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Density, Biomass and Length-Weight Relationship of Brown Trout (*Salmo trutta* Linnaeus, 1758) Population in the Çoruh River Basin, North-Eastern Anatolia, Turkey

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ABSTRACT

This study was conducted to determine density, biomass and length-weight relationship for brown trout (*Salmo trutta* Linnaeus, 1758) caught from removal method based on single-pass electro fishing. A total area of 2.176 ha was sampled and 167 fishes were caught using electro fishing between May 2012 and June 2013 in Tortumkale Stream of Çoruh River, Turkey. Mean density and biomass of brown trout were found to vary between 106-167 fish ha^{-1} and 4.76-10.64 kg ha^{-1}, respectively. The length-weight relationship exponent b values ranged from 3.0672 to 3.3158 and indicated positive allometric growth. Length-weight relationship in between stations were statistically significant (r^2>0.9917, p<0.05). Our results revealed that the density of the brown trout population in Tortumkale Stream is at a level indicating a risk of extinction of this species. Density of brown trout occuring in restricted habitats was reduced in Tortumkale Stream; especially it causes reduction of their population overfishing on adult individuals in reproduction season and lower of recruitment to the population. Also, biotic and abiotic effects of rainbow trout (*Oncorhynchus mykiss*), aquacultures activities, recreational fisheries, barriers of dam construction and irrigation canals, pollution from domestic, industrial and agricultural waste contribute to the destruction of the habitat of brown trout.

Keywords: Brown trout, growth, density, biomass, Turkey

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Kuzeydoğu Anadolu Çoruh Nehri Havzası'ndaki Kırmızı Benekli Alabalık (*Salmo trutta* Linnaeus, 1758) Populasyonunun Yoğunluk, Biyokütle ve Boy-Ağırlık İlişkisi

Öz: Bu çalışma, tek avlı ayrılmaya dayalı metot uygulanarak avlanan kırmızı benekli alabalığın (*Salmo trutta* Linnaeus, 1758) yoğunluk, biyokütle ve boy-ağırlık ilişkisini belirlemek için yürütülmüştür. Toplam 2,1760 ha alan, Kuzey Doğu Anadolu Çoruh Nehri Havzası'nın Tortumkale Çayı'nda; Mayıs 2012-Haziran 2013 tarihleri arasında elektroşokla 167 adet balık avlanmıştır. Ortalama yoğunluk ve biyomas değerleri sırasıyla 106-167 adet/ha ve 4,76-10,64 kg/ha arasında değişmiştir. Boy-ağırlık ilişkisinin b değerleri 3.0672-3.3158 arasında değişmiş ve pozitif allometrik büyüme gözlemlenmiştir. İstasyonlar arasındaki boy-ağırlık ilişkisi istatistikî olarak önemlidir (r^2>0,9917, p<0,05). Elde edilen sonuçlar doğrultusunda kırmızı benekli alabalık türünün, yok olma riski altında olduğunu tespit edilmiştir. Tortumkale Çayı'nda sınırlı habitatlarda yaşayan kırmızı benekli alabalığın yoğunluğu; özellikle populasyona yeni birey katılımının azalması ve üreme döneminde yetişkin bireyler üzerine aşırı av baskısı nedeniyle azalmıştır. Aynı zamanda, gökkuşağı alabalığı akuakültür aktivitelerinin biyotik ve abiyotik etkileri, rekreasyonel balıkçılık, baraj yapılar ve sulama kanalları bariyerleri ile evsel, endüstriyel ve tarımsal atıklardan kaynaklanan pollusyon kırmızı benekli alabalığın habitatlarını tahribine katkı sunmaktadır.

Anahtar kelimeler: Kırmızı benekli alabalık, büyüme, yoğunluk, biyomas, Türkiye

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Introduction

Brown trout, also known as redpoints trout (*Salmo trutta* Linnaeus, 1758), is a species of the Salmonidae family and is widespread throughout the world including the freshwaters of Turkey. This species has also been registered in Europe, North

Africa, the Middle East and the western regions of Asia (Alp et al. 2005). Therefore, in addition to being a global species, brown trout has a significant place in sports fishing, commercial fishing and aquaculture (Özvarol et al. 2010). From an ecological and economic perspective, *S trutta* is considered the most important native fish species found in the freshwaters of Turkey. They live in clear, clean, cool and oxygen-rich rivers with waterfalls at 50 to 2300 m altitude and a maximum temperature of 20°C (Duman et al. 2011).

In Turkey, the stocks and population of brown trout have been gradually reduced and the species is at the risk of extinction due to environmental factors such as pollution, construction, destruction of stream beds and spawning areas; attempts to restock with other fish species such as rainbow trout (*O. mykiss*); and fishing activities using illegal and prohibited equipment (Arıman and Kocaman 2003; Kocabaş et al. 2013). However, there is a lack of both direct and indirect data concerning the population of brown trout in rivers in Turkey and their preservation status that would allow an accurate assessment regarding their risk of the extinction of the subspecies (Smith and Darwall 2006; Tarkan et al. 2008). Therefore, brown trout is listed as DD = Data Deficient in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Smith and Darwall 2006).

The fluctuation of the fish population is really important for stock assessment and management. In this way, a major decline and rise in the population density or the population biomass can be detected, and appropriate management strategies can be adopted (Chen et al. 2004). A relatively simple and inexpensive method to assess the welfare of lentic systems is to monitor the density and biomass of fish population (Platts and Mchenry 1988; Bohlin et al. 1989).

Length-weight relationship (LWR) are important and have many applications in fish stock assessments, biomass estimations, ecological studies and modeling aquatic ecosystems (Froese 2006). In addition, the LWR is important in terms of providing for the estimation of weight from the length and the calculation of condition indices as well as providing general information about the morphology of populations in different habitats and their life cycles (Petrakis and Stergiou 1995; Froese et al. 2011).

Many scientists in various regions of the world have been studied the density and biomass of trout populations per surface unit in rivers (Almodovar and Nicola 1998; Maia and Valente 1999; Dikov and Zivkov 2004; Vlach et al. 2005; Zanetti et al. 2010; Kolev 2010, 2012). There are some studies for the LWR regarding brown trout in freshwater of different geographic regions of Turkey (Ölmez et al. 1998; Kocaman et al. 2004; Alp and Kara 2004; Arslan et al. 2004; Alp et al. 2005; Arslan et al. 2007; Gülle et al. 2007; Özvarol et al. 2010; Kocabaş et al. 2011; Kocabaş et al. 2012; Yıldırım et al. 2012; Başusta et al. 2013) although little work has been done in Turkey on brown trout populations parameters (i.e. density and biomass) (Korkmaz et al. 1998; Korkmaz 2005). But still there is no detailed information about the density and biomass of brown trout. With this respect it is needed to establish the status of fish density and biomass in a number of localities and stream profiles in North-eastern Anatolia, Turkey.

In addition, there were no studies and data about density, biomass and the LWR of brown trout in Tortumkale Stream. This study investigated density, biomass and LWR of brown trout living in Tortumkale stream in Çoruh River, Turkey to provide a basis for future studies on this population.

Material and Methods

Tortumkale Stream is one of the most important tributaries of Tortum Stream (Figure 1). Tortum Stream, which arise from Dumlu Mountain of the eastern Mescit Mountains is in Erzurum province. It has an average of 50 km length and fast-flowing river systems. The Tortum Stream is the most important resource landslides which feeds the lake and regarded as one of the world's largest waterfall. There are four trout farm, four stone quarries, two concrete plants as well as use of irrigation water and three Hydroelectric Dams Project are not completed on the Tortum Stream and its tributaries (Köktürk and Atamanalp 2015).

The stream substratum is constituted of rocks, boulders of various size, pebbles and sand in the lower section. The bottom of site 1 was covered with partially-large stony and rocky structures, and the banks were stony riverbeds; the bottom of site 2 was covered with large stony and rocky and typically fast flowing soft-water, and the banks were un-wooded and rocky; the bottom of site 3 was covered with large stony and big rocky, and the banks were occasionally tree roots and typically fast flowing soft-water.

This study was carried out in Tortumkale Stream. Three sampling sites were assigned on Tortumkale Stream for fish sampling, water samples and measuring water characteristics. These sampling sites in Tortumkale Stream were selected according to habitat structure, depth, water velocity, size and structure of substratum (Hankin 1984). Some physical and chemical characteristics of sampling sites were shown in Table 1.

Table 1. Some physical and chemical characteristics of sampling sites (±SD).

Sampling times and sites		pH	Water Temperature (˚C)	Dissolved Oxygen (mg L^{-1})	Water Velocity (m sec^{-1})	Conductivity (µmhos/cm)	Depth (m)	Width (m)	Area (ha)
May 2012	1	7.6±0.14	6.9±0.29	9.4±0.08	1.25±0.02	829±89.60	0.55±7.74	6.0±2.78	0.120
	2	7.4±0.45	7.10±0.40	9.5±0.33	1.30±0.14	724±87.26	0.60±7.87	6.5±2.64	0.130
	3	8.0±0.30	7.80±0.25	9.7±0.22	0.60±0.15	526±96.25	0.65±7.83	9.0±1.89	0.180
August 2012	1	7.1±0.47	15.4±0.60	7.5±0.57	1.10±0.18	811±76.02	0.44±6.46	6.0±1.75	0.120
	2	7.8±0.40	16.7±0.39	7.3±0.53	1.20±0.21	688±72.43	0.50±6.07	8.0±1.32	0.160
	3	7.4±0.60	17.1±0.49	8.2±0.42	0.50±0.18	547±69.04	0.55±7.26	7.5±1.00	0.150
November 2012	1	7.5±0.27	12.2±0.29	7.7±0.39	1.20±0.29	823±67.67	0.40±5.27	7.7±2.25	0.154
	2	7.6±0.27	13.4±0.40	7.8±0.33	1.30±0.18	726±76.29	0.45±6.29	6.7±1.89	0.134
	3	7.1±0.43	11.6±0.41	8.0±0.37	0.60±0.18	644±89.15	0.40±6.18	8.7±1.25	0.174
March 2013	1	8.2±0.47	5.40±0.55	9.9±0.29	1.20±0.35	786±50.77	0.62±6.07	6.0±1.80	0.120
	2	7.7±0.25	4.90±0.46	9.8±0.36	1.30±0.21	665±65.85	0.55±6.74	6.5±2.00	0.130
	3	7.5±0.66	6.10±0.39	9.5±0.49	0.70±0.18	595±84.72	0.50±6.18	9.0±1.32	0.180
June 2013	1	7.4±0.43	12.4±0.33	7.8±0.43	1.30±0.29	791±81.66	0.70±5.52	6.0±1.50	0.120
	2	7.3±0.37	14.8±0.40	6.7±0.21	1.20±0.35	711±99.30	0.65±5.64	7.1±1.50	0.142
	3	7.8±0.63	15.7±0.52	7.1±0.18	0.70±0.29	575±89.08	0.60±5.18	8.1±1.04	0.162
Total Sampling Area (ha)									2.176

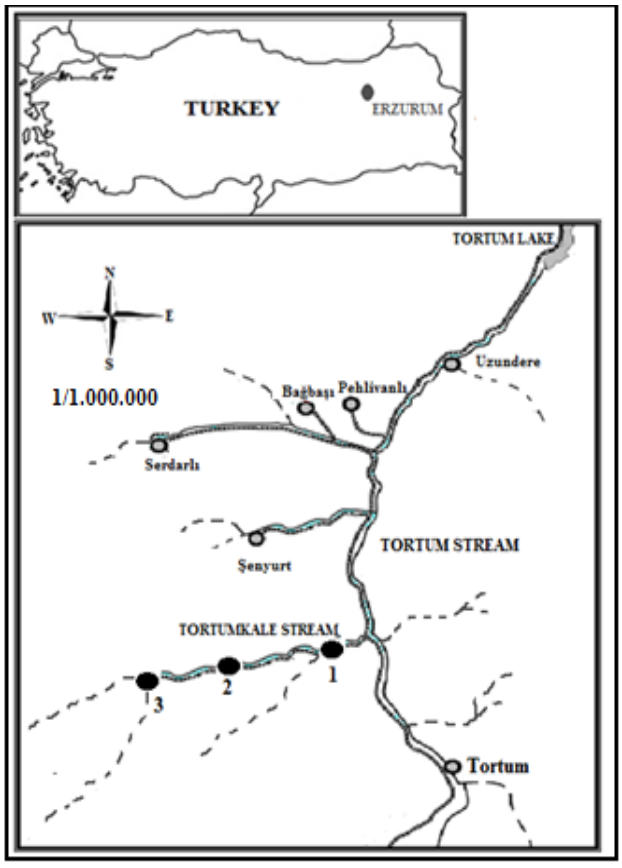

Figure 1. Study area and sampling sites.

Fish samples in Tortumkale Stream were collected approximately every 3 months between May 2012 and June 2013 by electro fishing. A pulsed DC current of 2 amperes at 500-750 volts was used in electro fishing, the current being supplied by a generator. The electro fishing team consisted of three experienced crew members with one using the anode and the other two using dip nets to capture the fish. Each sampling site was closed at both ends with 8-10 mm mesh size nets (Lacroix 1989) and the fishing was carried out in an upstream direction. Then, we took the measurement per 10-20 m to determine the mean stream depth (m) and width (m) (Neves and Pardue 1983).

Due to the rocky and stony of stream bed could be made only single-pass electro fishing in selected sampling sites (Seber 1973). Fish caught in each sample site were anesthetized with MS 222 and mortality was not observed. Fish caught were placed into different plastic buckets for 40 liters. Then, fish were numbered, measured and weighted to the nearest 1 mm (total length) and 1gr, respectively. To conserve as live the fish, fresh brook water was added into plastic buckets from time to time until experimental treatments were finished. Fish caught were returned to the water as alive in accordance with permission. This procedure was repeated in all sampling dates. In order to determine fish density (population size) and biomass in each sampling site, it was used removal method based on single-pass electro fishing (Seber 1973). Removal method based on single-pass electro fishing is described as:

$$\widehat{N}_i = \frac{C_i}{\hat{p}}$$

$\widehat{N}_i$ is population density; $\hat{p}$ is the catch efficiency ($\hat{p} = 1 - \hat{q}$) ; C_i is total catch. $i = 1, \ldots, n$. Catch efficiency value (0.63) has been used that calculated for brown trout in the Hatila Creek having similar bottom structure and habitat (Korkmaz et al. 1998). Catch efficiency may also vary as a function of fish size, operator skill, electrofisher settings, habitat, temperature and fish abundance (Van Dishoeck 2009). The sampling variance and an approximate 95

percent confidence interval of $\hat{N}_i$ were estimated from the following equations, respectively.

$$S^2_{\hat{N}_i} = \hat{N}^2.\hat{q}.\frac{(1+\hat{q})}{n}.\hat{p}^3 + \hat{N}.\frac{\hat{q}}{\hat{p}} + \hat{N}.\hat{q}^2.\frac{(5+\hat{q})}{n}.\hat{p}^4 \text{ and } \hat{N}_i \pm S_{\hat{N}_i}$$

The biomass or standing crop ($\hat{B}$) was estimated by $\hat{B} = B.\left(\frac{\hat{N}_i}{N}\right)$.Where, B is the total weight of fish caught and N is the total number of fish caught. Values of density and biomass of fish per unit area where catches were estimated by $\hat{N}/A$ and $\hat{B}/A$, respectively. Where A is area of sampling sites (ha) (Bohlin et al. 1989).

The LWR $W = a \times L^b$, was transformed into its logarithmic expression: $LogW = loga + b \times logL$. The parameters a and b were calculated by least-squares regression for sampling sites. The LWR curves were compared between all sampling sites. The significance of the regression was assessed by analysis of variance (ANOVA), and the variation in b values from 3 were controlled by the t-test for evaluating growth curve. When the b value in the LWR was equal to or did not show statistically significant deviation from 3, the growth was isometric, whereas the positive or negative allometric growth occurred when the b value deviated significantly from 3 (Ricker 1975).

Results

A total area of 2.176 ha was sampled by single pass electro fishing and 167 brown trout were caught from the Tortumkale Stream between May 2012 and June 2013. The results of single pass electro fishing together with estimates of brown trout density and biomass at the three sampling sites and sampling periods are presented in Table 2.

Table 2. Stock density and biomass obtained from sampling sites in the Tortumkale Stream.

Sampling times and sites		C_i	B	$\hat{N}_i$ (fish)	$\hat{N}_i \pm S_{\hat{N}_i}$	$\hat{B}$ (g)	$\hat{N}_i$ (fish ha^{-1})	$\hat{B}$ (kg ha^{-1})
May 2012	1	21	1414.03	34	34±11	2289.38	283	19.08
	2	11	428.14	18	18±8	700.59	127	4.93
	3	4	103.45	7	7±6	181.04	43	1.12
	Mean	12	648.54	19.67		1057.00	151	8.38
August 2012	1	10	578.65	16	16±8	925.84	133	7.72
	2	8	653.45	13	13±7	1061.86	100	8.17
	3	9	352.94	15	15±8	588.23	83	3.27
	Mean	9	528.35	14.67		858.64	106	6.38
November 2012	1	15	1416.52	25	25±10	2360.87	162	15.33
	2	11	302.02	18	18±8	494.21	134	3.69
	3	6	346.15	10	10±6	576.92	57	3.32
	Mean	11	688.23	17.67		1144.00	118	7.44
March 2013	1	19	1267.95	31	31±11	2068.76	258	17.24
	2	14	1062.77	23	23±9	1745.98	144	10.91
	3	9	340.22	15	15±8	567.03	100	3.78
	Mean	14	890.31	23.00		1460.59	167	10.64
June 2013	1	14	552.21	23	23±9	907.20	192	7.56
	2	10	411.11	16	16±8	657.78	123	5.06
	3	6	179.83	10	10±6	299.72	56	1.67
	Mean	10	381.05	16.33		621.57	123	4.76

According to these results, mean density and biomass of brown trout were found to vary between 106-167 fish ha^{-1} and 4.76-10.64 kg ha^{-1}, respectively. Mean density was as follows: 151 fish ha^{-1} at the May 2012; 106 fish ha^{-1} at the August 2012; 118 fish ha^{-1} at the November 2012; 167 fish ha^{-1} at the March 2013; 123 fish ha^{-1} at the June 2013. Mean biomass was as follows: 8.38 kg ha^{-1} at the May 2012; 6.38 kg ha^{-1} at the August 2012; 7.44 kg ha^{-1} at the November 2012; 10.64 kg ha^{-1} at the March 2013; 4.76 kg ha^{-1} at the June 2013.The lowest and the highest density and biomass values were observed during May 2013 in 3rd sampling site and 1st sampling site.

The sample size (n), ranges of total length and total weight, parameters a and b of the LWR, 95% confidence intervals of a and b, the determination coefficient (r^2), and growth type are given in Table 3. According to sampling sites, captured fish were measured as 16.0 ± 0.50 cm, 66.19 ± 6.34 g; 14.6 ± 0.63 cm, 52.91 ± 6.73 g; 13.2 ± 0.76 cm, 38.89 ± 6.21 g (S.D.), respectively (Table 3).

The LWR were found as $W = 0.0092*L^{3.1158}$ (r^2=0.9931) at 1st sampling site, $W = 0.0105*L^{3.0734}$ (r^2=0.9927) at 2nd sampling site and $W = 0.0104*L^{3.0672}$ (r^2=0.9917) at 3rd sampling site (Figure 2-4). The LWR exponent *b* values ranged from 3.06 to 3.31.The *b* values of the LWR from Tortumkale Stream were significantly different from 3.0 ($p<0.05$) and indicated positive allometric growth. All values of the coefficient of determination (r^2) varied between 0.9917 and 0.9931 (Table 3).

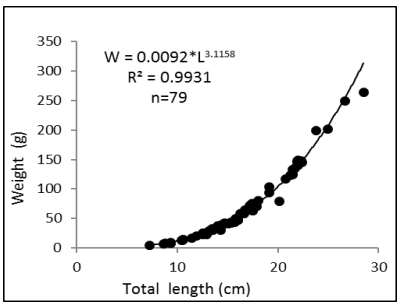

Figure 2. The LWR in brown trout (1st sampling site).

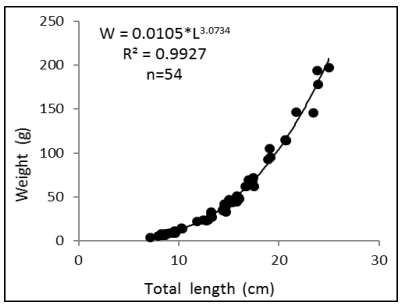

Figure 3. The LWR in brown trout (2nd sampling site).

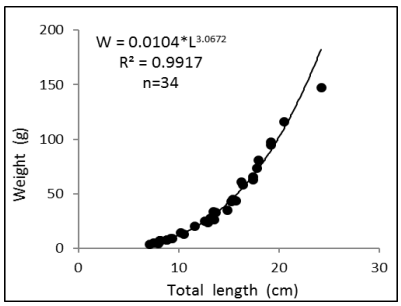

Figure 4. The LWR in brown trout (3rd sampling site).

Table 3. Descriptive statistics and estimated parameters of the LWR for brown trout according to the sampling sites.

Sampling sites	N	L±SD (cm)		W±SD (g)		Regression parameters		95% Cl of a	95% Cl of b	r^2	t-test	GT
		Min.	Max.	Min.	Max.	a	b					
1	79	7.2-28.5 (16.0±0.50)		5.02-264.31 (66.19±6.3)		0.0092	3.3158	-2.107 to 1.967	3.057 to 3.175	0.9931	p<0.05	A+
2	54	7.1-25.0 (14.6±0.63)		4.02-197.34 (52.91±6.7)		0.0105	3.0734	-2.064 to -1.895	2.999 to 3.146	0.9927	p<0.05	A+
3	34	7.1-24.2 (13.2±0.76)		4.02-197.34 (38.89±6.2)		0.0104	3.0672	-2.094 to -1.871	2.966 to 3.168	0.9917	p<0.05	A+
Overall	167	7.1-28.5 (14.99±0.3)		4.02-264.31 (56.34±3.9)		0.0090	3.115	-2.107 to -1.966	3.057 to 3.174	0.993	p<0.05	A+

N=sample size, *L*=total length (cm), *W*=total weight (g), *a*=intercept, *b*=slope, *Cl*=confidence intervals, r^2=coefficient of determination, *GT*=growth type, *A*+=positive allometric

Discussion

This study reveals density, biomass and LWR of brown trout in the Tortumkale Stream. The LWR were studied to give information on the growth condition of fish and to find out whether the fish grows isometrically or allometrically (Tesch 1971). According to sampling period, the lowest mean density and biomass values were observed during August 2012 with 106 fish ha^{-1} and 6.38 kg ha^{-1} whilst the highest mean density and biomass values were observed during March 2013 with 167 fish ha^{-1} and 10.64 kg ha^{-1}. Water temperature is the most important factor that influences on population parameters ($\widehat{N}_i$ and $\widehat{B}$). The reason for the decrease in total fish density and biomass during the sampling period can be explained by seasonal changes occurred in the water temperature.

The LWR exponent b values for all the species were within the limits (2-4) reported by (Tesch 1971; Bagenal and Tesch 1978) for most fishes. The LWR exponent *b* values ranged from 3.06 to 3.31. Exponent *b* was very close to values cited in the literature, 2.828 to 3.027 in Ceyhan, Seyhan and Euphrates Basins by Alp and Kara (2004), 2.89 to 3.04 in Kan Stream, Çoruh Basin by Arslan et al. (2004), 2.971 to 3.009 in Fırnız Stream by Alp et al. (2005), 2.997 to 3.106 in West Karadeniz Basin by Gülle et al. (2007), 3.008 to 3.166 in Coruh Basin by Özvarol et al. (2010) and 2.93 to 3.07 in Upper Coruh River by Yıldırım et al. (2012). However, quite different estimations were also noted. Kocaman et al. (2004) calculated the exponent for brown trout as 2.590 in Teke Stream (Erzurum). In Hatila Brook by Ölmez et al. (1998), in Aksu Stream by Arslan et al. (2007), in Uzungöl Stream by Kocabaş et al. (2011; 2012) and Munzur River by Başusta et al. (2013) reported values of 2.9056, 2.932, 2.788 to 2.949, 2.919 and 2.87 for brown trout which are much closer to the result obtained in the current study.

The *b* value has been shown to vary according to season, habitat, gonad development, sex, diet, feeding, stomach fullness, and spawning period

(Bagenal and Tesch 1978). In addition, changes in the *b* value result from several other factors such as sampling time and method, differences in length between the caught species, weight distribution and the ecological status of their habitat (Moutopoulos and Stergiou 2002).

The brown trout density and biomass in the Tortumkale Stream are > 19 to 612 fish ha^{-1} and 0.52 to 56.23 kg. ha^{-1} in Hatila Brook by Korkmaz et al. (1998), 1000 to 2000 fish ha^{-1} and 23.04 to 27.04 kg. ha^{-1} in Lima River by Maia and Valente (1999), 914.8 fish ha^{-1} and 61.258 kg. ha^{-1} in Veleka River by Dikov and Zikov (2004), 28 fish ha^{-1} and 2.55 kg. ha^{-1} in Kadıncık Brook by Korkmaz (2005), 108 to 608 fish ha^{-1} and 2.77 to 26.77 kg. ha^{-1} in some Bulgarian Rivers by Kolev (2010) and 630 fish ha^{-1} and 21.73 kg. ha^{-1} in Bunayska River by Kolev (2012). However, values of the brown trout density and biomass range from 1163.48 to 3135.08 fish ha^{-1} and 67.26 to 208.22 kg. ha^{-1} in Gallo River by Almodovar and Nicola (1998), 6076 fish ha^{-1} and 278.6 kg. ha^{-1} in Úpoř Brook by Vlach et.al. (2005) and 2580 fish ha^{-1} and 100.15 kg. ha^{-1} in river basins of Cagliari province by Zanetti et.al. (2010) were higher than our results.

These differences can be said to be caused by the fact that selective fishing or catch efficiency. The variable catch efficiency is not only dependent on the characteristics and habits of fish populations but also on factors related to the design and implementation of the sampling and on the physical, chemical and environmental characteristics of the habitat (Bravo et al. 1999).

The results of this study concerning the density, biomass and length-weight relationship of the brown trout population will not only assist fishery biologists in the sustainable management of this species, but also contribute to the introduction preventive measures for the preservation of stocks, and provide a baseline for future studies.

