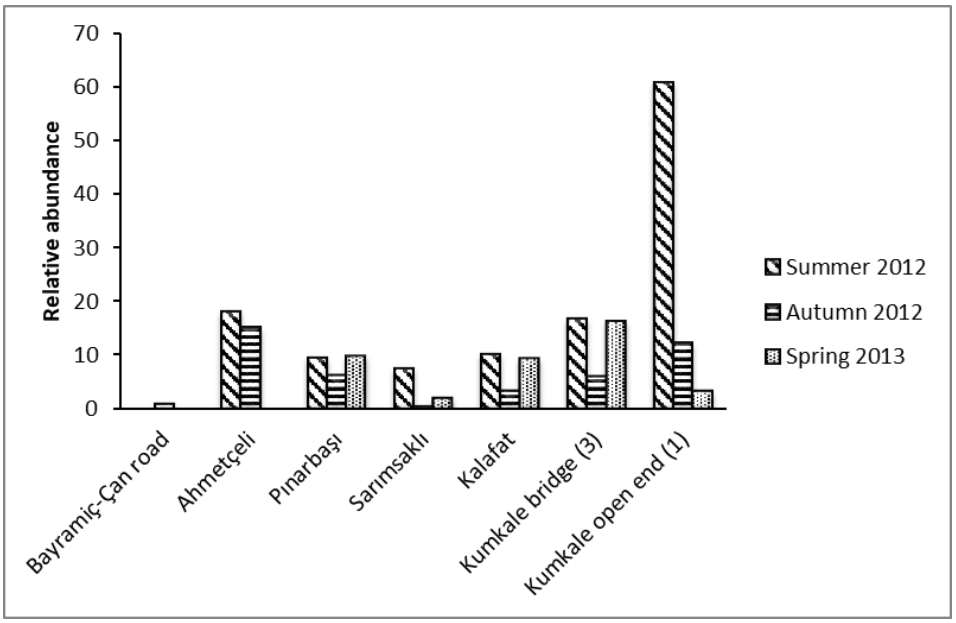


Figure 2. The distribution and relative abundance of C. gibelio specimens according to seasons and the station.

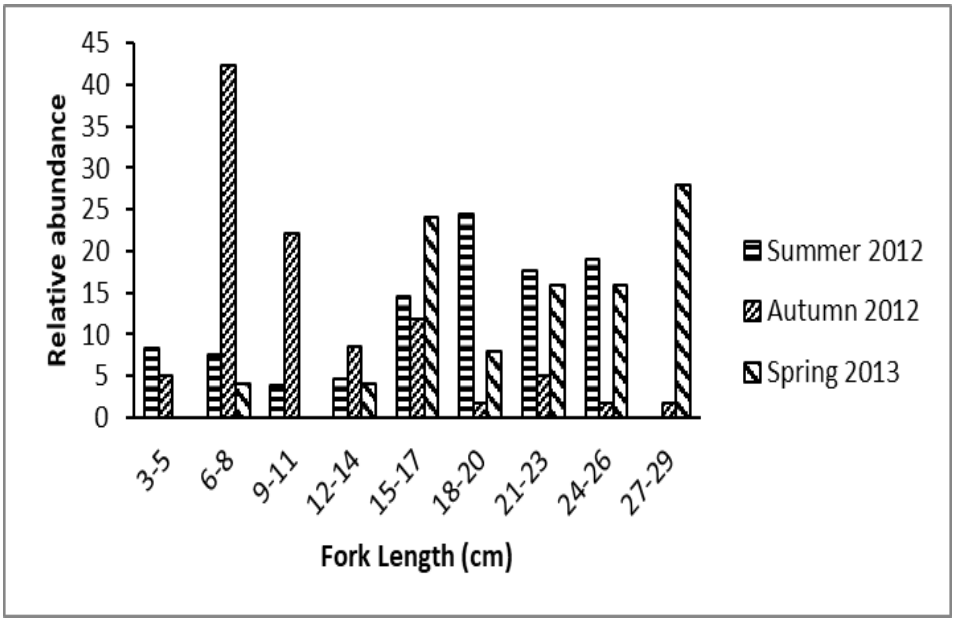


Figure 3. The distribution and relative abundance of the length groups according to the seasons.

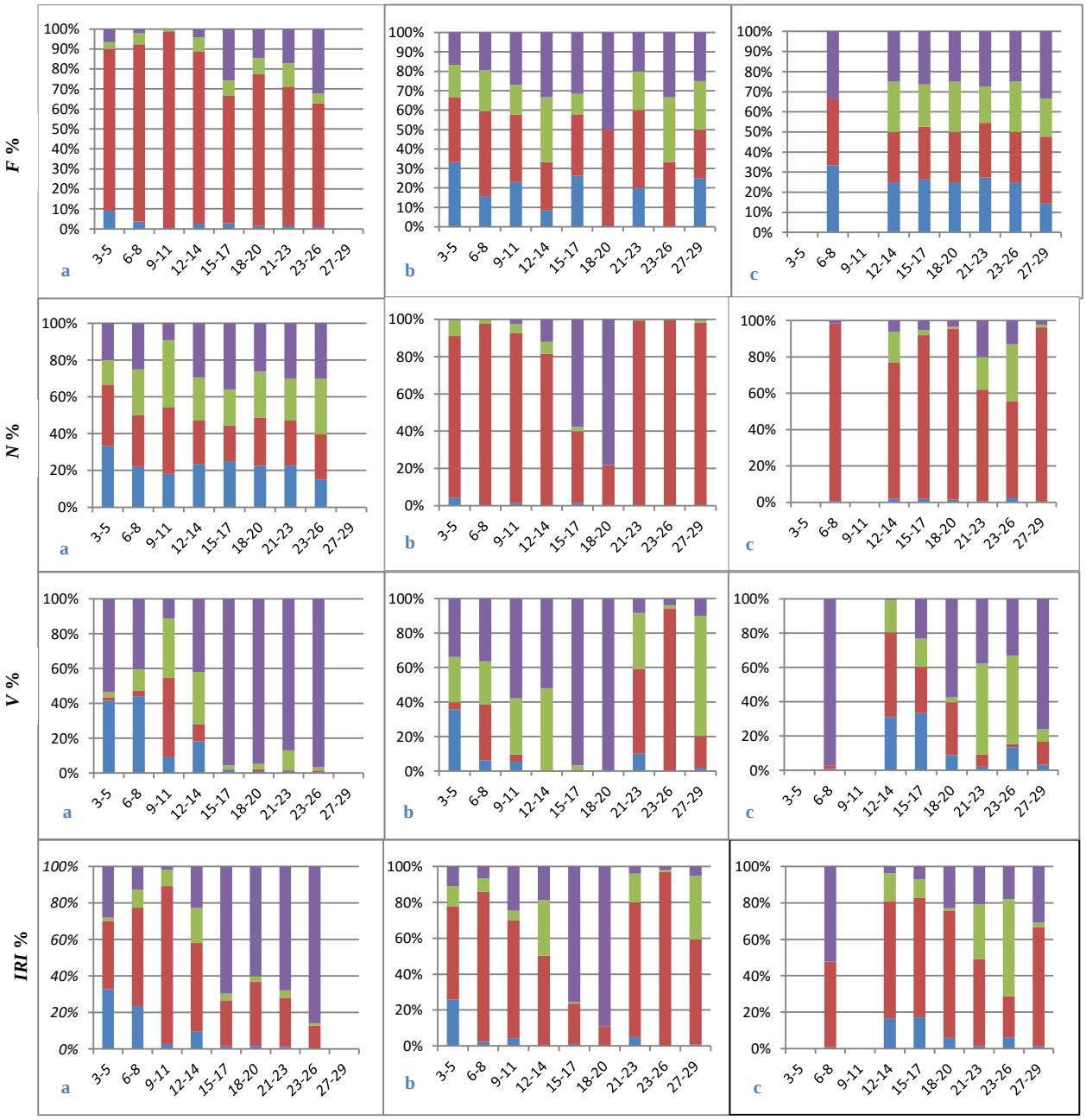
Table 1. Shannon-Wiener Diversity Index (SWI) and Vacuity Index (VI) values according to the length groups.

Sampling Season	Length Groups (cm)																	
	3-5		6-8		9-11		12-14		15-17		18-20		21-23		24-26		27-29	
	SWI	VI	SWI	VI	SWI	VI	SWI	VI	SWI	VI	SWI	VI	SWI	VI	SWI	VI	SWI	VI
Summer 2012	2.23	9.1	2.51	0	2.34	20	2.46	33.3	1.76	55.6	2.26	37.5	1.86	34.8	1.39	44	-	-
Autumn 2012	1.69	33.3	2.05	0	2.23	23.1	1.78	20	1.56	14.3	0.89	0	1.7	-	1.47	0	1.57	0
Spring 2013	-	-	1.39	0	-	-	1.41	0	2.19	16.7	1.64	0	2.04	0	1.99	25	1.63	0

Gut Contents

The gut contents of *C. gibelio* consisted of siliceous algae, green algae, vascular plants, pine pollen, amphipods and chironomids (Table 2). Algae took an important part in the gut contents by frequency and abundance. As members of Bacillariophyceae, *Navicula* sp., *Fragilaria* sp. and partly *Cocconeis* sp. taxa were dominant organisms in nearly all gut contents. Some animal groups such as Oligochaeta members could not include in the gut content analysis due to rapid digestion. However, there were encountered Oligochaeta setae in nearly every size group in every season.

When the gut contents were grouped as four-diet categories (detritus, periphyton, macrophyte, and macroinvertebrate) periphyton dominate nearly all length groups except 15-20 cm length groups in autumn according to N% values (Figure 4). Particularly, macroinvertebrates were low values both abundance and volume in > 20 cm length specimens in autumn. The V% values of macroinvertebrates were low in <15cm and >20 cm specimens in spring and autumn, respectively.



Fifure 4. The occurrence (F %), number (N %), volume (V %) and Index of Relative Importance (IRI %) of prey items in the gut contents, in terms of the length groups and seasons (a: Summer 2012; b: Autumn 2012; c: Spring 2013. Blue: detritus; red: periphyton; green: macrophyte; purple: macroinvertebrate)

Table 2. Seasonal (SUM: Summer 2012; A: Autumn 2012; SP: Spring 2013) IRI % values of length groups.

		Seasonal *IRI* % Values of Length Groups									
		3-5		**6-8**			**9-11**		**12-14**		
	Prey Items	**SUM**	**A**	**SUM**	**A**	**SP**	**SUM**	**A**	**SUM**	**A**	**SP**
Cyanobacteria	*Oscillatoria* sp.		1.82		14.14			2.33			
Heterokontophyta	*Amphora* sp.	1.19	0.33	1.29	0.23		2.34	2.70	0.40	0.80	
	Cocconeis sp.	0.34		3.90	1.34	2.0	7.30	10.25	2.50	10.45	2.8
	Cyclotella sp.								0.19		
	Cymatopleura sp.	0.17		0.74	0.08		0.10		0.45		
	Cymbella sp.	0.22		5.26	0.19	0.7	15.65	1.17	5.54	3.52	
	Diatoma sp.	0.04		0.09	0.06		0.01				
	Fragilaria sp.	0.01	0.91	0.07	8.64		0.08		0.07	0.16	
	Fragilaria sp. (chain)	13.48	0.54	17.49	1.26	16.8	2.37	15.04	18.58	0.24	54.8
	Gomphonema sp.	0.35	1.95	0.12	3.96			2.18	0.13		
	Gyrosigma sp.	0.34	0.40	0.25	0.07	0.7	12.07	1.20	4.18	0.66	
	Licmophora sp.		1.44		6.70			1.10			
	Melosira sp.	5.82		8.79	0.30		12.49	0.93	5.95	2.44	
	Navicula sp.	8.98	41.69	11.54	43.19	15.4	16.94	28.45	6.71	31.54	6.1
	Neidium sp.								0.06		
	Nitzschia sp.	2.88		1.73			16.55		0.15		
	Pinnularia sp.		2.34	0.004	0.76			0.05	0.20	0.16	
	Rhoicosphaenia sp.	2.28	0.33	0.94	0.40		0.07	0.05	0.11		
	Stephanodiscus sp.	0.03	0.35	0.41	0.003				1.23	0.16	
	Ulnaria sp.	0.95		1.10	0.07	10.7	0.02	0.26	0.66		
	Vaucheria sp.				0.31						
Chlorophyta	*Ankistrodesmus* sp.										0.6
	Chlorella sp.				1.53						
	Chlorophyta			0.03							
	Cladophora sp.	1.10		2.06	0.56		2.70	0.46	6.80		
	Closterium sp.	0.19		0.06	0.15	0.8	0.70				
	Conjugatophyceae										
	Cosmarium sp.	0.02		0.02					0.02		
	Microspora sp.										
	Oedogonium sp.	0.01		0.19	0.05		3.51	0.03	0.16	0.25	
	Pandorina sp.										
	Pediastrum sp.			0.10	0.04				1.08		
	Scenedesmus sp.	0.01			0.02			0.06			
	Spirogyra sp.				5.78			1.002			
	Stigeoclonium sp.	0.39	0.73	0.06	0.99		0.33	0.61	0.38		0.7
	Ulothrix sp.				0.01						
	Zygnema sp.							0.14			
Animal and Plant Prey	Amphipoda	23.94		7.99	5.79		1.49	18.23	19.50	9.73	
	Chironomidae	0.11				52.3		0.25			
	Copepoda		11.04							0.56	
	Crustacea				0.29						
	Insecta	3.91		2.46							
	Keratella sp.								0.46		
	Nematoda										
	Ostracoda			1.94	0.53			6.21	2.72	8.31	
	Plant (seed)				0.01						
	Pollen	0.20	0.27	0.04	0.004		0.02				
	Plant	0.23	10.15	7.59	0.13		2.12	0.13	11.98		14.8
	Fish egg	0.04		0.10	0.04				0.03		3.6
	Animal Detritus	15.78		19.07					0.80		15.5
	Digested Detritus	0.41		0.02			0.32				
	Other organisms	16.58	25.71	4.41	2.39	0.7	2.44	4.37	8.97	0.13	1.2
	Cystic material				0.001				0.02		
	Bryozoa			0.03			0.38	2.82		30.87	
	Cladocera			0.10							

Table 2. (Continous)

		Seasonal *IRI* % Values of Length Groups													
		15-17			18-20			21-23			24-26			27-29	
	Prey Items	SUM	A	SP	SUM	A	SP	SUM	A	SP	SUM	A	SP	A	SP
Cyano-bacteria	*Anabaena* sp.				0.003										
	Merismopedia sp.				0.02										
	Oscillatoria sp.	0.001		1.28	0.002										
Heterokontophyta	*Amphora* sp.	3.94	3.65	0.02	3.29	1.82		2.35		0.07	0.01			0.17	0.1
	Cocconeis sp.	1.49	10.53	0.29	7.03	3.64	3.1	6.96	5.18	2.72	2.66	5.96	0.5		0.5
	Cyclotella sp.	0.01													
	Cymatopleura sp.	0.09	0.03	0.32	0.03		0.2	0.15		0.42	0.01			0.23	
	Cymbella sp.	0.34	1.22	2.02	6.38			3.09	0.27	0.37	0.23	12.8		1.48	0.3
	Diatoma sp.	0.01		0.36	0.003			0.01			0.001				
	Epithemia sp.				0.07										
	Fragilaria sp.		0.09	0.33	0.14			0.001							
	Fragilaria sp. (chain)	0.04	1.51	28	3.11	1.84	45.5	0.76	6.38	37.5	5.02	4.32	6.4	1.22	21.7
	Gomphonema sp.	0.01	0.21		0.08			0.04	0.11		0.02				
	Gyrosigma sp.	2.15	0.28	1.65	2.56			2.28	1.04		0.06	0.79		1.25	
	Melosira sp.	0.49	0.84	8.73	2.85			1.63	16.39	5.36	0.93	34.9	4.9	27.8	37.3
	Meridion sp.				0.004						0.003				
	Navicula sp.	8.7	3.34	16.6	5.32	1.82	15.3	6.34	44.46	0.86	1.12	38.2	6.4	26.1	3.2
	Nitzschia sp.	1.32		0.03	0.76			1.31			0.29				0.02
	Pinnularia sp.	0.01	0.11		0.02			0.003			0.002				0.03
	Rhizosolenia sp.				0.0001										
	Rhoicosphaenia sp.	0.72	0.72		0.10	1.82	2.03	0.39	0.87		0.06				
	Stephanodiscus sp.	0.13		0.01	0.60			0.03			0.01		1.6		0.2
	Surirella sp.				0.001										
	Ulnaria sp.	5.46		5.90	2.80		3.5	1.66	0.16	0.38	1.95		2.5		2.3
Chlorophyta	*Ankistrodesmus* sp.	0.003			0.001										
	Chaetomorpha sp.				0.02										
	Chlorophyta							0.03			0.01				
	Cladophora sp.	0.01			1.04			2.22		3.35	0.54				
	Closterium sp.	0.003		0.03	0.01			0.02							
	Conjugatophyceae				0.10			0.26		9.26	0.01		7.3		0.2
	Cosmarium sp.	0.01			0.09			0.003			0.01				
	Microspora sp.	0.13			0.13			0.08			0.07				
	Oedogonium sp.	0.01			0.51			0.05	15.81	0.66	0.01		3.4	1.65	
	Pandorina sp.				0.002										
	Pediastrum sp.	0.02			0.005				0.15						
	Scenedesmus sp.		0.03		0.001			0.001			0.0004				
	Spirogyra sp.			0.09				0.01		3.29					1.4
	Stigeoclonium sp.	0.02	0.08		0.24			0.07	0.18		0.03			0.21	
	Ulothrix sp.				0.35			0.003							
	Ulvales							0.05							
	Zygnema sp.				0.01			0.15							
Charophyta	*Mougetia* sp.				0.01										
Animal and Plant Prey	Amphipoda	51.62	38.70	0.38	21.45	76.1	0.9	52.42	4.04	0.46	59.97	2.003	13.9	0.22	27.5
	Chironomidae	0.91	0.0003	2.26	1.34		21.3	0.25						4.87	1.8
	Copepoda	0.52	0.0003	0.27	2.02	0.02		0.27			0.10				
	Crustacea	0.12	0.11		0.18			0.80			3.83				
	Gastropoda	0.001			0.01						0.08				
	Insecta	0.03	0.26	0.36	0.38			0.20		9.35	0.55				0.5
	Nematoda				0.003			0.0001							
	Ostracoda	16	36.47		34.48	12.9		13.79			20.89				1.03
	Pollen	0.005		0.01	0.11		0.1	0.17			0.25	1.12			
	Plant	3.80	0.14	10.1	0.38		1.4	0.95		13.7	0.39		42.8		0.98
	Fish egg	0.45		3.65	0.28		0.7	0.08		10.9	0.47		3.96	0.11	0.04
	Animal Detritus	0.01		1.67	0.002			0.001					3.7		
	Digested Detritus	0.77			0.07		1.97	0.23		1.19	0.14		1.6		
	Other organisms	0.59	1.09	15.5	1.58		3.9	0.86	4.95	0.15	0.20		0.9	1.02	1.1
	Plant (circle shaped)	0.06			0.03			0.001			0.06				
	Cystic material			0.23	0.01			0.02						0.12	
	Bryozoa		0.56											33.5	

Ontogenetic diet shifts

The ontogenetic diet shift indicated seasonal variation taking into consideration *IRI* % values. There was a significant difference among *IRI* % of various length groups in all three seasons (*X=27.003, P<0.01, df=7; X=20.603, P<0.05, df= 8; X=14.073, P<0.05, df= 6*). Heterekontophyta members were the most common food groups in nearly all length groups. While the small specimens (3-4 cm) feed on mostly Heterokontophyta members, the larger specimens consumed mostly animal foods such as Amphipoda and Ostracoda in all the seasons. Particularly, the *IRI %* value of Amphipoda members was more than 50 % in the large specimens in summer. *Navicula* sp. was the highest *IRI %* in the 3-11 cm *FL* group in autumn. According to three season data, the critical length group shifting diet from the herbivorous to carnivore dominated feeding strategy is 12 cm *FL*. However, the *IRI* % of Amphipoda members were 23.9 %, 18.2 %, and Copepoda members 11.0 % in autumn, Chironomidae members were high (52.3 %) in spring in smaller than 12 cm *FL* group (Table 2).

Relative gut length and feeding strategy

The mean relative gut length of *C. gibelio* is 3.47 ± 0.85 with the range of 1.22-5.66 (in summer) 2.60 ± 0.73 with the range of 1.03-3.84 (in autumn) 3.52 ± 0.56 with the range of 2.12-4.56 (in spring). *C. gibelio* specimens have omnivore feeding strategy according to their length groups. (Table 3). When group's specimens that were smaller than 12.0 cm length collected in three seasons combined, the mean *RGI* value of this combined group was 2.51 ± 0.77 that means carnivorous dominant omnivorous feeding strategy. The *RGI* values of 12.1-17.9 cm and larger than 18.0 cm length groups combined in three seasons were 3.38 ± 0.67 and 3.66 ± 0.69 respectively.

Table 1. Taking into consideration *RGI* types of feeding in different length groups (O: Omnivorous; H: Herbivorous; C: Carnivorous; N: Number of specimens; GL: Gut length; FL: Fork length; SD: Standart Deviation).

Length groups (cm)	N	Summer (GL/FL±SD)		N	Autumn (GL/FL±SD)		N	Spring (GL/FL±SD)	
3-5	11	2.47±0.97	O-C	3	1.55±0.34	C-O	-	-	-
6-8	10	2.69±0.43	O	25	2.47±0.81	C-O	1	3.16	O
9-11	5	3.15±0.92	O	13	2.40±0.56	C-O	-	-	-
12-14	6	3.65±0.75	O	5	2.85±0.36	O	1	3.13	O
15-17	20	3.52±0.73	O	7	3.24±0.26	O	6	3.21±0.79	O
18-20	23	3.69±0.74	O-H	1	3.33	O	2	3.67±0.95	H-O
21-23	22	3.70±0.78	O-H	3	3.01±0.38	O	4	3.58±0.71	O
24-26	25	3.73±0.76	O-H	1	3.04	O	4	3.81±0.36	H-O
27-29	-	-	-	1	3.62	O	7	3.64±0.19	O

Trophic level

The trophic level of all *C. gibelio* specimens ranged from 2.03 to 3.34. The trophic level was low and more or less steady state in spring comparing to summer and autumn. The trophic level is increasing at larger than 12 cm *FL* specimens both in the spring and summer seasons. The trophic level of larger than 20 cm *FL* specimens decreased dramatically in autumn. The minimum and maximum *TL*s of all groups are 2.06-3.29, 2.03-3.34 and 2.07-2.79 in summer, autumn, and spring, respectively (Figure 5).

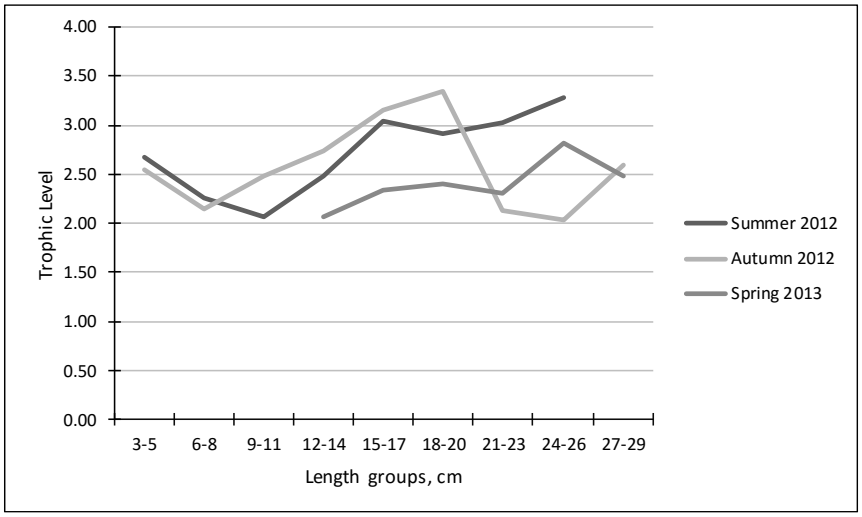


Figure 4. Trophic level of the length groups.

Discussion

C. gibelio were observed only the lower part of the Bayramiç Dam along the River Karamenderes. The spatial distribution of *C. gibelio* had seasonal variation along the River Karamenderes. While the specimens larger than 13.6 cm *FL* were abundant at the river mouth station, the specimens smaller than 8.8 cm *FL* were rich at the upper sites just below the Bayramiç dam in the summer and spring. The smaller specimens might escape from the Bayramiç Dam, which is regularly fished by aquaculture activities and the most available habitat for *C. gibelio* might be at the river mouth conditions.

A previous study based on a gravimetric method indicates that small specimens mostly feed on diatoms and large specimens consume animal materials such as copepods, beetles and chironomids in a Brackish Water Body in Southern Siberia (Rogozin et al. 2011). Another study, which is based on *F* % and *N* % indicates that small specimens consume zooplanktonic organisms such as copepods and cladocerans in Gelingüllü reservoir, Turkey (Kırankaya 2007). In addition, the phytoplankton was recorded as low frequency comparing to other food organisms in all age groups in Gelingüllü reservoir (Kırankaya 2007). There are no previous study recording gut contents as *IRI* % values, however, the results obtained from abundance and frequency in Karamenderes River were apart from the results of the previous records. Periphyton was important diet as frequency and abundance, as well as *IRI* %, in almost all length groups.

The amount of animal food might be enough only large specimens who are more capable to collect the animal materials comparing to smaller ones, so the smaller ones have to change their feeding characteristics into algae because of competition. This finding was supported by our high diet diversity in the gut contents of smaller specimens in Karamenderes River. There is no diet diversity in *C. gibelio* feeding patterns in the previous study, and our results indicated a decrease in Shannon diversity in food components of >12 cm *C. gibelio*. This indicated that gibel carp has a wide plasticity in every length group and this wide range on the capability of resource use give them a high advantage for surviving in even very limited resource conditions. In the other views, the food selection of specimens might be related to the abundance of resource users. While the abundance of large Gibel carp specimens were abundant in summer, the small ones were abundant in autumn and spring. In addition, the high amount of large specimens might exploit all favorable animal materials in summer.

In Karamenderes River, feeding intensity of *C. gibelio* had a seasonal variation with high feeding intensity reported by Kırankaya (2007) and Bobori et al. (2012). In general, the feeding intensity estimated based on a number of empty gut indicates seasonal variation, with low in hot summer season because of increasing enzyme activities and digestion metabolism. The seasonal and size depend variation in the feeding intensity might be related with the diet types. For example, in summer the specimens smaller than 8 cm *TL* feed mostly on plant materials and likely in autumn the large specimens consumed plant material. It is important that the digestion of plant material is hard when comparing to animal material and the retention duration of plant materials is longer than that of animal materials in the gut (Nikolsky 1978). In addition, the smaller specimens might feed on relatively small food and the retention duration of small animal materials as a diet would be relatively shorter time (Labropoulou et al. 1997) in gut content comparing to large animal materials which are presumably consumed by larger specimens. In other words, the feeding intensity might be explained by fish abundance. In small and large specimens was low abundance in summer and autumn, respectively (Figure 3). This indicates that when the population of *C. gibelio* low density, they prefer the most available and abundant plant materials as food. The small specimens mostly feed on siliceous algae and probably feed on the mats of periphyton, which are more abundant in the shallow, pooled, high vegetated stony and macrophytes dominant microhabitats along the river. This kind of habitat might be more suitable for small specimens serve them both food and shelter for escaping their predators. Yalçın Özdilek and Jones (2014) stated that the filamentous algae were important food components for about middle size (13.5-21.1 cm in FL) *C. gibelio* living in Karamenderes. However, *IRI* % results indicate that 12-21 cm length group members feed on animal prey items in overall stations in Karamenderes river in this study. This study supports that *C. gibelio* is an opportunistic feeder and the plasticity in its feeding strategy might be seasonal and ontogenetic.

C. gibelio has an omnivorous feeding strategy as indicated many studies (Specziár et al. 1997; Balık et al. 2003; Rybczyk 2006; Kırankaya 2007; Yılmaz et al. 2008; Yalçın Özdilek and Jones 2014; Partal and Yalçın Özdilek 2017). The trophic level based on *IRI* % and *RGI* data support this finding. The ontogenetic diet patterns taking into consideration, there was a conflict between *IRI* % and *RGI* results. Taking into consideration *RGI* carnivorous dominant feeding strategy was observed in smaller than 12 cm *FL* specimens' particularly in summer and autumn and herbivore dominant omnivore strategy was observed in larger than 18 cm *FL* specimens particularly in summer and spring. The *IRI %* values are indicative

of instant feeding, so animal materials are digested faster, especially in hot summer and autumn seasons (Windell 1978).

The increase in the trophic level with increasing of the fish length was found in many studies as a natural process (Weber and Brown 2013). In this study, the finding that large specimens have high trophic level in spring and summer is an anticipated result. However, a decrease in the trophic level of larger than 20 cm in *FL* might be explained by food availability. During this season because of competition (Yalçın Özdilek 2017) and limited resources (Akbulut et al. 2009), the large specimens may supply their requirements with foods that have lower energy but that are more abundant in surroundings.

