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Determination of Bacterial Contamination and Antibiotic Resistance of the Bacteria in the Some Trout Farm Hatcheries in the Eastern Black Sea Region of Turkey

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ABSTRACT

This study was carried out to reveal bacterial contamination and antibiotic resistance profiles of isolated bacteria in the hatchery systems of trout farms located in the Eastern Black Sea region of Turkey. Inlet water, egg, alevin and fry samples of 11 different trout farms were examined in terms of bacterial contamination in four different basins. After sampling, isolated bacteria were identified by making phenotypic and genotypic analyses. The majority of a total of 43 strains belongs to the genera *Aeromonas* and *Pseudomonas*, but also *Lelliottia* sp., *Bacillus* sp. and *Lactococcus lactis* were isolated from hatchery systems. Considering all basins, except for the 2nd basin, the highest antibiotic resistance of bacteria was against Ampicillin. The lowest antibiotic resistance percentages were determined against gentamicin and enrofloxacin. As a result of the research, the detection of different bacteria in the samples taken from the inlet water of fish farms showed presence of different bacteria contaminating the hatchery water. It thereby stressed the need for improved hygiene measures in these farms.

Keywords: Antibiotic, bacteria, trout, hatchery

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Türkiye'nin Doğu Karadeniz Bölgesinde Bulunan Bazı Alabalık Çiftliklerinin Kuluçkahanelerinde Bakteri Kontaminasyonu ve Bakterilerin Antibiyotik Direncinin Belirlenmesi

Öz: Bu çalışma, Türkiye'nin Doğu Karadeniz bölgesinde bulunan alabalık çiftliklerinin kuluçkahane sistemlerinden izole edilen bakterilerin kontaminasyonu ve antibiyotik direnç profillerini ortaya çıkarmak için yapılmıştır. 11 farklı alabalık çiftliğinin giriş suyu, yumurta, alevin ve yavru örnekleri, dört farklı havzada bakteriyel kontaminasyon açısından incelenmiştir. Örneklemeden sonra izole edilen bakteriler fenotipik ve genotipik analizler yapılarak teşhis edilmiştir. Toplam 43 suşun çoğunluğu *Aeromonas* ve *Pseudomonas* cinsinlerine aittir, fakat aynı zamanda *Lelliottia* sp., *Bacillus* sp. ve *Lactococcus lactis*, kuluçka sistemlerinden izole edilmiştir. 2. havza hariç tüm havzalara bakıldığında bakterilerin en yüksek antibiyotik direnci Ampisiline karşı olmuştur. En düşük antibiyotik direnç yüzdeleri gentamisin ve enrofloksasine karşı belirlenmiştir. Araştırma sonucunda, balık çiftliklerinin giriş sularından alınan örneklerde farklı bakterilerin tespiti, kuluçkahane suyunu farklı bakterilerin kontamine ettiğini göstermiştir. Böylelikle bu çiftliklerde hijyen önlemlerinin iyileştirilmesi ihtiyacı belirlenmiştir.

Anahtar kelimeler: Antibiyotik, bakteri, alabalık, kuluçkahane

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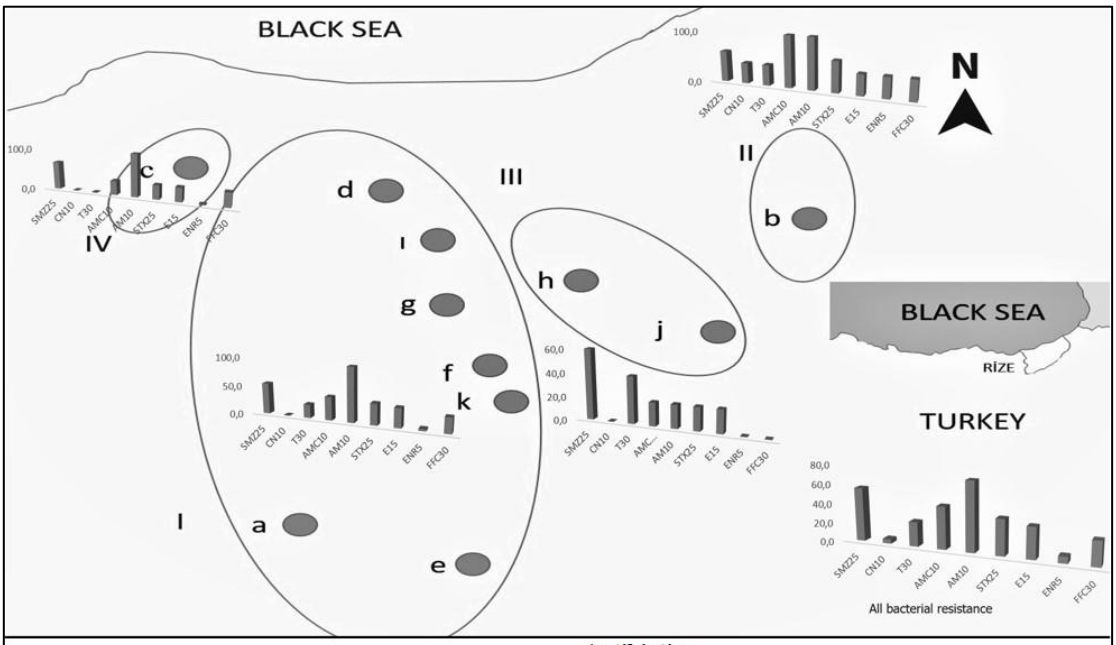
Introduction

The trout farming in Turkey was grown rapidly due to the technical and scientific advances occurred in the aquaculture and increased demand. Nowadays, trout farming can be done in land-based concrete and soil pools, as well as in net cages in sea, dams, lakes, and ponds. In Turkey, the rainbow trout (*Oncorhynchus mykiss*), the brook trout (*Salvelinus fontinalis*) and the Black Sea trout (*Salmo* sp.) are widely grown species. The trout production was approximately 44.553 tonnes in the year 2000 but reached about 109.657 tonnes in 2017, increasing the amount of production more than a hundred percent in 17 years. It takes place on the first with the largest production among the cultivated species in Turkey (Gün and Kızak 2019).

Rize is located in the Eastern Black Sea Region of Turkey (Figure 1). The most prominent livelihood of the city is tea agriculture and agricultural products. Besides agriculture, fishing has an important place in the city due to the city's seashore. Rize has a mountainous structure covered with the forest areas. Therefore, the annual average of areal precipitation in the region is higher than the country average and because of these reasons; Rize is also an affluent city in water resources (Gedik et al. 2010).



Basin	Antibiotics								
	SMZ25	CN10	T30	AMC10	AM10	STX25	E15	ENR5	FFC30
I	53.3	0	23.3	40.0	93.3	36.6	33.3	3.3	26.6
II	60.0	40.0	40.0	100	100	60.0	40.0	40.0	40.0
III	60.0	0.0	40.0	20.0	20.0	20.0	20.0	0.0	0.0
IV	66.6	0.0	0.0	33.3	100	33.3	33.3	0	33.3
All Bacteria	**55.8**	**4.6**	**25.5**	**44.1**	**72.0**	**37.2**	**32.5**	**6.9**	**25.5**

Figure 1. Study area, four different basins and antibiotic resistances of the bacteria (%). Ampicillin (AM, 10µg), gentamicin (CN, 10 µg), oxytetracycline (T, 30 µg), amoxicillin/clavulanic acid (AMC,10µg), enrofloxacin (ENR, 5µg), trimethoprim/sulfamethoxazole (TMP-SMZ, 25µg), florfenicol (FFC, 30µg), sulfamethoxazole (SMZ, 25µg) and erythromycin (E, 15µg).

In this context, it is known that there are 40 registered fish farming facilities in Rize, 34 of which have hatcheries. Some of these farms have impossibilities arising from small production capacities, lack of technical staff and some of them are family businesses. For these reasons, proper disinfection cannot be performed during the fertilization and incubation periods of the fish eggs. Although the failure to carry out disinfection processes in hatcheries is not considered an economic loss by the owners, this situation will cause economic damage to the business regarding the future mass losses. Moreover, disinfection must be carried out since it is known that it may affect other facilities producing in the same source and biome in the water source (Kayış 2019).

Common pathogens in trout incubation systems are viruses (IPNV, VHSV) (Işıdan 2006; Yılmaz et al. 2011), fungi (*Saprolegnia* sp. and *Saprolegnia parasitica*) (Ural et al. 2011), usually protozoan parasites (*Ichthyophthirius multifilis*, *Trichodina* sp., *Ichthyobodo necator*, etc.) (Balta et al. 2008), and bacterial pathogens (*Flavobacterium psychrophilum*, *Renibacterium salmoninarum*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Yersinia ruckeri*, *Lactococcus garviae*). *Flavobacterium psychrophilum* and *R. salmoninarum* have been reported as bacteria isolated from hatchery systems (Evelyn et al. 1986; Brown 1997). However, experimental studies have been conducted with *A. hydrophila*, *Pseudomonas* spp. and *Y. ruckeri*, various diseases have been reported in the contamination of these bacteria with eggs. Kayış et al. (2014) have provided contamination to the healthy trout eggs of the mentioned bacteria and reported that *Pseudomonas* species caused sac deformations and *L. garviae* caused haemorrhages in the alevins and juveniles. In another study in which *A. hydrophila* was contaminated with eggs belonging to *O. mykiss* and

S. fontinalis, pathogenicity was investigated from the egg stage to the larval stage. In the study, *A. hydrophila* was reported to cause of blue sac disease and severe mortality in fish (Kayış et al. 2015).

Materials and Methods

In this study, a total of 5 samplings were carried out between November 2016 and April 2017 from 11 different trout farms in Rize. Egg, alevin and fry individuals of 3 different trout taxa sampled (*O. mykiss*, *Salmo* sp. and *S. fontinalis*) and the inlet water samples of these farms were used. For this purpose, different fish farms in four different basins were selected in Rize. The locations of the farms in the basins are given in Figure 1.

Sterilized 10 ml of glass tubes were used for water samples. At the same time, eggs, alevin and fry samples were sampled with 10 ml sterile glass tubes in each farm. The water temperatures and pH values of each farm sampled were recorded (Isolab portable pH and temperature measuring device). In the samples, Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) (Merck) were used as a medium to detect the presence of bacteria, and Anacker Ordal Broth (AOB) medium was used to detect bacteria of the genus *Flavobacterium*. Also, Glutamate Starch Phenol Red (GSP) Agar was used to distinguish bacteria belonging to the genus *Aeromonas* and *Pseudomonas* (Austin and Austin 2007).

All of the samples obtained from the farms were cultured in TSB and AOB media to determine bacterial contamination. Then, TSA medium was used to purify cultures (Lasee 1995). Also, TSA medium was used mostly for the primary cultivation of alevin and fry samples.

The colony shape and colours of bacteria were examined with Gram staining, motility, oxidase, and catalase tests. Colonies forming yellow and purple colours in GSP Agar were considered to be *Aeromonas* and Pseudomonas, respectively (Cappuccino and Sherman 1992). Hence, an antibiogram test was performed to determine the antibiotic resistance and sensitivity of the isolated bacteria. The pure cultures obtained were stored in tubes containing glycerol at -80°C.

Isolated Gram-negative bacteria were cultured in TSB medium for DNA isolation and centrifuged at 3800 × g for 6 minutes. 100 µl of distilled water was added to the pelleted part in the Eppendorf tubes and boiled for 10 minutes at 100°C. Then after, it was centrifuged at 10000 × g for 2 minutes, and the supernatant was stocked at -20°C. For Gram-positive bacteria, DNA isolation kit (Qiagen, Netherlands) was used. The specific universal primers (27 F 5 'AGA GTT TGA TCC TGG CTC AG-3', 1492 R 5 'GTT TAC CTT GTT ACG ACT T-3') were used for the 16S rRNA region, for the genetic identification of bacteria. As a result of the PCR process, the 1465-bp-length products obtained were purified with the NucleoSpin PCR purification kit (Macherey-Nagel) and sent to the sequence (ABI PRISM 310 genetic analyzer, Applied Biosystems). The results were compared with the sequences available in the National Center for Biotechnology Information (NCBI).

Different antibiotics including ampicillin (AM, 10µg), gentamicin (CN, 10 µg), oxytetracycline (T, 30 µg), amoxicillin/clavulanic acid (AMC,10µg), enrofloxacin (ENR, 5µg), trimethoprim/sulfamethoxazole (STX, 25µg), florfenicol (FFC, 30µg), sulfamethoxazole (SMZ, 25µg) and erythromycin (E, 15µg) were used for determination of the bacterial resistances. Determination of the antibiotic resistance, bacteria inoculated on TSA medium. After then, the colonies on the TSA medium transferred to Mueller Hinton Agar medium. The density of the bacteria was determined as the McFarland 0.5 standard. All process was carried out aseptically according to Clinical and Laboratory Standards Institute (CLSI 2018) guidelines. Antibiotic discs placed on the medium with bacteria and the plates were incubated at 22 ±2ºC for 18-36 h. The resulting zone diameters were recorded as resistant (R) or sensitive (S), according to CLSI (2018) directive.

Results

The bacterial species identified after cultured from the samples taken from the farms were given in Table 1. Bacteria belonging to the genus *Aeromonas* and *Pseudomonas* were generally found in the inlet waters of the farms. *Lelliottia* spp. were only isolated from one farm. No *Flavobacterium* spp. were found in any fish farms. It was observed that bacteria isolation was highest in fry individuals (16 strains). The total number of bacteria isolated was recorded as 43, consisting of intake water (9 strains) and eyed eggs (8 strains), fertilised eggs (6 strains) and alevin (4).

When examined on basin basis, pH values average I, II, III and IV were measured as 6.79, 5.8, 6.2, and 6.29, respectively. In the first basin, the lowest pH value was recorded in the farm (ı) with 5.7, while the highest value was recorded in the farm (e) with 7.2. In the basin, average temperature value was measured as 9.01ºC. The only farm in the 2nd basin, the water temperature was 5ºC, and the pH value was 5.8. The average water temperature and pH values of the farms in the 3rd

basin were recorded as 6.2 and 10.5ºC, respectively. In the farm located in the last basin, the water temperature was recorded as 10ºC and the pH value was 6.29.

Table 1. Isolated bacteria and their hosts/samples. (B) basin, (F) farm, (IW) inlet water, (FE) fertilised egg, (EE) eyed egg.

B	F	Bacteria	Fish/Samples	IW	FE	EE	Alevin
(I)	**a**	*Lelliottia* sp.[1]	Water	+			
		Pseudomonas sp.[2]	*Salmo* sp.			+	
		Aeromonas tecta[3]	*Salmo* sp.				
		Aeromonas sobria [4a,b]	*O. mykiss*/*Salmo* sp.				
		Shewanella sp.[5]	*Salmo* sp.				
	d	*Aeromonas sobria*[6]	*O. mykiss*		+		
			Salmo sp.		+		
			S. fontinalis		+		
	e	*Pseudomonas* sp.[7]	*O. mykiss*	+		+	
		Pseudomonas fluorescens[8]	*O. myksis*				
		Aeromonas sp.[9]	*O. mykiss*			+	
		Aeromonas sobria[10]	*O. myksis*				
		Aeromonas hydrophila[11]	Water	+			
	f	*Aeromonas encheleia*[12]	*Salmo* sp.		+		
		Pseudomonas sp.[13]	*O. mykiss/Salmo* sp				
	g	*Pseudomonas* sp.[14]	*Salmo* sp.			+	+
		Aeromonas encheleia[15]	Water	+			
		Aeromonas sp.[16]	*Salmo* sp.				
	ı	*Aeromonas sobria*[17]	Water	+			
		Pseudomonas sp.[18]		+			
	k	*Aeromonas hydrophila*[19a,b]	Water/*O. mykiss* *Salmo* sp.	+			
		Aeromonas encheleia[20]			+		
		Bacillus sp.[21]	*Salmo* sp.				
(II)	**b**	*Aeromonas tecta*[22]	*O. mykiss*			+	
		Pseudomonas sp.[23]	*S. fontinalis/O. myksis*			++	
		Aeromonas tecta[24]	*Salmo* sp.			+	
		Lactococcus lactis[25]	*O. mykiss*				+
(III)	**h**	*Aeromonas* sp.[26]	*O. mykiss*/Water	+			
		Arthrobacter sp.[27]	*O. mykiss*				
	j	*Aeromonas tecta*[28]	Water	+			
		Pseudomonas sp.[29]	*O. mykiss*				
(IV)	**c**	*Aeromonas hydrophila*[30]	*Salmo* sp.		+		
		Pseudomonas fluorescens[31]	*O. mykiss*				+
		Enterobacteriaceae bacterium [32]	*O. mykiss*				+

Accession number of the bacteria; [1]W295477, [2]MW295473, [3]MW295469, [4a]MT730017, [4b]MT730018, [5]*Shewanella* sp., [6]MW295496, [7]MW295465, [8]MW295475, [9]MW295494, [10]MW295496, [11]MW295479, [12]MW295468, [13]MW295473, [14]MW295475, [15]MW295485, [16]MW295489, [17]MW295496, [18]MW295488, [19a]MT730015, [19b]MT730015, [20]MW295486, [21]MW295490, [22]MW295467, [23]MW295465, [24]MW295467, [25]MW295471, [26]MW295482, [27]MW295493, [28]MW295484, [29]MW295492, [30]MT730013, [31]MW295475, [32]MW295476.

The gas problem was observed in *O. mykiss* fry infected with *A. sobria* bacteria taken from samples belonging to the one farm (farm a). Also, deformities were found in the individuals of the same fish species infected with *Pseudomonas* sp. (Figure 2).

Considering all basins, except for the 2nd basin, the highest antibiotic resistance of bacteria was against Ampicillin. The lowest antibiotic resistance percentages were determined against gentamicin and enrofloxacin. The antibiotic resistances of the bacteria are given in Figure 1.

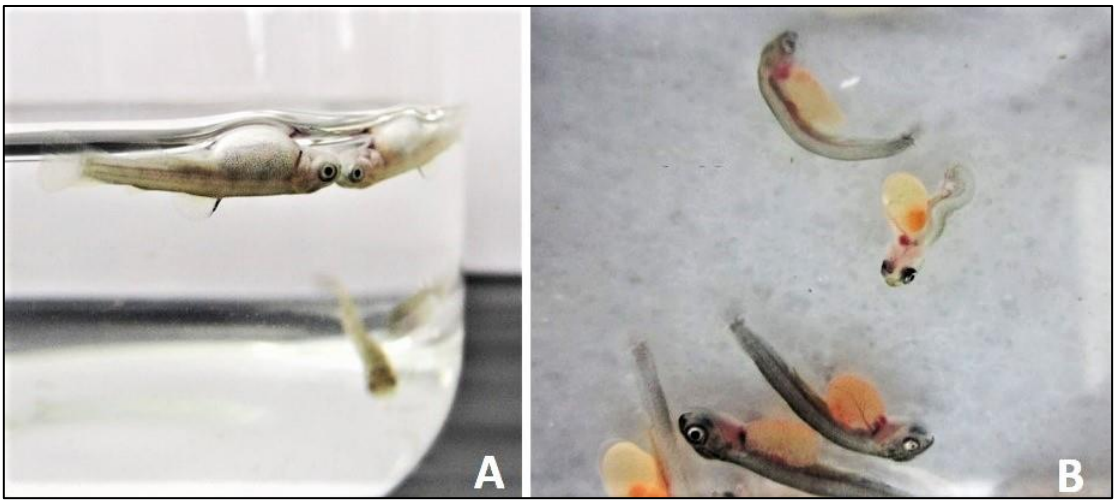


Figure 2. The gas problem infected with *A. sobria* (A) and deformations infected with *Pseudomonas* sp. on *O. mykiss* (B) sampled from (a) farm located in basin (I).

Discussion

In aquaculture, water quality is a very decisive criterion among different criteria such as fish, feed, personnel and transportation. In many countries, reports are requested for the physicochemical values of water to be used in fish farms. An assessment of the microbiological quality of water is not generally required in these application guidelines. However, previous studies have revealed the necessity of microbiological examination of the inlet waters of fish farms. In this sense, it has been reported that *A. salmonicida*, *A. hydrophila* and *A. sobria* species are isolated from the inlet water from different fish farms belonging to the Black Sea Region of Turkey (Onuk et al. 2017). Similarly, in the present study, different species belonging to the genus *Aeromans* were found in the inlet water in 6 of 11 farms. Also, *Pseudomonas* sp. and *Lelliottia* sp. were found in the inlet water samples.

The presence of bacteria belonging to the genus *Aeromonas* and *Pseudomonas* are frequently reported in all aquatic systems worldwide (Hanninen and Siitonen 1995; Fiorentini et al. 1998; Gavriel et al. 1998; Mena and Gerba 2009). In this respect, it is normal to isolate the bacteria belonging to these species from aquatic organisms and many foods. Among bacterial fish pathogens, motile *Aeromonas* species (*A. hydrophila, A. cavia, A. sobria, A. media*) and *A. salmonicida*, which is a furunculosis disease agent, have been reported from many fish species worldwide. The pathogenic species of bacteria of the genus *Pseudomonas* in fish continue to be included in the literature with a new one every day (Altınok et al. 2006; Altınok et al. 2007). This shows that the elimination of these pathogens in trout farming systems is an application that will play a key role in preventing future losses. In this context, the isolation of species belonging to *Aeromonas* and *Pseudomonas* genus in almost every farms in addition to the inlet water in eggs, alevin and fry samples in the farms sampled in the study presented may cause severe mortalities that may occur. Another threat is that bacteria are mostly isolated from fry samples. This also shows that contamination of the mentioned bacteria occurred during the cultivation process.

In general, many *Lactococcus* species bacteria are isolated from plant origins. The bacteria belonging to the genus *Bacillus* are generally isolated from the soil. On the other hand, they can be found in the normal flora of vegetables, water and some other live species (Barrie et al. 1994). The genus *Lelliottia* has been frequently reported, mostly from plant organisms. It can be stated as a common result that these bacteria were isolated from different farms in the samples of inlet water, alevin and fry in this study. Considering the origins of these bacteria, their vegetative, aquatic or soil origin make it natural to have the possibility of contamination to incubation systems. However, looking at the fish disease records of these genera, the genus *Lactococcus* is a severe disease factor especially with *L. garviae* species. Also, *Bacillus mycoides* emerges as the causative agent of the disease isolated from sturgeon and it is characterized by gas syndrome in fish in Turkey (Kayış et al. 2017). The genus *Lelliottia* can be considered as a genus whose pathogenicity should be investigated in fish diseases. The fact that some of the bacteria isolated are of vegetable origin suggests that measures should be taken to prevent plant origin contamination to the incubation systems.

Water quality criteria are important factors for the virulence of pathogenic fish disease factors. For example, *F. psychrophilum* causes disease in fry individuals at temperatures level below 10ºC. On the other hand, *Flavobacterium columnare* shows

efficiency at water temperatures of 10ºC and above. When this study presented is examined in this respect. However, Anacker Ordal media a selective medium for *Flavobacterium* species, was used, no bacteria belonging to the *Flavobacterium* genus were found in the samples. This situation can be explained by the fact that water temperature values were 6.5 and 5ºC in two farms while temperature was 10ºC and/or above in other farms.

Resistance against antibiotics of bacteria isolated from aquaculture basins has been revealed in many studies. Antibiotic resistance in bacteria is expressed as natural or acquired. Due to the drugs used for the treatment of diseases, the level of resistance increases in bacteria that have taken place in the environment. In this study, the highest antibiotic resistance was determined against ampicillin. This can be explained by the natural resistance of Gram-negative bacteria to ampicillin. Also, the lowest antibiotic resistance was determined against gentamicin. This antibiotic is the most effective on Gram-negative aerobic bacteria (Gür 1996). We determined about 40% resistance of the bacteria isolated in the 2nd basin against this antibiotic. *Lactococcus lactis* was the only gram-positive bacteria isolated from all basins. Therefore, there was a resistance to gentamicin in this basin. Also, the low resistance to ampicillin in the 3rd basin can be explained by the same reason.

As a result of the present study, detection of different bacteria in the samples taken from the inlet water of fish farms showed that the water was contaminated. For this reason, before fish farms are established, it can be recommended that they obtained microbiological analysis in the water source they intend to use. It can be thought that the analysis to be made will guide the detection of pathogens in the water and the determination of the measures to be taken. Instead of many microbiologically contaminated hatcheries in different basins, an equipped hatchery can be established to for facilitating microbiological contamination. Increasing bacterial pathogens in terms of quality and quantity of eggs, alevin and fries have shown inadequacy of hygiene measures in aquaculture facilities. In this context, fish farms should review their hygiene measures and follow this issue with a more effective process. Different levels of resistance to antibiotics in different basins are due to bacterial diversity and contamination. For this reason, the veterinary opinion should be taken on the use of antibiotics, and appropriate antibiotics should be used against the target bacteria.

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Melatonin'in Tatlısu İstakozu (*Pontastacus leptodactylus* Eschscholtz, 1823) Yavrularında Gelişim, Hayatta Kalma Oranı ve Bağışıklık Yanıtı Üzerindeki Etkisi

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ÖZ

Bu çalışmanın amacı melatoninin, *Pontastacus leptodactylus* yavrularının (II-IV. dönem) gelişim, hayatta kalma oranı ve bağışıklık yanıtı (total hemosit sayısı) üzerindeki etkilerini araştırmaktır. Melatonin dozları 0, 0,5, 1, 2, 4 mg / kg oranında deneme yemine ilave edilerek 4 tekerrürlü 5 ayrı grup şeklinde deneme grupları oluşturulmuştur. Denemeler, II. Dönem *P. leptodactylus* yavruları ile kurulmuş ve 60 gün süre ile besleme yapılmıştır. Deneme süresince günde bir kez %50 oranında su değişimi ve sürekli bir havalandırma sağlanmıştır. Stok yoğunluğu her deneme tekerrürü için 20 yavru olarak belirlenmiştir. Yavrular, günde bir kez canlı ağırlıklarının %5'i oranında beslenmiştir. Yavruların başlangıç ortalama ağırlık ve total boyları 34 ± 4 mg ve 11,6 ± 0,4 mm olarak belirlenmiştir. Deneme sonunda en yüksek ortalama total boy 22,0 ± 3,6 mm olarak 2 mg/kg melatonin içeren grupta, en iyi ortalama canlı ağırlık 216 ± 116 mg olarak 4 mg/kg melatonin grubunda, en iyi yaşama oranı %44,4 ± 13,9 olarak 2 mg / kg melatonin doz grubunda ve en yüksek ortalama total hemosit miktarı ise 2,27 ± 0,49 x 10^6 hücre/ml olarak yine 2 mg/kg melatonin doz grubunda elde edilmiştir. Bununla birlikte gruplar arasındaki farklar istatistiki açıdan önemli bulunmamıştır ($p > 0,05$). Sonuç olarak, bu çalışmada *P. leptodactylus* yavrularının gelişim, hayatta kalma oranı ve bağışıklık yanıtı üzerinde melatonin katkılı yemler ile beslemenin önemli bir etkisinin olmadığı belirlenmiştir.

Anahtar kelimeler: Kerevit, *P. leptodactylus*, melatonin, hayatta kalma oranı, gelişim

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Effect of Melatonin on Development, Survival Rate and Immune Response in the Juveniles of Freshwater Crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823)

Abstract: The aim of this study was to investigate the effects of melatonin on growth, survival and immune response (total hemocyte counts) of *Pontastacus leptodactylus* juveniles (stage II-IV). With this aim, five separate groups with 4 replicates were formed by adding melatonin at the doses of 0, 0.5, 1, 2, 4 mg/kg to the trial feed. The experiments was conducted using stage II *P. leptodactylus* juveniles which fed for 60 days. During the experiments, a 50% water change once a day and continuous ventilation was provided. Stock density was determined as 20 juveniles for each replicate tank. The juveniles were fed at rate of 5% of their body weight once a day. The average initial weight and total length of juveniles were 34 ± 4 mg and 11.6 ± 0.4 mm, repectively. At the end of the experiment, the maximum average total length (22.0 ± 3.6 mm) was in the group fed with 2 mg/kg melatonin containing diet, the best average live weight (216 ± 116 mg) was in the group fed with 4 mg/kg melatonin containing diet, the highest survival rate (44.4 ± 13.9%) was in the 2 mg/kg melatonin group and the highest average total hemocyte count (2.27 ± 0.49 x 10^6 cells/ml) was in the 2 mg/kg melatonin group. However, the differences between the groups were not statistically significant ($p > 0.05$). As a result of this study, it was determined that feeding with melatonin supplemented feed have no significant effect on growth, survival and immune response of *P. leptodactylus* juveniles.

Keywords: Crayfish, *P. leptodactylus*, melatonin, survival rate, growth

Alıntılama
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Giriş

2017 yılında dünyada toplam su ürünleri üretimi 92,5 milyon ton olarak gerçekleşmiştir. Bu üretimin 15,2 milyon tonluk bölümünü kabuklu üretimi (avcılık ve yetiştiricilik) oluşturmaktadır. Kabuklu üretiminin 8,4 milyon tonu yetiştiricilikten, 6,8 milyon tonu avcılıktan elde edilmiştir. 15,2 milyon tonluk toplam kabuklu üretiminin ise yaklaşık 1,2 milyon tonluk bölümünü sadece tatlısu ıstakozları oluşturmuştur. Bu üretiminin sadece 86 bin tonu avcılıktan elde edilirken, çok büyük bir bölümü yetiştiricilikten sağlanmıştır (FAO 2019). Ülkemizde kerevit üretimi sadece doğal stoklardan avcılığa dayanmaktadır ve 2018 yılında 524 ton olarak gerçekleşmiştir (TÜİK 2019).

Türkiye'deki doğal kerevit türü (*P. leptodactylus*), yüksek tüketici talebi nedeniyle Avrupa'daki en önemli tatlı su istakozu türlerindendir (Harlioğlu vd. 2012). Astacid kerevitlerin kültürü çoğunlukla yarı entansif sistemlerde yapılmakta ve edile edilen II. dönem yavrular doğal toprak havuzlara ya da suni havuzlara stoklanmaktadır. Bununla birlikte, ılık su kerevitleriyle kıyaslandığında Astacid kerevitlerde yavrulardaki hayatta kalma oranları çok düşüktür (Mazlum 2007). Bu nedenle, *P. leptodactylus*'larda yavruların hayatta kalma oranlarının ve gelişimlerinin artırılması konusundaki çalışmalara ihtiyaç duyulmaktadır.

Son yıllarda su ürünlerinde büyümeyi ve yaşama oranını arttırmaya yönelik yapılan çalışmalarda; hormonlar, antibiyotikler, iyonoforlar, probiyotikler ve prebiyotikler yem katkı maddeleri olarak kullanılmaktadır. Dekapod krustaselerinde yapılan çalışmalarda, melatonin ve serotonin gibi biyolojik aminlerin üreme ve glikoz dengesi dahil olmak üzere birtakım fizyolojik aktivitelerin düzenlenmesinde etkili oldukları tespit edilmiştir (Fingerman 1997; Reddy ve Pushpalata 2007).

Balıklarda, epifiz bezinde triptofandan ve retinadan sentezlenen melatonin hormonu, biyolojik saatin düzenlenmesi ve mevsimsel değişikliğe olan adaptasyon gibi birçok fonksiyona sahiptir (Kırım vd. 2006). Melatoninin, ot sazanı (Kezuka vd. 1988), deniz levreği (Sánchez-Vázquez vd. 1997) ve kerevit (*Procambarus clarkii*)'de mevsimsel üreme aktivitesini düzenlediği bildirilmiştir (Agapito vd. 1995). Yine *P. clarkii*'de aktiviteyi ve metabolit seviyelerini etkilediği, bununla birlikte etkilerin hayvanların aktivite evresi ve melatonin uygulamasının zamanlaması ile değişebileceği, omurgalılarda olduğu gibi omurgasızlarda da ritmik süreçleri yöneten karmaşık sistemin bir bileşeni olabileceği bildirilmiştir (Tilden vd. 2003). Ayrıca balıklarda, pineal bezden salgılanan melatoninin, immün fonksiyonlar için temel bir eser element olan çinko seviyelerini düzenlediği ileri sürülmektedir (Kırım vd. 2006). Melatoninin çipura (*Sparus aurata*)'da bağışıklık ile ilgili genlerin (mainly IL-1b and IRF-1) olumlu yönde düzenlenmesini sağladığı bildirilmiştir (Cuesta vd. 2008). Yengeç (*Eriocheir sinensis*) de gelişim ile ilgili genlerin ekspresyonunu artırarak uzuv rejenerasyonu sağlayabildiği, sindirim enzim aktivitesini artırdığı, bağışıklık tepkisini güçlendirdiği, özellikle antioksidan kapasiteyi güçlendirdiği bildirilmiştir (Zhang vd. 2018). Kabuklulardaki doğuştan gelen immün sistem, infeksiyöz mikroorganizmalara karşı temel savunma mekanizmasıdır. Bu savunma sisteminde kan hücreleri hemositler önemli bir rol oynamaktadır (Lee ve Söderhäll 2002). Hemositler kaya ıstakozlarında veya diğer ıstakozlarda stres veya sağlık durumunun değerlendirilmesinde yararlı araçlar olabilir (Jussila vd.1997).

Bu çalışmada melatoninin *P. leptodactylus* yavrularının gelişim, hayatta kalma oranı ve bağışıklık sistemi (total hemosit sayısı) üzerindeki etkileri araştırılmıştır.

Materyal ve Metot

Çalışma, 2016 yılında Eğirdir Su Ürünleri Araştırma Enstitüsü Müdürlüğü bünyesinde yer alan Kerevit Araştırma Merkezi kuluçkahanesinde yürütülmüştür. Yumurtalı kerevitler, 2016 yılı Nisan ve Mayıs aylarında Eğirdir Gölü'nden kerevit sepetleri ile yakalanmış ve 2-3 hafta boyunca kapalı devre sistemdeki kültür koşullarına adapte edilmiştir. Anaçlar bu aşamada günde bir kez çiğ patates, alabalık peleti ve çiğ balık etiyle beslenmiştir. Yumurtalı anaçların adaptasyonunda 280 x 60 x 60 cm ebatlara sahip polyester tanklar kullanılmış, tanklara saklanma ihtiyaçlarını karşılamak için 8 cm çapında ve 12 cm uzunluğunda PVC borular yerleştirilmiştir.

Yavrular mayıs ayı sonu ile haziran ayının ilk haftası boyunca elde edilmiştir. Bu aşamada anaç ve yavruların yaklaşık olarak 15 gün birlikte kalmaları sağlanmıştır. Haziran ayının ilk haftasından sonra yavrular bağımsız yaşam evresine (II. dönem) geçmişlerdir. Dışardan besleme bu dönemde başlamış ve yavrular ilk olarak dondurulmuş *Artemia* nauplii, *Daphnia* sp. ve *Chrinomus* sp. ile 15 gün boyunca beslenmiştir. *Daphnia* sp. ve *Chrinomus* sp., açık alandaki tanklarda üretilmiş ve belli aralıklarla süzülüp hasat edilerek, -20 °C 'de saklanmıştır. Bu dönemde denemeye başlamadan önce yavruların yavaş yavaş granül yemlere alışmaları sağlanmıştır. Ardından, başlangıç ortalama ağırlıkları 34 ± 4 mg ve total boyları 11,6 ± 0,4 mm olan, yumurtadan çıkarak I. döneme geçen, 8-10 gün içinde kabuk değiştirip II. döneme ulaşarak annelerini tamamen terkeden ve beslenmeye başlayan (Balık, 1993) II. dönem

yavrular ile deneme başlatılmıştır. Deneme süresince yem artıkları, kopan uzuv parçaları, ölü yavrular ve dışkılar ortamdan sifonlama ile uzaklaştırılmıştır.

Deneme yemlerinin hazırlanması

Deneme yemleri, bir yem firması tarafından soğuk ekstrüzyon tekniği ile üretilmiştir. Melatonin, karides yemine, üretim aşamasında ilave edilmiştir. Deneme yemleri, %55 ham protein, %10 ham yağ, %9 ham kül, %1,4 ham selüloz ve 1,76 ham lif içermektedir. Hammadde kaynağı olarak balık, buğday ve krill unları, balık yağı ve hidrolizatı, bazı mikro algler, vitamin ve mineral premiksleri kullanılmıştır. Deneme yemlerinin hazırlanmasına geçilmeden önce melatonin hormonunun; (N-[2-(5-methoxy-1H-indol-3-yl) ethyl]), etil alkol ile çözünmesi sağlanmış, yeterli düzeyde distile su ilave edilerek homojen bir karışım elde edilmiş ve daha sonra, yeme ilave edilerek her bir doz grubu (0, 0,5, 1, 2 ve 4 mg melatonin/kg yem) için 5 farklı deneme yemi üretilmiştir. Deneme yemleri, granül şeklinde olup ebatları 800-1200µ olarak ayarlanmıştır. Deneme süresince yavru kerevitler, günde bir kez canlı ağırlıklarının %5'i oranında (Mazlum vd. 2011) beslenmiştir.

Deneme planı

Deneme tam tesadüfe bağlı deneme planına göre oluşturulmuş olup, 4 tekerrürlü 5 grup olarak planlanmıştır: Grup 1 (Kontrol, melatoninsiz yem), grup 2 (0,5 mg melatonin /kg yem), grup 3 (1 mg melatonin /kg yem), grup 4 (2 mg melatonin /kg yem), grup 5 (4 mg melatonin /kg yem) 60 gün boyunca sürdürülen denemede 60 x 40 x 30 cm ebatlarına sahip şeffaf plastik kaplar kullanılmış olup, her birine barınak malzemesi olarak delikli tuğlalar yerleştirilmiştir. Deneme boyunca, günde bir kez %50 oranında su değişimi ve havalandırma sağlanmıştır. Stoklama yoğunluğu her tekerrür için 20 yavru, olarak ayarlanmış olup tüm denemede toplam 400 adet yavru kullanılmıştır.

Yavrularda ağırlık ve total boy ölçümleri

Denemenin başlangıcında, 30. ve 60. (deneme sonu) günlerde yavrularda ağırlık ve total boy ölçümü yapılmıştır. Total boy (TL) ölçümü: (rostrumun ucundan telsonun son uzantısına kadar olan kısım), dijital kumpas ile ağırlık ölçümleri ise 0,001 g hassasiyetindeki terazi ile yapılmıştır.