This study found that the density of the brown trout population in Tortumkale Stream is at a level indicating a risk of extinction of this species. The main causes were the wastewater from the surrounding trout farms, rainbow trouts escaping from farm and overfishing. Other factors contributing to the destruction of the habitat of brown trout are considered to be the destruction of breeding areas due to the ongoing construction of a dam in the area; contamination from domestic, industrial and agricultural waste; and changes in the water regime throughout the year. Native brown trout is an ecological and economic asset and is among the significant gene sources of Turkey. We believe that relevant institutions and organizations should implement strict regulations to preserve this species.

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Main Growth Parameters of a Threatened Species *Chondrostoma holmwoodii* (Boulenger, 1896) from Tahtalı Reservoir, İzmir, Turkey

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ABSTRACT

A diverse genus of the Cyprinidae family, genus *Chondrostoma* Agassiz, 1832 has a wide distribution. More than half of the species distributes in Turkey, however there is little biological information about them. The aim of this study is to investigate the population parameters of Eastern Aegean Nase *Chondrostoma holmwoodii* and to evaluate the risks for the species in Tahtalı Reservoir. Fish sampling was carried out at 8 different sampling points at Tahtalı Reservoir in 2014 with multimesh gillnets. Population parameters such as age and sex composition, length frequence analysis and von Bertalanffy growth function were investigated. A total of 215 specimens of *C. holmwoodii* was sampled. Total length of the specimens varies between 4.3 - 28.2 cm and total weight 1.05 - 271 g. Age composition of the sampled specimens varies between 0 to V and most of the specimens were age-III. The von Bertalanffy growth parameters for *C. holmwoodii* was estimated as; L_∞= 395.30 mm (SD=63.80), K=0.17 (SD=0.05) and L_0=46.45 mm (SD=9.41). In conclusion, main problem for *C. holmwoodii* population in Tahtalı Reservoir is predation risk by *Perca fluviatilis* Linnaeus, 1758. In order to offer conservation strategies for *C. holmwoodii* Tahtalı Reservoir population, diet, population structure, and life history traits of *P. fluviatilis* need to be studied in detail.

Keywords: Eastern Aegean Nase, *Chondrostoma holmwoodii*, endemic fish, von Bertalanffy.

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Nesli Tehlike Altında Olan *Chondrostoma holmwoodii* (Boulenger, 1896)'nin Tahtalı Baraj Gölü'ndeki Büyüme Parametreleri

Öz: Cyprinidae familyasına ait *Chondrostoma* Agassiz, 1832 cinsi geniş dağılım alanına sahiptir. *Chondrostoma* türlerinin çoğu Türkiye'de dağılım göstermesine rağmen biyolojileri hakkında yeterince bilgi bulunmamaktadır. Bu bağlamda *Chondrostoma holmwoodii*'nin Tahtalı Baraj Gölü'ndeki popülasyon parametrelerini incelemek ve mevcut popülasyonun risklerini değerlendirmek bu araştırmanın amacını oluşturmaktadır. Balık örnekleri 2014 yılında Tahtalı Baraj Gölü'nde belirlenen sekiz farklı örnekleme noktasından çokgözlü ağlar ile örneklenmiştir. Yaş ve cinsiyet kompozisyonu, boy frekans analizi ve von Bertalanffy büyüme fonksiyonu gibi popülasyon parametreleri araştırılmıştır. *C. holmwoodii*'nin total boyu 4,3 – 28,2 cm ve toplam ağırlığı 1,05 - 271 g arasında değişen 215 bireyi örneklenmiştir. Örneklenen bireylerin yaş kompozisyonu 0 ile V arasında değişirken popülasyondaki baskın yaş III olarak tespit edilmiştir. Ayrıca von Bertalanffy büyüme parametreleri; L_∞ = 395,30 mm (SS = 63,80), K = 0,17 (SS = 0,05) ve L_0 = 46,45 mm (SS = 9,41) olarak tespit edilmiştir. Sonuç olarak, Tahtalı Barajı Gölü'ndeki *C. holmwoodii* popülasyonu için temel tehditin *Perca fluviatilis* Linnaeus, 1758'in tür üzerindeki predasyon baskısı olduğu görülmüştür. *C. holmwoodii*'nin Tahtalı Baraj Gölü popülasyonu için koruma stratejileri belirlenirken *P. fluviatilis*'in diyet, popülasyon yapısı ve yaşam öyküleri de incelenmelidir.

Anahtar kelimeler: Kababurun balığı, *Chondrostoma holmwoodii*, endemik balık, von Bertalanffy

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Introduction

A diverse genus of the Cyprinidae family, genus *Chondrostoma* Agassiz, 1832 distributed throughout south and central Europe, Anatolia, the Caucasus and Mesopotamia (Elvira 1997; Durand et al. 2003; Robalo et al. 2007). Complicated phylogenetic

relationships of the genus seem have been recently investigated by Robalo et al. (2007) and five new genera are described, namely *Achondrostoma*, *Iberochondrostoma*, *Pseudochondrostoma*, *Protochondrostoma* and *Parachondrostoma*. With the description of two new species from Turkey (Küçük et al. 2017) and accounting *C. fahirae* (Ladiges, 1960), *Chondrostoma* sensu stricto comprises 22 species (Robalo et al. 2007; Küçük et al. 2017).

A total of 12 species distributed in Turkey (Krupp 1985; Küçük et al. 2007; Freyhof and Özuluğ 2009; Küçük et al. 2013; Kuru et al. 2014; Küçük et al. 2017) however there is little biological information about them. To our knowledge, length-weight relationship and growth of *Chondrostoma regium* (Heckel, 1843), *Chondrostoma holmwoodii* (Boulenger, 1896), *Chondrostoma kinzelbachi* Krupp 1985 and *Chondrostoma meandrense* Elvira, 1987 are present (e.g. Yüce et al. 2015; Oymak 2000; Suiçmez et al. 2011; Gürleyen et al. 2012; Özcan and Balık 2011; Balık et al. 2007; İlhan et al. 2010; Yerli et al. 2016).

Evaluation of life history traits of a threatened species can be an important tool to identify population trends, leading to take successive management actions for conservation (Park et al. 2003; Riberio et al. 2008). They are also valuable tool for fisheries biologists to determine the effects of exploitation on economic fish species (Jennings et al. 1999) or by policy makers to determine the time/limits of fishing activities (Redpeth et al. 2009). In addition to these, life history traits are being used in order to predict the probability of success of invasive/introduced species (Rosecchi et al. 2001; Copp et al. 2016).

Growth is a critical trait causing determination of population demography, evolution and community interactions (Urban 2007). However, some serious critics about the model has been reviewed (Roff 1980), the von Bertalanffy growth function is the most commonly used growth function in fisheries analyses (Haddon 2011).

Tahtalı Reservoir is an important drinking water supply for İzmir province and in order to keep water quality of the lake in the desired limits, alternative land use politics have been investigated (Ay 2001). In addition to these, lake is also highly protected for fisheries activities. A total of 8 species distributes in the lake (OSİB SYGM 2015). One of the residents of the lake, *C. holmwoodii* is listed under Vulnerable (VU) status and it has been reported that population size and also mature individuals are decreasing (Freyhof 2014)

Aim of this study is to investigate the population parameters of the threatened species *C. holmwoodii* in Tahtalı Reservoir, located in Küçük Menderes River Basin, İzmir, Turkey, report growth parameters for the species and compare results with other studies.

Materials and Methods

Fish sampling was carried out at eight sampling stations at Tahtalı Reservoir. Sampling was conducted at three different seasons, spring, summer and fall in 2014. Multimesh gillnets with 12 different mesh size ranging between 5 mm to 55 mm were used according to modified TS EN 14757. Dead specimens following sampling were fixed in a 4 % formalin solution and transferred to alcohol after fixation. Insize digital caliper was used to measure the total length (TL; cm) and digital scale to measure weight (W; g). Sex determinations were done with visual examination of the gonads of the sampled specimens.

Age-Length

Scales between lateral line and dorsal fin were used for determination of age. More than 25 % of the sampled specimens (n:54), covering total length range of the sample were aged by three independent readers. The multinomial logistic regression model (Gerritsen et al. 2006) from 'nnet' package (Venables and Ripley 2002) developed for R 3.4.0 (R Core Team 2014) was used to predict ages of the unaged specimens.

Growth Model

Due to low sample size of small specimens, von Bertalanffy growth function by von Bertalanffy (1938) was used in where t_0 is replaced by L_0;

$$E\{L|t\} = L_\infty - (L_\infty - L_0)\, e^{-Kt}$$

In the equation, $E\{L|t\}$ is the mean length at age t and L_∞, K, L_0 are the coefficients to be estimated from the function, they represent asymptotic length (L_∞), relative growth coefficient (K) and theoretical length when fish age is zero (L_0).

Results from the model were then bootstrapped and given in histograms with confidence intervals. All analyses were conducted with FSA package (Ogle 2014) developed for R 3.4.0 (R Core Team 2014).

Results

A total of 215 specimens of *C. holmwoodii* was sampled. Total length of the specimens varies between 4.3 cm and 28.2 with a mean value of 19.17 cm (SD=0.42) and total weight (W) varies between 1.05 and 271 g. According to a subset of 92 specimens, female/male ratio is 1.00/0.94 and 16 of

them were identified as immature. Deviation from 1:1 ratio is not significant at p<0.05.

Age-Length

Age of all specimens were predicted from the aged specimens (n:54). Age composition of the sampled specimens varies between 0 to V. Total length variability of the age groups is illustrated at Figure 1 and given in detail in Table 1.

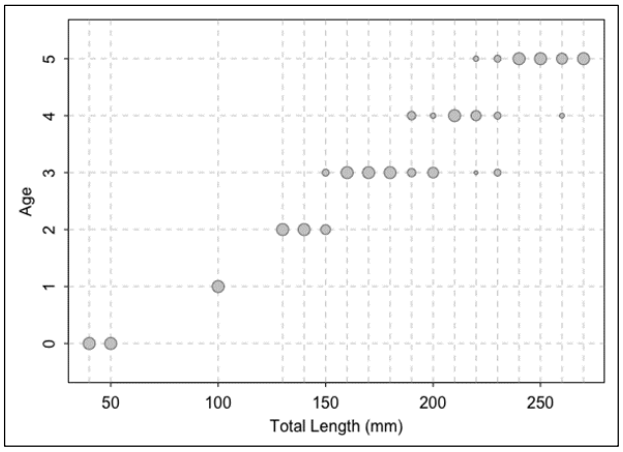

Figure 1. Age-Length of the *C. holmwoodii* in Tahtalı Reservoir (Dot size indicate percentages)

Table 1. *C. holmwoodii* mean total lengths at age

Age	n	Mean TL (cm)	*SD*	*SE*
0	13	63.5	19.8	5.48
I	1	104.0	NA	NA
II	29	148.0	6.33	1.18
III	71	189.0	24.16	2.87
IV	48	219.0	16.11	2.33
V	53	249.5	13.83	1.90

Most of the specimens (33%) were age-III with a mean total length of 189 mm (SD=24.16). Mean length of second lowest age, which was found out to be age-0, was 63.5 mm (SD=19.8). Only one specimen was found to be age-I. Total length of the 29 (6 %) age-II specimens were 148 mm with a standard deviation of 6.33 mm. Mean total length of age IV and V specimens were found out to be 219 (SD=16.11) and 249.5 (SD=13.83) mm respectively.

Growth Model

The von Bertalanffy growth parameters for *C. holmwoodii*, Tahtalı Reservoir population were estimated as: L_∞= 395.30 mm (SD=63.80), K=0.17 (SD=0.05) and L_0=46.45 mm (SD=9.41). Parameter estimates are summarized in Table 2.

Bootstrapped von Bertalanffy growth parameter results were given as histograms in Figure 2 with confidence intervals. Residuals of the model fit with frequencies shown in Figure 3.

Relationships between parameter estimates are as follows; $K - L_\infty$ =-0.99, $L_\infty - L_0 = 0.54$, $K - L_0 = -0.64$. Low correlation values indicate less dependent parameter estimates.

Table 2. von Bertalanffy growth paramater estimates for *C. holmwoodii*

	Estimate	SE	t value	p
L_∞	395.3004	63.7983	6.196	< 0.001
K	0.1758	0.0512	3.433	< 0.001
L_0	46.4474	9.4087	4.937	< 0.001

Discussion

Reviewing the extent literature is beyond the scope of this study however, using different parameterizations, controlling correlations between model parameters is gaining a momentum. Fitting fish growth data with a von Bertalanffy growth function is really hard, model fit can be inaccurate because of missing data (e.g. young classes) and characteristics of the model (Ogle 2016). In order to deal with lack of young fish classes (Age I), original version of the model where t_0 is replaced with L_0, is used in this study rather than Beverton (1954). According to Cailliet et al. (2006) L_0 has serious advantages over t_0, especially for evaluation of fit. According to an experimental study with *C. nasus*, length after 63 days of hatch is averagely 3.47 cm (Schludermann et al. 2009). Our estimate of L_0=4.65±0.94 cm seems to represent not the best but an acceptable fit.

Result of this study significantly differs from the results presented by İlhan et al. (2010). Authors reported $L_\infty = 29.19$ cm, t_0 = -3.258, k = 0.177 from a population whose fork length ranged between 13 – 24 cm. When we calculate L_0 from these values, it equals to 12.79 cm, which is larger than prediction of 4.65 cm. Also L_∞ results from this study is lower than prediction of 39.53 cm. These differences are probably due to sampling equipment selectivity. Specimens sampled in this study ranged from 4.3 cm to 28.2 cm and this range is probably giving a better estimation.

According to a study conducted with the same specimens allometric coefficient *b* of the *C. holmwoodii* was 3.04, the *b* value showed a significant deviation from value 3 ($p < 0.05$) indicating a positive allometry (Yerli et al. 2016). İlhan et al. (2010) reported that the *b* value for *C. holmwoodii* in Tahtalı Reservoir similar to this result (3.13). On the other hand, the *b* value of different species belonging to the genus *Chondrostoma* were 2.71, 2.76 and 3.24 for *C. meandrense*, *C. kinzelbachi* and *C. regium* respectively (Özcan and Balık 2011; Özcan and Altun 2016; Oymak 2000).

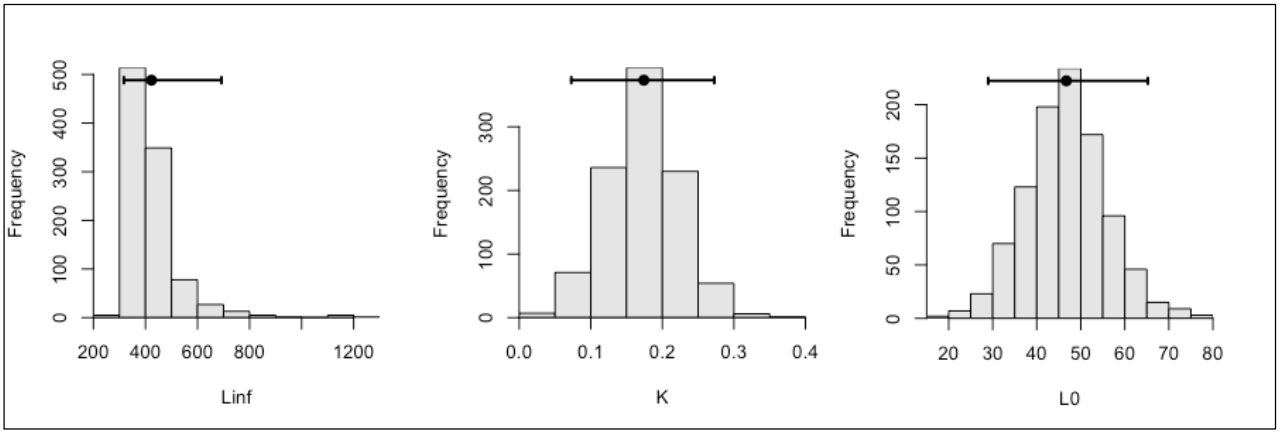

Figure 2. Frequency for bootstrapped von Bertalanffy growth parameters with confidence limits

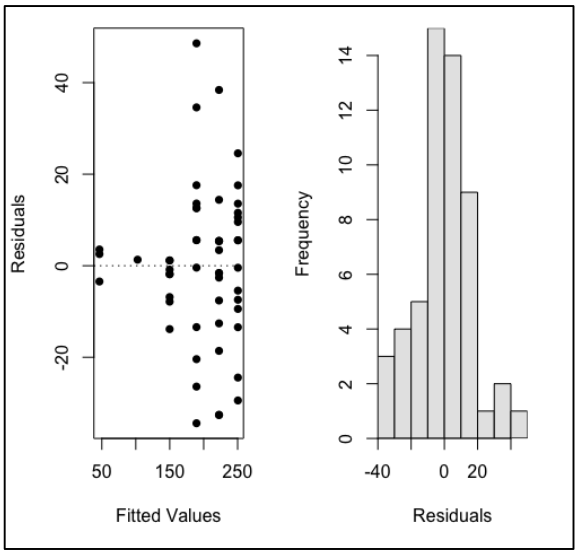

Figure 3. von Bertalanffy growth model fit with distribution of residuals

According to our observations, main problem for *C. holmwoodii* population in well protected Tahtalı Reservoir is predation risk by *Perca fluviatilis* Linnaeus, 1758. Multimesh gillnets sampled specimens from 4.3 cm to 28.2 cm; however, there is only one specimen between 10 – 13 cm range which falls to age I of the species. Similar observation was reported by İlhan et al. (2010). Lack of this age group might be due to evasion of young members of the population to deeper parts of the lake to avoid predation risk. Another possible explanation for this situation might be that this is a population response to decrease interspecific competition. However detailed predator-prey relationship studies need to be conducted in order to answer these questions and offer a conservation strategy.

In order to offer conservation strategies for *C. holmwoodii* Tahtalı Reservoir population, diet, population structure, and life history traits of *P. fluviatilis* need to be studied in detail. By this way, in addition to getting this important data about *P. fluviatilis*, its population size will be controlled too.

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Karakaya Baraj Gölü'ne Dökülen Han Çayı (Malatya, Türkiye)'nın Bazı Fiziksel ve Kimyasal Özellikleri

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ÖZ

Bu çalışmada, Karakaya Baraj Gölü'ne dökülen Han Çayı'nın bazı fiziksel ve kimyasal özelliklerinin araştırılması amacıyla Aralık 2013-Kasım 2014 tarihleri arasında 8 ay (Haziran-Eylül 2014 tarihleri arası çay kuruduğu için örnekleme yapılamamıştır) süreyle su örnekleri alınarak ölçüm ve analizler gerçekleştirilmiştir. Araştırma süresince su sıcaklığı, pH, elektriksel iletkenlik, çözünmüş oksijen ve akım değeri arazide yapılan ölçümlerle belirlenirken, toplam sertlik, tuzluluk ve organik madde titrimetrik metotla, asit kapasitesi, toplam azot, toplam fosfor, nitrit, nitrat, sülfat, silika ve kimyasal oksijen ihtiyacı gibi kimyasal parametrelere ait değerler laboratuvarda yapılan spektrofotometrik analizlerle tespit edilmiştir (APHA 1985). Araştırma sonuçları, Su Kirliliği Kontrol Yönetmeliğine Han Çayı'nın toplam fosfor hariç (II./III. sınıf) diğer bütün parametrelere ait değerlere göre I. sınıf su kalite özelliğine sahip olduğunu ortaya koymuştur. Araştırma sonuçları, Su Kirliliği Kontrol Yönetmeliğine göre Han Çayı'nın toplam fosfor hariç (II./III. sınıf) diğer bütün parametrelere ait değerler için I. sınıf su kalite özelliğine sahip olduğunu ortaya koymuştur.

Anahtar kelimeler: Fiziksel ve kimyasal özellikler, Han çayı, Karakaya baraj gölü

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Some Physical and Chemical Properties of Han Stream Spilled to Karakaya Dam Lake (Malatya, Turkey)

Abstract: In this study, some physical and chemical properties of Han Stream (Malatya) in relation to water quality was investigated. For this purpose water samples were collected at monthly for eight months interval between December 2013-November 2014 (June-September is dry period). Water temperature, pH, electrical conductivity, dissolved oxygen and flow rate were measured directly in study area whilst chemical analysis such as sulfate, silica, nitrite, nitrate, total nitrogen, total phosphorus, acide capacity and chemical oxygen demand were carried out in water quality laboratory by means of cell test through a spectrophotometer. Total hardness, salinity and organic matter were determined in accordance with methods in APHA (1985). According to the Water Pollution Control Regulation for Inland Water Resources, study results showed that Han Stream should be classified as class I (high quality water) in terms of all parameters except that total phosphorus values (II/III class, less polluted).

Keywords: Physical and chemical properties, Han stream, Karakaya dam lake

Alıntılama
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Giriş

Dünyadaki göller ve nehirler yaklaşık 105.000 km³ (toplam tatlı suların yaklaşık %0,3'ü), toplam kullanılabilir tatlı su miktarı ise 200.000 km³ (tüm tatlı suların %1'inden daha az) olarak tahmin edilmektedir. Kullanılabilir su miktarı, oldukça az olup dünyadaki toplam su miktarının yaklaşık %1'i kadardır (OECD 1982). Bu nedenle 1970'li yıllarda başlayan çevre hareketlerinin de etkisiyle doğal kaynakların sınırsız olmadığı, kaynaklarla kullanımlar arasında dengenin kurulması gerekliliği ön plana çıkmıştır.

Hızla artan dünya nüfusu, kentsel ve endüstriyel gelişmeler, sürekli daha fazla su gereksinimi doğurmakta ve bu durum yüzey su kaynaklarına verilen önemin artmasına neden olmaktadır. Dolayısıyla yüzey su kaynaklarının kalitesinin araştırılarak kullanım amaçlarının belirlenmesi ve her su kaynağı için uygun bir su kalite yönetiminin oluşturulması büyük önem arz etmektedir. Yüzey su

kaynaklarının sürdürülebilir kullanımını sağlamak ve ekolojik bütünlük çerçevesinde yüzey su kaynaklarının geleceğini garanti altına almak için, su kaynaklarının sürekli izlenmesi ve iyi yönetilmesi zorunludur (EC 2008). Bir izleme çalışmasında arzu edilen sonuçlara ulaşmak için ciddi bir izleme programı hazırlanması gerekir. İzleme programının tüm adımlarının doğru belirlenmesi ve programın titizlikle yürütülmesi durumunda, bir su kaynağının su kalite özellikleri ortaya konulur ve bu sayede kaynağın zarar görmesi önlenebilir hatta zarar görmüş bir ekosistem ıslah edilebilir. Bu durumda amaçlar doğrultusunda birkaç program tasarlanmalıdır.

EC (2008) ve Su Kirliliği Kontrol Yönetmeliği (SKKY 2015) hedefleri doğrultusunda tatlı su kaynaklarımızın özelliklerinin, kalite sınıflarının belirlenmesi ve gerekli önlemlerin alınması gerekmektedir. Son yıllarda Türkiye'de yapılan bilimsel araştırmalarda lotik sistemlerin su kalitesini belirlemeye yönelik çalışmaların (Bakan ve Şenel 2000; Boran ve Sivri 2001; Taşdemir ve Göksu 2001; Kayar ve Çelik 2003; Kara ve Çömlekçioğlu 2004; Tepe vd. 2006; Bulut vd. 2010; Tokatlı vd. 2016) artmasına paralel olarak tatlı su kaynakları yönünden zengin olan Doğu Anadolu Bölgesinde de birçok çalışma yapılmıştır (Şen vd. 1999, 2002, 2007; Şen ve Gölbaşı 2008; Varol ve Şen 2009; Alp vd. 2010; Gölbaşı 2014; Baytaşoğlu ve Şen 2015; Yiğiteli 2016).

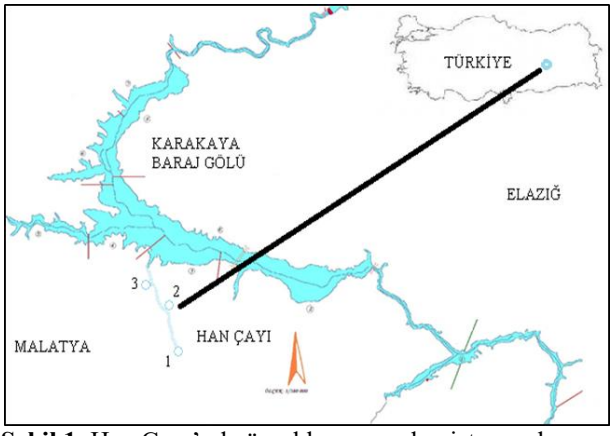
Elazığ ve komşu il sınırları içerisinde yer alan daha pek çok akarsuyun su kalite özellikleri henüz belirlenmemiştir. Yukarıda verilen literatür özetinden de anlaşıldığı üzere, bölgemizde yer alan akarsuların fiziksel ve kimyasal özelliklerinin ortaya çıkarılması için daha fazla araştırmaya ihtiyaç vardır. Bu amaç doğrultusunda Malatya il sınırları içinde yer alan Han Çayı'nın su kalite özellikleri bir yıl süreyle araştırılmıştır.

Materyal ve Metot

Karakaya Barajı; Diyarbakır ili Çüngüş ilçesi sınırları içinde, Fırat Nehri üzerinde, Güneydoğu Anadolu Projesi'nin bir parçası olarak elektrik enerjisi üretimi amacıyla 1976-1987 yılları arasında inşa edilmiştir. Baraj gölü Malatya-Elazığ-Diyarbakır, hidroelektrik santrali ise Çüngüş ve Doğanyol sınırında yer almaktadır (Frmartuklu 2017).

Karakaya Baraj Gölü birçok akarsu tarafından beslenmektedir. Bunlardan bir tanesi de çalışmanın gerçekleştirildiği Han Çayı'dır. Han Çayı Malatya'ya 16 km uzaklıktaki ekonomisi tarım ve hayvancılığa bağlı olan Hacıhaliloğlu Çiftliği Köyü dolaylarından çıkmaktadır. Uzunluğu yaklaşık 18 km olan çay Malatya'nın Hacıhaliloğlu Çiftliği Köyü, Bulgurlu Köyü, Yarımcahan Köyleri boyunca akıp Battalgazi ilçesine bağlı Çolakoğlu Köyü civarlarından Karakaya Baraj Gölü'ne dökülmektedir (Frmartuklu 2017).

Han Çayının su kalitesini belirlemek üzere çayın üst orta ve alt akarsu bölgelerini temsil eden 3 örnekleme noktası belirlenerek Aralık 2013-Kasım 2014 tarihleri arasında, Haziran, Temmuz, Ağustos ve Eylül ayları hariç (bu dönemler kurak geçmiştir) 8 ay boyunca aylık örnekleme yapılmıştır (Şekil 1).