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Turunçgil Kabuk Yağlarının Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Filetolarının Raf Ömrü Üzerine Etkileri

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ÖZ

Bu çalışmada farklı konsantrasyonlardaki (%0,5 ve %1) turunçgil kabuk yağlarının (limon, portakal ve bergamot) gökkuşağı alabalığı (*Oncorhynchus mykiss*) filetolarının raf ömrü üzerine etkileri araştırılmıştır. Örnekler kontrol grubu (*A*), %0,5 limon uçucu yağı ilave edilmiş (*B*), %1 limon uçucu yağı ilave edilmiş (*C*), %0,5 portakal uçucu yağı ilave edilmiş (*D*), %1 portakal uçucu yağı ilave edilmiş (*E*), %0,5 bergamot uçucu yağı ilave edilmiş (*F*) ve %1 bergamot uçucu yağı ilave edilmiş (*G*) olmak üzere yedi gruba ayrılmıştır. Balık örneklerinin mikrobiyolojik (toplam aerobik mezofilik bakteri, toplam psikrotrofik bakteri, laktik asit bakterileri, *Enterobacteriaceae)* kimyasal (pH, toplam uçucu bazik azotu, thiobarbitürik asit reaktif maddeler) ve duyusal (renk, koku, lezzet, genel kabul edilebilirlik) özellikleri incelenmiştir. Depolama süresi boyunca mikrobiyolojik ve kimyasal özellikler açısından kontrol grubu ile uygulama grupları arasında önemli değişiklikler tespit edilmiştir ($p<0,05$). En düşük ve en yüksek toplam aerobik mezofilik bakteri, toplam psikrotrofik bakteri, laktik asit bakteri ve *Enterobacteriaceae* sayıları sırasıyla 2,54-7,92, 2,69-8,03, 2,00-5,03 ve 2,00-4,09 olarak bulunmuştur. Çalışmadan elde edilen sonuçlara göre farklı konsantrasyonlarda turunçgil uçucu yağları ilavesinin bakteri sayısını önemli ölçüde azalttığı ve örneklerin bozulmasını geciktirdiği belirlenmiştir.

Anahtar kelimeler: Gökkuşağı alabalığı, turunçgil kabuk yağları, raf ömrü, kalite özellikleri

MAKALE BİLGİSİ

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Influences of Citrus Peel Oils on Shelf Life of Rainbow Trout (*Oncorhynchus mykiss*) Fillets

Abstract: In this study, the effects of different concentrations (0.5% and 1%) citrus peel oils (lemon, orange and bergamot) on shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets were researched. Samples were divided into seven groups: control (*A*), 0.5% [v/v] lemon EO added (*B*), 1% [v/v] lemon EO added (*C*), 0.5% [v/v] orange EO added (*D*), 1% [v/v] orange EO added (*E*), 0.5% [v/v] bergamot EO added (*F*) and 1% [v/v] bergamot EO added (*G*). Microbiological (total aerobic mesophilic bacteria, total psychrotrophic bacteria, lactic acid bacteria, *Enterobacteriaceae*), chemical (pH, thiobarbituric acid reactive substances and total volatile base nitrogen) and sensory (colour, odour, taste, general acceptance) properties of fish samples were investigated. During the storage period, significant changes were observed between the control group and treatment groups in terms of microbiological and chemical properties ($p<0.05$). The lowest and highest total aerobic mesophilic bacteria, total psychrotrophic bacteria, lactic acid bacteria and *Enterobacteriaceae* counts were found 2.54-7.92, 2.69-8.03, 2.00-5.03 and 2.00-4.09, respectively. According to the results obtained in this study, it was determined that the addition of citrus essential oils at different concentrations significantly reduced the number of bacteria and delayed the deterioration of the samples.

Keywords: Rainbow trout, citrus peel oils, shelf life, quality properties

Alıntılama
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Giriş

Beslenmede önemli bir yer tutan hayvansal kökenli proteinlerin temininde su ürünleri önemli kaynaklardan birisidir. Su ürünleri içerisinde de ilk sırayı balıklar almaktadır (Aras vd. 2000).

Dünyada ve Türkiye'de yetiştiriciliği en yoğun ve yaygın olarak yapılan alabalık türü gökkuşağı alabalığı (*Oncorhynchus mykiss*)'dır (Oğuzhan 2004; Sarıeyüpoğlu vd. 2017). Gökkuşağı alabalığı yüksek adaptasyon ve yemden yararlanma yeteneği, suni yöntemlerle yumurta alımının kolaylığı, kuluçka

sürelerinin kısalığı ve hastalıklara karşı dayanıklı olmasından dolayı tercih edilen bir türdür (Canyurt 1978, 1983; Emre ve Kürüm 1998; Oğuzhan 2004).

Gıdalarda sentetik ve doğal katkı maddeleri uzun yıllardan beri koruyucu olarak kullanılmaktadır. Ancak sentetik kökenli maddelerin sağlığa olan zararlı etkilerinden dolayı kullanımları sınırlandırılmış ve doğal katkı maddelerine olan yönelim her geçen gün artmıştır (Duman vd. 2012; Kuş 2012; Mutlu ve Bilgin 2016).

Su ürünlerinde doğal antioksidan kaynağı olarak çok sayıda bitki türü (biberiye, kekik, adaçayı, karanfil ve sarımsak) kullanılmıştır (Pazos vd. 2005; Serdaroğlu ve Felekoğlu 2005; Erkan 2012; Çetinkaya 2013; Mutlu ve Bilgin 2016).

Narenciye yağları da antimikrobiyal ve antioksidan aktivitesi yüksek yağlardandır. Limon (*Citrus limon*), portakal (*Citrus sinensis*) ve bergamot (*Citrus bergamia*) narenciye familyasından (*Rutaceae*) olup, esans yağ ve flavonoidler turunçgillerin kabuk kısmında yoğun bir şekilde bulunmaktadır. Bu kısımlar limonen ve linalool gibi uçucu yağları oldukça yüksek miktarlarda içermektedirler. Narenciye yağları; gıda, kozmetik, eczacılık, parfümeri ve kimya endüstrisi gibi birçok alanda yaygın bir kullanım alanına sahiptir. Özellikle de limon yağı gıda ve içecek endüstrisinde oldukça önemlidir. Limon ve bergamot yağı, antimikrobiyal ajan olarak başarıyla kullanılan uçucu yağlar arasında yer almaktadır. Portakal kabuk yağının önemli bileşeni olan limonenin antioksidan aktivitesinin oldukça yüksek olduğu yapılan çalışmalarla da bildirilmiştir (Turhan vd. 2006; Min-Hsiung 2008; Roberto vd. 2010; Sánchez-Gonzálezvd. 2011; Erhan ve Bölükbaşı Aktaş 2017).

Bu çalışmada, farklı konsantrasyonlardaki (%0,5 ve %1) turunçgil kabuk yağlarının (limon, portakal ve bergamot) gökkuşağı alabalığı (*O. mykiss)* filetolarının raf ömrü üzerine etkileri 9 günlük depolama süresi boyunca araştırılmıştır.

Materyal ve Metot

Araştırmada kullanılan gökkuşağı alabalıkları Artvin İli Şavşat İlçesindeki özel bir işletmeden, turunçgil kabuk yağları ise (limon, portakal ve bergamot) Kardelen firmasından (Kardelen Tarım Ürünleri Ltd., Ankara) temin edilmiştir. Yaklaşık 200-250 g ağırlığında toplam 56 adet balık satın alınmıştır. Balıklar buz içerisinde strafor kutularda soğuk zincire uygun olarak laboratuvara getirilmiştir. Laboratuvar ortamına getirilen balıkların başları kesilmiş, iç organları uzaklaştırılmış ve derili filetoları çıkarılmıştır. Elde edilen filetolar işleme hazır hale getirilmiş ve *A, B, C, D, E, F* ve *G* olmak üzere 7 gruba ayrılmıştır. *A*: kontrol grubu, *B*: %0,5 limon uçucu yağı ilave edilmiş, *C*: %1 limon uçucu yağı ilave edilmiş, *D*: %0,5 portakal uçucu yağı ilave edilmiş, *E*: %1 portakal uçucu yağı ilave edilmiş, *F*: %0,5 bergamot uçucu yağı ilave edilmiş ve *G*: %1 bergamot uçucu yağı ilave edilmiş örneklerden oluşmuştur.

Turunçgil kabuk yağları bir fırça yardımıyla filetoların her iki yüzeyine sürülmüştür. Filetonun önce bir yüzeyine, biraz kuruduktan sonra (5 dakika) ise diğer yüzeyine sürülmüştür. Daha sonra filetolar köpük tabaklar içine konularak streç filmle kaplanmış ve buzdolabı (4°C±1) koşullarında depolanmıştır. Filetolar depolamanın 0., 3., 6. ve 9. günlerinde mikrobiyolojik, kimyasal ve duyusal özellikleri bakımdan incelenmiştir. Araştırma iki tekerrürlü olarak yürütülmüştür.

Mikrobiyolojik Analizler

Mikrobiyolojik analizler petri yüzeyine yayma metodu kullanarak hesaplanmıştır. Balık örneğinden 25 g tartılmış, sterilstomacher torbaya alınmış ve üzerine 225 ml steril serum fizyolojik (%0,85 NaCl, Riedel-de Haën 13423) ilave edilerek stomacher cihazında (Lab Stomacher Blender 400-BA 7021 Sewardmedical, England) 2 dakika homojenize edilmiştir. *TAMB* ve *TPAB* sayımları için Plate Count Agar (*PCA*, Oxoid CM0325) kullanılmıştır. *TAMB* 30°C'de 2 gün, *TPAB* ise 7°C'de 7 gün inkübe edilmiştir. *LAB* sayımı için Man Rogosa Sharpe Agar (MRS, OxoidCM0361) kullanılmış ve ekimi yapılan petri plakları 30°C'de 2 gün inkübe edilmiştir. *Enterobacteriaceae*sayımı için *VRBD* (Violet Red Glucose) Agar (Oxoid CM0485) kullanılmış ve 30°C'de anaerobik şartlarda 2 gün inkübe edilmiştir (Gokalp vd. 2001).

Kimyasal Analizler

TVB-N (Toplam uçucu baz azotu) Analizi

Toplam Uçucu Bazik Azot (*TVB-N*) miktarı Malle ve Tao (1987) tarafından yapılan yönteme göre belirlenmiştir. Bu yöntemde 40 g balık örneğine 80 ml %7,5'luk *TCA* çözeltisi ilave edilmiş ve Ultra-Turrax (IKA Werke T 25, Germany) ile 1 dakika homojenize edilmiştir. Karışım 5 dakika santrifüj (400xg) edildikten sonra Whatman 3 (Whatman® Schleicher&Schuell CAT No:1001 125) filtre kağıdı kullanılarak süzülmüştür. Elde edilen 25 ml filtrata 5 ml %10'luk *NaOH* (Riedel-de Haën 06203) ilave edildi. Daha sonra distilasyon cihazına (BEHR S1 Steam Distillation Unit Labor-Technik GmbH, Dusseldorf/Germany) yerleştirilip erlen içerisine 10 ml %4'lük borik asit çözeltisi (H_3BO_3 Merck 1.00165.1000) ve 0,04 ml indikatör (0,1 g metil kırmızısı Merck 1.06076.0025)+0,1 g brom kresol yeşili (Merck 1.08121.0005)+100 ml etanol eklenmiş ve 50 ml distilat toplanıncaya kadar distilasyon yapılmıştır. Elde edilen distilat 0,1 N H_2SO_4 çözeltisi ile pembemsi renk oluşuncaya kadar titre edilmiş.

TVB-N miktarı titrasyonda harcanan H_2SO_4 çözeltisi (n) dikkate alınarak aşağıdaki formülle göre hesaplanmıştır.

TVB-N (mg/100g)= n x 16,8 mg azot

***TBARS* (Thiobarbitürik asit reaktif maddeler) Analizi**

TBARS değerinin belirlenmesi Lemon (1975) ve Kılıç ve Richards (2003) tarafından verilen yönteme göre yapılmıştır. Bu yöntemde 2 g örneğe 12 ml *TCA* çözeltisi [%7,5 *TCA* (Trichloroacetic Acid, Riedel-de Haën 27242) %0,1 *EDTA* (Ethylenediaminetetra-acetic Acid, Riedel-de Haën 34549), %0,1 Propil galat (Propyl Gallate, Fluka 48710-3 ml etanolde çözülür)] ilave edilmiş ve 15-20 sn ultra-turrax'da homojenize edildikten sonra Whatman 1 (Whatman® Schleicher&Schuell CAT No:1001 125) filtre kâğıdı kullanılarak süzülmüştür. Süzüntüden 3 ml alınarak deney tüpüne aktarılmış, üzerine 3 ml 0,02 M *TBA* (Thiobarbituric acid, Fluka 88481) çözeltisi eklenmiş ve karıştırılmıştır. Daha sonra deney tüpleri 100ºC'de 40 dakika su banyosunda bekletilerek, 5 dakika soğuk su içerisinde soğutulmuştur. 2000 g'de 5 dakika santrifüj (Hermle ZK 380, Germany) edildikten sonra spektrofotometrede (Shimadzu Corporation, Japan) 530 nm dalga boyunda absorbans okunmuştur. Standardın hazırlanmasında *TEP* (1,1,3,3-tetraetoksipropan) kullanılmış ve k (standart) değeri 0,06 olarak hesaplanmıştır. Sonuç µmol malonaldehit (*MA*)/kg olarak verilmiştir.

TBARS= ((absorbans/ k (0,006) x 2/ 1000 x 6,8) x 1000/örnek ağırlığı

pH Analizi

10 g balık eti örneğine 100 ml saf su eklenmiş ve 1 dakika ultra-turrax'da (IKA Werke T 25, Germany) homojenize edilmiştir. Daha sonra pH değerleri pH-metre (Schott Labstar pH, Germany) ile Gökalp vd (2001) tarafından belirlenen yönteme göre ölçülmüştür.

Duyusal Analiz

Duyusal analiz Kurtcan ve Gönül (1987) yöntemine göre yapılmıştır. Duyusal analiz için 5 kişilik panelist grubu oluşturulmuştur. Balık örnekleri yaklaşık 5 dakika mikrodalga fırında pişirildikten sonra panelistlere sunulmuş ve panelistler örnekleri renk, koku, lezzet ve genel kabul edilebilirlik açısından 1 ile 5 arasında değerlendirmiştir. Puanlamada; 5 çok iyi, 4 iyi, 3 normal, 2 kötü ve 1 çok kötü olarak değerlendirilmiştir.

İstatiksel Analiz

Araştırmadan elde edilen verilerin istatistiksel olarak değerlendirilmesinde SPSS 20.00 (SPSS, Inc., Chicago, IL,USA) paket programı kullanarak varyans analizi yapılmış ve önemli bulunan varyasyon kaynaklarına ait ortalamalar Duncan çoklu karşılaştırma testi ile test edilmiştir.

Bulgular

Mikrobiyolojik Analiz Sonuçları

Farklı konsantrasyonlarda turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı filetolarına ait mikrobiyolojik analiz bulguları Tablo 1'de verilmiştir.

Balık örneklerine ait en düşük *TAMB* sayısı (2,33±0,12 log kob/g) *C* örneğinde belirlenirken, en yüksek *TAMB* sayısı (7,92±0,19 log kob/g) A örneğinde saptanmıştır. Depolama süresince gruplar arasında önemli farklılıklar olduğu belirlenmiştir ($p<0,05$). *TMAB* sayılarının turunçgil kabuk yağları katkılı örneklerde kontrol grubuna daha düşük olduğu görülmüştür.

Toplam psikrotrofik bakteri sayısı depolama günlerine ve gruplara göre önemli farklılıklar göstermiştir ($p<0,05$). En yüksek psikrotrofik bakteri sayısı depolamanın son gününde *A* (8,03±0,02 log kob/g) örneğinde bulunurken, en düşük bakteri sayısı depolamanın 0. gününde *C* (2,69±0,11 log kob/g) örneğinde tespit edilmiştir.

Depolama süresince en yüksek laktik asit bakteri sayısı kontrol grubu örneklerinde (9. gün 5,03±0,12 log kob/g), en düşük bakteri sayısı ise turunçgil kabuk yağları katkılı gruplarda (0. gün 2,00±0,00 log kob/g)tespit edilmiştir ($p<0,05$). Depolama süresi boyunca tüm gruplarda *LAB* sayıları artmıştır.

Enterobacteriaceae sayısı tüm gruplarda günlere göre artış gösterirken, en yüksek değer depolamanın son gününde *A* (4,09±0,14 log kob/g) örneğinde saptanmıştır. Muhafaza süresince gruplar arasında önemli farklılıklar olduğu tespit edilmiştir ($p<0,05$).

Kimyasal Analiz Sonuçları

Farklı konsantrasyonlarda turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı filetolarına ait kimyasal analiz bulguları Şekil 1'de verilmiştir.

TVB-N değerlerinde depolama süresince önemli artışlar saptanmıştır ($p<0,05$). En fazla artış kontrol grubunda görülmüştür. Kontrol grubu örneklerde depolamanın 0. gününde T*VB-N* değeri 16,42 mg/100 g olarak bulunurken, depolama sonunda 26,24 mg/100 g'a ulaşmıştır. *TVB-N* değerinin turunçgil kabuk yağları katkılı örneklerde daha düşük olduğu belirlenmiştir. En düşük *TVB-N* değeri *C* grubunda 10,33 mg/100 g olarak tespit edilmiştir.

En yüksek *TBARS* değeri (5,53 µmol MA/kg) kontrol grubu (*A*) örneklerinde, en düşük *TBARS* değeri (1,21 µmol *MA*/kg) ise %1 limon uçucu yağı ilave edilmiş *C* grubunda belirlenmiştir. Muhafaza

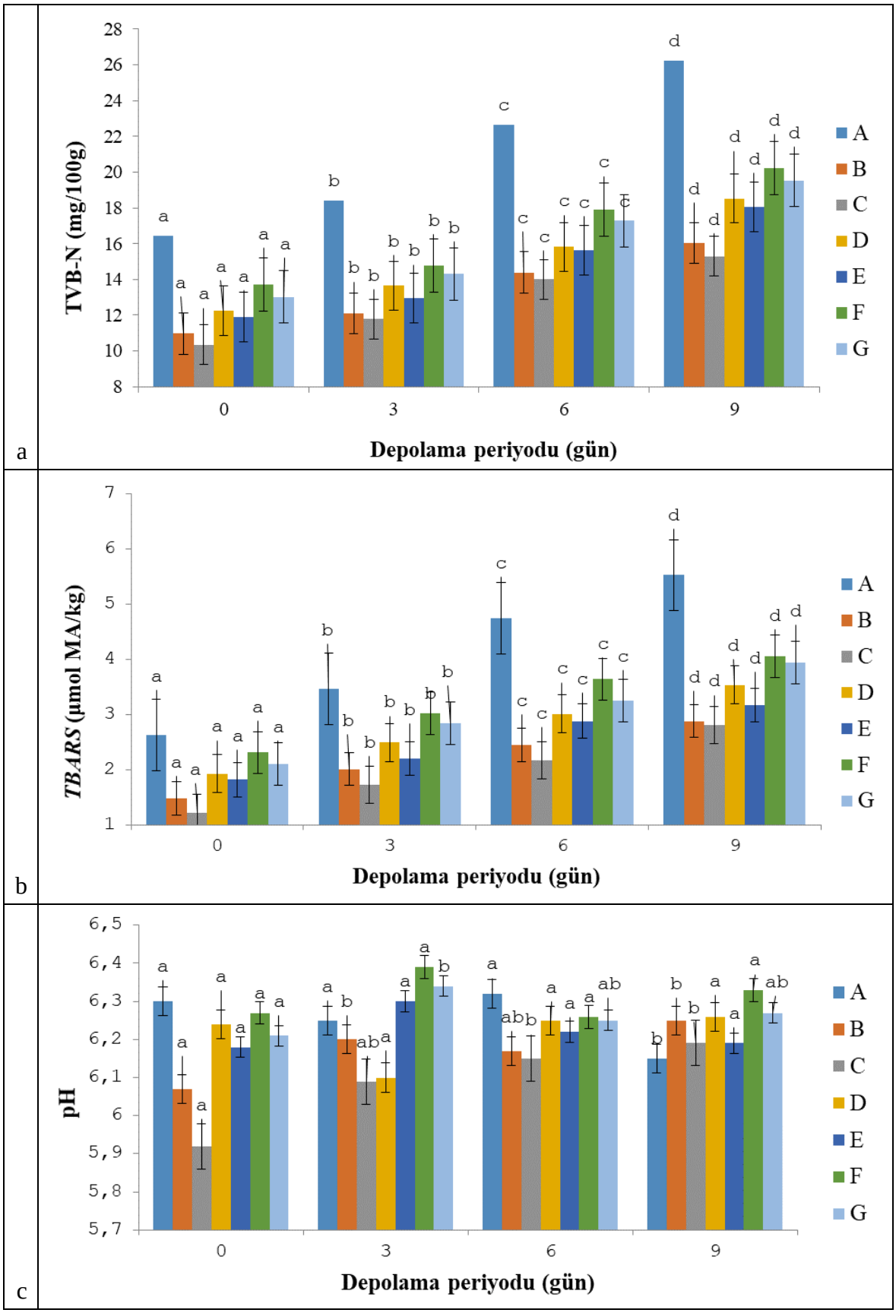
süresince bütün gruplarda zamana bağlı olarak artış görülmüştür. Muhafaza süresi boyunca *TBARS* değeri bakımında gruplar arasında önemli farklılıklar ($p<0,05$) saptanmıştır.

En yüksek pH değeri kontrol grubu örneklerde depolamanın 0. gününde 6,30 olarak ölçülmüştür. Depolama sonunda (9. gün) kontrol grubu örneklerde pH değerlerinde azalma görülmüştür. En düşük pH değeri *C* grubunda 5,92 olarak bulunmuştur. Muhafaza süresi boyunca pH değeri bakımında gruplar arasında önemli farklılıklar ($p<0,05$) tespit edilmiştir.

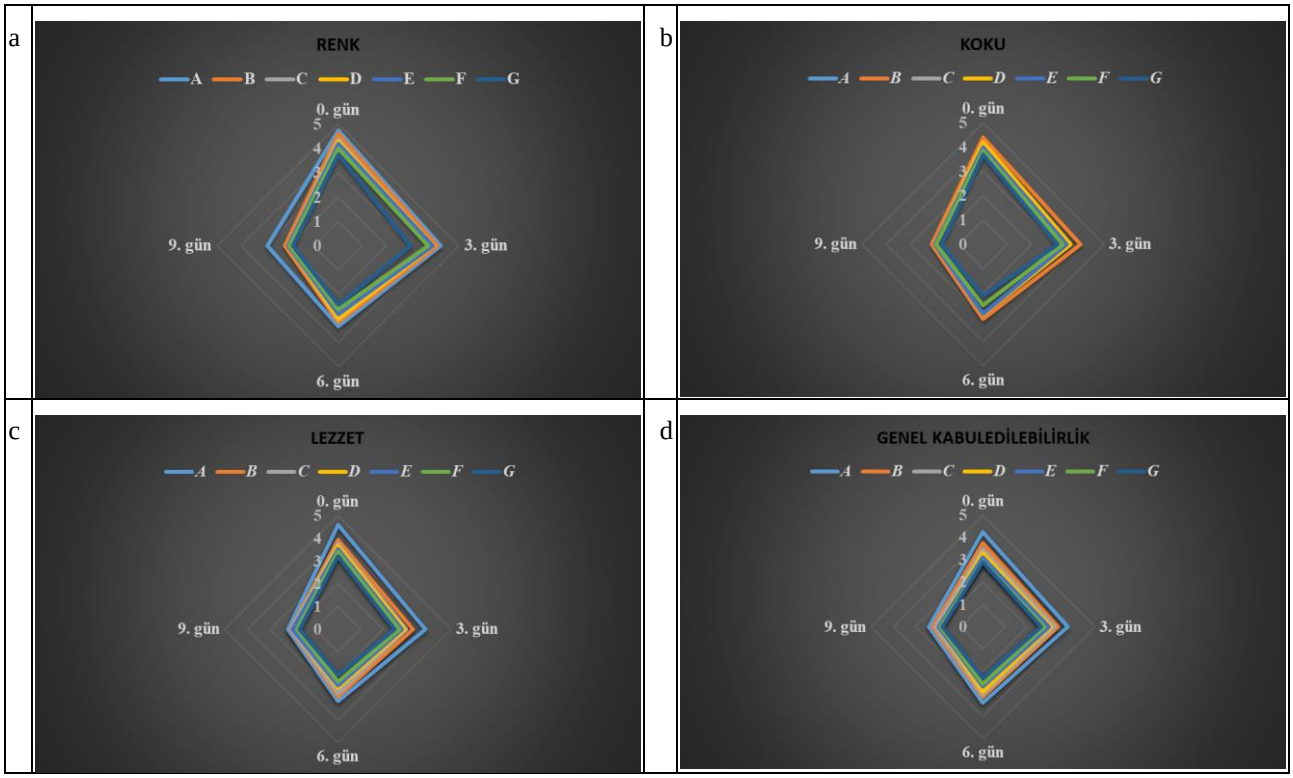
Çizelge 1. Farklı konsantrasyonlarda turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı örneklerinin mikrobiyolojik analiz sonuçları (log kob/g)

Örnek	Muhafaza Günleri	Toplam Aerobik Mezofilik Bakteri Sayısı	Toplam Psikrotrofik BakteriSayısı	Laktik Bakter Sayısı	*Enterobacteriaceae* Sayısı
A	0	4,07±0,03^{a}	4,36±0,06^{a}	2,16±0,06^{a}	2,00±0,00^{a}
	3	5,51±0,05^{b}	5.86±0,05^{b}	2,92±0,09^{b}	2,97±0,04^{b}
	6	6,91±0,08^{c}	7,02±0,02^{c}	3,31±0,14^{c}	3,55±0,13^{c}
	9	7,92±0,09^{d}	8,03±0,02^{d}	5,03±0,12^{d}	4,09±0,14^{d}
B	0	2,54±0,05^{a}	2,88±0,02^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	3	3,05±0,07^{b}	3,27±0,04^{b}	2,25±0,04^{bc}	2,25±0,04^{b}
	6	3.77±0,04^{c}	3,85±0,09^{c}	2,21±0,10^{b}	2,66±0,02^{c}
	9	4.60±0,14^{d}	4,99±0,07^{d}	2,40±0,02^{d}	2,81±0,06^{d}
C	0	2,33±0,02^{a}	2,69±0,11^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	3	2,96±0,05^{b}	3,12±0,04^{b}	2,11±0,04^{a}	2,10±0,08^{a}
	6	2,35±0,07^{c}	3,62±0,04^{c}	2,07±0,09^{a}	2,52±0,07^{b}
	9	4,12±0,04^{d}	4,28±0,02^{d}	2,66±0,14^{b}	2,76±0,07^{d}
D	0	2,74±0,04^{a}	3,11±0,03^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	3	3,26±0,09^{b}	3,70±0,05^{b}	2,66±0,07^{b}	2,48±0,09^{b}
	6	4,13±0,16^{c}	4,03±0,06^{c}	3,02±0,04^{c}	2,82±0,07^{c}
	9	4,88±0,04^{d}	5,29±0,07^{d}	3,30±0,03^{d}	2,95±0,05^{c}
E	0	2,58±0,28^{a}	3,01±0,03^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	3	3,13±0,07^{b}	3,37±0,06^{b}	2,53±0,07^{b}	2,35±0,05^{a}
	6	3,95±0,04^{c}	3,90±0,02^{c}	2,81±0,14^{c}	2,76±0,02^{c}
	9	4,73±0,08^{d}	5,10±0,02^{d}	3,07±0,05^{d}	2,85±0,05^{c}
F	0	3,02±0,04^{a}	3,51±0,06^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	3	3,63±0,07^{b}	4,02±0,04^{b}	3,07±0,04^{b}	2,92±0,05^{b}
	6	4,39±0,06^{c}	4,51±0,10^{c}	3,37±0,05^{c}	3,11±0,04^{c}
	9	5,15±0,06^{d}	5,68±0,03^{d}	3,72±0,08^{d}	3,36±0,04^{d}
G	0	2,91±0,04^{a}	3,31±0,04^{a}	2,00±0,00^{a}	2,00±0.00^{a}
	3	3,40±0,01^{b}	3,92±0,06^{b}	2,92±0,06^{b}	2,66±0,07^{b}
	6	4,20±0,08^{c}	4,33±0,07^{c}	3,21±0,08^{c}	3,01±0,04^{c}
	9	4,98±0,05^{d}	5,60±0,06^{d}	3,49±0,06^{d}	3,11±0,04^{c}

*Aynı sütundaki farklı harfler, Duncan çoklu karşılaştırma testine göre ortalamalar arasındaki önemli düzeydeki farklılıkları göstermektedir ($p<0,05$). Kısaltmalar; *A*: kontrol, *B:* %0,5 limon uçucu yağ katkılı örnek; *C*: %1 limon uçucu yağ katkılı örnek, *D*: %0,5 portakal uçucu yağ katkılı örnek, *E*: %1 portakal uçucu yağ katkılı örnek, *F:* %0,5 bergamot uçucu yağ katkılı örnek, *G*: %1 bergamot uçucu yağ katkılı örnek.



