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Aims and Scope

AQUATIC RESEARCH

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Editor in Chief: Prof. Nuray ERKAN

Address: Istanbul University, Faculty of Aquatic Sciences, Department of Seafood Processing Technology, Ordu Cad. No: 8, 34134 Fatih/Istanbul, Türkiye

E-mail: **nurerkan@istanbul.edu.tr**

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Pesticide accumulations in water and sediment of dam lakes located in Thrace part of Marmara Region (Turkey)

Cem Tokatlı

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Trakya University, Ipsala Vocational School, Department of Laboratory Technology, Edirne, Turkey

ORCID IDs of the author(s):
C.T. 0000-0003-2080-7920

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Correspondence:
Cem TOKATLI
E-mail: tokatlicem@gmail.com

ABSTRACT

Ergene River Basin, which is located in the north-west part of Turkey, is the most significant aquatic habitat of Thrace Region. In addition to the presence of important lentic ecosystems in the basin, there are also important natural and artificial lotic ecosystems, which are of great importance both for the natural life and for the local public. Thrace Region is one of the most important and fertile agricultural regions of our country and despite such a great importance of Ergene River Basin for Thrace Region, almost all the components of the watershed are being exposed to an intensive pollution by means of especially agricultural applications. In this research, the pesticide concentrations in water – sediment of most significant 6 dam lakes (Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes) located in Ergene River Basin were investigated. Water – sediment samples were taken in rainy season (spring) of 2018 from 15 stations and pesticide concentrations (174 pesticides varieties) were determined by using an LC/MS. In addition, the investigated locations were classified in terms of pesticide accumulations in water and sediment by using Cluster Analysis (CA). As a result of this research, 3 pesticide types in water and 18 pesticide types in sediment were detected. Carbendazim and forchlorfenuron-706 were recorded as the most dominant pesticide types for water samples and propiconazole and prochloraz were recorded as the most dominant pesticide types for sediment samples. The total pesticide contents determined in both water and sediment were found to be much higher in Altınyazı and Sultanköy Dam Lakes compared to the other investigated reservoirs. As a result of CA, 3 statistically significant clusters were formed both for water and sediment, which were named as “high contaminated zones”, "low contaminated zones" and "moderate contaminated zones".

Keywords: Thrace Region, Dam Lakes, Water – Sediment quality, Pesticides, Cluster Analysis

Introduction

Pesticides, which have become an integral part of the society, are widespread chemical compounds. They are used to increase the agricultural production in order to kill the pests including insects, rodents, fungi and weeds, which are damaging the agricultural crops. However, it is clearly known that, pesticides, which have long persistence in the environment, are potentially toxic to other organisms and dangerous for environment health, even at very low concentrations. Pesticides also tend to bio-accumulate and bio-magnify and are transferred to higher trophic levels through several food chains. As a result of this bio-magnification they may lead to toxicity in non-target organism and even in humans. Therefore, they need to be used safely and disposed of properly (Chopra et al. 2010, Ogbeide et al. 2015, Ccanccapa et al. 2016).

Ergene River Basin is the most significant river ecosystem of the Thrace Region and it is known to be exposed to a great agricultural and industrial pressure (Tokatlı 2015, 2017; Tokatlı and Baştatlı 2016). Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes were constructed by DSİ, on the Basamaklar, Poğaça, Teke, Şeytandere, Manastır and Süloğlu Streams respectively (DSİ, 2020). These reservoirs, which are located on the Ergene River Basin, are the most significant artificial lentic ecosystems of Thrace Region. As many freshwater ecosystems, these reservoirs are being adversely effected from agricultural and domestic pressure.

The main objective of this study was to determine the residues of 174 kinds of pesticides in the water and sediment samples of the most significant dam lakes located in the Thrace Region of Turkey.

Material and Methods

Study Area and Collection of Samples

Water and sediment samples were collected from 15 stations selected on the dam lakes in rainy (spring) season of 2018, when the precipitation and surface runoff have increased significantly in the basin. Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes and selected stations on the reservoirs are given in Figure 1.

Samples of water were collected 0.5 meter below the water surface in 1 liter precleaned bottles and they were kept at 4°C until the analysis. Samples of sediments were collected from the upper 10 centimeter of sediments by using an Ekman Grab in 1 liter sterile bottles and they were kept in dark and at 4°C until the analysis.

Pesticide Analysis

QUECHERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method has been applied for determination of pesticide residues in water – sediment samples (Schenck and Hobbs 2004). Chemical analysis were made by using a ZİVAK TANDEM GOLD LC-MS / MS device with detection limit of 10 ppt. Samples were analysed in Trakya University Technology Research and Development Application Center, which has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). All the element analyses were recorded by means of triplicate measurements.

Firstly, the samples were washed 3 times with distilled water and grinded in stainless steel blenders and made homogeneous. Other repeats of the same sample were also treated separately. 10 grams of analysis samples were weighed from these samples, and 100 mL of acetonitrile was added to it and it was broken down in the homogenizer. These samples, which will be homogenized by disintegration and placed in 50 mL balcony tubes, were centrifuged at 4000 rpm for 10 minutes. After taking 50 mL from the upper phase of the samples, Cleanert MAS - Q (NaAc: 1.5 gr, $MgSO_4$: 6 gr) kit was added to the new falt tubes for the cleaning stage and shaken for 1 minute. Samples were centrifuged again at 4000 rpm for 30 minutes. Then, the upper phase is filtered through a PTFE filter with a pore diameter of 0.22µm and transferred to the vials and injected into the LC – MS / MS device (Schenck and Hobbs 2004).

In addition, solutions of 25, 37.5, 50, 75, 100, 150, 200 ppb concentrations were prepared by diluting 100 µg / mL stock solutions in order to create calibration curves of pesticide standards. Calibration curves were drawn by analysing the prepared standard solutions. According to the quality control procedures, parameters such as laboratory and field blanks, matrix spikes were evaluated. The reliability of the sample preparation and calibration method was evaluated on the spiked samples. The calibrated midpoints (10,000 ppt) were spiked by using pesticide-free water, and then the QUECHERS stages were applied. According to the result of the analysis, the recoveries were determined between the rates of 80 – 120%. The list of pesticides investigated in the present research are given in Table 1.