Şekil 1. Han Çayı'nda örnekleme yapılan istasyonlar

Su örnekleri 1 litrelik polietilen şişeler kullanılarak yüzeyden dibe doğru şişelerde hava boşluğu kalmayacak şekilde suya daldırılarak alınmıştır. Örnekleme noktalarında sıcaklık, pH, elektriksel iletkenlik YSI 100 ölçüm cihazıyla, çözünmüş oksijen ise YSI 55 DO ölçüm cihazı ile yerinde ölçülmüştür. Akım değeri yüzdürme metoduyla belirlenerek formülle hesaplanmıştır. Alınan örnekler uygun şartlar altında laboratuvara ulaştırılarak analizleri gerçekleştirilmiştir. Laboratuvara getirilen su örneklerinde toplam sertlik (EDTA), organik madde miktarı (potasyum permanganat) ve tuzluluk (gümüş nitrat) titrimetrik metot ile tayin edilmiştir. Asit kapasitesi, toplam azot, toplam fosfor, nitrit, nitrat, sülfat, silika ve kimyasal oksijen ihtiyacına ait kimyasal parametrelerin analizi ise Merck test kitleri kullanılarak Su ve Atık su Analiz Fotometresi Merck Spectroquant Nova 60 A ile spektrofotometrik olarak tespit edilmiştir (APHA 1985).

Bulgular

Karakaya Baraj Gölü'ne dökülen Han Çayı'nın bazı fiziksel ve kimyasal özelliklerin ölçüm ve analizlerine ait minimum, maksimum ve hesaplanan ortalama değerlere ait veriler Tablo 1'de, aylık değişimlerine ait değerler grafiklerle Şekil 2'de ve istasyonlara göre mevsimsel ortalama değerler Tablo 2'de verilmiştir.

Tablo 1. Han Çayı'nda minimum, maksimum ve ortalama fiziksel ve kimyasal parametrelere ait değerler

Parametreler	İstasyon I	İstasyon II	İstasyon III
	Ort. ±SH (Min.-Maks.)	Ort. ±SH (Min.-Maks.)	Ort. ±SH (Min.-Maks.)
Sıcaklık (°C)	10,36±1,26 (5,6-16,0)	11,40±1,05 (7,2-16,3)	12,10±1,06 (7,7-16,8)
pH	7,10±0,30 (6,12-8,12)	7,18±0,32 (6,20-8,37)	7,10±0,30 (6,16-8,29)
Çözünmüş oksijen (mg O_2/L)	10,29±0,30 (8,7-11,4)	10,43±0,30 (8,8-11,5)	10,25±0,30 (8,6-11,2)
Elektriksel iletkenlik (µS/cm)	49,25±4,39 (37-72)	51,38±5,02 (38-75)	53,50±5,11 (40-77)
Akım (m^3/sn)	0,170±0,022 (0,120-0,274)	0,343±0,045 (0,140-0,517)	0,280±0,027 (0,142-0,383)
Tuzluluk (ppt)	1,58±0,19 (1,2-2,5)	1,88±0,19 (1,2-2,7)	1,76±0,20 (1,2-2,6)
Asit kapasitesi (m/mol)	3,04 (1,58-4,56)	2,33 (0,90-4,31)	2,35 (1,36-3,45)
Toplam sertlik (mg $CaCO_3$/L)	64,25±5,90 (39-97)	63,50±3,59 (49-80)	62,13±5,95 (36-86)
Organik Madde (mg O_2/L)	2,88±0,68 (1,00-6,40)	2,55±0,51 (1,00-5,40)	3,38±0,45 (1,60-5,40)
Sülfat (mg SO_4^{-2}/L)	15,75±2,34 (7-28)	13,63±2,15 (3-21)	15±1,93 (6-21)
Silika (mg SiO_2/L)	10,44±1,28 (5,35-14,90)	9,21±1,35 (5,35-14,30)	9,98±1,29 (5,35-14,40)
Nitrat (mg NO_3^-/L)	0,79±0,13 (0,3-1,5)	0,88±0,16 (0,3-1,3)	0,96±0,18 (0,3-1,7)
Nitrit (mg NO_2^-/L)	0,005±0,001 (0,002-0,007)	0,006±0,001 (0,002-0,009)	0,006±0,001 (0,003-0,009)
Toplam azot (mg N/L)	0,85±0,16 (0,4-1,7)	0,90±0,14 (0,4-1,5)	1,98±0,85 (0,5-7,1)
Toplam fosfor (mg P/L)	0,13±0,06 (0,02-0,55)	0,06±0,01 (0,02-0,11)	0,12±0,04 (0,02-0,36)
KOİ (mg/L)	12,25±1,67 (7-18)	9,25±1,08 (6-14)	10,75±1,21 (8-17)

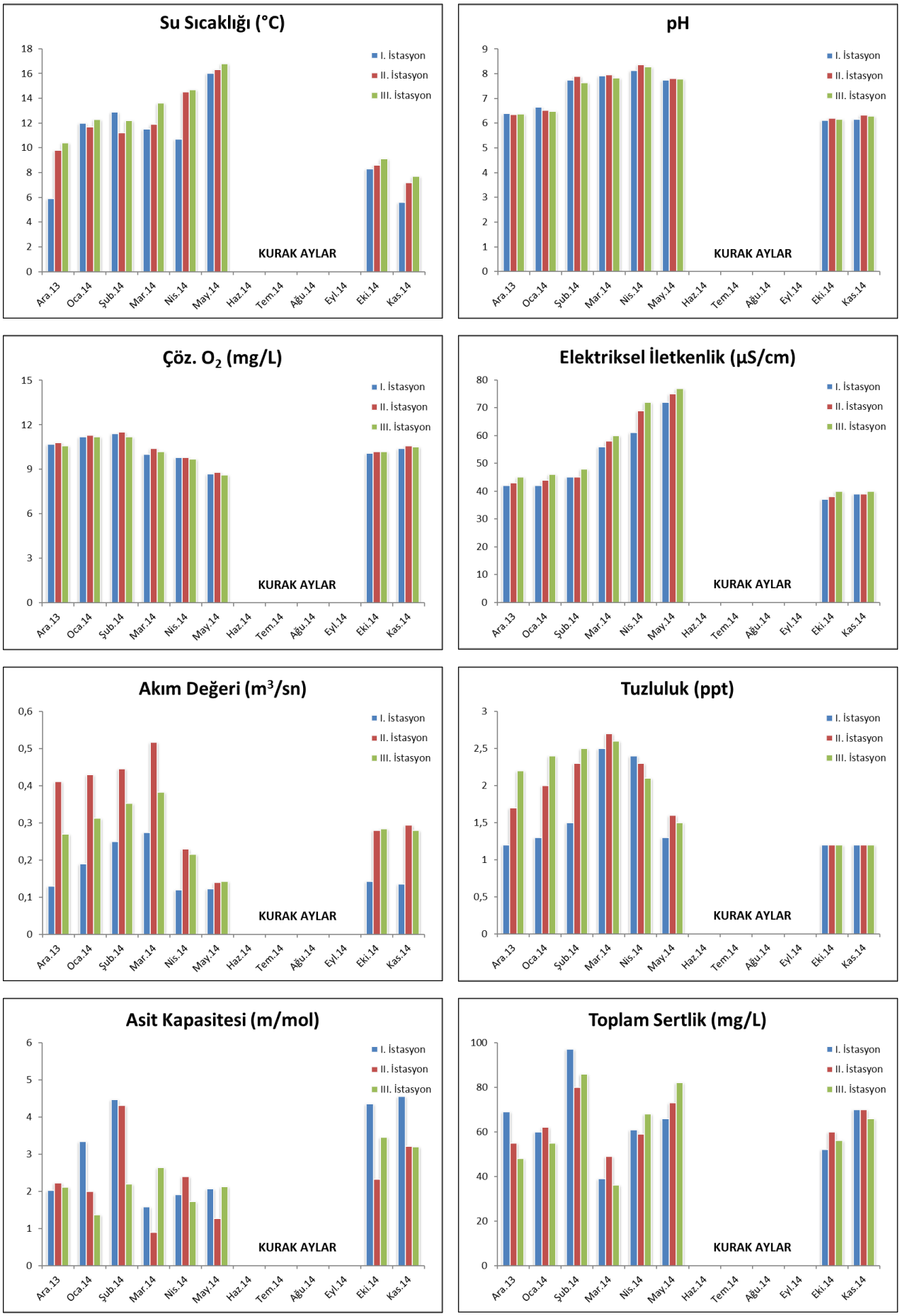
SH: Standart hata

Tartışma ve Sonuç

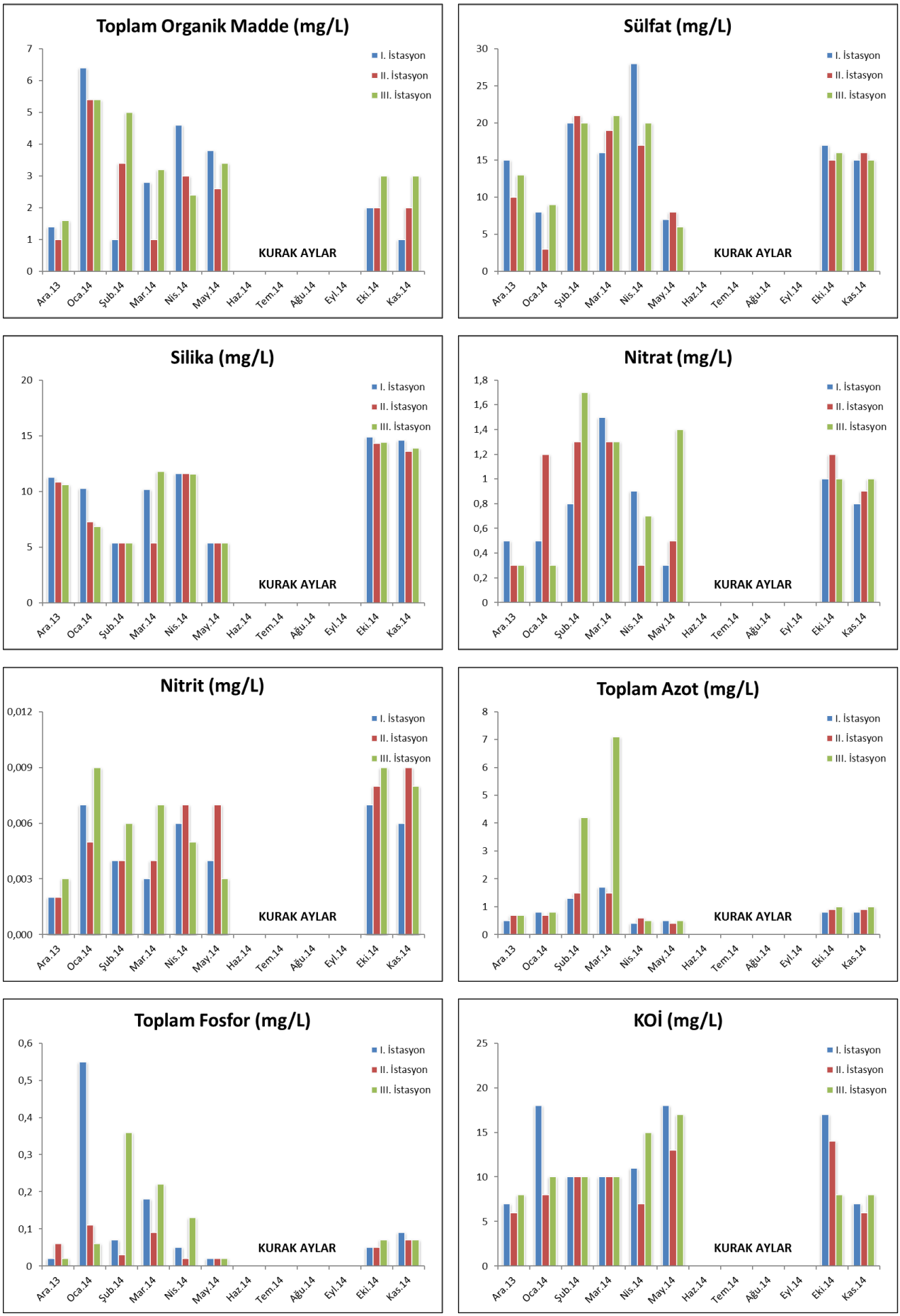
Araştırma süresince Han Çayı'ndaki tüm istasyonlarda tespit edilen toplam fosfor hariç tüm fiziksel ve kimyasal parametreler bakımından Han Çayı Su Kirliliği Kontrol Yönetmeliği (SKKY 2015)'ne göre I. sınıf su kalite özelliğine sahiptir (Tablo 1). Toplam fosfor değeri ise bu kriterlere göre I. ve III. istasyonlarda III. sınıf, II. istasyonda ise II. sınıf su karakterine sahiptir.

Akarsularda su sıcaklığının yüksekliğe, iklime, atmosfer şartlarına, akıntı hızına ve nehir yatağının yapısına göre değiştiği bildirilmiştir. Ayrıca akarsu yatağında gölge yapan bitkilerin bulunması, akarsu önünde oluşabilecek setler, soğuk su karışımları ve akarsu içine akan yeraltı sularının akarsularda su sıcaklığının değişmesinde etkili olduğu belirtilmiştir (USEPA 1997).

Yaptığımız çalışmada da Han Çayı'nda ölçülen su sıcaklıklarının normal olarak mevsimlere bağlı olarak azalıp arttığı tespit edilmiştir (Şekil 2). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler ilkbaharda III. istasyonda 15 ºC olarak, en düşük mevsimsel ortalama değer sonbaharda I. istasyonda 7 ºC olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek sıcaklık değeri III. istasyonda Mayıs ayında 16,8 ºC, en düşük sıcaklık değeri ise I. istasyonda Kasım ayında 5,6 ºC olarak kaydedilmiştir (Tablo 1). Özellikle kış aylarında yağan karların etkisiyle su sıcaklığı oldukça düşük ölçülmüştür. Benzer bulgular yurdumuzdaki pek çok akarsu için de rapor edilmiştir (Tepe vd. 2006, Şen ve Gölbaşı 2008; Tokatlı vd. 2016). Mart ayından itibaren havaların ısınmasıyla birlikte Han Çayı'nda su sıcaklığı artmaya başlamıştır. Haziran Temmuz, Ağustos, Eylül aylarında ise yüksek hava sıcaklıkları nedeniyle bölgede kuraklık olmuş ve akarsu kurumuştur.



Şekil 2. Han Çayı'nda fiziksel ve kimyasal parametrelere ait değerler



Şekil 2. Devamı

Tablo 2. Han Çayı'nda istasyonlara göre fiziksel ve kimyasal parametrelere ait mevsimsel ortalama değerler

	İstasyon I			İstasyon II			İstasyon III		
Parametreler	Kış	İlkbahar	Sonbahar	Kış	İlkbahar	Sonbahar	Kış	İlkbahar	Sonbahar
Sıcaklık (°C)	10,3±2,2	12,7±1,7	7,0±1,4	10,9±0,6	14,2±1,3	7,9±0,7	11,6±0,6	15,0±0,9	8,4±0,7
pH	6,9±0,4	7,92±0,1	6,14±0,02	6,92±0,5	8,04±0,2	6,26±0,06	6,83±0,4	7,97±0,2	6,22±0,06
Çözünmüş oksijen (mg O_2/L)	11,1±0,2	9,5±0,4	10,3±0,2	11,2±0,2	9,7±0,5	10,4±0,2	11,0±0,2	9,5±0,5	10,3±0,2
Elektriksel iletkenlik (µS/cm)	43±1,0	63±4,7	38±1,0	44±0,6	67±5,0	39±0,5	46±0,9	70±5,0	40±0,0
Akım (m^3/sn)	0,19±0,03	0,17±0,05	0,14±0,00	0,43±0,11	0,30±0,1	0,29±0,01	0,31±0,02	0,25±0,07	0,28±0,01
Tuzluluk (ppt)	1,33±0,1	2,07±0,4	1,20±0,0	2,00±0,2	2,20±0,3	1,20±0,0	2,37±0,1	2,07±0,3	1,20±0,0
Asit kapasitesi (m/mol)	3,28±0,7	1,85±0,1	4,46±0,1	2,84±0,7	1,52±0,5	2,77±0,5	1,89±0,3	2,16±0,3	3,32±0,1
Toplam sertlik (mg $CaCO_3$/L)	75,3±11,1	55,3±8,3	61±9,0	65,6±7,4	60,3±7,0	65±5,0	63±11,7	62±13,6	61±5,0
Organik Madde (mg O_2/L)	2,93±1,7	3,73±0,5	1,5±0,5	3,26±1,3	2,20±0,6	2,00±0,0	4,00±1,2	3,00±0,3	3,00±0,0
Sülfat (mg SO_4^{-2}/L)	14,3±3,5	17,0±6,1	16,0±1,0	11,3±5,2	14,6±3,4	15,5±0,5	14,0±3,2	15,6±4,8	15,5±0,5
Silika (mg SiO_2/L)	8,95±1,8	9,05±1,9	14,75±0,2	7,82±1,6	7,44±2,1	13,95±0,4	7,60±1,6	9,56±2,1	14,15±0,3
Nitrat (mg NO_3^-/L)	0,60±0,1	0,90±0,4	0,90±0,1	0,93±0,3	0,70±0,3	1,05±0,2	0,76±0,5	1,13±0,2	1,00±0,0
Nitrit (mg NO_2^-/L)	0,004±0,001	0,004±0,001	0,007±0,001	0,004±0,001	0,006±0,001	0,009±0,001	0,003±0,002	0,005±0,001	0,006±0,001
Toplam azot (mg N/L)	0,87±0,2	0,87±0,4	0,80±0,0	0,97±0,3	0,83±0,3	0,90±0,0	1,90±1,2	2,70±2,2	1,00±0,0
Toplam fosfor (mg P/L)	0,21±0,17	0,08±0,05	0,07±0,02	0,06±0,02	0,04±0,02	0,06±0,01	0,15±0,11	0,12±0,06	0,15±0,00
KOİ (mg/L)	11,7±3,3	13,0±2,5	12,0±5,0	8,0±1,2	10,0±1,7	10,0±4,0	9,03±0,7	14,0±2,1	8,0±0,0

Egemen ve Sunlu (1999) genel olarak kirlenmemiş bölgelerdeki akarsuların pH aralıklarının 6,5-8,5 arasında olduğunu ve gece oksidasyon yoluyla organizmaların ortama verdiği karbondioksit ve gün boyunca çözünmüş karbondioksitin akuatik bitkiler tarafından fotosentezde kullanılması sonucu pH'da inişler ve çıkışlar meydana gelebileceğini ifade etmişlerdir. Suyun pH'nı önemli ölçüde akarsu havzasının toprak yapısı ve jeolojisinin belirlediği bildirilmiş ve akarsu havzasının jeolojisine bağlı olarak akarsularda pH'nın genellikle 6,0-9,0 arasında değiştiği kaydedilmiştir. Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler ilkbaharda II. istasyonda 8,04 olarak, en düşük mevsimsel ortalama değer sonbaharda I. istasyonda 6,14 olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek pH değeri II. istasyonda Nisan ayında 8,37, en düşük pH değeri ise I. istasyonda Ekim ayında 6,12 olarak kaydedilmiştir (Tablo 1).

Soğuk sular daha fazla oksijen tutma kapasitesine sahip olduğundan akarsularda çözünmüş oksijen konsantrasyonlarının kışın daha yüksek, yazın ise daha düşük olduğu ve çözünmüş oksijen içeriğinin başlıca iklim şartları ve biyomas üretimi tarafından etkilendiği bildirilmiştir (Egemen ve Sunlu 1999). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler kış mevsiminde II. istasyonda 11,2 mg/L olarak, en düşük mevsimsel ortalama değer ilkbaharda I. ve III. istasyonlarda 9,5 mg/L olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek çözünmüş oksijen değeri II. istasyonda Şubat ayında 11,5 mg/L, en düşük çözünmüş oksijen değeri ise III. istasyonda Mayıs ayında 8,6 olarak kaydedilmiştir (Tablo 1).

Yeraltı sularının akarsu akımına birincil düzeyde katkıda bulunduğu düşük akım periyotlarında, mineralizasyondan dolayı akarsularda elektriksel iletkenliğin genel olarak yüksek olduğu, buna karşılık yağışların başlaması ve akımın artmasıyla birlikte iletkenliğin azaldığı tespit edilmiştir (Şen ve Gölbaşı 2008).

Araştırmamız süresince 3 istasyonda da elektriksel iletkenlik değerleri birbirlerine benzer olmuş ve 37-77 µS/cm arasında değişim göstermiştir (Şekil 2). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler ilkbaharda III. istasyonda 70 µS/cm olarak, en düşük mevsimsel ortalama değer sonbaharda I. istasyonda 38 µS/cm olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek elektriksel iletkenlik değeri III. istasyonda Mayıs ayında 77 µS/cm, en düşük elektriksel iletkenlik değeri ise I. istasyonda Ekim ayında 37 µS/cm olarak kaydedilmiştir (Tablo 1).

Akarsu havzasının yapısı, jeolojisi, bölgenin coğrafik ve klimatolojik özellikleri bir akarsuyun

akımı ile doğrudan ilişkilidir. Araştırmamızda da Han Çayı'nda akımın mevcut hava şartlarından oldukça etkilenmiş ve akım bulguları akarsu akışının yüksek veya düşük olmasını etkileyen en önemli faktörlerden birinin iklim olduğu bulgusunu desteklemiştir. Han Çayı'nda yağışların başladığı ve yüzey akışların olduğu aylarda akım değerinin artması söz konusu değerin artmasında en önemli etkenin yağışlar ve yüzey akışları olduğunu ortaya koymaktadır. Akım değerleri 0,120-0,517 m^3/sn arasında değişim gösterecek kadar düşük olmuştur (Şekil 2). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler kış mevsiminde II. istasyonda 0,429 m^3/sn olarak, en düşük mevsimsel ortalama değer sonbaharda I. istasyonda 0,139 m^3/sn olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek akım değeri II. istasyonda Mart ayında 0,517 m^3/sn, en düşük akım değeri ise I. istasyonda Nisan ayında 0,120 m^3/sn olarak kaydedilmiştir (Tablo 1).

Han Çayı'nın tuzluluk değerlerinin istasyonlara ve aylara göre değişimi model olarak birbirine benzemiştir. Araştırma süresince Han Çayı'nda analiz edilen tuzluluk miktarları 1,2-2,7 mg/L arasında değişim göstermiştir (Şekil 2). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değerler kış mevsiminde III. istasyonda 2,37 ppt olarak, en düşük mevsimsel ortalama değer sonbaharda tüm istasyonlarda 1,20 ppt olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek tuzluluk değeri II. istasyonda Mart ayında 2,7 ppt, en düşük tuzluluk değeri ise tüm istasyonlarda Ekim ve Kasım aylarında 1,2 ppt olarak kaydedilmiştir (Tablo 1).

Han Çayı asit kapasitesi değerleri 0,90 ile 4,56 m/mol arasında değerlerde kaydedilirken aylara göre artma ve azalmalar toplam sertlik değerleri ile paralel olmuştur (Şekil 2). Mevsimsel ortalamalara göre en yüksek ortalama değer sonbaharda I. istasyonda 4,46 m/mol olarak, en düşük değer ilkbaharda II. istasyonda 1,52 m/mol olarak ölçülmüştür (Tablo 2). İstasyonlara göre değerlendirildiğinde en yüksek değer 4,56 m/mol ile I. istasyonda Kasım ayında, en düşük değer 0,90 m/mol olarak II. istasyonda Mart ayında kaydedilmiştir (Tablo 1).

Wetzel ve Likens (1991) ile Egemen ve Sunlu (1999), sulardaki sertliğin büyük ölçüde toprak ve kayalara temas sonucu kalsiyum ve magnezyum tuzlarından ileri geldiğini ve karbonat, bikarbonat, sülfat, klorür ve mineral asitlerin diğer iyonları ile kombinasyon oluşturduğunu ifade etmişlerdir. Araştırmamızın bulguları akımın düşük olduğu aylarda toplam sertlik konsantrasyonunun arttığını, akımın yüksek olduğu aylarda ise toplam sertlik konsantrasyonunun azaldığını ifade eden Tepe vd. (2006)'nin çalışmasıyla uyum içerisinde olmuştur.

Egemen ve Sunlu (1999), suları sertlik derecesine göre 50 mg $CaCO_3$/L den küçük olan suların yumuşak, 50-150 mg $CaCO_3$/L arasında olan suların orta sert, 150-300 mg $CaCO_3$/L arasında olan suların sert ve 300 mg $CaCO_3$/L'den büyük olan suların ise çok sert su sınıfına girdiğini belirtmiştir. Araştırmamızda istasyonlarda belirlenen toplam sertlik değerleri birbirine yakın olurken (Şekil 2), minimum ve maksimum değerler 36-97 mg $CaCO_3$/L ve ortalama değer 63,29 mg $CaCO_3$/L olarak hesaplanmıştır. Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama değer I. istasyonda 75,3 mg $CaCO_3$/L olarak kış mevsiminde, en düşük mevsimsel ortalama değer ilkbaharda I. istasyonda 55,3 mg $CaCO_3$/L olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek toplam sertlik değeri I. istasyonda Şubat ayında 97 mg $CaCO_3$/L, en düşük toplam sertlik değeri ise III. istasyonda Mart ayında 36 mg $CaCO_3$/L olarak kaydedilmiştir (Tablo 1). Ortalama toplam sertlik değerleri dikkate alındığında Han Çayı orta sert su özelliğine sahiptir.

Han Çayı'nda organik madde miktarları istasyonlarda 1-6,4 mg O_2/L arasında kaydedilmiştir. Han Çayı'nda organik madde miktarı bütün istasyonlarda Ekim-Aralık ayları arasında kalan periyotta sürekli azalmıştır. Ocak ayında tekrar artmaya başlamış ve araştırma süresince en yüksek değerlerde kaydedilmiştir (Şekil 2). Mevsimsel ortalamalara göre en yüksek ortalama değer kış mevsiminde III. istasyonda 4 mg O_2/L olarak, en düşük değer sonbaharda I. istasyonda 1,5 mg O_2/L olarak ölçülmüştür (Tablo 2). İstasyonlara göre değerlendirildiğinde en yüksek organik madde değeri 6,4 mg O_2/L ile I. istasyonda Ocak ayında, en düşük değer 1 mg O_2/L olarak I. ve II. istasyonlarda Aralık, Şubat ve Mart aylarında kaydedilmiştir (Tablo 1).