Şekil 1. Farklı konsantrasyonlarda turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı örneklerinin kimyasal analiz sonuçları A: kontrol, B: %0,5 limon uçucu yağ katkılı örnek; C: %1 limon uçucu yağ katkılı örnek, D: %0,5 portakal uçucu yağ katkılı örnek, E: %1 portakal uçucu yağ katkılı örnek, F: %0,5 bergamot uçucu yağ katkılı örnek, G: %1 bergamot uçucu yağ katkılı örnek. Farklı harfler, Duncan çoklu karşılaştırma testine göre ortalamalar arasındaki önemli düzeydeki farklılıkları göstermektedir ($p<0,05$).



Şekil 2. Farklı konsantrasyonlarda turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı örneklerinin duyusal analiz sonuçları *A*: kontrol, *B*: %0,5 limon uçucu yağ katkılı örnek; *C*: %1 limon uçucu yağ katkılı örnek, *D*: %0,5 portakal uçucu yağ katkılı örnek, *E*: %1 portakal uçucu yağ katkılı örnek, *F:* %0,5 bergamot uçucu yağ katkılı örnek, *G*: %1 bergamot uçucu yağ katkılı örnek.

Tartışma ve Sonuç

Bu çalışmayla farklı konsantrasyonlarda (%0,5 ve %1) turunçgil kabuk yağları (limon, portakal ve bergamot) ilave edilen gökkuşağı alabalığı filetolarının buzdolabı (4°C±1) şartlarındaki raf ömrü araştırılmıştır.

Mikrobiyolojik Değişimler

Gıdaların mikrobiyolojik yükü, gıdanın kalitesi ile doğrudan ilişkilidir. Su ürünlerinde raf ömrünün belirlenmesinde mikrobiyolojik analizler en sık kullanılan yöntemlerden biridir (Mutlu ve Bilgin 2016). 9 günlük depolama süresi boyunca örneklere *TMAB*, psikrotrofik, *Enterobacteriaceae* ve *LAB* analizleri yapılmıştır. Mikrobiyolojik analiz sonuçlarına göre en düşük bakteri sayısı %1 limon uçucu yağı ilave edilmiş *C* grubu örneklerinde tespit edilmiştir. Depolama süresi boyunca örneklerin bakteri sayıları artış göstererek, bu artışın istatiksel olarak önemli olduğu ($p<0,05$) bulunmuştur.

Taze balıklarda bulunmasına müsaade edilen toplam bakteri sayısı değeri 7 log kob/g'dır (ICMSF 1986). Bu değere *A* grubu örnekler muhafazanın 9. gününde ulaşırken, diğer gruplar depolama periyodu sonunda bu değerin altında bulunmuştur (Tablo1). Toplam aerobik bakteri sayısının kontrol grubunda depolamanın 9. gününde tüketilemez değere (7,92 log kob/g) ulaştığı saptanmıştır. Can vd. (2011), aynalı sazan (*Cyprinus carpio carpio* L., 1758) balıklarını Öjenol ile farklı sürelerde salamura yaparak, 4°C' de 9 gün süreyle depolamışlar ve *A* grubu (6 saat) örneklerinde depolamanın 9. gününde *TMAB* sayısı 6,19 log kob/g iken, *B* (12 saat) ve *C* (24 saat) grubu örneklerinde sırasıyla 3,03 ve 2,13 log kob/g olarak bulmuşlardır. Bu çalışma sonucu mevcut çalışmayla farklılıklar göstermektedir. Bu durumun uygulanan esansiyel yağ ve balık türünün farklı olmasından dolayı kaynaklandığı düşünülmektedir. Duman vd. (2012) biberiye ve kekik uçucu yağlarının kerevitlerin (*Astacus leptodactylus* Esch., 1823) raf ömrü üzerine mikrobiyolojik etkisinin araştırıldığı çalışmalarında, kontrol grubuna oranla mikrobiyal yükün biberiye ve kekik esansiyel yağı katkılı örneklerde daha düşük olduğunu belirtmişlerdir. Bu çalışma sonuçları yapılan bu çalışma ile benzerlik göstermiştir.

Psikrotrofik bakteri sayısı kontrol grubunda başlangıçta 4,36 log kob/g olarak saptanmıştır. Bu değer muhafazanın 6. gününde yükselerek tüketilemez (7,02 log kob/g) değerine ulaşmış, diğer gruplar depolama periyodu sonunda bu değerin altında belirlenmiştir (Tablo 1). Özyılmaz (2007), farklı dozlarda (5, 20, and 35 µl) püskürtme tekniği ile kekik esansiyel yağı uygulamasının gökkuşağı

alabalığı (*O. mykiss*, Walbaum, 1972) filetolarının psikrofil bakteri faaliyetlerini önemli ölçüde yavaşlattığını belirtmiştir. Emir Çoban vd. (2018) dondurulmuş gökkuşağı alabalığı (*O. mykiss*) filetoları üzerinde yaptıkları bir araştırmada psikrofilik bakteri sayılarının karanfil yağı (%0,5 ve %1v/w) katkılı örneklerde, kontrol grubuna göre daha düşük olduğunu tespit etmişlerdir. Bu çalışma sonuçları mevcut çalışma ile benzerlik göstermiştir.

Kontrol grubunda *LAB* depolamanın 0. gününde 2,16 log kob/g ve depolama sonunda ise 5,03 log kob/g olarak saptanmıştır. Muhafaza süresince örneklerin laktik asit bakteri sayıları artış göstererek, bu artışın istatiksel olarak önemli olduğu ($p<0,05$) belirlenmiştir. *LAB* sayılarının turunçgil kabuk yağları katkılı örneklerde daha düşük olduğu görülmüştür. Duman vd. (2012) laktik asit bakterileri üzerinde biberiye ve kekik esansiyel yağlarının etkili olduğunu bildirmişlerdir. Patır vd. (2015) farklı oranlarda asetik asit ile (%2 ve %4) hazırlanan alabalık (*O. mykiss*) marinatları üzerine %0,1 ve %0,5'lik eugenolün etkisinin araştırıldığı çalışmada laktik asit bakteri sayılarının Eugenol katkılı örneklerde, kontrol grubuna kıyasla daha düşük olduğunu saptamışlardır. Mevcut bu çalışma sonuçları araştırmacıların bulgularıyla benzerlik göstermiştir.

Depolama süresince en yüksek *Enterobacteriaceae* bakteri sayısı kontrol grubu (*A*) örneklerinde, en düşük bakteri sayısı ise %1 limon uçucu yağı ilave edilmiş *C* grubunda bulunmuştur. Muhafaza süresi boyunca tüm gruplarda *Enterobacteriaceae* sayıları artış göstermiş, ancak en fazla artış kontrol grubunda tespit edilmiştir. *Enterobacteriaceae* bakteri sayılarının turunçgil kabuk yağları katkılı örneklerde daha düşük olduğu belirlenmiştir. Mutlu ve Bilgin (2016) sıcak dumanlanmış gökkuşağı alabalığı (*O. mykiss*) ile yapmış oldukları çalışmalarında zeytin yaprağı ve yağ gülü ekstraktlarının *Enterobacteriaceae* sayısı üzerine etkili olduğunu bildirmişlerdir. Aynı şekilde Tassou (1996), limon ve kekik esansiyel yağlarını uygulanmış çipura (*Sparus aurata*) filetolarında enterobakteri sayısının kontrol grubuna kıyasla daha düşük olduğunu rapor etmişlerdir.

Kimyasal Değişimler

Balık ve balık ürünlerinin tazeliğin belirlenmesinde çok fazla kullanılan kimyasal parametrelerden birisi de *TVB-N* değeridir (Köse ve Koral 2005; Oğuzhan 2011; Mutlu ve Bilgin 2016). Depolama periyodu boyunca en yüksek *TVB-N* değeri kontrol grubu örneklerinde, en düşük ise %1 limon uçucu yağı ilave edilmiş *C* grubu örneklerinde saptanmıştır (Şekil 1a). Depolama süresi boyunca tüm gruplarda *TVB-N* değeri artış göstermiş, en fazla artış kontrol grubunda belirlenmiştir. *TVB-N* değerlerinin turunçgil kabuk yağları katkılı örneklerde daha düşük olduğu görülmüştür. Benzer sonuçlar Can vd. (2011) tarafından da bulunmuştur. Özpolat vd. (2017), farklı dozlardaki biberiye esansiyel yağlarının gökkuşağı alabalığı üzerine antimikrobiyel ve antioksidan etkilerini araştırdıkları çalışmalarında, *TVB-N* değerindeki yükselişin en fazla kontrol grubunda, en az yükselişin ise %2 oranında biberiye uygulanan grupta olduğunu ve tüm gruplarda muhafaza süresi ile birlikte *TVB-N* değerinin istatistiki açıdan önemli derecede yükseldiğini belirlemişlerdir ($p<0,05$). Güran vd. (2015) kekik, karanfil ve biberiye yağları uygulanmış palamut (*Sarda sarda* Bloch, 1793) balıkları ile yaptıkları çalışmalarında *TVB-N* değerlerinin kontrol grubunda, diğer örneklere kıyasla daha yüksek olduğunu rapor etmişlerdir.

TBARS değeri, lipit oksidasyonunun derecesinin belirlenmesinde yaygın olarak kullanılan önemli bir kalite kriteridir (Günlü 2007; Oğuzhan 2011). Muhafaza süresine bağlı olarak kontrol grubu ve esansiyel yağ uygulanan gruplarda *TBARS* değerlerinde artışlar tespit edilmiştir. Depolama periyodu boyunca en yüksek *TBARS* değeri kontrol grubu örneklerinde belirlenirken, en düşük %1 limon uçucu yağı ilave edilmiş *C* grubu örneklerinde saptanmıştır (Şekil 1b). Fernandez-Lopez vd. (2005), *TBA* değerlerinin biberiye, limon ve portakal yağları katkılı köftelerde kontrol grubuna kıyasla daha düşük olduğunu ve esansiyel yağların muhafaza süresine de önemli etkilerinin ($p<0,05$) olduğunu vurgulamışlardır. Emir Çoban ve Can (2013) gökkuşağı alabalıkları (*O. mykiss)* ile yürüttükleri bir çalışmalarında farklı konsantrasyonlarda (%0,5 ve %1) biberiye esansiyel yağını kullanmışlar ve *TBA* değerlerinin kontrol grubunda, biberiye yağı ilaveli örneklere kıyasla daha yüksek olduğunu vurgulamışlardır. Her iki çalışmadaki sonuçlar, bu çalışmadan elde edilen değerlerle benzerlik göstermiştir. Duman vd. (2012) biberiye ve kekik uçucu yağları uygulanmış kerevitler (*A. leptodactylus* Esch., 1823) ile yaptıkları çalışmalarında, en yüksek *TBA* değerinin kontrol grubunda, en düşük ise kekik esansiyel yağı uygulanmış örneklerde olduğunu vurgulamışlardır.

Balık etinde pH değerinin 6,0 ile 6,5 arasında değişkenlik gösterdiği ve bu değerin muhafaza süresince bozulma ile birlikte yükseldiği ve tüketilebilirlik sınır değeri olarak da 6,8-7,0 kabul edildiği çeşitli araştırıcılar tarafından bildirilmiştir. pH değeri her zaman kesin bir kriter olmayıp duyusal, kimyasal ve mikrobiyolojik testlerle de desteklenmesi gerekmektedir (Baygar vd. 2004; İnal 2007; Özpolat vd. 2017). Depolama süresi boyunca tüm grupların pH değerlerinin dalgalanmalar

gösterdiği ve istatistiksel olarak pH değerleri arasındaki farkın önemli olduğu bulunmuştur ($p<0,05$). Yerlikaya vd. (2005) yapmış oldukları çalışmalarında hamsi köftelerin pH değerlerinin depolama süresince arttığını rapor etmişlerdir. Duman vd. (2012) kontrol örnekleri ile biberiye ekstraktı uygulanmış örnekler arasında pH değeri bakımından istatistiksel olarak önemli farklılıklar olmadığını bildirmişlerdir. Alçiçek (2011) sıvı tütsülenmiş gökkuşağı alabalığı üzerinde yürüttükleri çalışmalarında kontrol grubu ile farklı konsantrasyonlarda kekik yağı uygulanmış gruplar arasında pH değeri bakımından istatistiksel açıdan önemli farklılıklar olduğu saptanmıştır ($p<0,05$).

Duyusal Değişimler

Panelistler tarafından tüm kriterler (renk, koku, lezzet ve genel kabul edilebilirlik) açısından en fazla kontrol (*A*) grubu örnekleri beğenilirken, en az %1 bergamot uçucu yağı ilave edilmiş *G* grubu örneklerinin beğenildiği belirlenmiştir (Şekil 2). Kullanılan ekstraktların balıkta alışılmadık bir lezzet, koku vermesi ve dolayısıyla genel kabul edilebilirlik açısından tercih etmedikleri düşünülmektedir. Tüm parametreler açısından gruplar arasındaki farkın istatistiksel olarak önemli olduğu tespit edilmiştir ($p<0,05$). Çalışmaya paralel olarak Duman vd. (2012) duyusal analiz sonuçlarına göre panelistlerin en çok kontrol grubu örneklerini beğenirken, en az kekik ilaveli grubu beğendiklerini bildirmişlerdir. Mutlu ve Bilgin (2016) tarafından yapılan başka bir çalışmada ise gül ekstraklı grubun en beğenilmeyen grup olduğu vurgulanmıştır. Emir Çoban vd. (2018) dondurulmuş gökkuşağı alabalığı (*O. mykiss*) filetoları ile yürütülen bir araştırmada karanfil yağı uygulanmış örneklerin duyusal parametreler (koku, renk, tekstür) açısından kontrol grubuna kıyasla daha fazla beğenildiğini rapor etmişlerdir. Sıcak tütsülenmiş gökkuşağı alabalığı (*O. mykiss*) ile yürütülen bir çalışmada balıklar karanfil, sarımsak ve kekik uçucu yağları ilave edilerek buğday proteini gluteninden filmleri ile kaplanmış, vakum paketlenmiş ve +2±2 °C'de depolanmıştır. Uçucu yağlar ilave edilerek kaplama uygulanmış örneklerin kaplama uygulanmamış örneklere kıyasla duyusal (genel görünüş, koku, tat ve doku) açıdan daha çok beğenildiğini bildirmiştir (Akçay 2012). Emir Çoban ve Tuna Keleştemur (2017) tarafından yapılan bir çalışmada *Zataria multiflora* Boiss. uçucu yağının farklı konsantrasyonlarını (%0, %0,2 ve %0,4) kullanarak ürettikleri yayın balığı (*Silurus glanis*, Linnaeus, 1758) burgerlerinde duyusal kriterler (koku, tat, tekstür, genel kabuledilebilirlik) bakımından en yüksek puanı %0,4 uçucu yağ ilave edilerek üretilen burgerlerin aldığı rapor edilmiştir.

Sonuç olarak; turunçgil kabuk yağlarının gökkuşağı alabalığı filetolarının kimyasal ve mikrobiyolojik özellikleri üzerine olumlu etki yaptığı ve raf ömrünün arttırılmasında pozitif sonuçlar verdiği tespit edilmiştir.

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Potential Distribution of the Amphibian Pathogen, *Batrachochytrium dendrobatidis* in the Eastern Black Sea Region of Turkey

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ABSTRACT

Although *Batrachochytrium dendrobatidis*, pathogen for amphibians, has been reported in Anatolia, its geographical distribution, as well as its impact on the amphibians in Turkey, remained obscure. In this study, 62 adult individuals belonging to ten different species (*Pelodytes caucasicus*, *Rana dalmatina*, *Rana macrocnemis*, *Bufo bufo*, *Bufo verrucosissimus*, *Bufotes variabilis*, *Hyla savignyi*, *Pelophylax ridibundus*, *Ommatotriton ophryticus*, *and Mertensiella caucasicus*) were collected from five wetland habitats in Eastern Black Sea Region of Turkey. The prevalence and the intensity of *B. dendrobatidis* infections in all the individuals were investigated by using quantitative Real-time-PCR technique and the presence of *B. dendrobatidis* infection was reported for the first time in 13 of the 62 individuals collected from 10 amphibian species from Eastern Black Sea Region of Turkey. The intensity of *B. dendrobatidis* infection ranged from 403.520 to 534.280 genomic equivalents (*GE*) was detected. The highest *GE* between amphibian species were determined in *P. caucasicus* (534.280 *GE*) in Uzungöl (Çaykara-Trabzon) and *B. bufo* (504.00 *GE*) in Lake Karagöl (Şavşat-Artvin).

Keywords: Chytridiomycosis, *Batrachochytrium dendrobatidis*, Anatolia, Amphibia

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Doğu Karadeniz Bölgesinde Amfibi Patojeni *Batrachochytrium dendrobatidis*'in Potansiyel Dağılımı

Öz: Amfibi patojeni *Batrachochytrium dendrobatidis* Anadolu'da rapor edilmesine rağmen, hem coğrafik dağılımı hem de Türkiye amfibileri üzerindeki etkisi hala belirsizdir. Bu çalışmada, Doğu Karadeniz Bölgesindeki beş sulak alandan on farklı amfibi türlerine (*Pelodytes caucasicus*, *Rana dalmatina*, *Rana macrocnemis*, *Bufo bufo*, *Bufo verrucosissimus*, *Bufotes variabilis*, *Hyla savignyi Pelophylax ridibundus*, *Ommatotriton ophryticus*, *Mertensiella caucasicus*) ait 62 ergin birey toplandı. Kantitatif Real-time PCR tekniği ile tüm bireylerde *B. dendrobatidis* enfeksiyonlarının prevalansı ve yoğunluğu ile araştırıldı ve Doğu Karadeniz Bölgesi'ndeki 10 amfibi türünden toplanan 62 bireyin 13' ünde *B. dendrobatidis* enfeksiyonu varlığı ilk kez rapor edildi. Enfeksiyon yoğunluğu 403,520-534,340 genomik eşdeğerler arasında değiştiği belirlendi. Amfibi türler arasında en yüksek genomik eşdeğer Uzungöl' deki *P. caucasicus* (534,280) ve Karagöl'de *B. bufo* (504,00) saptandı.

Anahtar kelimeler: Chytridiomycosis, *Batrachochytrium dendrobatidis*, Anadolu, Amphibia

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Introduction

Infectious diseases are one of the factors implicated in the declines and extinctions of amphibians in worldwide. *Batrachochytrium dendrobatidis* (*Bd*) is a fungus that colonizes amphibian skin and the associated disease, chytridiomycosis, can disturb cutaneous respiration and osmoregulation and result in the death of the host (Carver et al. 2010). Differences in ecological factors such as host population density, habitat, and age structure may influence the rate at which chytrid spreads through the environment (Daszak et al. 1999). According to Ron (2005), *Bd* was predicted to spread in Anatolia, but the geographic distribution of *Bd* and its effect on Turkish amphibians is poorly understood (Farrer et al. 2011). Though 26 Turkish amphibian species are listed in the International Union for Conservation of Nature (IUCN) Red List,

investigation of the decrease in frog population has become mandatory (Başkale et al. 2013). Only two works were carried out on the distribution of *Bd* in Turkey. Previously, Göçmen et al. (2013) reported that one of two *P. bedriagae* specimens from Göynük Canyon (Antalya) was found as positive for *Bd*. Erişmiş et al. (2014) reported *Bd* infecting wild *P. ridibundus*, *H. orientalis*, *B. variabilis* as well as endemic Beysehir frogs *P. caralitanus* in West Anatolian Region and the District Lakes of South Western Turkey.

Thirty-six amphibian species were recorded in Turkey. Due to Turkey's geographical position, different species spread in different regions and they are exposed to a great number of threats (Şekercioğlu et al. 2011). This includes a number of restricted and rare amphibian species such as *Rana tavasensis* (Franzen et al. 2008), *R. holtzi* (Yildiz and Göçmen 2012), *R. macrocnemis* (Veith et al. 2003), *P. caralitanus* (Bülbül et al. 2011). If such species were susceptible to the fungal infection, the local and isolated populations might easily become extinct. Hence, the location of pathogens and susceptible to species are needed to be determined in Turkey. Management strategies for the containment of *Bd* spreading include the detection of wild and captive populations infected with chytrid disease, the identification of infected geographical areas, and the control of infected animal's movement from one location to another.

Therefore, the main objective of the present study was to determine *Bd* infected amphibian species through quantitative polymerase chain reactions (*qPCR*) (Kriger et al. 2006; Hyatt et al. 2007) in the Eastern Black Sea Region of Turkey.

Materials and Methods

The study was carried out in 6 different areas [Uzungöl (Çaykara-Trabzon, *UZL*), Karagöl (Şavşat-Artvin, *KRL*), Sahara Natural Park (Şavsat-Artvin, *SNP*), Ardeşen (Rize, *ARD*), Lake Şavsat (Şavşat-Artvin, *SVT*)] at 39 to1876 m elevation (*E*) in the eastern Black Sea region (*EBS*) of Turkey (Figure 1).

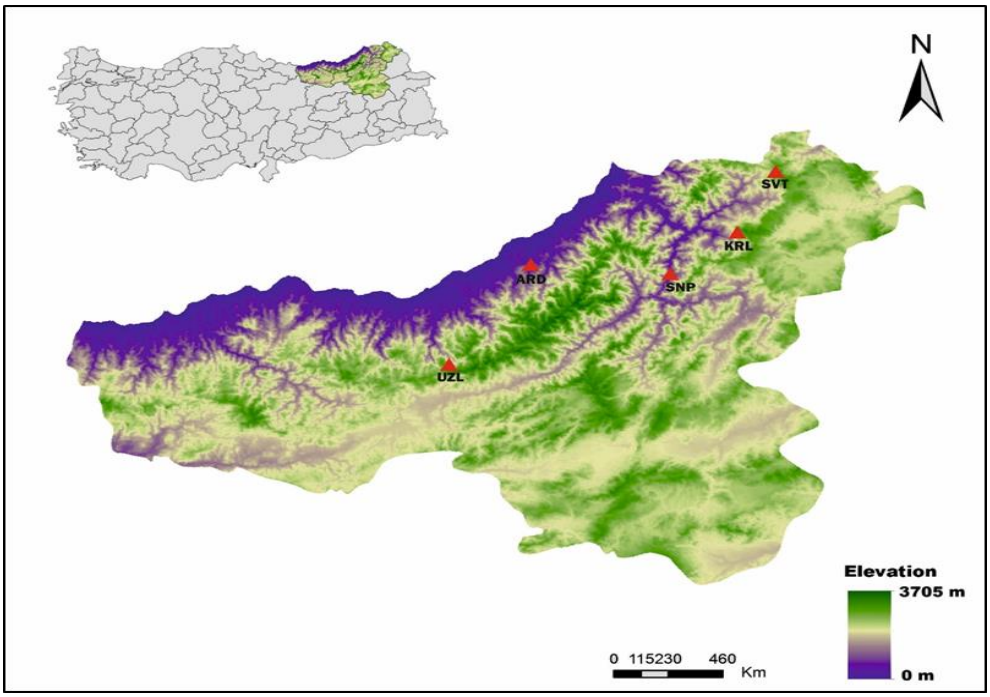

Figure 1. Map showing the collecting sites.

The specimens were collected during the summer season of 2014 (June through August). Air temperature, water pH, and humidity (H) were recorded during the fieldwork (Table 1). For each captured animal, surveyors recorded its GPS coordinates. To prevent the transfer of infected materials among sites, we rinsed all equipment with 5% bleach before entering each location. All of the frogs were handled with latex gloves and gloves were discarded after examination of each animal (Bai et al. 2010). The distribution of zoosporangia of *Bd* was studied in collected 10 species [*P. caucasicus* (*Pc*), *R. dalmatina* (*Rd*), *R. macrocnemis* (*Rm*), *B. bufo* (*Bb*), *B. verrucosissimus* (*Bv*), *B. variabilis* (*Bvs*), *H. savignyi* (*Hs*) *P. ridibundus* (*Pr*), *O. ophryticus* (*Oo*), *M. caucasicus* (*Mc*)].

To determine whether the animal was infected with *Bd* or not by *PCR* analysis, tissue samples were collected using swab method with a sterile cotton tip swab to take the keratinized tissues where *Bd* zoospores were highly concentrated (Marantelli et al. 2004). During *Bd* sampling process, each individual

was swabbed 30 times. We followed the standardized sampling protocol detailed by Hyatt et al. (2007). Samples were stored in 95% ethanol and were kept on ambient temperature (≥10°C) in the field and transported back to the laboratory and stored in a −80 °C freezer (Hyatt et al. 2007). The intensity of infection in all samples was determined by using qPCR (Boyle et al. 2004; Hyatt et al. 2007), with the modifications of methods described by Boyle et al. (2004). It was extracted nucleic acids using 50 µl PrepMan Ultra (Applied Biosystems), and the tip of the swab was used instead of a toe. To ensure the integrity of our results, a negative control (dH_2O) was run in triplicate on every 96-well *PCR* plate (Kriger et al. 2006). We constructed a standard curve to determine the zoospore load. A standard curve was constructed from the control reactions containing 100, 10, 1 and 0.1 *Bd* zoospores and the concentration determined for the test samples expressed as the number of zoospore equivalents. The intensity of infection was measured as the number of genome equivalents (*GE*) per swab, calculated by multiplying the *GE* values generated during the *qPCR* by the dilution factor of the template *DNA*. Swabs were categorized as *Bd* positive at ≥1 *GE* and as *Bd*-negative at <1 *GE* (Kriger et al. 2006; Hyatt et al. 2007; Anna et al. 2011; Erismis et al. 2014). All analyses were performed in triplicate. The percentages of infected individuals and *GE* were not compared among within sites or the species due to the low statistical power of small sample sizes for each species at a site. In the localities with positive *Bd*, we used the zonal statistic routine to extract from the digital maps the environmental variables values from each point (ArcView 3.2, Spatial Analyst). These values were also used to run Principal Component Analysis (*PCA*; implemented in XLSTAT v.3.0) to visualize the degree of clustering in environmental space among *EBS* region of Turkey where *Bd* was found.

Results

We swabbed 62 individuals from the 8 genera, including 10 species that occur in *EBS* region of Turkey. The prevalence and the intensity of *Bd* infections in all the individuals were investigated by using quantitative real-time-*PCR* technique and the presence of *Bd* infection was reported for the first time in 13 of the 62 individuals collected from 10 amphibian species from *EBS* Region of Turkey (Figure 2, Table1).

Bd was not detected only in *ARD* region. We determined the presence of *Bd* infection in 13 out of 62 (20.9%) samples comprising six species: *Pelobates caucasicus* (*Caucasian type-specific), Bufo bufo, B. verrucosissimus, Bufotes variabilis, Pelophylax. ridibundus, Ommatotriton ophryticus* (Table 1).

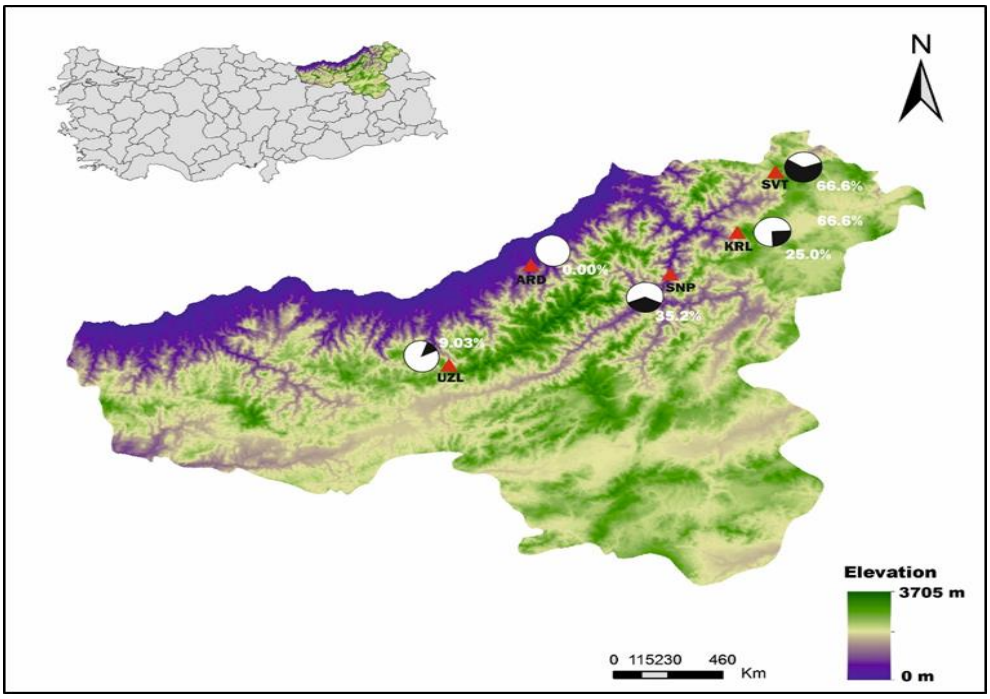

Figure 2. Map of *Bd* prevalence on EBS of Turkey. EBS from all states sampled tested positive and negative for *Bd*, Positive (black) and negative (white) proportions by the state were indicated by pie charts.