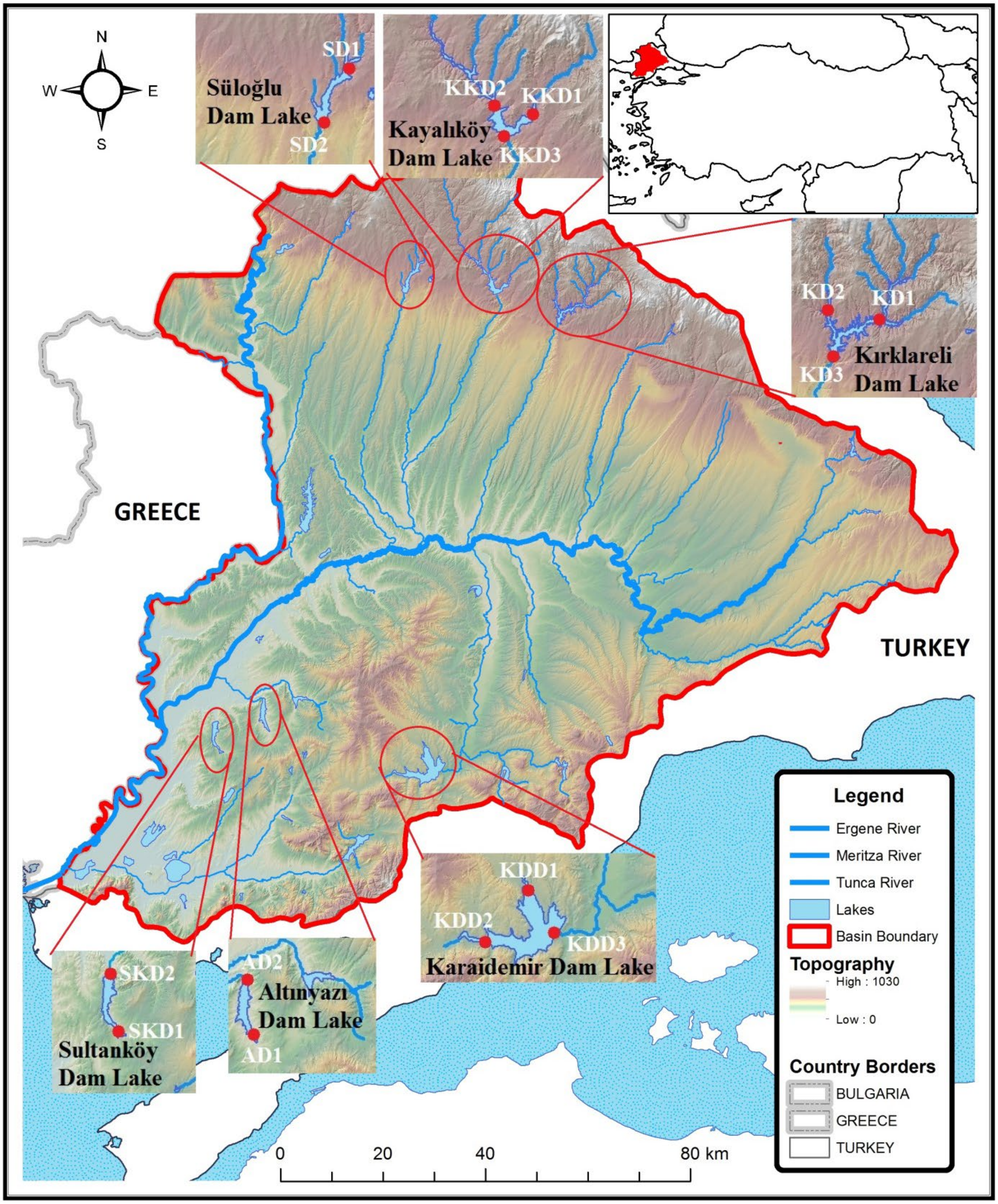

Figure 1. Study area and selected stations on the reservoirs

Table 1. Names of investigated pesticides

Acephate	Dimethoate	Ipconazole-713	Prothioconazole -734
Acetamiprid	Dimoxystrobin-688	Iprovalicarb	Pymetrozine
Aldicarb	Diniconazole	Isoprocarb	Pyracarbolid
Aldicarb sulfone	Dinotefuran	Isoproturon	Pyraclostrobin
Aldicarb sulfoxide	Diuron	Kresoxim-methyl	Pyridaben
Ametryne	Emamectin-Benzoate	Linuron	Pyrimethanil
Aminocarb	Epoxiconazole	Mandopropamid	Pyriproxyfen
Amitraz	Etaconazole	Mefenacet	Quinoxyfen
Azoxystrobin	Ethiofencarb	Mepronil	Rotenone-739
Benalaxyl-M	Ethirimol	Metalaxyl	Secbumeton
Bendiocarb	Ethofumasate	Metconazole -718	Siduron
Benfurocarb	Etoxazole	Methabenzthiazuron-719	Simetryn
Benzoximate	Famoxadone	Methamidophos	Spinetoram-741
Bifenazate	Fenamidone	Methiocarb	Spinosad A
Bitertanol	Fenarimol	Methoprotryne	Spirodiclofen
Boscalid	Fenazaquin	Methoxifenozide	Spiromesifen
Bromuconazole	Fenbuconazole	Metobromuron	Spirotetramat
Bupirimate	Fenhexamid	Metribuzin	Spiroxamine
Buprofezin	Fenobucarb	Mevinphos	Tebuconazole
Butocarboxim	Fenproprimorph	Mexacarbate	Tebufenozide
Butoxycarboxim	Fenuron	Monocrotophos	Tebufenpyrad
Carbaryl	Fibronil	Monolinuron	Tebuthiuron
Carbendazim	Fluazinam	Myclobutanil	Terbumeton
Carbetamide	Flubendiamide -695	Neburon	Terbutryn
Carbofuran	Fludioxonil	Nuarimol	Tetraconazole
Carbofuran-3-hydroxy	Flufenacet	Omethoate	Thiabendazole
Carboxin	Flufenoxuron	Oxadixyl	Thiacloprid
Carfentrazone Ethyl	Fluometuron	Oxamyl	Thiamethoxam
Chlorfluazuron	Fluoxastrobin-698	Paclobutrazol	Thidiazuron-747
Chlorotoluron	Fluquinconazole -699	Penconazole	Thiobencarb-748
Chloroxuron	Flusilazole	Pencycuron	Thiofanox
Clethodim -682	Flutolanil-703	Phenmedipham	Thiophonate Methyl
Clofentezine	Flutriafol	Picoxystrobin	Triadimefon
Clothianidin	Forchlorfenuron-706	Piperonyl butoxide	Triadimenol
Cyazofamid	Formetanate-hydrochloride	Pirimicarb	Trichlorfon
Cycluron	Fuberidazole-707	Prochloraz	Tricyclazole-753
Cyproconazole	Furalaxyl	Promecarb	Trifloxystrobin
Cyprodinil	Furathiocarb	Prometon	Triflumizole
Cyromazine	Hexaconazole	Prometryn	Triflumuron
Desmedipham	Hexaflumuron	Propamocarb-hydrochloride	Triticonazole
Dicrotophos	Hexythiazox	Propargite	Vamidathion
Diethofencarb	Hydramethylnon	Propham	Zoxamide
Difenoconazol	Imazalil	Propiconazole	
Diflubenzuron	Indoxacarb	Propoxur	

Statistical Analysis

"PAST" package statistical program was used for applying Cluster Analysis (according to Bray Curtis) to detected chemical data in water and sediment samples in order to classify the investigated dam lakes and selected stations on the reservoirs according to similar water – sediment quality characteristics.

Results and Discussion

According to detected data, among the investigated 174 kinds of pesticides, 3 kinds of pesticides residues were observed in water samples (Acetamiprid, Carbendazim and Forchlorfenuron-706) and 18 kinds of pesticide residues were observed in sediment samples (Acetamiprid, Azoxystrobin, Carbendazim, Cyproconazole, Difenoconazol, Dinotefuran, Epoxiconazole, Fluquinconazole -699, Imazalil, Metalaxyl, Picoxystrobin, Prochloraz, Propiconazole, Prothioconazole -734, Pyraclostrobin, Tebuconazole, Thiacloprid and Thiamethoxam). The mean values of pesticide concentrations are given in Table 2 and 3. The proportional values of pesticides for all the investigated reservoirs and the mean values of the total pesticide loads are given in Figure 2.

Table 2. Mean pesticide accumulations in waters of reservoirs (ppb)

Reservoir	Station	Pesticide	Residue	Reservoir	Station	Pesticide	Residue
Kırklareli Dam Lake	KD1	Carbendazim	0.14	**Kayalıköy Dam Lake**	KKD1	Carbendazim	0.12
		Forchlorfenuron-706	0.26		KKD2	Carbendazim	0.23
	KD2	Carbendazim	0.20			Forchlorfenuron-706	0.55
		Forchlorfenuron-706	0.41		KKD3	Carbendazim	0.66
	KD3	Carbendazim	0.15			Forchlorfenuron-706	0.45
		Forchlorfenuron-706	0.25	**Süloğlu Dam Lake**	SD1	Carbendazim	0.15
Sultanköy Dam Lake	SKD1	Carbendazim	0.30			Forchlorfenuron-706	0.26
		Acetamiprid	0.02		SD2	Carbendazim	0.29
		Forchlorfenuron-706	0.30			Forchlorfenuron-706	0.23
	SKD2	Carbendazim	0.40	**Karaidem ir Dam Lake**	KDD1	Carbendazim	0.12
		Acetamiprid	0.03			Forchlorfenuron-706	0.40
		Forchlorfenuron-706	0.43		KDD2	Carbendazim	0.31
Altınyazı Dam Lake	AD1	Carbendazim	0.58			Forchlorfenuron-706	0.68
		Forchlorfenuron-706	0.83		KDD3	Carbendazim	0.13
	AD2	Carbendazim	0.05			Forchlorfenuron-706	0.22

Table 3. Mean pesticide accumulations in sediment of reservoirs (ppb)

Reservoir	Station	Pesticide	Residue	Reservoir	Station	Pesticide	Residue
Karaidemir Dam Lake	KDD1	Carbendazim	4.97	**Altınyazı Dam Lake**	AD1	Carbendazim	0.02
		Acetamiprid	0.05			Imazalil	7.58
		Azoxystrobin	1.43			Azoxystrobin	3.12
		Epoxiconazole	15.59			Prochloraz	8.02
		Prochloraz	15.00			Tebuconazole	2.40
		Tebuconazole	3.14			Propiconazole	2.25
		Propiconazole	2.15		AD2	Carbendazim	5.68
	KDD2	Carbendazim	0.90			Thiamethoxam	0.50
		Acetamiprid	0.09			Acetamiprid	1.54
		Metalaxyl	0.26			Thiacloprid	0.40
		Azoxystrobin	36.99			Cyproconazole	9.95
		Epoxiconazole	60.57			Azoxystrobin	56.14
		Prochloraz	393.91			Epoxiconazole	53.66
		Tebuconazole	69.11			Tebuconazole	288.49
		Propiconazole	30.65			Prochloraz	4633.08
		Difenoconazol	7.19			Propiconazole	49.04
		Picoxystrobin	1.65			Difenoconazol	136.82
		Pyraclostrobin	25.79			Picoxystrobin	9.76
	KDD3	Carbendazim	1.23	**Sultanköy Dam Lake**	SKD1	Carbendazim	0.44
		Acetamiprid	0.14			Acetamiprid	0.63
		Azoxystrobin	17.24			Imazalil	5.79
		Epoxiconazole	3.38			Azoxystrobin	6.90
		Prochloraz	12.19			Prochloraz	202.90
		Tebuconazole	5.84			Tebuconazole	11.83
		Propiconazole	3.70			Prothioconazole -734	366.09
Süloğlu Dam Lake	SD1	Carbendazim	0.50			Propiconazole	362.54
		Imazalil	0.96		SKD2	Carbendazim	0.14
		Azoxystrobin	4.40			Acetamiprid	0.10
		Prochloraz	13.76			Imazalil	5.08
	SD2	Carbendazim	0.43			Azoxystrobin	4.52
		Imazalil	2.11			Fluquinconazole -699	119.64
		Azoxystrobin	4.18			Tebuconazole	64.69
		Prochloraz	4.99			Prochloraz	1287.10
Kayalıköy Dam Lake	KKD1	Carbendazim	0.49			Prothioconazole -734	196.76
		Imazalil	5.67			Propiconazole	195.43
		Azoxystrobin	3.00	**Kırklareli Dam Lake**	KD1	Dinotefuran	1.38
	KKD2	Carbendazim	0.40			Carbendazim	0.45
		Imazalil	8.00			Imazalil	5.24
		Azoxystrobin	3.48			Azoxystrobin	3.85
		Prochloraz	34.85		KD2	Dinotefuran	1.05
		Propiconazole	4.30			Carbendazim	0.56
	KKD3	Carbendazim	0.92			Imazalil	18.48
		Acetamiprid	0.09			Azoxystrobin	4.91
		Azoxystrobin	7.53		KD3	Dinotefuran	2.05
		Prochloraz	29.76			Carbendazim	0.38
		Propiconazole	4.46			Imazalil	21.93
						Azoxystrobin	28.70