Yavrularda yaşama oranlarının tespiti

Denemenin 30. ve 60. günlerinde yavrulardaki hayatta kalma (yaşama) oranı belirlenmiştir. Yaşama oranı, aşağıdaki formülle hesaplanmıştır:

$$YO\ (\%) = \frac{Ns}{Nb}\ x\ 100$$

YO = Yaşama Oranı
Ns = Deneme sonundaki kerevit sayısı
Nb = Deneme başlangıcındaki kerevit sayısı

Bağışıklık yanıtı üzerine olan etkilerin tespiti

Deneme sonunda her tekerrür için 4-5 yavrudan ayrı ayrı olmak üzere her gruptan yaklaşık 20 adet yavrunun kanı (hemolenf) ayrı ayrı alınarak hemolenfteki toplam hemosit miktarları belirlenmiştir. Yavrulardan hemolenf örnekleri alınmadan önce, stresi engellemek için, yavruların bulunduğu su, buz aküsü ile soğutulmuştur. Hemolenf örnekleri kerevit yavrularının 2. yürüme bacaklarının tabanından alınmış (Sepici-Dinçel vd. 2013) ve her örnek sadece bir yavrudan 1 ml'lik enjektör yardımıyla elde edilmiştir. Hemositlerin parçalanmasını ve pıhtılaşmayı engellemek için enjektöre önce 0,9 ml %4'lük formalin çekilmiş, daha sonra üzerine 0,1 ml hemolenf alınmış ve karıştırılmıştır. Daha sonra, bu karışımda (formalin+hemolenf) ışık mikroskonunda Thoma lamı ile toplam hemosit sayımı yapılmıştır (Miller ve Stanley 2000; Evans 2003; Ward vd. 2006). Hemositler mikroskop altında sayılmış ve toplam hemosit sayısı (THC) formülle hesaplanmıştır

$$\text{THC Toplam hemosit sayısı /ml} = \frac{Toplam\ hücre\ sayısı \times 2 \times 10 \times 1000}{16}$$

(Sepici-Dinçel vd. 2013).

Su kalitesi parametrelerinini ölçümü

Deneme süresince su sıcaklığı (termometre ile), çözünmüş oksijen (Oksijen metre ile) ve pH (PH metre ile) günlük olarak, amonyak, nitrat ve nitrit miktarı (Spektrofotometrik yöntemle) haftada bir kez ölçülmüştür.

Verilerin değerlendirilmesi

Verilerin normal dağılımı Kolmogrov Smirnov testi ile analiz edilmiştir. Normal dağılım gösteren parametrik verilerde grupların karşılaştırılması Tek Yönlü Anova testi, normal dağılım göstermeyen non-parametrik veriler ise Kruskal–Wallis sıralamalı tek–yönlü varyans analizi ile yapılmıştır. Gruplar arasında farklar için verinin normal dağılım sonucuna göre Duncan veya Tukey çoklu karşılaştırma testleri uygulanmıştır (Özdamar 2011). Ağırlık, boy ve hayatta kalma ile ilgili sonuçlar, ortalama ± standart sapma ($\bar{X} \pm SS$), toplam hemosit sayıları ise ortalama ± standart hata ($\bar{X} \pm SH$) şeklinde verilmiştir. Tüm istatistiki testlerde önem seviyesi α=0,05 olarak kabul edilmiştir. Veriler IBM SPSS Statistics v.25 ve Microsoft Excel 2013 paket programları ile işlenmiş ve değerlendirilmiştir.

Bulgular

Melatoninin gelişim üzerindeki etkisi

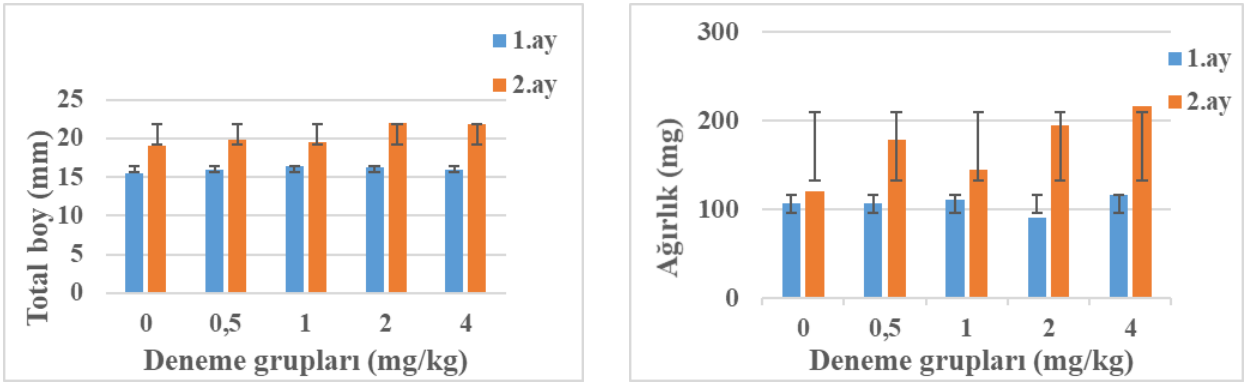
Denemenin 30. ve 60. günlerinde yapılan ölçümlerde boy ve ağırlık değerleri açısından gruplar arasında istatistiki açıdan önemli bir fark bulunmamıştır ($p > 0,05$) (Tablo1, Şekil 1).

Tablo 1. Melatonin dozlarına göre yavrularda aylık ortalama total boy (mm) ve ağırlık (mg) değerleri
Table 1. Average monthly total height (mm) and weight (mg) values of offspring according to melatonin doses

Melatonin Doz Grupları (mg/kg)	Ortalama Total Boy (mm)			Ortalama Ağırlık (mg)		
	Başlangıç Boyu	30. gün	60. gün	Başlangıç Ağırlığı	30. gün	60. gün
	$\bar{X} \pm SS$	$\bar{X} \pm SS$	$\bar{X} \pm SS$	$\bar{X} \pm SS$	$\bar{X} \pm SS$	$\bar{X} \pm SS$
Kontrol	$11,6 \pm 0,4^{b}$	$15,5 \pm 1,9^{a}$	$19,1 \pm 2,4^{a}$	34 ± 3^{b}	107 ± 38^{a}	120 ± 38^{a}
0,5	$11,6 \pm 0,4^{b}$	$15,9 \pm 1,9^{a}$	$19,9 \pm 2,4^{a}$	34 ± 3^{b}	107 ± 37^{a}	178 ± 60^{a}
1	$11,6 \pm 0,4^{b}$	$16,4 \pm 2,0^{a}$	$19,6 \pm 0,1^{a}$	34 ± 3^{b}	110 ± 48^{a}	144 ± 06^{a}
2	$11,6 \pm 0,4^{b}$	$16,3 \pm 2,2^{a}$	$22,0 \pm 3,6^{a}$	34 ± 3^{b}	90 ± 38^{a}	195 ± 93^{a}
4	$11,6 \pm 0,4^{b}$	$15,9 \pm 2,2^{a}$	$21,9 \pm 3,7^{a}$	34 ± 3^{b}	116 ± 34^{a}	216 ± 116^{a}

Boy ve ağırlık bazında aynı satırda farklı harflerle gösterilen ortalamalar arasındaki fark istatisitiki olarak önemlidir ($p < 0.05$). Aynı sütunda tek bir ölçüm gününde (Başlangıç, 30 ve 60. günler) deneme gruplarına ait ortalamalar arasındaki fark istatistiki olarak önemli değildir ($p > 0,05$).

The difference between the means shown in different letters on the same line is statistically significant ($p < 0.05$) on the basis of height and weight. The difference between the means of the experimental groups on a single measurement day (beginning, 30th and 60th days) in the same column is not statistically significant ($p > 0.05$).



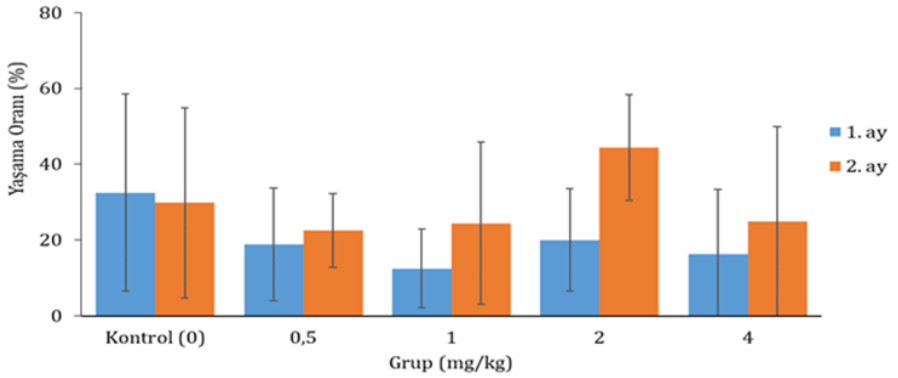
Şekil 1. Yavrularda melatonin dozlarına göre aylık ortalama total boy (mm) ve ağırlık (mg) değerleri
Figure 1. Average monthly total height (mm) and weight (mg) values according to melatonin doses in offspring

Melatoninin hayatta kalma oranları üzerindeki etkisi

Kerevit yavrularında yeme melatonin ilavesinin hayatta kalma oranlarında olumlu bir etkisinin olmadığı bulunmuştur ($p > 0,05$) (Tablo 2, Şekil 2).

Tablo 2. Farklı dozda melatoninli yemlerle beslenen yavrularda aylık yaşama oranları (%)
Table 2. Monthly survival rates (%) of offspring fed different doses of melatonin containing feeds

Melatonin Doz Grupları (mg/Kg)	1. Ay	2. Ay
Kontrol (0)	$32,5 \pm 26,0$	$29,8 \pm 25,1$
0,5	$18,8 \pm 14,9$	$22,5 \pm 9,8$
1	$12,5 \pm 10,4$	$24,4 \pm 21,4$
2	$20,0 \pm 13,5$	$44,4 \pm 13,9$
4	$16,3 \pm 17,0$	$25,0 \pm 25,0$



Şekil 2. Melatonin doz gruplarına göre yavrularda aylık hayatta kalma oranları (%)

Figure 2. Monthly survival rates (%) in offspring according to melatonin dose groups

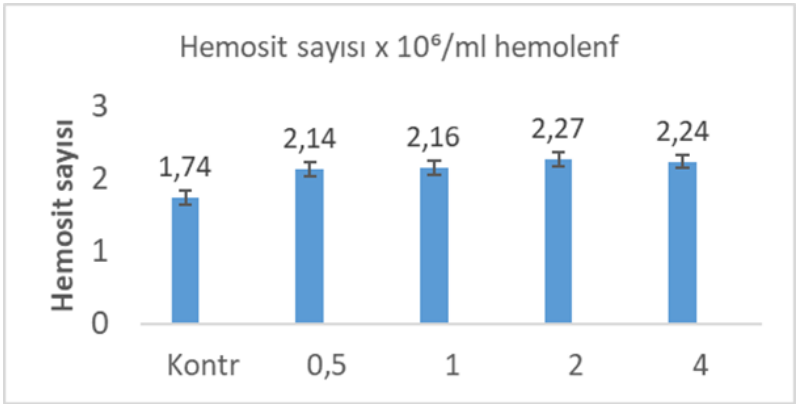
Melatoninin bağışıklık sistemi (toplam hemosit sayısı) üzerindeki etkisi

Deneme sonunda deneme gruplarında belirlenen toplam hemosit sayıları arasında istatistiksel olarak önemli bir fark bulunmamıştır ($p > 0,05$) (Tablo 3, Şekil 3).

Tablo 3. Gruplara göre hemolenfteki toplam hemosit değerleri (x 10^6 hücre / ml)

Table 3. Total hemocyte values in hemolymph by groups (x 10^6 cells / ml)

Melatonin Deneme Grupları (mg/kg)	Total Hemosit Sayıları $\bar{X} \pm SH$
Kontrol Grubu	1,74 ± 0,34
0,5	2,14 ± 0,39
1	2,16 ± 0,73
2	2,27 ± 0,49
4	2,24 ± 1,06



Şekil 3. Deneme sonunda hemolenfteki total hemosit sayıları (x 10^6 hücre/ml)

Figure 3. Total hemocyte counts in hemolymph at the end of the trial (x 10^6 cells / ml)

Deneme süresince tespit edilen su kalitesi parametreleri

Deneme süresince su sıcaklığı, çözünmüş oksijen ve pH günlük olarak, amonyak, nitrat ve nitrit miktarı haftada bir kez ölçülmüş ve ortalama değerler Tablo 4'de verilmiştir.

Tablo 4. Deneme boyunca tespit edilen su kalitesi parametreleri (Ortalama ± SS)

Table 4. Water quality parameters during the trial (Mean ± SD)

pH	Sıcaklık (C°)	Oksijen (mg/l)	NO_3-N (mg/l)	NO_2-N (mg/l)	NH_4-N (mg/l)
8,37 ± 0,14	20,3 ± 2,01	7,6 ± 1,14	4,26 ± 1,84	0,03 ± 0,02	0,07 ± 0,04

Tartışma ve Sonuç

Çalışmada yeme farklı dozlarda ilave edilen melatoninin, kerevit yavrularının deneme sonu final ağırlık ve total boyları üzerinde önemli bir etkisi bulunmamış olup gruplar arasında ortaya çıkan farkların istatistiki açıdan önemli olmadığı görülmüştür ($p > 0,05$). Singh vd. (2012) ise melatoninin nil tilapialarında 25 µg/L dozunda 3 hafta boyunca yetiştiricilik suyuna ilavesini takiben, spesifik gelişim oranı üzerinde baskılayıcı bir etkisi olduğunu bildirmişlerdir. Benzer şekilde, havuz balıklarında (*Carassius auratus*) da 10 µg/g vücut ağırlığı/g dozunda melatonin kullanımının vücut ağırlığında azalmaya neden olduğu bildirilmiştir (López-Olmeda vd. 2006; De Pedro vd. 2008).

Kabuklulardaki doğuştan gelen immün sistem, infeksiyöz mikroorganizmalara karşı temel savunma mekanizmasıdır. Bu sistem patojenler de dahil olmak üzere yabancı materyalleri tanımak, yok etmek için hızlı ve etkin bir sistemdir ve erken aşamada enfeksiyonları sınırlamaya yardımcı olan ilk savunma hattıdır. Bu savunma sisteminde kan hücreleri hemositler önemli bir rol oynamaktadır (Lee ve Söderhäll 2002). Hemolenfteki toplam hemosit sayısı (THC), diferansiyel hemosit sayısı (DHC), hemolenf pıhtılaşma süresi, bakteriyemi ve lizozomal membran stabilitesi bağışıklık tepkisinin göstergesi olarak değerlendirmek için araçlar olarak kullanılabilir (Sang ve Fotedar 2009). Hemositler kaya ıstakozlarında veya diğer ıstakozlarda stres veya sağlık durumunun değerlendirilmesinde yararlı araçlar olabilir (Jussila vd.1997).

Çalışmamızda, yeme melatonin ilavesinin kerevit yavrularında hemosit sayıları üzerinde önemli bir etkisi bulunmamıştır ($p > 0,05$). Buna karşın yengeçlerde (*Eriocheir sinensis*) de bağışıklık tepkisini güçlendirdiği bildirilmiştir (Zhang vd. 2018). Benzer şekilde, Randall vd. (1991), gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) melatoninin immün sistem ve özellikle hücresel bağışıklığı direkt ve indirekt yollarla etkilediğini belirtmişlerdir. Ayrıca yine melatoninin çipuralarda (*Sparus aurata*) bağışıklık ile ilgili genlerin (mainly IL-1b and IRF-1) olumlu yönde düzenlenmesini sağladığı bildirilmiştir (Cuesta vd. 2008). Çalışmada bağışıklık tepkisi ile ilgili sadece toplam hemosit sayısına bakılabilmiştir. Oysa bağışıklık tepkisinin diğer göstergeleri olan diferansiyel hemosit sayısı, hemolenf pıhtılaşma süresi, bakteriyemi ve lizozomal membran stabilitesi gibi immün sistem yanıtlarının da gelecek çalışmalarda ortaya çıkarılması yararlı olabilir.

Sonuç olarak, kerevit yavrularının (II.-IV. dönem) yemlerine 0,5, 1, 2, 4 mg/kg oranında meletonin ilavesi yapılarak beslemenin ağırlık ve boy kazancı, hayatta kalma oranı ve bağışıklık tepkisi (Total hemosit sayısı) üzerinde önemli bir etkisinin olmadığı saptanmıştır. Özellikle gelişim ve sağ kalım üzerindeki etkilerinin ortaya çıkmasına engel olarak kerevitlerde görülen ve önemli bir sorun teşkil eden kanibalizmin rolünü düşündürmektedir. Bununla birlikte, gelecekte yapılacak çalışmalarda melatoninin kerevitlerde kıskaç rejenerasyonu, sindirim enzim aktiviteleri, antioksidan kapasitesi ve biyoritim üzerindeki etkilerinin araştırılması yararlı olacaktır.

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Rotifer Diversity in Coal Mine Generated Pit Lakes of Raniganj Coal Field Area, West Bengal, India

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ABSTRACT

The research looks at the rotifer diversity in five different coal mine generated pit lakes from Raniganj Coal Field Area (RCF), West Bengal, India. The collection methodology was involved monthly sampling (n = 120) to analyze the rotifer diversity using surface hauling with standard plankton net of mesh size 55 µm and water column at different depths (20 cm to 50 cm) for the periods of two years (February 2018 – January 2020). Analyzes of some limnological parameters and macrophytes were also performed following standard protocol. Statistical analysis based on the physicochemical parameters showed that Harabhanga and Dhandardihi 1 Pit Lakes were more similar while Dhandardihi 1 Pit Lake and Dhandardihi 2 Pit Lake were more alike in terms of rotifer community structure. Seventeen taxa of rotifers under the five families were found with varying densities and diversity indices. The highest diversity was observed in the Searsole Pit Lake, and the dominant species was *Keratella tropica* Apstein. The five pit lakes can be separated from each other based on the variations in rotifer diversity and water quality parameters, advocating the implementation of limnological management. Our results indicated different abiotic and biotic variables influencing the rotifer assemblages and diversity of the pit lakes studied.

Keywords: Rotifer, pit lake, limnological variables, density, macrophyte

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Introduction

Members of the phylum Rotifera, a significant part of the aquatic metazoans, can be observed in almost any form of aquatic habitats having worldwide distribution and comprise two main groups: Bdelloidea and Monogonta while the taxon Seisonacea covers marine species (Segers 2007, 2008; Fontaneto and De Smet 2015). The morphological peculiarities and high richness of rotifers are key aspects in many investigations on freshwater ecosystems (Sharma and Michael 1980). The descriptive features of the rotifers were applied by many scientists for ecological analysis (Bai et al. 2006) and also for the water quality assessment. Besides, the impact of various abiotic parameters affecting the biological environment is also mirrored in population patterns and shifts in the rotifers' species architecture and functions (Chovanec et al. 2002; Dong 2004). The rotifer diversity is considerably large globally with at least 544 taxa reported from the Oriental region (Segers 2001, 2002, 2008). Approximately, 500 rotifer species have been reported from Indian water bodies and about 1700 of them have been reported from various regions of the world (Kiran et al. 2007). A substantial number of studies on the faunal diversity of Rotifera from aquatic biomes of the protected parts of the region of Northeastern India is seen in Indian literature. While taxonomic analyses of Indian rotifers were started more than a century ago, there has been still insufficient data on rotifer biodiversity in Indian aquatic bodies (Sharma and Sharma 2011). About 303 rotifer species has been reported from the Northeast India (Sharma and Sharma 2019). Rotifers play a significant part in the cycle of freshwater fish breeding, as it is an important live food for larval and early juvenile fish stages (Velasco-Santamaría and Corredor-Santamaría 2011; Shil et al. 2013).

Therefore, the nutrient dynamics and freshwater production are significantly governed by the rotifers (Snell 1998; Lin et al. 2005). Evaluation of the rotifers' diversity is key to demonstrate their importance in sustaining freshwater habitats (Gannon and Stemberger 1978; Dumont 1983). A negligible quantity of work was performed about the population ecology, habitat distribution of the zooplankton community in multiple wetlands, lakes and ponds in India (Sinha et al. 1994; Kumar 2001; Khan 2003; Bhalla et al. 2012; Deepthi and Yamakanamardi 2014). Furthermore, less number of data supporting the comprehensive review of rotifers with reference to the various physicochemical parameters in the man-made pit lake ecosystem is known. Though pit lakes are prevalent in several regions of India, these aquatic ecosystems have not been adequately studied. Water chemistry can vary significantly in different types of mining activities. Relatively high conductivity is observed in these huge water bodies (Ciszewski et al. 2013; Wołowski et al. 2013; Sienkiewicz and Gasiorowski 2015; Geller et al. 2013). These lakes formed by mining operations or extraction appear to be huge in-depth with sloppy sides (Blanchette and Lund 2016). Remarkably few biotic investigations were executed in these water bodies (Ferrari et al. 2015) since these researches are complicated to perform and needs specialized methodologies (Woelfl and Whitton 2000). Researchers have, therefore, started to find out different ecological components in these ecosystems, biotic succession and population dynamics (Geller et al. 2013; Wołowski et al. 2013; Sienkiewicz and Gasiorowski 2015; Vucic et al. 2019). The available information is inadequate to identify the trophic position of planktonic species in the mine lakes food system, which is different from that of more traditional lakes. Previous research has found that a few organisms dominate these pit lakes, like *Brachionus* sp., *Cephalodella* sp., *Rotaria* sp., *Elosa* sp., etc. (Deneke 2000). The work can demonstrate the application of rotifers as water quality indicators (Saksena 1987), thereby facilitating the monitoring of the resources of the aquatic ecosystem in the region (Kar 2014). The present work is a groundbreaking attempt which offers the first comprehensive evaluation of the rotifer ecology from the pit lake ecosystem of RCF. In addition to identifying and listing the inventory of different rotifer organisms from five distinct pit lakes, the findings of this analysis would also illustrate the variability of rotifers from the RCF. This study also highlights the association between aquatic macrophytes and rotifer assemblages in the pit lake ecosystem for the first time. To explore the ecosystem framework and its interaction with the limnological characteristics of these regions, some experimental works are required to establish a theoretical foundation for ecological regeneration, fair use and conservation of pit lake resources and an awareness of a healthy aquatic ecosystem. The finding represents valuable information to the diversity of Indian Rotifera. This result also allows us to enhance our understanding of rotifers' ecology in the pit lake system and unearth important connections between rotifer species and their ecosystem. The aims of this study were to (i) examine the spatial variability of rotifer populations in relation to various biotic and abiotic factors in the pit lake ecosystem created by the coal mine, West Bengal, (ii) provide and contribute to fill the research gap concerning the composition of rotifer communities in pit lakes, and (iii) assess the ecological integrity of the studied lakes utilizing pertinent data gathered during this research. This data will support the development of a comprehensive management strategy for developing in these water bodies a foundation for sustainable water protection and the development of fish stocks.

Materials and Methods

Study Sites

The sampling was made for two years running between February 2018 and January 2020, using five pit lake habitats: Harabhanga (H) Pit Lake, Dhandardihi 1 (D1) Pit Lake, Dhandardihi 2 (D2) Pit Lake, Searsole (S) Pit Lake and Dalurbandh (Dal) Pit Lake under RCF located in West Bengal, India. The detailed study area is represented in Table 1 and Figure 1.

Table 1. Physiography properties of the study sites

Study sites	Block	Mean Depth	Location
HARABHANGA (H)	RANIGANJ	~ 22.86 m	23°36'39.65"N, 87°3'49.18"E
SEARSOLE (S)	RANIGANJ	~ 31.50 m	23°37'35.38"N, 87°6'9.88"E
DHANDARDIHI 1(D1)	ANDAL	~ 30.48 m	23° 37' 26.58 " N, 87° 9 '22. 85" E
DHANDARDIHI 2(D2)	ANDAL	~ 25.91 m	23° 37'41.14"N, 87° 9'26.20"E
DALURBANDH(Dal)	PANDABESWAR	~ 22.86 m	23°42'48.68"N, 87°15'35.70"E

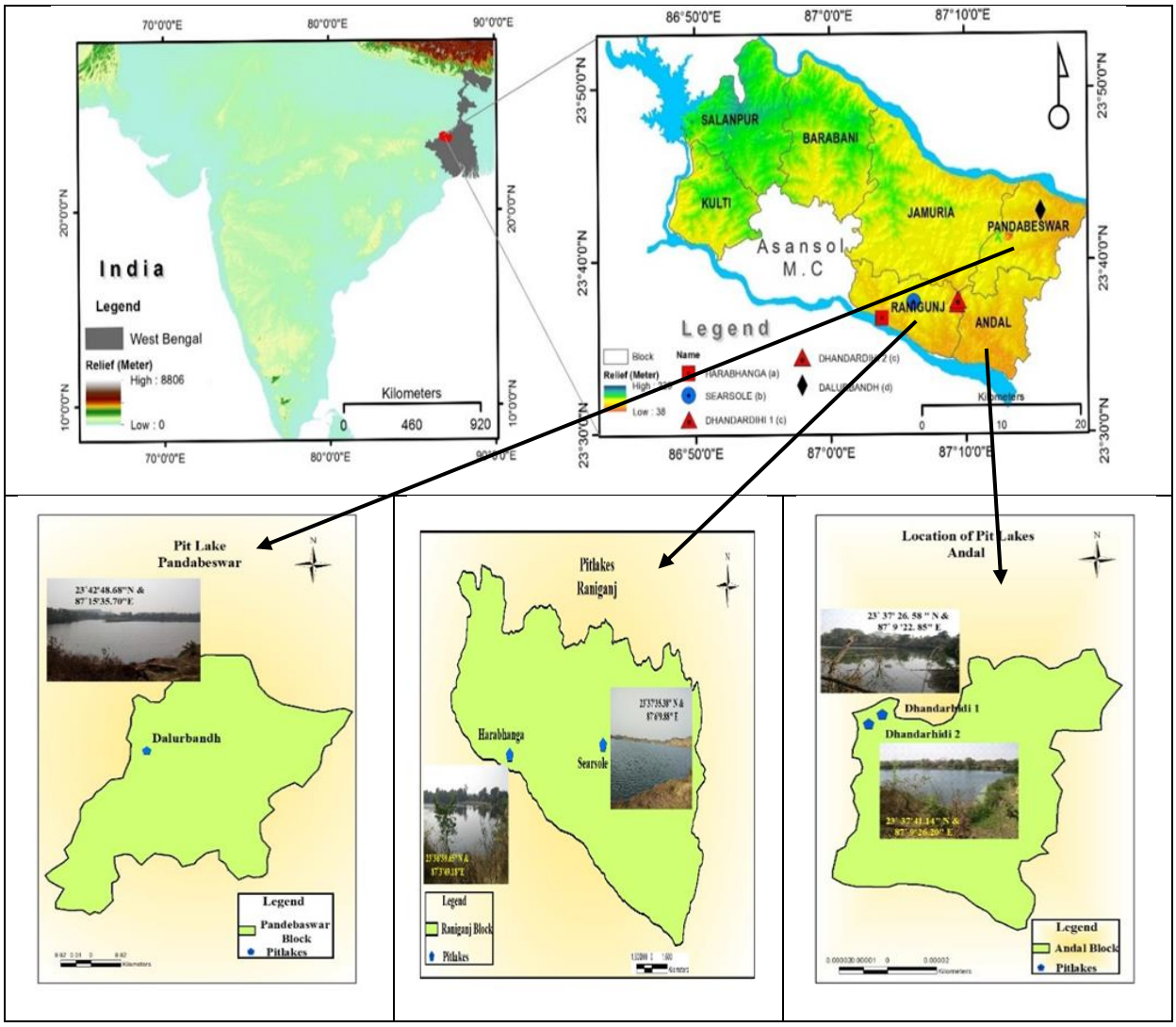


Figure 1. Map showing the study sites

Rotifer and Water Sampling

Random stratified sampling was implemented in compliance with the plankton selection standards (n= 24 per site) (Edmondson 1959; Battish 1992). The methodology involved monthly sampling of the mentioned sites to analyze the zooplankton using surface hauling with standard conical plankton net of mesh size 55 µm, 50 cm diameter and water column at surface water between 20 and 50 cm. The plankton net was drawn for a specific sample at a distance of between 5 and 10 m from the bank of the pit lakes. The equal force was exerted in the process of pulling the net across the water column to ensure a steady flow of water through the net. For a specific sample, a repetition of at least four hauls was conducted from four different locations of the investigated water bodies. This was packed in plastic containers after the plankton was harvested from the sites (100 mL), filtered and were fixed and preserved in a neutral formalin solution (4%) (May and O'Hare 2005).

The density and composition were calculated using the Sedgwick-Rafter counting chamber and compound microscope (Olympus Magus, Ch20i) (×10 and ×40) for identification up to species level. The rotifer species count was consequently represented in a unit liter of sample. The photos of the respective individuals were taken during the evaluation of the specimens in the collections to evaluate and validate the identity (Edmondson 1959; Battish 1992; Sharma 1998). Shannon and Weaver diversity index (Shannon and Weaver 1963), evenness index (Pielou 1966), and species richness index (Margalef 1958) were determined. Such indices help to interpret a specific ecosystem's population framework. The similarity index (Jaccard 1901) was also determined. Analyzes of some limnological variables from the same pit lakes were also made. Water temperature, pH, salinity, total dissolved solids (TDS) and conductivity were taken *in situ* using a multi-parameter probe (Oakton PCSTestr 35). Dissolved oxygen (DO) was measured following Winkler method (APHA 2005) and for the other variables such as total alkalinity, hardness, turbidity, biochemical oxygen demand (BOD), nitrate, and phosphate, the samples were evaluated in

the laboratory following the standard protocol (APHA 2005).

Macrophyte Analysis

Aquatic macrophyte abundance was reported at each site on a five-point scale (1: scarce or non-vegetable, 2: many individuals, 3: tiny clusters of vegetation, 4: consistent vegetation, and 5: 100% covers) by visual assessment and walking along the embankment of the studied pit lakes during sampling (Stefanidis and Papastergiadou 2010).

Statistical Analysis

Hierarchical cluster analysis (HCA) focused on physicochemical parameters and rotifer density was provided in dendrogram to demonstrate the association among study areas using PAST statistical software (Hammer et al. 2001). The non-parametric Spearman correlation was done to portray the association between the physicochemical and biological variables using SPSS statistical software. The Mann–Whitney test and Kolmogorov–Smirnov test were performed to establish the differences in the rotifer community. Canonical correspondence analysis (CCA) was also performed between the limnological variables and different indices as the response variables. PAST statistical software was used to conduct all the statistical analyses.

Results

The rotifer community of the five pit lakes consisted of 17 rotifer taxa (Table 2). Dhandardihi 1 and Searsole Pit Lakes represented the highest number of species (11), Harabhanga Pit Lake by 10 species, while Dhandardihi 2 and Dalurbandh Pit Lakes by 8 and 9 respectively. *Brachionus angularis*, *B. calyciflorus* Pallas, 1766, *B. falcatus* Zacharias, 1898, *B. forficula* Wierzejski, 1891, *B. diversicornis* Daday, 1883, *Keratella tropica* Apstein, 1907 and *Filinia longiseta* Ehrenberg, 1834 were observed in all the study sites. The species richness was the lowest in Dhandardihi 1 Pit Lake, with 8 species while it was the highest in Dhandardihi 2 Pit Lake and Searsole Pit Lake with 11 species (Table 2) each. The number of rotifer organisms in the samples ranged from 57 to 153 individuals / L (mean 98.40 ± 1.65 SE; n = 120 samples). The relative abundance was varied in different study sites (Figure 2). The rotifer per samples in the Harabhnaga Pit Lake was: ranges 120–153; mean 140.10 ± 1.16 SE; n = 24; in Dhandardihi 1 Pit Lake: ranges 60–80; mean 68.30 ± 0.73 SE; n = 24; in Dhandardihi 2 Pit Lake: ranges 38–65; mean 58.72 ± 0.75 SE; n = 24; in Searsole Pit Lake: ranges 95–125; mean 113.06± 0.99 SE; n = 24 and in Dalurbandh Pit Lake: ranges 80-120; mean 109.48 ± 1.44 SE; n = 24. In Harabhanga, Dhandardihi 1 and Dalurbandh Pit Lakes, *K. tropica* was the dominant species (Figure 2), whereas in Searsole Pit Lake, *Brachionus diversicornis* was the dominant one. In Dhandardihi 2 Pit Lake, *B. forficula* was the dominant one. The Harabhanga Pit Lake showed the highest rotifer abundance accounting for 31.27% of total rotifer and the lowest in Dhandardihi 2 Pit Lake (11.58%) (Figure 2).

Different ecological indices values including the Shannon and Weaver diversity index (H′), Pielou's evenness (E) and Margalef's richness index (D) are represented in Table 3. The results showed that the Shannon and Weaver index and Pielou's evenness were the highest in Searsole Pit Lake. These indices are useful for understanding the community structure of a given ecosystem. The Jaccard's similarity index (SJ) for the study sites was compared and highlighted in Table 3 to contrast the similarities between the rotifer compositions among the study sites. Both the Harabhanga and Searsole Pit Lakes showed the highest similarity in terms of rotifer composition (0.429) while least similarities between Dhandardihi 1 and Searsole Pit Lakes (0.318), and between Dhandardihi 1 and Dalurbandh Pit Lakes (0.318) also.

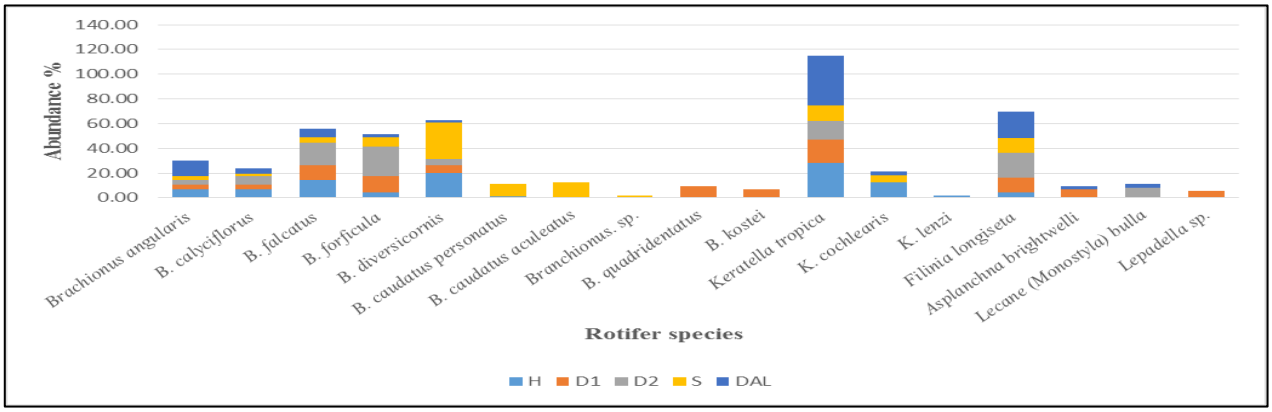


Figure 2. The abundance (%) of different rotifer species at study sites

Table 2. Inventory of rotifer taxa collected in the five pit lake habitats (n=24 per habitat)

Sl. no.	Scientific name	Acronym	(H)	(D1)	(D2)	(S)	(Dal)
	Family: Brachionidae		10	11	8	11	9
1	*Brachionus angularis* Gosse, 1851	Ba	+	+	+	+	+
2	*B. calyciflorus* Pallas, 1766	Bc	+	+	+	+	+
3	*B. falcatus* Zacharias, 1898	Bf	+	+	+	+	+
4	*B. forficula* Wierzejski, 1891	Bfo	+	+	+	+	+
5	*B. diversicornis* Daday, 1883	Bd	+	+	+	+	+
6	*B. caudatus personatus* Ahlstrom, 1940	Bcp	+	-	-	+	-
7	*B. caudatus aculeatus* Hauer, 1937	Bca	-	-	-	+	-
8	*Branchionus* sp.	Bs	-	-	-	+	-
9	*B. quadridentatus* Hermann, 1783	Bq	-	+	-	-	-
10	*B. kostei* Shiel, 1983	Bk	-	+	-	-	-
11	*Keratella tropica* Apstein, 1907	Kt	+	+	+	+	+
12	*K. cochlearis* Gosse, 1851	Kc	+	-	-	+	+
13	*K. lenzi* Hauer, 1953	Kl	+	-	-	-	-
	Family: Filiniidae						
14	*Filinia longiseta* Ehrenberg, 1834	Fl	+	+	+	+	+
	Family: Asplanchnidae						
15	*Asplanchna brightwelli* Gosse, 1850	Ab	-	+	-	-	+
	Family: Lecanidae						
16	*Lecane* (*Monostyla*) *bulla* Gosse, 1851	Lb	-	-	+	-	-
	Family: Lepadellidae						
17	*Lepadella sp.*	Le	-	+	-	-	-

'+' represents present of species and '-' represents the absence of species

Table 3. Different indices reflecting the community structure of the study sites

Ecological indices	**Study sites**				
	H	**D1**	**D2**	**S**	**DAL**
Shannon and Weaver diversity index (H')	1.978	2.285	1.921	2.559	1.783
Pielou's evenness index (E)	1.978	2.194	2.128	2.457	1.783
Margalef's richness index (D)	1.769	2.323	1.724	2.089	1.946

Study sites	**Jaccard's similarity index**				
	H	**D1**	**D2**	**S**	**DAL**
H					
D1	0.333				
D2	0.389	0.368			
S	**0.429**	**0.318**	0.368		
DAL	0.400	**0.318**	0.389	0.381	

Physicochemical -Parameters of Water

Mean values (with standard deviation) of physicochemical parameters obtained from the different pit lakes were given in Table 4. The water temperature was recorded to be highest in Dhandardihi 2 Pit Lake (32.20°C) and lowest in Harabhanga Pit Lake (28.13°C). The pH was recorded highest in Dhandardihi 2 Pit Lake (8.10±0.24), whereas lowest in Searsole Pit Lake (6.14 ± 0.06). TDS and conductivity were recorded highest from Searsole Pit Lake (581.33 ± 11.85 and 949±5.29 respectively). Total alkalinity was lowest at Harabhanga Pit Lake (22.22 ± 6.41), while the mean total alkalinity was highest in Dhandardihi 1 Pit Lake (28.00 ±4.67). Mean total hardness was recorded highest from Searsole Pit Lake (405 ±5). DO content showed highest in Dalurbandh and lowest in Dhandardihi 1 Pit Lakes. BOD was highest in Searsole and lowest in Dhandardihi 1 Pit Lake. The highest phosphate content was observed at Harabhanga Pit Lake and lowest in Dhandardihi 2 Pit Lake. The lowest and highest nitrate values were recorded from Searsole and Dalurbandh Pit Lakes, respectively. The lowest and highest turbidity values were recorded from Dalurbandh and Dhandardihi 2 Pit Lakes, respectively.