Table 1. Prevalence (*Prev*) and Average Genomic Equivalents (*GE*) of *Bd* in Eastern Black Sea Region of Turkey

Species	Locality	Coordinates	*Air* ˚*C*	*H* %	*E* *(a.s.l)*	*N* *(+ve)*	*Prev.* *(%)*	*GEs*
Pc	*UZL*	40˚37′23″N - 40˚16′50″E	26.0	56.0	1164	12(2)	16.0	527±16
Rd	*UZL*	40˚36′24″N - 40˚18′48″E	19.5	78.0	1271	1(0)	-	-
Rm.	*UZL*	40˚35′18″N - 40˚21′19″E	18.5	82.0	1701	2(0)	-	-
Bb	*UZL*	40˚37′07″N - 40˚18′45″E	19.5	78.0	1272	4(0)	-	-
	KRL	40˚56′20″N - 43˚23′30″E	27.0	65.0	1600	4(1)	25.0	504.00
Bv	*UZL*	40˚37′11″N - 40˚17′36″E	19.5	78.0	1278	10(1)	10.0	436.00
Mc	*UZL*	40˚35′27″N - 40˚20′45″E	27.5	72.3	1702	3(0)	-	-
Bvs	*SNP*	41˚14′24″N - 41˚13′12″E	23.0	77.0	1876	2(1)	0.50	463.00
Hs	*ARD*	41˚11′13″N - 40˚59′19″E	28.0	68.0	39.0	2(0)	-	-
Pr	*SNP*	41˚14′24″N - 41˚13′12″E	23.0	77.0	1876	15(5)	33.3	490.00
	KRL	41˚18′32″N - 42˚28′57″E	26.0	65.0	1600	4(1)	25.0	480.00
Oo	*SVT*	41˚17′47″N - 42˚28′37″E	30.5	66.0	1409	3(2)	66.6	405±9.0
Total						62(13)	20.9	473.66±18.44

Sampling localities and examined frog species abbreviations used*; UZL:* Uzungöl/Trabzon, *KRL:* Karagöl/Artvin, *SNP:* Sahara Natural Park/Artvin, *ARD:* Ardeşen/Artvin, *SVT:* Savsat Lake/Artvin*; Pc: Pelodytes caucasicus, Rd: Rana dalmatina, Rm: Rana macrocnemis, Bb: B. bufo, Bv: Bufo verrucosissimus, Mc: Mertensiella caucasicus; Bv: B. variabilis, Hs: Hyla savignyi, Pr: Pelophylax ridibundus, Oo: O. ophryticus respectively*, and *H:* Humidity, *E:* Elevation. *GE* genomic equivalent (including positive samples and negative samples, *GE* represents the burden of infection with *Bd).*

Bd was detected at 4 of 5 sites in *EBS* Region of Turkey were not being specifying *Bd* may be due to the small number of samples (Figure 1). *B. bufo* were sampled at two locations (*UZL* and *KRL*), only one of these locations tested positive for *Bd.* Although *P. ridibundus* were sampled at two regions (*SNP* and *KRL*) but 6 tested positive for *Bd*. The population of both *P. ridibundus* and *B. bufo* (at *KRL*) with a prevalence of 25%. However, we detected *Bd* in only one specimen of *B. verrucosissimus* (n=10). Furthermore, we did not detect any *Bd* on *R. dalmatina, R. macrocnemis, B. bufo,* and *M. caucasicus* (at *UZL*), *H. savignyi* (at *ARD*). In addition, Northern banded newts (*O. ophryticus)* were notable for their highest *Bd* infection rate at *SVT* with the prevalence of 66.6 % than other frog species (Table 1). Therefore, the prevalence of *Bd* infection on the populations of 10 frogs species among *EBS* regions (*UZL, KRL, SNP, SVT*) did differ significantly ($\chi 2$ = 8.43, *df* = 3, $P_{0.05}$> 0.03).

We also detected the rate of *Bd* infection as the mean number of *GE* per sample in 3 replicates. The mean number of *GE* for individual positive samples ranged from 405±9.0 (for O. ophryticus at *SVT* region) to 527±16.0 (for *P. caucasicus* at *UZL* region). The highest intensity of zoospores was found at *P. caucasicus* (527±16.00) at *UZL* region followed by *B. bufo* (504.00) at *KRL* region (Table 1). However, the average GEs among the four regions (*UZL, KRL, SNP, SVT*) individuals of frogs infected by *Bd* were analyzed through multiple comparisons based on a Tukey-HSD post-hoc test, which indicated a not significant difference among them (F = 3.27, *df* = 3;9, p = 0.08). Increasing suitability for both prevalence and *GE* of *Bd* was widely distributed on *EBS* regions of Turkey but was detected lowest in the *ARD* region (Tablo 1).

Final map resulted with areas highly suitable for the presence of *Bd* (Niche Overlap Index (*NOI*) > 0.70) dispersed irregularly overall *EBS* Region of Turkey (Figure 3). There are localities with highly suitable for the fungus in north regions of phytogeographic provinces of Trabzon and Artvin (Northeast of Turkey). *NOI* was varied among regions and was represented by different percentages of covered surface. Areas with 0.70 > OI < 1 (highest suitability for chytrid development) covered only 32.72 % of the total surface while areas with 0.50 > OI < 0.70 covered 56.86 %. Areas with 0.00 > OI < 0.50 only 10.42 of the total surface of *EBS* region of Turkey (Figure 3).

With eigenvalues >1, Principal Component I was positively correlated with (1) Elevation, (2) mean annual temperature, (2) precipitation of wettest explaining 89.6% of the variance of the system. Principal Component II explained 29.0% and was

highly positively correlated only with seasonal temperature. The environmental variables in the localities where *Bd*. was found show that suitable locations for the fungus are possible across a wide range of habitats (Table 1). In the localities with known presence of the fungus, the annual mean temperature ranged from 5℃ (*UZL* and *SVT*) to 23.5℃ (*SNP* and *SVT*), moreover in areas where the OI = 0.89. In addition, our analysis shows that the presence of the fungus in *EBS* region of Turkey is related to precipitations between 68 mm (*UZL* and *SVT*) and 235 mm (*UZL* and *SVT*).

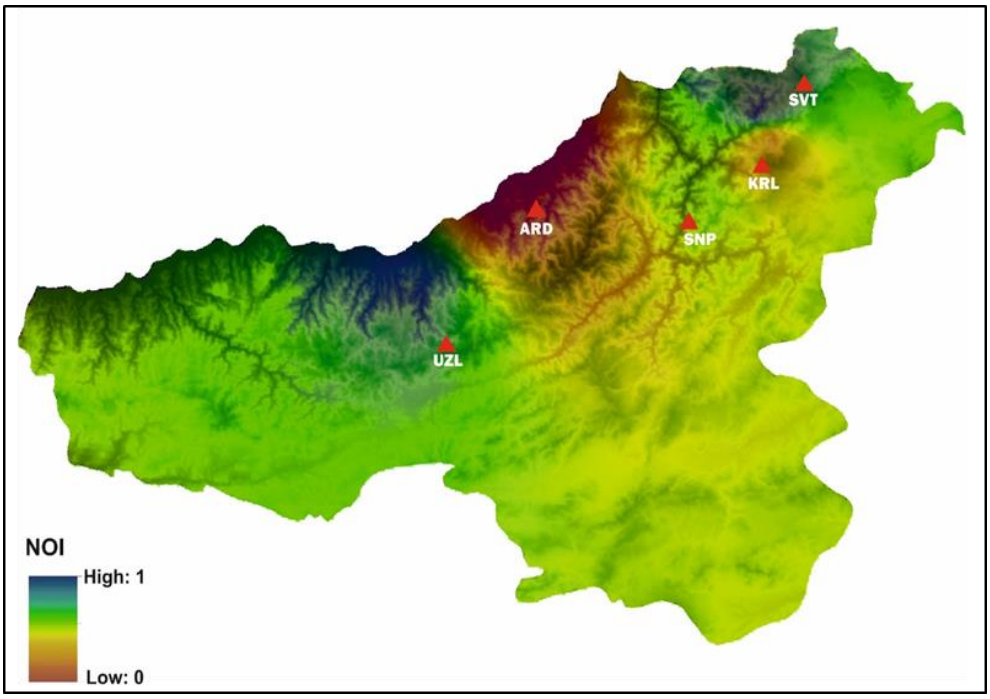

Figure 3. Niche Overlap Index (*NOI*) map of the potential distribution of *Bd* in *EBS* region of Turkey

Discussion

Distribution models showed that *Bd* has likely already spread to most climatically suitable regions (Fisher et al. 2009). Turkey's diverse regions have different climates because of irregular topography. Black Sea region has an oceanic climate (Köppen climate classification: *Cfb*), wet and humid (summer 23°C, winter 7°C) (Sensoy et al. 2008). Our previous study suggests that the Anatolian climate is indeed favorable for the spread of chytridiomycosis (Erismis et al. 2014). Intercalarily, *Bd* has been found in five mainlands including North and South America, Europe, Oceana, Africa, and Asia. However, there have only been two published studies up to date describing the presence of *Bd* in Anatolia at the crossroads of three continents. Therefore, it is not surprising that the *Bd* present in Anatolia.

In this study, a total of 62 specimens from 8 genera, including 10 species was sampled. We detected the presence of *Bd* infection in 13 of 62 (20.09%) samples comprising four species: *P. caucasicus* (*Caucasian type-specific), B. bufo, B. verrucosissimus, P. ridibundus, O. ophryticus* (Table 1). *Bd* was detected at 4 of 5 sites. We detected *Bd* in only one from ten swamp samples of *B. verrucosissimus*. We did not detect any *Bd* on *R. dalmatina, R. macrocnemis, B. bufo* (at *UZL*), *B. variabilis, H. savignyi* and *M. caucasicus*. In addition, Northern banded newts (*O. ophryticus)* were notable for their higher *Bd* infection rate at *SVT* prevalence of 75% than other frogs species. *P. ridibundus* were sampled at two locations (*SNP* and *KRL*) but 6 tested positive for *Bd.* We found a moderate *Bd* rate (20.09 %, *N* = 62) and low zoospore loads (473.66±18.44). The number of zoospores increases during infection. Low numbers may represent an earlier stage of infection, but the shedding of the skin may also contribute to low counts. We observed a widespread prevalence of *Bd* zoospore in apparently healthy adult amphibians in the study areas. Recent work suggests that *Bd* may produce tiny, non-pathogenic resting spores that attach to the amphibian skin surface but without causing disease (Di Rosa et al. 2007). The competing hypothesis contends that chytrid is endemic to many regions and that climate or other factors have altered the host-pathogen relationship, resulting in the recent outbreaks of chytridiomycosis (Morehouse et al. 2003; Weldon et al. 2004). As is also known adults may be protected by acquired immunity (Richmond et al. 2009) and thus may clear or prevent infections more efficiently than juveniles that are naive to *Bd,* infects some amphibian species with little negative effects on the host and do not die therefore may serve

as reservoirs of the disease (Mazzoni et al. 2003). Many amphibian species such as *Xenopus spp*, *R. catesbeiana*, and *B. marinus* carry this disease, also terrestrial species of anurans have been observed with *Bd*, suggesting frog to frog transmission is possible (Kriger et al. 2007).

Differences in morbidity and mortality in experimentally infected amphibians indicate that *Bd* virulence can vary between strains of the same and different lineages. Increased *Bd* growth rate, zoospore production, and sporangial size in pure culture, have been linked with increased host mortality and immunosuppressive activity (Fisher et al. 2009). Our studies suggested that amphibians can evolve resistance to *Bd* and may have the ability to coexist with the disease. The Eastern Black Sea region has unique reptile fauna. These regions are the corridors of the species coming from the Caucasus and the south. The high Anatolian diagonal mountains are a barrier to colonization (Ansell et al. 2011). This study showed that Anatolian diagonal mountains are not a barrier for the colonization of *Bd* in Anatolia. If amphibians can evolve resistance to *Bd* and may have the ability to coexist with the disease, testing for the presence of *Bd* should be mandatory other regions of Turkey.

In conclusion, the uncertain distribution and potential impact of *Bd* presence in Turkey require additional investigation before accurate evaluations can be made. Standardized field surveillance methods and laboratory diagnostic techniques are needed to more carefully investigate. The presence, distribution, virulence to native species and clade membership of *Bd* in Turkey must be verified before its potential impact on Anatolian amphibians can be accurately predicted.

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Dietary Protein Requirements of Zebrafish (*Dania rerio*)

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ABSTRACT

Zebrafish (*Danio rerio*) with an initial weight of 88.61±0.82 mg were fed eight isoenergetic diets containing dietary protein levels ranging from 20 to 55 % by 5 % increments. Each diet was feed in triplicate of fish for 6 weeks. Specific growth rates (*SGR*) at week 2 and 4 were quadratically affected by the treatments but this trend disappeared at the end of the experiment. Dietary protein levels linearly reduced the values of daily feed intake, feed conversion ratio and protein efficiency rate. The whole body dry matter, ash and lipid concentrations linearly decreased with dietary protein levels whereas whole body protein was quadratically affected. The second order polynomial and two break point linear models (*TBPLM*) were used to estimate dietary protein requirements. The later model generated lower residual sum of squares when SGR_{Week4} and SGR_{Final} values were used as a response. Minimum dietary protein requirements for SGR_{Week4} and SGR_{Final} were estimated by the *TBPLM* as 27.69 and 28.93 % respectively. Briefly, results of the study suggest a minimum dietary protein requirement of zebrafish is about 29 % for maximum growth rate.

Keywords: Zebrafish, dietary protein, growth, feeding

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Zebra Balığının (*Dania rerio*) Diyetsel Protein Gereksinimi

Öz: Ortalama başlangıç ağırlığı 88,61±0,82 mg olan zebra balıkları (*Danio rerio*) protein düzeyi % 20-55 arasında değişen sekiz adet izoenerjitik yemle beslenmiştir. Her bir deneme yemi üç tekrarlı olarak 6 hafta boyunca balıklara verilmiştir. Spesifik büyüme oranı (*SGR*) 2. ve 4. haftalarda kuadratik olarak etkilenirken, bu eğilim deneme sonunda kaybolmuştur. Protein düzeyleri arttıkça yem tüketimi, yemden yararlanma oranı ve protein etkinlik oranı doğrusal olarak düşmüştür. Tüm vücut kuru madde, kül ve lipit konsantrasyonları diyetsel protein düzeyinin artışı ile doğrusal olarak düşmüş, vücut protein düzeyi ise kuadratik olarak etkilenmiştir. Diyetsel protein gereksinimlerini tahmin etmek için, ikinci derece regresyon ve iki kırıklı linear model (*İKLM*) kullanılmıştır. 4. hafta ve deneme sonu *SGR* değerleri kullanıldığında *İKLM* daha düşük kalıntı kareler toplamı vermiştir. 4. hafta ve final *SGR* oranlarına göre, *İKLM* minimum protein gereksinimlerini sırasıyla, % 27,69 ve % 28,93 olarak tahmin etmiştir. Kısaca, çalışma bulguları zebra balıklarının maksimum büyüme için minimum protein gereksinimlerinin yaklaşık % 29 olduğunu göstermektedir.

Anahtar kelimeler: Zebra balığı, diyetsel protein, büyüme, yemleme

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Introduction

Zebrafish is used in a wide range of scientific disciplines as a model animal. Basic culture requirements particularly nutritional needs of zebrafish however are still incomplete (Lawrence 2007; Ulloa et al. 2014). Existing literature about zebrafish nutrition has dealt with some topics including the evaluation several diet types and protein sources (*Artemia*, paste liver, flake, commercial trout and experimental diets) in terms of reproductive and growth performance (Markovich et al. 2007; Siccardi III et al. 2009; Smith Jr et al. 2013), biotin requirements (Yossa et al. 2014) and effects of dietary carbohydrate levels on growth and nutrient utilization performance and hepatic transcriptome by sexes (Robison et al. 2008), although there are some others.

Dietary protein level in fish is considered as one of most important criterions since it is most the expensive nutrient and affects a number of functions from molecular level to growth related traits (Lawrence 2007; NRC 2011; Ulloa et al. 2011; Ulloa

et al. 2014). Despite its fundamental importance in nutritional physiology, dietary protein requirement of juvenile zebrafish has been studied recently by Fernandes et al. (2016), who estimated the minimum dietary requirements between 37.6 and 44.8% for maximum weight gain and protein retention using a four-parameter saturation kinetics model (*SKM*) and broken line model (*BLM*). O'Brine et al. (2015) also studied protein and lipid requirements of older zebrafish (*ca.* 4 months) and reported using ANOVA that diet with 32% dietary and 8% lipid can be sufficient for growth. Growth rate of zebrafish can vary greatly by laboratories, populations and batches (Eaton and Farley 1974), plus the estimations dietary requirements of fish are subjected to huge variations due to the selected statistical model and response variables (Hernandez-Llamas 2009; NRC 2011). Therefore, a six-week feeding trial with juvenile zebrafish from 42 to 84 days post hatching was planned to estimate dietary protein requirements.

Materials and Methods

Fish and rearing system

The experiment was carried out at the Kepez Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. A total of 720, 35day post hatching zebrafish (pink type) were randomly allocated in groups of 30 across 24, 10L tanks. Fish were acclimated for a week and fed a commercial rainbow trout diet with 60 % protein and 10 % lipid and 150-300 µm particle diameter (Bioaqua, Çamlı Yem, İzmir, Turkey). The average individual weight per tank was 88.61 ± 0.82 mg and the age was 6 weeks.

The experimental tanks were connected to a recirculation system. Daily water renewal rate of the system was 30 %. Each tank was given 100 mL/min of water and provided with aeration using one air stone. Average water temperature, oxygen, pH, NH_4-N and NO_2-N concentrations in the system over the experiment were checked twice a week and were 24.87±0.49°C, 7.65±0.06 mg/L, 8.52±0.06, <0.02 mg/L and 0.013±0.003 mg/L, respectively. A natural photoperiod was applied as 13-14 h L: 11-10 h D.

Fish were biweekly weighed in bulk after an anesthetization with ethylene glycol monophenyl ether (0.3 mL/L). Feed was withheld on the weighing days. Feed particle diameters were 300-500, 500-800 and 800-1000 µm during 0-2, 2-4 and 4-6 weeks of the experiment. Fish were fed *ad libitum* by hand twice a day at 09:00 and 16:00 h. Each feed was tried in triplicated tanks and was carefully administered until the feeding activity ceased. At the start of the experiment, a composite sample of five fish per tank were taken for initial body composition whereas at the end of the experiment, all fish per tank were sacrificed by an overdose of ethylene glycol monophenyl ether (1.2 mL/L) for final proximate analysis.

Experimental diets

Diets were formulated based on dry matter basis using the linear method in Winfeed 2.8 (Winfeed Ltd., Cambridge, UK). Eight isoenergetic diets (18 MJ/kg gross energy (*GE*)) were formulated to provide crude protein (*CP*) levels from 20 to 55 % by 5 % increments (Table 1). The dietary protein level was increased by adjusting the fraction of the fish meal in the diet. Fish meal was used were used as primary protein source whereas a 1:1 mixture of soybean meal and corn gluten meal was used as secondary protein source. Wheat starch and sunflower oil served as carbohydrate and lipid sources, respectively.

All the dietary ingredients were ground with a hammer mill (Kocamaz Machine, Model KT-20C, İzmir, Turkey), weighed at predetermined levels, thoroughly mixed and then extruded into 2 mm using a pasta machine (model P3, La Monferrina, Italy). The resulting material was air dried at a room.

Calculation and chemical analysis

Daily feed intake (*DFI* g/kg *MBW* / day) = (dry matter intake / $MBW^{0.8}$) / day

Metabolic body weight (*MBW*) = (Geometric mean of initial weight (*IW*) and *final weight* (*FW*))$^{0.8}$

Specific growth rate (*SGR*) = 100 × [(*ln FW* – *ln IW*)/day

Daily feed intake (mg/kg $MBW^{0.8}$/day^{-1}) = (dry feed intake / $MBW^{0.8}$) / days

Feed conversion ratio (*FCR*) = dry matter intake / weight gain

Protein efficiency ratio (*PER*) = weight gain / protein fed

Daily nutrient intake (g/kg $MBW^{0.8}$/day^{-1}) = [(protein, energy intake / $MBW^{0.8}$) / days.

Daily nutrient gain (g/kg $MBW^{0.8}$/day) = [(final body weight ×final body nutrient) – (initial body weight × initial body nutrient)] / $MBW^{0.8}$ / days.

Nutrient retention (%) = 100 × (daily nutrient gain / daily nutrient intake).

Fish samples were stored at -20 °C until analysis. Prior to analysis, they were chopped into very tiny pieces using knife. Proximate analysis, except crude lipid, of experimental diets and fish were performed according to the methods of AOAC (1990): dry matter at 104 °C till constant weight, ash content by incineration in a muffle furnace at 600 °C for 2 h; CP (N×6.25) by the Kjeldhal method after acid digestion.

Lipid was determined with ether-extraction using an automatic extraction system (ANKOMXT15 Extractor, ANKOM Technology, Macedon, USA).

Statistical analysis

Polynomial contrasts were used to detect linear and quadratic effects of dietary protein levels on the observed response variables. Significant treatment effects were considered at P≤0.10. Statistical analyses were conducted in JMP v.8.0 (SAS Institute Inc. 2008). To estimate dietary protein requirements for average SGR_{Week4} and SGR_{Final}, two models were tested using GRAPHPAD PRISM 5 for Windows (GraphPad Software, San Diego, CA, USA): second order polynomial regression and two-break points non-linear model (*TBPLM*). The latter is a combination of conventional broken line model (Hernandez-Llamas 2009) with a negative linear regression at the right side of the response curve. The optimum dietary protein levels were defined based on the model fitting best in terms of the residual sum of squares (Hernandez-Llamas 2009).

The equations of second order polynomial regression (1) and *TBPLM* (2, 3 and 4) are given below.

$$y = i_1 + b_1 x + b_2 x^2 \quad (1)$$

where $i_1$is intercept, b1 and b2 is are the regression coefficients (Shearer 2000).

$$y = i_1 + b_1 x \quad \text{if } x < x_{\text{bp}}, \quad (2)$$

$$y = y_{max} + b_2 x \quad \text{if } x \geq x_{\text{bp}}, \quad (3)$$

$$y = i_2 + b_3 x \quad \text{if } x > x_{\text{bp}} \quad (4)$$

where i_1 and b_1 are parameters describing the positive linear relation, y_{max} is the maximum response and i_2 and b_3 are parameters of negative linear relation. To assume a constant response, the slope at the plateau (b_2) was set at zero.

Table 1. Formulation and nutrient composition of experimental diets (% dry matter)

Ingredients	20P	25P	30P	35P	40P	45P	50P	55P
Fish meal	19.87	26.49	30.90	37.51	41.92	48.54	52.95	59.56
Soybean meal	2.14	3.06	3.67	4.59	5.20	6.12	6.73	7.65
Corn gluten meal	2.14	3.06	3.67	4.59	5.20	6.12	6.73	7.65
Wheat starch (Cooked)	64.15	54.83	48.61	39.28	33.07	23.74	17.53	8.20
Sunflower oil	8.21	7.53	7.08	6.40	5.94	5.26	4.81	4.13
MCP[1]	2.24	2.07	1.96	1.80	1.68	1.52	1.41	1.24
Mineral mixture[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin mixture[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
CMC[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alpha cellulose[5]	0.00	1.72	2.87	4.59	5.73	7.45	8.60	10.32
Nutrient levels (% dry matter)								
Dry matter	91.41	91.74	91.64	91.89	92.31	92.24	92.66	92.49
Crude ash	5.75	6.73	6.94	7.63	8.21	8.98	9.71	10.51
Crude lipid	10.26	10.02	10.32	10.94	10.62	9.97	10.28	10.34
Crude protein	20.38	26.22	28.93	34.94	39.56	44.20	49.72	56.88
Gross energy (*MJ*/kg)	19.82	19.69	19.70	19.81	19.74	19.47	19.58	19.62
Protein energy ratio (g/*MJ*)	10.28	13.32	14.69	17.64	20.04	22.70	25.39	28.99

Results

All experimental groups more than tripled their initial weights during the 6-week experiment (Table 2). There was a weak quadratic effect of dietary protein levels on 4th week weight (quadratic, P=0.104) but it disappeared at the final. *SGR* values at week 2 and 4 were quadratically affected by the treatments (quadratic, P=0.025 and P=0.060 respectively), which also vanished at the end of the experiment (linear, P=0.666 and quadratic, P=0.213) (Table 2). Dietary protein levels had a strong linear effect on daily feed intake, *FCR* and *PER* (linear, P=<0.001).

The second order polynomial model generated 0.0148 and 0.0293 of residual sum of squares for SGR_{Week4} and SGR_{Final} respectively, whereas the *TBPLM* yielded lower levels with 0.0128 and 0.0248. Minimum dietary protein requirements for SGR_{Week4} and SGR_{Final} were estimated by the *TBPLM* as 27.69 and 28.93% respectively (Figure 1).

Table 2. Growth, and nutrient utilization performance of zebrafish fed varying dietary protein levels

Diets	*IW* (mg/ fish)	*W* at 2nd week (mg/fish)	*W* at 4th week (mg/ fish)	*W* at final (mg/fish)	*SGR* at 2nd week (%/day)	*SGR* at 4th week (%/day)	*SGR* at final (%/day)	Daily feed intake (g/ kg^{-} $MBW^{0.8}$ /day)	*FCR*	*PER*
20P	86.81	117.70	195.29	293.86	2.34	2.90	2.90	42.74	2.00	2.46
25P	88.32	122.92	203.80	300.03	2.54	2.99	2.91	38.70	1.81	2.12
30P	86.36	123.64	203.25	310.87	2.76	3.06	3.05	37.66	1.66	2.08
35P	88.61	122.80	205.94	312.20	2.50	3.01	3.00	34.89	1.56	1.83
40P	90.25	126.40	208.53	320.32	2.60	3.00	3.02	32.53	1.44	1.76
45P	88.64	120.27	197.80	291.18	2.35	2.87	2.83	28.44	1.37	1.66
50P	88.36	120.66	201.11	306.55	2.39	2.94	2.96	29.55	1.35	1.50
55P	91.57	120.73	198.78	306.74	2.13	2.77	2.88	24.80	1.16	1.52
Pooled SEM	2.532	3.512	5.647	10.08	0.152	0.094	0.075	0.764	0.053	0.059
P values	Linear	0.768	0.844	0.471	0.109	0.170	0.666	<0.001	<0.001	<0.001
	Quadratic	0.136	0.104	0.277	0.025	0.060	0.213	0.114	0.017	<0.001

IW; initial weight, *W*; weight, *SGR*; specific growth rate, *MBW*, metabolic body weight, *FCR*; feed conversion rate, *PER*; protein efficiency ratio, Pooled *SEM*, standard error of the means.

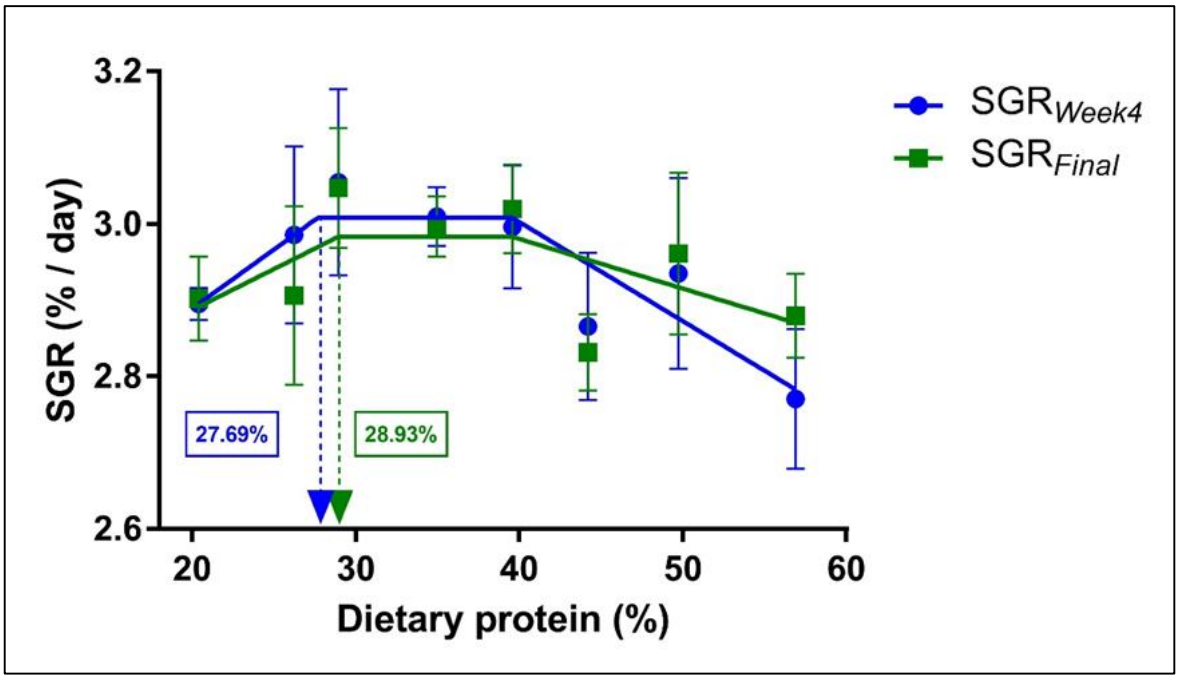

Figure 1. Effect of dietary protein levels on SGR_{Week4} and SGR_{Final} values in zebrafish. Values are represented as the mean *SEM* of three replicates. *SGR*; specific growth rate.

The whole body dry matter, ash and lipid concentrations linearly decreased with dietary protein levels (P= <0.001) whereas whole body protein was quadratically affected (P=0.050) (Table 3).