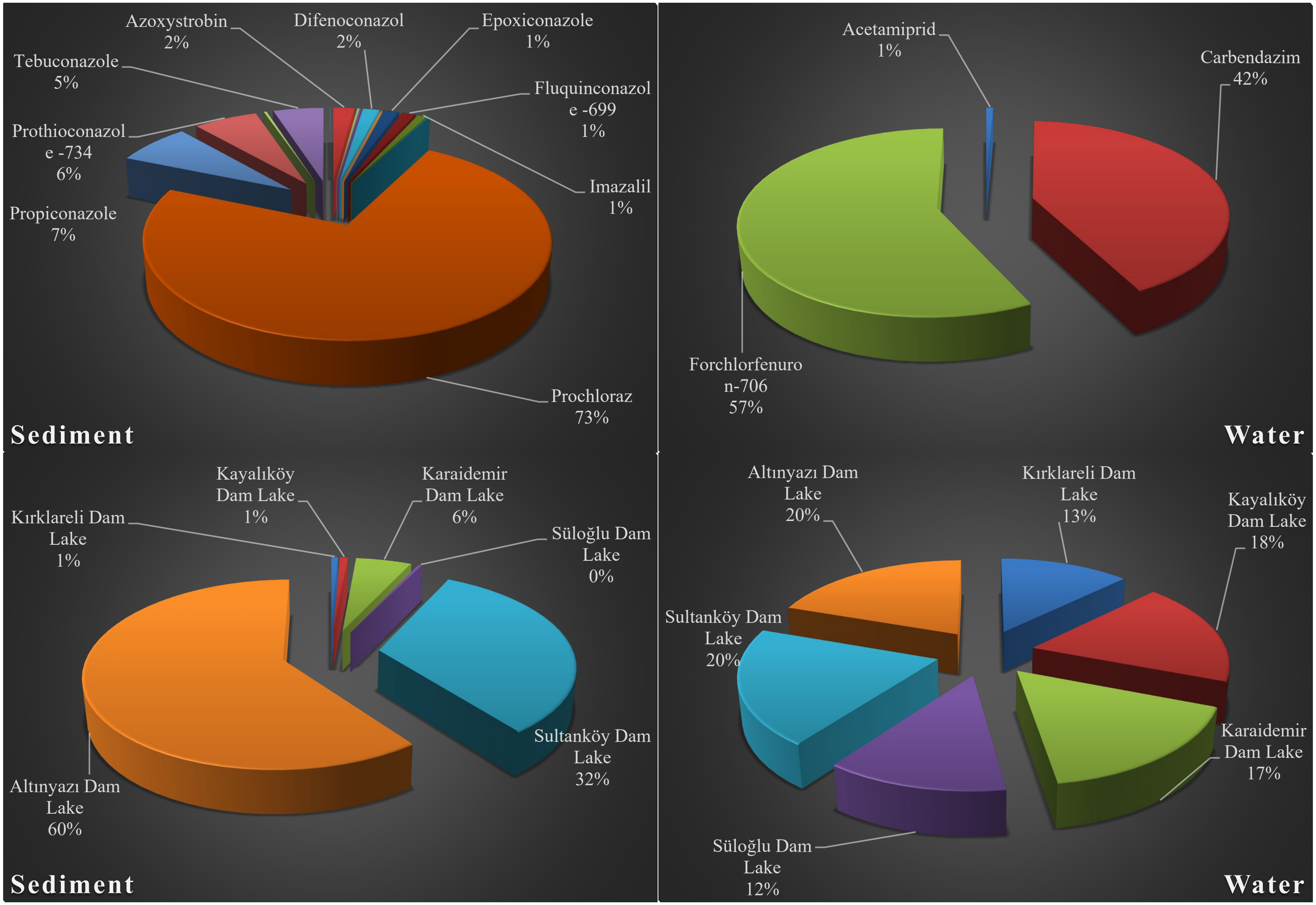

Figure 2. Pesticide rates (upside) and rates of total pesticide residues (downside)

Cluster Analysis (CA), which is an unsupervised multivariate statistical technique, is used to classify the objects into clusters based on their similar characteristics (Belkhiri and Narany 2015, Tiri et al. 2017). In this investigation, CA was used to define the similar groups among the investigated locations according to accumulation levels of pesticides in water and sediment samples.

The diagrams of CA calculated by using pesticide concentration levels in water and sediment are given in Figure 3. According to the results of CA both for water and sediment, a total of 3 clusters were identified as "high contaminated zones", "moderate contaminated zones" and "low contaminated zones". In terms of recorded pesticide residues in water, higher risk cluster was formed by the stations of KKD2, KKD3, KDD2, SKD2 and AD1; moderate risk cluster was formed by the stations of SD1, SD2, SKD1, KD1, KD2, KD3, KDD1 and KDD3; lower risk cluster was formed by the stations of KKD1 and AD2. In terms of recorded pesticide residues in sediment, higher risk cluster was formed by the stations of KDD2, SKD1, SKD2 and AD2; moderate risk cluster was formed by the stations of KKD2, KKD3, SD1, SD2, AD1, KDD1 and KDD3; lower risk cluster was formed by the stations of KKD1, KD1, KD2 and KD3.

As a result of this study, it was determined that pesticide concentration levels recorded in the Altınyazı and Sultanköy Dam Lakes, which are located in the downstream of Ergene River Basin, were found to be in quite high levels. A total of 3 pesticide varieties were determined in water and a total of 18 pesticide varieties were determined in sediment. As a result of this research, forchlorfenuron-706 was found as the most common pesticide type in water samples and prochloraz was found as the most common pesticide type in sediment samples (Figure 2). Although the Forchlorfenuron-706 was found almost all the surface waters, it was not found in surface sediments. And although the prochloraz were found almost all the surface sediments, it was not found in surface waters. As it is clearly known that the waters are much more affected by instantaneous discharges, agricultural practices and precipitation than the sediments. Therefore, the sediments are used as a much more useful indicator than the waters in order to detect the long-term effects in aquatic ecosystems (Tokatlı, 2019; Ustaoğlu and Tepe, 2019; Ustaoğlu and Islam, 2020). Although the evaluation of waters is quite practical and widespread in especially periodic aquatic ecosystem assessment studies, use of the data determined in sediment samples in especially single season studies as in the present application is much more useful in terms of reflecting the effects of long-term contamination.

Pesticide contamination in water of reservoirs were found as Sultanköy > Altınyazı > Kırklareli > Süloğlu > Kayalıköy > Karaidemir in terms of dam lakes and forchlorfenuron-706 > carbendazim > acetamiprid in terms of pesticide type. Pesticide contamination in sediment of reservoirs were found as Altınyazı > Sultanköy > Karaidemir > Kayalıköy > Kırklareli> Süloğlu in terms of dam lakes and prochloraz > propiconazole > prothioconazole-734 > tebuconazole > azoxystrobin > difenoconazol > epoxiconazole > fluquinconazole-699 > imazalil > pyraclostrobin > carbendazim > picoxystrobin > cyproconazole > dinotefuran > acetamiprid > thiamethoxam > thiacloprid > metalaxyl in terms of pesticide type (Figure 2).

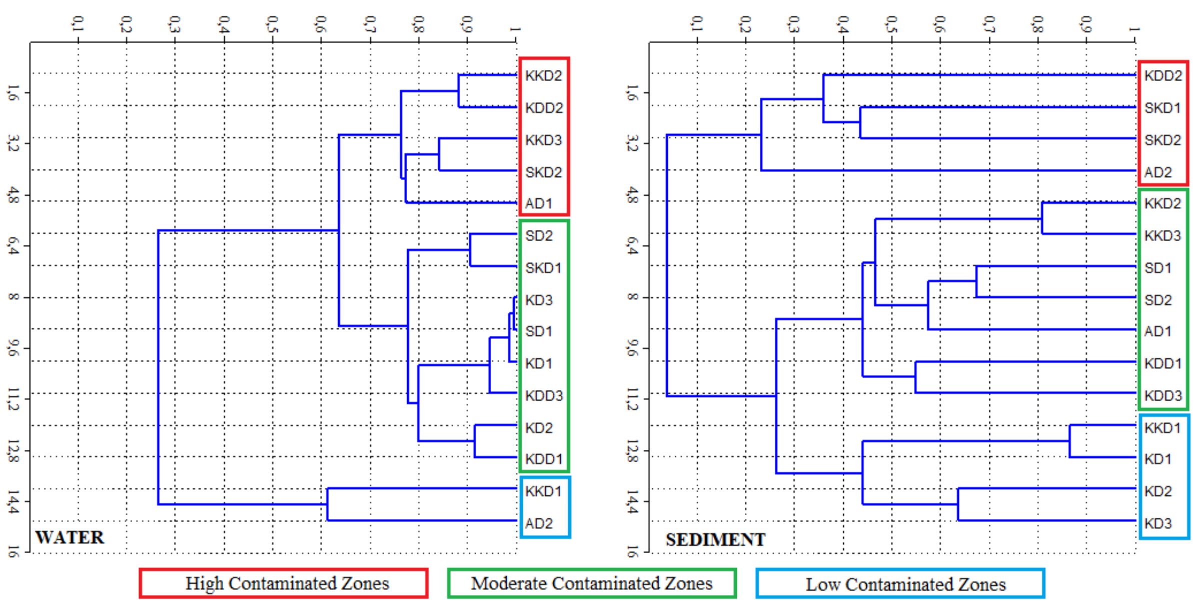

Figure 3. CA diagrams of investigated locations for water (left) and sediment (right)