Table 4. The physicochemical parameters recorded at five different pit lakes of the study area

Sites	Temp (°C)	pH	TDS mg/l	CON µS/cm	TH mg/l	TA mg/l	SAL mg/l	DO mg/l	BOD mg/l	PHOS mg/l	NIT mg/l	TUR NTU
H	28.13 ± 4.40	6.97 ± 0.12	184 ± 6.24	186.66 ± 4.14	219.77 ± 3.80	22.22 ± 6.41	211.33 ± 4.73	5.08 ± 0.52	2.28 ± 0.11	3.40 ± 2.91	1.33 ± 1.27	5.27 ± 1.80
D1	31.77 ± 3.49	7.85 ± 0.37	249.88 ± 13.42	258.33 ± 5.78	83.11 ± 5.67	28.00 ± 4.67	330 ± 15.13	4.25 ± 0.32	2.19 ± 0.13	1.60 ± 1.43	1.79 ± 1.00	5.98 ± 0.95
D2	32.20 ± 3.36	8.10 ± 0.24	250.78 ± 5.67	244.17 ± 21.36	189.56 ± 13.54	23.55 ± 0.38	192.33 ± 20.55	4.60 ± 0.36	2.37 ± 0.14	0.835 ± 0.50	1.34 ± 0.21	21.33 ± 3.27
S	28.9 ± 3.56	6.14 ± 0.06	581.33 ± 11.85	949 ± 5.29	405 ± 5	27.5 ± 2	412 ± 2	5.87 ± 0.70	3.26 ± 0.04	2.47 ± 0.26	0.91 ± 0.39	4.56 ± 0.86
DAL	29.73 ± 2.80	7.29 ± 0.60	308.44 ± 2.91	448.41 ± 8.07	204.22 ± 2.77	27.11 ± 1.68	247 ± 3	6.46 ± 0.81	2.94 ± 0.46	2.60 ± 1.64	2.02 ± 1.03	2.09 ± 0.09

TDS – Total dissolved solids, CON – Conductivity, TH – Total hardness, TA – Total alkalinity, SAL – Salinity, DO – Dissolved oxygen, BOD – Biochemical oxygen demand, PHOS – Phosphate, NIT – Nitrate, TUR – Turbidity.

Aquatic Macrophytes

During the investigation 20 aquatic macrophytes comprising 19 families were recorded (Table 5). Maximum macrophytes vegetation was found in the Harabhanga Pit Lake. Among the macrophytes, *Hydrilla verticillata* was the most abundant submerged macrophytes, *Azolla pinnata* was the most abundant free floating macrophyte while *Ipomoea aquatica* was the most abundant floating macrophytes, and *Marsilea minuta* was the most abundant rooted floating type macrophytes.

Statistical Analysis

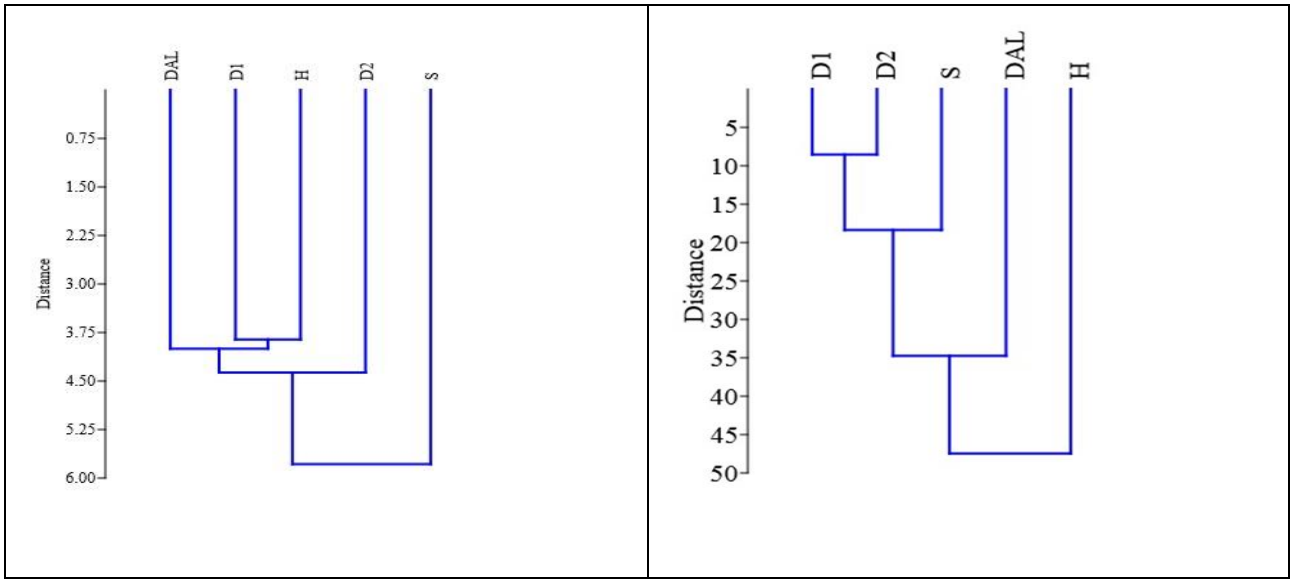
Hierarchical cluster analysis based on the different physicochemical factors placed Dhandardihi 1 and Harabhanga Pit Lakes in a cluster (D1+H) while the remaining lakes viz., Dhandardihi 2, Searsole and Dalurbandh did not form any separate cluster. Here Dalurbandh Lake being more similar to (D1+H) united to forma (D1+H+DAL) cluster. Next Dhandardihi 2 Lake joined individually to make a (D1+H+DAL+D2) cluster and finally the least similar Searsole Lake united with (D1+H+DAL+D2) cluster to complete the linkage (Figure 3a). However, when the analysis was determined regarding the zooplankton abundance, it showed different results (Figure 3b) that Dhandardihi 1 and Dhandardihi 2 Pit Lakes together formed a cluster (D1+D2), whereas other lakes did not make any separate cluster among them. Here Searsole Lake being more alike with (D1+D2) compared to other individual lakes, it joined to form a (D1+D2+S) cluster. With this cluster next closer Lake Dalurbandh joined and finally least similar Harabhanga Lake in rotifer community united to complete the linkage. *Brachionus calyciflorus* showed a significant negative correlation with the conductivity, salinity and alkalinity ($r= -0.900$; $p< 0.05$).

Table 5. The table presents the list of aquatic macrophytes observed at the study sites along with their ecotype

Sl. No.	Scientific name	Family	Habitat	D1	D2	S	D	H
1	*Chara* sp.	Characeae	S	-	-	-	-	+
2	*Eichhornia crassipes*	Pontederiaceae	FF	+	-	-	+	+
3	*Enydra fluctuans*	Asteraceae	F	-	+	+	-	-
4	*Hydrilla verticillata*	Hydrocharitaceae	S	+	-	+	+	+
5	*Ipomoea aquatica*	Convolvulaceae	F	+	-	+	-	+
6	*Nymphaea* sp.	Nymphaeaceae	F	-	-	+	-	+
7	*Nymphaea nouchali*	Nymphaeaceae	F	-	-	-	+	+
8	*Phragmites karka*	Poaceae	E	-	-	-	-	+
9	*Potamogeton* sp.	Potamogetonaceae	S	-	-	+	+	+
10	*Trapa natans*	Lythraceae	F	-	-	-	-	+
11	*Vallisneria spriralis*	Hydrocharitaceae	S	-	-	+	+	+
12	*Ceratophyllum demersum*	Ceratophyllaceae	S	-	-	+	+	+
13	*Lemna minor*	Lemnaceae	FF	+	-	+	+	+
14	*Spirodela polyrrhiza*	Araceae	FF	+	-	+	+	+
15	*Azolla pinnata*	Salviniaceae	FF	+	+	+	+	+
16	*Ludwigia perennis*	Onagraceae	FC	+	-	-	+	+
17	*Ottelia alismoides*	Hydrocharitaceae	S	-	-	+	-	+
18	*Hygrophila auriculata*	Acanthaceae	E	-	-	+	-	+
19	*Ipomoea carnea*	Convolvulaceae	S	+	-	+	-	+
20	*Marsilea minuta*	Marsileaceae	RF	+	+	+	+	+

Table 5. D1 – Dhandardihi 1, D2 – Dhandardihi 2, S – Searsole, D – Dalurbandh, H – Harabhanga. E – emergent, S – submerged, FF – free floating, F – floating, FC – floating and creeper. "+" represents present and "–" represents absent.

Brachionus forficula showed significant positive correlation with the turbidity (r= 0.900; p<0.05) while negative correlation with the phosphate (r= -0.900; p<0.05); *Brachionus falcatus* showed significant negative correlation with the conductivity and salinity (r= -0.900; p<0.05). *Brachionus diversicornis* significantly negatively correlated with the nitrate (r= -0.900; p<0.05); *Brachionus caudatus personatus* showed significant positive association with the hardness (r= 0.894; p<0.05) and significant negative correlation with both the pH and nitrate (r= -0.894; p<0.05). *Lecane* (*Monostyla*) *bulla* showed significant negative correlation with the temperature (r= -0.894; p<0.05) (Table 6). Mann–Whitney (p=0.0001) and Kolmogorov–Smirnov test (p= 0.001) demonstrated that there was a significant difference regarding the abundance of different rotifers taxa between the studied pit lakes.



(a) **(b)**

Figure 3. Hierarchical cluster analysis of the study sites based on the (a) physicochemical criteria and (b) rotifer community structure. H – Harabhanga Pit Lake, D1 – Dhandardihi 1 Pit Lake, D2 – Dhandardihi 1 Pit Lake, S – Searsole Pit Lake, DAL – Dalurbandh Pit Lake

Table 6. Spearman correlation matrix showing significant correlations between the physicochemical parameters and the biotic community

Rotifer	Temp	pH	TDS	CON	HARD	ALK	SAL	DO	BOD	PHOS	NITR	TUR
Ba	.205	-.103	-.308	-.154	-.051	-.205	-.103	.410	-.205	.718	.616	-.564
Bc	-.200	.300	-.700	-.900*	-.100	-.900*	-.900*	-.100	-.400	.300	.100	.300
Bf	-.200	.700	-.700	-.900*	-.500	-.600	-.900*	-.600	-.600	-.300	.100	.800
Bfo	-.100	.600	-.100	-.200	-.500	.200	-.200	-.800	-.300	-.900*	-.200	.900*
Bd	.500	-.700	0.000	.100	.600	.100	.500	-.100	.100	.200	-.900*	0.000
Bcp	.224	-.894*	.224	.224	.894*	-.112	.447	.335	.447	.447	-.894*	-.335
Bca	0.000	-.707	.707	.707	.707	.354	.707	.354	.707	0.000	-.707	-.354
Bs	0.000	-.707	.707	.707	.707	.354	.707	.354	.707	0.000	-.707	-.354
Bq	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354
Bk	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354
Kt	.100	.100	-.400	-.300	-.200	-.300	-.300	.300	-.300	.600	.700	-.400
Kc	.205	-.872	-.051	-.051	.872	-.462	.154	.564	.308	.872	-.564	-.564
Kl	.354	-.354	-.707	-.707	.354	-.707	-.354	0.000	-.354	.707	-.354	0.000
Fl	-.600	.600	.400	.300	-.500	.200	-.200	.200	.200	-.400	.800	-.100
Ab	.447	.335	-.112	.224	-.671	.671	.335	-.224	-.447	-.112	.783	-.112
Lb	-.894*	.671	.224	-.112	-.335	-.335	-.671	.112	.224	-.447	.447	.224
Le	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354

*Correlation is significant at the 0.05 level (2-tailed). For rotifer, acronym see Table 2.

Canonical Correspondence analysis (CCA) diagram showed a correlation between the different diversity indices and species richness with the different water quality parameters. The two canonical axes explain more than 98% of variations of the dataset with the Eigenvalues being 0.0026 and 0.0004, respectively. From the CCA diagram, it can be observed that Shannon and Weaver index (H') was positively correlated with salinity, hardness, TDS, conductivity and BOD. Evenness (E) showed an affinity with the turbidity whereas species richness (D) was linked with nitrate (Figure 4). Also, CCA reveals the influence of different aquatic macrophytes on the rotifer community structure (Figure 5). The two canonical axes explain about 72% of variations of the dataset. From the CCA diagram, it is observed that *B. caudatus personatus*, *B. caudatus aculeatus, B. diversicornis, K. cochlearis* and *K. lenzi* are influenced by *Enydra fluctuans, Chara* sp., *Trapa natans, Ludwigia perennis* and *Ottelia alismoides*. *Keratella tropica, Brachionus angularis* and *Lecane (Monostyla) bulla* are influenced by *Nymphaea nouchali* and *Ceratophyllum demersum*. *Filinia longiseta* and *B. calyciflorus* are influenced by *Azolla pinnata*. *B. falcatus* are influenced by *M. minuta*, *Phragmites karka* and *H. verticillata*. *Asplanchna brightwelli* is influenced by *Eichhornia crassipes, Spirodela polyrrhiza* and *Ludwigia perennis*. *Brachionus forficula, B. quadridentatus, Lepadella* sp., and *B. kostei* are influenced by *Ipomoea carnea* and *Ipomoea aquatica.*

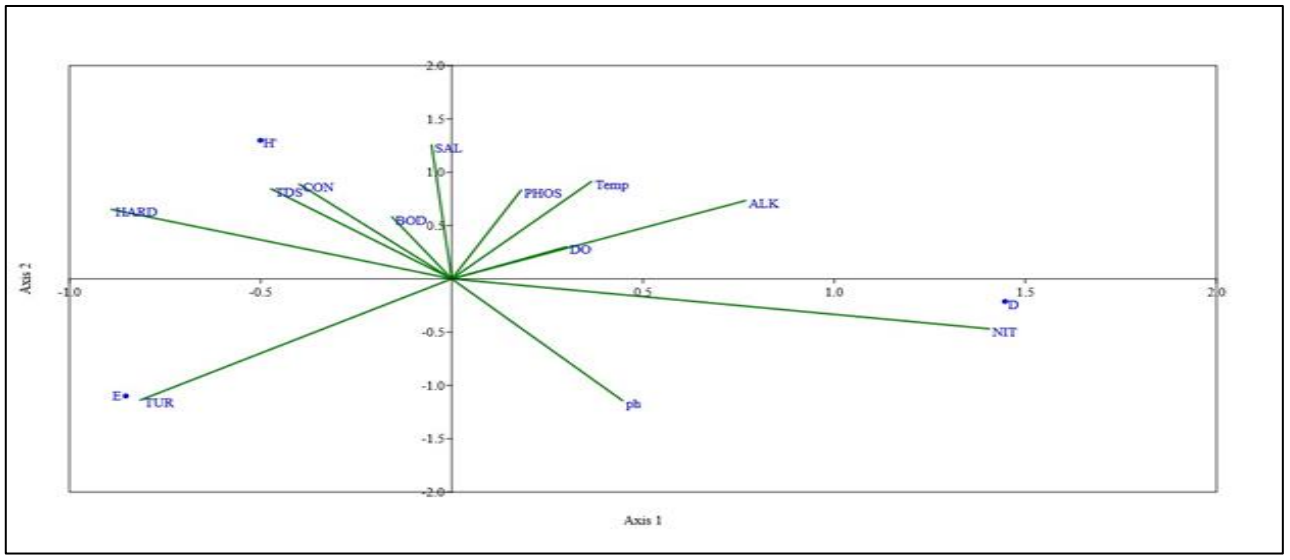


Figure 4. CCA biplot of the Shannon and Weaver diversity index (H'), evenness index (E) and species richness (D) as dependent variables against the physicochemical parameters shows a strong correlation. Temp – Temperature, TDS – Total dissolved solids, CON – Conductivity, TH – Total hardness, Alk – Total alkalinity, SAL – Salinity, DO – Dissolved oxygen, BOD – Biochemical oxygen demand, PHOS – Phosphate, NIT – Nitrate, TUR – Turbidity.

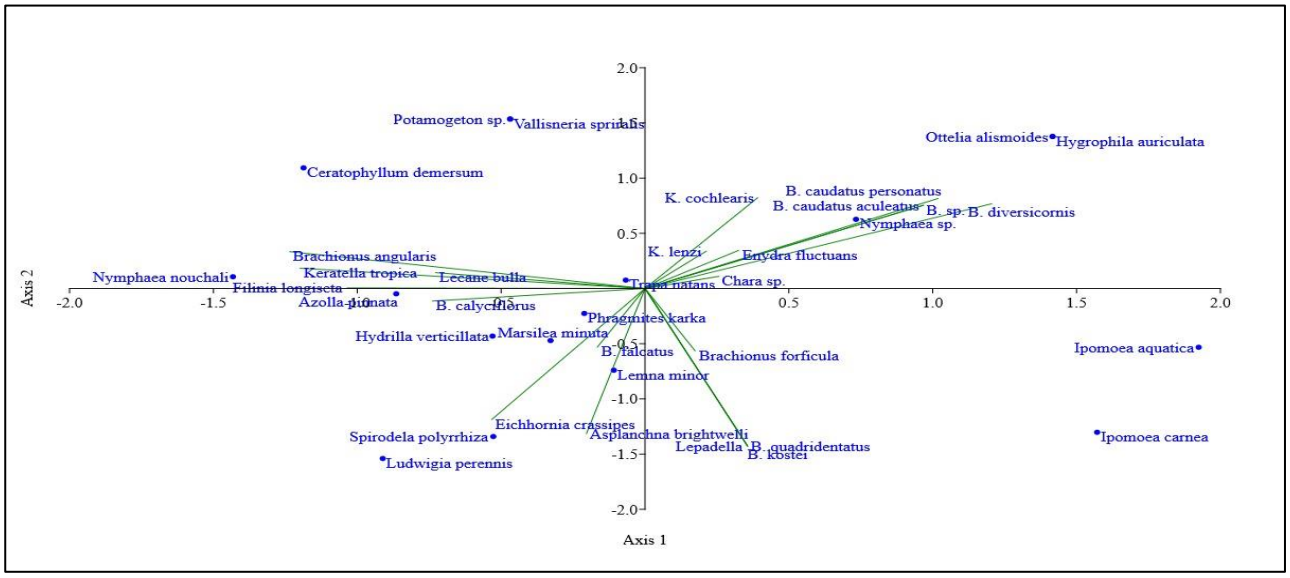


Figure 5. CCA biplot showing the relationship between aquatic macrophytes and rotifer communities

Discussion

Six different genera of rotifers have been identified during the study, where the genus *Brachionus* is most speciose while *Keratella* is least speciose. The lower alkalinity level in this study showed the strong buffering potential of the surface water of the pit lakes. At Harabhanga Pit Lake the lower DO value was liable for its higher alkalinity. Specific causes of hardness appears to be the presence of calcium and magnesium ions in water. The usage of soap, detergents and other cleaning products for washing practices in the Searsole Pit Lake contributed to a greater hardness level than other locations. In some sampled Pit Lakes (Harabhanga, Searsole and Dalurbandh) elevated phosphate concentration was found, suggesting enrichment of the nutrients. This in turn leads to greater plankton abundance which is reflected in our study. This culminated in relatively large nitrate levels, as the Harabhanga, Searsole and Dalurbandh Pit Lakes specifically receive domestic sewage through drainage systems from the nearby household. The rotifers' correlation tests against the environmental variables indicate a strong positive connection to the TDS, conductivity, hardness, salinity, DO, BOD, phosphate, nitrate, and turbidity as per this study. Several surveys across the globe have recorded the density of the rotifers and other zooplanktons with different relevant physicochemical parameters of different water bodies, including eutrophic freshwater lakes (Anton-Pardo et al. 2016; Sharma et al. 2016; Gupta and Devi

2014; Sharma and Pachuau 2013) as well as in the gravel-pit lakes (Vucic et al. 2019). However, it is possible that this is the pioneer work about the rotifer composition of the coal mine generated pit lake ecosystem in RCF. The important finding of this research is the mean density of rotifers is 98.40 ind/l, which is quite low. This may appear to be the condition of the studied pit lakes. Such a trend was also apparent in many reports on the prevalence of zooplankton and limnological variables encountered throughout the globe (Joniak and Kuczyńska-Kippen 2016; Burdis and Hirsch 2017). The younger pit lakes and the harsh environmental conditions resulted in less species diversity compared to elderly normal lakes. During the succession of this ecosystem when the community will reach its climax, diversity may increase (Lipsey 1980; Ejsmont-Karabin 1995; Hindák and Hindáková 2003). Pawlikiewicz and Jurasz (2017) found that Shannon diversity index value was increased along with the size of a water body. Mimouni et al. (2018) have described water body size as one of the significant local variables that influences variability like the zooplankton population. However, it was not noticed during this investigation. In Searsole and Dhandardihi 1 Pit Lakes, Shannon diversity index value was found to be higher compared to the other pit lakes tested, as these two pit lakes are relatively older and ecological succession got adequate time than the other studied pit lakes. The correlation found between the rotifer density and physicochemical variables like temperature, pH, conductivity, hardness, salinity, phosphate, nitrate and turbidity reflects the changes of rotifers' population composition mostly. In the Dhandardihi 2 Pit Lake, high pH illustrated the resalinization of the lakes linked to the freshwater inputs. The role of pH in structuring the rotifer community has appeared in the correlation and CCA analysis. Several experiments have demonstrated the vulnerability of the freshwater rotifers to elevated pH values (Bērzinš and Pejler 1987). Besides the geographical influences, the variations in the composition of the rotifers are probably related with the water quality variables such as nitrate and phosphate content, turbidity and temperature regime, as it is obvious from the observation of tropical lakes across the globe (Burdis and Hirsch 2017). This study also showed that *L.* (*Monostyla*) *bulla* is positively correlated with the temperature. The correlation study represents the sensitivity of a single zooplankton to the environmental factors (water quality parameters) seemed to be different a little from those observed in Manipur, Maharashtra, and Tamil Nadu because of the habitat conditions and the limnological variables of the pit lakes (Gupta and Devi 2014; Sharma et al. 2016; Rajagopal et al. 2010; Shinde et al. 2012). Previous findings indicated that the frequency and distribution of individual rotifer were generally affected by the trophic environment (Duggan et al. 2001; Wen et al. 2011). Throughout this analysis, *Brachionus* spp. dominated rotifer assemblages. *Brachionus* spp. are stated to be the most common rotifer in eutrophic lakes as they are strong pollution tolerant (Tasevska et al. 2012). Ismail and Adnan (2016) further reported that *Brachionus* spp. are one of the potent trophic indicators, as they are less influenced by algal bloom. The comparatively large rotifer abundance observed in both Harabhanga and Searsole Pit Lakes may have been correlated with macrophyte cover. During field sampling, large submerged macrophyte cover was recorded in these pit lakes. In a few experiments in other temporary and permanent waterways and stormwater storage systems, a clear beneficial association between zooplankton richness and macrophyte abundance has been demonstrated (Mimouni et al. 2018; Sun et al. 2019). Feasible macrophyte impacts that might drive richness of zooplankton species involve ecological gradients along with food availability and low visibility of predators (Mimouni et al. 2018). Many reports support this observation (Basu et al. 2000; Kuczyńska-Kippen and Nagengast 2003). In comparison, reports suggest that planktonic organisms such as *Keratella* spp. and *Brachionus* spp. can sometimes be bound to macrophytes (Green 2003). Submerged macrophyte vegetation is a very significant biotic factor for sustaining healthy lake ecosystem. Although several studies have examined the relationships in different lake environments between macrophytes and zooplankton, no research has yet been performed in these pit lakes formed by open cast mining operation. Taking the findings of this report as a foundation, further assessment of these pit lakes may be conducted to determine the overall ecological health and integrity. The present findings of correlation between the zooplankton assemblages and their responses to various biotic and abiotic factors will certainly pave the way for estimating the susceptibility of the native species of these major water bodies to specific kinds of stress. In turn, to conserve and allow judicious use of such tremendous water bodies, improved monitoring of these lake ecosystems would be required to take necessary measures towards their management and conservation. These findings showed the rotifer assemblages and their diversity at the different coal mine generated pit lakes.

The findings of this analysis show that the distribution and abundance of various rotifer organisms were determined by specific water quality parameters and aquatic vegetation. Despite the

heavily impacted environment of the studied pit lakes, several littoral species contributed to the total zooplankton diversity and densities, emphasizing the role of aquatic vegetation in providing habitat for many zooplankton species. This study marks an important contribution on the diversity of freshwater zooplankton of India in general and that of the pit lakes of West Bengal as well as the tropics and subtropics in particular. Using the outcomes of this study as a framework, subsequent monitoring of the pit lakes concerned can be pursued to evaluate the ecological quality and integrity of the aquatic community. Despite this report's limitations, this analysis still presents a holistic basis for further studies related to rotifer distribution in the RCF regions' pit lakes. This can provide a vital framework for local and national resource protection and fisheries management. The output of this reseach can be utilized for the effective pit lake management strategy that includes involoving ecologists in the construction of pit lakes, prioritizing ecosystem development and proactive treatment in mine closure preparation and eventually providing residents with post-mining alternatives for their livelihood.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Chlorella sorokiniana' nın İzolasyonu, Moleküler Tanılanması, Fototrofik, Miksotrofik ve Heterotrofik Üretimi

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ÖZ

İzmir'in Gümüldür bölgesinden alınan su örneğinde, seyreltme ve dökme plaka yöntemleri kullanılarak mikroalg izolasyonu yapılmıştır. Işık mikroskobuyla morfolojik olarak değerlendirilen türün *Chlorella* sp. olduğu saptanmıştır. Moleküler yöntemlerle mikroalg DNA'sı izole edilerek 16S ve 18S rRNA gen bölgeleri PCR'da çoğaltılmıştır. Bu dizinin sekanslanması ve filogenetik olarak değerlendirilmesi sonucu *Chlorella sorokiniana* olduğu belirlenmiştir. Aksenik *C. sorokiniana* elde etmek için santrifüj ile yıkama, antibiyotik ile muamele, agar ortamında büyütme ve tek hücre izolasyonu gibi farklı yöntemler kullanılarak aksenikleştirme işleminden başarılı sonuçlar elde edilmiştir. Fototrofik *C. sorokiniana*' dan elde edilen biyokütle (0,19 g L^{-1}) ve spesifik büyüme hızı (0,78 $gün^{-1}$), miksotrofik *C. sorokiniana*' dan elde edilen biyokütle (0,31 g L^{-1}) ve spesifik büyüme hızı(1,3 $gün^{-1}$), heterotrofik *C. sorokiniana*' dan elde edilen biyokütle (0,6 g L^{-1}) ve spesifik büyüme hızı (2,52 $gün^{-1}$) olarak belirlenmiştir. Elde edilen bulgulara göre aksenik mikroalg *C. sorokiniana*' nın farklı üretim koşullarındaki biyokütle verimliliği şu şeklide sıralanabilir: heterotrofi>miksotrofi>fototrofi.

Anahtar kelimeler: *Chlorella sorokiniana*, moleküler tanılama, fototrofi, miksotrofi, heterotrofi

MAKALE BİLGİSİ

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Isolation, Molecular Identification, Phototrophic, Mixotrophic and Heterotrophic Production of *Chlorella sorokiniana*

Abstract: Microalgae was isolated using dilution and pouring plate method from the sea water sample taken from the Gumuldur region of Izmir. The species was identified as *Chlorella* sp. through morphologic evaluation under light microscope. Microalgae DNA was isolated through molecular methods and 16S and 18S rRNA gene regions were amplified by means of PCR. As a result of sequencing and phylogenetic evaluation of the gene regions, it was determined that the microalgae was *Chlorella sorokiniana*. Different methods such as centrifuge washing, antibiotic treatment, growth on agar and single cell isolation used to obtain axenic *C. sorokiniana* yielded successful results. Biomass and specific growth rate of phototrophic *C. sorokiniana* (0.19 g L^{-1} and 0.78 $days^{-1}$, respectively), mixotrophic *C. sorokiniana* (0.31 g L^{-1} and 1.3 $days^{-1}$, respectively) and heterotrophic *C. sorokiniana* (0.6 g L^{-1} and 2.52 $days^{-1}$, respectively) were determined. According to the findings obtained, the biomass productivity of axenic *C. sorokiniana* under different culture conditions can be ordered as: heterotrophy > mixotrophy > phototrophy.

Keywords: *Chlorella sorokiniana*, molecular identification, phototrophy, mixotrophy, heterotrophy

Alıntılama
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Giriş

Dünyada 30.000' den fazla mikroalg türü vardır ve bunlardan sadece 100' e yakını ekonomik açıdan değerlendirilmektedir (Sasson 1997; Borowitzka 1992). Mikroalgler, gelişmiş ülkelerde, pigmentler gibi yüksek katma değerli bileşiklerin elde edilmesinde, gıda endüstrisi ve sağlık amaçlı gıda üretiminde; gelişmekte olan ülkelerde ise atık arıtımı ve proteince zengin gıda ve yem katkısı üretimini birleştiren küçük ölçekli projeler ile büyük ölçekli atık su arıtımında kullanılırlar (Sasson 1997). Mikroalglerin kimyasal komposizyonu türe ve kültür koşullarına göre değişmekle (Zhu 2015) birlikte genellikle

protein (%10-63) (Becker 2007; Miao ve Wu 2004) karbonhidrat (%6-57) (Yeh vd. 2010; Hariskos ve Posten 2014) ve lipitler (%4-55) (Becker 2007; Miao ve Wu 2004) gibi primer metabolitlerden oluşur.

Mikroalgler, genellikle ototrofik olarak yaşarlar ve fotosentez yaparak, karbondioksit, su ve güneş ışığını biyokütleye dönüştürürler (Pragya vd. 2013; Zhu 2015). Fakat bazı mikroalg türleri enerji ve karbon kaynağı olarak organik substratları kullanarak heterotrofik ve miksotrofik olarak da gelişebilirler (Chen 1996; Lee 2001). Örneğin, *Chlorella vulgaris* (Mitra vd. 2012), *Chlorella sorokiniana* (Wang vd. 2012), *Chlorella zofingiensis* (Liu vd. 2011), *Haematococcus pluvialis* (Kobayashi vd. 1992), *Spirulina platensis* (Marquez vd. 1993) ve *Botryococcus braunii* (Zhang vd. 2011) ototrofik, heterotrofik ve miksotrofik şartlar altında gelişebilmektedir (Kim vd. 2013).

Heterotrofik kültürler, ışıksız koşullar altında tek karbon kaynağı olarak organik karbonu kullanırlar. Miksotrofik kültürler ise ototrofik ve heterotrofik beslenmenin birleşimi bir karakteristik gösterirler, karbon kaynağı olarak hem organik hem de inorganik karbonu kullanırlar (Kim vd. 2013; Chen vd. 2011). Heterotrofik ve miksotrofik büyümede genellikle organik karbon kaynağı olarak glikoz, galaktoz, mannoz, fruktoz, sükroz ve laktoz kullanılmaktadır (Abreu vd. 2012). Organik karbondan elde edilen enerji, hücre sentezinde kullanılırken, ışık enerjisinden dönüştürülen kimyasal enerji depolanmaktadır (Chojnacka ve Marquez-Rocha 2004). Mikroalg kültür koşullarına bağlı enerji ve karbon kaynakları ve metabolit yolağına göre pH değişimi Tablo 1' de gösterilmektedir.

Tablo 1. Ototrofik, heterotrofik ve miksotrofik şartlarda enerji ve karbon kaynağı, pH değişiminin özeti

Table 1. Summary of energy and carbon source, pH change in autotrophic, heterotrophic and myxotrophic conditions

Kültür tipi	Enerji kaynağı	Karbon kaynağı	Metabolizma
Ototrof	Işık	İnorganik	$H_2O+HCO_3^- \rightarrow C(\text{biyokütle})+1/2O_2+3OH^-$: pH artar.
Heterotrof	Organik	Organik	$(1+a)CH_2O+O_2 \rightarrow C(\text{biyokütle})+aCO2+(1+a)H_2O$: pH azalır.
Miksotrof	Işık ve organik	İnorganik ve organik	$bHCO_3+cCH_2O \rightarrow (b+(c-a))C(\text{biyokütle})+3OH^-+aCO_2$: pH değişkendir.

Ototrofik mikroalgler, inorganik karbonu kullanırlar ve hidroksil üreterek pH'ı yükseltirler. Heterotrofik mikroalgler, organik karbonu kullanırlar ve CO_2 üreterek pH'ı düşürürler. Miksotrofik mikroalgler eş zamanlı olarak hem organik hem de inorganik karbonu kullanırlar ve pH değeri değişkenlik göstermektedir. (Elcik ve Çakmakçı 2017).

Literatür taraması sonucu ototrofik, heterotrofik ve miksotrofik mikroalg büyüme hızı sırasıyla 0,2-0,7 gün^{-1}, 0,4-0,9 gün^{-1} ve 0,3-0,6 gün^{-1} arasında değişmektedir. Heterotrofik mikroalglerin büyüme hızının, diğer kültür tipleriyle kıyaslandığında daha yüksek olduğu görülmektedir. Heterotrofik büyüme şekli özellikle değerli kimyasalların ve farmasötiklerin üretimi için uygundur.

Yapılan bu çalışmada, yerel bir kaynaktan izole edilen örneğin morfolojik ve moleküler yöntemler kullanılarak tür teşhisi yapılmış ve *Chlorella sorokiniana* olduğu saptanmıştır. Biyoteknolojik olarak önemli bir tür olan *C. sorokiniana* fototrofik, miksotrofik ve heterotrofik üretim potansiyeli açısından değerlendirilmiştir. Sonuç olarak en verimli üretim yöntemi heterotrofik şartlar altında sağlanmıştır.

Materyal ve Metot

Bu çalışmada kullanılan *Chlorella sorokiniana* İzmir'in Gümüldür bölgesinden izole edilmiştir. ESW (Enriched Seawater) ortamı kullanılarak türün kültürü yapılmıştır (Tablo 2) (Provasoli 1963,1968; McLachlan 1973).

ESW ortamı hazırlamak için 1L steril edilmiş deniz suyuna 20 mL/L zenginleştirilmiş çözelti (Enrichment Sewater Solution) eklenerek pH 7,8 olarak ayarlanmıştır. Heterotrofik ve miksotrofik kültürlerde karbon kaynağı olarak glukoz (3 g/L) kullanılmıştır.

Kültürü yapılan mikroalg örneği düzenli aralıklarla kültür ortamları ile seyreltilerek ve dökme plaka yöntemi kullanılarak izole edilmiştir. Kültüre alınan örnek agar (%1,5) besiyerine ve yatık agara çizgi ekim metodu uygulanarak saklanmıştır (Sukatar 2002). Saflaştırılan tür, 25^{o}C'de ve sürekli aydınlatmada (Wiselight marka 24W day-light) steril agar üzerinde inkübasyona bırakılmıştır.

Tablo 2. ESW (Zenginleştirilmiş Deniz Suyu) ortamının hazırlanışı

Table 2. Preparation of ESW (Enriched Seawater) medium

Kimyasal	**Miktar**	**Son konsantrasyon**
$NaNO_3$	2,35 g/L	34 mM
Na_2gliserofosfat.$5H_2O$	0,35 g/L	1,6 mM
ES Fe Çözeltisi	163 mL/L	
P-II Metal Çözeltisi	163 mL/L	
Tris	1 g/L	
Vitamin B12	1,5 mL/L	
Biotin Vitamin Çözeltisi	1,5 mL/L	
Tiamin Vitamin Çözeltisi	1,5 mL/L	
ES Fe Çözeltisi		
Kimyasal	**Miktar**	**Son konsantrasyon**
$FeCl_3.6H_2O$	0,35 g/500 mL	1,8 mM
$Na_2EDTA.2H_2O$	0,30 g/500 mL	1,6 mM
P-II Metal Çözeltisi		
Kimyasal	**Miktar**	**Son konsantrasyon**
$Na_2EDTA.2H_2O$	0,1 g/100 mL	0,27 mM
H_3BO_3	0,114 g/100mL	1,8 mM
$MnSO_4.H_2O$	16,4 mg/100 mL	0,097 mM
$ZnSO_4.7H_2O$	2,2 mg/100 mL	0,007 mM
$CoCl_2.6H_2O$	0,48 mg/100 mL	0,002 mM
Vitamin Çözeltisi		
Kimyasal	**Miktar**	
Vitamin B12	0,0135 g/100 mL	
Biotin	0,0025 g/100 mL	
Tiamin	0,11 g/100 mL	

Saflaştırılan mikroalgin morfolojik tayini için ışık mikroskobu (Olympus CH40) kullanılmıştır. Moleküler tür tayini için mikroalg DNA'ları, ZRFungal/Bacterial DNA Kiti kullanılarak saflaştırılmıştır. 50-100 mg agar yüzeyinden alınan mikroalg hücresi 200 µl ultra saf su içerisinde porselen boncukların olduğu tüpe alınarak üzerine lizis solüsyonu eklendikten sonra hücrelerin parçalanması için maksimum hızda 5 dk vorteks yapılmıştır. Lizis tüpü 10.000 x g de 1 dk santrifüjlendikten sonra Spin filter tüpüne aktarılan süpernatant 7.000 rpm de 1 dk santrifüj edilmiş ve DNA Binding Tamponu eklenerek toplama tüpüne ilave edilmiştir. Zymo-Spin Column toplama tüpü içerisine yerleştirilen ve 10.000 × g 1 dk santrifüj edilen Zymo-Spin Column yeni toplama tüpü içerisine yerleştirilir ve filtre üzerine DNA Pre-Wash Tamponu ilave edilip, 10.000 × g 1 dk santrifüjlendikten sonra 500 µl DNA Wash Tamponu ilave edilir ve sonra tekrar aynı şekilde santrifüjlenir. Zymo-Spin Column 1,5 ml steril ependorf içerisine dikkatlice yerleştirilir ve üzerine DNA Elution Tamponu ilave edilerek 10.000 × g 30 saniye santrifüjlenerek filtrede tutulan DNA'nın ependorf içerisine toplanması sağlanmıştır. Mikroalg genlerinin spesifik bölgelerinin PCR (HelixAmp™ HyberSense DNA polimeraz (Nanohelix) kiti) yöntemiyle amplifikasyonları için mikroalglerin gen bölgelerine özgül primer dizileri 16S rRNA 359F (5'-GGG GAA TYT TCC GCA ATG GG-3') -781R(a) (5'-GAC TAC TGG GGT ATC TAA TCC CAT T-3') ve (b) (5'-GAC TAC AGG GGT ATC TAA TCC CTT T-3') ve ayrıca 18S ribozomal küçük alt birim rRNA SSU-F (5'-TGG TTG ATC CTG CCA GTA G-3', SSU-R; 5'-TGA TCC TTC CGC AGG TTC AC-3') kullanılmıştır (Nübel, 1997; Boutte vd. 2006; Yıldırım vd. 2014). PCR cihazı HelixAmp™ HyberSense DNA polimeraz kiti kullanılarak; ilk denatürasyon adımı 95 °C de 2 dk olarak tek adımda başlatılmış ve 35 döngü 95 °C de 30 s, bağlanma ısısı (359F-781R (a, b)) için 65°C ve SSUF-R için 54 °C sıcaklıkta 40 s de, 72 °C de 40 s den sonra son uzama adımı, 1 döngü olarak 72 °C de 5 dk olacak şekilde ayarlanmıştır. DNA ve PCR ürünleri %1 agaroz jel elektroforezin de 1x-Tris-borik asit-EDTA (TBE) tamponu içerisinde 5 V/cm yürütüldükten sonra SYBR safe ile boyanan jel UV görüntüleme cihazı ile görüntülenmiştir.