Daily protein and energy intakes by zebrafish quadratically decreased in response to dietary protein (linear, P=<0.001; quadratic, P=<0.010) (Table 4). On the other hand, no effect of dietary protein levels was observed on daily protein gain. Dietary protein levels linearly decreased daily energy gain and energy retention of zebrafish (linear, P=<0.001), whereas quadratically decreased protein retention (linear and quadratic, P=<0.001).

Table 3. Whole body compositions of zebrafish fed varying levels of dietary protein (%)

Diets		Whole body dry matter	Whole body ash	Whole body lipid	Whole body protein
Initial		27.01	2.79	7.49	14.49
20P		30.95	3.09	9.82	16.14
25P		30.43	3.03	9.08	16.38
30P		29.96	3.09	8.55	16.14
35P		29.69	2.73	8.31	15.77
40P		30.09	2.92	8.24	16.37
45P		28.77	2.69	7.09	15.77
50P		28.55	2.62	7.07	16.17
55P		28.95	2.78	7.11	16.86
Pooled *SEM*		0.351	0.084	0.296	0.302
P values	Linear	<0.001	<0.001	<0.001	0.433
	Quadratic	0.227	0.117	0.049	0.05

Pooled *SEM*; standard error of the means

Table 4. Nutrient utilization of zebrafish fed graded levels of dietary protein

Diets		Daily protein intake (g/kg $MBW^{0.8}$/day)	Daily energy intake (*kJ*/kg $MBW^{0.8}$/day)	Daily protein gain (g/kg $MBW^{0.8}$/day)	Daily energy gain (*kJ*/kg $MBW^{0.8}$/day)	Protein retention (%)	Energy retention (%)
20P		8.71	926.89	3.60	176.29	41.42	19.06
25P		10.15	830.39	3.70	170.57	36.45	20.54
30P		10.89	809.35	3.81	170.57	34.96	21.09
35P		12.19	752.09	3.64	162.44	29.84	21.61
40P		12.87	695.66	3.87	167.92	30.10	24.17
45P		12.57	600.37	3.40	137.56	27.04	22.92
50P		14.69	624.19	3.71	148.02	25.34	23.79
55P		14.11	526.05	3.82	149.34	27.11	28.41
Pooled *SEM*		0.272	16.379	0.156	7.044	1.578	1.153
P values	Linear	<0.001	<0.001	0.744	<0.001	<0.001	<0.001
	Quadratic	0.001	0.074	0.76	0.471	<0.001	0.317

Pooled SEM; standard error of the means, MBW; metabolic body weight

Discussion

The responses of zebrafish to dietary protein levels in the present study displayed some differences from those of the previous studies (Fernandes et al. 2016; O'Brine et al. 2015). This could be resulted from several factors including growth depensation in zebrafish, differences in strain and in number of sexes in experimental tanks and maturational stages as underlined previous authors (Biga and Goetz 2006; Eaton and Farley 1974). Since we did not define maturational situation and sexes of the individuals in the present study, we were unable to conclude their contributions to the differences in our results and those of O'Brine et al. (2015) and Fernandes et al. (2016).

*SGR*s of zebrafish reared on increasing levels of dietary protein were affected as early as 2nd week of the study with a significant quadratic trend, but with a lower rate during the later periods. This could be resulted from that the fish were not able to totally adapted to the experimental conditions even after a week of acclimation period. The SGR responses were abated but with still a significant quadratic trend at 4th week, and became insignificant at the final, suggesting a decrease at the intensity of growth response with ages to dietary protein level. Although difficult to compare the results of this study with those of O'Brine et al. (2015) who used a higher range of dietary protein levels between 32 and 75%, no significant treatment effect on growth rate of about 4-month-old zebrafish was determined. The impacts of developmental stages on zebrafish growth rate has been previously underlined (Eaton and Farley 1974). Yet, we used SGR_{Week4} and SGR_{Final} values as

response variables to estimate the dietary protein requirements. The *TBPLM* estimated the requirements for SGR_{Week4} and SGR_{Final} as 27.69 and 28.93% respectively without a considerable change with fish size. Dietary protein requirement levels of zebrafish estimated here are consistent with those of omnivorous species such as common carp and goldfish reported by NRC (2011) and Ulloa et al. (2011). But, our findings are lower than those levels of 37.6 and 44.8 % for zebrafish by Fernandes et al. (2016), who used average estimated values of *SKM* and *BLM* based on weight gain and protein retention. The model with two breaks used in the present study was previously employed by Klatt et al. (2016) for estimation of lower and upper critical dietary concentrations of methionine+cysteine for juvenile turbot (*Psetta maxima*). The second order polynomial model is widely used in estimation of nutrient requirements of aquaculture species (Shearer 2000), but the *TBPLM* fitted better in the present study in terms of residual sum of squares, suggesting that it can be used in future studies as an alternative model for determination of minimum nutrient requirements. When it comes to right side of the curve, the present model estimated an inhibition dietary protein level of 39.56%. However, since the right side of the curve did not display a clear descending trend, a great caution should be exercised before a definite conclusion is reached in terms of inhibition level of dietary protein. The descending trend at the right side of *SGR*s curve is inconsistent with previous observations in zebrafish (Fernandes et al. 2016; O'Brine et al. 2015), who found a plateau at high protein levels. We can conclude that our *SGR* data appears to be suitable for estimation of only minimum dietary protein using the *TBPLM* model level but not for the inhibition level. Yet, care should be exercised that dietary protein levels above about 45% may lead to a reduction in growth performance of juvenile zebrafish, at least in the studied weight ranges.

Feed consumption of fish linearly decreased with the increase of dietary protein levels. This is consistent with the results reported by Akpınar et al. (2012) and Fernandes et al. (2016), who observed an inverse relation between feed intake and dietary protein in juvenile shi drum (*Umbrina cirrosa*) and zebrafish. This phenomenon could be attributed to compensatory response to get more protein in fish fed lower dietary protein levels, as argued by several authors (Akpınar et al. 2012; El- Dakar et al. 2011; Fernandes et al. 2016; O'Brine et al. 2015). Therefore, at restricted feeding regimes at the estimated requirement level in this study fish may not meet their daily protein requirements and significant attention should be paid to feeding levels in zebrafish laboratories. Our results related with *FCR* showed a quadratic decrease in response to the increase in dietary protein, being consistent to a certain degree with those of Fernandes et al. (2016), who observed an improvement in feed efficiency up to 35% protein level, then a plateau.

A quadratic decrease in *PER* with increasing dietary protein level was the case in the present study. This suggests that zebrafish did not use increasing dietary protein particularly at above requirement levels for protein synthesis as indicated several fish species including Arctic charr, *Salvelinus alpinus* (Gurure et al. 1995), *Zacco barbata* (Shyong et al. 1998), marbled spinefoot rabbitfish, *Siganus rivulatus* (El- Dakar et al. 2011) and tiger puffer, *Takifugu rubripes* (Kim and Lee 2009).

The effect of dietary protein levels on whole body compositions of zebrafish was a significant linear decrease in dry matter, crude ash and lipid whereas no change in crude protein in the present study. Our dry matter results are consistent with those of Fernandes et al. (2016), but this was not the case in the whole body protein which displayed an increase with dietary protein levels in their study. Although no clear consensus about the effects of dietary protein levels on the proximate compositions of fish in the literature, Gurure et al. (1995) found a decrease in dry matter and crude lipid concentrations in Arctic charr with dietary protein levels, being fully in parallel with our findings. Higher lipid concentrations in zebrafish on lower dietary protein levels could be a result of higher depositions of energy due to higher feed consumption.

Expectedly, daily protein intake of zebrafish increased with dietary protein level. Similar results were also recorded by other authors in different fish species including zebrafish (Akpınar et al. 2012; El-Dakar et al. 2011; Fernandes et al. 2016). However, this trend was not reflected to daily protein gain, which in turn resulted in a significant quadratic decrease in protein retention in response to increasing levels of protein as was the case in *PER* values. Although the protein retention data are in harmony with those by Fernandes et al. (2016) at a certain degree, daily protein gains are inconsistent with the findings of these authors. Our daily energy intake and gain values displayed a linear decrease with dietary protein levels but energy retention showed an inverse trend, being partly in parallel with the results of Fernandes et al. (2016).

In conclusion, the results of the present experiment show that zebrafish growing from 85 and 300 mg require minimum 29% dietary protein level in their diets including about 10% lipid or 19.5 MJ/kg gross energy when fed *ad libitum*. Further studies are required to determine the effects of

varying dietary protein to energy ratios at different feeding levels.

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Zooplankton Fauna of Abant Lake: Past and Present

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ABSTRACT

Observing the changes of zooplanktonic organisms over the years can give us consistent information about the limnological conditions of the present and future. Our aim in this study was to determine current conditions of zooplankton fauna of Abant Lake, which was studied seasonally, and could provide resources for future studies. The samples of zooplankton were collected horizontally and vertically from Abant Lake seasonally, between 2015 -2016 from seven stations. At the end of the study, a total of forty-nine zooplankton species were identified. Of these, 33 belonged to Rotifera, 14 to Cladocera and 2 to Copepoda. In addition, twelve species of Rotifera, and six species of Cladocera were found as new records for the Abant Lake.

Keywords: Rotifera, Cladocera, Copepoda, Abant Lake

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Abant Gölü Zooplankton Faunası: Geçmiş ve Bugün

Öz: Zooplanktonik organizmaların yıllar içerisindeki değişimlerini izlemek, gölün bugünkü ve gelecekteki limnolojik durumu hakkında tutarlı bilgiler verebilir. Bu çalışmada ki amacımız, Abant Gölü'nün zooplankton faunasının güncel durumunu belirlemek ve gelecekteki çalışmalara katkı sağlamaktır. Zooplankton örnekleri, Abant Gölü'nden 2015-2016 yılları arasında mevsimsel olarak, farklı habitatlarda ki yedi istasyondan yatay ve dikey olarak alınmıştır. Çalışmanın sonunda 33 tür ile Rotifera, 14 tür ile Cladocera ve 2 tür ile Copepoda grubuna ait toplam kırk dokuz zooplankton türü tespit edilmiştir. Bu çalışma ile birlikte, 12 Rotifera türü ile 6 Cladocera türü Abant Gölü zooplankton faunası için yeni kayıt olarak bulunmuştur.

Anahtar kelimeler: Rotifera, Cladocera, Copepoda, Abant Gölü

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Introduction

Lake studies provide a practical and useful structure for community studies because they can give important information to the researchers about climate change and ecosystem structure etc. (Olden et al. 2006). Zooplanktonic organisms include both predator and prey organisms. They are primary consumers in aquatic ecosystems, especially in lakes and constitute a major food source for their predator like macroinvertebrates, fish and birds. Zooplankton considered as indicators in lakes because of their pivotal role in aquatic food webs (Jeppesen et al. 2011).

It is known that zooplankton distribution, richness, and composition are affected by biotic parameters like the presence of predators, trophic structure, habitat differences etc. and abiotic parameters such as temperature, salinity, etc. (Kaya et al. 2010; Gürbüzer et al. 2017). For example, one of the most reliable predictors of eutrophic condition is total phosphorus (Filstrup and Downing 2017). It is known to affect species richness in zooplankton communities where richness decreases with an increasing total phosphate (Jeppesen et al. 2000). Since rotifers are opportunistic species they become dominant taxa when water quality deteriorates

(Gannon and Stremberger 1978). It is also thought that observing the changes of zooplanktonic organisms over the years can give us consistent information about the limnological conditions of the present and future.

Abant Lake is located in the west coast of the Black Sea Region and west of the city Bolu. It has been declared as a National Park in 1988 by The Ministry of Culture and Tourism. The area has warm temperate, fully humid and warm summer according to Köppen-Geiger Climate Classification (Kottek et al. 2006). It is located at 1340 m above sea level, and surface area of lake is 125 ha (Akşıray 1959, Erinç et al 1961) and the maximum depth is 18 m (Çelekli and Külköylüoğlu 2006). Abant Lake gets attention of scientists because the lake and its surroundings have the rich flora and fauna (Dügel et al. 2008, Karakaya et al. 2011, Atıcı and Tokatlı 2014).

There are available several zooplankton studies about Abant Lake and the first one was conducted in 1970 by Margaritora and Cottarelli which was later followed by extensive studies of Altındağ (1999), Altındağ and Yiğit (2000) and Özdemir Mis et al. 2017. Our aim in this study was to determine current conditions of zooplankton fauna of Abant Lake, which was studied seasonally, and could provide resources for future studies.

Materials and Methods

The samples of zooplankton were collected from Abant Lake seasonally, between 2015 -2016 from seven stations in different points (Figure 1) and coordinates of the sampling stations were given in Table 1. Samples were collected horizontally and vertically using Hydro-Bios Plankton Net (mesh size 55µ and 25 cm in diameter) and immediately fixed with 4% formalin. To identify zooplankton species, various resources were used which included the studies of Ward and Whipple (1945), Kolisko (1974), Koste (1978), Harding and Smith (1974), Nogrady and Pourriot (1995), Segers (1995), De Smet (1996), and Smirnov (1996). In the present study, all the identified taxa were checked from several checklists from Ustaoğlu 2004, Ustaoğlu et al. 2012, and Ustaoğlu 2015.

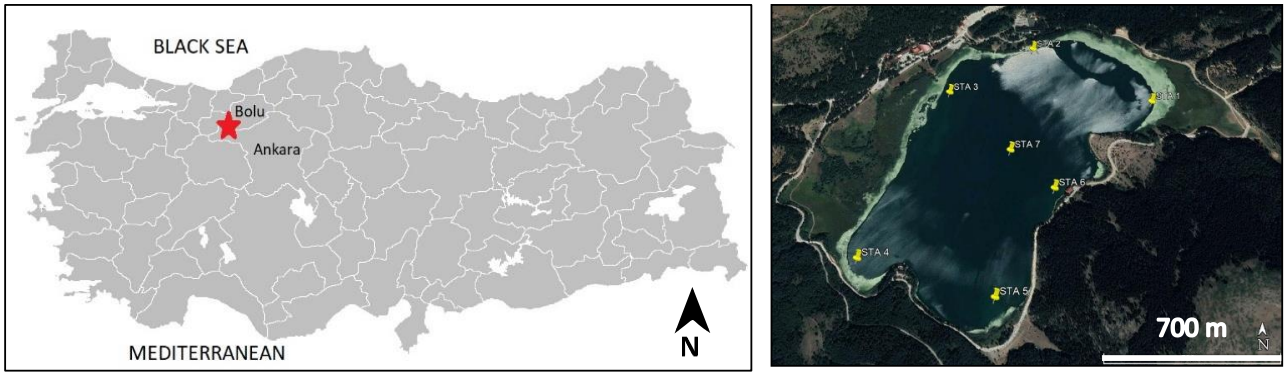

Figure 1. Study area and sampling stations

Table 1. Coordinates of the sampling stations

Station Number	Coordinates
STA 1	40° 36' 31"N / 31° 17' 21"E
STA 2	40° 36' 40"N / 31° 16' 55"E
STA 3	40° 36' 34"N / 31° 16' 39"E
STA 4	40° 36' 6"N / 31° 16' 22"E
STA 5	40° 35' 59"N / 31° 16' 48"E
STA 6	40° 36' 15"N / 31° 17' 00"E
STA 7	40° 36' 24"N / 31° 16' 55"E

Results

In this study, a total of forty-nine zooplankton species were identified. Of these, 33 belonged to Rotifera, 14 to Cladocera and 2 to Copepoda. The species list of Abant Lake was presented in Table 2. 12 species of Rotifera and 6 species of Cladocera are new records for the Abant Lake.

Rotifers *Kellicottia longispina* and *Keratella cochlearis* were found during all seasons, while *Asplanchna priodonta*, *Synchaeta pectinata* were found during three seasons and other taxa were identified only during two seasons or less. Cladocerans were seen during all seasons except winter period, *Alona guttata*, *Bosmina longirostris*, *Chydorus sphaericus* and *Daphnia longispina* were found during three seasons. Copepods were represented by two taxa *Acanthodiaptomus denticornis* and *Megacyclops viridis* and *A. denticornis* was found during all seasons except winter period like cladocerans.

When seasonal zooplankton changes were considered it was seen that, species richness was the highest in autumn (twenty-five taxa), followed by summer and spring (twenty-four and twenty-three taxa, respectively). In the winter season, only six

species were observed all of which belonged to the phylum Rotifera.

When zooplankton taxon frequencies were analyzed, rotifers were found to have the highest taxa percentage with 67%, while copepods had the lowest one with 4%.

Table 2. Species list of Abant Lake, past and present studies

Species	Present Study							
ROTIFERA	Winter	Spring	Summer	Fall	MC1970	A1999	AY2000	ÖM2017
Ascomorpha ecuadis (Petry, 1850)						+	+	
Asplanchna girodi Guerne, 1888			+		+	+	+	
Asplanchna priodonta Gosse, 1850		+	+	+		+		
Cephalodella gibba (Ehrenberg, 1830)*				+				
Collotheca mutabilis (Hudson, 1885)*			+					
Collotheca ornata (Ehrenberg, 1830)			+	+		+	+	
Collotheca pelagica (Rousselet, 1893)			+			+	+	
Collotheca sp.								+
Colurella adriatica Ehrenberg, 1831						+	+	
Conochilus hippocrepis (Schrank, 1803)			+	+		+	+	+
Conochilus unicornis (Rousselet, 1892)			+	+	+			+
Euchlanis dilatata (Ehrenberg, 1830)				+		+	+	
Filinia longiseta (Ehrenberg, 1834)	+					+	+	
Filinia terminalis (Plate, 1886)	+				+			
Gastropus stylifer Imhof, 1891				+		+	+	+
Kellicottia longispina (Kellicott, 1879)	+	+	+	+	+	+	+	+
Keratella cochlearis (Gosse, 1851)	+	+	+	+	+	+	+	+
Keratella quadrata (Müller, 1786)	+	+			+	+	+	+
Keratella tropica (Apstein, 1907)*			+					
Lecane bulla (Gosse, 1851)			+					+
Lecane closteracerca (Schmarda, 1859)*		+		+				
Lecane hamata (Stokes, 1896)						+	+	
Lecane luna (Müller, 1776)								+
Lecane lunaris (Ehrenberg, 1832)		+				+	+	+
Lecane stenroosi (Meissner, 1908)*		+						
Lepadella acuminata (Ehrenberg, 1834)*				+				
Lepadella ovalis (Müller, 1786)*		+						
Lepadella patella (Müller, 1773)*				+				
Lophocharis salpina (Ehrenberg, 1834)	+					+	+	
Mytilina ventralis (Ehrenberg, 1830)*		+		+				
Notholca squamula (Müller, 1786)		+					+	
Philodina megalotrocha Ehrenberg, 1832*		+						
Platyias quadricornis (Ehrenberg, 1832)								+
Polyarthra dolicoptera Idelson, 1925		+				+	+	
Polyarthra vulgaris Carlin, 1943		+		+		+	+	
Squatinella mutica (Ehrenberg, 1832)*				+				
Synchaeta litoralis Rousselet, 1902						+	+	
Synchaeta pectinata Ehrenberg, 1832		+	+	+		+	+	+
Trichotria pocillum (Müller, 1776)		+				+	+	
Trichotria tetractis (Ehrenberg, 1830)*		+						

Table 2. Continued

Species	Present Study							
CLADOCERA	Winter	Spring	Summer	Fall	MC1970	A1999	AY2000	ÖM2017
Acroperus harpae (Baird, 1843)					+			
Alona affinis (Leydig, 1860)					+			
Alona costata Sars, 1862					+			
Alona guttata Sars, 1862*		+	+	+				
Alonella excisa (Fischer, 1854)							+	
Alonella exigua (Lilljeborg, 1853)								+
Alonella nana (Bairs, 1850)		+					+	
Bosmina longirostris (Müller, 1785)		+	+	+			+	+
Ceriodaphnia quadrangula (Müller, 1785)			+	+				+
Ceriodaphnia reticulata (Jurine, 1820)*				+				
Coronatella rectangula Sars, 1862*			+					
Chydorus sphaericus (O.F. Müller, 1776)		+	+	+			+	
Daphnia hyalina Leydig 1860					+			
Daphnia longispina (O.F. Müller)		+	+	+			+	+
Diaphanosoma brachyurum (Lievin, 1848)			+				+	+
Macrothrix hirsuticornis Norman & Brady, 1867*			+	+				
Macrothrix laticornis (Jurine, 1820)*			+					
Pleuroxus truncatus (O.F. Müller, 1785)				+	+			
Polyphemus pediculus (Linnaeus, 1758)			+				+	+
Scapholeberis mucronata (O.F. Müller, 1758)					+			
Sida crystallina (O. F. Müller, 1776)							+	
Simocephalus serrulatus (Koch, 1841)*		+						
COPEPODA								
Acanthodiaptomus denticornis (Wierzejski, 1887)		+	+	+	+		+	+
Megacyclops viridis (Jurine, 1820)			+		+			+
Thermocyclops dybowskii (Lande, 1890)								+

(+: present, *: new records for region, MC1970: Margaritora and Cottarelli 1970; A1999: Altındağ 1999; AY2000: Altındağ and Yiğit 2000, ÖM2017: Özdemir Mis et al. 2017)

Discussion

In freshwater ecosystems, zooplankton community have an important role in water quality (Moss et al. 2003), and rotifers can also be used for this purpose (Gutkowska et al. 2013; Apaydın Yağcı et al. 2017). Rotifers are thought to become the dominant taxa in many lakes over time (Wen et al. 2011) and they can adapt to the degraded situation better than other similar taxa. According to Saksena (1987), rotifers are the dominant group in freshwater ecosystems and as being compatible with this result, in our study, rotifer percentages (67%) were found to be much higher than cladocerans (29%) and copepods (4%), a situation similar to other Mediterranean freshwater lakes (Saler 2017). *K. longispina*, *K, cochlearis* and *K. quadrata* known as cosmopolitan (Segers 2007) and they were observed by all researchers.

The first study on zooplankton community in Abant Lake was carried out in 1970 (Margaritora and Cottarelli) and six Rotifera, (Dumont and De Ridder 1987), six Cladocera and two Copoepoda taxa were described. All rotifer species were also found in this study (Table 2). The most important difference between these two studies is the number of species found. The low number of zooplankton species, especially rotifers, maybe resulting from the sampling procedures like using a broader mesh size, taking one sample, etc. The cladoceran species, *Scapholeberis mucronata* (O.F. Müller, 1758), *Daphnia hyalina* Leydig 1860, *Alona costata* Sars, 1862, *Acroperus harpae* (Baird, 1843) and *Alona affinis* (Leydig, 1860) were not found except *P. truncatus*.

Other detailed studies were carried out by Altındağ (1999) and Altındağ and Yiğit (2000). In these studies, rotifera phylum and zooplankton composition of Abant Lake were revealed. However, in our study *Lecane hamata* (Stokes 1896), *Colurella adriatica* Ehrenberg, 1831, *Synchaeta littoralis*

Rousselet, 1902 and *Ascomorpha ecuadis* were not observed despite being present in Altındağ (1999) and also *Alonella excisa* (Fischer, 1854) and *Sida crystallina* (O. F. Müller, 1776) were not observed despite being present in Altındağ and Yiğit (2000). Altındağ (1999) also pointed out that *Asplanchna girodi* is synonymous with *A. brightwellii* and we also described this taxon as *A. girodi*.

The most recent work regarding Abant Lake is of a field study of Özdemir Mis et al., which were conducted in between 2002-2003 yet published in 2017. In this study, twenty-one taxa were determined and among them, 12 belonged to Rotifera, 6 to Cladocera and 3 to Copepoda. Of these 21 taxa, 16 were mutual with our result (Table 2). *A. denticornis* has been found since 1970 in Abant Lake. Zooplankton differences between ours and this study were *Lecane luna* and *Platiyas quadricornis* from Rotifera, *Alonella exigua* from Cladocers and *Thermocyclops dybowskii* from Copepoda fauna.

Diversity of observed planktonic organisms can be affected by the sampling method (for example plankton net size and shape, etc.) therefore zooplankton density and composition differences can be due to the differences in sampling procedures (Gutkowska et al. 2012). Considering previous studies, it is easy to conclude, species richness is increased in the past decades especially for rotifers and cladoceran taxa. However, it is not clear whether there is an increase in species richness, because the methods used were not compatible with each other. In this study, we believe high numbers of identified separate species compared to past stem from vertical and horizontal sampling in different habitats. We think that using standardized and commonly accepted sampling methods will allow our predictions regarding future community compositions to be more accurate.

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Fossil and Recent Distribution and Ecology of Ancient Asexual Ostracod *Darwinula stevensoni* (Ostracoda, Crustacea) in Turkey

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ABSTRACT

In order to determine distribution, habitat and ecological preferences of *Darwinula stevensoni*, data gathered from 102 samples collected in Turkey between 2000 and 2017 was evaluated. A total of 1786 individuals of *D. stevensoni* were reported from eight different aquatic habitats in 14 provinces in six of seven geographical regions of Turkey. Although there are plenty of samples from Central Anatolia Region, recent form of the species was not encountered. Unlike recent, fossil forms of species were encountered in all geographic regions except Southeastern Anatolia. The oldest fossil record in Turkey was reported from the Miocene period (ca 23 mya). Species occurred in all climatic seasons in Turkey. *D. stevensoni* showed high optimum and tolerance levels to different ecological variables. Results showed a positive and negative significant correlations of the species with pH ($P<0.05$) and elevation ($P<0.01$), respectively. It seems that the ecological preferences of the species are much wider than previously known. Our results suggest that if *D. stevensoni* is used to estimate past and present environmental conditions, attention and care should be paid on its ecology and distribution.

Keywords: Ecologic preference and characterization, seasonality, stevensoni

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Eski Aseksüel Ostrakod *Darwinula stevensoni*'nin (Ostracoda, Crustacea) Türkiye'deki Ekolojisi, Fosil ve Güncel Dağılımı

Öz: *Darwinula stevensoni*'nin dağılımını, habitat ve ekolojik tercihlerini belirlemek için 2000 ve 2017 yılları arasında Türkiye'den toplanan 102 örnekten elde edilen veriler değerlendirilmiştir. Toplam 1786 *D. stevensoni* bireyi Türkiye'nin yedi coğrafi bölgesinin altısında bulunan 14 ildeki sekiz farklı sucul habitattan rapor edildi. İç Anadolu bölgesin de bol miktarda örnek olmasına rağmen, türün güncel formuna rastlanılmadı. Güncel formdan farklı olarak, türün fosil formu ile Güneydoğu Anadolu dışındaki tüm coğrafi bölgelerde karşılaşılmıştır. Türkiye'deki en eski fosil kayıt Miosen döneminden (yaklaşık 23 milyon sene önce) rapor edilmiştir. Türkiye'deki tüm mevsimlerde tür bulunmuştur. *D. stevensoni* farklı ekolojik değişkenlere yüksek optimum ve tolerans seviyeleri göstermektedir. Tür pH ile pozitif ($P<0,05$) fakat yükseklik ile negatif ($P<0,01$) anlamlı korelasyon göstermektedir. Görülmektedir ki türün ekolojik tercihleri daha önce bilinenden daha geniştir. Sonuçlar, *D. stevensoni*'nin güncel ve geçmiş çevre koşullarını tahmin etmek için kullanılması halin de ekolojisine ve dağılımına dikkat edilmesi gerektiğini göstermektedir.

Anahtar kelimeler: Ekolojik tercih ve karakterizasyon, mevsimsellik, stevensoni

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Introduction

Ostracods are small (0.3-5 mm long), bivalved (carapaces) aquatic creatures that are widely distributed in a variety of marine and non-marine environments (Meisch 2000). They show species-specific responses to the changes in different ecological conditions; therefore, they can be used as bioindicator species to estimate possible environmental deterioration (Benson 1990; Külköylüoğlu 1999). Also, because of the easily fossilization of calcium carbonated carapaces, they are commonly used in biostratigraphy, paleobiology, paleoclimatology, paleolimnology and paleoecology studies (Ruiz et al. 2013). In this sense, the autecology of individual species has an important role since ecology of recent species help paleontologists to widen their perceptions to understand types of

paleoenvironmental conditions based on fossil ostracods (Carbonel et al. 1988).

The genus *Darwinula* is the type genus of the family Darwinulidae that includes only the type species *Darwinula stevensoni*. Because of the absence of males in fossils (since Mesozoic) and in living populations, superfamily Darwinuloidea have survived asexually over 200 million years (Martens et al. 2003). Similar to bdelloid rotifers (Mark Welch and Meselson 2000; Mark Welch et al. 2004) and oribatid mites (Maraun et al. 2004), darwinuloid ostracods (Schön and Martens 2003) have also been suggested as one of the "putative ancient asexual" groups in animal kingdom. However, although its persisting asexuality is known, a few reports of rare males of the species by Turner (1895) and Brady and Robertson (1870) and one male of *Vestalenula cornelia* (Smith et al. 2006) has also been questioned within the family. Subsequently, Martens and Schön *(2008)* indicated *D. stevensoni* as a strong candidate in darwinuloids to being a true ancient asexual. In support of this view, Schön et al. (2009) stated that occasional males may be produced in many asexual species because of mutations in the regulatory cascade controlling sex and the males produced are not functional. The presence of female fossils dating back to 25 million years (Straub 1952) and the absence of functional or atavistic males in recent and fossil species (Schön et al. 2009) enforce the ancient asexuality of *D. stevensoni*.