Although some stations were close to the limit value, it was determined that almost all the investigated stations in dam lakes of Ergene River Basin have I. Class water quality in terms of total pesticide concentrations (Turkish Regulations 2015). The investigated station of KKD1 (Kayalıköy Dam Lake), KDD2 (Karaidemir Dam Lake) and AD1 (Altınyazı Dam Lake) have II. Class water quality in terms of total pesticide concentrations (Turkish Regulations 2015). In a study performed in Thrace Region of Turkey, in contrast to the results of the present study, Meriç Delta was declared as low contaminated area in terms of organochlorine pesticide residues (Erkmen and Kolankaya 2006).

In a study performed in Thrace Region, pesticide accumulations in water and sediment of Meriç River Basin were investigated. According to the results of this research, as similar to the present study, carbendazim was found as the most dominant pesticide type for the system. And Meriç River Basin was declared as III. – IV. Class (polluted – high polluted) in terms of total pesticide accumulations in water (Tokatlı et al., 2020). If we compare the present data with the results of this investigation, it can be clearly understood that the pesticide contamination levels of potamic habitats in Thrace Region are significantly higher than the artificial lacustrine habitats.

A number of studies conducted in different parts of the world, in different habitats and by different researchers have clearly revealed that, pesticides even in trace doses are significant contaminants for natural ecosystem and significant toxicants for all the biological organisms (Ogunfowokan et al. 2012, Masia et al. 2013, Wang et al. 2013). Agricultural activities carried out in the Ergene River Basin have been generally performed in the form of monoculture applications for many years. This situation causes the agricultural pests to have significant resistance gains over time and to increase the amount and quantity of pesticides used by the local producers every year. Especially in the Meriç – İpsala Plain, paddy farming has been going on without leaving fallow the soil and without changing the type of agricultural crop since about 1950-1960. In this study, the highest pesticide accumulations were determined in the Altınyazı and Süloğlu Dam Lakes, which are located on the downstream of Ergene River Basin and in the middle of Meriç – İpsala Plain. This situation causes the agricultural pests to have significant resistance gains over time and to increase the amount and quantity of pesticides used by the local producers every year.

In a few socio-economic and socio-ecological studies conducted in the region, it has been revealed that the environmental sensitivity and environmental awareness of the local people is low and rice producers have performed paddy cultivation for many years (Tokatlı et al. 2013, Tokatlı and Gürbüz 2014, 2015). In another socio-economic study conducted in the region, it has been revealed that many rice producers living in Edirne Province have performed paddy cultivation for more than 30 years (Helvacıoğlu et al. 2015).

The detected data of this study clearly reveals the danger of monoculture agricultural applications around the region. It was also revealed that agricultural runoff is a major contamination source for all the artificial lentic components of the Ergene River Basin and overuse of pesticides may cause significant health problems not only for the ecosystem but also for the local people in the near future.

Conclusions

In this study, pesticide accumulations in water and sediment of Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes, which are located in the Ergene River Basin, were investigated. As a result of this study, agricultural pressure on the abiotic components of the reservoirs was clearly revealed. Altınyazı and Sultanköy Dam Lakes were found to be the most polluted ecosystems among the investigated artificial lentic habitats. Total pesticide contents of waters were found as Sultanköy > Altınyazı > Kırklareli > Süloğlu > Kayalıköy > Karaidemir and total pesticide contents of sediments were found as Altınyazı > Sultanköy > Karaidemir > Kayalıköy > Kırklareli> Süloğlu respectively. Forchlorfenuron-706 (in water) and prochloraz (in sediment) were found to be the most commonly used pesticide variety in the region. Although the reservoirs have I. – II. Class water quality in terms of total pesticide concentrations, in general, pesticide residues in sediments of investigated dam lakes were found to be in quite high levels. Also the applied CA was grouped 15 stations into 3 clusters of similar sediment quality characteristics; "high contaminated zones", "moderate contaminated zones" and “low contaminated zones” both for water and sediment. For the protection and improvement of the quality of these significant lentic ecosystems, monoculture agricultural practices should be changed and the farmers should be encouraged to polyculture applications. Also over use of fertilizers and pesticides should be prevented by providing environmental awareness for local people.

Compliance with Ethical Standard

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

Ethics committee approval: All authors declare that this study does not include any experiments with human or animal subjects.

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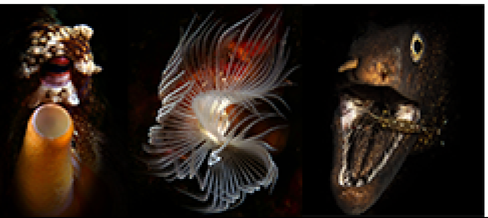

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Determination of serotypic differences of *Lactococcus garvieae* isolates obtained from rainbow trout farms

Şükrü Önalan[1], Muhammet Arabacı[1], Haşmet Çağırgan[2]

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[1] Van Yuzuncu Yil University, Fisheries Faculty, Van, Turkey
[2] Ege University, Fisheries Faculty, İzmir, Turkey

ORCID IDs of the author(s):
Ş.Ö. 0000-0003-0058-5232
M.A. 0000-0002-2462-644
H.Ç. 0000-0003-3038-6154

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Correspondence: Şükrü ÖNALAN
E-mail: sukruonalan@yyu.edu.tr

ABSTRACT

This study aimed to determine the serotypic differences between *Lactococcus garvieae* strains isolated from rainbow trout obtained from different fish farms in Turkey. For this purpose, some phenotypic properties of isolates were determined and then ELISA test was performed to determine serotypic differences. It was determined that all 22 *L. garvieae* isolates used in the study gave a cream colored, bright, rounded smooth S type colony in Triptic Soy Agar (TSA) and all strains were nonmobile in the native examination. Morphologically, all isolates were found to be Gram positive, nonmobile, α-hemolytic, to have a growth of 0-6.5% NaCl salinity, 21, 37 and 45°C temperature, and to be oxidase and catalase negative. After examining the biochemical made with API Rapid ID 32 Strep test, it was observed that two *L. garvieae* isolates were different from the other isolates in terms of sucrose use and one *L. garvieae* isolate was different from other isolates in terms of maltose profile. According to the results of ELISA test used to determine the serotypical differences of the isolates, two *L. garvieae* isolates were serotypically different from the other isolates and *L. garvieae* isolates used in the study formed two different serotypic groups.

Keywords: Rainbow trout, *L. garvieae*, Serotype, ELISA

Introduction

Rainbow trout, is the most cultured fish species in the world, because of its adaptability to environmental conditions, its ability to benefit from natural and artificial feed, and its resistance to diseases (Edwards, 1978).

Bacterial infections have an important place in fish diseases, and in the last decade Gram positive cocci have been identified as important fish pathogens. Many epidemic and sporadic diseases caused by Gram positive pathogens have been reported in various parts of the world (Arda et al., 2002). Japan, Singapore, Australia, Israel, Italy, Spain, France, South Africa and the United States are among the countries affected by outbreaks caused by Gram positive cocci (Eldar et al., 1999). *Lactococcus garvieae*, *Yersinia ruckeri* and *Listonella anguillarum* have been reported to be among the most common pathogens in rainbow trout (Çağırgan, 2009).

Lactococcosis is one of the most serious diseases that causes economic loss among the other diseases caused by Gram positive bacteria. Lactococcosis is a septicemic disease that causes economic loss in many fish species, especially rainbow trout, when the water temperature reaches 15°C in the summer months (Diler et al., 2002; Çağırgan, 2004; Balta and Balta, 2019).

In bacterial fish diseases, vaccination is one of the preventive measures. It is important to obtain and investigate different serotypes of the same bacterial species for vaccination studies. In this way, vaccines with high protection can be developed.

This study aimed to detect serological differences between *L. garvieae* strains identified as pathogens causing economic losses in our country.

Material and Methods

Sampling

240 rainbow trouts (*Oncorhynchus mykiss*) (20-300g) used in the study were taked from 30 active rainbow trout farms registered at the Ministry of Agriculture and Forestry in Van, Bitlis, Muş and Hakkari. While choosing the fish, pools were visited with the owner of the farm and care was taken to choose the fish that showed symptoms of disease. In the study, four different *L. garvieae* were isolated from the rainbow trout farms. 18 different *L. garvieae* isolates which were cultured were added to the study. The study was carried out with a total 22 *L. garvieae* isolates.Sampling studies were carried out in June, July, August and September 2014. For the sampling, a 45-liter capacity container (Rubbermaid) was used.