PCR sonuçlarının dizi analizi İzmir İleri Teknoloji Enstitüsü Biyoteknoloji ve Biyomühendislik Merkezi Araştırma Laboratuarı' nda (ABI3130XL, 16 KAPİLLER SİSTEM)

yapılmıştır. Sonuçlar NCBI-Blast programı yardımıyla değerlendirilmiştir.

Hücre konsantrasyonunu tespit etmek ve büyüme grafiklerini çıkarabilmek için gün aşırı örnek alınmıştır. 2,5 mL mikroalg örneğinin 2 mL'si optik yoğunluk (OD) ölçümünde ve geri kalanı Neubauer sayım kamarası yardımıyla mikroskop altında sayılmıştır. *C. sorokiniana*'nın OD değeri 600 nm'de Ultraspec 1100 pro markalı spektrofotometre kullanılarak ölçülmüştür.

Spesifik büyüme hızlarının ve ikilenme sürelerinin hesaplamaları Becker (1995)' in formülüne göre yapılmıştır.

$$\mu = \frac{lnx2 - lnx1}{\Delta t}$$

μ, spesifik büyüme hızı; x2, t2 zamanındaki hücre konsantrasyonu; x1, t1 zamanındaki hücre konsantrasyonu; Δt = t2-t1, DT, ikilenme süresi

$$DT = \frac{ln2}{\mu}$$

Aksenik kültür izolasyonu için 100 µg. mL^{-1} ampisilin, 25 µg. mL^{-1} kanamisin, 25 µg. mL^{-1} streptomisin ve 25 µg. mL^{-1} gentamisin içeren antibiyotik kokteyli 0,2 µm steril filtreden geçirilerek kullanılmıştır. Antibiyotik uygulamasından önce *C. sorokiniana* örneği 4500 rpm' de 5 dk süreyle santrifüj işlemiyle steril kültür ortamı ile 3 kere yıkanmıştır. İşlem sonrasında kalan pellet, 5 ml taze ESW ortamıyla homojenize edilerek 200 µl antibiyotik kokteyli eklenmiş ve 24 saat bekletilmiştir. Kültür taze ESW ortamına alınarak antibiyotiksiz agara çizgi ekim yöntemiyle transfer edilmiştir. Santrifüj işlemiyle bakteri sayısı azaltılan kültür aynı zamanda 5 ml taze ESW ortamına transfer edilmiş ve 25 µl örnek antibiyotikli agar üzerine çizgi ekim yöntemiyle ekilmiştir. Hem antibiyotikli hem de antibiyotiksiz agar üzerinde oluşan mikroalg kolonileri steril edilmiş kürdan yardımıyla teker teker toplanıp 3 mL steril sıvı ortam içeren test tüplerine alınmıştır. Yoğun ışık altında inkübatörde inkübasyona bırakılmıştır.

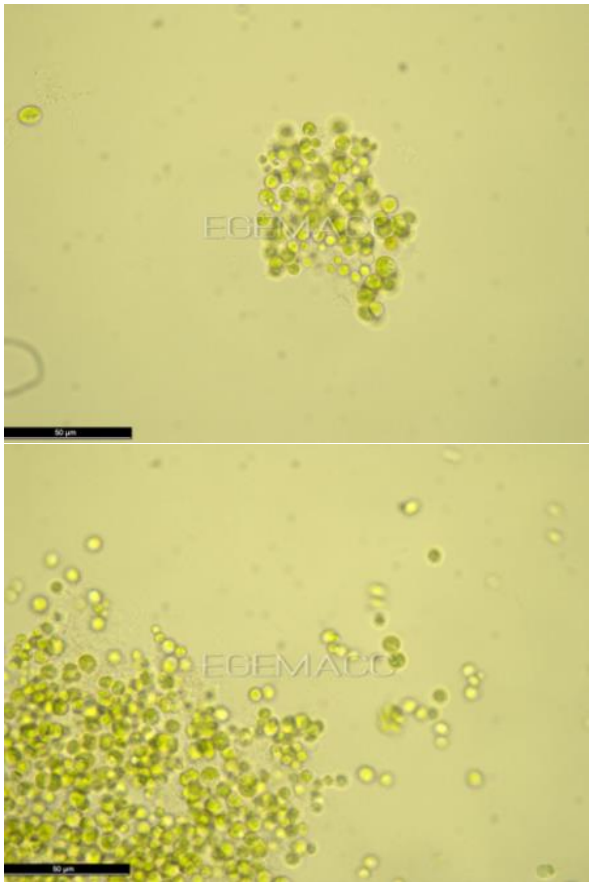
Yoğunlaşan kültürlerin akseniklik testi için 100 µL örnek "Nutrient Agar" üzerine yayma ekim yöntemiyle transfer edilerek 30ºC' de 2-3 günlük inkübasyona bırakılmıştır.

Bulgular

Chlorophyta grubunun üyeleri genellikle 2-10 µm çapındaki boyutlara sahiptir. Hücreler küresel ya da elipsoid şekillidir. Ayrıca hücrelerinde tek nukleus ve bir kromatofor vardır, tek tek olduğu gibi koloni de meydana getirebilirler. Vejatatif safhada kamçıları olmayan, hareketsiz alglerdir. Kloroplastlarında bulunan pirenoidlerde nişasta oluştururlar. Nişastayı sitoplazma yerine kloroplastlarında oluşturdukları için diğer alg gruplarından ayrılırlar. Bu grup üyeleri hem denizlerde hem de tatlı sularda yayılış gösterir (Güner ve Aysel 1991).

Mikroalg taksonunun sınıflandırılmasında AlgaeBase web sitesi temel alınmıştır (AlgaeBase 2021).

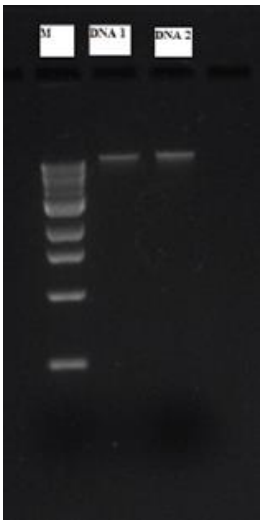
Domain	: Eukaryota
Kingdom	: Plantae
Subkingdom	: Viridiplantae
Infrakingdom	: Chlorophyta infrakingdom
Phylum	: Chlorophyta
Subphylum	: Chlorophytina
Class	: Trebouxiophyceae
Order	: Chlorellales
Family	: Chlorellaceae
Genus	: Chlorella



Şekil 1. *Chlorella* sp.'nin ışık miskoskobu görüntüsü 60X
Figure 1. Light microscope image of *Chlorella* sp. 60X

Chlorophyta üyelerinin morfolojik olarak birbirine çok benzemesi sebebiyle moleküler olarak tanılamayı da zorunlu kılmıştır. Les Algues D'eau Douce, Initiation à la systématique (Bourrelly 1966/70) mikroalg kataloğu yardımıyla ışık mikroskobuyla morfolojik olarak *Chlorella* sp. Beyerinck [Beijerinck], 1890 olduğu tespit edilen

türün (Şekil 1) DNA'sı ZRFungal/Bacterial DNA Kiti ile izole edilmiştir (Şekil 2).



Şekil 2. *Chlorella sp.*' nın elde edilen DNA' sının agoroz jel üzerindeki görüntüsü Marker (M) 1kb DNA ladder (10,0 kb, 8 kb, 6,0 kb, 5,0 kb, 4,0 kb, 3,0 kb, 2,0 kb, 1,5 kb, 1,0 kb ve 0,5 kb); 1(a)-2(b)

Figure 2. The image of DNA obtained from *Chlorella sp.* on agarose gel Marker (M) 1kb DNA ladder (10.0 kb, 8 kb, 6.0 kb, 5.0 kb, 4.0 kb, 3.0 kb, 2.0 kb, 1.5 kb, 1.0 kb and 0.5 kb); 1 (a) -2 (b)

359 F-781R(a) için bağlanma sıcaklığı 62 ºC, 359 F-781R(b) için Nanohelix PCR kitinde 65 ºC derece kullanılarak optimizasyonları yapılmıştır.

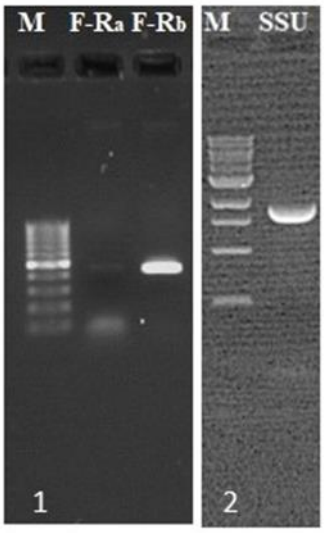
Şekil 3' de elde edilen PCR sonuçlarına göre 359F-781R(a) primeriyle çoğaltılan DNA silik bir bant oluştururken, 359 F-781R(b) primeriyle çoğaltılan DNA, belirgin bir bantlaşma göstermiştir. PCR da çoğaltılan 18S rRNA gen bölgesi de Şekil 3 de belirtilmiştir.

Dizi analizi sonuçları NCBI-Blast programına göre;

359F-781R (a) *Auxenochlorella protothecoides* 16S small subunit ribosomal RNA gene, partial sequence; chloroplast, AY553213.1; türüne maksimum %90 benzerlik göstermiştir.

359F-781R (b) *Chlorella sorokiniana* plastid DNA small subunit (16Slike) ribosomal RNA, X65689.1; türüne maksimum %97 benzerlik göstermiştir.

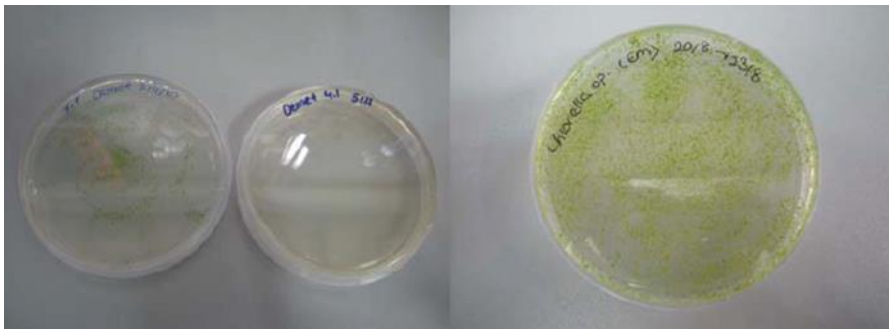
SSUF-SSUR *Chlorella sorokiniana* isolate 34-2 18S ribosomal RNA gene, KU948991.1 türüne maksimum %97,88 benzerlik göstermiştir (Tablo 3).



Şekil 3. PCR sonuçları, 1: Marker (M);356F-781R(a) (F-Ra); 356F-781R(b) (F-Rb); 2: Marker (M); SSUF-SSUR (SSU)

Figure 3. PCR results, 1: Marker (M): 356F-781R (a) (F-Ra); 356F-781R (b) (F-Rb); 2: Marker (M); SSUF-SSUR (SSU)

Akseniklik kontrolü için yoğunlaşan kültürler "Nutrient Agar" üzerinde temiz bir görüntü oluşturmuştur. Mikroalg dışında bir mikroorganizma üremediği gözlenmiştir (Şekil 4). Ayrıca kültürün optik yoğunluğu (OD), bulanıklığı, pH değişimi ve Faz-Kontrast mikroskobuyla kontaminasyon içerip içermediği kontrol edilmiştir.



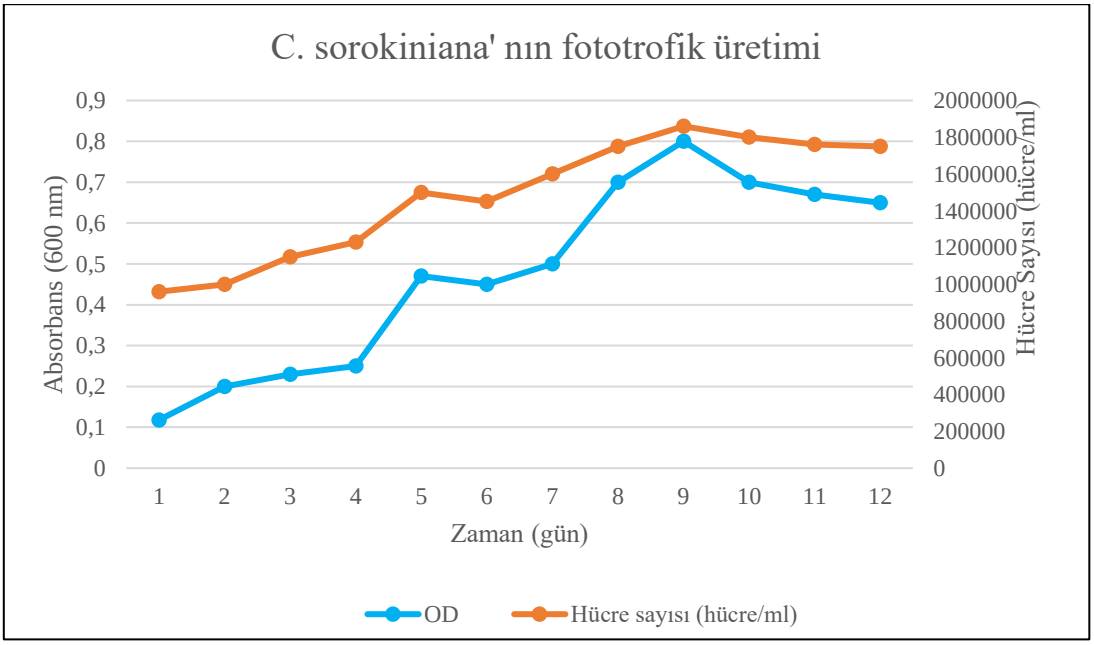
Şekil 4. Aksenik *Chlorella sorokiniana* kültürü

Figure 4. Axenic *Chlorella sorokiniana* culture

Tablo 3. Çoğaltılan gen bölgelerinin NCBI daki nükleotid dizileri ile uyumu (BLAST 2021)

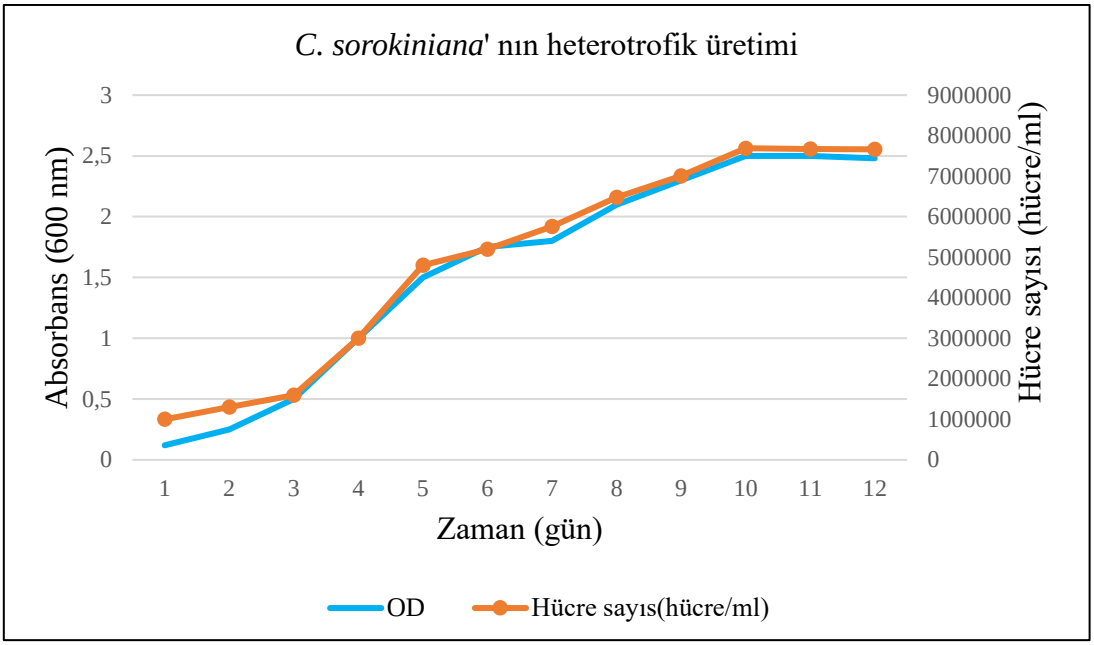
Table 3. Compatibility of the amplified gene regions with the nucleotide sequences in NCBI (BLAST 2021)

Tanımlama	Maksimum skor	Skor	Sorgulama kılıfı	E değeri	Yüzde tanımlama	Erişim numarası
Chlorella sp. EGEMACC40 18S small subunit ribosomal RNA gene, partial sequence	1794	1794	1	0.0	%100,00	JQ981943.1
Chlorella sorokiniana isolate 34-2 18S ribosomal RNA gene, partial sequence	1622	1622	0,96	0.0	%97,88	KU948991.1
Chlorella sp. YACCYB97 18S ribosomal RNA gene, partial sequence	1607	1607	0,97	0.0	%97,57	MH619545.1
Pseudochlorella pringsheimii 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene, complete sequence	1605	1605	0,97	0.0	%97,47	KY364701.1
Chlorella sp. QUCCCM33 18S ribosomal RNA gene, partial sequence	1605	1605	0,97	0.0	%97,47	KM985401.1
Chlorella sorokiniana strain SAG 211-31 18S ribosomal RNA gene, complete sequence	1605	1605	0,97	0.0	%97,47	KF673387.1
Chlorella sp. ZJU0209 18S ribosomal RNA gene, partial sequence	1605	1605	0,97	0.0	%97,47	JX097061.1
Chlorella sorokiniana 18S rRNA gene, strain Prag A14	1605	1605	0,97	0.0	%97,47	X74001.1
Chlorella sorokiniana strain Icheon4 18S ribosomal RNA gene, partial sequence	1604	1604	0,97	0.0	%97,47	KF864476.1



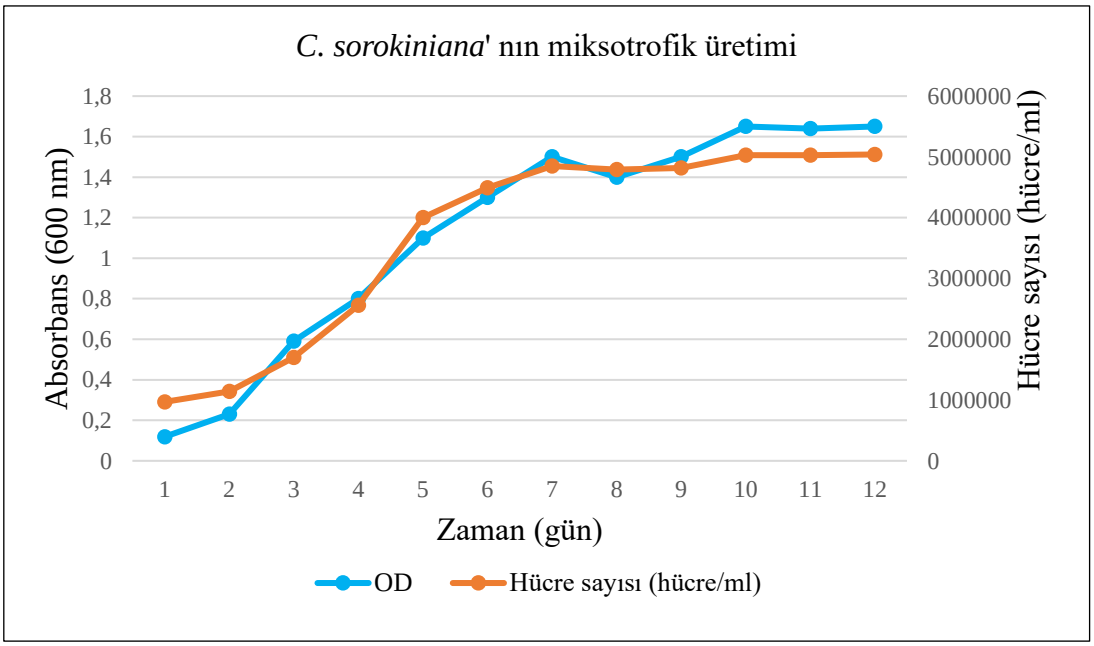
Şekil 5. *Chlorella sorokiniana'* nın 12 günlük fototrofik koşullar altında optik yoğunluk ve hücre sayısı ölçüm sonuçları

Figure 5. Optical density and cell number measurement results of *Chlorella sorokiniana* under 12-day phototrophic conditions



Şekil 6. *Chlorella sorokiniana'* nın 12 günlük heterotrofik koşullar altında optik yoğunluk ve hücre sayısı ölçüm sonuçları

Figure 6. Optical density and cell number measurement results of *Chlorella sorokiniana* under 12-day heterotrophic conditions



Şekil 7. *Chlorella sorokiniana'* nın 12 günlük miksotrofik koşullar altında optik yoğunluk ve hücre sayısı ölçüm sonuçları

Figure 7. Optical density and cell number measurement results of *Chlorella sorokiniana* under 12-day myxotrophic conditions

Pigment farkından dolayı OD'nin birbiri ile karşılaştırılamayacağı düşünülürse biyokütle artışı esas alınmıştır. 12 günlük deney süresince Şekil 5, 6 ve 7'de gösterildiği gibi *C. sorokiniana'* nın hücre sayısı başlangıçtaki hücre sayısına göre fototrofik üretimde yaklaşık 2 kat, heterotrofik üretimde 7,5 kat ve miksotrofik üretimde ise 5,2 kat artmıştır. Bu üç üretim şekline göre *C. sorokiniana*'nın spesifik büyüme hızı ve ikilenme süresi Tablo 4' te verilmiştir.

C. sorokiniana fototrofik, heterotrofik ve miksotrofik üretim açısından değerlendirildiğinde maksimum büyümenin 8. gün olduğu bulunmuştur. Aksenik *C. sorokiniana'* nın farklı üretim yöntemleri karşılaştırıldığında en iyi üretim yönteminin (Şekil 6) heterotrofik üretim yönteminde olduğu belirlenmiştir.

Tablo 4. Fototrofik, miksotrofik ve heterotrofik üretimi yapılan *C. sorokiniana*'nın spesifik büyüme hızı (μ değeri) ve ikilenme süreleri

Table 4. Specific growth rate (μ value) and doubling times of *C. sorokiniana* produced under phototrophic, myxotrophic and heterotrophic conditions

	Spesifik büyüme hızı (gün^{-1})	İkilenme süresi (gün)
Fototrofik üretim	0,069	10,05
Miksotrofik üretim	0,108	6,93
Heterotrofik üretim	0,247	2,56

Tartışma ve Sonuç

Son yıllarda yenilenebilir enerji kaynaklarına ve ekonomik üretim yöntemlerine olan ilgi artmıştır. Alglerin bu enerji kaynakları arasındaki yerini alma nedeni ise hızlı büyüme özelliğine sahip olmaları, ortam koşullarındaki değişimlere dayanıklı ve toleranslı olmaları sayılabilir. Mikroalgler genelde fotosentetik organizmalar olarak değerlendirilir. Ancak yapılan çalışmalar çoğu türün heterotrofik ve miksotrofik büyüme yeteneği olduğunu göstermiştir (Droop 1974). Ayrıca bu heterotrofik türler neredeyse alglerin bütün taksonomik sınıflarında bulunurlar. Geçtiğimiz elli yıl içerisinde geliştirilen fermantasyon teknolojisi ile büyük miktarlarda maya ve bakterinin endüstriyel üretimi yapılabilmektedir ve bu teknoloji ile heterotrofik algal üretim kolayca yapılabilir. Diğer mikroorganizmalar için kullanılan fermantasyon tankları aynı zamanda heterotrofik alg üretimi içinde uygundur. Buna ek olarak hücrenin büyümesi ve biyokimyasal içeriğini etkileyen bazı parametreler de kontrol edilebilir. Ayrıca verimliliği arttırma maliyetleri azaltma metotları ile ilgili zengin bir endüstriyel mikrobiyolojik bilgi kaynağı da vardır.

Bulut (2009) tarafından *Chlorella vulgaris'* in fototrofik üretimi sonucu elde edilen maksimum kuru ağırlık 0,19 g. L^{-1} olarak belirlenmiştir. Başka bir çalışmaya göre heterotrofik olarak üretilen; *Chlorella sorokiniana'* dan 1 g L^{-1} h^{-1}, *Nitzschia alba'* dan 0,8 g L^{-1} h^{-1}, *Arhtrospira* sp.*'* dan 1,2 g L^{-1} h^{-1} biyokütle elde edilmiştir (AlgaePARC 2021). Kim vd. (2013) tarafından yapılan bir çalışmada *C. sorokiniana*' nın spesifik büyüme hızı fototrofik, heterotrofik ve miksotrofik koşullarda sırasıyla 0,24 gün^{-1}, 0,53 gün^{-1} ve 0,44 gün^{-1} olarak bildirilmiştir. Ayrıca aynı çalışma sonuçlarına göre *C. sorokiniana*' nın nitrojen ve fosfor giderim hızı heterotrofik üretimin fototrofik üretimden iki kat daha yüksek olduğu görülmüştür. Heterotrofik mikroalglerin organik substratları ve besinleri giderim hızı yüksek olduğu için ileri atık su arıtımında iyi bir seçenek olabilir. Baytut vd. (2014) Türkiye' nin alg florasında *C. sorokiniana* Shihira & Kraus (A102) ilk kez 2014 yılında kayıt altına almıştır. *C.sorokiniana* tatlı sularda yaşayan bir yeşil mikroalg olmasına (Kim vd. 2016) rağmen Chen vd. (2013) yerel izolatları olan *C. sorokiniana* CY1 türünü %20 deniz suyu ortamında yetiştirerek yağ miktarını % 61 ve biyokütle miktarını ise 3g/L olarak belirlemiştir.

Sonuç olarak, bu çalışma ile ülkemiz sularında yayılış gösteren bir yerel mikroalg izole edilmiş, moleküler yöntemlerle tanımlanmış laboratuvar koşullarında kültürü yapılmıştır. NCBI gen bankasına kayıt ettirilen mikroalg için JQ981943.1 aksesyon numarası alınmıştır. Tür tayini yapılan mikroalg örneği Ege Üniversitesi Mikroalg Kültür Koleksiyonu' na (EGE-MACC 40) eklenmiştir. *C. sorokiniana* biyokütle verimliliği açısından değerlendirilerek bu tür için üç üretim şeklinden en iyi sonuç heterotrofik koşullar altında sağlanabilmiştir. Bu çalışma ile elde edilen verilerin de ileride yapılması planlanan büyük ölçekli üretime temel oluşturabilecek ön çalışma niteliği taşıdığı düşünülmektedir.

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Effects of Aquacultural Practices on the Sediment Characteristics of Certain Type of Earthen Fishponds

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ABSTRACT

The effect of aquacultural practices on the bottom sediment quality of six selected earthen fishponds in Ife North Local Government Area of Osun State was investigated for a period of two years. The fishponds were grouped with regard to fertilization practice and water flowage regime into three sets comprising two fertilized non-flow-through ponds (FNF); two fertilized flow-through ponds (FF) and two unfertilized flow-through ponds (NFF). The investigated sediment quality parameters include color and textural composition, salinity parameters, major ions, organic parameters and heavy metals using standard methods. The parameters were not statistically different ($P > 0.05$) for the three sets of fishponds with the exception of calcium which was significantly available in the fertilized flow-through pond. The fertilized ponds were however richer in nutrient and of better drainage quality than the unfertilized ponds. The parameters with higher mean in the fertilized ponds (FNF and FF) were 16% higher on average and flow-affected parameters were 67% higher on average in the flow-through ponds (FF and NFF), of which 7.00-fold higher lead concentration contributed most to this situation. Of these parameters, cations, anions, micronutrients were found to be of highest mean concentration in fertilized flow-through ponds. However, the presence of significant levels of calcium ions as well as minimal accumulation of clay, silt and nutrients in fertilized flow ponds made this fish culture method most suitable.

Keywords: Nutrient, sediment salinity, fish culture, drainage, heavy metal

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Introduction

The pond bottom sediment is the storehouse in the pond ecosystem. Hence good bottom sediment and high-water quality are essential for successful pond management (Muendo et al. 2014). Many chemical and biological processes which occur on its surface greatly influence the water quality and the productivity of fish culture ponds (Boyd 1995). Factors associated with aquaculture practices such as liming, fertilization, exogenous feeding of fish, fish excrement, dead fish and increased vegetation also cause organic accumulation in the pond ecosystem and affect the physical and chemical properties of the bottom sediment (Boyd et al. 2002). These pollutants are preserved in the sediment over a long period dependent on their chemical persistence and physico- /bio- chemical characteristic of the sediment (Singare et al. 2011). The resultant poor sediment properties that arise through the accumulation of organic matter, nitrogen and phosphorus (Jamu and Piedrahita 2001) can lead to low dissolved oxygen, high un-ionized ammonia, high pH levels and high biological oxygen demand, leading to deterioration of pond water quality and low fish yield (Ludwig 2002). Furthermore, the decomposition processes occurring in aquatic sediments helps to recycle nutrients and during the process, elements such as nitrogen, phosphorus, iron, cobalt, and copper are interchanged between sediment and the overlying water (Tsadu 1998).

Poor fish yield is the effect of reduced pond depth (or available space for fish growth), increased microbial activities and large fluctuations in water temperature, as well as increased susceptibility of fish to diseases associated with organic matter accumulation in the pond sediment (Rahman et al.

2004; Muendo et al. 2014). The proffered solution to these problems has been removal and disposal of such pond sediment to natural system which also constitute threat to the ecosystem of the fish farm (Rahman et al. 2004). This study therefore seeks for appropriate pond management/fish culture method that would reduce organic matter accumulation in fishpond. Consequently, the study aimed at assessing the effect of pond fertilization and water flowage on the sediment characteristic of certain types of fish ponds of a commercial farm in order to ascertain the stability of the fish ponds.

Materials and Methods

Study Area

The fish ponds assessed belong to Niger Feeds and Agricultural Operations Limited (NIFAGOL), which is a commercial fishing company in Yakoyo-Origbo, Ife North Local Government Area (LGA), Osun State, Nigeria. The LGA includes primarily rural and semi-urban villages and is roughly situated on a general elevation scale of 250 to 265 m above the sea, spanning between latitudes 07 ° 25′N to 07 ° 40′N and longitudes 004 ° 25′E to 004 ° 30′E. The Shasha River (one of the largest bodies on the southwest) drains the LGA along with other water bodies such as swamps, lakes, streams and rivers of minor significance (Adedeji 2011).

The studied fish farm (NIFAGOL) established in 1984, consists of 18 ponds of varying sizes (ranging from 432 m^2 to 6383 m^2), each of which is rectangular in shape and usually shallow (Adedeji 2011). Water supply to the ponds comes from a reservoir (2 hectare surface area) located within the farm. The aquaculture activities on the farm were in semi-intensive form in which some ponds received organic fertilizers in the form of chicken drop and cow dung and the rest receiving inorganic fertilizers (NPK). Fish stocked in all ponds were supplemented by additional feed (pelleted feed) at a rate of 3% of their body weight twice a day. The feed was produced locally out of a combination of corn, soybeans, fishmeal, millet, palm kernel cake, groundnut cake, kernel-palm oil and the brewer waste. The water retention period for the fertilized non-flow-through ponds was six months and the unfertilized flow-through ponds were not drained. Fish stock density in all the culture ponds was 3 fish/m^2. In the majority of ponds, *Clarias gariepinus* are monocultured and it was polycultured with *Oreochromis niloticus* in the flow-through ponds (Table 1).

Sample Collection and Analysis

Of the eighteen ponds in NIFAGOL fish farm, only ten ponds were operational during the study. Sediment samples for sediment characteristic determinations were collected from six (Table 1) of these ten ponds bimonthly over an annual seasonal cycle from November 2006 to October 2007. The analysis composed of t hree sets of ponds, based on existing cultural practice, with regard to fertilizer treatment and water flow. The first sets of two ponds were fertilized non-flow-through ponds (FNF) and received organic and inorganic fertilizers. The second sets of two ponds were fertilized flow-through ponds (FF) and received organic and inorganic fertilizers. The third sets of two ponds were unfertilized flow-through ponds (NFF) and received no fertilizer (Adedeji 2011). Sediment samples were taken bimonthly using an improvised mud grabber (metallic plates of cross-sectional area of 15.2 by 15.2 cm).

The sediment samples were transferred to the laboratory, air dried and crushed with a pestle in a porcelain mortar. Then, they were sieved through a 2 mm mesh, before the analysis of selected physico-chemical parameters, carried out based on Boyd (1995) and Chapman (1996). Colour, particle size analysis, textural composition was done according to procedure of Bouyoucos (1962) and Shields et al. (1966). pH, organic matter, nitrate, magnesium, sodium, potassium, aluminium ion acidity, hydrogen ion acidity, pH, sulphate and phosphate levels in the sediment were determined according to Boyd (1995) and Ademoroti (1996). While some selected heavy metals (nickel, manganese, lead, copper, arsenic, iron, cobalt and chromium) were assessed by digesting 5 g of the sediment sample with dilute double acid solution (0.05 N HCl + 0.025 N H_2SO_4) (Boyd 1995). The resultant solution from the digest was analyzed for heavy metals using atomic absorption spectrophotometer (AAS PG-990 model) at appropriate wavelengths (Boyd 1995). The metals analyzed were nickel (232.0 nm), manganese (279.5 nm), lead (283.3 nm), copper (324.7 nm), arsenic (193.7 nm), iron (248.3 nm), cobalt (240.7 nm), and chromium (357.9 nm). Data obtained subjected to two-way analysis of variance (ANOVA) using SPSS software (Version 21; SPSS Inc. 2012) with fertilization and water flowage as the main factors and season (Dry and Rainy Season) as sub-factor.

Table 1. Description of fish ponds in NIFAGOL Farm

Variable	Unit	1	2	3	4	5	6
Year of impoundment		1984	1984	1984	1984	1984	1984
Outline shape	**-**	**Rectangular**	**Rectangular**	**Rectangular**	**Rectangular**	**Rectangle**	**Rectangle**
Dam size	**-**	**Small**	**Small**	**Small**	**Small**	**Small**	**Small**
Apporx. Surface Area	m^2	5550	3445	1325	4875	2275	1135.5
Apporx. Volume	m^3	13875	8612.5	1987.5	12187.5	3412.5	1703.25
Stock density	At 3 fish/m^2	16650	10335	3975	14625	6825	3305
Water Retention	Month(s)	6 (Non flow-through)	6(Non flow-through)	Flow through	Flow through	Flow through	Flow through
Cropping frequency	/ year	Twice	Twice	Not fixed (As required)	Not fixed	Not fixed	Not fixed
Fertilization Before stocking		NPK or Organic fertilizer	NPK or Organic fertilizer	NPK or Organic fertilizer	Not fertilized	Not fertilized	NPK or Organic fertilizer
Feeding frequency	/ day	Twice	Twice	NA	NA	NA	NA
Modifications done after impoundment		Refilling of caves and Excavating using bulldozer	Refilling of caves and Excavating using bulldozer	NA	NA	NA	NA
Duration of culture	Month(s)	6	6	Thru' out the year	Thru' out the year	Thru' out the year	Thru' out the year
Type of feed used		Pellets	Pellets	Pellets	Pellets & Brewer's waste	Pellets	Pellets
Type of fish being reared in the pond		*Clarias sp*	*Clarias sp*	*Clarias*/(Brooders)	*Tilapia & Clarias*	*Channa & Clarias*	*Channa & Clarias*
Al (mT)		254/255	256/255	250/251/254	256/257/259	258/258	254/256
Latitude (^{0}N)		07^0 32.379′ $07^0 32^0 402'$	$07^0$32.413′ $07^0$32.432′	$07^0$32.462′ $07^0$32.468′ $07^0$32.433′	$07^0$32449′ $07^0$32434′ $07^0$32471′	$07^0$32.412′ $07^0$32.425′	$07^0$32423′ $07^0$32395′
Longitude(^{0}E)		$004^0$26.786′ $004^0$26.769′	$004^0$26.770′ $004^0$26.742′	$004^0$26.770′ $004^0$26.781′ $004^0$26.801′	$004^0$26.842′ $004^0$26.812′ $004^0$26.790′	$004^0$26.826′ $004^0$26.856′	$004^0$26.793′ $004^0$26.800′

The means were separated using Tukey post-hoc test and differences were consider significant at significance level of 0.05. Inter-relationship among/between the three sets of ponds studied was determined using PAST (Paleontological Statistics) Statistical software version 2.12 (Hammer et al. 2001).

Results

The sediments of the investigated ponds showed various colors (Table 2) ranging from reddish brown, grayish brown, yellowish brown, dark brown to olive yellow. The sediments of the fertilized ponds ranged from greyish brown to very dark brown whereas the non-fertilized ponds ranged from yellow to red with very weak red and olive yellow coloration in April and June 2007 respectively (Table 2). As a result, the non-fertilized ponds' sediment had the lowest hue of 2.5 YR, whereas the fertilized ponds' sediment hues were typically 10 YR with a low of 5 YR. However, at the end of the fish culture period, the non-fertilized pond (precisely pond 6) had darker sediment.

Each of the investigated factors (fertilization and flowage) had effect on 12 out of 24 parameters considered (Table 3). The 12 parameters with higher mean in the fertilized ponds (FNF and FF) were on the average 1.16 times (16 percent) (1.01–1.47) higher than the unfertilized pond. Among these were the nutrient parameters (Nitrate, phosphate and organic matter/carbon) (1.05 times) and associated percentage silt and clay as well as few heavy metals (Ni, As, Cr) (1.13 times) and cations (Na^+, $Mg^{2+,}$ H^+) (1.31times) (Table 3). Flowage, on the other hand, had effect on both cations and anions (K^+, Ca^{2+}, Al^{3+}, SO_4^{2-}) (1.32 times), Heavy metals/Micronutrients (Fe, Mn, Cu, Co, Pb) (2.34 times) and percentage sand. These parameters were on the average 1.67 (67%) (1.01 – 7.00 times) higher in the flow-through ponds (FF and NFF) than non-flow-through ponds (FNF) with lead (7.00 times) having the greatest contribution to flowage effect (Table 3). Aluminum ion, potassium, lead and iron were discovered to have higher mean of approximately 17 percent (1.01-1.56 times) in non-fertilized flow-through ponds than fertilized flow-through (Table 3).

With the exception of copper and manganese, the heavy metals were mostly less than 1 mg/100 g and their order of dominance was Mn > Cu > Fe > As> Ni > Pb > Co > Cr. While the cationic order of dominance was in two patterns with either calcium or hydrogen ion dominating in each case. The order was $Ca^{2+} > H^+ > Mg^{2+} > Al^{3+} > Na^+ > K^+$ (ponds 2, 3, 4, 5 and 6) and $H^+ > Ca^{2+} > Mg^{2+} > Al^{3+} > Na^+ > K^+$ (pond 1). The anionic order of dominance was also in two slightly different pattern due to sulphate or phosphate ion dominance, resulting in $SO_4^{2-} > PO_4^{3-} > NO_3^{2-}$ (ponds 2, 3, 5 and 6) or $PO_4^{3-} > SO_4^{2-} > NO_3^{2-}$ (ponds 1 and 4).