D. stevensoni is a small sized (0.6-0.8 mm in length) (Meisch 2000) ostracods. Carapace of species is characteristically cigar shaped with unequal valves (Figure 1). Right valve extends left valve on all sides except hinge. The posterior margin of carapace is wider than anterior. The widening of posterior part is due to the developing of a brood chamber since species is viviparous parthenogenetic unlike most of other ostracods (Cypridoidea and Cytheroidea) (Rossetti and Martens 1996). Generally, species obtain about ten eggs in this brood chamber, but this number can be changed up to 13 (Külköylüoğlu pers. obs.). Later, juveniles may have more than one molting stage within brood space until hatched. Muscle scars that control the opening and closing of valves arranged in a characteristic circular rosette shape, which includes 9 - 12 spots. Additionally, species do not swim because of the absence of natatory setae on the second antennae of species and so it is a typical benthic form (for more taxonomic remarks see Rossetti and Martens 1996; Meisch 2000).

D. stevensoni showed a cosmopolitan distribution (Meisch 2000) except from Antarctic region, Pacific region and Oceonic Islands (Martens et al. 2013). Van Doninck et al. (2003a) presented the global distribution of species (cf. Figure 2 in this paper). Species has been collected in lotic, lentic and interstitial habitats and it is ecologically characterized as thermoeuryplastic, oligorheophilic, titanoeuryplastic and mesohalophilic (Meisch 2000). Until now, the ecology of *D. stevensoni* has not been widely evaluated except some papers partially discussed its status in local and/or regional perspectives (e.g., Ranta 1979; Rossetti and Martens 1996; Gandolfi et al. 2001a, 2001b; Van Doninck et al. 2003a; Rossi et al. 2002, 2004; Higuti et al. 2009a; Van den Broecke et al. 2013). Therefore, the aims of the present study are to (i) determine geographic and local distribution of both fossil and living populations of the species among different aquatic habitats, (ii) estimate ecological preferences of *D. stevensoni* in Turkey, and (iii) evaluate species ecological tolerance and optimum ranges for those of particular environmental variables.

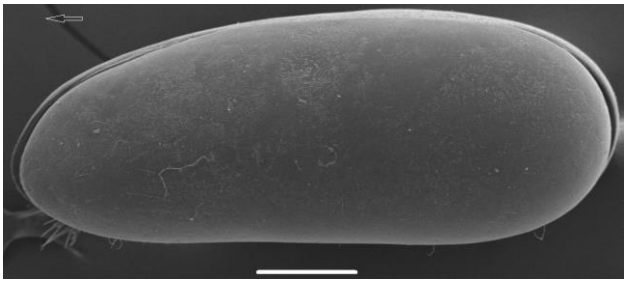

Figure 1. *Darwinula stevensoni*. Left valve external view (Scale bar 100 μm).

Materials and Methods

Study sites and Sampling

A total of 102 samples from 14 provinces (72 samples from Bolu, 1 in Gaziantep, 2 in Ordu, 3 in Adıyaman, 3 in Burdur, 2 in Hatay, 3 in Mardin, 2 in Muş, 4 in Kütahya, 1 in Mersin, 2 in Sakarya, 2 in Isparta, 3 in Antalya and 2 in Muğla) of Turkey were collected between the years 2000 and 2017 (Figure 2, Appendix). All the measurements were taken *in situ* before ostracods were collected to prevent the mixing of water and to obtain the actual values of variables. Ostracod samples were collected with a standard sized (200 μm) d-frame hand net and fixed in 70% ethanol.

Physico-chemical variables (*pH*, dissolved oxygen (*DO, mg/L*), percent oxygen saturation *(%DO*), water temperature (*Tw, °C*), electrical conductivity (*EC, μS/cm*), salinity (*Sal. ‰*), total dissolved solids (*TDS, mg/L*) and redox potential (*ORP, mV*)) were measured by a YSI-85 model of oxygen-temperature and HI-98150 pH-ORP meter from sapling sites in Bolu and Ordu where geographic data (coordinates and elevation) was recorded with a Garmin GPS-12XL. In Gaziantep and Hatay provinces, Tw, pH, EC and salinity values

were obtained with a Delta OHM pH/conductivity meter while air temperature was recorded with a Testo 410-2 anemometer, and coordinates and elevation with a Garmin GPS-45. The measurements for the rest of the provinces were done by YSI Professional Plus and Testo 410-2 anemometer and a Garmin etrex Vista H GPS (for elevation and coordinates).

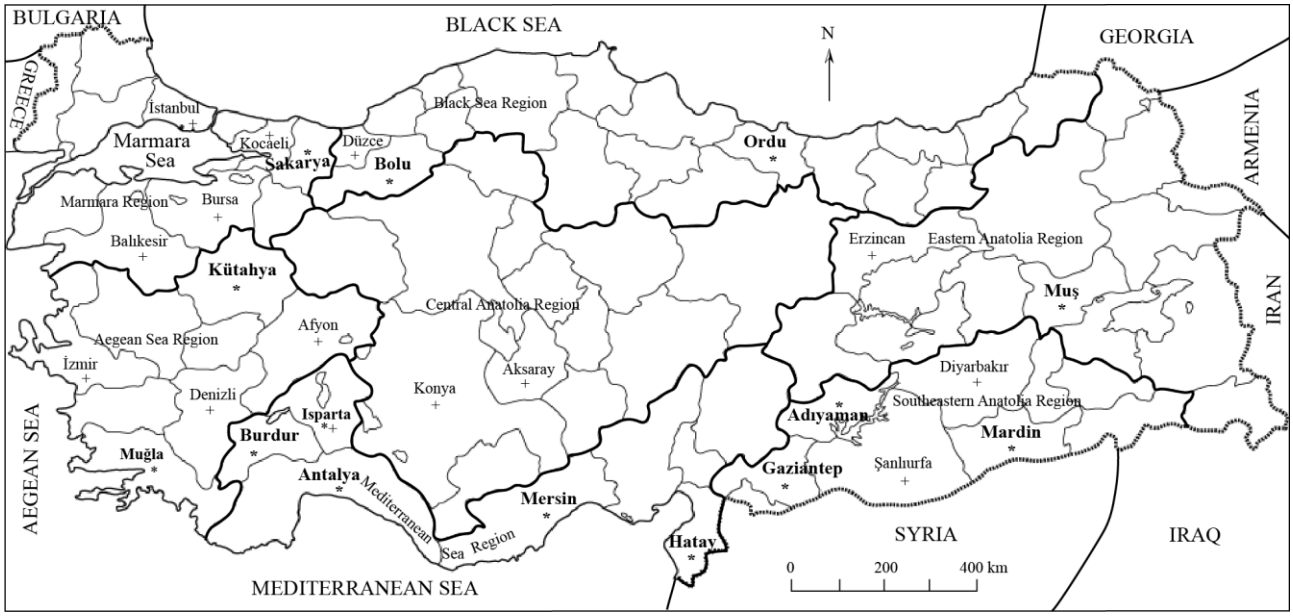

Figure 2. Distribution of living *D. stevensoni* in 27 provinces of Turkey. * indicates provinces (in bold) sampled during the current study, + represents the species previously reported in the literature. *+ displays the species records in this study and previously as well. Citations for the previous reports; İstanbul (Külköylüoğlu pers. obs. unpublished data), Kocaeli (Akdemir pers. obs. unpublished data), Bursa (Altınsaçlı and Griffiths 2001a); Balıkesir (Altınsaçlı and Griffiths 2001b); Düzce (Gülen 1985); Afyon (Gülen 1985); Denizli (Altınsaçlı and Mezquita 2008); İzmir (Meriç et al. 2010); Isparta (Özuluğ et al. 2001); Konya (Akdemir 2004); Aksaray (Altınsaçlı 2004); Şanlıurfa (Özuluğ and Dökümcü 2014); Diyarbakır (Gülen et al. 1996; Akdemir and Külköylüoğlu 2011); Erzincan (Akdemir and Külköylüoğlu 2014).

In the laboratory, samples were filtered through four standardized sieves (0.5, 1.0, 1.5 and 2.0 mm mesh size) under tap water and then specimens were separated from sediment under stereomicroscope and fixed in 70% ethanol for further studies. Taxonomic identification was done according to the carapace and soft body parts dissected in lactophenol solution by using taxonomic key of Meisch (2000) under a light microscope (Olympus BX-51). According to Meisch (2000), *D. stevensoni* was ecologically characterized for salinity limnetic (freshwater) range (<0.5 ‰), oligohaline (0.5-5 ‰), mesohaline (5-18 ‰), polyhaline (18-30 ‰), euhaline (30-40 ‰) and hyperhaline (≥40 ‰) and water temperature (cold stenothermal, oligothermophilic, mesothermophilic (between two previous), polythermophilic and warmstenothermal). On the other hand, because this ecological characterization does not classify water temperature ranges for freshwater habitats, we followed the offering of Chu et al. (2009) and Olivero-Sheldon et al. (2014). The ranges as very cold (<12.8 °C), cold (<18 °C), cold cool (>18 at <21 °C) and warm (>21 °C) were used. All the specimens were kept in Limnology Laboratory of Bolu Abant İzzet Baysal University, Turkey.

Statistical Analyses

The tolerance (t_k) and optimum (μ_k) estimates of the species for different ecological variables were calculated by using C2 software after using a transfer function of weighted averaging regression (Juggins 2003). A non-parametric Spearmen Rank Correlation was applied to see the the levels of correlations between species and different variables (IBM-SPSS Statistics version 21).

Results

We encountered 1786 individuals of *D. stevensoni* from eight different aquatic habitats in 14 provinces (Figure 3). These provinces were found in six geographical regions of Turkey except Central Anatolia Region (Figure 2). The highest (1117) and lowest (1) individual numbers were reported in dams and pond, respectively. Although the number of lakes and springs sampled were approximately 9 and 6 times more than the number of troughs, respectively, the number of individuals found in troughs (277) are more than the number of individuals in both habitats (234) (Figure 3). In general, *D. stevensoni* was mostly encountered in May, December, and January months of the seasons (spring and winter) when it was collected in all months of summer and autumn.

Accordingly, we found the species from all the four seasons in Turkey. The distribution of fossil forms of species throughout Turkey were given in the Figure 4. Accordingly, fossil records indicated that the species has been known from Miocene (about 25 mya) in Turkey.

The optimum and estimated tolerance levels of *D. stevensoni* for eight different variables are given in Table 1. *D. stevensoni* showed positive and negative significant correlations with *pH* ($r = 0.218$, $N = 82$, $P<0.05$) and elevation ($r = -0.280$, $N = 101$, $P<0.01$), respectively.

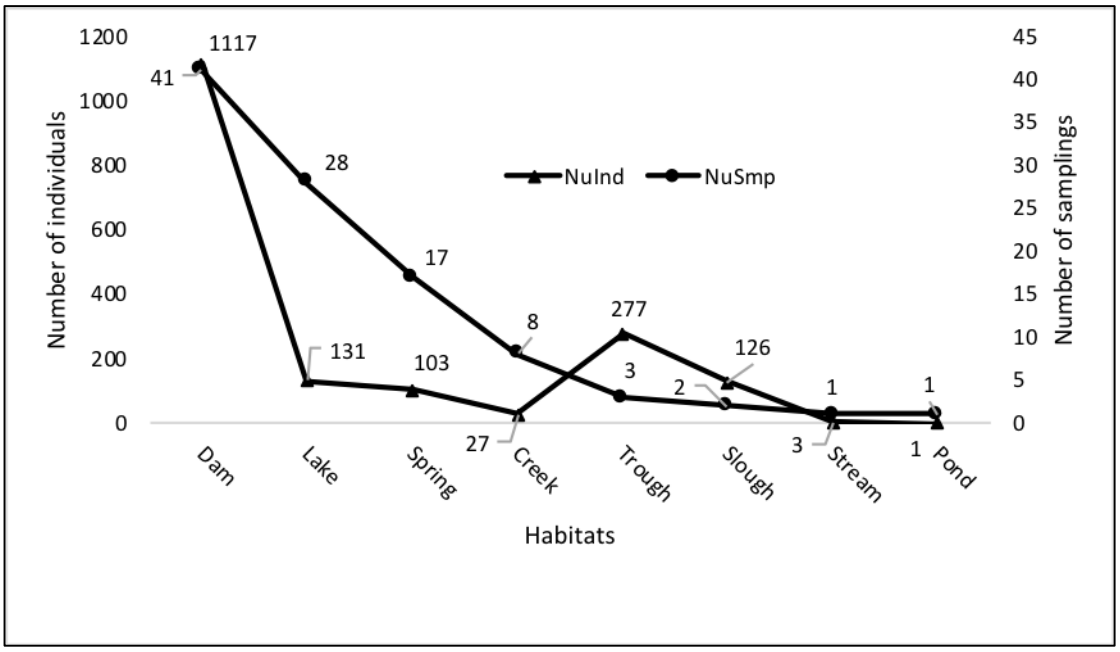

Figure 3. Sampling numbers (NuSmp) of eight different habitats and individual numbers of *D. stevensoni* (NuInd) in these habitats

Table 1. The optimum (μk) and estimated tolerance (tk) levels of *D. stevensoni* for *pH*, dissolved oxygen (*DO*), electrical conductivity (*EC*), water temperature (*Tw*), oxidation and reduction potential (*ORP*), elevation (*Elev*), salinity (*Sal*) and total dissolved solids (*TDS*). Abbreviations: Count, the number of sampling sites where species found; Max, the maximum number of individuals of concerned species among sampling sites and N_2, Hill's coefficient value that indicates the measure of effective number of occurrences.

				pH		DO		EC		Tw	
	Count	Max	N_2	μk	tk	μk	tk	μk	tk	μk	tk
Darwinula stevensoni	101	214	17.38	6.82	3.37	9.17	3.68	351.62	168.67	19.44	6.22
				ORP		Elev		Sal		TDS	
	Count	Max	N2	μk	tk	μk	tk	μk	tk	μk	tk
	101	214	17.38	114.92	82.56	708.62	232.33	0.13	0.07	142.00	104.31

The minimum and maximum values of *pH* (6.90-10.60), *DO* (0.32-18.31 mg/L), *DO%* (3.30-171.50 %), *EC* (21-844 *µS/cm*), *Tw* (6.10-31 °C), *Ta* (13-40.20 °C), *ORP* (-107.27-240.60 mV), *Elev* (39-2163 m a.s.l.), *Sal*. (0-0.42 ‰) and *TDS* (0.06-503.17 mg/L) of sampling sites where *D. stevensoni* collected. Accordingly, the species lives in waters with fresh to slightly brackish water ranges and it is characterized as a meso-polythermophilic.

Discussion

Along with the results of the present study and literature, recent living forms of the species has been now recorded from 27 provinces located in all seven geographic regions of Turkey (Figure 2). On the other hand, the fossil forms of the species *D. stevensoni* were only reported from 20 provinces covering six geographic regions of Turkey except Southeastern Anatolia. More than half of these 20 provinces were located in the western parts of

Turkey (Figure 4). Of these provinces, in Sakarya, dead specimens of *D. stevensoni* were taken from superficial sediments at 18 and 6 m depth of Lake Sapanca by Nazik et al. (2011) but they did not specify the age of these sediments. Meisch (2000) indicated that fossil record is known from Mid-Oligocene (ca 28 mya) to present but oldest fossil record in Turkey has been reported from the Miocene period (ca 23 mya) (see Figure 4). These data actually indicate the lack of paleontological studies dealing with ostracods in other parts (north, south, and east) of Turkey. As a result, *D. stevensoni* (in fossil and recent forms) has been reported in 44.4% of 81 provinces, Turkey. Among these provinces, eleven have fossil and recent forms and of the rest, 16 and nine provinces have only recent and fossil forms, respectively (Figures 1 and 3). Accordingly, with the current study, geographical distribution of *D. stevensoni* has been now expanded throughout Turkey.

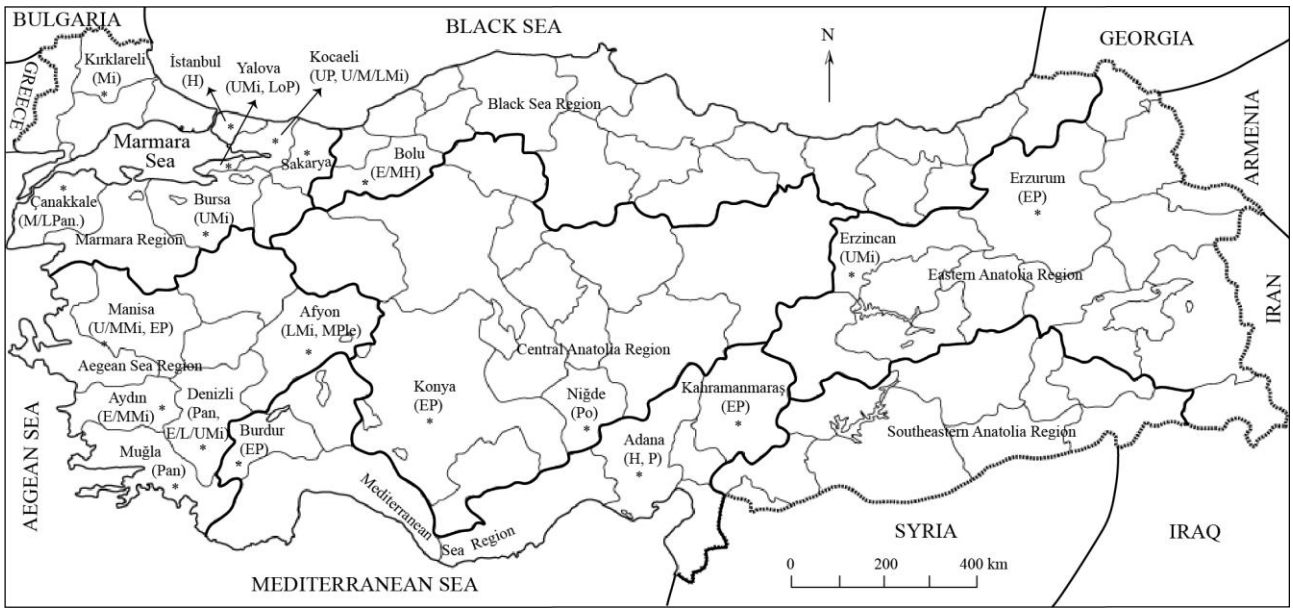

Figure 4. Fossil reports of *D. stevensoni* from Kırklareli (Witt 2011), İstanbul (Şafak et al. 1999; Meriç et al. 2000; Şekeryapan 2011), Yalova (Rückert-Ülkümen and Yiğitbaş 2007), Kocaeli (Matzke-Karasz and Witt 2005; Rückert-Ülkümen et al. 2006; Schneider et al. 2005), Sakarya (Nazik et al. 2011), Bursa (Freels 1980; Franz et al. 2006; Meriç et al. 2009; Nazik et al. 2011), Çanakkale (Atay and Tunoğlu 2002), Afyon (Demirer et al. 2017), Manisa (Witt 2003), Aydın (Tuncer and Tunoğlu 2015), Denizli (Gökçen 1979a, 1979b; Freels 1980; Şafak 2010), Muğla (Gökçen 1979a, 1979b), Burdur (Freels 1980), Adana (Nazik et al. 1992, 1999), Kahramanmaraş (Freels 1980), Konya (Freels 1980), Niğde (Nazik and Gökçen 1989), Erzincan (Freels 1980), Erzurum (Freels 1980) and Bolu (Tunoğlu et al. 2012) provinces in Turkey. Abbreviations: Early (E), Middle (M), Late (L), Lower (Lo), Upper (U), Miocene (Mi), Pannonian (Pan), Pontian (Po), Pliocene (P), Pleistocene (Ple), and Holocene (H).

Darwiula stevensoni prefers ponds, lakes and slow flowing streams (Meisch 2000). Szlauer-Łukaszewska (2014) and Ruiz et al. (2013) suggested *D. stevensoni* as a lake littoral species and benthos of shallow aquatic bodies, respectively. Pérez et al. (2011) and Marchegiano et al. (2017) indicated its negative correlation with depth. Contrary to common belief that species is generally found in shallow aquatic bodies, it has been reported from 0 to a maximum 20 m depth in literature (Meisch 2000; Pérez et al. 2010; Lorenschat and Schwalb 2013; Lorenschat et al. 2014) but species had a low optimum (2.3 m) and tolerance (3.8 m) levels to depth (Lorenschat and Schwalb 2013). The occurrence of species in the water bodies with a maximum 1 m depth herein supports the common occurrence of species in shallow waters although it has been encountered in a maximum of 20 m depth.

Additionally, species was commonly found in dam, lake, and springs (Figure 3). These habitats are generally permanent almost throughout the year. This confirms the suggestions of Palacios-Fest (2002-2003) as the occurrence of the species might be associated with long term water permanence. In addition, Escrivà et al. (2014) emphasized that *D. stevensoni* was one of the most common species in reservoirs. Until now, it has been reported from variety of habitats, such as ponds, slow flowing streams, lakes, springs, rivers, cenotes, troughs, coastal lagoons, wetlands (slough), artificial dam lakes, marshes, interstitial ground water, hot springs, reed beds, rice field, peat bogs, ditch and canal (Gülen 1985; Mezquita et al. 1999a, 1999b; Meisch 2000; Laprida et al. 2006; Higuti et al. 2009b; Pieri et al. 2009; Pérez et al. 2011; Akdemir and Külköylüoğlu 2014; Escrivá et al. 2014; Mazzini et

al. 2014; the current study). Based on the data available, one may consider the fact that species seems to have high levels of plasticity for habitats as proposed by Ranta (1979). As seen in Figure 3, our results also reinforced the plasticity of species for habitats. This means that there are no specific habitat preferences of species, which may present certain conditions. However, highest numbers of individuals per site were reported from an artificial trough (92) that is followed by slough (63) and dam (27) (Figure 3). These habitats are sensitive to the outside effects of anthropogenic, seasonal and climatic changes (Külköylüoğlu et al. 2013; Uçak et al. 2014). Besides to habitat plasticity of the species, it has been collected from the habitats with sapropel (mud), sand, rocks, stones and gravel sediment types (Altınsaçlı and Griffiths 2001b; Szlauer-Łukaszewska and Kowaluk-Jagielska 2011; Lorenschat and Schwalb 2013) that reinforce the proposal of Ranta (1979). In addition, Külköylüoğlu and Vinyard (2000) reported it from muddy and sandy sediments with high dissolved oxygen concentration.

The reproduction periods of the species show slightly different patterns. For example, reproduction of the species takes from May to October (Meisch 2000) while it takes from March to September in a temperate pond in Belgium (Van Doninck et al. 2003b). These previous authors were also reported species from January to November in this temperate pond. Besides, Külköylüoğlu (1999) collected species from February to November from springs of Nevada. Martín-Rubio et al. (2005) reported species from Lake Caicedo de Yuso-Arreo (Spain) in January, February, March, April, June - August, and November when Scharf and Viehberg (2014) encountered species in February, April, June, July, September and October in Germany. The occurrence of the species in April from Lake Meyil (Konya, Turkey) (Akdemir 2008) and from May to January herein is now supported previously reported reproduction period of species. The occurrence of species in all climatic seasons and almost in all months in Turkey supports the founding of species throughout the year (Hiller 1972; Altınsaçlı and Griffiths 2001a). Martens and Tudorance (1991) also pinpointed that *D. stevensoni* is a perennial species in a tropical Ethiopian lake. Therefore, *D. stevensoni* is showed as a eurychronal species. All the data provided in here enforces its life cycle with about one or more years, during which two or more generations are produced. In each generation, females can carry maximum 11-12 embryos within their brood pouch (Van Doninck et al. 2003b; Gandolfi et al. 2001b). However, Horne et al. (1998) observed the presences of 15 juveniles in brood cavity. Accordingly, the species seems to have characteristics of K-selected or r-K continuum species (Van Doninck et al. 2003b). Furthermore, when comparing Darwinulids with other ostracods, they generally have low fecundity (Geiger 1998; Van den Broecke et al. 2013) and produce less eggs (0.02-0.07 layed eggs per day (Gandolfi et al. 2001b) and maximum 20 eggs per generation (Ranta 1979) that lower the number of cell division and thus the mutation rate falls in *D. stevensoni* (Van Doninck et al. 2003b).

Martens and Tudorance (1991) recorded the escape of species from the places with high temperature values in a tropical Ethiopian lake. Indeed, this observation was actually supported by the studies of Van Donnick et al. (2003a) that the species survival was shown to decrease with increasing temperature. Besides, Pérez et al. (2011) reporting a negative correlation between *D. stevensoni* and temperature confirmed the previous observation. When we look at the optimum and tolerance values of the species for water temperature 16.4-1.2 °C (Mezquita et al. 2005), 20.6-5.3 °C (Lorenschat and Schwalb 2013) and 19.44-6.22 °C (this study) (Table 1) it appears that species can tolerate a broad temperature range from cold to warm waters. Along with these information, wide temperature ranges of species from 4 (in subarctic) to 35 °C (Van Doninck et al. 2003b; Külköylüoğlu 2013) support the suggestion of Gandolfi et al. (2001b) and Anàdon et al. (2012) who characterized the species as eurythermal (tolerating and adapting to wide range of temperature) and thermoeuryplastic (a wide range of temperature tolerance), respectively.

When we look at the literature for the species occurrence patterns in different areas, it appears that its occurrence was reported to be positively related to biological oxygen demand (*BOD*), ammonium content (Mezquita et al. 1999a), *DO*, *pH* (Martens and Tudorance 1991), low (Rieradevall and Roca 1995) or highest water temperatures (Escrivá et al. 2014), warm water, carbonated water rich with sulfate and chloride (Mezquita et al. 1999b), but the relationship with iron content was negative (Iglikowska and Namiotko 2012). The result of the previous studies supports the suggestion of Külköylüoğlu and Vinyard (2000) as *D. stevensoni* prefers less saline waters. Similarly, Van Doninck et al. (2003a) suggested that survival of *D. stevensoni* is declined with increasing in salinity. Besides, Pérez et al. (2011) reported the species tolerating electrical conductivity optima at <700 µS/cm when Mezquita et al. (2005) and Lorenschat and Schwalb (2013) announced the optimum and tolerance level of

species for electrical conductivity as followings 3.09 ± 0.39 mS/cm and 239.1 ± 35.3 µS/cm (for salinity 0-0.04 ‰), respectively. The low optimum and tolerance levels of species for EC (351.62 ± 168.67) and salinity (0.13 ± 0.07) herein (Table 1) strengthened these previous findings. On the other hand, Martens (1990) noted the presence of species in the East African Lake Shala with 16–21 g/L salinity. Recently, Mischke et al. (2014) indicated the overcoming of species to large specific conductivity fluctuation but suggesting low optimum and tolerance of the species in 3.164 µS/cm and 0.916 µS/cm of EC values. The minimum and maximum value of electrical conductivity 21–9600 (after 15 days 100 % mortality observe) µS/cm (the current study; Gandolfi et al. 2001a) and salinity range 0-15 ‰ (the current study; Meisch 2000) for the species in literature strengthen the tolerating ability of species to salinity and EC fluctuations. Consequently, all of them fortify the euryhaline characteristics of species (Gandolfi et al. 2001b) and the presence of species from distilled water to sea water (Van Doninck et al. 2003a).

The positive correlation of *D. stevensoni* with *pH* herein endorses the recommendation of Rossetti et al. (2004). They found a close association between species and *pH* in eutrophic freshwater wetlands of northern Italy. In contrast, negative correlation was recorded between the species and *pH* (Pérez et al. 2011; Marchegiano et al. 2017). However, the high optimum and tolerance levels of species for *pH* as following 7.74 ± 0.40 (Mezquita et al. 2005) and 8.62 ± 0.26 (Lorenschat and Schwalb 2013) were announced from several different aquatic bodies. When we compile all data and compare optimum and tolerance (6.82 ± 3.37, Table 1) levels of the species along with min/max values (5.5–10.60) (Ruiz et al. 2013; the current study) for *pH*, it can be clearly seen that the *D. stevensoni* is of wide ranges of *pH* tolerance.

Additionally, species was negatively correlated with elevation in the current study. Possible effects of elevation on the physico-chemical characteristics of the aquatic bodies are widely discussed (Reeves et al. 2007; Rogora et al. 2008). Accordingly, it seems that elevation can be effective on the abundance but its effect may not be significant on the occurrence and distribution of species at high elevations. This is because it has been reported from sea level (Külköylüoğlu pers. obs.) to 4000 m a.s.l. (Laprida et al. 2006). Indeed, generally individual numbers larger than 100 were found from the sampling sites between 700-900 m a.s.l. and at lowest elevation when the range is 39 (122 individuals) – 2163 (5 individuals) m a.s.l in the present study.

Ranta (1979) delineated that *D. stevensoni* prefers highly oxygenated waters to aerate its eggs in brood chamber as stated by Külköylüoğlu and Vinyard (2000) and Rossetti et al. (2004). The high optimum and tolerance of species for dissolved oxygen 8.4 ± 2.1 mg/L in Mezquita et al. (2005), 7.9 ± 3.1 mg/L in Lorenschat and Schwalb (2013) and herein (see Table 1) support the proposal of Ranta (1979). On the other hand, Escrivà et al. (2014) proposed preferences of species for lowest dissolved oxygen. Although species might die under oxygen depletion (Rieradevall and Roca 1995), species can live over one month (38 days) under hypoxic conditions (ca. 0.12 ml/L oxygen) in laboratory conditions (Rossi et al. 2002). The minimum and maximum DO ranges of the species (0.32-18.31 mg/L) herein indicate that species may tolerate from low to high oxygen concentrations. Moreover, there is no any studies dealing the number or quality of eggs of species in low and high DO concentration and so the proposal of Ranta (1979) may be acceptable until otherwise stated. In addition, minimum and maximum values of ambient air temperature (12-40.20 ºC) (Horne 2007; the current study), calcium (5.25-80.80 mg/L) (Higuti et al. 2009a; Pérez et al. 2015) and magnesium (2.30-100.80 mg/L) (Holmes 1997; Pérez et al. 2015) contents of water bodies where species collected.