Phenotypic Determination of Isolates

Bacteria isolation

Bacteriological samples from anterior kidney were streaked onto TSA. Petri plates were incubated at 21°C for 7 days in a cooled incubator. The colonies formed by the bacteria in the medium where reproduction was observed during the incubation period were examined in terms of morphological features such as color, shape and brightness (Austin and Austin, 1999). Gram staining of the growing bacteria (Beşe, 1993), Oxidase (Beşe, 1974) and Catalase (Aydın, 1992) tests were applied. For hemolysis test, 5% sheep blood agar was streaked onto and classified according to zone areas (Buller, 2004).

Salinity tolerance test

To determine the physiological properties of the isolates, the tolerance of different salinity ratios was tested by the method of Konemann (1992). For this purpose, sterile TSA containing 4% and 6.5% NaCI was prepared and inoculated petri plates were incubated at 21°C for 7 days. Isolates were evaluated as positive and non-growth isolates as negative.

Temperature tolerance test

The temperature tolerance tests of the isolates were applied according to Konemann (1992). The bacteria which had been planted in the TSA prepared sterile in order to determine the growth abilities at different temperatures were determined after 7 days incubation at 21, 37 and 45°C. Growth isolates were evaluated as positive and non-growth isolates as negative.

Determination of serotypical differences

ELISA test was used to determine the serotypic properties of *L. garvieae* isolates. ELISA test is carried out using two different methods, direct and indirect ELISA. Since the antigen are obtained from *L. garvieae* isolates and tested with the known antibody, indirect ELISA method was used in the study (Baraketi et al., 2020).

Antigen production

L. garvieae isolates growth in TSB were produced by incubating at Todd Hevith Broth (THB) for 24 hours at 25°C. After adding 5% formaldehyde (Sigma-F8775), it was inactivated by keeping it at 4°C for 24 hours. It was centrifuged at 2500 rpm for 15 minutes (Inovia-Ino 3H). After the bacterial precipitate obtained was washed three times with PBS, antigen density was prepared by adjusting it to 0.6 ±0.010 optical density (OD) at 630 nm and used as antigen in ELISA test (Eyngor et al., 2004).

Immunization of rabbits

The anti *L. garvieae* antibody used in the study was prepared for the rabbit by intravenous inoculation of *L. garvieae* isolate 12. *L. garvieae* isolates were used as antigens in the ELISA test. The antibody from adsorbed isolate 12 was used against other isolates. Adsorption was performed by the method reported by Eyngor et al. (2004).

ELISA test

U-based, 96-well polystyrene ELISA plates (Costar) were used for the ELISA test. All of the antigens obtained from *L. garvieae* isolates were coated with ELISA plates at a determined dilution rate and two parallel. The last two wells were used as negative controls.

The U-based ELISA plate was coated with PBS and with prepared antigen 50 µL to each well with isolate 6 of *L. garvieae* standardized to OD_{630} 0.600 ±0.010. The antigen-coated plate was left overnight at 4°C. Two wells for each antigen were coated with antigen-free diluent. These wells were used as blank. To determine whether there was non-specific binding, control wells were created and the optimum concentration of reagents was determined by preliminary tests (Çağırgan, 2008).

Table 1. Antibody rates used in the study and format in ELISA plates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1:32000 anti *L. garvieae* sera											
B	1:16000 anti *L. garvieae* sera											
C	1:8000 anti *L. garvieae* sera											
D	1:4000 anti *L. garvieae* sera											
E	1:2000 anti *L. garvieae* sera											
F	1:1000 anti *L. garvieae* sera											
G	1:500 anti *L. garvieae* sera											
H	Uncoated Blank											

After washing the ELISA plate coated with antigen three times with PBS (pH 7.4), blocking was done and 100 µL blocking solution (PBS + 1.5% Bovine Serum Albumin) was added to the wells to fill the empty areas in the wells. They were left in the water bath for 1 hour. 1% BSA in 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000 ratios for all eyes coated with antigen (Antigen density OD_{630}: 0,600) after washing with PBS twice for two minutes serum was added, diluted with PBST (0.05% Tween 20 added PBS), and the serum of adsorbed rabbit anti *L. garvieae* with antigen 12 was added. ELISA plates were incubated in a humidified sandwich box in an incubator at 37°C for one hour. To see the effect of adsorption of homologous serum with *L. garvieae*, the antigen-coated non-adsorbed anti *L. garvieae* serum was added to the eyes coated with antigen 12 in the same proportions as in the adsorbed serum. After all the wells were washed three times for two minutes with PBST, 1:20000% BSA(1%) was added, and 50 µL of goat anti-rabbit (KPL, 74-1506) conjugate labeled with PBST was placed and then the plates were incubated at 37°C for one hour. After incubation, the plate was washed four times with PBST for three minutes 100 µL of TMB substrate (Sigma, T0440-100 mL) was added to all wells. After ten minutes of waiting at room temperature, the reaction was stopped by adding 50 µL of 10% sulfuric acid to the wells (Voller et al., 1978). The results were read with an ELISA microplate reader (Versamax, Molecular Devices, USA) at a wavelength of 450 nm.

Evaluation of serotypical differences

Standard deviation (STDDEVP) was calculated (Sümbüloğlu, 1985) and results were evaluated in the 95% (2δ) and 98% (3δ) confidence intervals in order to reveal the differences in absorbance values of the blank value of *L. garvieae* isolates applied in the ELISA test.

Results and Discussion

General Status of Farms

The production capacities of the rainbow trout farms ranged from 1 to 500 tons. During the sample collection season, the air temperature ranged between 19°C and 38°C, and the water temperature in the rainbow trout farms where sampling took place ranged from 13°C to 24°C.

Phenotypic Properties of L. garvieae Isolates

Bacterial colonies obtained after incubation in TSA medium were found to be pin sized, round, bright in appearance, have sharp borders and to form S-type colonies. Then the colonies taken from TSA were incubated at TSB for 24 hours at 21°C. TSA (Figure 1-A) and TSB (Figure 1-B) of *L. garvieae* isolates. After gram staining, it was observed that all of the isolates consisted of Gram positive cocci forming a short L-shaped chain painted with blue-purple color (Figure 1-C). The hemolysis properties of *L. garvieae* isolates obtained in TSA were examined in blood agar. After the 24-hour incubation period at 21°C in the blood agar, it was observed that all the isolates had α-hemolytic properties and there was a light green area around the colonies that developed on the medium (Figure 1-D).

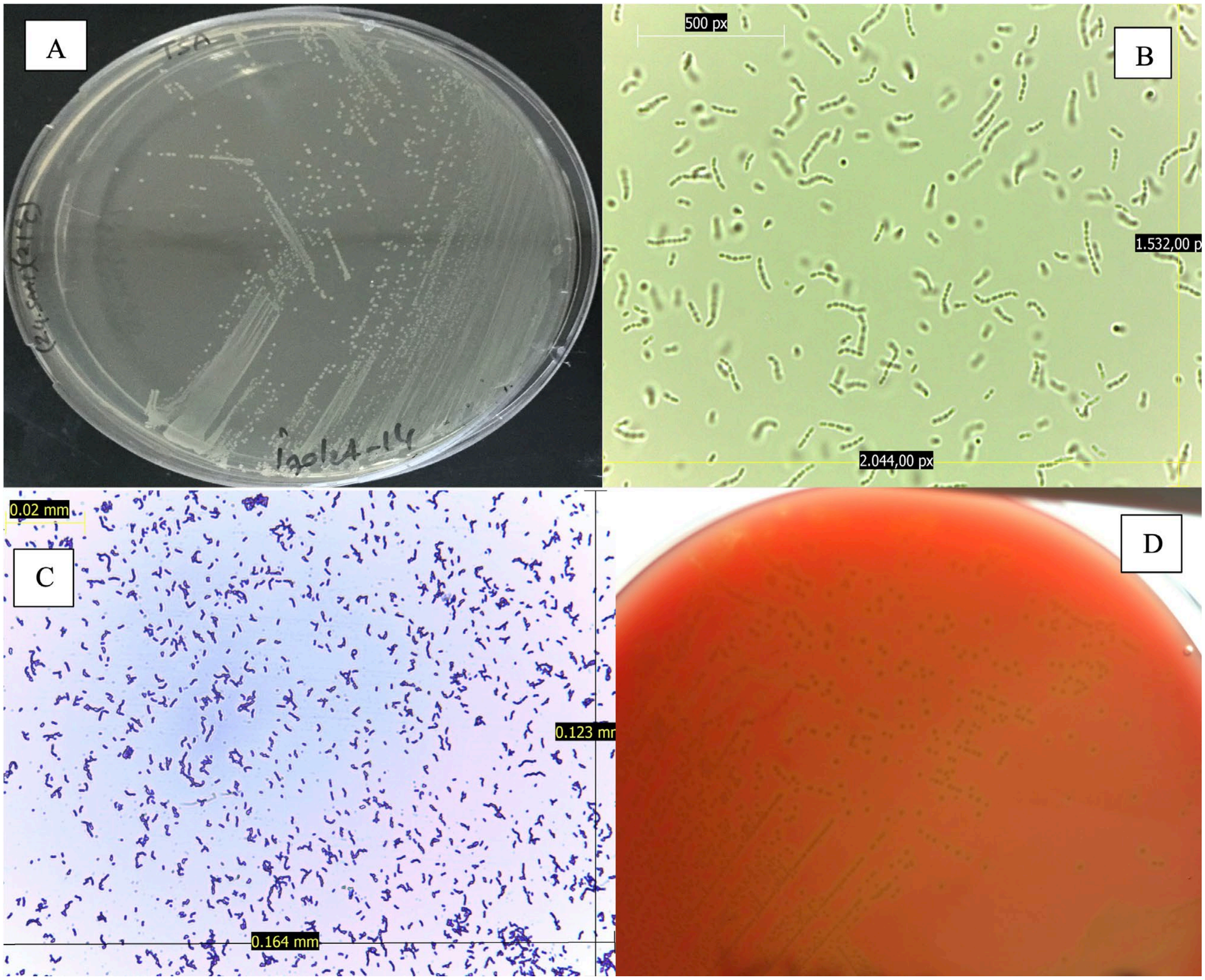

Figure 1. Phenotypic test results of *L. garvieae* isolates (A: TSA colony morphology, B: TSB bacteria type, C: Gram stain result, D: Hemolysis test result).