Sülfat değerleri kışın en düşük, ilkbaharda en yüksek değerinde kaydedilmiştir (Şekil 2). Değerler II. istasyonda diğerlerine göre daha düşüktür. II. istasyonda 3-21 mg/L arasında olup ortalama değer 13,63 mg/L olarak ölçülmüştür (Tablo 1). İstasyonlara göre en yüksek sülfat değeri 28 mg/L olarak I. istasyonda Nisan ayında kaydedilmiştir. Mevsimsel ortalama olarak en düşük değer kış mevsiminde 11,3 mg/L ile II. istasyonda; en yüksek değer ilkbaharda 17 mg/L ile I. istasyonda kaydedilmiştir (Tablo 2).

Silika en düşük kış mevsiminde, en yüksek sonbaharda tespit edilmiştir (Şekil 2) İstasyonlara göre en yüksek silika değeri I. istasyonda 14,9 mg/L olarak Ekim ayında kaydedilirken (Tablo 1), mevsimsel ortama olarak en yüksek silika değeri I. istasyonda sonbaharda 14,75 mg/L, en düşük silika değeri II. istasyonda ilkbaharda 7,44 mg/L olarak kaydedilmiştir (Tablo 2).

Nitrat değerleri 0,3-1,7 mg/L arasında değişim göstermiş ve I. ve II. istasyonlarda Mart ayında III. istasyonda ise Şubat ayında ölçülmüştür (Şekil 2). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama nitrat değeri ilkbaharda III. istasyonda 1,13 mg/L olarak, en düşük mevsimsel ortalama nitrat değeri kış mevsiminde I. istasyonda 0,60 mg/L olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek nitrat değeri III. istasyonda Mart ayında 1,7 mg/L, en düşük nitrat değeri ise 0,3 mg/L olarak kaydedilmiştir (Tablo 1).

Han Çayı'nda istasyonlarda belirlenen ortalama nitrit konsantrasyonu 0,003 mg/L- 0,009 mg/L arasında değişmiştir (Şekil 2). Han Çayı'nda yüksek nitrit değerlerinin Aralık ayı gibi soğuk bir ayda belirlenmiş olması, Emet Çayı'nda nitrit iyonu değerlerinin 0,01-0,17 mg/L arasında değiştiğini ve yüksek nitrit değerlerin çok düşük sıcaklıklarda ortaya çıktığını bildiren Tokatlı vd (2016)'nin bulgularıyla da uyum içerisindedir. Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama nitrit değeri sonbaharda II. istasyonda 0,009 mg/L olarak, en düşük mevsimsel ortalama nitrit değeri kış mevsiminde III. istasyonda 0,003 mg/L olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek nitrit değeri II. ve III. istasyonlarda sırasıyla Kasım ile Ocak ve Ekim aylarında 0,009 mg/L, en düşük nitrit değeri ise I. ve II. istasyonlarda Aralık ayında 0,002 mg/L olarak kaydedilmiştir (Tablo 1).

Han Çayı'nda istasyonlarda belirlenen toplam azot konsantrasyonunun ortalama değerleri I. ve II. istasyonlarda (sırasıyla 0,85 ve 0,90 mg/L) III. İstasyondakinden (1,98 mg/L) daha düşük çıkmıştır (Şekil 2). Toplam azot konsantrasyonlarının tüm istasyonlarda düzensiz olarak artıp azalması, toplam azot miktarı üzerinde etkili olan faktörleri belirsiz kılmıştır. Bununla birlikte en yüksek konsantrasyonun III. istasyonda Mart ayında 7,1 mg/L olarak, ancak en yüksek sıcaklıkların kaydedildiği Nisan ve Mayıs (16 ^{o}C) aylarında toplam azot değerleri en düşük değerlerde (0,4 mg/L) kaydedilmiştir (Tablo 1). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama toplam azot değeri ilkbaharda III. istasyonda 2,7 mg/L olarak, en düşük mevsimsel ortalama toplam azot değeri sonbaharda I. istasyonda 0,80 mg/L olarak kaydedilmiştir (Tablo 2).

Araştırma süresince Han Çayı'nda tüm istasyonlarda analiz edilen toplam fosfor miktarları 0,002-0,55 mg/L olarak ölçülmüştür (Şekil 2). Su Kirliliği Kontrol Yönetmeliği (SKKY 2015)'ne göre Ocak ve Şubat aylarında ölçülen maksimum toplam fosfor değerleri bakımından Han Çayı'nın II. istasyonda II. sınıf (0,11 mg/L) ve I. ve III. istasyonlarda ise III. sınıf (sırasıyla 0,55 ve 0,36 mg/L) su kalite özelliğine sahip olduğuna işaret etmektedir (Tablo 1). Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama toplam fosfor değeri kış mevsiminde I. istasyonda 0,21 mg/L olarak, en düşük mevsimsel ortalama toplam azot değeri ilkbaharda II. istasyonda 0,04 mg/L olarak kaydedilmiştir (Tablo 2).

Araştırma süresince Han Çayı'nda analiz edilen KOİ miktarları düşük (7-18 mg/1L) çıkmıştır (Şekil 2), bu değerler Su Kirliliği Kontrol Yönetmeliği (SKKY 2015)'ne göre Han Çayı'nın I. sınıf su kalite özelliğine sahip olduğunu ve organik bir kirliğin olmadığını ortaya koymuştur. Mevsimsel ortalama değerlere bakıldığında en yüksek ortalama KOİ değeri ilkbaharda III. istasyonda 14 mg/L, en düşük mevsimsel ortalama KOİ değeri kış mevsiminde II. istasyonda 8 mg/L olarak kaydedilirken (Tablo 2); istasyonlara göre en yüksek KOİ değeri I. istasyonda Ocak ve Mayıs aylarında 18 mg/L, en düşük KOİ değeri ise II. istasyonda Kasım ve Aralık aylarında 6 mg/L olarak kaydedilmiştir (Tablo 1).

Yapılan çalışma sonucunda, Han Çayı'nın Su Kirliliği Kontrol Yönetmeliği (SKKY 2015)'ne göre, toplam fosfor miktarı bakımından II. sınıf (az kirli su) ve tayin edilen diğer parametreler açısından I. sınıf (yüksek kaliteli su) su özelliğine sahip olduğu belirlenmiştir. Han Çayı'nın fiziksel ve kimyasal özellikleri, akarsu havzasının jeomorfolojisine ve yatağının morfometrik özelliklerine bağlı olarak akış hızına, taşıdığı su miktarına ve mevsimlere bağlı olarak değişiklik göstermiştir. Su kalitesi açısından bütün veriler değerlendirildiğinde, Han Çayı'nda kirliliğin şu anda bir öneminin olmadığı sonucuna varılmıştır.

Teşekkür

Bu çalışmayı, "Karakaya Baraj Gölü'ne Dökülen Han Çayı'nın Bazı Fiziksel ve Kimyasal Özelliklerinin Araştırılması" başlık ve 13.07 numarayla destekleyen Fırat Üniversitesi Bilimsel Araştırma Projeleri Yönetim Birimi (FÜBAP)'ne teşekkür ederim.

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Dietary Replacement Effects of Maize with Graded Levels of Melon Shell on Growth Performance of *Clarias gariepinus* Fingerlings (Burchell, 1822)

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ABSTRACT

A 60-day study was conducted to investigate the dietary effect of replacement of maize with graded levels of melon shell meal (MSM) on growth performance of *Clarias gariepinus* fingerling. Five isonitrogenous diets were formulated. MSM was added to the diet to replace maize at graded levels; 0%, 25%, 50%, 75% and 100%. Total of 150 fish (3.40±0.05g) were randomly assigned to the five treatment diets. Each treatment contained 10 fish per tank and each treatment was triplicated in a completely randomised design (CRD). Fish were fed twice daily at 5% body weight in equal proportions. Results showed significant differences ($P<0.05$) for measured growth parameters. Fish fed diet containing 100% MSM had the highest mean weight gain (70.70±4.62g), specific growth rate (*SGR*) (5.06±0.10%), best feed conversion ratio (*FCR*) (0.54±0.04%) and protein efficiency ratio (*PER*) (1.68±0.11%) compared to other treatments and the control while fish fed 25% *MSM* had the poorest values for *MWG* (39.88±4.37g), *SGR* (4.20±0.16), *FCR* (0.60±0.02) and *PER* (0.95±0.11). *MSM* inclusion in diet of *C. gariepinus* up to 100% enhanced growth.

Keywords: Melon-shell, Replacement, Maize, Growth, *C. gariepinus*

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Introduction

The consumption and demand for fish as a cheap source of animal protein is increasing in Africa. In most countries, vast majority of the fish supply comes from the rivers as captured fisheries. FAO (2004) in "The State of the World Fisheries and Aquaculture" concluded that developments in world fisheries and aquaculture during recent years have continued to follow the trends that were already becoming apparent at the end of the 1990s, as capture fisheries production is stagnating and aquaculture output is expanding faster than any other animal-based food sector. Thus development policies increasingly perceive aquaculture as means for economic growth and prospect for future fish supply (FAO 2004). According to FAO (2006), fish supplies from capture fisheries can no longer meet the growing global demand for aquatic foods. Hence, there is a need for a viable alternative fish production system that can sufficiently meet this demand, and aquaculture fits exactly into this role. As aquaculture production becomes more intensive in Nigeria, fish feed will be a significant factor in increasing productivity and profitability (Akinrotimi et al. 2007). Jamiu and Ayinla (2003) opined that feed management determines the viability of aquaculture as it accounts for at least 60% of the cost of fish production.

Maize is one of the conventional feedstuffs, thus a major source of metabolisable energy in most compounded diets for catfish as it is readily digestible by fish (Olurin et al. 2006). FAO (2005) reported that

maize, which is predominantly used for human consumption in Nigeria, is not provided in sufficient quantities. The use of maize in fish feeds is becoming increasingly unjustified in economic terms (Tewe 2004), because of the ever increasing cost. Therefore, there is a need to exploit cheaper energy sources to replace expensive cereals in fish feed formulation. For the purpose of nutritional and economic benefits, previous researchers have attempted to increase the use of non-conventional feed resources to replace conventional feed ingredients like maize and fishmeal in fish diet (Olatunde 1996; Baruah et al. 2003; Eyo 2005). To relieve the feed competition between man and animal and for profit maximization, melon shell appears to be very appropriate for this purpose.

Melon husks are shells that are discarded after processing or shelling of melon seeds (*Citrullus vulgaris*). Melon is a cucurbit crop belonging to the family cucurbitaceae (Abiodun and Adeleke 2010). Melon (seed) crops are grown, harvested and processed in large tonnage in Nasarawa Local Government Area of Nasarawa as well as Kaduna State, Nigeria. The seeds are removed from the fruit, washed, sun-dried and sold in large quantities (tonnage) annually for commercial purpose (as a special soup condiment). They are also used as domestic remedy for urinary tract infection, hepatic congestion, intestinal worms and abnormal blood pressure (Moerman 1998). The freshly shelled seeds were reported contained 34.24% crude protein, 45.95% fat, 7.18% crude fibre, 4.05% ash, 8.03% moisture and 0.56% carbonhydrate (Fagbohun et al. 2011). However, large quantities of the melon husks are discarded and burnt, which pollute the environment (Ogbe and George 2012). While the fisheries industry is threatened with acute shortage of conventional feed ingredients leading to low productivity, it may be possible to utilize melon husks as non-conventional source of feed ingredient for fish. The current study seeks to investigate the effect of varying dietary inclusion levels of *MSM* in replacement of maize on growth performance of *C. gariepinus.*

Materials and Methods

Experimental fish

Fingerlings of *C. gariepinus* were procured from Regina Pacie fish farm in Abakaliki and transported to the Department of Fisheries and Aquaculture laboratory in a 50 litre and opened at the top within 20-30 minutes. Fish were subsequently subjected to a 2 min bath with 0.05% potassium permanganate ($KMnO_4$) to prevent skin infections. The fish were acclimatized for two weeks in a tarpaulin tank (10m x10m x 2m) and fed *ad libitum* on a daily basis with commercial feed (Coppens International Helmond Netherlands) containing 45% crude protein.

Collection and processing of melon shell

Melon shells were collected from the rural women who process melon seeds for commercial purpose (also called "egusi" by the people); they were sundried (3-4 days) and milled to powdered form using manual grinding machine at Abakaliki main market to aid incorporation with other feed ingredient. The milled melon shell was incorporated directly with other finely ground feedstuffs, pelletized, sundried and individual diets were packed into separate bags and then stored in a cool and dry place.

Experimental Diet

Five (5) isonitrogenous diets (42% crude protein) were formulated to contain melon shell meal (*MSM*) at 0% (*D1*) as control diet, 25% (*D2*), 50% (*D3*), 75% (*D4*) and 100% (*D5*) in the diet of the experimental fish. Pearson's square method was used in feed formulation (De Silva and Anderson 1995). Feed ingredients for the experimental diet include soya bean meal (*SBM*), groundnut cake (*GNC*), maize meal (*MM*), fish meal (*FM*), melon shell meal (*MSM*), methionine, lysine, vitamin/mineral premix, oil, salt and starch (binder) (Table 1). The formulation was based on gross proximate composition of the ingredients.

Table 1. Gross composition of experimental diets (g/2000g) containing melon shell meal fed to *Clarias gariepinus*

Ingredient	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
FM	548	548	548	548	548
SBM	390	390	390	390	390
GNC	402	402	402	402	402
MM	460	345	230	115	-
MSM	-	115	230	345	460
BM	40	40	40	40	40
Vit. premix	50	50	50	50	50
Methionine	15	15	15	15	15
Lysine	15	15	15	15	15
Starch	50	50	50	50	50
Vegetable oil	20	20	20	20	20
Salt	10	10	10	10	10

FM - Fish meal, *SBM* - Soybean meal, *GNC* - Groundnut cake, *MM* - Maize meal, *MSM* - Melon shell meal, *BM* - Bone meal, *Vt* - Vitamin premix.

Proximate Composition of MSM and Experimental diets

Samples of MSM and experimental diets were sent to the International Institute for Tropical Agriculture (IITA) Laboratories Ibadan. Samples

were analysed chemically according to the official methods of analysis described A.O.A.C. (2000). All analysis was carried out in triplicate. Proximate composition of the MSM and experimental diets are presented in Table 2. Result showed that MSM has 14.88% CP, 7.16% moisture, 6.98% ash and 5.67% crude fibre.

Table 2. Proximate composition of experimental diets and *MSM* (%)

Parameters	***MSM***	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
Moisture	7.16	5.46	5.40	5.37	5.08	5.00
Crude fat	3.16	4.29	4.25	4.37	4.16	4.15
Crude ash	6.98	10.24	10.73	11.35	10.83	11.33
Crude fibre	5.67	2.66	2.71	2.57	2.65	2.71
Crude protein	14.88	43.27	43.97	44.25	44.28	44.38
NFE	62.15	34.08	32.94	32.09	33.00	32.43
G. Energy (kj/g)	15.76	17.15	18.00	17.97	18.05	17.97

G. Energy = Gross Energy. *NFE* - Nitrogen Free Extract = 100 - (*C. fat + C. P +C. Fibre + Ash + Moisture*), *MSM* = Melon Shell Meal

Experimental design

A total of one hundred and fifty (150) fingerlings with an average weight 3.40±0.50 g were randomly assigned to fifteen experimental aquaria tanks (1m x 1m x 1m). Fish were subjected to five test diets with varying dietary inclusion levels of melon shell as treatments. Each treatment contained 10 fish and was triplicated in a completely randomised design (*CRD*). Fish were fed twice daily (9:00 am and 4:00pm) at 5% body weight for 60 days. Total fish weight in each tank was determined at two weeks intervals and the amount of diet was adjusted accordingly.

Water quality management

Water in the experimental tank was removed every three days and replaced with fresh water in order to prevent water fouling and also remove fish faecal waste. Physicochemical analysis of water was measured once a week during the study (Table 3). The following parameters were tested; water temperature, pH, and total dissolved solids (Turbidity) and dissolved oxygen (*DO*) using a water testing kit (Pro-lab™, Florida).

Table 3. Water quality parameters of the experimental tank water.

Parameter	DO (mg/l)	pH	Temp. (°C)
D1	6.83±0.17[a]	6.67±0.17[a]	27.67±0.33[a]
D2	6.83±0.17[a]	6.83±0.17[a]	27.33±0.33[a]
D3	6.67±0.17[a]	6.50±0.29[a]	28.00±0.00[a]
D4	6.67±0.17[a]	6.67±0.17[a]	27.67±0.33[a]
D5	6.36±0.15[a]	6.32±0.08[a]	28.67±0.33[a]

Means within column with different superscripts are significantly different (P<0.05).

Growth parameters

Growth were following Iheanacho et al. (2018) and Ogunji (2004);

Mean Weight Gain (g) (*MWG*)

$$MWG = \frac{WT2 - WT1}{N}$$

Where *Wt1* = initial mean weight of fish at time T1
Wt2 = final mean weight of fish at time T2
N = Number of days

Feed Conversion Ratio (g) (*FCR*)

$$FCR = \frac{weight\ of\ feed\ given\ (g)}{Fish\ weight\ gain}$$

Relative Conversion Ratio *(RCR)*

$$RCR\ (\%) = \frac{(Wf - Wi)}{Wi} x100$$

Wf = final average weight at the end of the experiment
Wi = initial average weight at the beginning of the experiment

Protein Efficiency Ratio (*PER*)

$$PER = \frac{Fish\ weight\ gain(g)}{Protein\ intake\ (g)}$$

Where;

$$Protein\ intake = \frac{\binom{\%\ protein}{in\ feed}\ x\ \binom{total\ diet}{consumed}}{100}$$

Specific Growth Rate (*SGR*)

$$SGR = \frac{100(\log_e Wf) - (\log_e Wi)}{Time\ (days)}$$

Wf = final average weight at the end of the experiment
Wi = initial average weight at the beginning of the experiment
Loge = Natural Logarithm reading

Survival rate (%)

$$Survival\ Rate\ (\%) = \frac{\binom{Number\ of\ fish}{that\ survived}\ x\ 100}{\binom{Total\ number\ of}{fish\ stocked}}$$

Statistical Analysis

Data collected from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS package version 20 and the differences among treatments were separated using Duncan multiple range test (Duncan 1955).

Results

Water Quality

Water quality parameters recorded during the experiment are presented in Table 3. There was no significant difference (P>0.05) in all the parameters. The range for pH was observed to be between 6.32±0.08 to 6.83±0.17. Dissolved oxygen ranged between 6.36±0.15mg/L - 6.83±0.17 mg/L, while temperature between 27.33±0.33 ^{o}C - 28.67±0.33 ^{o}C.

Growth parameters and feed utilization

Results on growth performance are presented in Table 4. Significant differences (P<0.05) were seen among the treatments in almost all growth indices expect for survival rate. Mean weight gain (*MWG*) of the fishes differ significantly (P<0.05) from each other. D5 had the highest *MWG* of 70.70±4.62g which was significantly higher (P<0.05) than other treatments. This was followed by D4, (54.72±4.40g), D3, (44.66±3.38g), D1 (42.14±2.31g) and D2 (39.88±4.37g). The highest *FCR* was recorded in D1 (0.62±0.14) and the least value was observed in T5 (0.54±0.04) which are not significantly different (P>0.05) from other treatments. *SGR* values differed significantly among the treatments (P<0.05). D5 had the highest *SGR* of 5.06±0.10 which differed significantly (P<0.05) from other treatments. This was followed by D4 (4.63±0.15g) and D1 (4.57±0.26g) respectively. D2 and D3 had the lowest with *SGR*s 4.26±0.16 and 4.27±0.08 respectively. *PER* differed significantly among the treatments (P<0.05). D5 showed the highest *PER* of 5.06±0.10% being significantly (P<0.05) from the other diets. This was followed by D4 (1.30±0.10%), D1 (1.24±0.20%), D3 (1.06±0.09%) and D2 (0.95±0.11%). There were no significant differences (P>0.05) between *PER* values of D1, D2 and D3. There were no significant differences (P>0.05) in survival rates of the fish fed different inclusion levels of *MSM*. The highest survival rate was recorded in D2 (96.67±3.33%) followed by D1 (93.33±3.33%) and the least in D3 (86.67±3.33%) and D4 (86.67±6.67%). The final length of fish differed significantly with the highest in D5 (25.03±1.04) and the least in D3 (19.27±0.64).

Discussion

Water Quality

The values for physico-chemical parameters observed in current study were within the tolerance range of *C. gariepinus* (Table 3) and agree with the findings of Adekoya et al. (2004). Bhatnagar et al. (2004) reported that the levels of temperature as (25-30^{o}C) is ideal for culture of *C. gariepinus*. Adekoya et al. (2004) also recommended dissolved oxygen level of between 4-8mg/L. Bhatnagar et al. (2004) reported dissolve oxygen level greater than 5 mg/L support good fish production. Bhatnagar et al. (2004) also suggested that 1-3 mg/L has sublethal effect on growth and feed utilization; 0.3-0.8 mg/L is lethal to fishes and oxygen concentration above 14mg/l is lethal to fish fry, and gas bubble disease may occur. Santhosh and Singh (2007) reported that the suitable pH range for fish culture is between 6.7 and 9.5 and ideal pH level is between 7.5 and 8.5 and above and below this could be stressful to the fishes.

Growth Performance

The present study investigated the effect of replacement of maize with *MSM* on the growth performance and feed utilization of African catfish *(C. gariepinus).* Among the diets, T5 (100% *MSM*) recorded the best growth performance which reflected in the values obtained for *MFWG, FCR, SGR,* and *PER* (Table 4). While T5 (100% *MSM*) recorded the highest weight gain, lowest *FCR*, highest *SGR* and the highest *PER* among the experimental diets, this result collaborates the findings of Iheanacho et al. (2017) who reported increased growth when Nile tilapia (*Oreochromis* niloticus) juveniles were fed melon seed peel based diet in 56 days feeding trial. Orire and Ricketts (2013) reported better growth performance and feed utilization efficiency when *O. niloticus* were fed melon seed peel based diet. Nwanna et al. (2009) reported poor feed utilization of potato peel based diet as dietary carbohydrate source in the diet of *C. gariepinus.* The findings of the present study differ from the report made by Jesu et al. (2008) on the utilization of various dietary carbohydrate levels by the freshwater catfish *Mystus montanus*. The disagreement may be due to the higher protein content of *MSM* (14.88%) when compared to 8.11% in potato peel. Values for *FCR, MFWG, SGR, PER* and mortality) were optimal and similar to the result reported by Amanat and Nasser (2001). Diet 2 (25% *MSM*) recorded the poorest growth response indicating poor feed utilization by the fish. This was expressed in the values of its biological evaluation such as high *FCR*, low *MWG, SGR* and *PER* values (Table 4). The results of this study disagree with the observation made by Nwanna et al. (2009), who reported that very high inclusion levels of unconventional dietary carbohydrate sources often result in poor performance of the fish. The growth response of the control diet (T1, 0%*MSM*) was the second to least which was in agreement with the reports of Solomon et al. (2007) and Nwanna et al. (2009). Fagbenro et al. (2000) reported a comparable performance of Acha (*Digitaria exilix*) meal with maize and sorghum meal as dietary carbohydrate source for Nile tilapia. Nwanna (2003) showed the

Table 4. Growth performance of *C. gariepinus* fingerling fed graded level of melon shell based

Parameter	D1	D2	D3	D4	D5
IW(g)	3.50±0.09^{a}	3.45±0.03^{a}	3.70±0.47^{a}	3.61±0.17^{a}	3.57±0.21^{a}
IL (cm)	6.30±0.15^{b}	6.13±0.12^{b}	7.00±0.35^{ab}	6.80±0.40^{ab}	7.07±0.12^{a}
FW(g)	46.57±1.74^{bc}	43.33±4.40^{c}	48.38±3.97^{bc}	58.33±4.41^{b}	74.27±4.74^{a}
FL (cm)	22.00±0.84^{b}	21.80±0.25^{bc}	19.27±0.64^{c}	22.40±1.00^{b}	25.03±1.04^{a}
MWG (g)	42.14±2.31^{bc}	39.88±4.37^{c}	44.66±3.83^{bc}	54.72±4.40^{b}	70.70±4.62^{a}
FCR	0.62±0.14^{a}	0.60±0.02^{a}	0.59±0.07^{a}	0.55±0.16^{a}	0.54±0.04^{a}
PER (%)	1.24±0.20^{b}	0.95±0.11^{b}	1.06±0.09^{b}	1.30±0.10^{ab}	1.68±0.11^{a}
SGR (%/day)	4.57±0.26^{ab}	4.20±0.16^{b}	4.27±0.08^{b}	4.63±0.15^{ab}	5.06±0.10^{a}
Survival rate (%)	93.33±3.33^{a}	96.67±3.33^{a}	86.67±3.33^{a}	86.67±6.67^{a}	90.00±0.00^{a}

Means within rows with different superscripts are significantly different (P<0.05).
IW = Initial weight, *IL* = Initial length, *FW* = Final weight, *FL* = Final length, *MWG* = Mean weight gain, *FCR* = Food conversion ratio, *PER* = Protein efficiency ratio, *SGR* = Specific growth rate.

effectiveness of replacing maize with acha in the diets of Nile tilapia (*Oreochromis niloticus*). Nwanna et al. (2004) also reported that replacing maize with a non-conventional carbohydrate source tamarind (*Tamarindus indica)* resulted in good growth performance of catfish and improved economic returns.

Our results showed that *C. gariepinus* effectively utilized feed formulated with different dietary inclusion levels of *MSM* based diet especially diet 5 (100% *MSM*). This implies that *MSM* is an agro waste product to efficiently replace maize as carbohydrate source in the diet of *Clarias gariepinus*. Fish farmers are encouraged to explore this opportunity as it will reduce drastically the cost of fish production and enhance growth of fish as well.