The above-mentioned information and wide range of environmental variables for species confirm the presence of all characteristics of the idea called "general purpose genotype" (GPG) in *D. stevensoni* (Rossi et al. 2002; Van Doninck et al. 2002). GPG emphasizes the production of different phenotypes by a genotype across a wide range of environmental conditions that allow species survive with high fitness in a wide range of habitats (Baker 1965; Geiger et al. 1998). This character of *D. stevensoni* reinforce the idea of Vandel (1928) as that "parthenogenetic (i.e., ancient asexual *D. stevensoni* herein) forms can be found in much wider areas" and referring to its long living without sex. Accordingly, Külköylüoğlu (2013) called the species as "cosmoecious species" to distinguish it from other species because of its wide geographical distribution and with relatively wide ecological tolerance ranges in variety of aquatic habitats. This view implies to take attention of scientists who want to use *D. stevens*oni as a potential bioindicator species to estimate past conditions and to determine water quality values of the present habitats. Additionally, species is ecologically characterized as stated by Meisch (2000), thermoeuryplastic and it encountered from freshwater range to mesohaline range.

As mentioned above, fossils of the species are known from Miocene and distribution of fossils

forms are mostly known from western parts of Turkey. Indeed, occurrences of both living and fossil forms partially overlap in some regions, indicating the long lasting surviving possibilities of the species in these regions. On the other hand, considering the fact that the species has not been found from hundreds of recent and paleontological samples, we may assume that *D. stevensoni* has not been able to reach to these regions. We believe that absence of the species from these samples may also be related to several other a/biotic factors but it is also possible that this is just a matter of time. Besides, as seen from Figures 1 and 2, as much as contemporary studies on living recent forms, paleontological studies are far away from understanding of their distribution in Turkey (if not the whole world). Thus, our study strongly suggests the need for future studies not only to understand for the distributional patterns of *D. stevensoni* but also other ostracods found from 4000 m below sea level to ca. 6000 m a.s.l.

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Appendix

Sampling numbers (SpNu), name of the county, habitat type, date and number of individuals of *D. stevensoni* (Abundance). na means not available. Habitat types: 1, Lake; 2, Creek; 3, Trough; 4, Dam; 5, Stream; 6, Pond; 7, Spring and 8, Slough.

SpNu	County name	Habitat type	Date	Abundance
1	Bolu	4	30.06.2000	2
2	Bolu	4	28.07.2000	1
3	Bolu	4	28.07.2000	3
4	Bolu	4	31.08.2000	3
5	Bolu	4	29.09.2000	2
6	Bolu	4	29.09.2000	2
7	Bolu	4	29.09.2000	3
8	Bolu	4	29.09.2000	4
9	Bolu	4	29.09.2000	4
10	Bolu	4	29.09.2000	8
11	Bolu	4	29.09.2000	34
12	Bolu	4	29.10.2000	2
13	Bolu	4	29.10.2000	2
14	Bolu	4	29.10.2000	3
15	Bolu	4	29.10.2000	3
16	Bolu	4	29.10.2000	3
17	Bolu	4	29.10.2000	28
18	Bolu	4	29.10.2000	201
19	Bolu	4	26.11.2000	32
20	Bolu	4	31.12.2000	1
21	Bolu	7	31.05.2001	10
22	Bolu	4	30.06.2001	50
23	Bolu	4	26.07.2001	2
24	Bolu	4	26.07.2001	6
25	Bolu	4	26.07.2001	10
26	Bolu	4	26.07.2001	14
27	Bolu	4	26.07.2001	22
28	Bolu	4	26.07.2001	32
29	Bolu	4	26.07.2001	101
30	Bolu	7	28.08.2001	1
31	Bolu	4	28.08.2001	19
32	Bolu	4	28.08.2001	101
33	Bolu	4	30.09.2001	3
34	Bolu	4	30.09.2001	101
35	Bolu	4	30.09.2001	101
36	Bolu	1	6-7.10.2001	1
37	Bolu	1	13.10.2001	1
38	Bolu	1	13.10.2001	3
39	Bolu	1	14.10.2001	1
40	Bolu	4	30.10.2001	1
41	Bolu	4	30.10.2001	101
42	Bolu	4	30.10.2001	101
43	Bolu	1	11.11.2001	1
44	Bolu	4	30.11.2001	1
45	Bolu	4	30.11.2001	2
46	Bolu	4	30.11.2001	3
47	Bolu	1	31.05.2002	1
48	Bolu	1	31.05.2002	3
49	Bolu	1	30.07.2002	1
50	Bolu	1	29.08.2003	8
51	Bolu	1	31.08.2003	1
52	Bolu	1	26.10.2003	1
53	Bolu	1	26.10.2003	1
54	Bolu	1	31.10.2003	1
55	Bolu	1	31.10.2003	2
56	Bolu	1	31.10.2003	3
57	Bolu	7	15.11.2003	6
58	Bolu	1	30.11.2003	2
59	Bolu	7	13.12.2003	1
60	Bolu	7	17.01.2004	6
61	Bolu	1	29.05.2004	1
62	Bolu	1	29.05.2004	1
63	Bolu	1	28.07.2004	1
64	Bolu	1	28.07.2004	1
65	Bolu	7	27.08.2004	1
66	Bolu	7	27.08.2004	2
67	Bolu	1	30.08.2004	1
68	Bolu	7	18.09.2004	5
69	Bolu	1	25.09.2004	2
70	Bolu	7	17.10.2004	2
71	Bolu	7	13.11.2004	1
72	Ordu	3	15.06.2010	1
73	Ordu	1	15.06.2010	77
74	Gaziantep	7	21.07.2010	9
75	Adıyaman	7	17.07.2012	52
76	Adıyaman	6	18.07.2012	1
77	Adıyaman	4	19.07.2012	2
78	Hatay	2	01.08.2012	5
79	Hatay	2	06.08.2012	2
80	Burdur	7	31.08.2012	1
81	Mardin	2	14.08.2013	1
82	Mardin	3	14.08.2013	62
83	Mardin	5	15.08.2013	3
84	Muş	7	18.08.2013	1
85	Muş	1	19.08.2013	5
86	Sakarya	7	10.05.2014	1
87	Sakarya	8	10.05.2014	122
88	Kütahya	4	21.09.2014	3
89	Kütahya	8	21.09.2014	4
90	Kütahya	2	21.09.2014	9
91	Kütahya	3	21.09.2014	214
92	Mersin	7	06.10.2015	3
93	Antalya	2	17.08.2017	6
94	Isparta	1	18.08.2017	2
95	Antalya	2	19.08.2017	2
96	Burdur	1	21.08.2017	3
97	Muğla	1	22.08.2017	2
98	Muğla	2	25.08.2017	1
99	Antalya	2	13.10.2017	1
100	Isparta	1	16.10.2017	2
101	Burdur	1	19.10.2017	3
102	Bolu	7	na	1

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Çayköy Deresi (Eğirdir-Isparta)'ndeki Eğirdir Siraz (*Capoeta pestai* Pietschmann, 1933)'larının Helmint Parazitleri Üzerine Bir Araştırma

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ÖZ

Bu çalışmada, Çayköy deresi (Eğirdir-Isparta)'nden elektroşokerle avlanan *Capoeta pestai* (Pietschmann, 1933)'nin helmint parazitleri araştırılmıştır. Araştırma süresince toplam 150 birey incelenmiştir. Çalışma sonucunda konak balıkta Platyhelminthes grubundan bir tür digenean *(Allocreadium isoporum* Loos, 1894) ve Nemathelminthes grubundan bir tür nematod (*Rhapdochona denudata*, Dujardin, 1845) bulunmuştur. Her mevsimde örneklenen bireylerden toplam 188 *A. isoporum* ve 592 adet *R. denudata* olmak üzere toplam 780 adet parazit tespit edilmiştir. Her iki parazitin de mevsim, cinsiyet, boy ve yaş değişkenlerine göre; yaygınlık, ortalama yoğunluk, bolluk ve toplam parazit sayıları belirlenmiştir.

Anahtar kelimeler: Çayköy Deresi, *Capoeta pestai*, helmint parazitler

MAKALE BİLGİSİ

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A Research on Helminth Parasites of *Capoeta pestai* (Pietschmann, 1933) from Çayköy Stream (Eğirdir-Isparta)

Abstract: In this study, the helminth parasites of *Capoeta pestai* (Pietschmann, 1933) that was caught by electro shocker in Çayköy stream (Eğirdir-Isparta) has been investigated. Throughout study, 150 individuals have been taken into consideration. As a result, digenean belongs to Platyhelminthes group and nematod belongs to Nemathelminthes have been determined. 188 *A. isoporum* Loos, 1894 *and* 592 *R. denudata*, Dujardin,1845 have been obtained from individuals that was sampled in every season. Mean intensity, total number, abundance and prevalence of both parasites has been set in regard to season, host size, age and sexes.

Keywords: Çayköy Stream, *Capoeta pestai*, helminth parasite

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Giriş

Su ürünleri içinde balık gerek sayısal ve gerekse ekonomik yönden önemli bir yer tutmaktadır. Ülkemizdeki tatlı sularda yaşayan balık tür sayısı da son yıllarda yapılan revizyonlarla 27 familyaya ait 92 cins ve 371 türe ulaşmıştır. Bu türlerin yarısından fazlasının Cyprinidae familyası üyesi oldukları saptanmıştır. Bunların 19'unu *Capoeta* cinsine ait türler oluşturmaktadır (Kuru vd. 2014).

Gerek ülkemizde ve gerekse dünya balık üretiminde yetiştiricilikle avcılık arasındaki istihsal oranları oldukça biribirine yaklaşmıştır (Anonymous 2016). Sürekli yapılan bilimsel çalışmalar sayesinde gerek tüketimlik ve gerekse hobi anlamında birçok tür yetiştiricilik yelpazesinde yerini almaktadır. Doğal yaşam biçiminden koparılan bu türlerin gerek beslenme ve gerekse sağlık anlamında problemleri olmakta ve bununla ilgili çözümler için çalışmalar yapılmaktadır. Sucul ekosistemlerde besin zincirinin son basamaklarında yer alan ve insanlar için önemli bir protein kaynağı olan balıklar, farklı parazit türlerine konaklık ederler. Bu parazitler ise balıklarda ciddi hastalıklara ve ekonomik kayıplara neden olabilirler. Parazitler doğal balık populasyonlarında az düzeyde görüntü zararlarına neden olduğu gibi, yetiştirilen balıklarda önemli hastalıkların oluşmasına zemin hazırlayabilir; patolojik değişimlere, sağlıksız ve pazar değeri kayıplarına yol açabilirler (Arslan vd. 1995; Scholz 1999). Yine bazı nematod ve cestodların insan sağlığına zarar verebildiği bilinmektedir (Olson 1986).

Özellikle Eğirdir Gölü ülkemizin tatlı su faunası için oldukça önemli bir göldür. Eğirdir Gölü'ne yapılan plantasyonlardan dolayı bugün 7 tür yerli, 6 tür de yabancı olmak üzere 13 tür balık bulunmaktadır (Yerli vd. 2013). 1915-2007 yılları arasında Eğirdir Gölün'deki ihtiyofaunal değişimlerin değerlendirildiği bir çalışmada Eğirdir bıyıklısı (*Capoeta pestai*)'nin kritik düzeyde bulunduğu ifade edilmiştir (Küçük vd. 2009). Hem korunması ve hem de üretilmesi konusunda çalışmaların yapıldığı göl ve gölü besleyen akarsu habitatlarına uyum sağlayan Capoeta cinsine ait *C. pestai* türüne yönelik birçok çalışma yapılmıştır (Küçük vd. 2007; Özen vd. 2008; Ayyıldız vd. 2015; Emre vd. 2016; Demir 2017). Ancak bu türün parazitleri üzerine detaylı herhangi bir çalışma yapılmamıştır. Çalışmamızın amacı Çayköy Deresinde yaşayan bu konaktaki metazoan parazit faunasını mevsimsel olarak bazı değişkenlere göre incelemektir.

Materyal ve Metot

Balık materyalinin avlandığı Çayköy Deresi, Eğirdir Gölü su toplama havzası (drenaj alanı) içindedir. Dere, Eğirdir Gölü'nün güneydoğusundaki Yılgıncak Köyü yakınlarından başlayıp, Çayköy'e kadar devam eden ve derin olmayan bir vadi içerisinde bulunur (37º 49′ 24′′K-030º 56′ 46′′D) (Şekil 1). Çayköy'den sonra Aksu HES çıkış suyu ile birleşerek yapay bir kanal ile Eğirdir Gölü'ne boşalır. Kış ve bahar aylarında 1,5-5 m^3 su taşıyabilir. Yaz aylarında suları $1m^3$ altındadır.

Çalışma için Çayköy deresinden ilk balık örnekleri ilkbahar mevsiminde alınmıştır. Balıklar elektroşokerle avlanmıştır. Araştırma süresince, toplam 150 balık bireyi incelenmiştir. Mart 2013-Şubat 2014 tarihleri arasında avlanan balıkların öncelikle total boy ve ağırlıkları ölçülmüştür. Daha sonra deri, yüzgeçleri ve gözler incelenip ektoparazit taraması yapılmıştır. Bu işlem tamamlandıktan sonra, ilk önce balığın solungaçları ile iç organları çıkartılmış ve stereo mikroskop altında parazitler aranmıştır. Bulunan parazitlerin tür, yerleşim ve sayıları kaydedilip, türlere göre petri kaplarına konulmuştur. Parazitlerden bir kısmı hemen ve canlı olarak incelemeye tabii tutulmuştur. Diğerleri ise daha sonraki çalışmalar için % 70'lik etil alkolde saklanmıştır. Öte yandan Nematodlar gliserin alkolde temizlenmiş ve % 70'lik alkolde fikse edilmişlerdir. Parazitlerin teşhisleri Bychovskaya-Pavlovskaya 1962, Gussev 1985, Gussev vd. 1987, Markevic 1951'e göre yapılmıştır. Parazitlerin boyama ve tespit işlemlerinde Fernando vd. (1972)'nin geliştirdiği yöntemden yararlanılmıştır. Konak balık örneklerinin yaş tayinleri için otolitlerden faydalanılmıştır (Murray 1994; Campana vd. 2003; Walsh ve Maloy 2008). Ayrıca, Parazit enfeksiyonlarının mevsimlere ve cinsiyetine göre değişimleri Quantitative Parasitology 3.0 (Rózsa vd. 2000; Reiczigel ve Rózsa 2005) programına göre sınanmıştır. *C. pestai'*de bulunan bir tür parazitin SEM görüntüleri alınmıştır. Görüntüleme işlemleri Akdeniz Üniversitesi Tıp Fakultesi Elektron Mikroskop Görüntü Analiz Ünitesi (TEMGA)'nde yapılmıştır.

Şekil 1. Çayköy Deresi

Şekil 2. *Capoeta pestai*

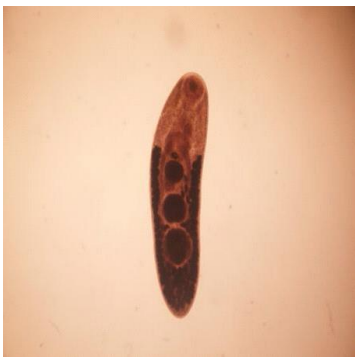
Bulgular

Mart-2013/Şubat-2014 tarihleri arasında mevsimsel olarak, Çayköy deresinden (Eğirdir-Isparta) elektroşokerle yakalanan *C. pestai* türüne ait 150 bireyden 93'ü erkek, 57'si ise dişi bireylerden oluşmuştur. Yapılan parazit muayenelerinde Platyhelminthes grubundan bir tür digenean *(Allocreadium isoporum,* Loos, 1894) ve Nemathelminthes grubundan bir tür nematod (*Rhapdochona denudata,* Dujardin, 1845) bulunmuştur (Şekil 3, 4 ve 5). Dört mevsimdeki muayenelerde toplam 188 *A. isoporum,* 592 adet *R. denudata* paraziti saptanmıştır. *C. pestai* türünde karşılaşılan her iki parazit türlerine ait mevsimsel yaygınlık (%), ortalama yoğunluk, ortalama bolluk ve toplam parazit sayıları Çizelge 1'de verilmiştir. Buna göre *A. isoporum* için en yüksek yaygınlık (%52,5),

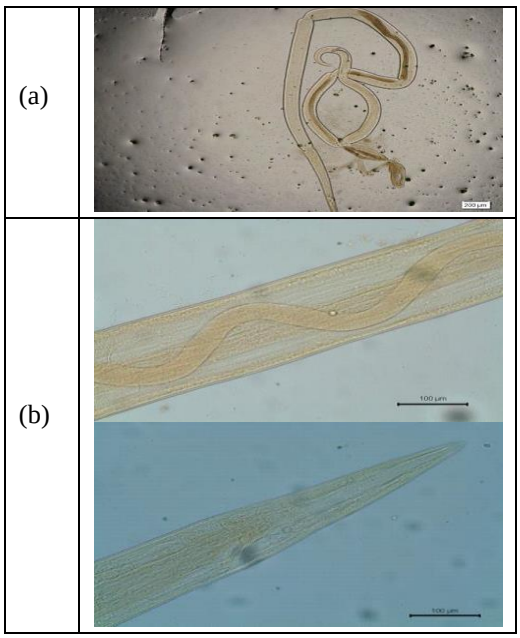
ortalama yoğunluk (4,57) ve ortalama bolluk (2,4) değerleri ile kış mevsiminde bulunmuştur. Buna karşın; *R. denudata* için ise, en yüksek yaygınlık (%60) kış, ortalama yoğunluk (9,09) yaz ve ortalama bolluk ise yine kış mevsimlerinde bulunmuştur**.**

Çizelge 1. *C. pestai* türündeki mevsimsel digean ve nematod gruplarına ait parazitik enfeksiyon değerleri.

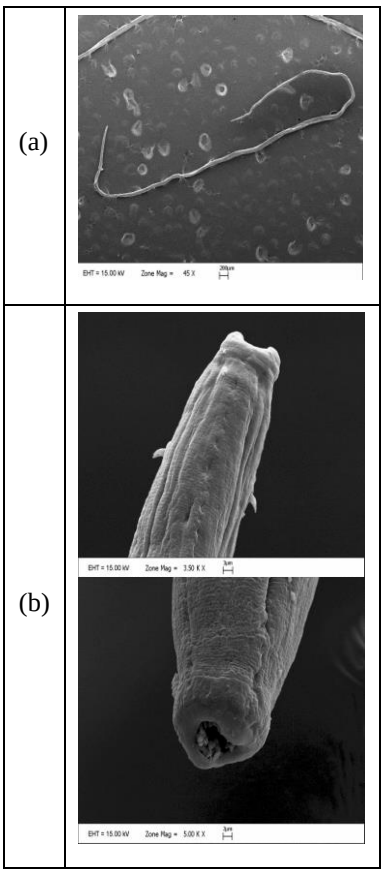
Mevsimler	Türler	İncelenen balık sayısı	Enfekte balık sayısı	Yaygınlık (%)	Ortalama Yoğunluk	Ortalama Bolluk	Toplam Parazit Sayısı
İlkbahar	*A.isoporum*	(n:42)	22	52,4	2,23	1,17	49
	R.denudata	(n:42)	24	57,1	6	3,43	144
Yaz	*A.isoporum*	(n:33)	6	18,2	2,67	0,48	16
	R.denudata	(n:33)	11	33,3	9,09	3,03	100
Sonbahar	*A.isoporum*	(n:35)	8	22,9	3	0,69	27
	R.denudata	(n:35)	18	51,4	8,11	4,17	146
Kış	*A.isoporum*	(n:40)	21	52,5	4,57	2,4	96
	R.denudata	(n:40)	24	60	8,42	5,05	202



Şekil 3. *C. pestai*'de bulunan *Allocreadium isoporum*'un genel görünüşü (x40).



Şekil 4. (a) *R. denudata'*nın genel görünüşü, (b) anteriör kısmı.



Şekil 5. *C. pestai*'de bulunan Nematoda (*Rhapdochona denudata*)'nın SEM görüntüleri (a) Genel görünüm, (b). Anterior.

C. pestai türünde dört mevsim boyunca yapılan çalışmalarda *A. isoporum* paraziti 57 dişi balıkta değerlendirilmiştir. En yüksek yaygınlık (%) 78,9 ortalama yoğunluk 4,93 ve 3,89 ortalama bollukla kış mevsiminde bulunmuştur. *R. denudata* için ise en

yüksek yaygınlık (%) 73,7 ile dişilerde kış mevsiminde, ortalama yoğunluk 14,50 ve 7,25 ortalama bollukla yaz mevsiminde en yüksek değerler bulunmuştur. Buna karşılık tüm mevsimlerde toplam 93 erkek balık örneğinde de değerlendirme yapılmıştır. Buna göre; *A. isoporum* için en yüksek yaygınlık (%) 48,40 erkeklerde ilkbaharda, ortalama yoğunluk 3,67 ile kış ve 1,13 ortalama bollukla ilkbahar mevsiminde yüksek değerler bulunmuştur. Buna karşılık, *R. denudata* için ise en yüksek yaygınlık (%) 58,06 erkeklerde ilkbaharda, ortalama yoğunluk 11,4 ile ve 5,43 ortalama bollukla kış mevsiminde yüksek değerler bulunmuştur. *A. isoporum* ve *R. denudata'*ya ait mevsim ile konak cinsiyetlerine göre dağılımlarına ait veriler Çizelge 2'de verilmiştir.

Çizelge 2. *C. pestai* türünün cinsiyetlere göre mevsimsel digenean ve nematod gruplarına ait parazitik enfeksiyon değerleri.

Mevsimler	Cinsiyet	Türler	İncelenen balık sayısı	Parazitli balık sayısı	Yaygınlık (%)	Ortalama Yoğunluk	Ortalama Bolluk	Toplam Parazit Sayısı
İlkbahar	Dişi	*A.isoporum*	(n:11)	7	63,6	2	1,27	14
		R.denudata	(n:11)	6	54,5	2,83	1,55	17
	Erkek	*A.isoporum*	(n:31)	15	48,4	2,33	1,13	35
		R.denudata	(n:31)	8	58,06	7,06	4,1	127
Yaz	Dişi	*A.isoporum*	(n:8)	2	25	4	1	8
		R.denudata	(n:8)	4	50	14,5	7,25	58
	Erkek	*A.isoporum*	(n:25)	4	16	2	0,32	8
		R.denudata	(n:25)	7	28	6	1,68	42
Sonbahar	Dişi	*A.isoporum*	(n:19)	4	21,1	2,75	0,58	11
		R.denudata	(n:19)	9	47,4	8,78	4,16	79
	Erkek	*A.isoporum*	(n:16)	5	31,3	3,2	1	16
		R.denudata	(n:16)	9	56,3	7,44	4,19	67
Kış	Dişi	*A.isoporum*	(n:19)	15	78,9	4,93	3,89	74
		R.denudata	(n:19)	14	73,7	6,29	4,63	88
	Erkek	*A.isoporum*	(n:21)	6	28,6	3,67	1,05	22
		R.denudata	(n:21)	10	47,6	11,4	5,43	114

A. isoporum'un mevsimlere göre konak yaş grupları değerlendirildiğinde, ilkbahar döneminde III. Yaş grubuna ait balıkların A. isoporum ile enfazla enfekte olduğu görülmüşütür. (Çizelge 3). Diğer değerlendirme sonuçları aynı Çizelge'de verilmiştir. Yine boya göre değerlendirme de Çizelge 4'de gösterilmiştir. Buna göre ilkbahardaki 14,1-18,0 cm boy grubunda daha fazla örneğin *A. isoporum* ile enfekte olduğu görülmüştür. Ayrıca mevsimsel olarak değerlendirilen örneklemelerin boya göre değerlendirmeleri Çizelge 4'de verilmiştir.

C. pestai için mevsimsel olarak yapılan örneklemelerde nematod grubundan *R. denudata* parazitine tüm mevsimlerde rastlanmıştır (Çizelge 1,2 ve 5,6). Çizelge 5'de yaş grubuna göre yapılan değerlendirmede en fazla enfekte balık sayısının kış mevsiminde III yaşındaki bireylerde olduğu görülmüştür. Yine Çizelge 6'da boya göre yapılan değerlendirmede ise 14,1-18,0 grubundaki konak örnekleinin daha fazla enfekte olduğu görülmüştür. Çizelge 5 ve 6'da *Capoeta pestai*'de bulunan *R. denudata*'nın konak boy ve yaş gruplarına göre dağılımları ile ilgili diğer sonuçlar verilmiştir.

Çizelge 3. *C. pestai*'de bulunan *Allocreadium isoporum*'un konak yaş gruplarına göre değerlendirilmesi.

Yaş Grupları	O	I	II	III	IV	V	VI	VII	VIII	IX	X
İlkbahar											
İncelenen Balık Sayısı	2	2	6	15	10	3	1	0	3		
Enfekte Balık Sayısı	0	0	2	10	6	2	1	0	1		
Yaygınlık (%)	0	0	33,3	66,7	60	66,7	100	0	33,3		
Ortalama Yoğunluk	0	0	1,5	2	2	4,5	3	0	2		
Ortalama Bolluk	0	0	0,5	1,33	1,2	3	3	0	0,67		
Toplam Parazit Sayısı	0	0	3	20	12	9	3	0	2		
Yaz											
İncelenen Balık Sayısı	8	12	3	4	3	2	1	0	0		
Enfekte Balık Sayısı	1	3	0	0	1	1	0	0	0		
Yaygınlık (%)	12,5	25	0	0	33,3	50	0	0	0		
Ortalama Yoğunluk	2	3	0	0	1	4	0	0	0		
Ortalama Bolluk	0,25	0,75	0	0	0,33	2	0	0	0		
Toplam Parazit Sayısı	2	9	0	0	1	4	0	0	0		
Sonbahar											
İncelenen Balık Sayısı	2	4	4	3	4	6	3	0	8	0	1
Enfekte Balık Sayısı	1	0	1	0	1	1	2	0	3	0	0
Yaygınlık (%)	50	0	25	0	25	16,7	66,7	0	37,5	0	0
Ortalama Yoğunluk	3	0	1	0	3	3	2	0	4,33	0	0
Ortalama Bolluk	1,5	0	0,25	0	0,75	0,5	1,33	0	1,63	0	0
Toplam Parazit Sayısı	3	0	1	0	3	3	4	0	13	0	0
Kış											
İncelenen Balık Sayısı	5	8	6	11	3	3	0	0	8	0	0
Enfekte Balık Sayısı	0	1	2	9	2	3	0	0	4	0	0
Yaygınlık (%)	0	12,5	33,3	81,8	66,7	100	0	0	50	0	0
Ortalama Yoğunluk	0	2	6,5	3,67	6	3	0	0	6,75	0	0
Ortalama Bolluk	0	0,25	2,17	3	4	3	0	0	3,38	0	0
Toplam Parazit Sayısı	0	2	13	33	12	9	0	0	27	0	0

Çizelge 4. *C. pestai*'de bulunan *Allocreadium isoporum*'un konak boy gruplarına göre değerlendirilmesi.

Boy Grupları (cm)	**I (6,0-10,0 cm)**	**II (10,1-14,0 cm)**	**III (14,1-18,0 cm)**	**IV (18,1-22,0 cm)**	**V (22,1-28,0 cm)**
İlkbahar					
İncelenen Balık Sayısı	2	8	17	15	0
Enfekte Balık Sayısı	0	2	12	8	0
Yaygınlık (%)	0	25	70,6	53,3	0
Ortalama Yoğunluk	0	1,5	2,08	2,63	0
Ortalama Bolluk	0	0,38	1,47	1,4	0
Toplam Parazit Sayısı	0	3	25	21	0
Yaz					
İncelenen Balık Sayısı	7	14	7	5	0
Enfekte Balık Sayısı	1	3	1	1	0
Yaygınlık (%)	14,3	21,4	14,3	20	0
Ortalama Yoğunluk	2	3	1	4	0
Ortalama Bolluk	0,29	0,64	0,14	0,8	0
Toplam Parazit Sayısı	2	9	1	4	0
Sonbahar					
İncelenen Balık Sayısı	0	9	6	12	7
Enfekte Balık Sayısı	0	1	1	4	2
Yaygınlık (%)	0	11,1	16,7	33,3	28,6
Ortalama Yoğunluk	0	1	3	3,5	3
Ortalama Bolluk	0	0,11	0,5	1,17	0,86
Toplam Parazit Sayısı	0	1	3	14	6
Kış					
İncelenen Balık Sayısı	6	10	15	5	4
Enfekte Balık Sayısı	0	1	11	5	4
Yaygınlık (%)	0	10	73,3	100	100
Ortalama Yoğunluk	0	2	4,18	4,6	6,25
Ortalama Bolluk	0	0,2	3,07	4,6	6,25
Toplam Parazit Sayısı	0	2	46	23	25

Çizelge 5. *Capoeta pestai*'de bulunan *Rhapdochona denudata*'nin yaş gruplarına göre değerlendirilmesi.