Salinity Tolerance Test Results

In determining the salinity tolerances of *L. garvieae* isolates, single colonies obtained from the isolates were added to TSA containing 4% and 6.5% NaCl. After the 7-day incubation period at 21°C, all of the isolates showed growth in TSA prepared at 4% and 6.5% salinity (NaCl) rates (Figure 2).

Temperature Tolerance Test Results

The isolates were incubated at temperature ranges of 21°C, 37°C and 45°C. After the incubation period, all of the isolates were observed to grow at 21, 37 and 45°C. Therefore, it was understood that all the isolates used in the study were able to tolerate these temperature ranges (Figure 3).

Evaluation of Serotypical Differences

The differences between the isolates were evaluated according to the adsorbance data obtained after the ELISA test. While determining the serotypical differences between the isolates, standard deviation (STDDEVP) was calculated in order to reveal differences in absorbance values whose Blank value was decreased in ELISA test. The anti *L. garvieae* antibody used in the study was prepared by intravenous inoculation of *L. garvieae* strain no. 12 to the rabbit, and the agglutination titer of the serum obtained was 1:1024. 2 sigma value was deducted from the average of the adsorbance values and the results were evaluated in the excel program. The results were evaluated within the 95% (2δ) and 98% (3δ) confidence intervals. The graphic obtained according to the adsorbance values given by *L. garvieae* isolates after the ELISA test is given below (Figure 4).

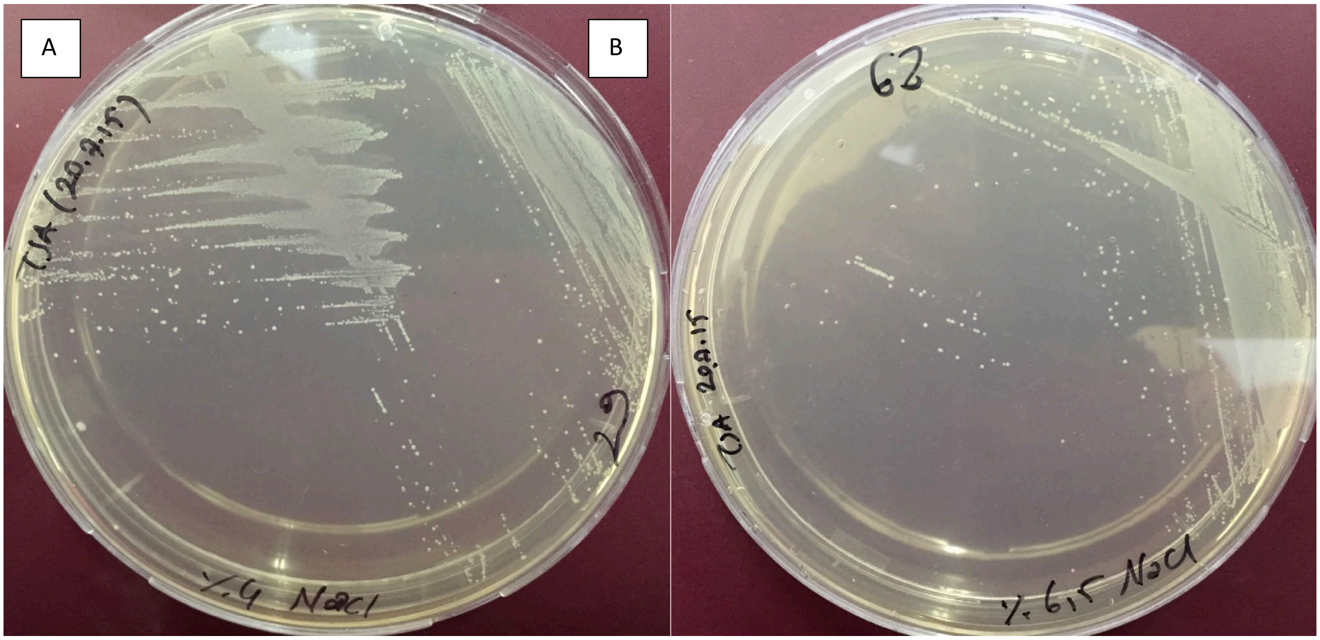

Figure 2. TSA medium image of *L. garvieae* isolates at 4% (A) and 6.5% (B) salinity

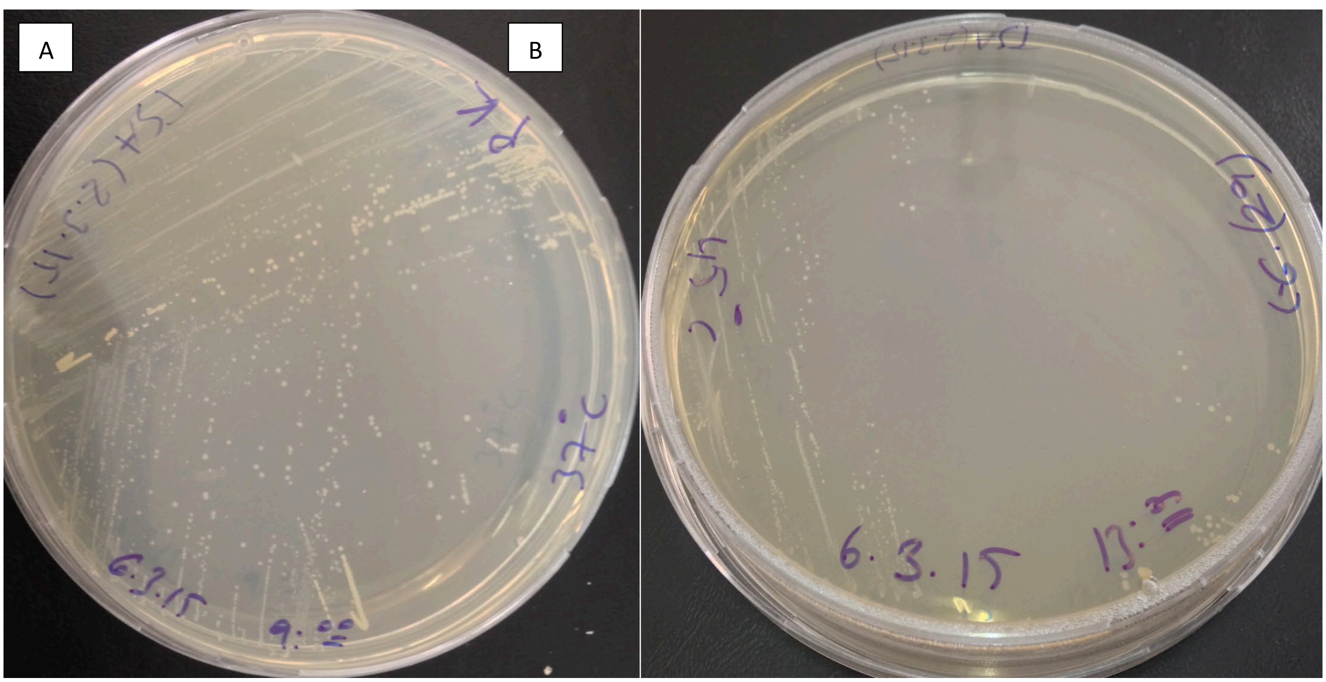

Figure 3. TSA medium image of *L. garvieae* isolates at 37 ºC (A) and 45 ºC (B) temparature.