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Effect of Different Feed and Stocking Density on Survival and Growth Performance of *Astacus leptodactylus* (Esch., 1823) Juveniles

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ABSTRACT

The aim of this study was to investigate the combined effects of two stocking densities (650 and 1300 juveniles/m^2) and diets (live feed - *Daphnia magna* and commercial trout feed) on survival rate and growth performance of *Astacus leptodactylus* juveniles. Juveniles in the second developmental stage were used in the experiment. Final survival rate and growth paremeters of juveniles were evaluated at the end of experiment (90 days). The results of this study indicated that diets were not found effective on survival rates, though increasing stock densities reduced survival rates. The best final survival rate as 21.99 %, was achieved in the group fed with live feed and kept under the 650 juveniles/m^2 stocking density, whereas the lowest survival rate values as 10.84 % was obtained in the group fed with trout pellet and kept under the 1300 juveniles/m^2 stocking density. At the end of experiment, the highest final body weight was found in group of at 650 juveniles/m^2 stocking density and fed with trout feed. The result of our study also revealed that stocking density had a significant effect on survival rates of crayfish juvenile fed with both feeds.

Keywords: *Astacus leptodactylus,* feeding, stocking density, survival rate, growth

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Farklı Yem ve Stok Yoğunluğunun *Astacus leptodactylus* (Esch., 1823) Juvenillerinin Hayatta Kalma ve Büyüme Performansı Üzerine Etkisi

Öz: Bu çalışmada, iki farklı yem (canlı yem-*Daphnia magna* ve ticari alabalık yemi) ve stok yoğunluğunun (650 ve 1300 juvenil/m^2) *Astacus leptodactylus* yavrularının hayatta kalma ve büyüme performansı üzerine kombine etkileri araştırılmıştır. Denemede II. dönem yavrular kullanılmıştır. Deneme sonunda, yavruların hayatta kalma ve büyüme parametreleri değerlendirilmiştir. Bu çalışmanın sonuçları, diyetlerin hayatta kalma oranları üzerinde etkili olmadığını, ancak artan stok yoğunluğunun yaşama oranlarını azalttığını göstermiştir. En iyi yaşama oranı % 21,99 olarak 650 juvenil/m^2 stok yoğunluğunda canlı yemle beslenen grupta sağlanırken, en düşük yaşama oranı ise % 10,84 olarak 1300 juvenil/m^2 stok yoğunluğunda alabalık yemi ile beslenen grupta elde edilmiştir. Denememe sonunda en yüksek vücut ağırlığı, 650 juvenil/m^2 stok yoğunluğunda alabalık yemi ile beslenen grupta bulunmuştur. Çalışmamız sonucunda ayrıca, yavru kerevit beslenmesinde her iki yem ile beslenen yavrularda hayatta kalma oranları üzerinde stok yoğunluğunun önemli bir etkiye sahip olduğu görülmüştür.

Anahtar kelimeler: *Astacus leptodactylus,* besleme, stok yoğunluğu, yaşama oranı, büyüme

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Introduction

The narrow-clawed crayfish, also known as Turkish crayfish, *Astacus leptodactylus*, is the native freshwater crayfish species of Turkey (Holdich 2002). *A. leptodactylus* has a widespread distribution in lakes and ponds in many parts of the country. Its

distribution area was considerably expanded in Turkey after 1985 because of its commercial importance and declined catches from traditional fisheries. Although the domestic demand for crayfish was very low in Turkey, this country has been the main supplier of *A. leptodactylus* to Western Europe from 1970 to until 1986 (Harlıoğlu 2004, 2008). Until 1984, *A. leptodactylus* had an important role as an export product. But after 1985, Crayfish production declined dramatically (from 5000 tonnes to 200 tonnes) (Bolat 2001). The main cause of the decrease in crayfish production was the outbreak and spread of crayfish plague, caused by the funguslike organism, *Aphanomyces astaci*, excessive crayfish harvesting, pollution of water in lakes and overfishing (Bolat 2001; Svoboda et al. 2012, 2014).

Crayfish culture can vary in intensity, from extensive to intensive rearing systems. However, intensive (completely closed) crayfish culture is not practically used in Europe. As a matter of fact, this culture has not proved economically profitable due to high mortality caused by cannibalism amongst cultured crayfish and to high production costs (Kozák et al. 2015). Several environmental factors, such as water temperature, water quality, nutrition, light intensity, photoperiod and density, affect growth and survival of juveniles in intensive crayfish culture (Köksal 1985, 1988; Köksal et al. 1992; Sáez-Royuela et al. 2007; Erol et al. 2010). Among these factors, nutrition is one of the most important factors directly affecting the growth of crayfish (Taugbol and Skurdal 1992; Austin et al. 1997; Savolainen et al. 2004; Mazlum 2007; Sáez-Royuela et al. 2007). Several variables (water quality, the quality and amount of food, food supply frequency, its availability to the crayfish) have played significant roles in crayfish culture since the crayfish are totally dependent on supplemental food in intensive culture systems.

Several studies have been carried out under controlled conditions to evaluate the effect of feed on growth and survival of crayfish juveniles and a wide variety of foods have been tested in juveniles from the onset of exogenous feeding. *Artemia*, *Chlorella*, chironomid larvae, *Daphnia*, earthworms, fresh fish meat, aquatic plants and vegetables have been tried by Köksal (1985), Celada et al. (1989, 1993), Taugbol and Skurdal (1992), Köksal et al. (1992), Blake et al. (1994), Oliveira and Fabiao (1998), Verhoef et al. (1998), Verhoef and Austin (1999), Zaikov et al. (2000), Sáez-Royuela et al. (2001, 2007), Savolainen et al. (2003), González et al. (2008). Besides the exogenous feeding, commercial feeds for crustacean and fish species (Taugbol and Skurdal, 1992; Celada et al. 1993; Sáez-Royuela et al. 1995, 2001; Mazlum 2007) and experimental dry pellets for crayfish and crustaceans (Celada et al. 1989; Savolainen et al. 2004; Ulikowski et al. 2006), have been used in feeding of crayfish juveniles. The results in these studies carried under controlled conditions indicated that the survival and growth of crayfish juveniles have shown a great variability. In addition, it was also stated that the high mortalities were observed in the first period of the independent life of juveniles (Sáez-Royuela et al. 1995; Ulikowski et al. 2006).

In addition to feeding, stocking density is another important factor affecting crayfish growth and survival (Naranjo-Paramo et al. 2004; Ulikowski et al. 2006; Mazlum 2007). Studies under controlled conditions indicated that crayfish growth and survival decreased with increasing stocking density (Naranjo-Paramo et al. 2004; Savolainen et al. 2004; Ulikowski and Krzywosz 2004; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009; González et al. 2010). In addition, effects of aggressiveness and cannibalism rise with increasing stocking density, resulting in reductions in survival and growth rates, as well as lack of chelae (Savolainen et al. 2004; Mazlum 2007; González et al. 2010).

Several studies have evaluated the effects of different stocking densities on growth and survival of *A. leptodactylus* (Köksal 1985; Ulikowski and Krzywosz 2004; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009). However, little is known about the combined effect of feeding and density on the growth and survival of *A. leptodactylus* juveniles. The aim of this study was to evaluate the impact of feed type and stocking density on growth and survival of *A. leptodactylus* juveniles.

Materials and Methods

Experimental conditions

This study was carried out in the hatchery of Eğirdir Fisheries Research Institute in 2008. The material for the study consisted narrow-clawed crayfish, *A.leptodactylus* juveniles.

The juveniles were reared from ovigerous females of *A. leptodactylus* kept under the laboratory conditions. Ovigerous females were obtained from Lake Eğirdir in April and May 2008 and were placed in breeding tanks in order to obtain the juvenile crayfish. The eggs hatched in the second week of June. Stage 2 juvenile crayfish were fed with *Artemia* nauplii and *Chlorella* sp. during one week period. The juveniles with mean weight (35±0.005 mg), total length (11.67±0.47 mm) and carapace length (5.87±0.35 mm) were used in the experiment lasting for 90-days period.

Experimental design

The experimental design consisted of two different feed types (live *Daphnia magna* and commercial trout feed) and two different stocking densities (650 juveniles/m^2 and 1300 juveniles/m^2) in triplicates. The stage 2 juveniles were randomly stocked into 12 rearing tanks with a bottom area 1.5 m^2 (975 and 1950 juveniles in each tank at lower and higher stocking density, respectively).

Experimental groups: (D650) crayfish fed with *D. magna* and stocked 650 juveniles/m^2; (D1300) crayfish fed with *D. magna* and stocked 1300 juveniles/m^2; (T650) crayfish fed with trout feed and stocked 650 juveniles/m^2; (T1300) crayfish fed with trout feed and stocked 1300 juveniles/m^2.

The experiment was conducted for 90 days. The measurements of carapax, total length, and weight of the juveniles were performed both at beginning (100 individuals) and at the end of the experiment (all individuals). The number of surviving juveniles in each tank was counted at the end of experiment. The specific growth rate (*SGR*, %/day) and survival rate were calculated as follows:

$$SGR(\%/day) = \frac{100(lnW_t - lnW_0)}{t}$$

W_t and W_0 are the final and initial mean weights of juveniles, respectively, and t is the time in days (t=90 day).

$$Survival\ rate(\%) = \frac{final\ number\ of\ crayfish}{initial\ number\ of\ crayfish} x100$$

Experimental tanks and water quality management

Twelve circular fiberglass tanks with 1.5 m^2 bottom area in flow-through system were used in the experiment. Severel bricks (29x19x13.5cm) with 42 holes were placed in each tank as shelter. Each tank had its own water inlet and outlet. The quality parameters of the incoming water were: pH 7.57, calcium 54.04 mg/L, magnesium 58.66 mg/L, total hardness 338.65 mg/L, O_2 4.99 mg/L. One third of the water in each tank was exchanged every day and aeration was continuously supplied with an airstone in each tank. During the experimental period, water temperature, dissolved oxygen concentration and pH were measured once daily in each tank with a WTW 340i. The temperature ranged from 17 to 19 ^oC; dissolved oxygen from 6 to 7 mg/L and pH from 7.5-8.

Feed materials and feeding

Two different feed types as *D. magna* and commercial trout feed (800-1000µ) were used in the experiment. Commercial trout feed was obtained from a feed manufacturing company. *D. magna* were cultured in outdoor ponds. The proximate composition of commercial trout feed and *D. magna* is shown in Table 1. Moisture contents were detected with an automatic moisture analyzer (AND MX-50). The crude protein contents according to Kjeldahl method (Nx6,25) (AOAC 2000a), crude lipid contents by Bligh and Dyer (1959)'s method and crude ash contents according to (Lovell 1981) were done. Crude fibre content was determined according to Standard Association of Official Analytical Chemists (AOAC) methods (AOAC 2000b).

During the experiment, crayfish juveniles were fed daily *ad libitum* with the experimental diets and waste of feed and feces were removed from the tanks by siphoning.

Table 1. Proximate composition (%) of *Daphnia magna* and commercial trout feed used in the experiment

Parameter	*Daphnia manga*	Trout feed
Crude protein (%)	42.05	55
Crude lipid (%)	16.2	13
Crude cellulose (%)	13.5	1.5
Crude ash (%)	14.7	12

Data collection and statistical analysis

The weight and length of crayfish were measured at the beginning and end of the experiment. Total body length and carapace length were measured with digmatic calliper (to the nearest 0.01 mm) and weight measurement were performed by using digital scale with 0.01 g sensitivity. Significant differences among treatment groups were tested by one-way ANOVA and Duncan's multiple range tests at 5% level of significance by applying SPSS (version 11) software.

Results

At the end of 90-days experiment, it was determined that the mean survival rates, final weights, total lengths, carapace lengths and specific growth rates of *A. leptodactylus* juveniles ranged 10.84-21.99 %, 367-653 mg, 23.71-28.68 mm, 12.24-14.70 mm, 2.61-3.22 %, respectively (Table 2). It was seen that the difference between the values of average length and weight obtained from all groups was statistically significant ($P<0.05$). The juveniles fed with trout feed showed a better growth compared to the juveniles fed live feed. The best growth performance was seen on juveniles with low density and with fed trout feed. Both final weights and total lengths in juveniles were negatively affected by increased stocking density.

The highest values (3.22 % and 3.07 %) in specific growth rate were obtained in the groups fed with trout pellet, whereas the lowest values (2.64 % and 2.61 %) were obtained in the groups fed with live feed. It was shown that *SGR* was statistically

different between trout feed and live feed groups, but stocking density formed in the relevant groups did not exhibited significant differences on *SGR*.

Survival rates varied from 10.84 % to 21.99 %. Survival of the juveniles decreased with increased stocking density (Table 2). The best survival rate as 21.99 %, was achieved in the group fed with live feed and kept under the low stocking density, whereas the lowest survival rate values as 10.84 % was obtained in the group fed with trout pellet and kept under the high stocking density. The survival rates of reduced with increasing stocking density. The results indicated that stocking density had a significant impact on the survival rate of crayfish juveniles.

Table 2. Final survival and growth indices of juvenile crayfish in the end of experiment ($\bar{X}$ ±SD).

Parameters	Experimental groups			
	D650	D1300	T650	T1300
Total length (mm)	24.04±3.23^{c}	23.71±3.13^{d}	28.68±4.55^{a}	27.71±4.46^{b}
Carapax length (mm)	12.39±1.72^{c}	12.24±1.80^{d}	14.70±2.57^{a}	14.09±2.38^{b}
Weight (mg)	367±1.181^{c}	368±0.168^{d}	653±0.321^{a}	570±0.341^{b}
SGR (%/day)	2.64±0.005^{b}	2.61±0.16^{b}	3.22±0.13^{a}	3.07±0.006^{a}
Survival rate (%)	21.99±2.32^{a}	14.25±1.38^{b}	20.67±3.13^{a}	10.84±2.53^{b}

Mean values in rows with different superscripts are significantly different (P<0.05).

Discussion

Several researchers stated that the juvenile astacids had the poor survival or growth rates when they were fed dry diets as the only food from the onset of exogenous feeding (Taugbol and Skurdal 1992; Ulikowski et al. 2006; Sáez-Royuela et al. 2007; González et al. 2009). For instance, a mortality of 83-90 % in noble crayfish juveniles were recorded with feeding a dry pellets (Taugbol and Skurdal, 1992). They observed that when juveniles were fed with fish and potatoes in addition to dry pellets, mortality was reduced to about 70 %. Similarly, Sáez-Royuela et al. (2007) recorded a mortality of 88.7 % after 100 days when juveniles did not receive live feed. According to the results of these studies, the researchers advised to use the live feed as supplement from the onset of external feeding to guarantee the viability of juvenile crayfish. González et al. (2008), supplementing a dry diet with live *Artemia* nauplii in excess, obtained good results (1283 mg weight, 4459 % weight gain and 3.82 % *SGR*) for juvenile *Pacifastacus leniusculus*. González et al. (2011) indicated that the juvenile crayfish (*P. leniusculus*) receiving decapsulted *Artemia* cysts up to day 50 as supplement to a dry diet showed a faster growth (averaging 13.8 mm carapace length, 610 mg weight 3.05 % day^{-1} *SGR*) at the end of the experiment. In contrast to these results, the commercial trout feed used in the present study resulted in a better growth performance (28.68 mm total length and 653 mg alive weight) than live feed. However, similar survival rates (20.67-21 %) were obtained with both live feed and commercial trout feed. These results suggest that good growth can be achieved by using manufactured diets. This diet or one of similar composition may also constitute a good reference for future dietary studies. Similarly, Verhoef et al. (1998) using a variety of natural and an artificial diet, found similar results for juvenile yabbies *Cherax destructor*. The researchers indicated that the commercial diet used in their study produced growth rates similar to those obtained by feeding live or frozen zooplankton.

Köksal (1985), obtained relatively good survival and growth rates (44.23 % and 430.64-476.16 mg respectively) after 90 days in *A. leptodactylus* juveniles fed trout pellet and flamentous green algae. In our study performed during the same period, the growth of crayfish was better than that reported by Köksal (1985), but the survival rates of juveniles were lower. This may be due to the higher stocking density. In 1985, the same researcher, using different food type in feeding of stage 4 juveniles of *A. leptodactylus* (mean total length 21.1 mm) at the same density, obtained total lengths of 29.2-37.4 mm, weights of 623-1216 mg and survival rates of 50.6-72.4 % after 60 days. In the present experiment, stage 2 juveniles (mean size 11.67 mm) reached 23.71-28.68 mm length and 367-653 mg weight after 90 days. However the survival rates of juveniles were lower the levels reported by Köksal (1985). It is noteworthy that initial sizes used for this study (stage 4 juveniles) were higher than size in our experiment (stage 2 juveniles). The lower survival rates may be due to the initial size of the juveniles used in the experiment. The second-stage that the juveniles become independent from the mother is critical period for crayfish and the highest mortality during the rearing of crayfish occurs in this period but fell considerably during ensuing periods (Ulikowski et al. 2006).

In the present study, crayfish juveniles fed trout feed showed a higher specific growth rate compared to juveniles fed live feed. Sáez-Royuela et al. (2007) indicated that *P. leniusculus* juveniles fed a dry diet (for salmonids) supplemented with live feed (*Artemia* nauplii or *Daphnia*) had a higher *SGR* value (2.83) those fed a dry diet as the sole food (2.24). Ulikowski et al. (2006) used experimental pellets in the feeding of juvenile crayfish and obtained *SGR* ranging from 1.3 (for the third month) to 6.4 % (for the first month) for *A. leptodactylus*. In a similar study of the same species by Harlıoğlu (2009), *SGR* values were found to be 2.41-2.48 % for *A. leptodactylus* when crayfish were provided with natural food. Zaikov et al. (2000), used four different diets for *A. leptodactylus* during up to 1 month of age under 4 diet variants: zooplankton, feed mixture, meat and soyabean meal, and reported the best results of *SGR* in *A. leptodactylus* fed with zooplankton diet (3.56 %) and fed mixture diet (3.15 %). González et al. (2008), supplementing a dry diet with live *Artemia* nauplii in excess, obtained 3.82 % *SGR* for juvenile *P. leniusculus*.

It has been reported that increased stocking density has a certainly negative impact on crayfish growth and survival in previous studies (Ulikowski and Krzywosz 2004; Savolainen et al. 2004; Ulikowski et al. 2006; Mazlum 2007; González et al. 2010). Savolainen et. al. (2004) indicated that final mean weight and mean length in *P. leniusculus* were decreased with increasing stocking densities. The researchers also predicted a reduction of crayfish survival 11 % when stocking density was doubled. The decrease rates of survival when stocking density was doubled in our study for *A. leptodactylus* juveniles were much higher (35.2 % for the live feed groups and 47.5 % for the trout feed groups). The reason of this might be that we had a higher initial stocking densities. In *P. leniusculus*, González et al. (2010) reported 86.33 % and 39.3 % survival in the lowest and highest stocking densities (100 and 1000/m^2) respectively, after 100 days. Ulikowski et al. (2006) reported the high mortality rate of 48-77 % in *P. leniusculus* and *A. leptodactylus* at different stocking densities (600, 1200 juveniles/m^2). Harlıoğlu (2009) reported survival and *SGR* for the same species from 34,3 % to 56 % and 1.10 to 1.40 respectively at different stocking densities, ranging from 234 juveniles/m^2 to 937 juveniles/m^2. Köksal (1985) reported survival rates of 22.5-58 % and at different stocking densities (130-260 juveniles/m^2) in *A. leptodactylus*. The length of weight of juveniles ranged from 20.13 to 20.9 mm and from 201.33-210.5 mg after 45 days, respectively. Ulikowski and Krzywosz (2004) obtained good results in *A. leptodactylus* after four-week rearing period. The survival was 70 % (initial density: 300 juveniles/m^2), 58% (initial density: 600 juveniles/m^2) and 47.8% (initial density: 1200 juveniles/m^2). Harlıoğlu (2009) obtained 57.3-62.6%, 51.3-56.6%, and 42-44.3% survival for *A. leptodactylus* at stocking densities of 234, 468, and 937 juveniles/m^2, respectively. Mazlum (2007) reported good growth rate and survival for newly hatched third instars *A. leptodactylus* at stocking densities of 50, 100 and 200/m^2. In the present study, growth and survival of crayfish juveniles were affected by stocking density; they decreased as initial stocking density increased. This is in accordance with results on *P. leniusculus* (Savolainen et al. 2004; González et al. 2010), *A. leptodactylus* (Ulikowski and Krzywosz 2004; Mazlum 2007). Harlıoğlu (2009) stated that crayfish exhibit density dependent growth even in situations where sufficient food resources are available. The results obtained in our study also support this view. The values of growth obtained in our study for *A. leptodactylus* were similar to those achieved in other studies with similar or even much lower stocking densities; *P. leniusculus* in densities 100-800 individuals/m^2 (Savolainen et al. 2004), *A. leptodactylus* and *P. leniusculus* in densities 600-1200 individuals/m^2 (Ulikowski et al. 2006), for *P. leniusculus* in densities 100-1000 individuals/m^2 (González et al. 2010). Regarding the survival, the values obtained in our experiment was much lower than the levels of the same species reported by other authors (Köksal 1985; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009). The poor survival rates may be related to the initial stocking density and initial weight and length of the juveniles. In addition, this is due to the food, experimental conditions, and the number of the available shelters.

In conclusion, the crayfish juveniles fed with trout feed exhibited better growth performance than those fed with live feed. This suggest that it might be better to develop an artificial diet for use in the intensive culture of juvenile astacid juvenile crayfish. In addition, the survival of juvenile crayfish were negatively affected by increased stocking density. The best survival rate was obtained at 650 juveniles/m^2 stocking density. This study has clearly demonstrated that stocking density had a significant effect on survival rates of crayfish juveniles.

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Growth Performance and Substrate Preference of Juvenile Mexican Dwarf Orange Crayfish (*Cambarellus patzcuarensis*) in Different Substrate Types

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ABSTRACT

Substrate is very important factor on growth and survival for crayfish in their entire life. Survival rate and growth of juveniles of the Mexican dwarf orange crayfish (*Cambarellus patzcuarensis*) were evaluated in aquaria with six different substrates covering their bottom. The six different substrates were plastic mesh, pebble, sand, basalt and calcite and bare glass without a cover as a control group. The study was carried out in two different experimental areas. In the first experiment, ten (mean body weight 0.25±0.01 g) juveniles were stocked in aquaria with above mentioned six substrates and each substrate in three replicates. After 100 days, the best weight gain was observed in the group raised on basalt substrate ($P<0.05$). In the second experiment, juveniles were individually placed in a hexagonal glass aquarium divided into six sections each containing one of the same substrates used in the first experiment was used for substrate preference. Each individual was filmed for 24 h, and the time spent in each compartment was registered. Basalt was the most preferred substrate for Mexican dwarf orange crayfish ($P<0.05$). The preference of basalt substrate can be explained by its proximity to the natural substrate of this species.

Keywords: Cambaridae, habitat variation, growth, survival, pet trade.

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Farklı Substrat Tiplerinde Genç Meksika Cüce Portakal Kerevitlerinin (*Cambarellus patzcuarensis*) Büyüme Performansı ve Substrat Seçimi

Öz: Substrat, kerevitlerin yaşamlarının tüm evrelerinde büyüme ve yaşama oranı açısından oldukça önemli bir faktördür. Jüvenil Meksika cüce portakal kerevitlerinin (*Cambarellus patzcuarensis*) yaşama oranları ve büyümeleri, zemininde altı farklı substrat bulunan akvaryumlarda incelenmiştir. Substratlar sinek teli, çakıl, kum, basalt, kalsit ve kontrol grubu olarak ta boş cam şeklinde belirlenmiştir. Çalışma iki farklı deneme ortamında yürütülmüştür. İlk denemede yukarıda bahsedilen altı substrat bulunan akvaryumlara üç tekrarlı olmak üzere, her birine onar adet birey (ortalama ağırlık 0,25±0,01 g) stoklanmıştır. 100 günün sonunda en iyi ağırlık artışı, bazalt üzerinde beslenen grupta gözlenmiştir ($P<0,05$). İkinci denemede, substrat seçimi için jüveniller, tabanı altıya bölünmüş ve her bir bölümde ilk denemeyle aynı substratlar bulunan altıgen şeklindeki bir akvaryuma bireysel olarak stoklanmıştır. Kerevitlerin her biri 24 saat izlenmiş ve her bölümde geçirilen süreler kaydedilmiştir. Bazalt, Meksika cüce portakal kerevitleri için en çok tercih edilen substrat olarak bulunmuştur ($P<0,05$). Bu substrat türünün sıklıkla tercih edilmesi, türün doğal substratına yakınlığı ile açıklanabilir.

Anahtar kelimeler: Cambaridae, habitat değişimi, büyüme, yaşama oranı, evcil hayvan ticareti.

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Introduction

Nowadays, aquaristic is a popular hobby of millions supporters worldwide. In the United States, 95.5 million freshwater fish and 9.5 million marine fish are reported to be handled in aquariums in 2016 (APPA 2016). It is also indicated that there are 36 million aquarium fish as a result of surveys conducted with 4,000 people in England (PFMA 2016).

A wide range of freshwater invertebrates are also kept in captivity (Chucholl 2013; Patoka et al. 2014). Alternative species are being introduced to aquarium sector speedily, including freshwater decapods such as shrimps, crayfish and crabs (Faulkes 2015;

Patoka et al. 2015). The interest in crayfish is increasing even though shrimps have seen more popular among these living groups (Türkmen and Karadal 2012a). Aquarists interest in crayfish species for many causes such as their interesting behaviours, individual personalities, attractive colours and patterns. Therefore, mostly exotic crayfish species are favoured for home aquariums, including *Cherax quadricarinatus, C. destructor, C. cainii, C. peknyi, C. gherardiae, C. holthuisi, Cambarellus patzcuarensis, Procambarus clarkii, P. alleni* (Kawai et al. 2015) and many others, also they are becoming more commonly seen for sale in aquarium stores and websites (Türkmen and Karadal 2015).

The global achievement of the freshwater crayfish farming in recent years is based on the selection of species with sufficient characteristics for both cultural and commercial purposes (Viau and Rodríguez 2010). Healthy and peaceful crayfish species are preferred for ornamental purposes by skilled aquarists. Species found in the aquarium trade include many crayfish species from Parastacidae and Cambaridae family, including Mexican dwarf orange crayfish, *C. patzcuarensis* (Kwang et al. 2010). *C. patzcuarensis* comes from Lake Pátzcuaro, a volcanic crater lake in Michoacán which is located in southwest of the major city of Morelia, Mexico. Wild individuals of this crayfish are mostly tan, brown, and rust colour (Pottern 2007). However, the orange colour morph of the dwarf species originated in the Netherlands (Dost 2013). Mexican dwarf orange crayfish is suitable for smaller sized aquaria (attaining a maximum length of 4 cm). Unlike most crayfish, this species does not destroy plants because of their peaceful nature (Rohmann 2010). *C. patzcuarensis* is not only sold in Turkey, but is an extremely common pet in other countries, including the United States (Faulkes 2015), the United Kingdom (Peay et al. 2010), Germany (Chucholl 2013), Greece (Papavlasopoulou et al. 2014), the Czech Republic (Patoka et al. 2014), and Slovakia (Lipták and Vitázková 2015). Further, this species is listed on the IUCN red list of endangered species (Alvarez et al. 2010). Like many crayfish, this species exhibits mortalities during juvenile stages because of some reasons, including vulnerability in the moulting process and cannibalism. However, crayfish need substrate or shelters for providing themselves from external dangers.