For 13 of the investigated parameters, the pattern of seasonal variations in the three sets of ponds seemed to be similar. Phosphate, sulphate, chromium, pH (water/$CaCl_2$) and aluminum/hydrogen ion showed higher mean values in the rainy season for all ponds.

Whereas potassium, nitrate, organic matter/carbon, nickel, manganese and lead had higher mean values in the dry season (Table 4). The percentage sand and clay content of the sediment, as well as the sodium level, showed significant seasonal variation, with the highest mean for these parameters recorded in fertilized non-flow-through ponds (FNF) (Table 4). The effect of pond fertilization was further observed in the mean values of percent silt, percent clay, sodium, magnesium, nitrate, organic matter/carbon, nickel, copper and iron which were higher in the fertilized non-flow-through ponds during the dry season. Similarly, during the rainy season, the percentage sand, pH (water), and phosphate had the highest mean in FNF. Seasonal variation due to flowage was observed for calcium, manganese, and cobalt levels in sediment, which were highest in fertilized flow-through ponds (FF) during the dry season and sulphate during the rainy season. Furthermore, during the dry season, the highest detectable level of lead (0.24 mg/100g) was found in the non-fertilized flow-through pond. The overall t-test of the seasonal variation patterns of the investigated parameters confirmed significant variation for phosphate, nickel and manganese concentrations in the ponds' sediment, with phosphate concentration depicting highly significant differences (Table 5). Moreover, the concentration of phosphate, sulphate, nickel, manganese, lead and copper in the sediment were higher than the levels measured in reservoir inlet water (Table 6). Whereas water soluble and essential nutrients like sodium, potassium, calcium, nitrate and arsenic were not as concentrated in the sediment.

Based on the mean values of the studied variables, the Euclidean distance (similarity index) was used to test for correlation between the sets of ponds. The index revealed that the flow-through ponds (FF and NFF) were more similar than the fertilized ponds (FF and FNF). Furthermore, cluster analysis of the pond sets based on the mean values of the investigated parameters produced two clusters, with flow-through ponds (FF and NFF) separated from non-flow-through ponds (FNF) (Figure 1).

Table 2. Monthly variation in colour and textural composition of the investigated NIFAGOL fish pond sediments over the period of study

Cultural Practice	Parameters	FNF		FF		NFF	
Sampling Station		**1**	**2**	**3**	**4**	**5**	**6**
Month							
November 2006	Value / Chroma	3 / 2	NOT In Use	4 / 4	3 / 2	2.5 / 2	NOT In Use
	Hue (YR)	5		5	5	5	
	Color	Dark Reddish Brown		Reddish Brown	Dark Reddish Brown	Dark Reddish Brown	
	Sediment Type	Clayey Sand		Silty Sand	Silty Sand	Silty Sand	
February 2007	Value / Chroma	3 / 3	5 / 3	4 / 2	2 / 2	5 / 8	3 / 6
	Hue (YR)	10	10	10	10	10	10
	Color	Dark Brown	Brown	Greyish Brown	Dark Brown	Yellowish Brown	Yellowish Brown
	Sediment Type	Silty Mud	Silty Mud	Silty Sand	Silty Sand	Sandy Mud	Silty Sand
April 2007	Value / Chroma	NOT In Use	NOT In Use	2 / 2	3 / 3	3 / 3	6/ 8
	Hue (YR)			10	10	10	2.5
	Color			Very Dark Brown	Dark Brown	Dark Brown	Olive Yellow
	Sediment Type			Sandy Mud	Sand	Silty Sand	Silty Mud
June 2007	Value / Chroma	3 / 3	NOT In Use	5 / 2	3 / 6	5 / 2	7 / 6
	Hue (YR)	10		7.5	10	2.5	10
	Color	Dark Brown		Brown	Dark Yellowish Brown	Weak Red	Yellow
	Sediment Type	Silty Sand		Silty Sand	Silty Sand	Clayey Sand	Silty Mud
August 2007	Value / Chroma	NOT In Use	3 / 4	NOT In Use	NOT In Use	6 / 8	2 / 2
	Hue (YR)		10			10	10
	Color		Dark Yellowish Brown			Brownish Yellow	Very Dark Brown
	Sediment Type		Sand			Silty Mud	Silty Sand
October 2007	Value / Chroma	NOT In Use	3 / 6	3 / 6	3 / 6	3 / 6	2 / 2
	Hue (YR)		10	10	10	10	10
	Color		Dark Yellowish Brown	Dark Yellowish Brown	Dark Yellowish Brown	Dark Yellowish Brown	Very Dark Brown
	Sediment Type		Sand	Sand	Sand	Silty Sand	Sand

Table 3. Mean values of the pond sediment physico-chemical parameters based on cultural practice of investigated fish ponds in NIFAGOL Farm, Osun State, Nigeria, 2006-2007

Parameter	Pond						ANOVA	
	FNF		FF		NFF		F value	P
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.		
Sand %	39.00 - 89.00	68.17 ± 20.77	63.00 - 92.00	76.80 ± 9.27	39.00 - 92.00	70.69 ± 14.83	1.175	0.329
Silt %	3.00 – 31.00	14.50 ± 10.91	2.00 – 19.00	11.90 ± 5.86	2.00 – 31.00	13.58 ± 7.64	0.251	0.780
Clay %	8.00 – 36.00	17.33 ± 10.71	6.00 – 21.00	11.30 ± 4.55	6.00 – 36.00	15.73 ± 8.60	2.057	0.154
pH (Water)	6.20 – 7.40	6.88 ± 0.54	6.20 – 7.80	7.10 ± 0.53	6.20 – 7.80	6.96 ± 0.44	0.518	0.604
pH ($CaCl_2$)	5.90 – 7.10	6.53 ± 0.48	6.00 – 7.50	6.87 ± 0.53	5.90 – 7.50	6.67 ± 0.44	1.423	0.264
Hydrogen ion (meq/100g)	0.20 – 0.45	0.33 ± 0.10	0.10 – 0.75	0.32 ± 0.21	0.05 – 0.75	0.33 ± 0.19	0.036	0.964
Aluminium (meq/100g)	0.10 - 0.25	0.18 ± 0.05	0.10 - 0.30	0.20 ± 0.08	0.10 - 0.40	0.20 ± 0.08	0.651	0.532
Sodium (meq/100g)	0.14 – 0.67	0.30 ± 0.20	0.19 – 0.32	0.25 ± 0.05	0.14 – 0.67	0.26 ± 0.10	1.007	0.383
Potassium(meq/100g)	0.13 – 0.46	0.23 ± 0.13	0.12 – 0.49	0.23 ± 0.12	0.08 – 0.49	0.25 ± 0.11	0.671	0.523
Magnesium (meq/100g)	0.08 – 7.00	1.80 ± 2.60	0.03 – 2.56	0.85 ± 1.09	0.03 – 7.00	1.30 ± 1.81	0.686	0.515
Calcium (meq/100g)	4.00 – 7.20	5.30 ± 1.09a	5.20 – 10.00	7.17 ± 1.35b	4.00 – 10.00	5.30 ± 1.09ab	4.830*	0.019
Nitrate (%)	0.01 – 0.13	0.06 ± 0.05	0.01 – 0.11	0.06 ± 0.03	0.01 – 0.23	0.06 ± 0.05	0.004	0.996
Phosphate (ppm)	21.96 – 50.90	34.06 ±10.74a	19.32 – 30.06	26.55 ± 3.45b	19.32 – 55.40	30.67 ± 9.05ab	2.804	0.084
Sulphate (ppm)	21.96 – 78.93	43.26 ± 20.68	22.63 – 140.32	65.02 ± 37.73	21.96 – 149.72	61.25 ± 33.81	0.865	0.436
Organic Matter (%)	0.17 – 2.70	1.18 ± 1.02	0.17 – 2.10	1.11 ± 0.62	0.17 – 4.60	1.13 ± 0.98	0.003	0.997
Organic Carbon (%)	0 .10 – 1.57	0.69 ± 0.59	0.10 – 1.22	0.64 ± 0.36	0.10 – 2.67	0.66 ± 0.57	0.002	0.998
Nickel (mg/100g)	0.09 – 0.59	0.31 ± 0.17	0.02 – 0.53	0.17 ± 0.17	0.02 – 0.59	0.21 ± 0.15	1.582	0.230
Manganese (mg/100g)	2.48 – 11.45	6.97 ± 3.23	4.38 – 17.28	8.74 ± 4.01	2.48 – 17.28	8.11 ± 3.20	0.842	0.446
Lead (mg/100g)	ND – 0.02	0.02 ± 0.00	ND – 0.09	0.09 ± 0.00	ND – 0.24	0.14 ± 0.15	-	-
Copper (mg100g)	0.24 – 1.72	0.89 ± 0.56	0.02 – 1.92	1.03 ± 0.48	0.20 – 2.09	0.99 ± 0.47	0.138	0.872
Arsenic (mg/100g)	0.23 – 0.99	0.72 ± 0.28	0.19 – 0.83	0.47 ± 0.21	0.19 – 0.99	0.59 ± 0.24	1.811	0.189
Iron (mg/100g)	0.44 – 1.33	0.91 ± 0.43	0.46 – 1.40	0.91 ± 0.31	0.44 – 1.73	0.92 ± 0.33	0.048	0.953
Cobalt (mg/100g)	0.01 – 0.20	0.11 ± 0.07	0.07 – 0.22	0.14 ± 0.06	0.01 – 0.32	0.11 ± 0.08	0.665	0.525
Chromium (mg/100g)	0.02 – 0.14	0.07 ± 0.04	0.02 – 0.11	0.05 ± 0.03	0.01 – 0.14	0.06 ± 0.03	1.148	0.337

NB: Values in a row followed by different letters are significantly different ($P \leq 0.05$)

* = Significant

FNF – Fertilized Non flow-through pond

FF – Fertilized flow-through pond

NFF – Not fertilized flow-through pond

Table 4. Seasonal mean values of the sediment physico-chemical parameters based on aquacultural practice of the investigated fish ponds in NIFAGOL Farm, Osun State, Nigeria, 2006-2007

Parameter	Pond									ANOVA	
	FNF			FF			NFF				
	DS	RS	DS / RS	DS	RS	DS / RS	DS	RS	DS / RS	F value	P
Sand %	54.33 ± 21.57	82.00 ± 6.24	0.66	75.00 ± 2.31	78.00 ± 12.13	0.96	72.33 ± 7.57	63.43 ± 16.67	1.14	3.348*	0.056
Silt %	21.67 ± 11.37	7.33 ± 3.79	2.95	14.25 ± 3.40	10.33 ± 6.89	1.38	13.67 ± 5.03	15.14 ± 8.71	0.90	2.073	0.152
Clay %	24.00 ± 12.00	10.67 ± 3.06	2.25	10.75 ± 1.50	11.67 ± 5.96	0.92	14.00 ± 5.29	21.43 ± 9.86	0.65	3.395*	0.054
pH (Water)	6.57 ± 0.55	7.20 ± 0.35	0.91	7.00 ± 0.58	7.17 ± 0.53	0.98	6.87 ± 0.38	6.89 ± 0.28	1.00	0.762	0.480
pH ($CaCl_2$)	6.27 ± 0.47	6.80 ± 0.36	0.92	6.8 ± 0.62	6.92 ± 0.52	0.98	6.47 ± 0.40	6.61 ± 0.25	0.98	0.443	0.648
Hydrogen ion (meq/100g)	0.30 ± 0.10	0.37 ± 0.10	0.82	0.28 ± 0.21	0.35 ± 0.23	0.79	0.18 ± 0.08	0.40 ± 0.23	0.46	0.541	0.590
Aluminium (meq/100g)	0.17 ± 0.06	0.20 ± 0.05	0.83	0.20 ± 0.12	0.19 ± 0.05	1.04	0.28 ± 0.10	0.19 ± 0.09	1.46	2.377	0.119
Sodium (meq/100g)	0.42 ± 0.24	0.18 ± 0.04	2.36	0.25 ± 0.07	0.26 ± 0.04	0.97	0.22 ± 0.06	0.25 ± 0.05	0.86	4.587*	0.023
Potassium(meq/100g)	0.30 ± 0.17	0.16 ± 0.02	1.89	0.29 ± 0.16	0.20 ± 0.08	1.46	0.34 ± 0.01	0.26 ± 0.12	1.31	0.104	0.902
Magnesium (meq/100g)	2.74 ± 3.73	0.85 ± 0.54	3.24	0.09 ± 0.06	1.35 ± 1.18	0.07	1.02 ± 1.17	1.60 ± 2.18	0.64	1.450	0.258
Calcium (meq/100g)	5.60 ± 1.40	5.00 ± 0.87	1.12	7.40 ± 2.00	7.02 ± 0.90	1.05	5.27 ± 0.21	6.45 ± 1.56	0.82	0.761	0.480
Nitrate (%)	0.09 ± 0.06	0.03 ± 0.03	2.65	0.09 ± 0.02	0.04 ± 0.02	2.41	0.06 ± 0.04	0.06 ± 0.07	1.09	0.797	0.464
Phosphate (ppm)	27.29 ± 4.78	40.84 ± 11.31	0.67	23.94 ± 3.97	28.30 ± 1.68	0.85	24.93 ± 2.97	35.44 ± 11.40	0.70	0.937	0.408
Sulphate (ppm)	28.06 ± 5.80	58.47 ± 18.50	0.48	54.26 ± 57.43	72.20 ± 20.70	0.75	65.39 ± 73.03	68.48 ± 14.87	0.95	0.274	0.763
Organic Matter (%)	1.70 ± 1.25	0.66 ± 0.47	2.59	1.70 ± 0.34	0.71 ± 0.40	2.39	1.23 ± 0.76	1.08 ± 1.47	1.14	0.645	0.535
Organic Carbon (%)	0.99 ± 0.73	0.38 ± 0.27	2.57	0.99 ± 0.20	0.41 ± 0.24	2.39	0.72 ± 0.44	0.63 ± 0.85	1.14	0.634	0.541
Nickel (mg/100g)	0.38 ± 0.21	0.24 ± 0.13	1.55	0.30 ± 0.21	0.08 ± 0.07	3.80	0.21 ± 0.04	0.19 ± 0.12	1.10	1.093	0.355
Manganese (mg/100g)	9.26 ± 2.43	4.68 ± 2.10	1.98	10.26 ± 6.32	7.73 ± 1.40	1.33	9.99 ± 2.75	7.46 ± 1.99	1.34	0.223	0.802
Lead (mg/100g)	0.22 ± 0.00			0.09 ± 0.00			0.24 ± 0.00	0.03 ± 0.00	8.00		
Copper (mg/100g)	1.29 ± 0.49	0.49 ± 0.24	2.65	0.94 ± 0.80	1.09 ± 0.12	0.86	0.77 ± 0.52	1.11 ± 0.42	0.70	2.975	0.074
Arsenic (mg/100g)	0.72 ± 0.14	0.72 ± 0.43	0.99	0.54 ± 0.26	0.43 ± 0.18	1.27	0.62 ± 0.25	0.62 ± 0.22	1.00	0.123	0.885
Iron (mg/100g)	1.12 ± 0.37	0.70 ± 0.44	1.59	1.11 ± 0.44	0.78 ± 0.10	1.42	0.89 ± 0.13	0.94 ± 0.37	0.95	0.866	0.436
Cobalt (mg/100g)	0.12 ± 0.04	0.09 ± 0.10	1.33	0.16 ± 0.07	0.12 ± 0.05	1.33	0.09 ± 0.07	0.10 ± 0.11	0.88	0.396	0.678
Chromium (mg/100g)	0.07 ± 0.03	0.07 ± 0.06	0.95	0.03 ± 0.02	0.06 ± 0.03	0.49	0.04 ± 0.02	0.06 ± 0.04	0.63	0.360	0.702

* = Significant
FNF – Fertilized Non flow-through pond
FF – Fertilized flow-through pond
NFF – Not fertilized flow-through pond
DS – Dry Season
RS – Rainy Season

Table 5. Seasonal mean values of the sediment physico-chemical and heavy metal parameters of of the investigated fish ponds in NIFAGOL Farm, Osun State, Nigeria

Parameters	Dry Season	Rainy Season	t-test for Equality of Means	
	(Mean ± SD)	(Mean ± SD)	t	Sig. (2-tailed)
Sand %	68.00 ± 14.43	72.38 ± 15.30	-0.725	0.476
Silt %	16.30 ± 7.21	11.88 ± 7.62	1.469	0.155
Clay %	15.70 ± 8.59	15.75 ± 8.88	-0.014	0.989
pH (Water)	6.83 ± 0.50	7.08 ± 0.39	-1.209	0.238
pH ($CaCl_2$)	6.54 ± 0.52	6.79 ± 0.37	-1.219	0.234
Hydrogen ion (meq/100g)	0.26 ± 0.14	0.39 ± 0.20	-1.665	0.108
Aluminium (meq/100g)	0.22 ± 0.10	0.18 ± 0.05	0.636	0.531
Sodium (meq/100g)	0.29 ± 0.15	0.23 ± 0.05	0.997	0.342
Potassium(meq/100g)	0.30 ± 0.12	0.21 ± 0.09	1.995	0.057
Magnesium (meq/100g)	1.17 ± 2.18	1.46 ± 1.64	-0.289	0.775
Calcium (meq/100g)	6.22 ± 1.68	6.27 ± 1.33	-0.291	0.773
Nitrate (%)	0.08 ± 0.04	0.05 ± 0.05	1.847	0.077
Phosphate (ppm)	25.24 ± 3.80	32.52 ± 8.32	-3.244**	0.004
Sulphate (ppm)	49.74 ± 50.39	67.87 ± 17.79	-1.110	0.292
Organic Matter (%)	1.56 ± 0.75	0.92 ± 1.05	1.824	0.080
Organic Carbon (%)	0.91 ± 0.44	0.53 ± 0.61	1.825	0.080
Nickel (mg/100g)	0.30 ± 0.17	0.16 ± 0.12	2.384*	0.025
Manganese (mg/100g)	9.88 ± 4.06	6.90 ± 2.00	2.405*	0.024
Lead (mg/100g)	0.18 ± 0.08	0.03 ± 0.00	1.630	0.245
Copper (mg/100g)	1.00 ± 0.61	0.99 ± 0.39	0.023	0.982
Arsenic (mg/100g)	0.62 ± 0.22	0.55 ± 0.26	0.493	0.627
Iron (mg/100g)	1.05 ± 0.33	0.82 ± 0.31	1.607	0.121
Cobalt (mg/100g)	0.13 ± 0.06	0.11 ± 0.08	0.688	0.498
Chromium (mg/100g)	0.04 ± 0.03	0.07 ± 0.04	-1.423	0.167

*Significant ($P \leq 0.05$)
**Highly significant ($P \leq 0.01$)

Table 6. Mean values of pond sediment characteristic of the investigated fish ponds in NIFAGOL Farm, Osun State, Nigeria in comparison with the water supplying reservoir and desirable limits

Parameter	Pond			Reservoir's water quality	Desirable limits
	FNF	FF	NFF		
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D (mg/L)	Persaud et al., 1993 (ppm)
Sand %	68.17 ± 20.77	76.80 ± 9.27	70.69 ± 14.83	NA	
Silt %	14.50 ± 10.91	11.90 ± 5.86	13.58 ± 7.64	NA	
Clay %	17.33 ± 10.71	11.30 ± 4.55	15.73 ± 8.60	NA	
pH (Water)	6.88 ± 0.54	7.10 ± 0.53	6.96 ± 0.44	7.78 ± 0.42	
pH ($CaCl_2$)	6.53 ± 0.48	6.87 ± 0.53	6.67 ± 0.44	NA	
Hydrogen ion (meq/100g)	0.33 ± 0.10	0.32 ± 0.21	0.33 ± 0.19	NA	
Aluminium (meq/100g)	0.18 ± 0.05	0.20 ± 0.08	0.20 ± 0.08	NA	
Sodium (meq/100g)	0.30 ± 0.20	0.25 ± 0.05	0.26 ± 0.10	11.3 ± 1.8	
Potassium(meq/100g)	0.23 ± 0.13	0.23 ± 0.12	0.25 ± 0.11	10.2 ± 2.04	
Magnesium (meq/100g)	1.80 ± 2.60	0.85 ± 1.09	1.30 ± 1.81	1.62 ± 0.80	
Calcium (meq/100g)	5.30 ± 1.09	7.17 ± 1.35	5.30 ± 1.09	16.9 ± 4.4	
Nitrate (%)	0.06 ± 0.05	0.06 ± 0.03	0.06 ± 0.05	0.84 ± 0.10	
Phosphate (ppm)	34.06 ±10.74	26.55 ± 3.45	30.67 ± 9.05	1.18 ± 0.29	600 - 2000
Sulphate (ppm)	43.26 ± 20.68	65.02 ± 37.73	61.25 ± 33.81	15.20 ± 4.03	
Organic Matter (%)	1.18 ± 1.02	1.11 ± 0.62	1.13 ± 0.98	5.20 ± 1.75	
Organic Carbon (%)	0.69 ± 0.59	0.64 ± 0.36	0.66 ± 0.57	3.03 ± 1.02	1 - 10
Nickel (mg/100g)	0.31 ± 0.17	0.17 ± 0.17	0.21 ± 0.15	0.0 ± 0.0	16 – 75
Manganese (mg/100g)	6.97 ± 3.23	8.74 ± 4.01	8.11 ± 3.20	0.038 ± 0.033	460 – 1100
Lead (mg/100g)	0.02 ± 0.00	0.09 ± 0.00	0.14 ± 0.15	0.007 ± 0.019	31 – 250
Copper (mg/100g)	0.89 ± 0.56	1.03 ± 0.48	0.99 ± 0.47	0.005 ± 0.006	16 – 110
Arsenic (mg/100g)	0.72 ± 0.28	0.47 ± 0.21	0.59 ± 0.24	8.29 ± 3.52	6 – 33
Iron (mg/100g)	0.91 ± 0.43	0.91 ± 0.31	0.92 ± 0.33	NA	2 – 4
Cobalt (mg/100g)	0.11 ± 0.07	0.14 ± 0.06	0.11 ± 0.08	NA	50
Chromium (mg/100g)	0.07 ± 0.04	0.05 ± 0.03	0.06 ± 0.03	NA	26 – 110

FNF – Fertilized non flow-through pond
FF – Fertilized flow-through pond
NFF – Not fertilized flow-through pond
NA – Not Assessed

Discussion

Fish ponds are completely man-made environments, with constant additions of fertilizer and feed to increase the culture's productivity and profitability. The impact of management and feeding could cause major issues in fishponds because the majority of food that is not consumed by fish is available for the growth of algae and bacteria. As observed during the current study, a wide range of environmental factors operating in the fish pond system, such as liming, fertilization, feeding with exogenous feeds, aquatic animal feces, dead animals, and higher aquatic vegetation, had a significant impact on sediment characteristics.

These organic waste components darkened the pond sediment hence the colors recorded were generally dark indicating their reduced state (Boyd 1995). The olive yellow coloration in pond 6 could be attributed to low of aquacultural activities observed during the study period, and even the fact that it is flow-through, so a small amount of organic waste sinks to its bed. And since the water is not turbid, the light coloration could also be attributed to sediment transport caused by flow and exposure to direct sunlight. (Berkowitz et al. 2018). According to Aldorfer (1974), the color of sediments, could also be an indicator of the drainage pattern, so the observed sediment coloration of red, yellow and brown color

implies good drainage. While the grayish coloration observed in pond 3 in February indicated poor drainage, this was due to the pond being left almost stagnant for a long duration to fallow.

The average percentage clay was 20% which is an optimal state for bottom sediment in properly built ponds to minimize the risk of excessive seepage (Boyd 1995). The variation in textural composition observed during the study period, on the other hand, could be due to pond erosion and sedimentation. Sandy nature recorded, mostly during rainy months, in the ponds could be attributed to their susceptibility to erosion which could have prevented sedimentation of fine particles (silt and clay) and organic waste sink.

Conversely, highest percentage sand was also observed in the flow-through ponds as compared to the non-flow-through ponds. The highest percentage of silt recorded in the non-flow-through ponds further proved the tendency of organic waste to sink faster when the waterbody is stagnant. The accumulation of clay, silt and nutrients in the fertilized pond sediment has been linked to intensive management which may result in in pond depth and space reduction (Rahman et al. 2004). Despite this, only a minimal accumulation of silt and other nutrients was recorded in the fertilized flow-through ponds. Therefore, in order to minimize eutrophication during fish culture, fertilized flow-through production method would be most suitable. This was also confirmed by detection of lowest mean concentration of total phosphate in these set of ponds. Hartono et al. (2019) observed that continuous flow of water over fishpond sediment reduces phosphorus bonding energies, minimizing the rate of phosphorus adsorption by the sediment.

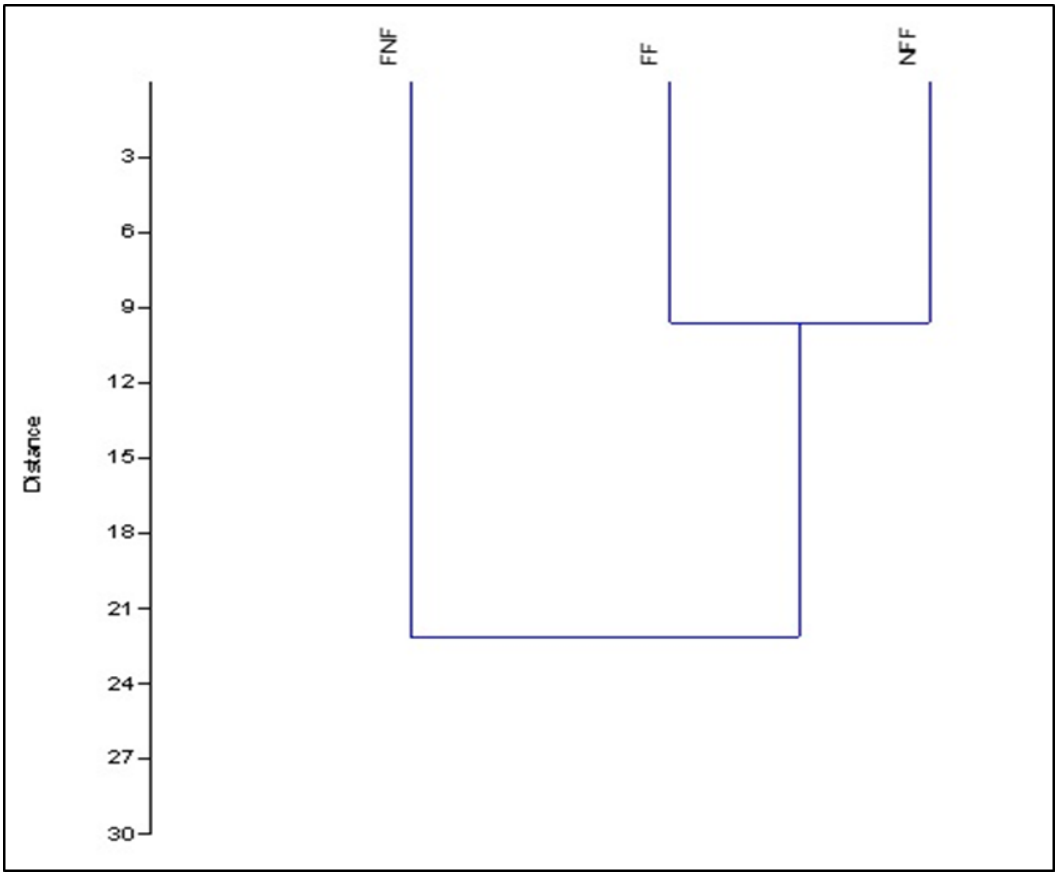

Figure 1. Cluster analysis showing the relationship between fishponds based on the sediment quality parameters studied

The pH of commercial fish farm sediment was on the average below 7.5 which could be attributed to the clayey nature of the sediment (Wurt and Masser 2004). However, the pH range was 6.5 to 7.2, suggesting that the sediments were medium acidic, slightly acidic, or neutral. This acidic condition is a common problem in pond aquaculture and liming of ponds has been the solution (Boyd and Tucker 1998). Acidity and pH of sediment are known to be caused by the exchangeable aluminium and hydrogen ions in the sediment. Therefore, based on the values of exchangeable aluminium ion recorded which ranged from 0.10 meq/g to 0.40 meq/g, the ponds had very low exchangeable acidity. The concentration of exchangeable aluminium ion was high, especially in the flow-through ponds, indicating a higher proportion of basic cations (calcium, magnesium, sodium and potassium). Whereas, the observed increase in hydrogen ion concentrations in the sediment during the rainy season has been attributed

to rising in-flow of floodwaters, as well as the subsequent re-cycling and settling of benthos materials (Boyd et al. 2002).

On the average, the pond sediment organic carbon recorded in this study fell within the usual range of 0.5% to 5% organic carbon (Boyd et al. 2002). Occasionally during the study period, the organic carbon in the sediment was less than 0.5 percent, which is very low and will not support good benthos growth. However, the lowest percentage of organic carbon and matter recorded in fertilized flow-through pond may be due to its existing flow and management process. In general, the sediments with mineral soil of low organic matter content are excellent condition for ponds with exogenous feeding.

The calculated carbon: nitrogen ratio ranged from 8.5: 1 to 16.0: 1, implying that these waterbodies might not be susceptible to anaerobic condition at the sediment-water interface (Boyd et al. 2002). Based on the Healey and Hendzel's nitrogen deficiency criterion, (C: N ratio < 9- No deficiency; 9-15 – moderate and >15 – severe) (Gautam and Bhattarai 2008), all the investigated waterbodies were not nitrogen deficient during the study period. However, pond 6 (Non-fertilized flow-through) undergone considerable nitrogen deficiency in April and October 2007, possibly due to the accumulation of stable organic matter that decomposes slowly. The source of these organic matter may be linked to the erosion influx that occurs at these times of year.

The phosphorus concentrations measured in this analysis were within the optimum range of 30-60 ppm (Munsiri et al. 1995). The low sediment phosphorus concentration observed during the rainy season may be attributed to seasonal mixing at the water-sediment interface, which results in the release of sediment phosphorus into the water (Gerhardt et al. 2010). The higher sulphate concentrations observed in flow-through ponds may be linked to erosion, which is the primary source of sulphur in non-acidic sulfate soils (Munsiri et al. 1995). Cation's concentrations of sediments in the present study were far below average range obtained from 358 freshwater fish ponds by Munsiri et al. (1995). Since acidic sediment usually contains little to no calcium carbonate, as seen in ponds located on calcareous soils, the low calcium level in the ponds confirmed the acidity of the sediments (Munsiri et al. 1995). Furthermore, the cationic hierarchy was such that calcium concentration was greater than sodium concentration in all ponds, which was responsible for the lower pH observed. As sodium is a known basic cation whose presence in high concentrations leads to high pH (Munsiri et al. 1995). The sodium adsorption ratios (SAR) as calculated were also quite low and generally below 0.50 which further confirmed the acidity of the sediment (Boyd et al. 2002).

The highest concentration of calcium in the fertilized flow-through ponds is also an advantage this method of production had over others as it connotes availability of notable level of calcium in the ponds. According to the literature, calcium plays an important role in reducing sodium and potassium ion loss from fish body fluid (Wurts and Durborow 1992). It also improves phosphorous availability for primary productivity (Wurts and Masser 2004), allows for the blocking of copper and zinc effects at the site of their toxic activity (Wurts and Perschacher 1994), and sedimentation of muddy water (Wurts 2002). Conversely, the high sodium levels observed in fertilized non-flow-through ponds are most likely due to significant loss of sodium and magnesium salt from the fishes' body fluid into the water (Wurts and Durborow 1992), which then settles into the bottom sediment.

The presence of the micronutrients such as iron, manganese, cobalt, copper and other heavy metals in the sediments have been connected to high pH and alkalinity which favors micro - nutrient precipitation. (Boyd 1995). Flowage, on the other hand, promoted the presence of 5 of the 8 investigated heavy metals, with high concentrations of these metals (Fe, Mn, Cu, Co, and Pb) in the flow-through ponds. The high Pb and Fe concentrations may be attributed to the material of pipe network used to supply water to the ponds. Nonetheless, their concentrations in these sediments, which were very low to low (based on the range developed by Munsiriet al. 1995), may be classified as non-toxic (MacDonald et al. 2000). Furthermore, with the exception of iron in all of the ponds, the majority of the heavy metals were within the suitable range for sediment. (Table 6).

The significant variations in clay, silt and nutrient parameters accumulation (phosphate, organic matter and carbon) based on flowage, as well as the significant availability of calcium ion in the fertilized flow-through ponds, revealed that revealed that this mode of fish culture is probably the most suitable one in the study area. As a result, more research should be done to determine the best water flow rate for the fertilized flow-through ponds.

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Assessment of Chromogenic Media in Bacterial Fish Pathogens

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ABSTRACT

Accurate and quick identification of bacterial fish disease has great importance for effective disease control and prevent economic loss in aquaculture facilities. Generally used agar media allow the growth of wide-range bacteria but new chromogenic media results in colored colonies that lead to presumptive identification without requiring biochemical or serological tests. In this study, the recently commercialized chromogenic media; chromID® CPS® Elite, chromID® CPS® Elite / Columbia CNA +5% sheep blood agar, chromID® *Vibrio*, chromID® MRSA Smart and chromID® *Staphylococcus aureus* Elite (BioMérieux, France) were tested with growth performance and colony coloration of bacterial fish pathogens. chromID® CPS® Elite and chromID® CPS® Elite / Columbia CNA +5% sheep blood agar were detected the most suitable culture media to grow for all five isolated fish pathogens (i.e., *Aeromonas hydrophila*, *Lactococcus garvieae*, *Pseudomonas fluorescens*, *Yersinia ruckeri* and *Vibrio anguillarum*). Of which, *L. garvieae* was the only one observed with green colonies. chromID® *Vibrio* designed for *V. parahaemolyticus* and *V cholerae* was lead dark pink/purple colonies by *V. anguillarum*. The chromID® MRSA Smart and chromID® *S. aureus* Elite were detected not effective for incubation of bacterial fish pathogens.

Keywords: Chromogenic media, fish diseases, fish pathogens, isolation

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Bakteriyel Balık Patojenleri için Kromojenik Besiyerlerinin Değerlendirilmesi

Öz: Akuakültür işletmelerinde etkin hastalık kontrolü ve ekonomik kayıpların önlenmesi için bakteriyel balık hastalıklarının doğru ve hızlı tanımlanması büyük önem taşımaktadır. Genel olarak kullanılan agar besiyerlerinde çok çeşitli bakteri türünün üremesi gözlenirken, yeni kromojenik besiyerleri, biyokimyasal ve serolojik test gerektirmeden renkli koloni görünümüyle tahmini ön tanımlama imkânı sunmaktadır. Bu çalışmada yakın zamanda ticarileşen kromojenik besiyerlerinde (chromID® CPS® Elite, chromID® CPS® Elite / Columbia CNA +5% sheep blood agar, chromID® *Vibrio*, chromID® MRSA Smart ve chromID® *Staphylococcus aureus* Elite (BioMérieux, France)) bakteriyel balık patojenlerinin üremesi ve koloni renklenmeleri tespit edilmiştir. ChromID® CPS® Elite ve chromID® CPS® Elite / Columbia CNA +5% sheep blood agarın test edilen 5 farklı bakteriyel balık patojeni (*Aeromonas hydrophila*, *Lactococcus garvieae*, *Pseudomonas fluorescens*, *Yersinia ruckeri* ve *Vibrio anguillarum*) için en uygun besiyeri olduğu, ancak sadece *L. garvieae*'nin yeşil koloni görünümü ile ürediği belirlenmiştir. *Vibrio parahaemolyticus* ve *Vibrio cholerae*'nın hızlı teşhisi için geliştirilen chromID® *Vibrio* besiyerinde *V. anguillarum* kolonileri koyu pembe/mor renkli olarak gözlenmiş, chromID® MRSA Smart ve chromID® *S. aureus* Elite besiyerlerinin bakteriyel balık patojenlerinin üremesi için uygun olmadığı belirlenmiştir

Anahtar kelimeler: Kromojenik besiyeri, balık hastalıkları, balık patojenleri, izolasyon

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Introduction

Along with the fastest growing food industry, aquaculture, and the increasing production rates, it is important to identify the fish diseases accurately and quickly that these diseases can cause great economic loss during the production cycle. Proper and quick identification is the most important step for diagnosis of the fish diseases.

The use of chromogenic media engenders the inhibition of nontarget organisms with differential and selective culture characteristics and target pathogens grow as colored colonies. The system of

these chromogenic media depends on the metabolism of pathogens or enzyme substrates (Perry 2017). chromID® CPS® Elite and chromID® CPS® Elite / Columbia CNA +5% sheep blood are designed for isolation of urine specimens, *Escherichia coli*, *Proteeae* and *Enterococcus*; chromID® *Vibrio* is for presumptive identification of *Vibrio parahaemolyticus* and *Vibrio cholerae*; chromID® MRSA Smart and chromID® *Staphylococcus aureus* Elite for the direct screening of methicillin-resistant *S. aureus* (MRSA) and *S. aureus* in clinical microbiology. It is important to utilize these media for quick presumptive identification of the bacterial fish disease by easier prediagnosis in order to prevent economic loss in aquaculture facilities.

There is no single media for isolation of bacterial fish pathogens. Besides the generally used culture media, some selective media are used for eliminating the growth of a wide range of bacteria. Thiosulfate Citrate Bile Sucrose (TCBS) Agar was originally designed for the isolation of *V. parahaemolyticus* and *V. cholerae* but it is also used for the isolation of some *Vibrio* species that are infectious for fish such as *V. alginolyticus*, *V. anguillarum*, *V. fischeri*, *V. harveyi*, *V. ordalii*, *V. splendidus* and *V. pelagus*. Waltman-Shotts medium has been devised in order to isolate another fish pathogen, *Yersinia ruckeri* with precipitation of insoluble calcium salts around the colonies depending on the Tween 80 hydrolysis (Austin and Austin 2007). Similarly, Yersinia CIN agar supplies selective cultivation of Yersinia species, commonly for *Y. pseudotuberculosis* and *Y. enterocolitica*, but also for isolation of infectious fish pathogen, *Y. ruckeri*. GSP agar is a selective agar base for detection of *Pseudomonas* and *Aeromonas* and has been using for isolation of infectious *Aeromonas* and *Pseudomonas* species in fish. The *Pseudomonas* selective agar base, Cetrimide agar has been especially used for the isolation of *Pseudomonas aeruginosa* by yellow/green colonial growth. Likewise, Eosin Methylene-Blue (EMB) agar is selective for *Enterobacteriaceae* family, coliform bacteria and especially for *E. coli* by greenish metallic sheen colored colonies caused by the lactose fermentation.