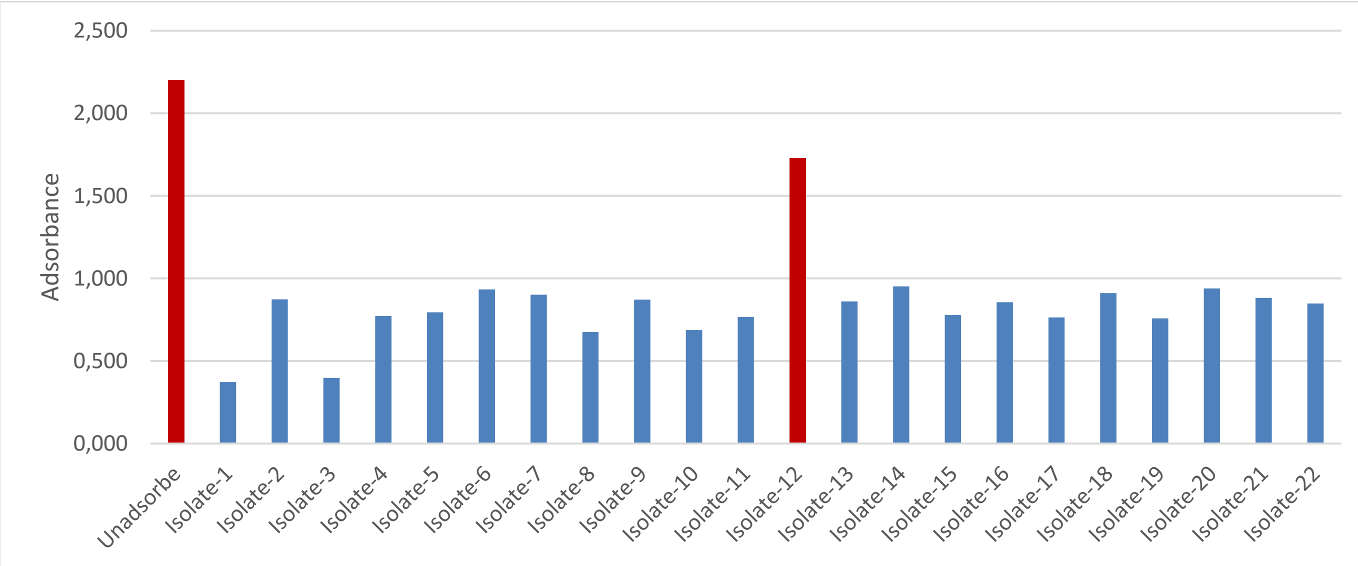

Figure 4. Groups obtained as a result of ELISA test of *L. garvieae* isolates.

According to the results obtained in the Excell program, an antibody prepared against isolate-12 was used as serum and adsorbed with isolate-12. The values obtained when the average was deducted from 2 sigma (0.49>) were evaluated as different serotypes in 95% confidence interval. According to Excel data; isolate-1 and isolate-3 are grouped as serotype-1. Other *L. garvieae* isolates were evaluated as serotype-2 and *L. garvieae* isolates were divided into two different serotypical groups. The serotypic groups obtained after the ELISA test are given below (Table 2).

Table 2. Serotypic groups obtained from *L. garvieae* isolates after ELISA test.

Serotip	***Isolate name***
Serotype 1	Isolate 1 and Isolate 3
Serotype 2	Other 20 isolates

In this study, the sample collection was carried out in the summer, when the water temperature was highest because 14°C water temperature is a critical water temperature for lactococcosis. During sampling, it was observed that the air temperature ranged between 19°C and 38°C, and the water temperature ranged between 13°C and 24°C. Previous studies have reported that infection occurs when the water temperature in lactococcosis exceeds 14-15°C (Ghittino and Muzquiz, 1998; Soltani et al., 2008), and the mortality rate increases when the water temperature exceeds 18°C (Munday et al., 1993; Pereira et al., 2004). It has also been stated that the lactococcosis agent can be isolated from the endophthalmic fluid in the winter months (Savvidis et al., 2007).

Lactococcosis is a bacterial infection that shows specific symptoms in fish and causes large economic loss. Four typical symptoms of lactococcosis have been observed in rainbow trout farms where lactococcosis is seen and isolated. Standing and standing alone on the water surface are symptoms of acidity, bilateral exophthalmos and color darkening.

The immunological techniques used for serotyping studies are based on antigen-antibody interaction. In these methods, specific antibodies can be detected using a known antigen or specific antigens using a known antibody (Altınışık, 2004). The ELISA test is the most sensitive among the methods used in serological studies (Erkan et al., 2011; Ürkü and Timur, 2014).

The ELISA test was used to determine the serotypic characteristics and differences of *L. garvieae* strains used in this study. Previous researchers also used the ELISA method to determine the serotypic properties of *L. garvieae* strains (Voller, 1978; Pozo, 2005; Kav and Erganiş, 2008). Some researchers used the agglutination test for diagnostic purposes (Kitao, 1982; Knappskog et al., 1993). It has been reported that the agglutination test has a disadvantage in terms of cross-reactions and that the ELISA test is fast and economical (Bortz, 1984).

In the study, isolates 19, 20, 21 and 22 were isolated from Van, Bitlis, Muş and Hakkari provinces. The other isolates were previously cultured. Knowing the field data of *L. garvieae* strains (19, 20, 21 and 22) obtained from rainbow trout farms showed importance in terms of evaluating the results obtained in the study. Mortality rates observed in rainbow trout farms where *L. garvieae* agents are isolated are as follows.

Table 3. Field data in rainbow trout farms with symptoms of lactococcosis.

	Water temperature (°C)	*Mortality rate (%)*	*Antibiotic application*	*Vaccine application*
Isolate-19	18-19	5	-	-
Isolate-20	21	< 0,01	-	+
Isolate-21	18,5	2	-	-
Isolate-22	15	2	Enrofloxacin	-

When, Four *L. garvieae* strains (isolates 19, 20, 21 and 22) with known pathogenicity in the field were evaluated together with the field data obtained as a result of the study we obtained the following results: In the four isolates (isolates 19, 20, 21 and 22) that we obtained in the study, deaths started to occur when the water temperature exceeded 14°C in the farm where the isolate 22, which appeared the most pathogenic, was obtained and immediately after the treatment with Enrofloxacin (also compatible with antibiogram results). In the 8 days infection, 2% of the fish died.

In the farm where isolate 20 was obtained, although the fish were not vaccinated, deaths did not start above 14°C water temperature, but when the water temperature exceeded 21°C, only 12 fish out of 500,000 fish were detected and lactococcosis were observed. Rainbow trout farms where isolates 19 and 21 are obtained are located in geographical regions close to each other. It was observed that the water temperature ranged between 18-19°C in both farms. According to the field data of these two rainbow trout farms, lactococcosis was observed to have different mortality rates in both farms where antibiotics and vaccines were not administered. According to field data, the different mortality rates in farms from which isolates 19 and 21 are obtained coincide with the data we obtained in the study.

Conclusions

In summary, according to the results of this study, *L. garvieae* strains 19 and 21 were evaluated as two different types of *L. garvieae* strains due to phenotypic and serotypic differences. While the field data is not known, Turkey isolated from various strains of 18 *L. garvieae* at different times. Therefore, the phenotypic and serotypic differences obtained in this study could not be evaluated together with the field data of these strains.

As a result of the study, it was found that isolating the differences in terms of pathogenicity in the same environmental conditions was important in terms of evaluating the data together. When the results of this study are evaluated together, these results are considered to be important in terms of preventing the damages caused by lactococcosis. The repetition and improvement of the methods used could also be an important step for future studies.

Compliance with Ethical Standard

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

Ethics committee approval: This study was carried out with the permission document obtained by Van Yuzuncu Yıl University, Animal Experts Local Ethics Committee dated 18/11/2013 and numbered 341.

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Multivariance analysis on the distribution of micro-macro elements and their derivates at Meriç River (Thrace Region, Turkey)

Menekşe Taş Divrik[1], Belgin Çamur Elipek[2], Burak Öterler[2], Timur Kırgız[2]

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[1] Sivas Cumhuriyet University, Sarkisla Asık Veysel Vocational School, Sarkisla, 58400, Sivas, Türkey

[2] Trakya University, Faculty of Science, Department of Biology, Hydrobiology Section, 22030, Edirne, Turkey

ORCID IDs of the author(s):
M.T.D. 0000-0002-4828-2575
B.Ç.E. 0000-0002-0954-8967
B.Ö. 0000-0002-9064-1666
T.K. 0000-0001-5967-0509

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Correspondence: Menekşe TAŞ DİVRİK
E-mail: menekse.tas@cumhuriyet.edu.tr

ABSTRACT

Meriç River, called international water, is one of the most important river systems in Thrace. As the river is an open ecosystem to intensive anthropogenic impacts from settlements, agricultural and industrial areas, monitoring its aquatic characteristics is very valuable in terms of maintaining its sustainable use. In particular, knowing the micro and macro element contents that play an important role on primary productivity in aquatic ecosystems will be very useful in predicting the eutrophication process. In this study some chemical analyzes (calcium, magnesium, chloride, nitrate nitrogen, nitrite nitrogen, sulphate, phosphate, cupper, iron, zinc) were carried out to determine the concentrations and distribution of some micro-macro elements and their derivates in Meriç River. Thus, it was aimed to determine the micro and macro element contents of different regions in the river, to compare the data with other studies in the region and to make suggestions on the sustainable use of the river. For this aim, samplings were done selected from eight stations located in Meriç River at Thrace region of Turkey between January and December 2011. Chemical analyzes of Ca, Mg, Cl, NO_3-N, NO_2-N, PO_4, SO_4 in water samples taken from the sampling stations by the Ruttner water sampler at monthly intervals were carried out in the laboratory using classical and spectrophotometric methods. The multivariance analysis (Bray-Curtis Cluster Index) was used to evaluate the similarities of sampling stations in terms of seasonal averages of these parameters. In order to determine the concentrations of some heavy metals (Cu, Fe and Zn), water samples taken by Ruttner sampler and sediment samples taken by Ekman grab at seasonal intervals were measured in flame atomic absorption spectrometry. The sampling stations were evaluated also statistically by using Bray-Curtis Cluster Index in terms of heavy metal contents of water and sediment. According to the result of statistical analysis, it was determined that the locations at lower Meriç River area are different from the upper area. Especially it was observed that the sampling locality after Ergene River added to Meriç River has very low quality level in terms of some chemical contents. It is thought that this may be due to the agricultural and industrial pollution load carried by the Ergene River. Therefore, it has been concluded that these locations must be evaluated in the studies of physicochemical evaluation of Meriç River.