Substrate type is important to stream communities (Francis and Kane 1995). Many decapod groups such as shrimps, crabs and crayfish are existed in the bottom of both lotic and lentic areas in inland waters (Zimmermann et al. 2016). These macroinvertebrates have some unique features such as tail flipping, punting and digging (Faulkes 2006). Bottom substrate of aquaria has been mixed by digging behaviours of crayfish and this causes the cycling of detritus. For this feature, crayfish are naturally considered keystone organisms to the ecological habitats (Kusabs et al. 2015). Although freshwater decapods are important organisms for inland streams, limited information is known about their substrate requirements in artificial environments.

Although Mexican dwarf orange crayfish is an important species for aquarium trade, there are no studies on their culture, growth and survival or substrate requirements. In the present study, effects of six different substrates on growth and survival of juvenile Mexican dwarf orange crayfish were investigated and their substrate preference was observed. The aim of these experiments was to test whether natural substrate of Mexican dwarf orange crayfish is important for its culture.

Materials and Methods

Rearing system and crayfish

The experiment was carried out in eighteen glass aquaria (1000 cm^2 of bottom surface) at a density of 10 crayfish per tank with three replicates for each substrate type. Each aquarium contained 2-cm-diameter PVC pipes in excess as refuge. Temperature was maintained at 24.5±0.7 ºC, and photoperiod was held at 14:10 (light/dark). The water in all aquaria was changed twice a week. Dissolved oxygen (WTW-Oxi 315), pH (Sartorius PT-10), ammonia (HANNA C205), total hardness (measured with Aquamerck® kit, 114652 total hardness test) and alkalinity (measured with Aquamerck® kit, 111109 alkalinity test) were determined in each aquarium at the beginning and at the end of the experiment before changing water and feeding the animals. Juveniles (0.25±0.01 g of body weight) of Mexican dwarf orange crayfish, *C. patzcuarensis* were used in this study. Crayfish were provided from a commercial facility in Antalya, Turkey, and transported to the Faculty of Fisheries, Ege University, Turkey. Prior to the start of the feeding trial, crayfish were transferred to non-substrated aquariums for 1 week for acclimation. Every day, all animals were fed near to satiation with commercial feed (Ecobio® 1.5 mm granules, 44% of protein) during the experiment.

Experimental design and substrate types

Six different substrates were tested in this study. Bare glass is non-substrated control group. Plastic mesh is similar to mosquito netting adhered to the bottom of the aquarium. Particle sizes of other substrates for pebble is 3-5 mm, sand is 800-1000 μm, calcite is 800-1000 μm with white colour and basalt is 300-500 μm with greyish black colour.

Particulates substrates (pebble, sand, basalt and calcite) covered the bottom of aquaria in the layer of 0.5 cm.

Evaluation of growth performance

Ten juveniles were placed in each aquarium, similar to densities used previous research carried out by Viau and Rodríguez (2010) with early juveniles of Australian red claw crayfish, *C. quadricarinatus*. Total length and weight measurements were carried out biweekly during 100-days experiment. All animals were dried with a paper towel to remove excess water before measurement process. Crayfish weights were individually measured with an electronic balance (Sartorius BL610, precision of ±0.01 g) and total lengths were measured with vernier caliper.

Substrate preference

In the substrate preference experiment, a hexagonal glass aquarium (60 cm of length of diagonal filled with 7 L of dechlorinated water) divided on the bottom into six equal sections each containing a different substrate. The substrates tested were the same substrates used in the growth performance experiment. Ten individuals of 0.25 g from the first experiment were tested and each individual was recorded for 24 h and the time spent in each compartment was assessed from recording. In the beginning of the video recording, each crayfish was placed in the center of the hexagonal aquarium. The movement of each juvenile in the experimental arena was recorded with a web camera (Piranha Tarantula) located directly above the center of the aquarium and connected to a PC (Figure 1). The experimental arena was placed in a closed room maintained at an ambient temperature of 24.4±0.6 ºC and a 14:10 (light/dark) photoperiod. During the light phase, the light source was a white lamp located above the aquarium and connected to a timer. During the dark phase, the light source was infrared LEDs located around the webcam. Animals were not fed during the experiment. The time spent in each substrate during the 24 h recording was calculated for each animal.

Statistical analysis

All data sets were examined to verify normality, independence and homogeneity of variance before further analysis was undertaken. All data were subjected to one-way analysis of variance (ANOVA) when interaction between the factors is found differences, Duncan's multiple range test was used to rank groups using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) statistical software (Zar 2001). All data are presented as mean ± standard error of the mean calculated from all replicates. In all tests, a significance level of $P<0.05$ was used.

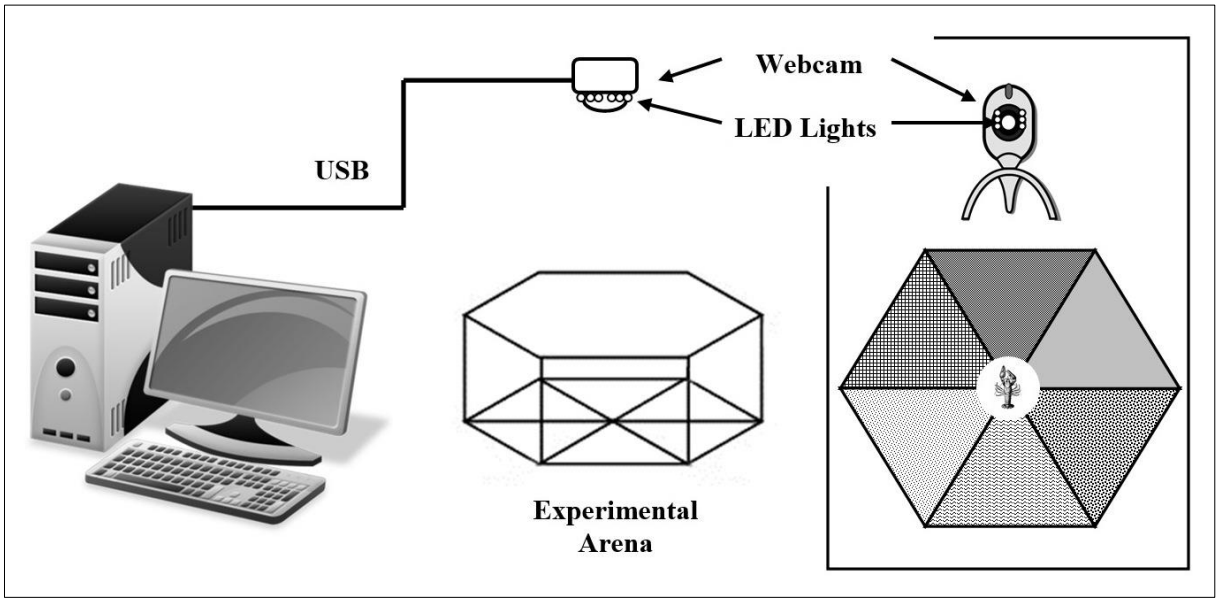

Figure 1. Scheme of the experimental area used for the substrate preference experiment

Results

The growth performance of Mexican dwarf orange crayfish kept in different substrates was given in Table 1. The final mean weight (*FMW*) of juveniles raised on the basalt substrate was significantly higher ($P<0.05$) than those all other substrates. Bare glass (control) was the lowest among the other substrates, except calcite ($P<0.05$). Pebble and sand groups were significantly higher than plastic mesh group which substrates causing intermediate effect for *FMW* ($P<0.05$). Differences among mean weights (final ranges for bare glass was

0.39±0.01 g and basalt was 0.53±0.01 g) emerged after 60 days (Figure 2). Enhancing of average total lengths (cm) of Mexican dwarf orange crayfish kept in different substrate types during 100-days was shown in Figure 3. The final mean total length (*FMTL*) of basalt and pebble groups were statistically greater than bare glass and calcite groups ($P<0.05$). There were no significant differences in carapace lengths. The survival rate was 100% in this study because of no animals died during the study period.

Table 1. Growth performance of Mexican dwarf orange crayfish after 100 days of substrate experiment (n=3)

Parameter	Bare glass	Fly mesh	Calcite	Sand	Pebble	Basalt
Initial mean weight (g)	0.25±0.01	0.25±0.01	0.24±0.01	0.25±0.01	0.25±0.01	0.25±0.01
Final mean weight (g)	0.39±0.01^{a}	0.43±0.01^{b}	0.41±0.01^{ab}	0.46±0.01^{c}	0.49±0.01^{c}	0.53±0.01^{d}
Initial mean total length (cm)	2.12±0.03	2.12±0.03	2.11±0.03	2.12±0.03	2.12±0.03	2.12±0.03
Final mean total length (cm)	2.45±0.02^{a}	2.50±0.03^{ab}	2.47±0.03^{a}	2.52±0.03^{ab}	2.55±0.02^{b}	2.58±0.03^{b}
Initial mean carapace length (cm)	0.73±0.01	0.73±0.01	0.72±0.01	0.73±0.01	0.73±0.01	0.73±0.01
Final mean carapace length (cm)	0.91±0.05	0.88±0.01	0.86±0.01	0.88±0.01	0.89±0.01	0.90±0.01

The water quality parameters did not change significantly ($P<0.05$) among treatments. The mean value of pH was 7.58±0.04, dissolved oxygen 5.82±0.21 ppm, ammonia values were below of 0.08 mg/L, alkalinity shows a range between 85 and 95 mg/L, hardness between 115 and 125 mg/L and nitrite values were in a range 0-0.02 mg/L. All juveniles significantly ($P<0.05$) preferred basalt among the five other substrates (Figure 4). Pebble and sand caused an intermediate effect. Glass, plastic mesh and calcite are the less preferred substrates ($P<0.05$)

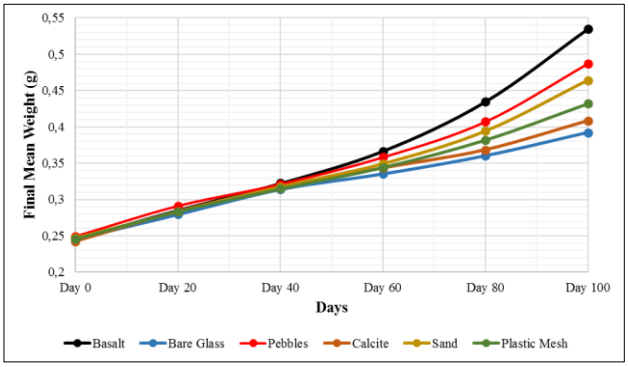

Figure 2. Gain of average body weight (g) of Mexican dwarf orange crayfish during 100 days of substrate experiment.

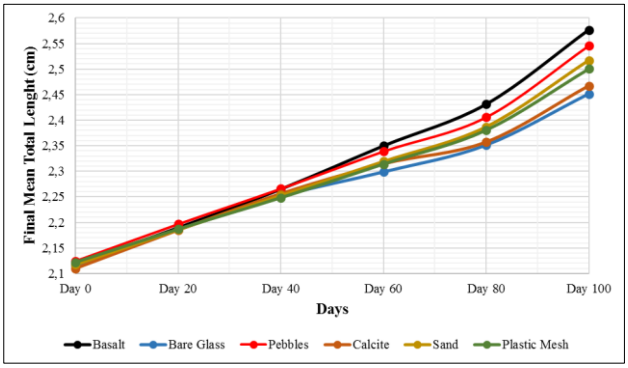

Figure 3. Gain of average total length (cm) of Mexican dwarf orange crayfish during 100 days of substrate experiment.

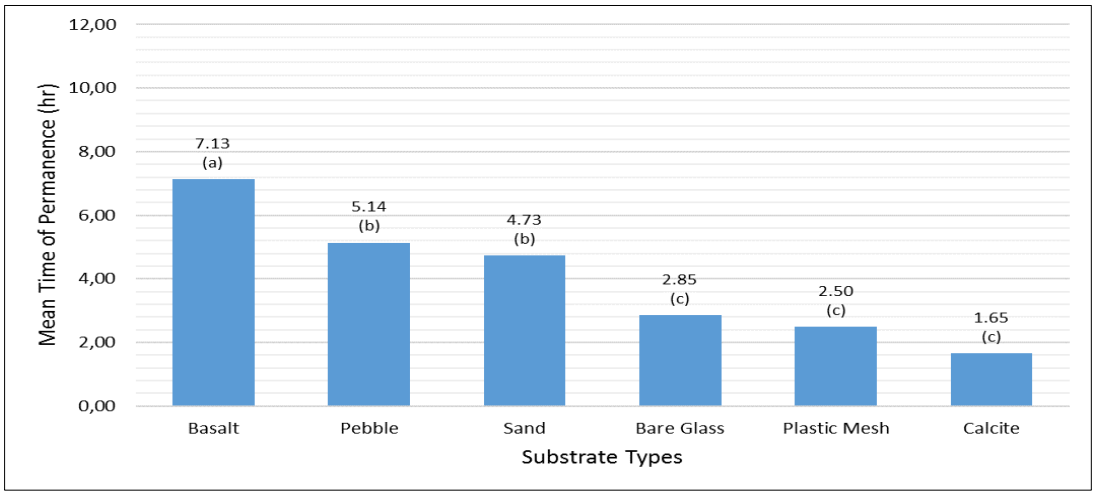

Figure 4. Spending time (h) in each substrate in substrate preference experiment (different letters indicate statistical differences at 5% among the treatment

Discussion

The results of the current growth experiment indicate that Mexican dwarf orange crayfish gained significantly higher weight when raised on basalt substrates. According to result of substrate preference experiment, crayfish spent more time on basalt substrate when given a choice of substrates. These results were pointed that the type of substrate is an important parameter for growth performance and selection of life habitats on this species.

Nocturnal macroinvertebrates like many crayfish can use the substrate as a shelter and hiding area during daytime (Barbaresi et al. 2007). Substrate presence in natural conditions of crayfish is very important factor for growth and other living features. Non-substrated artificial areas or poor sheltering may put the life of crayfish in danger. For example, unwanted situations such as decreasing growth performance and survival rates, harmful behaviours in vulnerable stages (juvenile stages or moulting) and increasing cannibalism can occur. Previous substrate selection studies carried out with different crayfish species such as signal crayfish, *Pacifastacus leniusculus* (Mason 1978; Savolainen et al. 2003), Australian red claw crayfish, *C. quadricarinatus* (Viau and Rodríguez 2010), red swamp crayfish, *P. clarkii* (Türkmen and Karadal 2012b) and blue tiger crayfish, *Cherax albertisii* (Karadal and Türkmen 2014) have clearly showed that crayfish on the bare glass (control) have the lowest growth and survival rate. These findings are supported by results of this present study.

Artificial substrates can be used to increase the growth performance of crayfish. Jones et al. (2002) showed that using synthetic elastic mat as a substrate on juvenile yabby, *C. destructor* was significantly improved some growth parameters, except survival. Nevertheless, synthetic substrates do not always provide advantages. Especially, feeding behaviours was often limited after these substrates were placed in the tanks. For instance, crayfish cannot reach the feed because of they flee under synthetic substrate covered whole bottom of the tanks or aquariums. So, if there is a natural substrate in the medium, the synthetic substrate may not be used. D'Abramo et al. (2006) observed the development of red swamp crayfish in pools with plastic net and natural substrates on the ground. They found that the daily average growth rate of the individuals in the pools with natural substrate was 48%, while plastic net group was 15%. A synthetic substrate (plastic fly mesh) was used in current study and it caused intermediate effect between natural substrates and bare glass on growth performance of Mexican dwarf orange crayfish as seen in Australian red claw crayfish studies by Viau and Rodríguez (2010).

According to Streissl and Hödl (2002), substrate selection affected by distribution and habitat variation. Previous studies have clearly stated that the effects of different substrates may vary species to species. Simon and Cooper (2014) pointed that large species prefer habitats with larger substrate particle size than small crayfish. Especially, juveniles and moulted individuals use substrate as refuge to defend themselves from predation (Jones and Ruscoe 2001; Molony and Bird 2005). Some natural substrate types including gravel, stones, sand and pebble were increased the growth rates of signal crayfish, Australian red claw crayfish, red swamp crayfish and blue tiger crayfish, respectively (Savolainen et al. 2003; Viau and Rodríguez 2010; Türkmen and Karadal 2012b; Karadal and Türkmen 2014). This could be explained by all four substrate types are found in the ecological environment of these crayfish species. Previous studies have stated that the importance of ensuring proper habitats for Australian red claw crayfish during the commercial culture to improve growth performance (Du Boulay et al. 1993; Karplus et al. 1995). Herrnkind and Butler (1986) declared that natural substrates have vital properties on growth performance of both freshwater and marine decapod species. Viau and Rodríguez (2010) noticed that it was feasible that a nutritional or behavioural response to a substrate similar to the one found in nature enhanced the growth of crayfish species. Our results indicated that Mexican dwarf orange crayfish kept in basalt attained higher body weights. Lava sediment overlie lacustrine clays oversaturated with in the Pátzcuaro region (Pola et al. 2014). In nature, this species is found in lava sandy areas (Dost 2013). So that the basalt is close to natural substrate of this species with its small particles and greyish black colour. Our findings for substrate preference of Mexican dwarf orange crayfish were supported by all these earlier researches carried out with various crayfish species.

The results of these experiments show that the natural substrate of Mexican dwarf orange crayfish is very important for their growth and survival. In conclusion, on the purpose of growing, preference and keeping of juvenile this dwarf species in aquarium systems, it is recommended to use of basalt as bottom substrate. However, further studies on the environmental requirements in aquarium systems of Mexican dwarf orange crayfish are needed to decrease aggressive interactions and cannibalism of this poorly known species.

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Isolation and Identification of *Streptococcus parauberis* From Freshwater Fish in Turkey

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ABSTRACT

Streptococcus parauberis is an alpha-hemolytic gram positive coccoid bacterium belonging to the *Streptococcaceae* family. This bacterium cause streptococcosis is a major disease in cultured fish due to the intensification of aquaculture and causes significant economic losses in fish farm industry. In our study, we isolated a total of 37 lactic acid bacteria from wild fish (*Sander lucioperca*, *Carassius gibelio*, *Cyprinus carpio*) of Lake Eğirdir and cultured fish (*Oncorhynchus mykiss*, Walbaum) of farm in Turkey. For the isolation of bacteria phenotypic and biochemical characteristics of the 37 isolates obtained from the colonies grown on MRS, TSA and M17 agar characterized by determining colony morphology, cell morphology, motility, gram staining and the production of cytochrome oxidase and catalase. Further biochemical characteristics were determined using conventional tests according to Bergey's Manual of Systematic Bacteriology. From the isolated lactic acid bacteria 25 of were identified as *S. parauberis*, 2 of as *Vagococcus* sp., 2 of as *Lactococcus garvieae* and 5 of as *Lactococcus lactis* by culture-based, biochemical test and 16Sr RNA gene sequencing techniques but 3 of them couldn't (F65, F49 and F50) identified. In this study, the main objective of us is to identify *S. parauberis* with conventional and 16Sr RNA gene sequencing techniques from wild fish in Lake Eğirdir and in cultured fish in fish farm of Egirdir-Turkey.

Keywords: 16Sr RNA, freshwater, molecular identification, fish disease, aquaculture.

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Streptococcus parauberis'in Türkiye'deki Tatlısu Balıklarından İzolasyon ve İdentifikasyonu

Öz: *Streptococcus parauberis* alfa hemolitik, gram pozitif kokoid bir bakteri olup *Streptococcaceae* familyasına aittir. Bu bakteri kültür balıklarında yoğun üretimden kaynaklı çok önemli bir hastalık olan streptokokozise neden olmaktadır ve balık çiftliği endüstrisinde önemli bir ekonomik kayba neden olmaktadır. Çalışmamızda Eğirdir gölü balıklarından (*Sander lucioperca*, *Carassius gibelio*, *Cyprinus carpio*) ve kültür balığı türü olan (*Oncorhynchus mykiss*, Walbaum)'dan 37 tane laktik asit bakterisi izole edilmiştir. Laktik asit bakterilerinin tanımlanması için MRS, TSA ve M17 agardan izole edilen 37 izolatın fenotipik ve morfolojik karakterleri koloni morfolojisi, hücre morfolojisi, hareketlilik, gram boyama, katalaz ve oksidaz üretimiyle yapılmıştır. Diğer biyokimyasal özellikler de Bergey's Manual of Systematic Bacteriology'e göre yapılmıştır. İzole edilen bakterilerinden 25 tanesi *S. parauberis*, 2 tanesi *Vagococcus* sp., 2 tanesi *Lactococcus garvieae* ve 5 tanesi de *Lactococcus lactis* olarak biyokimyasal yöntemler ve 16Sr RNA gen sekansıyla tanımlanmıştır fakat 3 tanesi (F65, F49 ve F50) tanımlanamamıştır. Çalışmamızda *S. parauberis*'in izolasyon ve identifikasyonu ilk olarak Türkiye'deki Eğirdir gölü balıklarından ve gökkuşağı alabalığından gerçekleştirildi.

Anahtar kelimeler: 16Sr RNA, Tatlısu, moleküler tanımlama, balık hastalığı, yetiştiricilik.

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Introduction

Aquaculture has become an economically important industry in the world which requires continued research with scientific and technical developments and innovation. So the purpose of global aquaculture is to maximize the efficiency of

production. The world aquaculture production in 2014 was approximately of 73.8 million tons, which represents around 41% of that obtained from extensive captures for human consumption. To meet the increased need of food in the World, there is an intensive production in fisheries and this makes inevitable the occurence of diseases (FAO 2016; Bondad-Reantaso et al. 2005; Kesarcodi-Watson et al. 2008; Subasinghe et al. 2009).

The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. It is important to point out that diseases classically considered as typical of freshwater aquaculture, such as furunculosis (*Aeromonas salmonicida*), bacterial kidney disease (BKD) (*Renibacterium salmoninarum*) and some types of streptococcosis are today important problems. Streptococcal infection is a disease affecting most of fish including wild and cultured fish (Toranzo et al. 2005).

The important fish pathogens which have importance are *Lactococcus garvieae (*synonym *Enterococcus seriolicida), Lactococccus piscium, S. iniae (*syn. *S. shiloi), S. agalactiae* (syn. *S. difficile*), *Streptococcus parauberis* and *Vagococcus salmonina*rum. Therefore, streptococcosis of fish should be regarded as a complex of similar diseases caused by different genera and species capable of inducing a central nervous damage characterised by suppurative exophthalmia and meningoencephalitis. While warm water streptococcosis (causing mortalities at temperatures above 15˚C) typically involves *L. garvieae, S. iniae, S. agalactiae* and *S. parauberis*, cold water streptococcosis (occurring at temperatures below 15 ˚C) is caused by *L. piscium* and *V. salmoninarum*. It is important to report that the etiological agents of warm water streptococcosis are considered also as potential zoonotic agents capable to cause disease in humans (Domenech et al. 1996).

Streptococcus is a genus of bacteria containing some species that cause serious diseases in a number of different hosts. This disease causes significant economic losses in the aquaculture industry in the United States of America, Japan, Israel, South Africa, Iran, Australia, Philippines, Taiwan, Bahrain, Turkey and other countries. Streptococcal disease in fish was first reported in 1957 (Hoshina et al. 1958; Baeck et al. 2006; Rahimi and Yadollah 2013).

S. parauberis seems to be endemic of cultured turbot (Toranzo et al. 1994). *S. parauberis* was first identified as a fish pathogen after an outbreak in 1993 in cultured turbot (*Scophthalmus maximus*) in Spain (Domenech et al. 1996). It was also responsible for streptococcosis in olive flounder (*Paralichthys olivaceus*) from a fish farm on Jeju Island, Korea during 2005 (Baeck et al. 2006). Prior to these reports, *S. parauberis* was known primarily as an etiologic agent of mastitis in dairy cows (Williams and Collins 1990).

To date, examples of Streptococcus species that have been associated with disease in fish include: *S. iniae, S. agalactiae, S. parauberis, S. dysgalactiae, S. faecium, S. milleri, S. uberis, S. ictaluri, S. phocae* and *S. faecalis* (Yang and Li 2009).

Unfortunately, conventional biochemical tests do not allow for the precise identification and classification of streptococcal isolates, because of differences in growth rates, inoculum levels, and incubation periods (Facklam and Elliott 1995). Consequently, the number and the nature of bacteria species associated with fish streptococcosis remains controversial (Romalde JL et al. 2008).

Molecular techniques to diagnose fish streptococcosis are powerful method have been applied. The techniques based on amplification of 16S rRNA (Zlotkin et al. 1998a,1998b; Nho et al. 2009; Lämmler 1998) seem to be of choice as a standard method for diagnosis of these gram positive cocci. In the case of *S. parauberis*, detection can be performed using the procedures that described for mammals by Lämmler et al. (1998) which combines PCR amplification and endonuclease restriction. Here we report *S. parauberis* from freshwater fish and Rainbow trout (*Oncorhychus mykiss*, Walbaum) which are important economically. Especially, rainbow trout faced with diseases due to the intensive production and culture condition. For hindered of these diseases the one of the natural solution is probiotic bacteria. During the bacterial detection for probiotic bacteria *S. parauberis* obtained in significant densities by biochemical methods. Molecular techniques to diagnose fish streptococcosis are powerful method have been applied. The techniques based on amplification of 16Sr RNA seem to be of choice as a standard method for diagnosis of these gram positive cocci. So we made the identification of these bacteria by 16Sr RNA gene sequence analysis. Because the fast and correct identification is very important in fish farm for hindered and transport of disease from one fish to another. In this study, the main objective of us is to identify *S. parauberis* with conventional and 16Sr RNA gene sequencing techniques in wild fish in Lake Eğirdir and in cultured fish in fish farm of Egirdir-Turkey.