The aim of this study was to test the growth and the selectivity of five commercially available chromogenic media (chromID® CPS® Elite / Columbia CNA +5% sheep blood, chromID® CPS® Elite, chromID® *S. aureus* Elite, chromID® *Vibrio*, and chromID® MRSA Smart) for the isolation and presumptive identification of *A. hydrophila, L. garvieae, P. fluorescens, V. anguillarum* and *Y. ruckeri* while comparing its specificity and sensitivity with generally used selective media.

Materials and Methods

Medium

The recently commercialized chromID® CPS® Elite / Columbia CNA +5% sheep blood (product no. 418229), chromID® CPS® Elite (product no. 418284), chromID® *S. aureus* Elite (product no. 419042), chromID® *Vibrio* (product no. 43761) and chromID® MRSA Smart (product no. 413050) were purchased from BioMérieux (France) and compared with routinely employed selective agar media **(**Thiosulfate Citrate Bile Sucrose (TCBS-Merck, Germany) Agar, Cetrimide Agar (Merck, Germany), Yersinia CIN Agar (Merck, Germany), EMB Agar (Merck, Germany), Glutamate Starch Phenol Red Agar - *Pseudomonas Aeromonas* Selective Agar (GSP, Merck, Germany) and Waltman-Shotts Medium for the isolation of *Y. ruckeri* (WS)) for detection and identification of infectious fish pathogens. In addition, comparison of the growth performance and advantages in isolation and identification were performed between new chromID® media and former media.

Microbial Strains

The bacterial fish pathogens previouslyisolated and identified from rainbow trout farms were used for all media. *A. hydrophila*, *L. garvieae*, *P. fluorescens*, *V. anguillarum* and *Y. ruckeri* were obtained from İzmir Katip Çelebi University, Faculty of Fisheries Fish Disease and Biotechnology Laboratory.

Inoculation of Isolates onto Culture Media

Each isolate was suspended in 1 ml of saline (0.85 %) until the turbidity reached at 0.5 McFarland standard (approximately 1.5 x 10^8 cfu/ml) then they were inoculated onto each medium type by using a loop. The petri dishes were incubated at 21°C. The growth was observed every 12, 24 and 48 hours of incubation. All strains were inoculated in duplicate on separate occasions.

Results

The growth of *A. hydrophila* on chromID® CPS® Elite was observed as pale brown colonies but no colonies grew on Columbia CNA +5% sheep blood agar, chromID® MRSA Smart, chromID® *S. aureus* Elite and chromID® *Vibrio* (Figure 1, Table 1). Yellowish/green colonies were observed on GSP agar which is selective for *Pseudomonas*/*Aeromonas* species. *L. garvieae* grew as green colonies on chromID® CPS® Elite /

Columbia CNA +5% sheep blood and chromID® CPS® Elite but was not recovered from chromID® MRSA Smart, chromID® *S. aureus* Elite and chromID® *Vibrio* (Figure 1, Table 1).

Table 1. Growth of different infectious fish pathogens on various media after 48 h of incubation at 21 °C

Species	Total number of isolates	Growth on chromID® CPS® Elite / Columbia CNA +5% sheep blood	Growth on chromID® CPS® Elite	Growth on chromID® *Vibrio*	Growth on chromID® MRSA Smart	Growth on chromID® *S. aureus* Elite
A. hydrophila	2	2	2	0	0	0
L. garvieae	21	21	21	0	0	0
P. fluorescens	2	2	2	2	0	0
V. anguillarum	6	6	6	6	0	0
Y. ruckeri	14	14	14	3	2	3

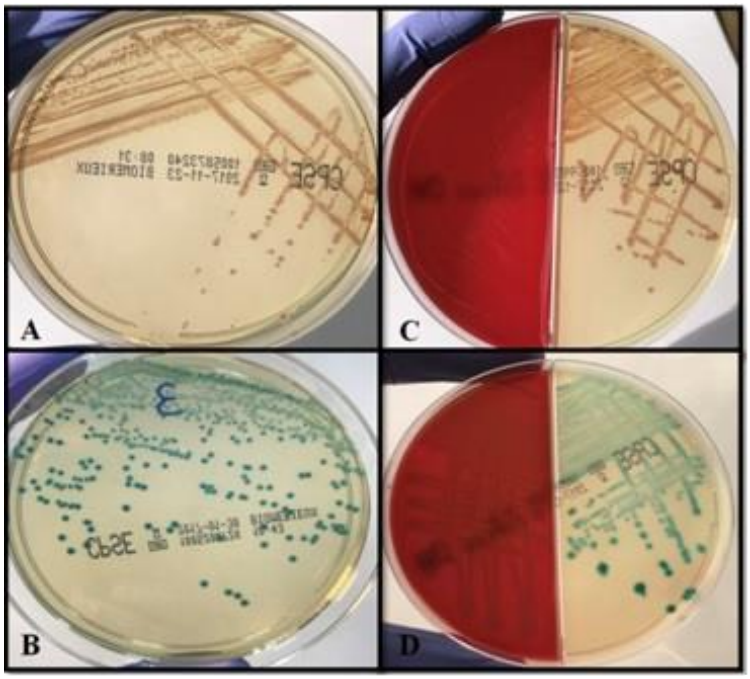

Figure 1. A: *A. hydrophila* on chromID® CPS® Elite; B: *L. garvieae* on chromID® CPS® Elite; C: *A. hydrophila* on chromID® CPS® Elite / Columbia CNA +5% sheep blood agar; D: *L. garvieae* on chromID® CPS® Elite / Columbia CNA +5% sheep blood agar

The colonial growth of *P. fluorescens* appeared on chromID® CPS® Elite / Columbia CNA +5% sheep blood and chromID® CPS® Elite, moreover on chromID® *Vibrio* as orange colonies (Figure 2, Table 1). No colonies were presented on chromID® MRSA Smart and chromID® *S. aureus* Elite. In addition, colonies were present on selective GSP and Cetrimide agar.

Vibrio anguillarum colonies were observed on chromID® CPS® Elite as pale brown colonies and on chromID® *Vibrio* as dark pink/purple colonies but there was no colonial growth on Columbia CNA +5% sheep blood agar, chromID® MRSA Smart and chromID® *S. aureus* Elite (Figure 2, Table 1). In addition, yellow colored colonies appeared on TCBS agar which is the selective agar for *Vibrio* species.

Pale brown colonies of *Y. ruckeri* were present on chromID® CPS® Elite but there was only weak growth on chromID® MRSA Smart and chromID® *S. aureus* Elite with pink pigmentation and on chromID® *Vibrio* with green pigmentation. There were no colonies detected on Columbia CNA +5% sheep blood agar (Figure 3, Table 1). The precipitation of insoluble calcium salts around colonies were observed on Waltman-Shotts Medium and dark pink/red colonies were detected on Yersinia CIN Agar.

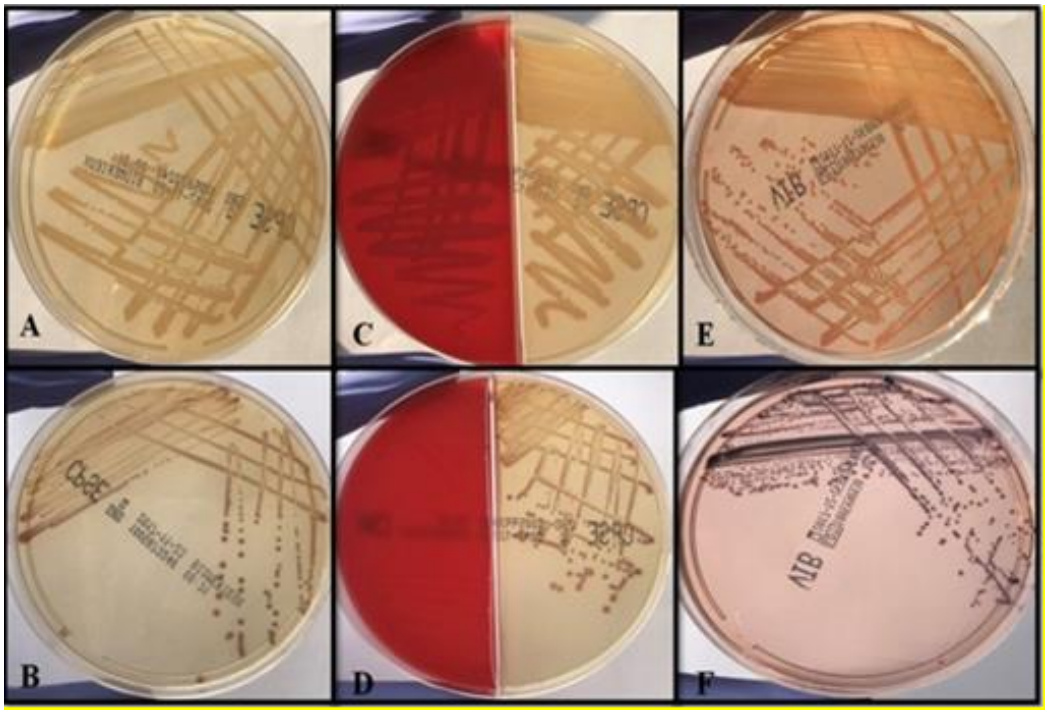

Figure 2. A: *P. fluorescens* on chromID® CPS® Elite; B: *V. anguillarum* on chromID® CPS® Elite; C: *P. fluorescens* on chromID® CPS® Elite / Columbia CNA +5% sheep blood agar; D: *V. anguillarum* on chromID® CPS® Elite / Columbia CNA +5% sheep blood agar; E: *P. fluorescens* on chromID® *Vibrio*; F: *V. anguillarum* on chromID® *Vibrio*

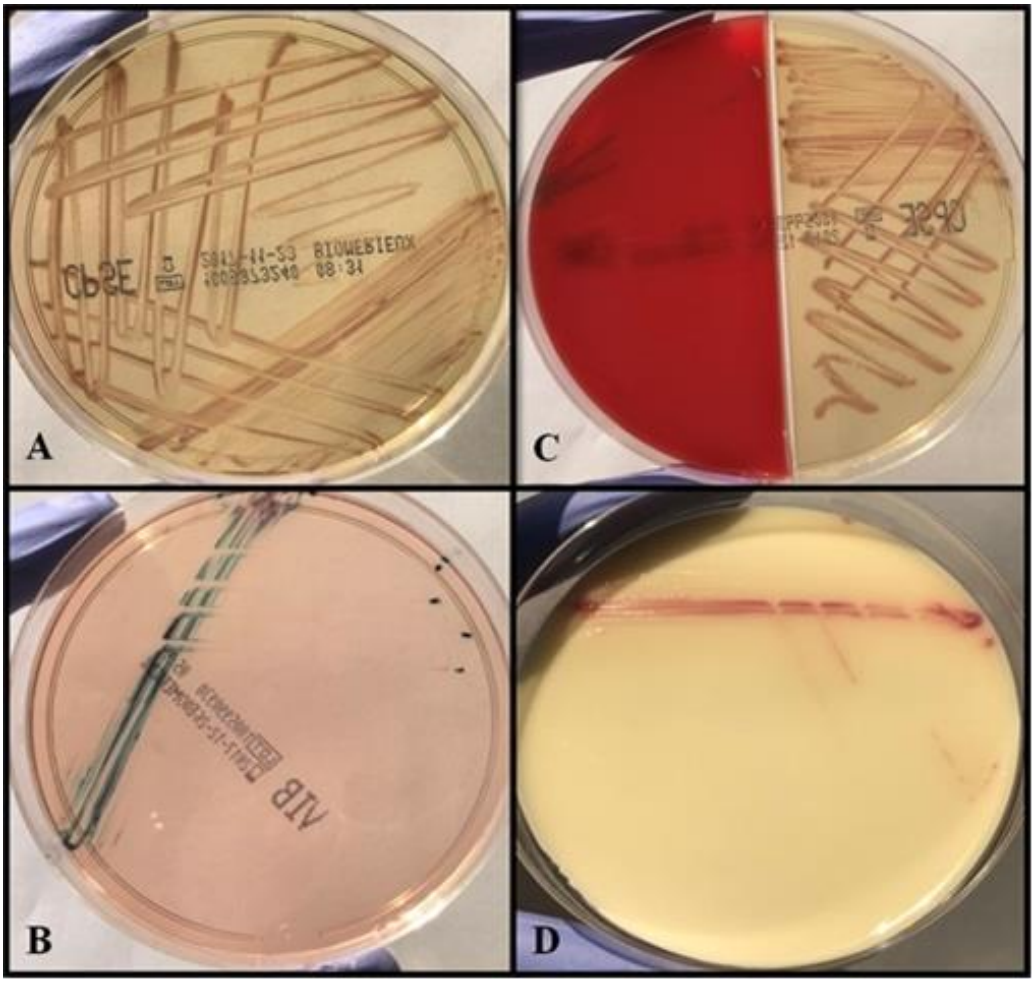

Figure 3. A: *Y. ruckeri* on chromID® CPS® Elite; B: *Y. ruckeri* on chromID® *Vibrio*; C: *Y. ruckeri* on chromID® CPS® Elite / Columbia CNA +5% sheep blood agar; D: *Y. ruckeri* on chromID® *S. aureus* Elite

Discussion

The bacterial fish pathogens were plated on 5 different commercialized media and compared to selective media in order to detect colony morphologies, growth performance and efficiency for detecting and identifying infectious fish pathogens.

Based on the colony characteristics, chromID® CPS® Elite agar has been recently commercialized and used for isolation and identification of urinary tract infections, especially *E. coli* in clinical microbiology. In addition, it is possible to detect *Enterococcus* spp., some members of *Proteeae* group and some members of *Enterobacteriaceae*, culture them and amplify antibiotic susceptibility tests. This media allows a preliminary identification for various microorganisms with genus or group level on the strength of colony appearance and Gram stain. Also, it is able to directly identify *E. coli* by the colony appearance without any requirement of Gram stain or biochemical testing (Yarbrough et al. 2016). The bacterial load of fish is less then urinary samples, it is likely that the selectivity of fish pathogens would be higher. In this study, all 5 bacterial fish pathogen grew on chromID® CPS® Elite, especially green colony was observed with *L. garvieae* which is one of Gram-positive bacteria but *Y. ruckeri* which belongs to the family *Enterobacteriaceae* showed no distinctive colony coloration. Moreover, we have tested chromID® CPS® Elite / Columbia CNA +5% sheep blood which contains two different media on the same agar plate in half. Columbia CNA +5% sheep blood agar is specific for the isolation and differentiation of Gram-positive microorganisms. Our results clearly showed that the chromID® CPS® Elite / Columbia CNA +5% sheep blood agar was highly selective to *L. garvieae*. Also, observing growth on both media on the same plate can lead to reliably compare the colony morphologies and coloration easily.

The members of the genus *Vibrio* have great importance because of the association with human disease as *V. cholerae* (the etiological agent of epidemic cholera) and *V. parahaemolyticus* (the cause of gastroenteritis associated with the consumption of seafood) (Farmer et al. 1985; Grimes 1991; Blanco-Abad et al. 2009; Eddabra et al. 2011). chromID® *Vibrio* was developed for determining *V. cholerae* and *V. parahaemolyticus* from other *Vibrio* species by the blue/green colonies of *V. cholerae* and pink colonies of *V. parahaemolyticus* on the medium. The TCBS *Vibrio* selective media was developed for isolation of pathogenic *Vibrio* species but it has poor sensitivity in natural specimens, labor-intensive and time-consuming. In addition, TCBS may not differentiate *V. cholerae* from *V. alginolyticus* or *V. parahaemolyticus* and *V. fluvialis* from *V. mimicus* or *V. vulnificus* (Eddabra et al. 2011). There is a slight selective advantage of chromID® *Vibrio* because of the color colonies. It has indicated that its sensitivity is equivalent to TCBS medium and two times more specific as well as no confirmation is needed (Eddabra et al. 2011). *Vibrio cholerae* appears as blue-green colonies because of beta galactosidase-produce and *V. parahaemolyticus* grows pink colonies based on arabinose assimilation on chromID® *Vibrio* media. Pinto et al. (2011) reported that *Vibrio* CHROMagar plates (CAV, PBI International, Milan, Italy) displayed higher specificity and accuracy than TCBS agar for isolating *V. parahaemolyticus* and *V. fluvialis.* Furthermore, unlike TCBS medium, the CAV medium was more efficient to isolate *V. parahaemolyticus* for DNA-based typing analysis. The fish pathogen *V. anguillarum* was tested on chromID® *Vibrio* media for the first time in this study and observed dark pink/purple colonies. The color characteristic of *V. anguillarum* was not clear; therefore, further studies are needed to certify the reason of dark pink/purple coloration.

The direct screening of methicillin-resistant *S. aureus* (MRSA) is practicable with using chromID® MRSA Smart in clinical microbiology. ChromID® *S. aureus* Elite is another media for the direct identification of *S. aureus* by spontaneous green coloration of glucosidase-producing colonies. *Staphylococcus* genus has been implicated as pathogen for fish and causing septicemia-like signs, exophthalmia and lesions around fins (Shotts and Teska 1989; Gil et al. 2000). Another important fish pathogen *L. garvieae* affects mainly rainbow trout farms that cause losses approximately 50% of the total production (Ghittino and Prearo 1992; Alrabadi 2012; Tanrıkul 2012; Balta and Dengiz Balta 2019). Also, infected or carrier fish may spread the disease to healthy rainbow trout and lead to serious economic damage (Algöet et al. 2009). Several antibiotic treatments were applying such as enrofloxacin, florfenicol and oxytetracycline in order to control the outbreaks in Turkey (Kayış et al. 2009; Ture and Boran 2015). The antimicrobial resistant bacteria and its potential transfer between animals and human are the main concern and the antibiotic treatment may be not efficient in fish farms (Cabello et al. 2013; Muziasari et al. 2014; Ture and Boran 2015). In this study, we aimed to use both media for isolation of *Staphylococcus* while inhibiting the growth of *L. garvieae* but none of the fish pathogens were able to

grow on chromID® MRSA Smart and chromID® *S. aureus* Elite. These results clearly show that both of these media are not suitable for isolation and identification of fish pathogens.

The use of chromogenic media brings some advantages such as easy detection of target pathogens and differentiation of mixed cultures. On the contrary, these products are more expensive than conventional media but the disadvantage may be compensated by the reduction of complementary reagents and different culture plates for suspected pathogens (Perry and Freydiére 2007). Yarbrough et al. (2016) reported that using chromID® agar decrease the laboratory costs with reducing standard media consumption such as inoculation on both blood agar and MacConkey agar plates and additional biochemical tests.

In conclusion, the bacterial fish pathogens grew well in chromID® CPS® Elite agar and chromID® CPS® Elite / Columbia CNA +5% sheep blood agar, *V. anguillarum*, *P. fluorescens* and *Y. ruckeri* grew well in chromID® *Vibrio* but pigment production was only observed for *V. anguillarum*. ChromID® CPS® Elite agar, chromID® CPS® Elite / Columbia CNA +5% sheep blood agar and chromID® *Vibrio* may be a practicable alternative to routinely employed chromogenic medium may be a feasible alternative to routinely used media. They are cost saving with reducing the number of agar plates and provide presumptive data by coloration specificity. Also in some cases, it is unnecessary to do confirmation tests such as Gram stain, oxidase test and determination of biochemical properties for presumptive identification.

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The Influence of Lake Level Fluctuations on Fisheries in Lake Van

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ABSTRACT

The impact of hydrological regimes on the productivity in lake ecosystems has received considerable attention in recent decades. This study tested the hypothesis that water level fluctuations influence the fisheries productivity of Lake Van. Monthly water level data from 2000 to 2014 were obtained from Hydroweb and fisheries data corresponding to the same time period were acquired from the Turkish Statistical Institute. In order to test whether water level fluctuations demonstrated significant concordance with fish landings, landings data as a dependent variable, and seasonal water level amplitude and mean annual water level as independent variables were used in a linear regression analysis. The regression analysis proved insignificant results. The general trend of a linear decline, with a rate of -575 tonnes per year, observed in the landings did not match the seasonal and inter-annual water level variations which occurred in the lake during the same period. The consistently declining yields might result from a prolonged overexploitation and/or a constant recruitment failure. The estimated values of the seasonal and inter-annual relative fluctuation index (0.402 and 0.097, respectively) were rather low indicating that Lake Van is hydrologically stable with limited aquatic/terrestrial transition zone interactions and a relatively low nutrient load.

Keywords: Lake Van, water level fluctuations, productivity, fisheries

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Su Seviyesi Değişimlerinin Van Gölü'ndeki Balıkçılığa Etkisi

Öz: Hidrolojik rejimlerin göl ekosistemlerindeki verimlilik üzerindeki etkisi son yıllarda büyük ilgi görmektedir. Bu çalışmada, Van Gölü su seviyesindeki değişimlerin balıkçılık üretimini etkilediğini öngören hipotez test edilmiştir. 2000-2014 yılları arasına ait aylık su seviyesi verileri Hydroweb'den, aynı döneme karşılık gelen balıkçılık verileri ise Türkiye İstatistik Kurumu'ndan alınmıştır. Su seviyesindeki değişimlerin, avlanan balık miktarları ile anlamlı bir ilişki gösterip göstermediğini test etmek için doğrusal regresyon analizinde bağımlı değişken olarak avcılık verileri ile bağımsız değişken olarak mevsimsel su seviyesi genliği (amplitüt) ve ortalama yıllık su seviyesi değerleri kullanılmıştır. Regresyon analizi sonucunda anlamlı bir ilişki bulunamamış, avcılık miktarlarında gözlenen yıllık -575 tonluk doğrusal düşüş trendi, aynı dönemde gölde meydana gelen mevsimsel ve yıllık su seviyesi değişimleriyle uyumluluk göstermemiştir. Av ürün miktarlarındaki kalıcı düşüşün sebebi, uzun süreli aşırı avcılık ve/veya stoka katılımda süregelen azalmalar olabilir. Mevsimsel ve yıllık bağıl dalgalanma endeks değerleri sırasıyla 0,402 ve 0,097 olarak hesaplanmıştır. Bu değerlerin oldukça düşük olması Van Gölü'nün hidrolojik olarak kararlı olduğunu, su/kara geçiş bölgesi etkileşimlerinin sınırlı düzeyde kaldığını ve göle nispeten düşük nütrient girişi gerçekleştiğini göstermektedir.

Anahtar kelimeler: Van Gölü, su seviyesi değişimleri, verimlilik, balıkçılık

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Introduction

Lake levels vary as a result of environmental and anthropogenic factors such as climate elements, tectonic activities, rivers, underground waters, infiltrations from lake floors and human exploitation of water resources (Zohary and Ostrovsky 2011, Shafaei and Kisi 2016). Water flowing into lakes is generally the main source of nutrient supply. In addition, seasonal water level fluctuations may influence the productivity and nutrient recycling (Kolding and van Zwieten 2012). Especially in relatively shallow and highly fluctuating lakes, seasonal changes in levels of water carrying nutrients from rivers or surrounding terrestrial ecosystems are important. In deeper lakes, water level fluctuations drive internal nutrient mixing (Zohary and Ostrovsky

2011). Furthermore, with changes in water level, interactions occur within the aquatic/terrestrial transition zone and lead to terrestrial originated accumulation of nutrient rich organic matter which also enhances the productivity in the lake system. In addition, water level fluctuations may impact lake ecosystems negatively by enhancing eutrophication and algal blooms. In short, seasonal and inter-annual water level fluctuations have a crucial impact on the productivity of lakes and fisheries landings (Gownaris et al. 2018). The degree to which water level fluctuations affect any given lake is highly dependent on that lake's average depth (Gownaris et al. 2018). Kolding and van Zwieten (2012) suggested an index called relative lake level fluctuation (*RLLF*), which uses the amplitude (i.e. the range between the maximum and minimum water level) and the average depth of the lake. This index is useful to categorize lakes as a proxy to productivity and classifies lake systems as high *RLLF* and low *RLLF*, and can reflect the system stability. The *RLLF* also serves as a proxy for nutrient input and fish production.

In this study, the hypothesis that water level fluctuations will demonstrate significant concordance with fish landings is tested. For this purpose, Lake Van, Turkey's largest lake, was chosen. Interestingly, only one fish species inhabits Lake Van, *Alburnus tarichi*, which is endemic and commercially exploited. The contribution of *A. tarichi* landings to Turkey's total inland capture fisheries is quite considerable. In 2019, for instance, the landings of *A. tarichi* were 9970 tonnes, and formed approximately 31% of the country's total inland fisheries production (Turkstat 2020). The aim of this study was to investigate whether there was a relationship between water level fluctuations and fish production in Lake Van for the period of fifteen years between 2000 and 2014. Another objective here is to apply the *RLLF* to Lake Van in order to clearly understand its system stability and to predict productivity.

Materials and Methods

Lake Van (Figure 1) is the world's largest soda lake. It is characterized by highly alkaline-saline water with a pH range of 9.7-9.9 and a 22g kg^{-1} salt content (Reimer et al. 2009). The lake itself is a closed basin with a volume of about 600 km^3, a maximum depth of 451 m and is located at an altitude of 1648 m above sea level. The only fish species living in Lake Van, *A. tarichi* is an anadromous fish which migrates to breeding grounds of freshwater inlets from April to July (Sarı 2008; Oğuz 2013). *A. tarichi* is a planktivorous fish and reaches sexual maturity at about three years old and has an average life span of seven years (Sarı 2008). The major part of the *A. tarichi* population lives in Lake Van, but it also inhabits the smaller lakes of Erçek, Nazik and Aygır in the Lake Van drainage basin (Figure 1) albeit in relatively small numbers (Şen et al. 2015).

Data on the water levels of Lake Van were obtained from Hydroweb, an internet database (http://hydroweb.theia-land.fr) which records time series of water levels of rivers and lakes in various places in the world by using satellite altimeter data (Crétaux et al. 2011). Water levels are given in meters above sea level (m asl). The Hydroweb water level data of Lake Van start in January 2000 and end in February 2015 and were recorded in intervals of roughly 30 days (Figure 2). Since the data for 2015 only consisted of the two first months of that year, they were excluded from the analysis. Lake Van fisheries data corresponding to the same time period of the available water level data (2000-2014) were acquired from the website of the Turkish Statistical Institute (http://www.turkstat.gov.tr) (Turkstat 2020).

In order to test whether water level fluctuations would demonstrate significant concordance with fish landings, a linear regression analysis was carried out with landings data as a dependent variable and hydrological variables as independent predictors. The hydrological variables were seasonal water level amplitude (WL_{amp}) and mean annual water level ($\overline{WL}_Y$). The value of WL_{amp} was calculated for each year as the difference between the maximum and minimum water levels recorded in that year. The $\overline{WL}_Y$ value for a given year was the mean of all water levels recorded for that year. Prior to the linear regression analysis, a correlation analysis was also done to check whether the two independent predictors were correlated with each other. A single-factor analysis of variance was also conducted to explore whether $\overline{WL}_Y$ values differed among the years from 2000 to 2014. Following the analysis of variance, Tukey's honestly significant difference (HSD) test (Sokal and Rohlf 2012) was used for pairwise comparisons among the years. All variables were checked for normality using the Shapiro-Wilk test and the homogeneity of variances was checked with Levene's test (Sokal and Rohlf 2012). Statistical analyses were performed with R software version 3.5.3 (R Core Team 2019) with a significance level set at 5%.

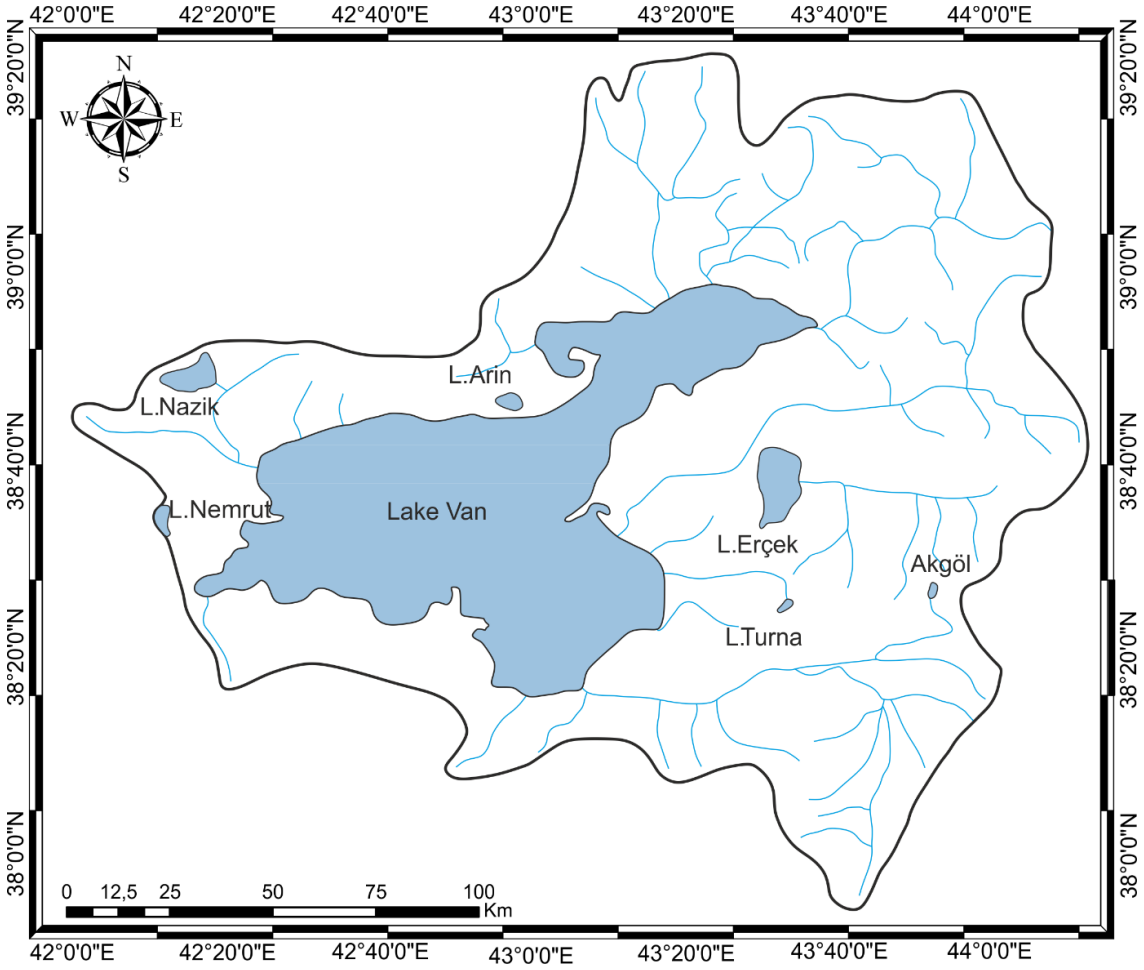

Figure 1. Lake Van and its water drainage basin

The relative lake level fluctuation index (*RLLF*) proposed by Kolding and van Zwieten (2012) is defined as the percentage ratio of the average lake level amplitude to the average depth of the lake. The average lake level amplitude, hence the *RLLF*, can be calculated from a time series of data in two different ways. One way is to calculate the average seasonal pulse amplitude, which is the average of the differences between the maximum (max) and minimum (min) water levels recorded within *i*th year. This form is called *RLLF-s*:

$$RLLF - s = \frac{\frac{1}{n} \times \sum_{i=1}^{n} \max(WL_i) - \min(WL_i)}{\text{Average depth}} \times 100.$$

Here n is the total number of years. So the numerator in the above equation is simply the average of the WL_{amp} values described previously. In the second form, referred to as *RLLF-a*, the average lake level amplitude is calculated as the average amplitude of inter-annual water levels, i.e. the average of the absolute differences between the mean water levels ($\overline{WL}$) of two sequential years (i and i+1):

$$RLLF - a = \frac{\frac{1}{n-1} \times \sum_{i=1}^{n-1} |\overline{WL}_i - \overline{WL}_{i+1}|}{\text{Average depth}} \times 100.$$

While the *RLLF-a* is used to show the long term inter-annual stability of a system, the *RLLF-s* indicates the average strength of the seasonal pulse with which different systems could be scaled in terms of stability over different time scales (Kolding and van Zwieten 2012). The value of average depth used for Lake Van in the calculations with the above equations is 170 m (Degens et al. 1984).

Results

Water levels of Lake Van from January 2000 to February 2015 obtained by using satellite altimeter data are presented in Figure 2. The minimum and maximum lake levels measured corresponding to 1646.41 and 1648.13 masl, were observed in November 2001 and June 2007, respectively.

The monthly variations in the water level of Lake Van during the fifteen-year period from January 2000 to December 2014 are presented in Figure 3. Although year to year variations exist (Figure 2), water levels on average rose from April through May and peaked in June and then started to decrease reaching the lowest level in December (Figure 3). Variances associated with the monthly average water level

values were homogenous (Levene's test). In other words, when all data were combined, year to year variations for each month were similar (Figure 3).

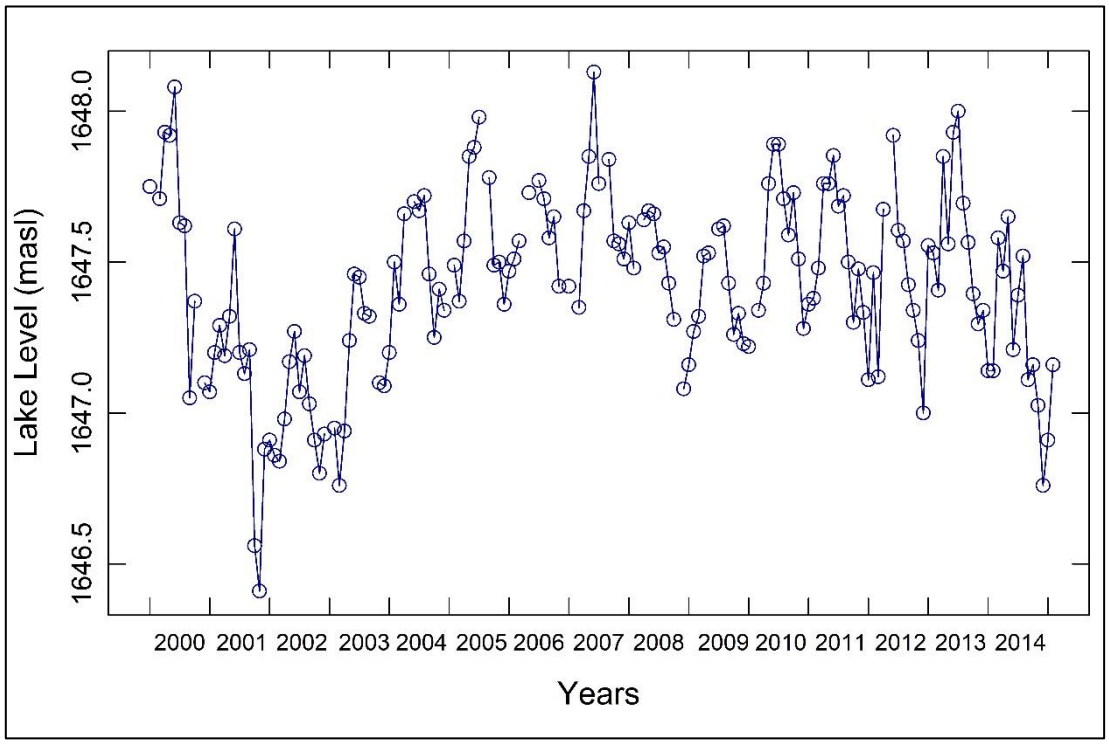

Figure 2. The Hydroweb water level data of Lake Van from January 2000 to February 2015 with approximately monthly intervals, obtained by using satellite radar altimeters

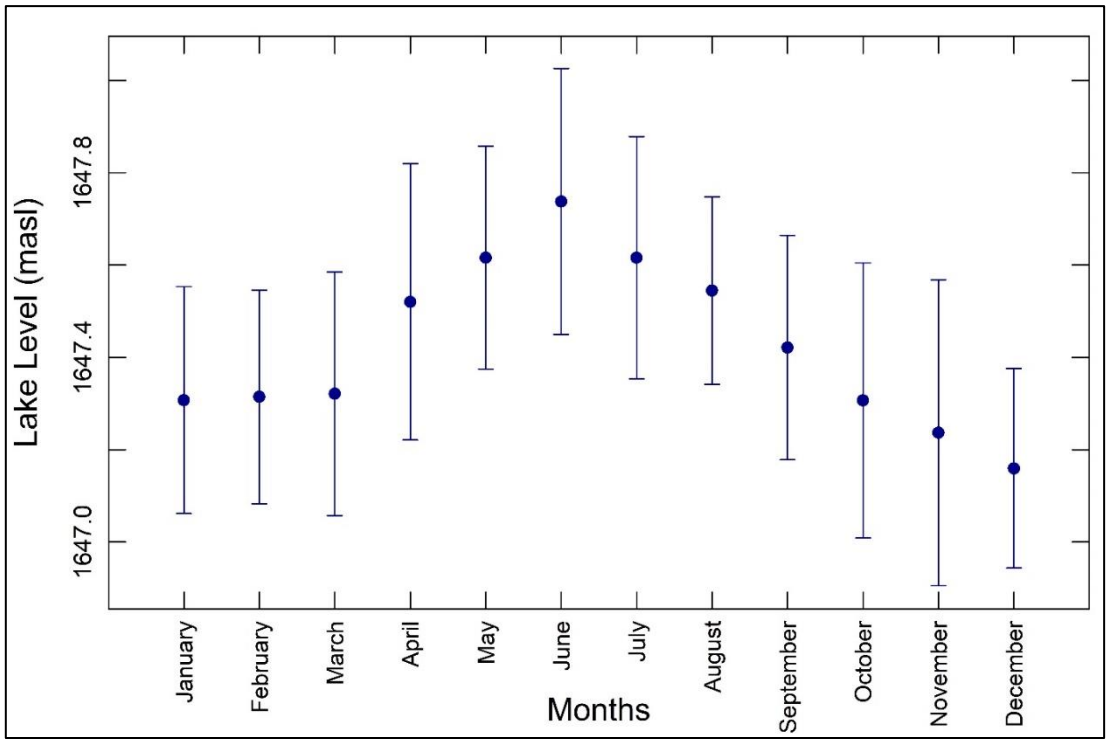

Figure 3. Monthly variations in the water level of Lake Van during the fifteen-year period from January 2000 to December 2014. The dots represent monthly mean values and the vertical bars denote ± standard deviations associated with those means

The linear regression analysis carried out with the landings data of *A. tarichi* as the dependent variable, and the WL_{amp} and $\overline{WL}_Y$ as the independent predictors did not yield any significant coefficients. Nor were the two independent predictors correlated with each other. The lack of concordance between any two of

these three variables can also be inferred from Figure 4 which depicts the year to year variations in the landings, WL_{amp} and $\overline{WL}_Y$ from 2000 to 2014 in Lake Van. During this time, the highest *A. tarichi* landing was observed in 2001 as 15848 tonnes. Thereafter the landings began to decrease linearly at a rate of -575 tonnes per year (linear regression analysis with an adjusted R^2 of 0.95) and the total catch amounted to only 8310 tonnes in 2014 (Figure 4). In contrast, there were no discernable linear or nonlinear trends in the time series data of the WL_{amp} and $\overline{WL}_Y$ (Figure 4). The analysis of variance revealed that the $\overline{WL}_Y$ values were not homogenous among the years from 2000 to 2014 and according to the follow-up Tukey's HSD test, three year groups; low, middle and high lake levels can be distinguished based on the pairwise comparisons of and $\overline{WL}_Y$ values. The low $\overline{WL}_Y$ years were 2001, 2002 and 2003, and the middle $\overline{WL}_Y$ years were 2004, 2009, 2012 and 2014. The remaining years were the high $\overline{WL}_Y$ years (Figure 4). The minimum and maximum values of $\overline{WL}_Y$ were 1646.99 and 1647.67 masl, respectively, and were observed in 2002 and 2007. However, the minimum and maximum WL_{amp} values were recorded in 2006 and 2001 as 0.35 and 1.05 m, respectively (Figure 4). The average values (and ± standard deviations) of the WL_{amp} and $\overline{WL}_Y$ were 0.68 (±0.203) m and 1647.44 (±0.209) m asl, respectively, over the fifteen years.