Yaş Grupları	O	I	II	III	IV	V	VI	VII	VIII	IX	X
İlkbahar											
İncelenen Balık Sayısı	2	2	6	15	10	3	1	0	3	0	0
Enfekte Balık Sayısı	1	2	3	11	4	2	1	0	0	0	0
Yaygınlık (%)	50	100	50	73,3	40	66,7	100	0	0	0	0
Ortalama Yoğunluk	1	3,5	1,33	9,82	1,5	8	2	0	0	0	0
Ortalama Bolluk	0,5	3,5	0,67	7,2	0,6	5,33	2	0	0	0	0
Toplam Parazit Sayısı	1	7	4	108	6	16	2	0	0	0	0
Yaz											
İncelenen Balık Sayısı	8	12	3	4	3	2	0	0	0	0	0
Enfekte Balık Sayısı	2	4	2	1	2	0	0	0	0	0	0
Yaygınlık (%)	25	33,3	66,7	25	66,7	0	0	0	0	0	0
Ortalama Yoğunluk	6,5	6,25	12	25	6,5	0	0	0	0	0	0
Ortalama Bolluk	1,63	2,08	8	6,25	4,33	0	0	0	0	0	0
Toplam Parazit Sayısı	13	25	24	25	13	0	0	0	0	0	0
Sonbahar											
İncelenen Balık Sayısı	2	4	4	3	4	6	3	0	8	0	1
Enfekte Balık Sayısı	0	2	3	0	2	5	3	0	3	0	0
Yaygınlık (%)	0	50	75	0	50	83,3	100	0	37,5	0	0
Ortalama Yoğunluk	0	7	4,67	0	7,5	8,4	5	0	15,33	0	0
Ortalama Bolluk	0	3,5	3,5	0	3,75	7	5	0	5,75	0	0
Toplam Parazit Sayısı	0	14	14	0	15	42	15	0	46	0	0
Kış											
İncelenen Balık Sayısı	5	8	6	11	3	3	0	0	8	0	0
Enfekte Balık Sayısı	2	1	2	11	2	2	0	0	4	0	0
Yaygınlık (%)	40	12,5	33,3	100	66,7	66,7	0	0	50	0	0
Ortalama Yoğunluk	35,5	2	5,5	7,09	8	3,5	0	0	4,25	0	0
Ortalama Bolluk	14,2	0,25	1,83	7,09	5,33	2,33	0	0	2,13	0	0
Toplam Parazit Sayısı	71	2	11	78	16	7	0	0	17	0	0

Çizelge 6. *Capoeta pestai*'de bulunan *Rhapdochona denudata*'nin boy gruplarına göre değerlendirilmesi.

Boy Grupları (cm)	**I (6,0-10,0 cm)**	**II (10,1-14,0 cm)**	**III (14,1-18,0 cm)**	**IV (18,1-22,0 cm)**	**V (22,1-28,0 cm)**
İlkbahar					
İncelenen Balık Sayısı	2	8	17	15	0
Enfekte Balık Sayısı	1	5	12	6	0
Yaygınlık (%)	50	62,5	70,6	40	0
Ortalama Yoğunluk	1	2,2	9,17	3,67	0
Ortalama Bolluk	0,5	1,38	6,47	1,47	0
Toplam Parazit Sayısı	1	11	110	22	0
Yaz					
İncelenen Balık Sayısı	7	14	7	5	0
Enfekte Balık Sayısı	2	5	3	1	0
Yaygınlık (%)	28,6	35,7	42,9	20	0
Ortalama Yoğunluk	6,5	8	12,33	10	0
Ortalama Bolluk	1,86	2,86	5,29	2	0
Toplam Parazit Sayısı	13	40	37	10	0
Sonbahar					
İncelenen Balık Sayısı	0	9	6	12	7
Enfekte Balık Sayısı	0	5	2	8	3
Yaygınlık (%)	0	55,6	33,3	66,7	42,9
Ortalama Yoğunluk	0	5,6	7,5	7,13	15,33
Ortalama Bolluk	0	3,11	2,5	4,75	6,57
Toplam Parazit Sayısı	0	28	15	57	46
Kış					
İncelenen Balık Sayısı	6	10	15	5	4
Enfekte Balık Sayısı	2	1	13	5	3
Yaygınlık (%)	33,3	10	86,7	100	75
Ortalama Yoğunluk	35,5	2	6,85	5	5
Ortalama Bolluk	11,8	0,2	5,93	5	3,75
Toplam Parazit Sayısı	71	2	89	25	15

Tartışma ve Sonuç

Ülkemizdeki endemik balıklardan *C. pestai* türünde bulunan helmint biyoçeşitliliğinin incelendiği bu çalışmamızda, *C. pestai* türünün % 58,0'nın en az iki tür parazitle enfekte olduğu saptanmıştır. Zayıf bir patojen olarak bilinen ve özellikle sazangiller ailesine mensup türlerde barsakta bulunan *A. isoporum* türü çalışmamızda bu konakta bulunmuştur. Bu konak için yeni kayıttır. *C. pestai* türünde ağırlıklı olarak yakalanan balıkların 1/3'ü dişi bireylerden oluşmuştur. En yüksek yaygınlık oranları ilkbahar ve kış örneklemelerinde bulunmuştur (% 52,5). En düşük ise yaz örneklemelerinde belirlenmiştir (% 18,2). Buna karşılık, III yaşındaki ilkbahar örneklerinde en fazla enfekte balığa rastlanmıştır. Moravec (1992) Tuna havzasındaki Rokytna Nehirinde *Leuciscus cephalus* konağına yönelik yaptığı çalışmada en yüksek yaygınlık oranını ilkbahar aylarında (%100), ay ortalama yaygınlık oranını ise % 73 olarak saptamıştır. Koyun (2001), Enne Baraj Gölünde yaptığı çalışmada *Alburnus alburnus* konağında Mayıs ve Haziran (1998)'da %15 ve %19; Nisan ve Mayıs (1999)'da ise %15 ile %10 yaygınlık oranında *A. isoporum* paraziti belirlemiştir. Aydoğdu vd. (2002), Doğancı Baraj Gölündeki *Barbus plebejus* konağının helminth faunasına yönelik yaptıkları araştırmada *A. isoporum* parazitine Mayıs ve Haziran aylarında %75 ve %33,3; Eylul ve Ekim aylarında %11,1 ve %20 ve toplamda ise %19,1 yaygınlık oranında rastlanılmıştır. Yine Raissy ve Ansari (2012) İran'da Arman Nehiri balıklarındaki parazitlere yönelik yaptıkları çalışmada *C. aculeata, C. damascina* ve *Barbus barbulus*'un barsaklarında yaygınlık oranları %2, %0,79 ve % 1,4 şeklinde bulmuşlardır.

Diğer yandan *C. pestai* konağında, nematod grubuna ait bir tür olan *Rhabdochona denudata* türü bulunmuştur. Bu tür de yeni kayıt özelliği taşımaktadır. Bu türle alakalı olarak ayrıca SEM çalışması yapılmıştır. *C. pestai*'de en yüksek yaygınlık oranı % 58,1 ile ilkbahar, en düşük ise %28 ile yaz örneklemelerindeki erkek bireylerde saptanmıştır. III. yaş gruplarında (kış) ve 14,1-18,0 boy aralığındaki bireylerde daha fazla enfekte olan bireylere rastlanmıştır. Aydoğdu vd., (2011) Antalya Körfezine dökülen akarsularda yaptıkları çalışmada sınırlı sayıdaki *Capoeta antalyensis*'te %86,6 yaygınlık oranında *R. denudata* tespit edilmiştir. Pazooki vd. (2012) İran-Kerman ilindeki dört ayrı su kaynağında avlanan *C. damascina*'da yaygınlık oranını %73,39; *Cyprinion watsoni*'da %88,60, *Schistura sargadensis*'da %2.94 ve *Channa gachua*'da ise % 13,63 şeklinde *R. denudata* bulmuşlardır. Koyun vd. (2015) Murat Nehirin'de yaşayan *B. lecerta* konağında bu nematod da sadece ilkbahar (% 23) ve yaz mevsimlerinde (% 16,7) yaygınlık oranı ile karşılaşılmıştır. Sonuç olarak yukarıda belirtildiği gibi; Çayköy Deresindeki *C. pestai*'de bulunan iki parazit türü konak için yeni kayıt özelliğini taşımaktadır. Bu nedenle, gerek mevcut alandaki ve gerekse Eğirdir Gölü'ndeki stoklara ait tehditlerin tespiti ve yine Türkiye'nin helmint biyoçeşitliliğinin belirlenmesi noktasında çalışmamız önem arz etmektedir.

Teşekkür

Bu makale Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsünde "Akdeniz Bölgesi'ndeki *Capoeta erhani, Capoeta pestai, Capoeta mauricii* Türlerinin Helmint Parazitlerinin Biyoçeşitliliğinin Araştırılması" başlıklı tezden üretilmiştir.

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Assessment of Fish Exports from Blantyre District, Southern Malawi

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ABSTRACT

This study presents findings on fish exports from Blantyre, which is the biggest commercial city in Malawi. A cross-sectional research design was used in the study in which fish quantities, species, processing methods, gender of fish exporters and export destinations were analysed. Non-probability data collection methods were used on the secondary data that was collected from Blantyre District Fisheries Office. Findings indicate that a total of 9596 kg (9.6 metric tonnes) of fish was exported within a period of six months in the year 2013 comprising all species available in the local markets and those endemic in Malawi's water bodies. Most fish were exported as sun-dried, para-boiled and smoked. Findings suggest that fish exports from Malawi are in their infancy, fluctuate, and are insignificant for making a positive and sustainable impact to the economy of the country. It is recommended that national and regional policies should be fully utilized to curb exports of fish in order to sustainably satisfy the local huge demand for fish. About 82.6% of the fish exporters were women underpinning the need for policy considerations that value their critical role in the fish export trade such as women empowerment.

Keywords: Fish exports, species, processing, destinations, gender

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Introduction

In Malawi, documented fish exports are estimated at 0.102 metric tonnes, accounting for a monetary value of MWK (Malawi Kwacha currency) 22,000 (US$ 250) in 1998 to 159.5 metric tonnes valued at MWK18.5 million (US$170,000) in 2002 (Commonwealth/GTZ 2007). The figures indicate that fish exports increased in the period of 1998 to 2002. However, the Government of Malawi (GoM 2012) reported that annually, an average of 86 tonnes of fish was exported between 2002 and 2010. Reports also indicated that quantities of annual fish exports within the same period greatly fluctuated due to dwindling fish harvests (Commonwealth/GTZ 2007; Donda and Njaya 2007; GoM 2011, 2012). Fish catches from Malawi were estimated at 70000 and 3000 metric tonnes per annum from capture fisheries and fish farming, respectively (GoM 2012). To the contrary, the domestic demand for fish in the country was estimated at around 110000 metric tonnes per year (GoM 2012) indicative of a supply deficit. Recent reports showed that Malawi informally exported fish to neighbouring Tanzania, Mozambique and Zambia between 2015 and 2016 with an estimated annual quantity of 24115.68 metric tonnes valued at 41.6 million dollars (Mussa et al. 2017). Such reports exist at a time when the country was in a dire shortage of fish supply with an average per capita annual fish consumption estimated at 5.6 kg, far below the 13-15 kg recommended by the World Health Organisation (Kapute 2017). The demand for fish in Malawi has in recent years tremendously increased due to increased population against the declining fish catches from the natural waters. In Malawi, fish provides the best and most affordable source of dietary animal protein to many people contributing about 28% of all dietary animal protein supply (Kapute 2017). Another concern is that there is little consideration about quality standards for fish trade in Malawi and clearly indicating that fish quality standards have not been well developed for exports to highly regulated markets (Kapute 2009; GoM 2012; Manyungwa-Pasani et al. 2017). The traders have thus, been

trading without proper licensing and no empowerment. In addition, there have been limited studies focusing on the exports of fish from Malawi. This study differs from earlier studies because it focuses on formal fish exports. The novelty of this study provides a foundation to the sustainability of the fish exports in order for the country to benefit from the trade and diversify the income generating activities in the fisheries value chain. In this study, exports of fish from Malawi were assessed in order to assist the government in establishing the magnitude of fish quantities exported, fish processing methods, fish species, gender of exporters and fish export destinations.

Material and Methods

Study area and data collection

Cross-sectional research design was used in the study (Bhattacherjee 2012) and fish export data were collected from Blantyre District Fisheries Office in southern Malawi. Blantyre is the commercial and industrial city of Malawi and forms the communication and transportation hub to all parts of the country as well as the neighbouring countries. Fish exports data are collected by the Malawi Department of Fisheries when issuing the phytosanitary certificates to fish exporters. Information that is contained in the phyto-sanitary certificates includes destinations, fisheries quality control unit, name and address of exporter, declared name and address of consignee, declared quality and name of fish product, scientific name of fish, number and description of packages, declared means of conveyance and treatment, among others. The Department of Fisheries started providing the phyto-sanitary certificates to Blantyre District Fisheries Office in March 2013. To ensure validity and reliability of the data collected, 10 duplicate sanitary certificates were collected from Blantyre District Fisheries Office before the actual data collection exercise. The collected duplicate Phyto-sanitary certificates were checked by qualified technical personnel in the Department of Fisheries and Aquatic Sciences at Mzuzu University, Malawi. Fish exports data for a period of six months, from May to October, 2013 were collected from a total of 217 individual traders using non - probability sampling technique i.e. all the data available were collected for the study (Ukaonu et al. 2011; Haambiya et al. 2013).

Species and quantity of fish exported

Fish species that an individual person could export were summarized as percentages instead of listing used in earlier studies (Jayalal and Ramachandran 2012). Data collected for this study did not indicate the exact quantities exported per species and as such, frequencies were used. The monthly fish exports were added for all months from May to October 2013, and quantities were also added to give a total quantity of fish exported within the period of study. The number of fish exporters were separately classified into three categories according to the quantity of fish exported and percentages of monthly quantities from total quantity exported were computed. The classes were: less than 50 kg ($fq<50kg$), between 50 and 100 kg ($50 < fq < 100$), and greater than 100 kg ($fq>100$), where fq is the fish quantity exported. The percentage of traders in each category was calculated. To determine fish processing methods used for fish destined for trading in the international markets, the proportion of each processed method of the fish species was summarized in the bar graphs to observe the frequently exported forms of processed fish.

Gender of fish exporters and destinations of fish exports

The percentages of male and female fish exporters were found for each month of the study to understand whether they were equal percentages for each month. All countries, which serve as markets for fish from Blantyre, were identified and frequencies of fish traders were calculated and recorded. This method was preferred due to its robustness in encompassing specific details without overlooking the importance of the details.

Statistical analysis

Data were analyzed in SPSS Software version 16.0. Chi – square (goodness of fit) test was used to test for the differences in the distributions of categories of fish exporters and their gender. The hypotheses were tested at 5% significance level. Graphs were generated using Microsoft Excel 2010.

Results

Quantity of fish exports

A total of 9596 kg of fish were exported in the period studied and the highest quantity was exported in the month of June (39.2%). It should be noted that although the percentages of fish exported per month were fluctuating, the trend showed an increase from 7.9%, 12.0% and 17.5% from July, August and September respectively (Figure 1).

There were significant differences among the three categories of fish exporters in all the months studied (Table 1). The months showing the degrees of freedom (df) of one suggest no fish exports of more than 100 kg.

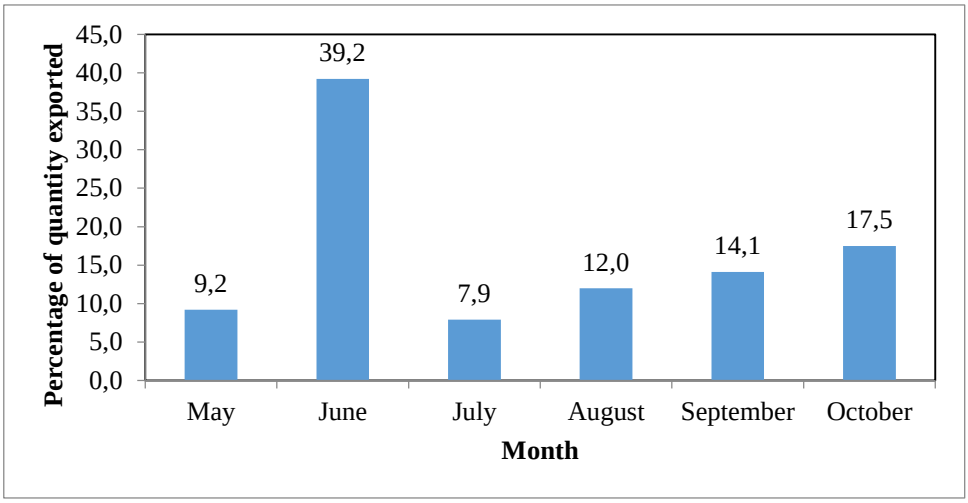

Figure 1. Percentage of fish exports from May to October 2013 from Blantyre

Table 1. Chi-square (goodness of fit) test results for the three categorised fish exporters

	May	June	July	August	September	October
$\chi^2 - value$	13.300[a]	28.374[a]	4.263[a]	9.143[a]	11.789[a]	12.250[a]
df	2	1	1	1	2	2
Asymp. Sig.	0.001	0.000	0.039	0.002	0.003	0.002

Values with similar superscripts in a row are significantly different (P < 0.05).

Fish species on international trade

Fish species that were exported are *Engraulicypris sardella* (Günther, 1868) (local name: Usipa), *Clarias gariepinus* (Burchell, 1822) (Catfish: Mlamba), *Oreochromis* species (Weyl et al 2010) (Chambo), *Oreochromis mossambicus* (Makakana), *Enteromius paludinosus* (Peters, 1852) (Matemba), and *Rhamphochromis* species (Mcheni). However, frequently exported species were *E. sardella* and *C. gariepinus* with more than 75% and 65% of the fish exporters respectively (Figure 2).

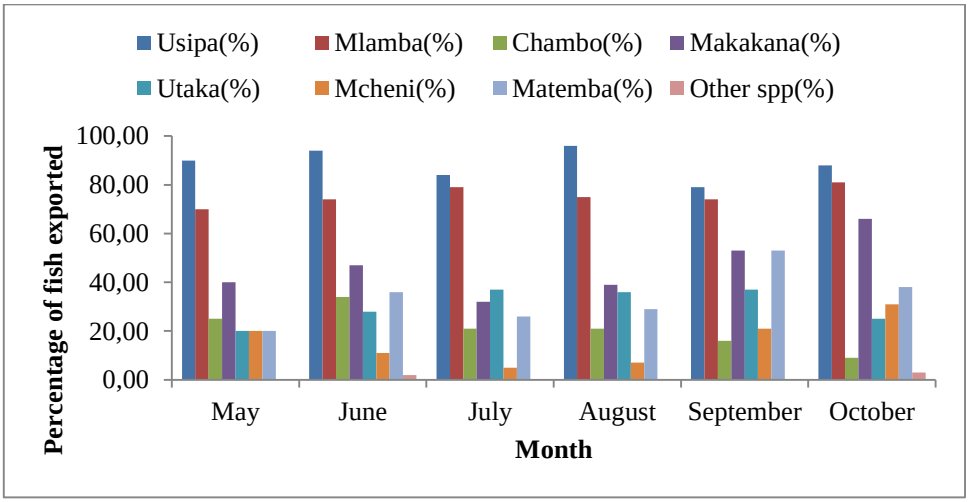

Figure 2. Percentages of fish exporters from Blantyre to various export destinations from May to October, 2013.

Fish processing methods

Observed fish processing methods were sun - dried and para - boiling for *E. sardella* (Figure 3).

Commonly, *Oreochromis* species (local name: Chambo), *Diplotaxodon spp*. (Ndunduma) and *C. garipinus* (Catfish) were exported in smoked form. A small quantity of *Oreochromis* species were also exported frozen while *Rhamphochromis* species (Mcheni) were exported in the smoked form compared to other forms (Figure 4).

Fishes such as *Barbus* species (Matemba), *Copadichromis* species (Utaka), *Bagrus* species (Kampango), *Buccochromis* species (Mbaba) were mainly exported in smoked and sun - dried forms (Figure 5).

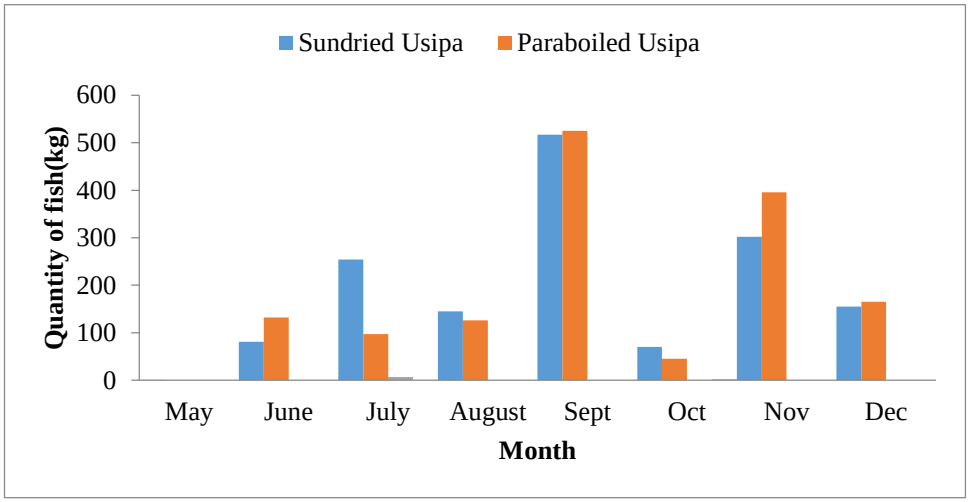

Figure 3. Processed form of exported *E. sardella*

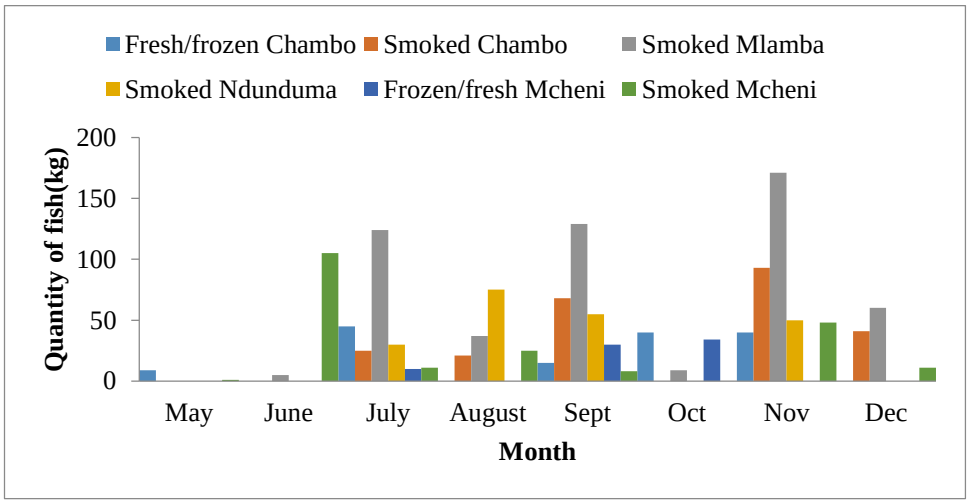

Figure 4. Processed form of *Oreochromis* spp. (Chambo), *Diplotaxodon* spp. (Ndunduma), *Rhamphochromis* spp. (Mcheni) and *Clarias* spp. (Catfish) exported from Blantyre.

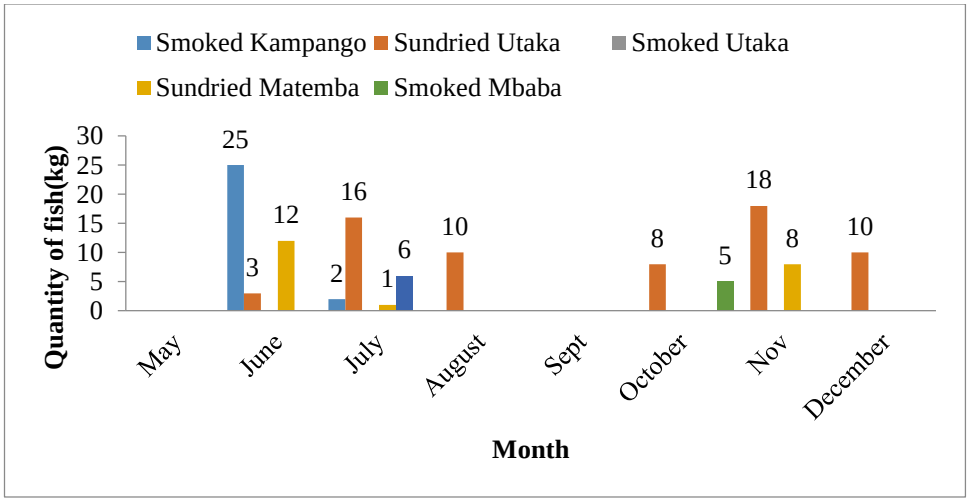

Figure 5. Processed form of *Copadichromis* spp. (Utaka), *Enteriomius* spp.(Matemba), *Bagrus meridionalis* (Kampango) and *Buccochromis* spp. (Mbaba) exported in 2013.

Export destinations of fish from Blantyre

Results show that the Republic of South Africa (n=214) is the main importer of fish from Malaŵi. Some fish were also exported to Botswana (n=1), Mozambique (n=1) and Vietnam (n=1) in the same period.

Contrary to the expectations, there was no export of fish to the neighboring countries like Tanzania, Zambia and Zimbabwe in the period under study (Table 2).

Table 2. Number of fish exporters from Blantyre in months of study

Month	Republic of South Africa (n=214)	Botswana (n=1)	Other (n=2)
May	20	0	0
June	98	0	1
July	19	0	0
August	27	1	0
September	19	0	0
October	31	0	1

Gender of fish exporters

About 13.8% of fish exporters were males and 86.2% were females with a significant difference (P<0.05) between the gender of fish exporters in the months of May (P = 0.000, June (P = 0.000), July (P = 0.000), August (P=0.001) and October (P=0.000) and the month of September showed gender difference was insignificant (P = 0.108) as reported in Table 3.

Table 2. Percentages and Chi - square (goodness of fit) test results for fish exporters.

Month	Male (%)	Female (%)	χ^2-value	Asymp. Sig.
May	5.0	95.0	16.200[a]	0.000
June	12.1	87.9	56.818[a]	0.000
July	5.3	94.7	15.211[a]	0.000
August	17.9	82.2	11.571[a]	0.001
September	31.6	68.4	2.579[b]	0.108
October	15.6	84.4	15.125[a]	0.000

Discussion

The study of fish exports from Blantyre District in Malawi was limited by the availability of sufficient data. Data that was available at the time of the study were for six months only. The findings in this study were consistent with earlier studies that fish quantities exported from Malawi have been low (Commonwealth/GTZ 2007; Donda and Njaya 2007; Kapute 2009; GoM 2011, 2012). Quantities as low as 9596 kg exported in six months from Blantyre District may imply that fish exports at the current rate cannot meaningfully contribute to the economy of the country. However, due to increased informal cross border trade as evidenced by the informal fish export of 24115.68 metric tonnes between 2015 and 2016 from Malawi to neighboring countries (Mussa et al. 2017), fish export quantities could be higher than reported. The inconsistences earlier reported by Kapute (2017) may rise due to poor monitoring of the fish exports by the Government of Malawi. The increasing trend of fish export quantities from May (9.2 %), July (7.9 %), August (12.0 %), September (14.1 %) and October (17.5 %) may be indicative of increased fish availability on the markets following the opening of the lakes following seasonal closure in November and/or December to March. Highest fish exports (39.2 %) in June may be due to traders turning up as production of *E. sardella* increased along with other species. This study revealed that *E. sardella* and *Copadichromis* species which were commercially - less valuable fishes (GoM 2012; Singini 2013) were the mostly exported by traders (at least 75% of the traders exported the two species). The fish processing methods such as sun-drying, smoking and para-boiling indicate that the exported fishes were destined for local markets (Kapute 2009; GoM 2012). The processing methods also suggest that the species of fish exported are small in size.

It was observed fish were mainly exported to the Republic of South Africa as earlier reported by GoM (2011). Republic of South Africa imported largest quantities of fish from Malawi probably due to many Malawians staying in that country as consumer studies in Johannesburg found that demand for fish

species depends on culturally acquired tastes (Kapute 2017). However, other studies have established reported fish exports to Zambia, Tanzania and Zimbabwe (Kapute 2017; Mussa et al. 2017) which were not found in the current study. The large percentage of female fish exporters (86.2%) and the monthly gender differences agree with the findings from Mussa et al. (2017) that a larger number (65.7%) of women from Malawi are involved in the informal cross border fish trade. The issue of women empowerment is therefore, crucial as 70% of women in the Southern African Development Community (SADC) are involved in the informal cross border trade (Mussa et al. 2017).

The study recommends that the Government of Malawi should make a balanced decision on policy issues regarding regulation of fish exports. This comes at a time when demand for fish on the local market in the country is not satisfied. Further, the government should consider exploring fish processing methods that ensure highest quality for exports to highly regulated markets. The more women (86.2%) than men (13.8%) who exported fish from Blantyre District provides insights on the unsustainability of fish exports. Therefore, a policy that considers mainstreaming of gender into the policy may ensure the sustainability of livelihoods of women in fish exports.

Acknowledgements

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