Material and Methods

Isolation of bacteria

Gut and spleen samples obtained from healthy rainbow trout (*O. mykiss*) in fish farm during summer in Isparta province, and gut and spleen samples of carp (*Cyprinus carpio*), silver crucian carp

(*Carassius gibelio*) and sander (*Sander lucioperca*) living in Egirdir lake were diluted for the isolation of lactic acid bacteria. Isolation sources and incubation conditions of bacterial isolates were given in Table 1. The isolates were inoculated in the selective mediums. All the fish were treated in 1 liter of water containing 0.5 ml phenoxyethanol for 2 min and killed. After one gram of sample obtained from the fish gut was placed in 10 ml PBS (phosphate-buffered saline) and was diluted 10^{-7} times, 0.1 ml dilutions were seeded on TSA (Tryptic Soy Agar-Merck 1.05458), MRS (De Man, Ragosa and Sharpe Agar- Merck 1.10660) and M17 (Merck 1.15108) agar and incubated at 22 °C under aerobic and anaerobic conditions for 24-48 hours. Then those whose morphologies resembling lactic acid bacteria were selected from petri dishes and stocked in 15 % of TSA at – 80 °C (Perez-Sanchez et al. 2011).

Table 1. Isolation sources and incubation conditions of bacterial isolates

Strain No	Fish	Organ	Medium	Aerobic/Anaerobic	Temperature	Incubation time
51	*C. gibelio*	Gut	MRS	Aerobic/Anaerobic	22˚C	24-48h
34	*Trout*	Spleen	M17	Aerobic/Anaerobic	22˚C	24-48h
57	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
35	*O. mykiss*	Spleen	M17	Aerobic/Anaerobic	22˚C	24-48h
61	*C. carpio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
56	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
73	*C. gibelio*	Gut	MRS	Aerobic/Anaerobic	22˚C	24-48h
42	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
28	*C. carpio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
23	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
48	*C. gibelio*	Gut	MRS	Aerobic/Anaerobic	22˚C	24-48h
52	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
41	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
46	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
43	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
64	*S. lucioperca*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
26	*S. lucioperca*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
22	*C. gibelio*	Gut	TSA	Aerobic/Anaerobic	22˚C	24-48h
58	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
62	*C. carpio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
59	*C. gibelio*	Spleen	M17	Aerobic/Anaerobic	22˚C	24-48h
47	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
44	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
45	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
63	*C. carpio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
19	*C. carpio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
18	*C. carpio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
25	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
24	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
60	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
31	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
55	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
32	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h
29	*C. gibelio*	Gut	M17	Aerobic/Anaerobic	22˚C	24-48h

Biochemical and phenotypic characterization

Phenotypic and biochemical characteristics of the 37 isolates obtained from the colonies grown on MRS, TSA and M17 agar characterized by determining colony morphology, cell morphology, motility, gram staining (Figure 1) and the production of cytochrome oxidase and catalase. Further biochemical characteristics were determined using

conventional tests according to Bergey's Manual of Systematic Bacteriology (Schleifer 1986).

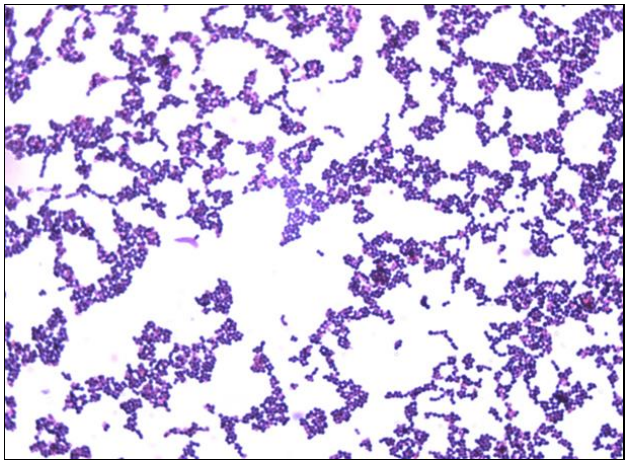

Figure 1. Gram staining of *S. parauberis* (F34) cultured on trypticase soy agar at 22˚C for 24h.

DNA isolation and molecular identification

DNA isolation was carried out with the rapid phylogenetic analysis (Liu 2000). For the molecular biological identification 16Sr RNA about 1.5 kb regions of the 37 isolates were amplified with 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers (216bp of 16S rRNA) using ARDRA PCR (Soto 2010). Representative amplification products of isolates were given in Figure 2.

Sequencing

The isolates were sequenced with the same primers used in PCR. The obtained sequence data were compared with the sequences in the GenBank database using the BLAST algorithm and then sent to NCBI (National Center for Biotechnology Information) to receive an access number.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *S. parauberis* isolates was performed with the disc diffusion method in accordance with guidelines for the Clinical and Laboratory Standards Institute (CLSI), and the following antibiotics (Oxoid) were tested with the disk diffusion method: doxcycline (30 µg), enoxacine (10 µg), erythromycin (15 µg), florfenicol (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), enrofloxacin (5 µg), oxytetracycline (30 µg), chloramphenicol (30 µg), vancomycin (30 µg), penicillin (10 µg) . Antimicrobial susceptibility test of isolates were given in Table 2.

Experimental infection

For experimental infection the method by Haines et al. (2013) used after a minor modification. Pathogenicity test of isolates was conducted using healthy rainbow trout (9-10 g). To determine if *S. parauberis* (F34 and F35) isolates from fish would produce infection in rainbow trout, a group of 20 fish (n=10/tank) was inoculated intraperitoneally with 1 x 10^5 bacteria by optical density in a final volume of 10 ul sterile PBS for each isolate. Animals were held in 2 (PVC π 150 cm=880 L) maintained at 16 °C, supplied with freshwater and aeration and monitored daily for clinical signs of streptococcosis for two weeks, including evidence of external hemorrhage, exophthalmia, lethargy, and loss of appetite. Two weeks after inoculation all fish were euthanized and the spleens were aseptically removed (Figure 3) macerated and suspended in liquid media for re-isolation as described above. After experimental infection agent of disease (*S. parauberis*) was obtained from kidney of fish that have signs of streptococcosis and from the results of bacterial examination made with API 20 strep test *S.parauberis* reisolated from diseased fish.

Figure 2. Rainbow trout (*O. mykiss*) showing clinical signs of streptococcosis a) Exopthalmus and haemorrhagic eyes, b) ascites, c) dark skin

Results

Upon the completion of the phenotypic tests, it was found that 25 of the 37 isolates belonged to the genus *Streptococcus*, 5 of them belonged to the genus *Lactococcus* and 2 of them belonged to the genus *Vagococcus*. After being amplified with universal primers definitive identification was made by sequence analysis. However, after the sequence analysis, while 34 of these 37 strains were identified, 3 were not (F65, F49 and F50). The identified 34 isolates with accession number obtained from genebank like as follows: F51 (Accession number KP137338), F34 (KP137328), F57 (KP137342), F35 (KP137329), F61 (KP137346), F56 (KP137341), F73 (KP137350), F42 (KP137331), F28 (KP137324), F23 (KP137320), F48 (KP137337), F52 (KP137339), F41 (KP137330), F46 (KP137335), F43 (KP137332), F64 (KP137349), F26 (KP137323), F22 (KP137319), F58 (KP137343), F62 (KP137347), F59 (KP137344), F47 (KP137336), F44 (KP137333), F45 (KP137334), F63 (KP137348) were identified as *S. parauberis*, F18 (KP137317), F19 (KP13731) as *L.*

garvieae, F24 (KP137321), F25 (KP137322) as *Vagococcus* sp. F60 (KP137345), F55 (KP137340), F29 (KP137325),F31 (KP137326),F32 (KP137327) as *L. lactis* subsp *lactis*.

Isolation sources and incubation conditions of bacterial isolates were given in Table 1. Bacteria isolated from fish were gram positive ovoid cells forming chains or single cells (Figure1) growth occured from 4˚C (except F34, F35 and F73) to 45˚C (except F23 and F48) with 0 to 6.5 % (except F24, F23, F48, F47, F22) NaCl. Antibiotic susceptibility test results are given in Table 2. Of the samples studied, except F18, F19, F26, F22 and F29 all isolates were sensitive to all antibiotics tested. Three isolates were multiple resistant against to 3 antibiotics which are F18 (en, o, c), F19 (en, o, c), F26 (do, sxt, c) respectively. The highest antibiotic resistance was to enoxacin, oxytetracycline and chloramphenicol. All the strains were sensitive to vancomycin and penicillin (except F29). In pathogenecity test, after inoculation with *S. parauberis* the 90-100 % of the fish species were died in two weeks.

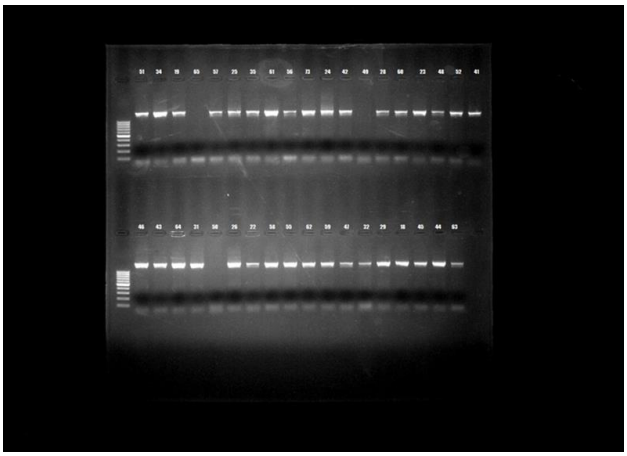

Figure 3. Representative amplification products of isolates (F51, F34, F57, F35, F61, F56, F73, F42, F28, F23, F48, F52, F41, F46, F43, F64, F26, F22, F58, F62, F59, F47, F44, F45, F63 are *S. parauberis*; F19 and F18 *L. garvieae*; F25 and F24 *Vagococcus* sp.; F60, F31, F55, F32, F29 are *L. lactis* subsp.*lactis*)

Table 2. Antimicrobial susceptibility test of isolates

Bacteria	do	en	e	flr	sxt	o	enr	c	v	p
F18	S	R	S	S	S	R	S	R	S	S
F19	S	R	S	S	S	R	S	R	S	S
F24	S	S	R	S	S	S	S	S	S	S
F25	S	S	S	S	S	S	S	S	S	S
F22	S	S	S	S	R	S	S	S	S	S
F26	R	S	S	S	R	I	I	R	S	S
F34	S	S	S	S	S	S	S	S	S	S
F42	S	S	S	S	S	S	S	S	S	S
F43	S	S	S	S	S	S	S	S	S	S
F47	S	S	S	S	S	S	S	S	S	S
F48	S	S	S	S	S	S	S	S	S	S
F51	S	S	S	S	S	S	S	S	S	S
F52	S	S	S	S	S	S	S	S	S	S
F57	S	S	S	S	S	S	S	S	S	S
F58	S	S	S	S	S	S	S	S	S	S
F59	S	S	S	S	S	S	S	S	S	S
F60	S	S	S	S	S	S	S	S	S	S
F29	S	S	S	S	S	S	S	S	S	R
F31	S	S	S	S	S	S	S	S	S	S
F32	S	S	S	S	S	S	S	S	S	S
F55	S	S	S	S	S	S	S	S	S	S

(*do:* doxycycline, *en:* enoxacin, *e:* erithromycin, *f:* florfenicol, *sxt:* trimethoprim *o*: oxytetracycline, *enr*: enrofloxacin, *c*: chloramphenicol (*ı*:intermediate sensistive, *s*:sensitive, *r*: resistance)).

Discussion

Initially, main objective of us was detect and isolate lactic acid bacteria with potential probiotic properties. So we isolated bacteria from healthy fish. But from the molecular results also we isolated *S. parauberis* that is a pathogenic bacteria for fish especially in rainbow trout. Streptococcosis is associated with acute and chronic mortality in many aquaculture species (Nho et al. 2009). The considerable diversity of *streptococcus* bacteria associated with fish may explain the difficulties encountered when identification procedures are based only on phenotypic characteristics. The identification schemes for the causative agents, based on biochemical and antigenic features can barely differentiate these bacterial pathogens from other low virulent gram-positive cocci such as *L. lactis*. The study has been conducted using conventional

methods and miniaturized systems and have given variable results. Thus, final identification of the bacteria requires the support of genetic data.

The PCR method can be employed as a supplementary and complementary test for definitive identification of the bacteria cultured from suspected samples. Isolates identified through phenotypic methods did not support the sequence results obtained through 16Sr RNA sequencing. This result indicated that conventional method are not enough or useful in the identification of *S. parauberis* or the other lactic acid bacteria isolates (Domenech et al. 1996; Haines et al. 2013; Park et al. 2013). In some studies, 16Sr RNA sequencing technique was used for identification of *S. parauberis* and lactic acid bacteria (Haines et al. 2013; Pourgholam et al. 2013; Didinen et al. 2014). So we can say that 16Sr RNA technique is very powerful for discriminating of lactic acid bacteria and also *S. parauberis*. Also in our study we identified *Vagococcus* sp isolated by Didinen et al. (2011) from Turkey and *L. garvieae* isolated by Diler et al. (2002) and *L. lactis* subsp. *lactis*.

Antibiotic resistance and sensitivity in lactic acid bacteria vary depending on the strains and the source of isolation (Salminen 1998). Antibiotic susceptibility test results are given in Table 2. Of the samples studied, except F18, F19, F26, F22 and F29 all isolates were sensitive to all antibiotics tested. All *S. parauberis* isolates were sensitive to vancomycin and erythromycin. This result was similar with (Haines et al. 2013; Pitkälä et al. 2008). The highest antibiotic resistance was to enoxacin, oxytetracycline and chloramphenicol.

S. parauberis isolates were obtained from apparently healthy rainbow trout and experimentally injected trout managed to demonstrate symptoms of streptococcosis such as exophtalmus, hemorrhage, erratic swimming, dark skin. Haines et al (2013) report that they isolated *S. parauberis* from healthy stripped bass as we did, but when they infected fish with *S.parauberis* they failed to demonstrate the signs of streptococcosis. This could be explained as; the fish species we obtained the bacteria may not be susceptible to disease. Also we can think that, in the facility where we obtained pathogen bacteria from healthy fish species may have been an infection caused by *S. parauberis* previously and became a porter.

According to our knowledge this is the first report for isolation and molecular identification of *S.parauberis* from wild fish and fish farm in Turkey. To date, *S. parauberis* have been isolated from salmon, rainbow trout (Kitao et al. 1981; Eldar et al.1995) mullet, golden shiner, pinfish, eel, sea trout, tilapia sturgeon, red drum (*Sciaenops ocellatus*), yellowtail (*Seriola quinquerodiata*) (Kusuda et al. 1991), rabbit fish (*Siganus canaliculatus*) sea bass (*Dicentrarchus labrax*); Japanese flounder (*Paralichthys olivaceus*)], ayu (*Plecoglossus altivelis*), barramundi (*Lateus calcifer*) and striped bass (Rahimi and Yadollah 2013). Due to the *S. parauperis* cause major disease in fish species we must alarm the relevant bodies against any disesae outbreaks and we need to take precautions in fish farming Turkey. So this study can be helpful in the prevent of disease outbreaks and may help the researchers in further scientific works.

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Re-description of the *Pseudamnicola lindbergi* Boettger 1957 (Gastropoda: Hydrobiidae)

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ABSTRACT

In this study, conchological and anatomical characters of the topotypes of *Pseudamnicola lindbergi* Boettger 1957 living in Elbistan, Pınarbaşı spring have been studied. Detailed male anatomy of the species was examined for the first time in which only shell morphology was given before. Findings were compared with the Boettger's original description and other *Pseudamnicola* spp. of Turkey.

Keywords: *Pseudamnicola lindbergi*, re-description, anatomy, topotype

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***Pseudamnicola lindbergi* Boettger 1957'nin Yeniden Tanımlanması (Gastropoda: Hydrobiidae)**

Öz: Bu çalışmada, Elbistan, Pınarbaşı su kaynağında yaşayan *Pseudamnicola lindbergi* Boettger 1957'nin topotip örneklerinin konkolojik ve anatomik karakterleri çalışılmıştır. Daha önce sadece kabuk morfolojisi gösterilen türün erkek bireylerinin ayrıntılı anatomisi ilk defa incelenmiştir. Bulgular Boettger'in orjinal tanımlamasıyla ve Türkiye'nin diğer *Pseudamnicola* türleriyle karşılaştırılmıştır.

Anahtar kelimeler: *Pseudamnicola lindbergi*, yeniden tanımlama, anatomi, topotip

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Introduction

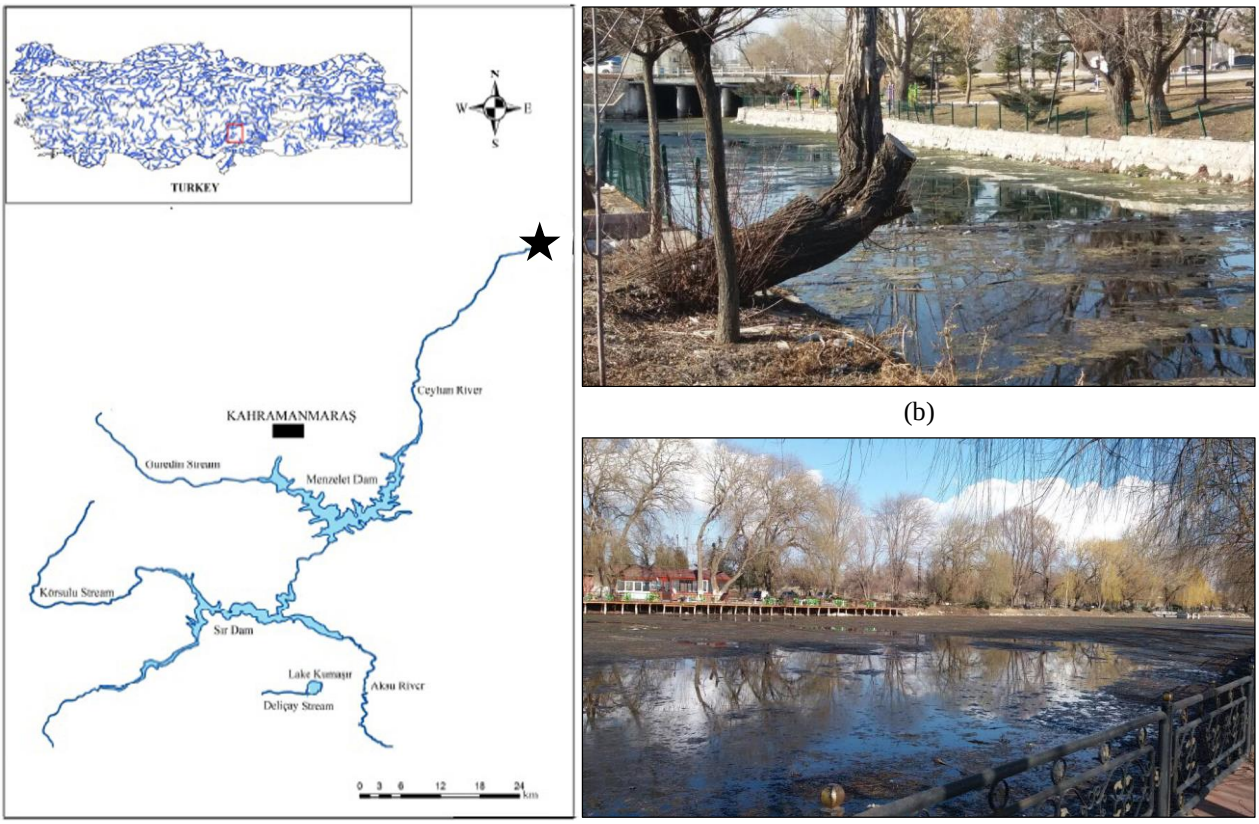
The genus *Pseudamnicola* is distributed in the West-Mediterranean, North-Africa, East-Mediterranean in Bulgaria, Greece, Turkey and Middle East (Glöer et al. 2010). In Turkey, thirteen species, two subspecies and one fossil species of *Pseudamnicola* have been reported so far. The represented species are; *Pseudamnicola macrostoma* Küster 1852, *Pseudamnicola kotschyi* Frauenfeld 1856, *Pseudamnicola elbursensis* Starmühlner and Edlauer 1957, *Pseudamnicola lindbergi* Boettger 1957, *Pseudamnicola geldiayana* Schütt and Bilgin 1970, *Pseudamnicola bilgini* Schütt and Şeşen 1993, *Pseudamnicola intranodosa* Schütt and Şeşen 1993, *Pseudamnicola vinarskii* Glöer and Georgiev 2012, *Pseudamnicola marashi* Glöer, Gürlek and Kara 2014, *Pseudamnicola merali* Glöer, Gürlek and Kara 2014, *Pseudamnicola goksunensis* Glöer, Gürlek and Kara 2014, *Pseudamnicola gullei* Glöer, Yıldırım and Kebapçı 2015, *Pseudamnicola kayseriensis* Glöer, Yıldırım and Kebapçı 2015, *Pseudamnicola natolica natolica* Küster 1852, *Pseudamnicola natolica smyrnensis* Schütt 1970 and *Pseudamnicola elongata* Taner 1973 (Geldiay and Bilgin 1969; Paydak 1976; Bilgin 1980; Yıldırım 1999; Glöer and Georgiev 2012; Glöer et al. 2014; Glöer et al. 2015). In addition, *Pseudamnicola elbursensis* Starmühlner and Edlauer 1957 and *Pseudamnicola kotschyi* Frauenfeld 1856 species were recorded from Southeastern Anatolia region by Paydak (1976: 31-35) but the existence of this Iranian species in Turkey is not certain. *Pseudamnicola macrostoma* Küster 1852 was

recorded from İzmir (Geldiay and Bilgin 1969; Bilgin 1980) and its drawings in the article seems to belong to member of the family Bithyniidae. I think these three species needs to be dissected again.

Pseudamnicola lindbergi was described by C. Boettger in 1957. Only shell morphology was included in the study and there is no information about the detailed anatomy of the species until today. This study is aimed to determine male anatomical characters of the *Pseudamnicola lindbergi* Boettger 1957 for the first time.

Materials and Methods

Samples were collected from Elbistan, Pınarbaşı spring originating from the Ceyhan river in 2015 (Figure 1). They were living on stones and aquatic plants. They preserved in plastic tubes including 80% ethanol. Dissections and measurements of the shells and genital organs were carried out using a stereo microscope (Olympus SZX7) and photographs were taken with a digital camera system (Olympus DP26). The topotypes are deposited in private collection of the author. Comparisons were made according to Boettger (1957).



(a) (b) (c)

Figure 1. Study area (a) Locality of the *Pseudamnicola lindbergi* (b-c) Photos of the Elbistan Pınarbaşı spring

Results

Genus: *Pseudamnicola* (Paulucci 1878)

Pseudamnicola lindbergi (Boettger 1957:69, abb:1)

Material examined: 16 exx. From type locality.

Type locality: Kahramanmaraş, Elbistan, Pınarbaşı spring, 16.07.2015, M.E.Gürlek leg. [1153 m asl, N 38°10'53.07″ E 37°13'08.50″]

Description: Shell height 2.81 mm, width 1.83 mm. Aperture height 1.34 mm width 0.97 mm (topotype). The yellowish shell conical with 4-4.5 whorls which are nearly straight with a weak suture. The umbilicus slit-like. Aparture ovoid, angled at the top and clear whitish peristome at the columella. Mantle and head dark colored, eye spots visible. The operculum dark yellow (Figure 2).

Male genital morphology: The whitish penis long, triangular and warped to the distal part.

Habitat: Natural spring water. The specimens were collected from stones and aquatic plants.

As a characteristic feature of the genus, the penis is seen in a triangular structure and has a conical shell shape. *Pseudamnicola lindbergi* is similar to other *Pseudamnicola* species living in Turkey as shell morphology especially *Pseudamnicola merali* and *Pseudamnicola kayseriensis*. But *P. lindbergi* has a different penis shape. Its penis is also triangular but warped to the distal part

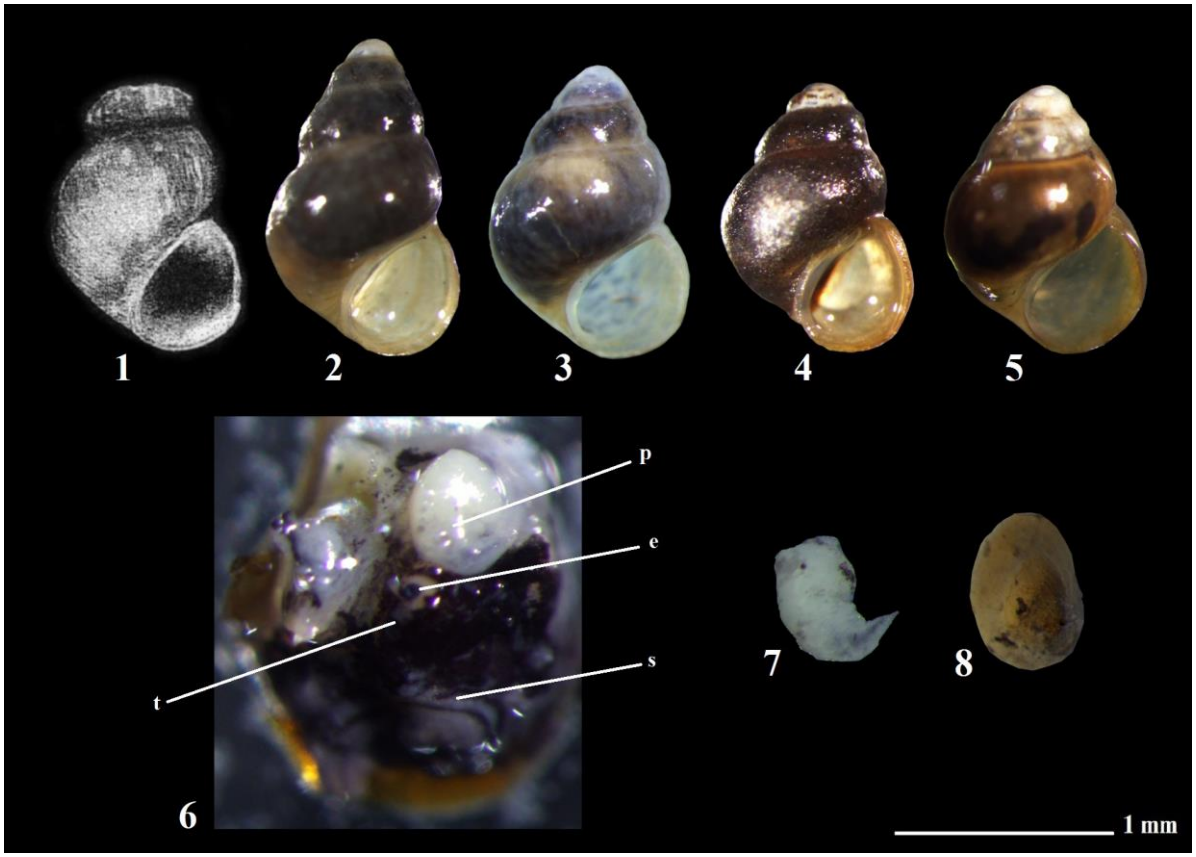


Figure 2. Shells and soft parts (1) Facsimile photo of the *Pseudamnicola lindbergi* Boettger (1957: 69). (2-5) topotypes of the *Pseudamnicola lindbergi* (6) Penis in situ (7) Penis (8) Operculum [Abbreviations: p = penis, e = eye spot, s = snout, t= tentacle]

Discussion

In the description of Boettger (1957), it is said that the apex is undergoing corrosion thats why the shell with 3 sutures (Figure 2, Figure 3). Corrosion was also found in the apexes of the topotypes, but not so much as in Boettger's type species. This explains why the topotypes sutures are 4-4.5 whorls. Götting (1963), recorded the *P. lindbergi* from Bursa Uludağ but Schütt and Bilgin (1970) reported that this species belongs to the genus *Bythinella* on the basis that the study of Schütt 1965. The reason of all this confusion can be that the photograph of the holotype which has corroded apex. Because the shell is similar to genus *Bythinella* in this photo.