The estimated values of *RLLF-s* and *RLLF-a* for Lake Van between 2000 and 2014 were 0.402 and 0.097, respectively.

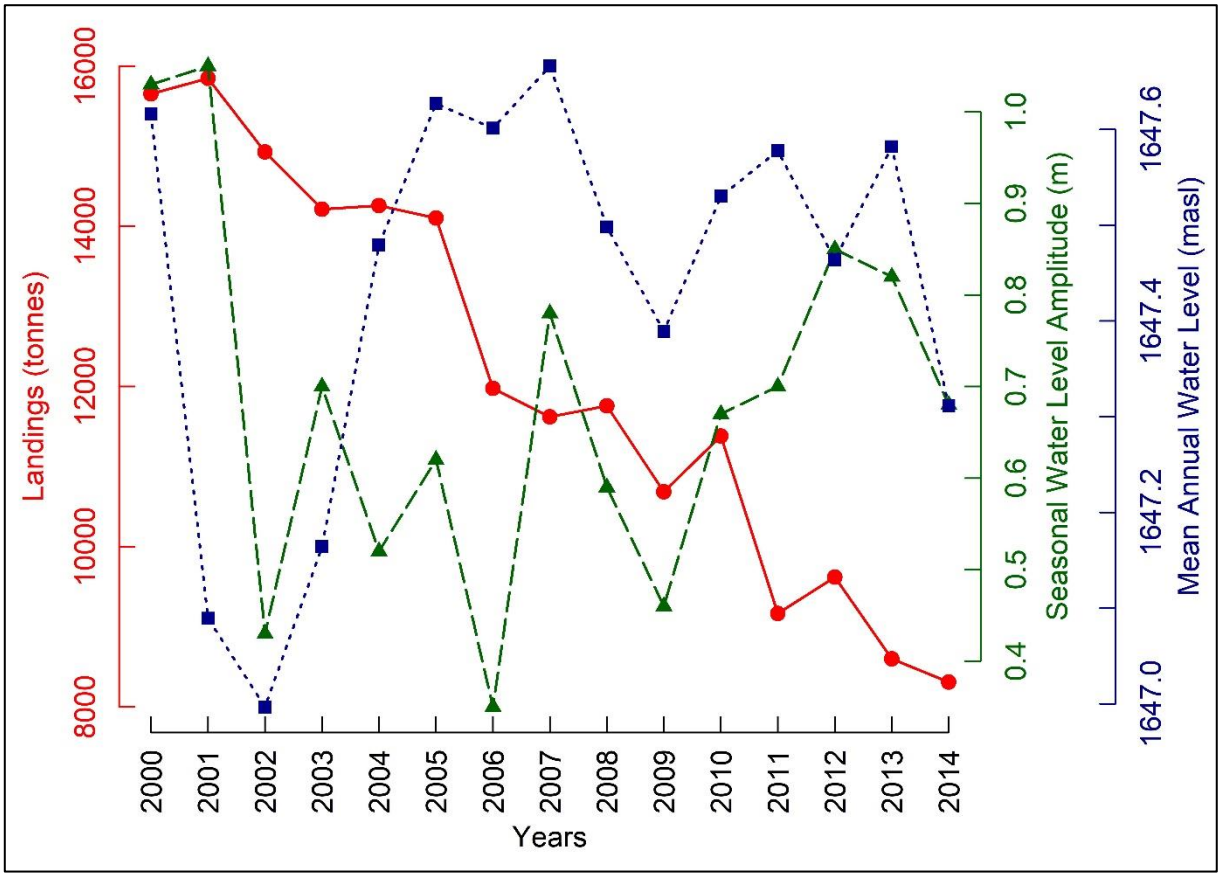

Figure 4. Variations in the landings of *A. tarichi* (red dots), seasonal water level amplitude (green triangles) and mean annual water level (dark blue squares) in Lake Van between 2000 and 2014

Discussion

Impact of water level fluctuations on the ecological processes in lake ecosystems, particularly regarding the biological productivity, has received considerable attention, especially in the last three decades (e.g., Coops et al. 2003; Leira and Cantonati 2008; Wantzen et al. 2008; Kolding and van Zwieten 2012; Gownaris et al. 2018). However, most of the research focused on effects of water level fluctuations on macrophyte communities in lakes and reservoirs (Leira and Cantonati 2008; Gownaris et al. 2018). Only a limited number of studies investigated the functional relationship between fisheries productivity and magnitude of water level fluctuations in lakes. Williams (1972) reported highly significant correlations between the catch per unit effort of the most common species in the commercial catch *Tilapia macruchir* and the water

levels two years previously in Lake Mweru in northern Zambia. Furse et al. (1979) providing no statistical results noted that species composition and magnitude of catches in the shallow Lake Chilwa in Malawi were related to the level of the lake. Davies and Sloane (1988) documented the impact of variations in lake water level on the population dynamics of two introduced trout species (*Salmo trutta* and *Oncorhynchus mykiss*) in the Great Lake in Tasmania. Tweddle and Magasa (1989) examined the catch data of three cichlid species (*Oreochromis* spp.) from a multigear (ring net, midwater and demersal trawl) fishery in Lake Malawi in Malawi and showed that the catches were related to changes in annual mean lake level three years previously. Kolding (1992) established a significant linear regression between annual commercial catch per boat and mean lake level of the previous year in Lake Turkana in Kenya. Karenge and Kolding (1995) found no significant correlation between the catch per unit effort estimates from the man-made Lake Kariba located between Zambia and Zimbabwe and mean annual lake water levels. However, the catch per unit effort values were highly significantly correlated to the lake's seasonal water level amplitude and the differences between the mean lake water levels of two sequential years (Karenge and Kolding 1995). In another man-made lake, Lake Nasser in Egypt, Agaypi (2000) used linear regression analysis and demonstrated a significant functional relationship between the log-transformed catch per unit effort of *Oreochromis niloticus* and the mean lake levels two years previously. Gownaris et al. (2017) regressed log-transformed annual catch data from Lake Turkana on seasonal water level amplitude and preceding year's lake level and obtained significant linear regression coefficients. According to Gownaris et al. (2017), the variations in Lake Turkana's fish stocks appeared to have been linked to changes in the lake's hydrology rather than to fishing pressure.

The present study is the first attempt to investigate water level fluctuations and their probable impact on capture fisheries production in Turkey's standing inland waters. The linear regression analysis carried out in this study attempting the relate the landings data of *A. tarichi* from Lake Van to the two independent hydrological variables, WL_{amp} and $\overline{WL}_Y$, was an approach similar to that of Gownaris et al. (2017). However, unlike the above mentioned works, the regression analysis did not yield any significant coefficients in the current investigation. Repeating the linear regression analysis with the mean lake levels of one or two previous years (i.e. $\overline{WL}_{Y-1}$ and $\overline{WL}_{Y-2}$) instead of $\overline{WL}_Y$ (Kolding 1992; Gownaris et al. 2017; Williams 1972; Agaypi 2000) did not change the outcome. The fisheries production from Lake Van between 2000 and 2014 appeared to be independent of the hydrological conditions prevailed in the lake in the same time period. The general trend observed in the landings was a linear decline and this linearly decreasing trend did not match the seasonal and inter-annual water level variations occurred in the lake during the studied period (Figure 4).

Caution has been suggested when using fisheries landings data from the Turkish Statistical Institute since the accuracy, precision, coverage and representativeness of these statistics have long been debated (Tıraşın and Ünlüoğlu 2012). Tıraşın and Ünlüoğlu (2012) stated that not only many fisheries scientists but also a considerable number of people involved in the fisheries sector are in a general consensus that the catch figures reported in these statistics are underestimates of the fish actually caught and that a substantial amount goes unreported. For example, illegal catches have routinely been taken in Lake Van during the annual spawning migration of *A. tarichi* to the freshwater inlets from April to July even though all fisheries activities have officially been banned in the entire lake between 15 April and 30 June every year since the late 1990s (Sarı 2008). It is very difficult to estimate the amounts of these unauthorized catches (Sarı 2008), any attempt to adjust the catch statistics is, therefore, also problematic. In spite of all valid concerns regarding the official catch statistics, Tıraşın and Ünlüoğlu (2012) acknowledge that they are still considered by many in the field as a useful index reflecting the overall variations in the stocks of major fisheries resources. As the demand for consumption of this fish did not change for the duration of the investigated time period, it is reasonable to assume that the total fishing effort in the lake did not vary substantially from one year to another either. Thus, the observed linear decay trend in *A. tarichi* landings from 2001 to 2014 depicts clearly the decline of the population in the lake during this time period. Such a consistent decline in a fish population often results from a prolonged overexploitation or in other terms overfishing. The overfishing problem in Lake Van was also stated by Sarı (2008) and Şen et al. (2015). Another likely explanation for the population decline might be a continued failure in recruitment of *A. tarichi* to Lake Van. Previous studies (Elp et al. 2006; Şen et al. 2015; Atıcı 2017) documented various destructive anthropogenic activities in the streams flowing into Lake Van such as sand extraction, pollution, construction of regulators and embankments, channeling water for irrigation which caused degradation and loss of the breeding grounds of this endemic species. According to Şen et al.

(2015), ongoing devastation of the breeding grounds has a more adverse impact on the *A. tarichi* population in Lake Van than the overfishing.

The general trend observed in monthly variations in the water level of Lake Van from the satellite altimeter data from January 2000 to December 2014 was that the lake's water level rose from April through May and peaked in June and then started to decrease (Figure 3) and the lowest level in December is congruent with the earlier studies describing the relationship between the prevailing climatic conditions in the region and lake water level (Kadıoğlu et al. 1997, Altunkaynak et al. 2003). Detailed description of seasonal dynamics of the meteorological processes (i.e. precipitation, evaporation, inflow etc) considered to be affecting the water level of Lake Van can be found in Kadıoğlu et al. (1997).

There is a growing evidence that water level fluctuations, particularly in tropical lakes and reservoirs, have an important role in the injection and re-suspension of nutrients, and accordingly has a crucial impact on the productivity of these water bodies (Kolding and van Zwieten 2012; Gownaris et al. 2018). The simple empirical index, *RLLF*, proposed by Kolding and van Zwieten (2012) to categorize lakes as a proxy to productivity may serve as a practical and useful means to provide some indication of the production in Lake Van. Reminding that the estimated values of *RLLF-s* and *RLLF-a* for Lake Van in the present study are 0.402 and 0.097, respectively. According to Kolding and van Zwieten (2012), *RLLF* values less than 1 indicates hydrologically stable lakes with low production per unit area. Based on these low *RLLF* values, the natural hydrological regime of Lake Van can be classified as steady meaning that the aquatic/terrestrial transition zone interactions in the lake are considered limited, and nutrient load is relatively low. In order to provide more insight for interpretation of the present *RLLF* estimates, Lake Van can be compared with some other studied lakes whose *RLLF* values are available (Table 1). Two larger and deeper lakes than Lake Van, Tanganyika and Malawi, and a slightly smaller but deeper one, Lake Kivu have low *RLLF* and production values comparable to those of Lake Van. Similar to Lake Van, all these three lakes were classified as steady or hydrologically stable by Kolding and van Zwieten (2012). On the other hand, Lake Nasser, the larger but much shallower lake has higher *RLLF* and production values. The remaining two smaller and shallower lakes (Lake Edward and Lake Mweru) were also associated with higher *RLLF* and production values. As Kolding and van Zwieten (2012) pointed out, the impact of water level fluctuations on productivity in the studied lakes (Table 1) is inversely proportional with the size and depth of the system.

Drastic recruitment failure due to the destruction of the breeding grounds in the connected streams and the strong overfishing occurring in Lake Van might have masked the relatively subtler effects of the lake level fluctuations on the fisheries productivity. However, studying the possible effects of variations in a lake's hydrology on the fisheries production may prove to be very useful in other lakes in Turkey. In addition, application of the simple empirical index, *RLLF*, in other Turkish lakes may serve as an easy and practical tool to get some indication of their productive status.

Table 1. Morphometric, hydrological and fish production data on some lakes

Lakes	Country	Area (km^2)	Average depth (m)	Landing (kg/ha)	*RLLF-s*	*RLLF-a*
Van	Turkey	3574	170	26.35	0.39	0.10
Malawi	Malawi, Tanzania, Mozambique	29600	290	9.09	0.30	0.10
Kivu	DR Congo, Rwanda	2693	240	27.79	0.14	0.06
Tanganyika	Zambia, Tanzania, Burundi, DR Congo	32900	580	22.20	0.14	0.04
Edward	DR Congo, Uganda	2150	34	68.95	5.60	1.43
Mweru	Zambia, DR Congo	2700	8	155.60	25.70	7.20
Nasser/Nubia	Egypt, Sudan	5248	25	57.16	27.50	7.14

Excepting Lake Van all information regarding the other lakes is quoted from Kolding and van Zwieten (2012). Data on area and average depth of Lake Van are from Degens et al. (1984). Amount of landing from Lake Van is the average of landings from last five years (2010-2014)

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Length-Weight Relationship of 15 Different Freshwater Fish Species in the Gediz River Basin (Turkey) Lentic System

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ABSTRACT

The aim of study is to determine the length-weight relationship of freshwater fish species in the Gediz River basin lentic system. Fish samples were collected between November 2016 and April 2017 using multi mesh gillnets and beach seine nets from six different locations in the Gediz River basin lentic system. Length–weight relationship was estimated for 15 fish species (*Luciobarbus lydianus, Barbus pergamonensis, Cyprinus carpio, Carassius gibelio, Petroleuciscus smyrnaeus, Alburnus battalgilae, Squalius fellowesii, Chondrostoma holmwoodii, Vimba vimba, Rhodeus amarus, Pseudorasbora parva, Atherina boyeri, Cobitis kurui, Gambusia holbrooki* and *Knipowitschia mermere*), belonging to 8 different families (Cyprinidae, Leuciscidae, Acheilognothidae, Gobionidae, Atherinidae, Cobitidae, Poeciliidae, Gobiidae). Computed exponent *b* and R^2 values ranged from 1.9348 to 4.3466 and 0.7072 to 0.9986, respectively. In the study, a longer maximum length value was determined for the two species than reported in the literature. In addition, this study presents the first records of *LWR* parameters for four endemic species in the basin.

Keywords: *LWR* parameters, lake, reservoir, West Anatolia, Gediz River

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Gediz Nehri Havzası Lentik Sistemindeki (Türkiye) 15 Farklı Tatlı Su Balığı Türünün Boy-Ağırlık İlişkileri

Öz: Çalışmanın amacı, Gediz Nehri lentik sisteminde yayılış gösteren tatlısu balık türlerinin boy-ağırlık ilişkilerini belirlemektir. Balık örnekleri Kasım 2016 ve Nisan 2017 tarihlerinde Gediz Nehri lentik sistemindeki (6 farklı lokalite), çokgözlü solungaç ve kıyı sürütme ağları ile toplanmıştır. Boy-ağırlık ilişkileri, 8 farklı aileye (Cyprinidae, Leuciscidae, Acheilognothidae, Gobionidae, Atherinidae, Cobitidae, Poeciliidae, Gobiidae) ait, 15 balık türü (*Luciobarbus lydianus, Barbus pergamonensis, Cyprinus carpio, Carassius gibelio, Petroleuciscus smyrnaeus, Alburnus battalgilae, Squalius fellowesii, Chondrostoma holmwoodii, Vimba vimba, Rhodeus amarus, Pseudorasbora parva, Atherina boyeri, Cobitis kurui, Gambusia holbrooki* and *Knipowitschia mermere*) için tahmin edilmiştir. Hesaplanan *b* ve R^2 değerleri sırasıyla, 1,9348 ile 4,3466 ve 0,7072 ile 0,9986 arasında değişmektedir. Çalışmada, iki tür için mevcut literatürde bildirilenden daha uzun bir maksimum uzunluk değeri tespit edilmiştir. Ayrıca bu çalışma havzadaki dört endemik tür için boy-ağırlık ilişkisi parametrelerinin ilk kayıtlarını sunmaktadır.

Anahtar kelimeler: Boy-ağırlık ilişkisi parametreleri, göl, rezervuar, Batı Anadolu, Gediz Nehri

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Introduction

Length and weight, both in population and individual basis, are two basic morphological characteristics in fish biology. The weight of fish is closely related to their length, thus it determines whether somatic growth is isometric or allometric. Length-weight relationship (*LWR*) is widely used for fisheries management and conservation (LeCren 1951; Pitcher and Hart 1982; Froese 2006; Froese et al. 2011). They are commonly used for weight estimation from the length of individual fish (Tsoumani et al. 2006) and for the calculation of condition factors when comparing observed and expected length–weight values (LeCren 1951; Froese 2006; Gaygusuz et al. 2013).

Turkey is geographically situated between two continents and is one of the few terrestrial parts of world with high biological diversity. Turkey's geography consists of the Anatolian and Thrace regions, but its ichthyofaunal richness originates

from the Anatolian region (Görür et al. 1984; Güçlü and Küçük 2015). Although scientific studies in Anatolia began in the second half of the 18th century, the basic biological information for the majority of freshwater fish species in Turkey is still missing (Güçlü and Küçük 2015).

Located in the Aegean Region of Turkey, Gediz River's length is second only to the Büyük Menderes River, which is flow in the south is roughly parallel with a distance of slightly more than a hundred kilometers. Length of the Gediz River is 401 km and has a stream catchment area of 17.500 km². River basin has been contaminated day by day, due to intensive, rapid and excessive industrial, domestic and agricultural expansion. However, the reserve suffers from water shortages due to large volume water demand of irrigation projects connected to the Demirköprü reservoir. High level of urbanization and industrialization throughout the basin also cause Gediz River to be exposed to severe pollution, especıally from sand and gravel quarries and the leather industry. These factors have caused the river's former rich fish reserves to become a thing of the past in today (Güçlü and Küçük 2015).

There is no ecological study (length–weight relationship) conducted with *Luciobarbus lydianus*, *Chondrostoma holmwoodii*, *Barbus pergamonensis* and *Cobitis kurui*, which are endemic fish fauna of the basin. Researches on the other endemics of the basin, *Petroleuciscus smyrnaeus*, *Alburnus battalgilae* and *Knipowitschia mermere* are also limited. In this study, we described the *LWR* parameters for 15 fish species (8 endemic, 3 natural and 4 invasive species) obtained from 6 different locations (including two lake and four reservoirs) in the lentic system of the Gediz River basin (Aegean Region, Turkey). The aim of this study was to produce *LWR* for species in Gediz River basin, which will be helpful for sustainable management of local fishery and developing of conservation programs in the region.

Materials and Methods

The study was carried out in 6 different locations including two lakes and four reservoirs (Küçükler, Afşar, Buldan and Demirköprü reservoirs, the Gölcük and the Marmara Lakes) in the lentic system of the Gediz River basin (Turkey) (Table 1). 1630 individuals were caught from 15 species, belonging to 8 families (Cyprinidae (4), Leuciscidae (5), Achelignothidae (1), Gobionidae (1), Atherinidae (1), Poeciliidae (1), Gobiidae (1) and Cobitidae (1)). Sampling was carried out in November 2016 and April 2017 (one fishing operation was carried out on the specified dates) with multi mesh gillnets (35x1.5 m and 35x6 m in size, 10, 15, 20, 40, 55, 70, 80 and 100 mm mesh size) according to modified TS EN 14757 and beach seine net (5 and 15 mm mesh size) in the study area. Family names were given taxonomically according to Stout et al. (2016) and Van der Laan (2017). Specimens were measured to the nearest 0.1 cm total length and weighted to the nearest 0.01 g total weight. The *LWR* was established using the exponential regression equation $W = aTL^b$, where *W* was the body weight in g, *TL* was the total length in cm, "*a*" is the intercept and "*b*" is the regression coefficient (Ricker 1975). The statistical significance level of the coefficient of determination (R^2) and 95% confidence intervals (95% CI) of *b* was also estimated (Zar 1999).

Table 1. Lentic sampling points in the Gediz River basin

Lokality	Code	Altitude	Coordinates
Küçükler reservoir (Uşak)	KR	1.241 m	38° 52′ 30′′ N- 29° 36′ 39′′ E
Afşar reservoir (Manisa)	AR	249 m	38° 14′ 20′′ N- 28° 36′ 29′′ E
Buldan reservoir (Denizli)	BR	481 m	38° 08′ 40′′ N- 28° 50′ 44′′ E
Demirköprü reservoir (Manisa)	DR	234 m	38° 39′ 40′′ N- 28° 21′ 01′′ E
Gölcük Lake (İzmir)	GL	1.052 m	38° 19′ 01′′ N- 28° 01′ 37′′ E
Marmara Lake (Manisa)	ML	76 m	38° 36′ 59′′ N- 27° 59′ 00′′ E

Results and Discussion

A total of 1630 specimens of 15 species belonging to 8 families were used for calculation of the *LWR*. Table 2 shows range of *TL* and *W*, parameters *a* and *b*, the 95% confidence limits of *b* and the regression coefficient (R^2). Eight of these 15 species were endemic. Computed *LWR* parameters for 4 endemic species (*L. lydianus*, *C. holmwoodii*, *B. pergamonensis*, *C. kurui*) are given for the first time. New maximum length values has been determined for *P. parva* (11.14 cm TL, Afşar reservoir) and *K. mermere* (3.74 cm TL, Marmara Lake) in the basin. The expected range of $2.5<b<3.5$ was confirmed for all species (Froese 2006). Positive or negative allometry indicates a rounder or slimmer body, respectively, whereas isometric

growth shows that the body grows in the same proportion in all dimensions (Jobling 2008). The values of parameter *b* varied from 1.9348 (*A. battalgilae*, Marmara Lake) to 4.3466 (*C. gibelio*, Demirköprü reservoir). The regression coefficient between length and weight (R^2) varied between 0.7072 for *C. kurui* (Marmara Lake) and 0.9986 for *V. vimba* (Marmara Lake).

Table 2. *LWR* parameters of fishes in lentic systems in Gediz River basin

Species	Loc.	*n*	*TL* range	*W* range	*a*	*b*	95% CI of *b*	R^2
				Cyprinidae				
L. lydianus	DR	14	9.53-11.03	9.12-14.20	0.0148	2.8459	2.8216-2.8621	0.9506
	AR	27	13.57-25.26	26.05-182.45	0.0134	2.9266	2.8984-2.9412	0.9761
B. pergamonensis	KR	16	12.89.16.89	23.67-50.21	0.0785	2.2511	2.2301-2.2732	0.9257
C. carpio	GL	9	14.89-20.85	47.35-159.13	0.0050	3.4250	3.3976-3.4501	0.9507
	BR	35	12.61-22.64	33.52-191.51	0.0291	2.7721	2.7623-2.7903	0.9751
	AR	12	12.85-25.50	34.84-339.57	0.0123	3.0752	3.0601-3.0934	0.9719
C. gibelio	GL	20	12.44-17.64	38.12-102.00	0.0144	3.1038	3.0812-3.1189	0.9373
	KR	15	19.52-20.61	121.69-145.10	0.0015	3.8076	3.7912-3.8220	0.8644
	BR	21	19.23-20.85	122.35-160.60	0.0025	3.6361	3.6203-3.6424	0.9134
	DR	5	19.72-20.03	124.60-134.50	0.0003	4.3466	4.3109-4.3821	0.9630
	ML	56	7.59-22.85	6.36-216.60	0.0167	3.0320	3.0192-3.0498	0.9849
	AR	15	14.15-21.21	50.78-172.97	0.0115	3.1229	3.1145-3.1387	0.9632
				Leuciscidae				
P. smyrnaeus	GL	85	5.29-8.24	2.36-10.98	0.0011	3.1597	3.1456-3.1721	0.9776
	ML	208	4.91-12.48	1.64-30.65	0.0075	3.3276	3.3040-3.3421	0.9830
	AR	307	5.25-13.42	2.08-35.15	0.0100	3.1655	3.1423-3.1875	0.9831
A. battalgilae	DR	34	12.01-18.56	15.11-76.88	0.0009	3.9075	3.8821-3.9206	0.9470
	ML	10	19.42-19.86	75.14-81.25	0.2353	1.9348	1.9175-1.9542	0.8759
S. fellowesii	KR	47	11.54-17.61	15.80-52.16	0.0312	2.6073	2.5901-2.6231	0.9210
	BR	14	12.84-19.14	18.90-56.34	0.0314	2.5971	2.5701-2.6124	0.9124
C. holmwoodii	AR	27	15.44-23.74	31.23-104.60	0.0041	3.2680	2.2431-2.2789	0.9880
V. vimba	ML	15	12.06-23.41	18.47-189.23	0.0032	3.4884	3.4686-3.5001	0.9986
				Acheilognothidae				
R. amarus	DR	25	3.48-5.81	0.46-2.79	0.0067	3.4511	3.4345-3.4704	0.9245
	ML	142	3.48-5.61	0.46-2.38	0.0081	3.3473	3.3223-3.3635	0.8919
				Gobionidae				
P. parva	DR	73	5.51-9.10	1.46-6.87	0.0094	3.0080	2.9802-3.0121	0.9434
	ML	122	4.63-9.67	0.91-8.04	0.0236	2.5294	2.5058-2.5421	0.8078
	AR	61	4.09-11.14*	0.80-15.47	0.012	2.9863	2.9740-3.0023	0.9880
				Atherinidae				
A. boyeri	DR	101	6.61-10.48	1.84-8.98	0.0029	3.4227	3.4006-3.4442	0.9637
	ML	19	5.93-7.44	1.37-2.66	0.0082	3.9208	3.9002-3.9445	0.7767
				Cobitidae				
C. kurui	BR	14	6.82-8.58	2.58-4.84	0.0206	2.5074	2.4790-2.5132	0.9539
	ML	15	5.22-7.22	1.14-2.30	0.0162	2.4941	2.4601-2.5147	0.7072
	AR	21	7.01-8.00	2.58-3.32	0.0066	3.0271	3.0178-3.0413	0.9258
				Poeciliidae				
G. holbrooki	ML	20	3.09-5.13	0.50-2.64	0.0090	3.4789	3.4640-3.4825	0.9589
				Gobiidae				
K. mermere	ML	25	2.53-3.74*	0.19-0.66	0.0082	3.4064	3.3814-3.4206	0.8723

Loc: lokality, *n*: number of individuals, *T*L: total length (cm), *W*: weigth (g), *New maximum length

According to Tesch (1971), the values *b* varies between 2 to 4, and mostly remained within the expected range of 2.5-3.5. Length-weight relationship parameters are affected by various factors such as season, number of individuals surveyed, habitat, gonad maturity, gender and stomach content (Bagenal and Tesch 1978). In particular, *b* values are considered to be high for two species (*b*= 4.3466 *C. gibelio* - Demirköprü reservoir, *b*= 1.9348 *A. battalgilae* Marmara Lake). Because the number of samples is small and therefore covers a narrow range of lengths (Froese 2006).

The comparison of the values obtained in the study with limited number of previous studies conducted in the basin is shown in Table 3. Differences with *b* values obtained in the other studies may be due to factors affecting fish growth, such as water quality and nutrient availability (Sparre et al. 1989). Another reason for such differences may be the differences in number of samples, sampling time and sampling methods of the species.

Table 3. Comparison of *LWR* parameters reported by different studies in the Gediz River basin lentic system

Species	Locality	Ref.	*n*	*TL* range	*W* range	*a*	*b*	R^2
A. boyeri	Marmara Lake	1	101	3.70-8.70	0.40-5.40	0.0084	2.908	0.971
	Marmara Lake	2	20	3.80-4.70	0.36-0.64	0.0010	2.580	0.880
	Demirköprü reservoir	2	41	3.90-13.60	0.40-16.50	0.0080	2.949	0.990
	Gediz estuary	3	121	3.20-10.139	0.24-7.29	0.0073	2.985	0.999
C. gibelio	Marmara Lake	1	2213	6.80-27.50	4.90-372.20	0.0173	2.974	0.976
	Buldan reservoir*[1]	4	2325	9.70-25.50	23.80-269.10	0.0310	2.870	0.985
C. carpio	Marmara Lake	1	120	11.30-49.00	24.00-1790.00	0.0310	2.796	0.979
V. vimba	Marmara Lake	1	79	14.20-24.90	36.30-236.90	0.0053	3.283	0.974
R. amarus	Marmara Lake	1	105	2.80-6.50	0.26-4.49	0.0089	3.328	0.972
P. parva	Marmara Lake	1	116	5.20-11.00	1.60-14.60	0.0121	2.929	0.983
G. holbrooki	Marmara Lake*[2]	1	5	2.60-3.90	0.20-0.80	0.0145	2.945	0.818
	Marmara Lake	5	35	-	-	0.0160	2.905	0.975
A. battalgilae	Marmara Lake	1	298	14.60-24.10	31.60-141.60	0.0102	2.997	0.876
P. smyrnaeus	Marmara Lake	1	87	4.40-13.80	1.30-45.70	0.0091	3.284	0.994
K. mermere	Marmara Lake	1	39	2.00-2.70	0.08-0.23	0.0069	3.429	0.849

Ref.: Reference, *[1] Fork length, *[2] *G.affinis*, 1.İlhan and Sarı 2015; 2. İnnal and Engin 2020; 3. Kara et al. 2017; 4. Sarı et al. 2008; 5. Kurtul and Sarı 2020

The results of the study provide useful information for the management and protection of endemic species that are particularly threatened by water pollution, habitat loss, river regulation, water extraction and invasive-alien fish inflows. Besides contributing the *LWR* knowledge of fish found in the inland waters of Turkey, this study will form an important basis for the work will be done in the future.

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Global Length–Length Relationships for Common Carp *Cyprinus carpio* (Cypriniformes: Cyprinidae)

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ABSTRACT

A review is provided of length–length relationships (LLR) for common carp (*Cyprinus carpio* L., 1758) at the global scale. In total, 16 studies were retrieved from a comprehensive literature search that provided LLR for *C. carpio* populations from 26 water bodies consisting of rivers, lakes and reservoirs across nine countries in four continents. There was large variation in LLR, which were available for all six possible combinations of total, fork and standard length, due to the wide range of fish sizes measured. This is the first study that provides LLR for *C. carpio* that can be used as a reference base for future age-growth and population dynamics studies on this species.

Keywords: Size, growth, population dynamics, invasive species, Turkey

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Sazan *Cyprinus carpio* (Cypriniformes: Cyprinidae) için Global Boy-Boy İlişkileri

Öz: Bu çalışmada küresel ölçekte sazanın *Cyprinus carpio* boy-boy ilişkilerinin bir derlemesi gerçekleştirilmiştir. Toplamda 16 çalışmada, dört kıtadan dokuz ülkedeki rezervuarlar, göller ve akarsuları içeren 26 su kütlesinden *C. carpio* popülasyonlarının boy-boy ilişkileri kapsamlı bir literatür taraması ile toplanmıştır. Çok geniş boy aralıklarının varlığı nedeniyle mümkün olan altı olası total, çatal ve standart boy kombinasyonları için boy-boy ilişkilerinde önemli varyasyonlar tespit edilmiştir. Bu çalışma, tür için gelecekte gerçekleştirilecek popülasyon dinamiği ve yaş-büyüme çalışmalarında referans olarak kullanılabilecek boy-boy ilişkilerini sağlayan ilk çalışmadır.

Anahtar kelimeler: Boy, büyüme, popülasyon dinamikleri, istilacı tür, Türkiye

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Introduction

Length–length relationships (LLR) are important in fish stock and population assessment (Ricker 1968) and for comparative population growth studies (Binohlan et al. 1998). The common carp *Cyprinus carpio* is one of the most widely distributed freshwater fishes in the world (Froese and Pauly 2019), and is a species of particular ecological relevance due to its dual status of both vulnerable in its native area of distribution (Freyhof and Kottelat 2008) and noxious in most of its non-native areas (Vilizzi 2012; Vilizzi et al. 2015a). A plethora of age-growth studies worldwide have provided length-at-age, weight–length relationships and condition factors for this species, and these were recently reviewed in Vilizzi and Copp (2017). This study provides LLR for *C. carpio* based on a similar, comprehensive literature review. Such information is timely, because there are currently no peer-reviewed based LLR for this species available from FishBase (Froese and Pauly 2019) that can be used as a reference base for age-growth and population dynamics studies on *C. carpio*.

Length-length relationships for *C. carpio* were retrieved from publications in the peer-reviewed and 'grey' literature (cf. conference proceedings). For each study providing LLR, the following were recorded: (i) number of fish; (ii) minimum and

maximum length type (if provided) used for the conversion [i.e. independent Y variable in the length–length equation $Y = a + bX$, where X is the dependent variable, and Y and X are either total length (TL), fork length (FL) or standard length (SL)]; (iii) parameters *a* and *b* of the length–length equation; (iv) coefficient of determination r^2; (v) water body and country of study; (vi) literature source. Whenever LLR were (also) provided for males and females separately, these were added to the database for completeness together with the LLR for the sexes combined.

In total, 16 studies were retrieved that provided LLR for *C. carpio* populations from 26 water bodies consisting of rivers, lakes and reservoirs across nine countries in Europe, Africa, Asia and Australasia (Table 1). Length–length relationships were available for all six possible combinations (i.e. as dependent/independent variables) of SL, FL and TL, with fish sizes ranging from 87 mm SL to 780 mm TL (Table 1). Overall, length–length equations, as described by parameters *a* and *b*, were quite different across studies and this was mainly related to the large variation in the range of fish sizes used for the LLR computations.

Table 1. Length–length relationships for common carp *Cyprinus carpio* worldwide grouped according to type of conversion (i.e. X → Y, where X is the predictor length type and Y is the response length type in the equation: $Y = a + bX$). TL = total length; FL = fork length; SL = standard length. For each water body, the following are provided: number of fish measured (*n*), minimum (min) and maximum (max) predictor length type (if provided), parameters *a* and *b*, coefficient of determination r^2, country, and source study. Decimal places are reported in all cases as per the original source.

n	Min (mm)	Max (mm)	a	b	r^2	Water body	Country	Source
TL → FL (FL = *a* + *b*TL)								
601	111	767	−12.167	0.929	0.999	Lower River Murray	Australia	(15)
35	207	598	0.661	0.8717	0.94	Anzali Wetland	Iran	(10)
77	115	780	−3.5872	0.9184	–	Hirfanlı Reservoir	Turkey	(8)
114	199	300	−6.622	1.020	0.978	K'sob Reservoir	Algeria	(9)†
36	203	380	−7.676	1.026	0.992	K'sob Reservoir	Algeria	(9)‡
19	106	320	0.065	0.887	0.998	Büyükçekmece Reservoir	Turkey	(12)
26	122	424	−0.598	0.9024	–	River Kızılırmak Basin	Turkey	(13)
20	140	180	−18.02	1.045	0.995	River Ganga	India	(7)§
TL → SL (SL = *a* + *b*TL)								
122	175	720	8.274	0.845	–	Lake Vransko	Croatia	(14)
148	–	–	−0.123	0.828	0.996	Hirfanlı Reservoir	Turkey	(17)
83	–	–	−0.104	0.829	0.995	Hirfanlı Reservoir	Turkey	(17)†
65	–	–	−0.109	0.825	0.997	Hirfanlı Reservoir	Turkey	(17)‡
114	199	300	−9.626	1.012	0.986	K'sob Reservoir	Algeria	(9)†
36	203	380	−3.768	0.970	0.990	K'sob Reservoir	Algeria	(9)‡
19	106	320	0.404	0.794	0.996	Büyükçekmece Reservoir	Turkey	(12)
26	122	424	−0.5260	0.817	–	River Kızılırmak Basin	Turkey	(13)
20	140	180	−40.12	1.115	0.982	River Ganga	India	(7)§
FL → SL (SL = *a* + *b*FL)								
160	100.1	438.2	−2.1977	0.8815	–	River Guadalquivir	Spain	(5)¶
26	–	–	−0.1474	0.8991	–	River Kızılırmak Basin	Turkey	(13)
FL → TL (TL = *a* + *b*FL)								
160	100.1	438.2	−2.8817	1.1058	–	River Guadalquivir	Spain	(5)
337	174.3	401.1	0.1584	1.0947	0.995	Gelingüllü Reservoir	Turkey	(4)
142	–	–	1.10	1.07	0.99	Altınkaya Reservoir	Turkey	(16)
65	–	–	0.80	1.08	0.99	Altınkaya Reservoir	Turkey	(16)†
77	–	–	1.26	1.07	0.99	Altınkaya Reservoir	Turkey	(16)‡
155	–	–	−0.02	1.09	0.99	Lakes Bafra Balık	Turkey	(16)
74	–	–	0.13	1.08	0.99	Lakes Bafra Balık	Turkey	(16)†
81	–	–	−0.16	1.09	0.99	Lakes Bafra Balık	Turkey	(16)‡
97	–	–	2.13	1.04	0.99	Derbent Reservoir	Turkey	(16)

(Table 1. continued)

n	Min (mm)	Max (mm)	a	b	r^2	Water body	Country	Source
49	–	–	1.93	1.05	0.99	Derbent Reservoir	Turkey	(16)†
48	–	–	2.02	1.04	0.99	Derbent Reservoir	Turkey	(16)‡
36	–	–	0.33	1.12	0.99	Lake Karaboğaz	Turkey	(16)
6	–	–	0.39	1.11	0.99	Lake Karaboğaz	Turkey	(16)†
30	–	–	−0.23	1.08	0.99	Lake Karaboğaz	Turkey	(16)‡
148	113	454	0.246	1.10	0.998	Hirfanlı Reservoir	Turkey	(17)
83	133	454	0.209	1.10	0.997	Hirfanlı Reservoir	Turkey	(17)†
65	113	425	0.274	1.10	0.998	Hirfanlı Reservoir	Turkey	(17)‡
42	119	217	−4.073	1.1815	−	Lake Ula	Turkey	(11)
120	–	–	0.1025	0.9612	0.998	Dahmouni Reservoir	Algeria	(2)
38	–	–	0.0915	0.969	0.9984	Dahmouni Reservoir	Algeria	(2)†
50	–	–	0.0974	0.9462	0.9979	Dahmouni Reservoir	Algeria	(2)‡
SL → FL (FL = *a* + *b*SL)								
602	87	647	8.667	1.097	0.995	Lower River Murray	Australia	(15)
148	−	−	0.025	1.09	0.997	Hirfanlı Reservoir	Turkey	(17)
83	−	−	0.058	1.09	0.996	Hirfanlı Reservoir	Turkey	(17)
65	−	−	−0.042	1.10	0.998	Hirfanlı Reservoir	Turkey	(17)
42	104	187	5.8308	1.0695	−	Lake Ula	Turkey	(11)
SL → TL (TL = *a* + *b*SL)								
12	117	409	0.2635	1.1937	0.999	Lake İznik	Turkey	(6)
49	104	740	1.9500	1.1233	0.997	Ömerli Dam	Turkey	(6)
42	93	172	2.7014	1.2645	−	Lake Ula	Turkey	(11)
10	222.0	253.0	60.6	0.93	0.53	Baghdad	Iraq	(1)§
10	213.0	259.0	118.3	0.68	0.58	Babil	Iraq	(1)§
10	221.2	248.6	249.1	0.10	0.02	Karbala	Iraq	(1)§
10	224.0	259.0	121.9	0.64	0.49	Al-Najaf	Iraq	(1)§
10	239.0	295.0	195.9	0.47	0.32	Dhi Qar	Iraq	(1)§
12	238.6	295.3	208.3	0.42	0.31	Al-Muthanna	Iraq	(1)§
12	209.0	256.0	194.8	0.47	0.97	Al-Basrah	Iraq	(1)§
100	–	–	0.30	1.18	0.99	Three Gorges Reservoir	China	(3)

Source: (1) Al-jebory et al. (2018); (2) Askri et al. (2013); (3) Xie et al. (2019); (4) Ekmekçi (1996); (5) Fernández-Delgado (1990); (6) Gaygusuz et al. (2006); (7) Kamboj and Kamboj (2019); (8) Kırankaya et al. (2014); (9) Mimeche et al. (2015); (10) Moradinasab et al. (2012); (11) Önsoy et al. (2011); (12) Saç and Okgerman (2016); (13) Sungur Birecikligil et al. (2016); (14) Treer et al. (1995); (15) Vilizzi (1997); (16) Yılmaz et al. (2010a); (17) Yılmaz, et al. (2010b).

† Parameter *a* multiplied by 10 (original measurements in cm). ‡ Males. § Females. SL measured at the hypural plate.

Discussion

This is the first study to provide a summary of LLR for *C. carpio* at the global scale, and together with the review by Vilizzi and Copp (2017) provides a comprehensive reference base for the 'vital statistics' (*sensu* Ricker and Foerster 1948) regarding the age and growth of this species. Based on a comparative evaluation of TL, FL and SL for three cyprinid fishes including *C. carpio*, TL was suggested to be the most reliable length measurement (Önsoy et al. 2011). At the same time, the proportion of age-growth studies for *C. carpio* using FL was found to be larger than those relying on SL and TL (Vilizzi and Copp 2017), with a strong bias towards the use of FL in studies from Anatolia (Vilizzi et al. 2015b) and the use of SL [cf. *longitudo corporis* (Balon 1957) or 'length to the base of *C*' (Berg 1964)] in studies from the former USSR. The LLR provided in the present study for all length type combinations as well as for a range of waterbody types (for which overall growth differences have been described by Vilizzi and Copp 2017), will help in the selection of the parameters 'best' suited to the *C. carpio* population(s) under

investigation, including studies on both young-of-year and adult fish.

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Mikroalgal Üretimlerde Kinetik Modelleme

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ÖZ

Tek hücreli, basit organizmalar olan mikroalgler, sahip oldukları karakteristik özellikleri sayesinde gıda, çevre teknolojileri, enerji, kozmetik, ilaç, akuakültür gibi çeşitli endüstrilerde yaygın olarak kullanılmaktadır. Mikroalglere ve uygulama alanlarına olan ilgi her geçen gün artış gösterse de endüstriyel çaptaki üretimlerde çeşitli sorunlarla karşı karşıya kalınabilmektedir. Organizmaların büyüme kinetiği ve hedef ürün eldesi proseslerdeki temel aşamalardan olup, bu aşamalarda meydana gelebilecek herhangi bir problem, sistemin tamamını olumsuz etkilemektedir. Bu problemleri önlemek için izlenebilecek yollardan biri, hücrelerin büyümesini ve ürün miktarını etkileyen parametrelerin kullanımıyla kinetik modeller geliştirilmesidir. Matematiksel modeller ile üretim sırasında elde edilen sonuçların sayısal olarak ifadesi sağlanmakta ve böylece ölçek büyütmede ve benzer proseslerde kullanılabilecek güvenilir veriler elde edilmektedir. Bu makalede, mikroalg hücrelerinin büyümesi ve ürün üretimine dair geliştirilen kinetik modeller substrat, ışık ve sıcaklık parametreleri açısından değerlendirilerek literatürde kullanılan modeller özetlenmiştir.

Anahtar kelimeler: Kinetik modelleme, mikroalg, ışık yoğunluğu, substrat, sıcaklık

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Kinetic Modelling of Microalgae Productions

Abstract: Being simple organisms, unicellular microalgae are commonly used in several industrial applications such as food, environmental technologies, energy, cosmetic, pharmaceutical and aquaculture due to their specific features. Although the interest in microalgae and their application areas are increasing day by day, various problems may be encountered for their industrial scale production. Varieties of problems may be faced in their industrial scale production despite the interest on microalgae and their application areas are increasing day by day, Growth kinetics of organisms and target product formations are the basic stages in the processes when any problem that may occur during these stages affects the entire system negatively. One of the ways to prevent these problems is to develop kinetic models by means of using parameters that affect the growth of cells and the amount of product. Numerical expression of the results gained during the tproduction is provided with mathematical models, and thus, reliable data that can be used in scaling up and similar processes are obtained. In this article, the models used in the literature are summarized by evaluating the kinetic models developed for the growth of microalgae cells and product production in terms of substrate, light and temperature parameters.

Keywords: Kinetic modelling, microalgae, light intensity, substrate, temperature

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Giriş

Biyolojik proseslerde hücrelerin büyüme karakteristikleri, ürün üretimi, substrat tüketimi gibi metabolik fonksiyonlar, üretime etki eden çeşitli parametrelerin değerlendirilmesiyle optimize edilmektedir. Uygun koşullarda gerçekleştirilen üretimler hem mevcut prosesin başarısını göstermekte hem de benzer proseslere model üretimler olarak kullanılabilmektedir. Üretim sırasında elde edilen sonuçların sözel ifadesi yerine sayısal olarak belirtilmesi sistemlerin verimliliğini tanımlamada daha gerçekçi sonuçlar ortaya çıkarmaktadır. Ayrıca benzer prosesler ya da ölçek büyütme işlemleri için gerekli verilerin kolay elde edilebilirliğini sağlamaktadır. Bu noktada hücrelerin davranışlarının matematiksel olarak ortaya konduğu kinetik modeller karşımıza çıkmaktadır.

Kinetik modelleme biyolojik bir prosesin nicel olarak ifadesidir. Matematiksel modeller prosesin kontrolüne yardımcı olurken, maliyeti düşürüp, ürün

kalitesini arttırmaktadır. Laboratuvar ölçeğinden ticari ölçeğe geçişte yaşanan en büyük sorunlardan biri, büyük ölçekteki optimum koşulların öngörülememesidir. Çevresel parametrelerin her birinin ayrı ayrı denendiği optimizasyon çalışmaları ise özellikle maliyet açısından büyük kayıplara yol açmakta ve gereksiz zaman almaktadır. Her parametrenin büyük ölçekte denenmesi yerine küçük ölçekte yapılan üretim sonuçlarından matematiksel modellerin oluşturulması ve bunların yeni üretimlere entegrasyonu çok daha avantajlı bir yöntemdir (Koutinas vd. 2012). Kinetik modellerde hücre davranışları zamana göre irdelenerek hücre büyüme hızı, ürün oluşumu, substrat tüketimi gibi mekanizmalar matematiksel olarak belirlenebilmektedir. Hücre davranışlarını etkileyen parametrelerin çok sayıda olduğu göz önüne alındığında çeşitli araştırmalar sonucunda tüm parametrelerin değerlendirildiği farklı modeller geliştirilmiş ve proseslere göre modifikasyonları yapılmıştır. Geliştirilen modeller temelde süreç ve sonuç odaklı olmak üzere iki ana gruba ayrılmaktadır. "Açıklayıcı" modeller biyolojik süreçteki mekanizmaların nicel olarak ifadesine dayanır. Metabolik yolaklar, ara aşamalar ve son ürüne giderken geçilen aşamaların matematiksel modellemesi yapılmaktadır. Bu modeller son derece karmaşık olmasından dolayı üzerinde modifikasyonlarla sadeleştirilmeye çalışılmaktadır. "Betimleyici" modeller ise sonuca odaklıdır. Bu modeller üretimin performansını öngörme yönünde geliştirilmiştir. Çoğu prosesin modellemesinde kolay ve amaca yönelik olmasından dolayı bu modeller kullanılmaktadır (James ve Boriah 2010; Lee vd. 2015; Huesemann vd. 2016).

Bu makalede, mikroalg hücrelerinin büyümesi ve ürün üretimine dair geliştirilen kinetik modeller substrat, ışık ve sıcaklık parametreleri açısından değerlendirilerek literatürde kullanılan modeller özetlenmiştir.

Mikroalgal Büyüme Hızının Kinetik Modellemesi

Mikroalgal biyokütle üretiminde geliştirilen modeller ışık yoğunluğu, sıcaklık, pH, çözünmüş oksijen ile karbonidoksit miktarı ve gerekli besinlerin konsantrasyonu gibi parametreleri içerdiğinden karmaşık modellerdir. Bu parametrelerin ayrı ayrı incelendiği modeller olduğu gibi birbirleriyle etkileşimlerini de göz önünde bulunduran çoklu faktör içeren modeller de kullanılmaktadır. Her ne kadar farklı model çeşitleri söz konusu olsa da temelde üretim süresince hücre davranışlarının iyi bir şekilde gözlemlenmesi gerekmektedir. Genel olarak mikroalgal üretimlerde hücre büyümesi 5 evreden oluşmaktadır (Şekil 1).

Şekil 1. Mikroalg kültürlerinde hücrelerin büyüme profili
Figure 1. Growth profile of cells in microalgae cultures

İlk aşama hücrelerin ortama ve koşullara uyum sağladığı "adaptasyon" evresidir. Bu aşamada hücre sayısında artış yoktur. Aksine adapte olamayan hücreler elimine olabilir. Optimum koşullar mevcut olduğunda 2-3 gün adaptasyon için yeterli süre olarak düşünülmektedir. Ardından ikinci evre hücre sayısında yüksek artışın görüldüğü "logaritmik" evredir. Koşullara adapte olan hücreler yüksek büyüme hızı göstererek yoğun hücre konsantrasyonuna ulaşılmaktadır. Bu aşama mikroalg türüne bağlı olarak farklı sürelerde görülebilmektedir. Takip eden süreçte hücre büyümesinin yavaşladığı ancak devam ettiği üçüncü evre gelmektedir. Hücre konsantrasyonundaki artış halen sürmektedir, ancak önceki aşamada olduğu kadar yüksek değildir. Ortamda maksimum hücre miktarına ulaşıldığı ve optimum koşullardan uzaklaşılmaya başlandığında hücreler "durgun"

evreye girmektedir. Bu evrede ortamdaki besin konsantrasyonu azalmış, hücreler arası rekabet artmış, yoğun mikroalg içeriği ışık iletimini engellemiş ve metabolik faaliyetler azalmıştır. Hücre ölümlerinin yanında çoğalmalar da devam etmektedir. Ayrıca, toplam hücre sayısında değişiklik olmaz ise hücreler daha çok sekonder metabolit üretimi yönüne ilerlemektedirler. Son aşama ise "ölüm" evresidir. Hücrelerin hızla öldüğü ve büyümenin neredeyse hiç olmadığı bu aşamaya gelindiğinde üretim sonlandırılmaktadır. Pek çok biyolojik proseste olduğu gibi mikroalgal biyokütle üretiminde de genel olarak görülen hücre büyüme profili bu şekildedir (Price ve Farag 2013; Lee vd. 2015).

Hücre büyümesi, ürün üretimi ve bunlar üzerine etki eden parametreler farklı modellerle ifade edilmektedir.

Substrata Bağlı Modelleme

Her organizmada olduğu gibi mikroalglerde de büyüme profilini etkileyen temel etmenlerden biri substrat konsantrasyonudur. Farklı mikroalg türleri için optimize edilen besin ortamlarında azot ve fosfat temel bileşenler olmak üzere bunlara ek olarak çeşitli iz elementler bulunmaktadır. Ayrıca hücrelerin gelişiminde en önemli faktör karbon kaynağıdır. Substrata bağlı olarak geliştirilen modellerden en yaygını Monod denkliğidir.

$$\mu = \frac{\mu_{max} \cdot C_S}{K_S + C_S} \quad (1)$$

μ, spesifik büyüme hızı (gün^{-1}, sa^{-1}); C_s, substrat konsantrasyonu (mg/L); μ_{max}, maksimum spesifik büyüme hızı (gün^{-1}, sa^{-1}); K_s, Monod doygunluk sabiti (mg/L).

Bu model, kültür ortamındaki besin bileşenlerinin tek başına büyüme üzerine etkilerini incelemekte olup, besin konsantrasyonunun düşük olduğu koşullar için kullanılmaktadır. Basit yapısı ve düşük besin konsantrasyonlarında doğru sonuçlar vermesi nedeniyle kullanımı yaygınlaşmıştır. Ancak yüksek substrat konsantrasyonlarında gerçekleşen inhibisyonun etkisini bu modelle görmek mümkün değildir. Bu sorunu önlemek için Monod eşitliğinin modifiye edilmesiyle oluşturulan modeller kullanılmaktadır. Bu amaçla belirlenen modellerden biri Haldane tarafından oluşturulmuştur (Zhang vd. 1999; Lee vd. 2015; Sachdeva vd. 2016).

$$\mu = \frac{\mu_{max} \cdot C_S}{K_S + C_S + \frac{C_S^2}{K_i}} \quad (2)$$

K_i, inhibisyon sabiti.

Eşitlik (1) ve (2) dışında substrat varlığı, yokluğu, besin kıtlığı ya da inhibisyon koşullarını göz önünde bulunduran çeşitli modeller ortaya konmuştur. Ancak bu modeller sadece bir substratın büyüme üzerine etkisini ifade ettiğinden, tek başına kullanımları güvenilir sonuçlar vermemektedir (Zhang vd. 1999; Mirzaie vd. 2016).

Hücrelerin gelişiminde ortamdaki substrat miktarından daha çok hücrelerin bu substratı kullanabilme potansiyelleri önemlidir. Bu noktada tek bir hücre içindeki maksimum besin miktarı olarak tanımlanan "hücre kota"sı devreye girmektedir. Monod benzeri modellerden farklı olarak bu modellerde belirli bir bileşen için hücrenin kotası değerlendirilir ve böylece ortamdaki besin eksikliğinde hücre büyüme davranışlarının ilerleyişi de incelenebilmektedir. Ancak hücre içerisindeki besin miktarının belirlenmesi çok zor bir yöntem olduğundan bu modellerin daha basitleştirilmiş modifiye halleri geliştirilmiştir. Bunların başında yaygın olarak kullanılan "Droop Model"i gelmektedir (Eşitlik 3). 1968 yılında M. R. Droop tarafından ortaya konan bu model, hücre büyüme hızı ile hücre içi besin konsantrasyonu arasındaki ilişkinin matematiksel ifadesidir (Droop, 1968). Modelde büyüme hızı her bir hücrede depolanan ortalama besin miktarı ile bağlantılıdır. Mikroalgler için Droop modeli özellikle doğal ortamda besin eksikliğinin yüksek olması dolayısıyla hücrelerin davranışlarını incelemek için kullanılmıştır. Ayrıca gerçekleştirilen üretimlerde azot ve fosfat eksikliğinde meydana gelen değişimlerin ortaya konmasında bu model ile gerçekçi sonuçlar elde edilmektedir (Lemesle ve Mailleret 2008; Packer 2014; Sachdeva vd. 2016).

$$\mu = \mu^{l}_{max}\left(1 - \frac{Q_{min}}{Q}\right) \quad (3)$$

μ^{l}_{max}, varsayımsal maksimum spesifik büyüme hızı (gün^{-1}, sa^{-1}); Q_{min}, minimum hücre içi besin kotası (g/g karbon); Q anlık hücre içi besin kotası (g/g karbon).

Mikroorganizmaların karmaşık yaşam döngülerini açıklamak için oluşturulan ve yaygın kullanılan bir diğer model "Lojistik" modeldir (Eşitlik 4). Lojistik model biyolojik proseslere kolaylıkla entegre edilebilmesi, substrat konsantrasyonundan bağımsız olması ve özellikle fotootrofik canlılar için kullanıldığında doğru modeller oluşturması sebebiyle tercih edilmektedir. Bu modelde hücre üretimini belirleyen temel nokta ortamın maksimum taşıyabileceği mikroalg popülasyonudur (Yang vd. 2011; Surendhiran vd. 2015).

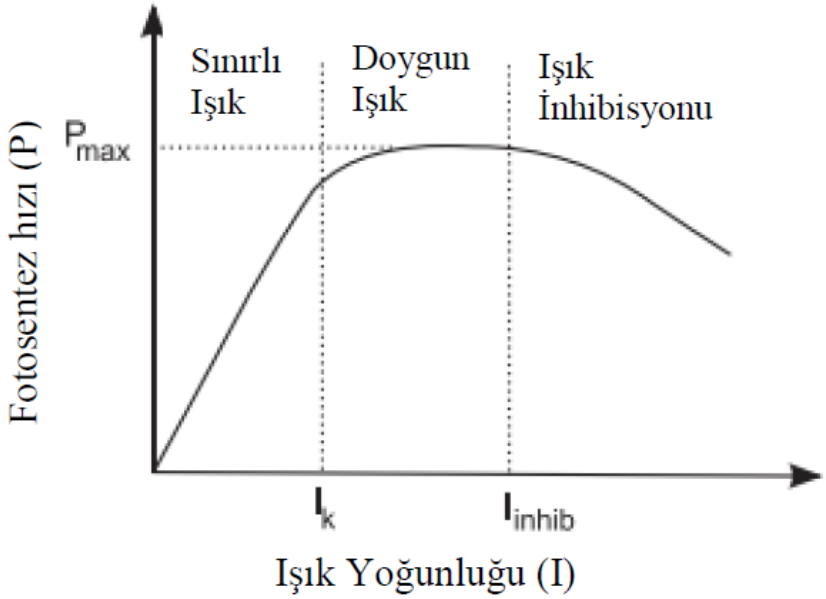
$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{X_m}\right) \quad (4)$$

dX/dt, mikroalg büyüme hızı; X, ortamdaki mikroalg konsantrasyonu (g/L); X_m, ortamın taşıyabileceği maksimum mikroalg konsantrasyonu (g/L).

Işığa Bağlı Modelleme

Substrat kaynağı tüm canlılar için primer yaşam gereksinimlerinden biridir. Ancak kompleks canlı metabolizması sadece substrattan değil canlı türüne göre pek çok bileşenden etkilenmektedir. Fotoototrofik canlılar için bu bileşenlerden en önemlisi ışık yoğunluğudur. Işık yoğunluğuna göre hücreler farklı davranışlar göstermektedir (Şekil 2) (Bechet vd. 2013; Lee vd. 2015).

- Düşük ışık yoğunluğunda; hücreler metabolik faaliyetlerini sürdürmek için gerekli enerjiyi üretemezler. Hücrenin fotosentetik aktivitesi düşüktür bu da besin miktarı yeterli olsa da yaşam döngüsünü zorlaştırır.
- Doygunluk noktasındaki ışık yoğunluğunda, hücrenin fotosentetik aktivitesi maksimuma ulaşmaktadır. Bu noktadan sonra artan ışık yoğunluğu fotosentez hızını arttırmaz. Hücreler ışığa doygun hale gelir ve metabolik aktiviteler en iyi düzeydedir.
- Yüksek ışık yoğunluğunda, fotosentezde etkili olan proteinlerin deaktivasyonu nedeniyle fotosentez hızında düşüşgörülmektedir. Bu noktadan sonra ışığın inhibeedici etkisi söz konusudur ve bu da "fotoinhibisyon" olarak tanımlanmaktadır (Bechet vd. 2013; Bernard vd. 2015; Lee vd. 2015).



Şekil 2. Işığın fotosentez hızı üzerine etkisi (Bechet vd. 2013).

Figure 2. Effect of light intensity on photosynthesis rate

Işığa bağlı mikroalg modellemelerinde yapılması gereken ilk aşama üretim için ortalama ışık yoğunluğunun hesaplanmasıdır. Bunun için farklı yöntemler mevcut olmakla birlikte yaygın olarak Beer-Lambert yasası kullanılır (Mirzaie vd. 2016).

$$I_i = I_0 + e^{-\varepsilon.X.d} \quad (5)$$

I_i, yerel ışık yoğunluğu (μmol foton/m^2.s); I_0, reaktör yüzeyindeki ışık yoğunluğu (μmol foton/m^2.s); ε, molar absorpsiyon katsayısı (L/g.cm); X, biyokütle konsantrasyonu (g/L); d, ışık yolu uzunluğu (cm).

Ortalama ışık yoğunluğu belirlendikten sonra hücre büyümesinin ışığa bağlı değişimini gösteren model kullanılmaktadır. Yaygın olarak rastlanan modellerden biri Monod-benzeri modeldir (Perez vd. 2008; Bechet vd. 2013; Mirzaie vd. 2016).

$$\mu = \frac{\mu_{max}.I}{I + K_I} \quad (6)$$

I, kültürün içindeki ortalama ışık yoğunluğu; K_I, ışık doygunluk sabiti

Bu model Monod eşitliğinden farklı olarak substrat konsantrasyonu yerine ışık yoğunluğunun etkisini incelemekte ve spesifike olarak ışık yoğunluğunun doygunluk noktasından düşük olduğu üretimlerin modellenmesinde tercih edilmektedir.

Işık yoğunluğunun etkisini gösteren Monod-benzeri model dışında deneysel olarak ortaya konmuş çok sayıda model mevcuttur. Yaygın olarak kullanılan diğer modeller Eşitlik (7), (8) ve (9)'da verilmiştir (Chalker 1980; Yuan vd. 2014; Lee vd. 2015).

$$\mu = \mu_{max} tanh \frac{I}{K_I} \quad (7)$$

$$\mu = \mu_{max}(1 - e^{-I/K_I}) \quad (8)$$

$$\mu = \mu_{max} \frac{I}{(K_I^m + I^m)^{1/m}} \quad (9)$$

m; şekil faktörü

Laboratuvar ortamında gerçekleştirilen, yoğun mikroalg kültürü içermeyen üretimlerde ışığın etkisinin görülmesi için Monod benzeri ya da hiperbolik tanjant modeli kullanılabilmektedir. Ancak çoğu mikroalgal biyoproses için düşük hücre konsantrasyonu yeterli olmamaktadır. Hücre konsantrasyonu arttıkça ışığın penetrasyonu, homojen dağılımı gibi özellikleri de değişmektedir (Grima vd. 1994; Lee vd. 2015; Huesemann vd. 2016). Bu tip kültürlerde ışık etkisini incelemek için yaygın olarak kullanılan model;

$$\mu = \mu_{max} \frac{I_{av}^n}{I_k^n + I_{av}^n} \quad (10)$$

I_k; Mikroalgin ışığa karşı afinitesi (µE/m^2.s), I_{av}; Kültürdeki ortalama ışık yoğunluğu (µE/m^2.s), n; Üssel katsayı

Modelde ifade edilen ortalama ışık yoğunluğu her üretim için değişmekte olup ışık yolunun uzunluğu, hücre konsantrasyonu ve yüzeydeki ışık yoğunluğu dikkate alınarak Eşitlik (11)'e göre hesaplanmaktadır (Grima vd. 1994; Lee vd. 2015; Huesemann vd. 2016).

$$I_{av} = \frac{I}{K_a p X}[1 - e^{-K_a p X}] \quad (11)$$

K_a, sönümleme sabiti (kg/m^3); p, fotobiyoreaktör içindeki ışık yolu uzunluğu (m); X, hücre konsantrasyonu (kg/m^3)

Önceki modellerin tamamında hücrelerin ortalama ışık yoğunluğunda üretildiği ve fotosentetik aktivitelerinin istenen oranda gerçekleştiği durumların nicel ifadesi üzerinde durulmuştur. Ancak kontrol altına alınamayan özellikle dış ortam koşullarında gerçekleştirilen üretimlerde ışık miktarı istenen düzeyden çok yüksek olabilir. Bu istenmeyen durumlarda ışık inhibisyonu sonucu fotosentez hızı düşmektedir. Bu üretimlerin hem sınırlı hem de yüksek ışık koşullarını değerlendirmek için kombine model geliştirilmesi avantajlı bir durumdur. Hem düşük ışığın sınırlandırıcı etkisini hem de yüksek ışığın inhibe edici etkisini birlikte gösteren karmaşık modeller bulunmaktadır (Steele 1962; Lee vd. 2015). Bunların içinde en yaygın kullanılanı Steele (1962) tarafından ortaya konan aşağıdaki modeldir;

$$\mu = \mu_{max} \frac{I}{I_{opt}} \mathrm{e}^{\left(1 - \frac{I}{I_{opt}}\right)} \quad (12)$$

Bu eşitlikte $\frac{I}{I_{opt}}$ ifadesi düşük ışık konsantrasyonlarında hücrelerdeki fotosentetik aktivitenin ve büyümenin sınırlanmasını ifade ederken, $e^{\left(1 - \frac{I}{I_{opt}}\right)}$ değeri yüksek ışık şiddetinde azalan fotosentetik aktiviteyi göstermektedir.

Sıcaklığa Bağlı Modelleme

Her biyolojik proseste olduğu gibi mikroalgal biyokütle üretiminde de sıcaklık optimizasyonu önemli parametrelerden biridir. Hücredeki metabolik faaliyetlerden sorumlu enzimler ve proteinlerin aktivitesi açısından optimum sıcaklık koşulları sağlanmalıdır. Sıcaklığın hücre büyümesine etkisinin incelendiği en basit modellerden biri Arrhenius benzeri sıcaklık eşitliğidir (Perez vd. 2008).

$$\mu = Aexp\left(-\frac{E_a}{RT}\right) - Bexp(-\frac{E_b}{RT}) \quad (13)$$

A ve B; frekans faktörü (sa^{-1}), R; gaz sabiti (kcal/mol), T; üretim sıcaklığı (K), E_a; hücre büyümesi için gerekli aktivasyon enerjisi, E_b; hücresel degredasyon için gerekli aktivasyon enerjisi.

Sadece sıcaklığın hücre büyümesi ve fotosentetik aktivite üzerine etkisinin incelenmesiyle bir model oluşturulsa da diğer faktörlerin de göz önünde bulundurulması daha doğru sonuç vermektedir. Işık ve sıcaklığın büyüme ve fotosenteze birlikte etkilerinin incelenmesi amacıyla Monod ve Arrhenius eşitlikleri birlikte kullanılarak Eşitlik (14) geliştirilmiştir (Goldman ve Carpenter 1974; Carcano 2010; Bechet vd. 2013).

$$\mu = \mu_{max} \exp\left(-\frac{E_a}{kT}\right) . \frac{I_{av}}{K + I_{av}} \quad (14)$$

K, ışık sabiti (µmol/m^{2}s); k, Boltzmann sabiti (J/kg)

Arrhenius benzeri modelin farklı mikroalg türleri ve üretimler için geliştirilmiş çok sayıda modeli mevcuttur. Ancak bu modeller yalnızca metabolik aktiviteler için optimum sıcaklık değerlerinin korunduğu prosesler için uygulanmaktadır. Ortam sıcaklığı yükseldikçe enzimlerin yapısında denatürasyon meydana gelmekte ve inaktif hale geçmektedirler. Bu sorunu önlemek için enzim degredasyonunu da içeren benzer modeller geliştirilmektedir (Eşitlik 15) (Bechet vd. 2013).

$$\mu(I) = \mu_{m,0}(I) \frac{\exp(-\frac{E_a}{RT})}{1 + Kexp(-\frac{E_a^i}{kT})} \quad (15)$$

Bu modelde Eşitlik (14)'den farklı olarak yüksek sıcaklıkta meydana gelen enzim denatürasyonunu ifade eden aktivasyon enerjisi "E_a'" bulunmaktadır.

Mikroalgin büyümesinde etkili olan faktörlerin bireysel olarak büyüme ve ürün oluşumu üzerine etkisinin incelenmesi kullanışlı bir yöntemdir, ancak bu faktörlerin birbirleriyle etkileşimleri de üreme kinetiğinde önemlidir. Özellikle açık sistemlerde gerçekleştirilen üretimlerde prosesin kontrolü zor olduğundan değişen parametrelerin üretim üzerindeki etkisi daha büyüktür. Bu yüzden birkaç faktörün etkisinin birlikte gösterildiği çoklu kombine modeller geliştirilmiştir. Bu modellerde azot, fosfat, CO_2, ışık

yoğunluğu ve sıcaklık parametrelerinden birkaçının etkisi birlikte incelenerek daha karmaşık modeller oluşturulmuştur. Bu modellerden bazıları Tablo 1'de gösterilmektedir.

Tablo 1. Mikroalgal hücre büyümesinde çoklu faktör etkisini gösteren modeller

Table 1. Models showing effects of multi level factors on the microalgal cell growth.

Mikroalgal büyüme modelleri	Parametreler	Denklem Numarası	Referans
$\mu = \mu'_{max,min}(1 - \frac{Q_{min,N}}{Q_N}, 1 - \frac{Q_{min,P}}{Q_P})$	Azot, Fosfat	(16)	Klausmeier vd. 2004
$\mu = \mu'_{max,min}(\frac{1 - \frac{Q_{min,N}}{Q_N}}{1 - \frac{Q_{min,N}}{Q_{max,N}}}, \frac{1 - \frac{Q_{min,P}}{Q_P}}{1 - \frac{Q_{min,P}}{Q_{max,P}}})$	Azot, Fosfat	(17)	Bougaran vd. 2010
$\mu = \mu_{max,min}\,(\frac{S_P}{K_{S,P}+S_P}, \frac{S_{CO_2}}{K_{S,CO_2}+S_{CO_2}})$	Karbondioksit, Fosfat	(18)	Spijkerman vd. 2011
$\mu = \mu'_{max,min}\left[1 - \left(\frac{Q_{N,min}}{Q_N}\right)^4, 1 - \left(\frac{Q_{P,min}}{Q_p}\right)^4\right].f(I_{av})$	Azot, Fosfat, Işık	(19)	Lee vd. 2015
$\mu = \mu_{max}.Q^{T-T_{ref}}(\frac{I}{K_I + I})(\frac{S_P}{K_{S,P}+S_P})(\frac{S_N}{K_{S,N}+S_N})$	Azot, Fosfat, Sıcaklık, Işık	(20)	Haario vd. 2009
$\mu = \mu_{max}.1.06^{T-20}(\frac{I_{av}^m}{K_I^m + I_{av}^m})(\frac{S_{CO2}}{K_{S,CO2} + S_{CO2} + \frac{S_{CO2}^2}{K_{I,CO2}}})$	CO_2, ışık, sıcaklık	(21)	Pegallapati ve Nirmalakhanda 2012
$\mu = \mu_m \frac{S_N}{S_N + K_{NA}}\left(\frac{S_{CO2}}{S_{CO2} + K_{CO2}}\right) f(\iota)$ $f(\iota) = \frac{I_{av}}{I_s}\exp(1 - \frac{I_{av}}{K_{s,\iota}})$	CO_2, ışık, azot	(22)	Jalalizadeh 2012

$\mu'_{max,min}$: Sınırlayıcı besin için sonsuz hücre kotasında varsayımsal maksimum büyüme hızı (gün^{-1}, sa^{-1}), Q_{min}: Hücre canlılığı için gerekli minimum besin kotası (g/g karbon), Q: Hücrenin besin kotası (g/g karbon), O_{max}: Hücre canlılığı için gerekli maksimum besin kotası (g/g karbon), N: nitrogen, P: phosphorus, S: Besin konsantrasyonu (mg/L), K_s: Monod yarı doygunluk sabiti (mg/L), $f(I_{av})$: Ortalama ışık yoğunluğu fonksiyonu, T: sıcaklık (°C), T_{ref}: Referans sıcaklığı (20 °C), K_I: Işık doygunluk sabiti (µmol/m^{2}s), K_{NA}: Sabit, K_{CO2}: CO_2 Doygunluk sabiti.

Mikroalgal Ürün Üretiminin Kinetik Modellenmesi

Açık ve kapalı olmak üzere çeşitli sistemlerde yetiştirilen mikroalglerde hedef ürün endüstriyel alana göre değişiklik göstermektedir. Yağ içeriğinin arttırılarak biyoyakıt olarak kullanımı, primer ve sekonder metabolit üretimi ile katma değeri yüksek ürünlerin eldesi, biyokütle miktarının artırımı ile yem endüstrisinde kullanımı gibi çalışmalar yaygın olarak karşılaşılan proses çıktılarındandır. İstenen amaç doğrultusunda mikroalgal biyokütleyi arttırmak kadar ürün üretimini de optimize etmek önemlidir. Bu nedenle hücre büyümesinin yanı sıra üretimin modellemesi de diğer prosesler ve mikroalgler için yol gösterici olmaktadır. Mikrobiyal prosesler için ürün üretiminin ifadesi için yaygın olarak kullanılan model "Luedeking-Piret" modelidir. Bu modelde ürün oluşumu biyokütle konsantrasyonu ve spesifik büyüme hızı ile doğru orantılıdır.

$$\frac{dp}{dt} = \alpha\frac{dx}{dt} + \beta x \quad (23)$$

$\frac{d_p}{d_t}$, ürün oluşum hızı; $\frac{d_x}{d_t}$, mikroalg büyüme hızı; α, ürün oluşum katsayısı; β, hücre büyümesinden bağımsız ürün oluşum katsayısı

Bu model hem kolay kullanımı hem de ürün çeşidine göre entegre edilebilirliği açısından yaygın kullanılmaktadır.

Mikroorganizmalarda üretilen ürünler hücre büyümesiyle ilişkili olması açısından 3 gruba ayrılmaktadırlar. İlk grupta, ürün üretimi hücre büyümesiyle doğrudan ilişkilidir. Bu gruptaki ürünler genel olarak hücre büyümesi için hayati önem taşıyan primer metabolitler olarak adlandırılmaktadır. İkinci grupta ise hücre büyümesiyle kısmen ilişkili olan ürünler bulunmaktadır. Hücrede büyüme görülmese de ürün üretilmeye devam eder, ancak maksimum ürün miktarı ve ürün kalitesi hücre üremesinin en

yüksek olduğu dönemde görülmektedir. Son grupta ise hücre büyümesinden tamamen bağımsız olarak üretilen ürünler görülmektedir. Genel olarak hücrenin strese girdiği koşullarda bir nevi savunma mekanizması oluşturmak için ürettiği ürünleri kapsayan bu grupta sekonder metabolitler mevcuttur. Luedeking-Piret modeli sahip olduğu katsayılar sayesinde bu 3 gruptan her biri için uyarlanabilir (Yang vd. 2011; Ronda vd. 2012; Vinayagam vd. 2014; Surendhiran vd. 2015).

- $\alpha=0$ ve $\beta\neq0$ → Ürün oluşumu hücre büyümesinden bağımsızdır;
- $\alpha\neq0$ ve $\beta\neq0$ → Ürün oluşumu hücre büyümesiyle kısmen ilişkilidir;
- $\alpha\neq0$ ve $\beta=0$ → Ürün oluşumu doğrudan hücre büyümesiyle bağlantılıdır.

Tartışma ve Sonuç

Mikroalg hücrelerinin büyüme kinetiği ve ürün üretimi, substrat konsantrasyonu, ışık yoğunluğu, sıcaklık, gaz konsantrasyonu gibi çeşitli faktörlerden etkilenen karmaşık bir prosestir. Literatürde bu faktörlerin etkisinin incelenmesi amacıyla çok sayıda kinetik modele rastlamak mümkündür. Geliştirilen modellerden sadece bir faktörün etkisinin incelendiği eşitlikler uygulama açısından kolaydır ancak ortaya çıkan sonuçlar değerlendirildiğinde daha doğru çıktılar için modifikasyon gereksinimi doğmaktadır. Bu nedenle, çoklu faktörlerin etkisinin incelendiği daha karmaşık ancak daha tutarlı sonuçlar veren modeller geliştirilmiştir. Gelecek çalışmalar için daha etkili modellerin ortaya konması mikroalg üretimlerinin daha verimli olmasını ve olası sorunların önlenmesini sağlayacaktır. Ayrıca mikroalg türleri doğada monokültür halinde değil diğer organizmalar ile birlikte yaşamaktadır. Bu yüzden, mikroalg davranışlarının daha doğru incelenmesi adına habitattaki diğer türlerin de etkisinin değerlendirildiği modeller üzerine çalışmalara yoğunlaşılmalıdır.

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ERRATUM

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Effects of Temperature Changes on the Spatial Distribution and Ecology of Ostracod (Crustacea) Species

Derya AKDEMİR, Okan KÜLKÖYLÜOĞLU

In this article, three expressions used in three places (pages 5, 6, 7) need to be corrected. These statements are inadvertently misspelled and do not correspond to the original sources. Wrong sentence and sentence corrected statements are shown below.

Wrong sentence (page 5): "*H. chevreuxi* known as a pure freshwater species showed the lowest tolerance for water temperature (tk= 1.29) and conductivity (tk = 75.85)."
Sentence corrected: "*H. chevreuxi* showed the lowest tolerance for water temperature (tk= 1.29) and conductivity (tk = 75.85)."

Wrong sentence (page 6): "In contrast, stenoecious species (e.g., *H. chevreuxi*) with a narrow tolerance levels to some of those environmental variables are of limited distributional ranges."
Sentence corrected: "In contrast, stenoecious species with a narrow tolerance levels to some of those environmental variables are of limited distributional ranges."

Wrong sentence (page 7): "According to Meisch (2000), the species can be considered as pure freshwater species and its co-occurrence with one or more halophilic ostracods (e.g., *Heterocypris salina*) indicates an increase in salinity levels of that water body."
Sentence corrected: "According to Meisch (2000), the species can also be found in pure freshwater habitats and thus its co-occurrence with one or more halophilic ostracods (e.g., *Heterocypris salina*) indicates an increase in salinity levels of that water body."