Ordo Monotocardia.
Fam. Hydrobiidae.

Pseudamnicola lindbergi nov. spec.

(Abb. 1.)

Schale klein, geritzt genabelt, mit korrodiertem Apex, die erhaltenen 3 Umgänge getürmt eiförmig; Farbe helloliv. Umgänge kräftig gewölbt, durch eine tiefe Naht getrennt, rasch zunehmend. Mündung schief gestellt, gerundet eiförmig, nach oben stumpf gewinkelt; Mundsaum zusammenhängend, scharf, innen durch eine schmale, dünne, weißliche Lippe verdickt. Spindelrand nur wenig zurückgebogen. Deckel weit in die Schale zurückgezogen, gerade noch sichtbar; scheint von dem allgemeinen Bau des Deckels bei dieser Gattung nicht abzuweichen.

Typus: Höhe 2·15 mm, Breite 1·55 mm; Höhe der Mündung 0·90 mm, Breite der Mündung 0·85 mm.

Fundort: Ausfluß aus der Höhle Punar Badi bei Elbistan (Vilayet Malatya).

Diese Art der Gattung *Pseudamnicola* PAULUCCI unterscheidet sich durch ihre mehr getürmte Gestalt, die trotz ihrer Korrosion gut erkennbar ist, von ihr verwandten Arten aus Syrien, nämlich den als „*Bithinia*" beschriebenen und sämtlich von Saida stammenden „Arten" *Pseudamnicola gaillardotii* (BOURGUIGNAT) (7, S. 147, Pl. 8 Fig. 10-11), *Pseudamnicola moquiniana* (BOURGUIGNAT) (7, S. 148-149, Pl. 8 Fig. 14-15), *Pseudamnicola putoniana* (BOURGUIGNAT) (7, S. 149, Pl. 15 Fig. 5-6) und *Pseudamnicola hebraica* (BOURGUIGNAT) (7, S. 181-182, Pl. 15 Fig. 7-9), deren artliche Verschiedenheit untereinander noch zu klären ist. Auch die kleinasiatischen Arten *Pseudamnicola pallida* (MARTENS), die bis nach Transkaspien reicht, und die von Bursa (Brussa) beschriebene *Pseudamnicola byzanthina* (KÜSTER) (16, S. 6, Taf. 11 Fig. 19-20) haben eine weit kugeligere Schale als die neu aufgestellte Species. Möglicherweise gehört zu ihr aber die nicht näher beschriebene Schnecke, die H. WAGNER 1940 als „*Pseudamnicola* sp.?" von Ceyhan angibt (31, S. 163). Die von demselben Autor gleichzeitig als „*Bythinella* sp?" aus dem Sulta suyu bei Hara im Vilayet Malatya erwähnte Form dürfte kaum zum *Pseudamnicola lindbergi* nov. spec.

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gehören, da sie H. WAGNER zu *Bythinella* MOQUIN-TANDON stellt und nicht zu der ebenfalls erwähnten Gattung *Pseudamnicola* PAULUCCI. Eine Nachprüfung von H. WAGNER's Material ist zur Zeit nicht möglich, in nomenklatorischer Hinsicht auch bedeutungslos. Immerhin ist zu bedenken, daß H. WAGNER unter „*Bythinella*" offenbar noch schlankere Formen als die hier neu beschriebene Art versteht, wie etwa seine *Bythinella soósi* H. WAGNER aus dem Issik-Kül (28, S. 132), die er mit „*Bithinia*" *longiscata* BOURGUIGNAT von Saida in Syrien (7, S. 148, 185, Taf. 8 Fig. 12-13) vergleicht.

Außer dem bereits beschriebenen Typus von *Pseudamnicola lindbergi* nov. spec. liegen von der Art noch 2 Paratypen vor. Aus dieser Serie von 3 Exemplaren ist das von mittlerer Größe zum Typus gewählt worden. Die beiden Paratypen gleichen dem Typus weitgehend. Auch bei ihnen ist der Deckel weit in die Schale zurückgezogen; er wurde nicht herauspräpariert, um die wenigen Exemplare nicht zu beschädigen. Die Paratypen sind ebenso stark korrodiert wie der Typus; auch bei ihnen sind nur etwa 3 Umgänge erhalten. Das größte Exemplar unterscheidet sich durch grünliche Färbung (Algenbewuchs) von den beiden anderen. Der kleinere Paratyp ist zusammen mit dem Typus im Naturhistoriska Museet in Lund niedergelegt. Das andere Exemplar befindet sich im Senckenbergischen Museum in Frankfurt am Main (SMF 132684).

Maße der Paratypen:

Paratyp 1 (SMF 132684): Höhe 2·25 mm, Breite 1·70 mm; Höhe der Mündung 1·05 mm, Breite der Mündung 0·90 mm.

Paratyp 2: Höhe 1·85 mm, Breite 1·35 mm; Höhe der Mündung 0·90 mm, Breite der Mündung 0·80 mm.

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Figure 3. The facsimile of the original description of *Pseudamnicola lindbergi* Boettger (1957: 68,70)

Another information that needs to be corrected, in the original description it is given that the type locality is in the province of Malatya ('Ausfluß aus der Höhle Punar Bachi bei Elbistan (Vilayet Malatya)') (in Turkish vilayet=province). But today Elbistan is not in Malatya, it is in Kahramanmaraş province borders.

There are many other endemic species with poorly known anatomical and morphological characters in Turkey. Its need to be redescriptions besides new descriptions to understand taxonomic situations of the species clearly.

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First Observation of European eel (*Anguilla anguilla*) As a Prey for Mallard (*Anas platyrhynchos)* in Gediz Delta Ramsar Area (İzmir Turkey)

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ABSTRACT

In this study, a European eel (*Anguilla anguilla* L. 1758) was found in stomach content of a drake mallard (*Anas platyrhynchos* L. 1758) in Gediz Delta Ramsar Area (Izmir, Turkey) in spite of there is no fish in the diet of dabbling ducks. With this new record for Turkey, European eel would be added to the prey list of mallard as a new species.

Keywords: *Anguilla anguilla*, *Anas platyrhynchos*, prey, predator, stomach content

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Avrupa yılan balığı *(Anguilla anguilla)* nın Gediz deltası Ramsar alanında (İzmir Türkiye) Yeşilbaş ördek *(Anas platyrhynchos)* midesinde rastlanması

Öz: Yüzücü ördeklerin genellikle balık yeme özellikleri olmamakla birlikte bu grup ördeklerden en büyüklerinden biri olan ve Gediz deltası Ramsar alanı civarında avlanan bir yeşilbaş ördeğin (*Anas platyrhynchos* L.1758) mide içeriğinde tatlısu yılan balığına (*Anguilla anguilla* L.1758) rastlanmış olup, Türkiye'de gerek ördeğin besin diyetinde gerekse de yılan balığının tatlısulardaki predatörleri listesine yeni bir tür eklenmiş olmaktadır.

Anahtar kelimeler: *Anguilla anguilla*, *Anas platyrhynchos*, av, predatör, mide içeriği

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Introduction

Anguilla anguilla has been shown to be distributed from North Cape in Northern Norway, southwards along the coast of Europe, all coasts of the Mediterranean and on the North African Coast (Tesch 2003).

Aves species like herons (*Ardea cinerea*), cormorant (*Phalarocorax carbo*) and common merganser (*Mergus merganser*) are known to be predators of eels (Hastie et al. 2008). Although there were small fish in the diet of diving ducks, feeding of mallard (*Anas platyrhynchos*) which is one of the biggest sized dabbling ducks (nearly 60-63 cm), is omnivorous type (Hocaoğlu 1992). It was reported that the feeding of nurturing ducks consists of 30% carnivorous 70% herbivorous and vice versa while in spawning period (Krapu and Reinecke 1992). The diet of omnivorous mallards consists of gastropods, crustaceans, worms, and insects (flies, Lepidoptera, dragonflies) and also small fishes (del Hoyo et al.1992; Snow and Perrins 1998) from animals. As plant materials seeds, thin roots from plants and the vegetative parts of aquatic and terrestrial plants (Swanson et al. 1985; Hocaoğlu 1992; Snow and Perrins 1998).

Case

Semelparous fish species *A. anguilla* has been added to the list of critically endangered fish species by IUCN, migrates between sea and freshwaters for reproduction purpose. The specimen (*A. anguilla*) which is the scope of this study, was found in

December 31st, 2016, from the stomach content of a drake mallard which was hunted in Gediz Delta Ramsar Area (Izmir, Turkey). The specimen was found partly digested with no head, no body part from the mid-point of coelomic cavity. The length of ruptured eel that partly digested in the mallard stomach was 16 cm. It was predicted that the eel was juvenile and its estimated length was 22-25 cm (Figure 1).

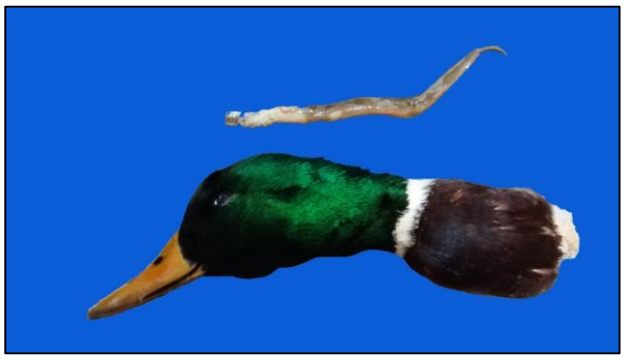

Figure 1. The European eel which was found in stomach from drake mallard (Same scale).

Discussion

The eel extracted from the stomach of mallard which is known as omnivorous and opportunistic (del Hoyo et al. 1992; Snow and Perrins 1998) was quite large in size (Figure 1) compared with sizes of foods in its normal diet (Krapu and Reinecke 1992; Hocaoğlu 1992).

So far, mallard has not been in the predator list of European eel *A. anguilla*. Also, for the first time, eel has been reported in the prey list of mallard which is known as an opportunistic animal and rarely prey with small fish (Swanson et al. 1985; del Hoyo et al. 1992; Snow and Perrins 1998).

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Management of Overfishing in the Inland Capture Fisheries in Nigeria

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ABSTRACT

Nigeria is endowed with extensive inland water bodies that are very rich in fish and shell fish many of which are of commercial importance. These diverse fish resources with immense potential plays contributing role to the food security and improving livelihoods of local fishers, but in recent years, there has been serious concern about the poor status of the inland capture fisheries. Overfishing has been recognized as a major threat to sustainability of the fisheries. Overfishing has brought about changes in species composition, decline yield and fish stocks are being threatened with depletion because of ineffectiveness and weak regulations. This paper elucidates and analyses the causes, signs and types of overfishing in the inland capture fisheries in Nigeria. It also highlights current management measures, reasons for management failure and some of the procedure that could be adapted for sustainable management of inland capture fisheries.

Keywords: Nigeria, inland, capture, fisheries, overfishing

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Introduction

Nigeria has a wide variety inland waters which are rich in many and diverse finfish and shellfish. There are several significant riverine systems with about two thirds of the country lies in the watershed of the Niger River, and other major river systems include the Benue River, Cross River, Anambra River, Imo River, Kwa Iboe River, Ogun River and Oshun River (Olopade et al. 2017) and innumerable small rivers.

The most prominent amidst the networks in Nigeria is the Niger – Benue system, whereas Lake Chad and kanji are the most important with regards to fish production (Eyo and Ahmed 2005). These water bodies have been exploited by artisanal fishers operating in rivers, streams, estuaries, wetlands, brackish water, natural and man-made lakes for variety of benefits, including food security, livelihoods and employment.

In Nigeria, the inland capture fisheries is small scale and have been the most neglected by the government notwithstanding the major and the significant contribution of the sub-sector. The inland fisheries give about 82% of domestic fish production (FDF 1994). The catch of the inland capture fishery is dominated by *Lates spp.*, *Tilapia spp.*, *Citharinus spp.*, *Chrysichthys spp.*, *Mormyrus spp.* and *Clarias spp.* (Arawomo 2004).

For decades, the catches from the inland water bodies steadily increased with the many fish stocks are now classified as overfished due to continuous overfishing and use of obnoxious fishing methods such as the use of small mesh, unselective fishing gear, fish poisons and explosive. The overexploitation of the finite resources has led to a drastic reduction in inland rivers and lakes fish production from 213,996 metric tonnes in 1998 to 181,268 and 194,226 metric tonnes in 2000 and 2001 respectively (Eyo and Ahmed 2005).

The status of the inland capture fisheries in Nigeria is largely a result of a failure of the present process of fisheries governance which pose significant threats to the management of fisheries resources. The lack of effective governance can be attributed to outdated, weak and inappropriate regulations and incoherent policy. There is, however,

a need to protect and sustainably manage inland capture fisheries in Nigeria that are essential to people. This paper examines causes, types and problems of overfishing in inland capture fisheries in Nigeria and subsequently recommending remedial actions.

Inland fisheries resources and potentials in Nigeria

Nigeria is watered from North to South and East to West by a network of rivers. Rivers Niger and Benue that form a big 'Y" right across the middle of Nigeria originate from outside the country (Ajai 2012). The rivers of Nigeria may be divided into four main groups: 1) the Niger-Benue system, 2) the rivers west of the lower Niger, 3) the rivers east of the lower Niger and 4) those flowing into Lake Chad. All other rivers flow across the territory to empty into Lake Chad in the North-East corner or the Atlantic Ocean in the South.

The major rivers make up about 11.5 percent and lakes and reservoirs about 1 percent of the total area of Nigeria (Ajai 2012). The total water bodies, including deltas, estuaries, etc., make up about 15.9 percent of the total area of the country. The fresh water components are within extensive river systems, lakes, flood plains and reservoirs scattered over the entire land surface area of over 4,212,500 ha (Ita 1993). The brackish water sector consists of estuaries, beach ridges, intertidal mangrove swamps, intersecting rivers and winding saline creeks covering a total area of 1,751,509 ha (mostly unexploited). The estuarine resources alone are found in the extensive mangrove ecosystem estimated to cover an area of about 858,000 ha (Ita 1993) (Table 1).

Table 1: Area of inland water surface

Inland Water Resource	Area (ha)
Freshwater Bodies (Basin & Flood plains)	3,221,500
Major Rivers	10,812,400
Major Lakes & Reservoirs	853,600
Deltas & Estuaries	858,000
Minor Reservoirs	98,900
Miscellaneous wetlands	4,108,100
Fish ponds	5,500
Total	19,958,000

Freshwater has potential resources of 226,550 mt for rivers/floodplains 160,000 mt for Lake Chad, 38,000 mt for Kainji Lake and 2.5 million mt for aquaculture (FISON 2004). Ita (1993) emphasized that Nigerian inland water bodies are presently producing lower than 50% of their conjectured potential fisheries yields. The freshwater fish faunas of Nigeria have not been well document but recent information on species diversity in Nigerian freshwater reported 260 fish species. Given the diversity of freshwater fish assemblages, levels of fisheries productivity, cultural norms, density of human population and socioeconomic conditions, it is not surprising that there is immense variation in how, why and the extent to which freshwater fishes and other aquatic animals are exploited. The capture from inland waters are multi-species and multi-gear with a great majority of fishers traditionally operate at small-scale level to exploit the natural fisheries resources in whatever means available to them.

According to FAO (1997) the inland water resource has the following potentials:

(1) food fisheries based on wild stocks;
(2) enhancement of food fisheries in smaller water bodies and reservoirs;
(3) recreational fisheries, which are becoming more common in many areas of the world, and, where they develop, tend to supplant commercial food fisheries; and locally very intense exploitation of juvenile or small adults for stocking into other water bodies and/or aquaculture ponds, or for the ornamental fish trade (FAO 1997).

Based on above the inland capture fisheries in Nigeria is under exploited and less developed compared to other sectors such as oil, agriculture, industry and trade in Nigeria because fishing activities do not contribute significantly to the country´s gross national product.

Causes of overfishing

The overfishing of Nigeria's inland capture fisheries began with introduction of synthetic fishing materials such as polyamide (nylon), polyester, polyethylene etc. Traditional materials for fishing have been replaced by modern ones which have increase fishing gears catchability and the most obvious effect is the depletion of some natural fish stocks. For example, the consequent of overfishing on a given species leads to immature populations with a fast turnover and high fecundity which result in a smaller and faster growing species as we are experiencing in most of inland waters in Nigeria. Kanji, Seisay and du Feu (1997) notice that a decline in mean sizes (mean length and weight) in fish species and changes in species composition due to other recruitment and ecosystem overfishing in Lake Kanji. Eyo (2004) reported a significant poaching of juvenile fishes on lake kanji by foreign fishermen who use gillnet and beach seine (Dala) less than 3 inches as specialized by the inland water decree. Therefore, there is the need for a new act which regulate the giving practice on Nigerian freshwater, which endured significant overfishing in current times (Eyo 2004).

Obnoxious fishing practices: This may involve fishing with explosives and poisons that damage the ecosystem, killing most of the non-target species and compromising any possibility of sustaining yields in the future. Some toxic plant parts and their active ingredients in Nigerian inland waters are well described by Udolisa et al. (1994) and in Lake Kanji basin by Reed et al. (1967). The use of obnoxious fishing practices has been seen as a challenge in conserving the kanji and Jebba lakes (Nwabeze and Erie 2013).

Understanding of the nature small scale fishery is currently the most neglected component in the inland fisheries management. The fishers by nature are fiercely competitive and independent and do not easily respect fishing regulations. This can be traced to their poor socio economic status in the society. Persistent poverty among the fishers aggravate illegal practices, overfishing and habitat destruction in order to survive (Lacanilao 1998).

Other causes of overfishing include:

i) Difficulties in regulating fishing areas due to lack of resources and tracking activity,
ii) There are little to no rules regarding fishing practices,
iii) Lack of knowledge regarding fish populations.
iv) Unreported fishing, which is nearly impossible to track, partly because of the diffuse and small-scale nature of individual fisheries, the lack of easily definable landings, and because much of the catch goes directly to domestic consumption (Welcomme 1976)

Types of overfishing

Overfishing of stocks is not only an ecological problem but also economical problem. Based on these there are four recognised types of over fishing;

i) Growth overfishing: this occurs where the young fish (recruits) are harvested at an average size that is smaller than the size that would produce maximum yield per recruit,
ii) Recruitment overfishing: This occurs where the parent stock is so reduced that not enough young are produced by the fishery to maintain itself,
iii) Ecosystem overfishing: This occurs where species distribution is drastically changed, altering the efficiency of the system. In this situation, the niche inhabited by originally abundant species is not fully taken up by other species, changing the system's productivity,
iv) Economic overfishing: This occurs where the cost of fishing effort is greater than the revenue generated from the fishing. As fish stocks decline, the effort required to catch a given quantity of fish increases.

Research have shown that all these various forms of overfishing have being recorded in the inland water bodies in Nigeria.

Signs of overfishing

Basically the declines in the size of annual catch is important sign that the stock size is overfished and also, the quantity of sexually mature adult fish the spawners because they determine how many offspring are produced. According to Ita (1993) signs of overfishing are as follows:

i) General decline in catch per unit effort and observation that the increase in fishing effort does not result in a proportional increase in the catch.
ii) Reduction in genetic diversity, especially when the stock size is greatly reduced from natural level.
iii) Decline in catches in larger mesh net, results into high catch in small mesh net.
iv) Low productivity of the littoral zone.
v) High concentration of fishermen per unit length of shore land per surface area.
vi) Rise in the cost of catching a unit weight of fish associated with increase in effort without relative increase in catch.
vii) Mass migration of non-indigene fishermen to other places.
viii) Decrease in market landing as compared to previous year or season.
ix) Increase in the price of fish in an area which could be a sign of low harvest resulting from overfishing.
x) When so fewer age groups are represented in catches (Rosenberg and Restrepo 1995).
xi) Frequent damage or destroy of passive fishing gears as result of competition for a dwindling resource base. This in turn has contributed to social unrest and inability of fishing community to organise for social, economic or political purposes (Bailey 1987).
xii) The contribution of capture inland fish to food security has been largely ignored and priorities switched to other sectors.

Management of Inland Capture Fisheries in Nigeria

As far as fisheries management is concerned in Nigeria, the system is divided into two namely, government and traditional institutions. Traditional management systems entail the regulation of fisheries activities and resources by traditional authorities or communities using common beliefs or norms, prescriptions, religious practices, behaviours, taboos, magic, languages and such systems are still operational in spite of the population growth, changes in legal systems, urbanization, commercialisation and technological change. In general, there are no customary restrictions to access water from large sources but customary law disapproves of the abuse of these rights. For examples fishing in rivers and streams is generally unrestricted but there may be a right to tribute by the local head fisherman or traditional leader, usually by strangers and in some cases tax is levied on fishing activities and freight traffic (Ramazzoti 2008), but if flood waters completely cover a person's land, the rights over the land belong to him (Ezeomah 1985).

Most traditional fishing methods and management patterns are still applicable at the present time. Property rights are the bases for traditional management systems (Hall 1999). These systems are usually well enforced, as they tend to be self-policed by fishermen. Traditional systems management focuses on resolving gear use or allocation problems. Access control is enforced by fishermen and by local moral and political authority. Supernatural sanctions are probably the most effective punishment for poachers. However, there are a number of obstacles that might face the encouragement to use them. Among these obstacles are the social change, poverty and educational levels of the fishers. And therefore, these resource users are losing control of traditional authority for management and less effective.

The management of water and fisheries resources in Nigeria is undertaken by all levels of government - federal, state and local governments which often lead to legislative overlaps and conflicts. The federal government is involved in fisheries management through the activities of the Federal Department of Fisheries. Federal involvement in inland fisheries management varies considerably with most inland waters in Nigeria are managed by the individual states within which the waters are located. When inland waters border more than one state, the states involved usually collaborate to determine how the water will be managed but each state normally has its own regulations. The inland fisheries decree was promulgated to harmonise the administration, management, protection and improvement of inland water fisheries through the use of decree. The roles of the decree included:

i. Provision for licensing and identification of fishing craft.

ii. Restriction on the use of certain fishing gear.

iii. Prohibition of obnoxious fishing methods.

iv. Prohibition of unauthorised export or import of live fish.

v. Protection of fish products from contamination and infection.

Although, some states in Nigeria have promulgated their fisheries edicts, some of which are yet to be effectively implemented (Ita 1993) and out of the 36 states, only 15 states have fisheries edicts, laws or regulations in place. However, where the framework is in place, the capacity to implement and enforce it is weak or nonexistent. Therefore the inland fisheries edicts is devoid of detailed regulations, outdated and not complemented by sufficient administrative structures, processes and institutions for its administration, largely due to lack of resources, weak database and weak law enforcement.

With the failure of centralized public sector control in fisheries management, collaborative management, or comanagement, has emerged as one of the most promising approaches to arrive at more sustainable forms of fisheries management. Cogovernance or Comanagement arrangements range from those that are largely consultative, to those in which the fishers design, implement, and enforce laws and regulations with advice from the government (Berkes 1994). In Nigeria, this type of management is commonly seen in communal water bodies. A typical example is being practised by the Argungu Emirate council of Kebbi state, where the use of gillnet and cast net are prohibited (Eyo and Ahmed 2005); this is to conserve the resource for the popular Argungu festival and in communal fish shelter popularly called acadja widely spread in most inland water bodies in Nigeria (Olopade et al. 2008).

Reasons for management failure of overfishing in inland fisheries in Nigeria

For many years, authorities have been attempting to control fishing with a variety regulating instrument in order to conserve the stocks. However, many of these measures fail because of the following:

1. To date, regulating overfishing have been very difficult. Enforcement tend to be weak because fisheries department in Nigeria is understaffed and underfunded.
2. "Tragedy of the common" (Hardin 1968); in Nigeria, most inland capture fisheries are generally considered as common property

and some are entirely open-access to the effect that no one feels ownership or responsibilities for damage and in addition to short sightedness and greed of the fishers.

3. Failure to incorporate both scientific uncertainty and measurement error in stock assessments (Hilborn and Peterman 1995).
4. Lack of political will to implement scientific regulations and other recommendation measures.
5. Lack of know regarding fish populations.
6. The low level of organisation of small-scale fishers in most fishing settlements or villages has hindered their participation in fisheries governance
7. Lastly, most inland water bodies and landing sites are largely in remote areas making them inaccessible for proper management.

Discussion

Control of fishing effort is a basic tool of fisheries management. Although both the government and tradition institutions and lately comanagement are involved in the management of inland capture fisheries in Nigeria. The governments retain rights over resources, or lease or cede them to local communities. In order to reduce or totally eradicate the challenges being faced in the management of overfishing in inland capture fisheries and ensure sustainability and increase in fish production. The following is recommended:

1. Education is considered as a key component in the effective management systems of all successful fisheries. Fishers must be educated to use prescribed mesh sized net, so that under sized fishes would not be killed.
2. Existing illegal and indiscriminate fishing practice must be strictly checked by imposing stringent laws and penalties.
3. Government should allocate more policy attention and resources to develop inland capture fisheries in Nigeria.
4. Small scale fisheries development requires special support from government. An integrated approach through, and with the participation of, fishing communities is often the best way of channelling technical, financial and other form of assistance.
5. Restocking and stock enhancement programs should be introducing in most inland water by releasing reared juveniles into open waters has proven to be very effective for some species.

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