Keywords: Meriç River, Nutrients, Water pollution, Environmental parameters, Multivariance analysis

Introduction

The rapid development of various industries, dense pesticide usage, agricultural activities and uncontrollable anthropogenic deposits affect freshwater sources negatively. Over the past years, the harmful effects of water contamination to ecosystem caused by various anthropogenic activities have been discussed from different perspectives.Water resources are often threatened by industrial wastes, mines, and urban and agricultural pollutants that contain materials other than organic contaminants. While some elements cause eutrophication in aquatic ecosystems, the others like heavy metals can deposited in the sediment. Thus, the usage of water resources becomes limited and the bioaccumulation potential of some heavy metals in aquatic organisms leads to important environmental hazards (Pourkhabbaz, 2018).

Some elements (C, H, O, N, K, P, Mg, S) and their derivatives (nitrite, nitrate, sulphate, phosphate) are necessary for the growth of plants. They are called macro elements and they can enter into aquatic ecosystems in many ways (Çepel, 1996; Bolat & Kara, 2017). Especially nitrogen and phosphorus are necessary for the biochemical cycle but their excessive amounts lead to eutrophication in aquatic ecosystems (Alkan et al., 2013). Also, some elements (Fe, Cl, Cu, Mn, Zn, Mo, B, Ni) are called micro elements. Although these are necessary to aquatic plants, their high concentrations lead to toxic effects (Çepel, 1996; Bolat & Kara, 2017). High concentration levels of micro and macro elements play an important role in pollution of aquatic ecosystems (Webber, 1981; Alkan et al., 2013). Also, some micro elements can accumulate in sediment, lead to deterioration of water quality, and they reach all organisms by the food web (Seven et al., 2018).

The most widely used definition of water quality is that water resources have suitable characteristics on chemical, physical and biological properties for designated usage. Some of the most important chemical characteristics of water are macro and micro elements such as plant nutrients and heavy metals. Plant nutrients such as nitrogen and phosphorus (from agricultural activities such as fertilization and animal feeds) can enter river ecosystems at excessive rates. In addition, industrial and domestic pollutants contribute to the entering of macro and micro elements into the river ecosystem (Thangamalathi & Anuradha, 2018). Inorganic pollutants from these activities are usually substances of mineral origins with metals and their salts (Wong, 2012). Inorganic pollutants as material can be found naturally in ecosystems but anthropogenic activities have been lead to increase their concentrations and numbers in aquatic environments (Thangamalathi & Anuradha, 2018). These inorganic substances also enter the environment through, as well as natural processes, different anthropogenic activities such as mine drainage, smelting, metallurgical and chemical processes and they can be toxic due to the accumulation in the food chains (Salomons et al., 1995; Thangamalathi & Anuradha, 2018).

Meriç River is one of the largest rivers in Turkey. Meriç River Basin known as "Evros River" in Greek and "Maritsa River" in Bulgarian is located in Turkey, Greece and Bulgaria. Major tributaries of Meriç River are the rivers Arda (Bulgaria, Greece and Turkey), Tundja-Tunca (Bulgaria and Turkey), Erithropotamos (Bulgaria and Greece), and Ergene (Turkey). Meriç River is called as "International River" because it forms the border between Turkey and Greece; it is also called the "Transboundary River" because it crosses the border between Bulgaria and Turkey (Yanık, 1997). The water of the river is mostly used for agricultural irrigation in the area, while the delta is suitable for fishing (ORSAM, 2011). Unfortunately the increase of industrial activities and intense agricultural applications due to the rapid developments of urbanization in the area has caused lot contaminants to be added to the river. The Ergene River, which is one of the most important tributaries of the Meriç River, flows through settlements, agricultural and industrial areas to the river before joining (Anonymous, 2012). In this study, concentrations of some micro-macro elements and their derivates along the Meriç River in the Thrace region of Turkey and their local distributions were investigated. For this purpose, while the seasonal averages of Ca, Mg, Cl, NO_3-N, NO_2-N, PO_4, SO_4 analysis in water samples taken from eight locations at monthly intervals in the river were evaluated, heavy metal concentrations (Cu, Fe, Zn) were analysed and evaluated from the samples taken from water and sediment at seasonal intervals from the same locations. While the water and sediment quality levels were evaluated in terms of chemical contents, the obtained results of the chemical analysis were analysed statistically also using multivariance analysis method (Bray-Curtis Cluster Analysis). In addition, the chemical changes in the river over time were evaluated by comparing them with other studies performed in the Meriç River.

Material and Methods

Meriç River starts in Bulgaria and, after forming the Turkey-Greece border it flows in to the Aegean Sea (Figure 1). The Meriç River Basin, including its main tributeries Arda and Tunca that mainly lies in the Bulgarian territory, and Ergene River is added to the basin in the Turkish territory. Meriç River has a drainage area of approximately 52,600 square kilometers (Bulgaria contains 65% of this total area, Greece 87% and Turkey 28%) (UNECE, 2009).

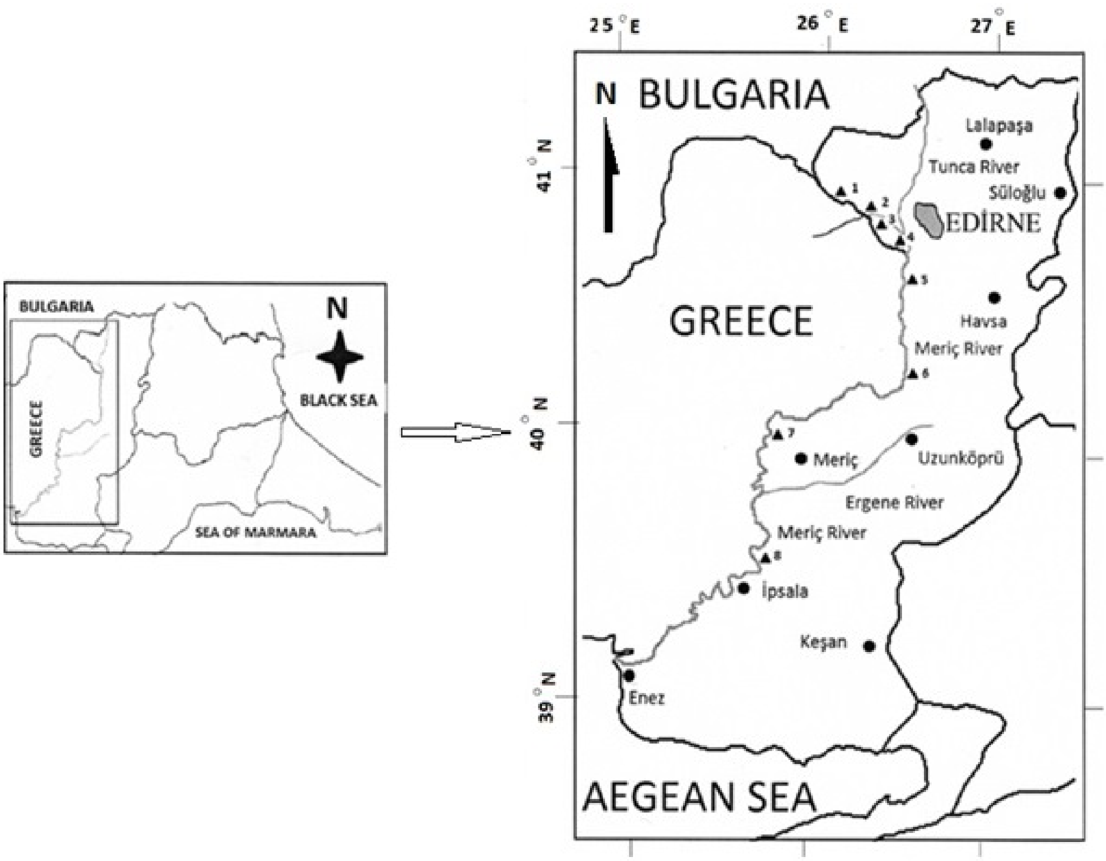

Figure 1. Location of Meriç River and sampling stations

Table 1. Location knowledges of the sampling stations

Station No	Station Knowledge	Coordinates	
1st	Meriç River enters in to Turkey	41º 42' 59" N	26º 22' 15" E
2nd	Some industrial facilities area	41º 41' 17" N	26º 24' 56" E
3rd	Arda River joint Meriç River	41º 39' 89" N	26º 32' 93"E
4th	Tunca River joint Meriç River	41º 37' 57" N	26º 34' 84 "E
5th	Tatarköy Village/Agricultural area	41º 34' 69" N	26º35' 83" E
6th	Saçlımüsellim Village/Agricultural area	41º 25' 17" N	26º 37' 77" E
7th	İpsala/Agricultural area	41º 14' 99" N	26º 21' 29" E
8th	Ergene River joint Meriç River	40º 59' 36" N	26º 20' 77" E

In this study, water samples to determine the concentrations of Ca, Mg, Cl, NO_3-N, NO_2-N, PO_4, SO_4 were taken from a total of 8 stations along Meriç River at monthly intervals between January and December 2011. The obtained values for these chemical parameters were used to calculate the seasonal averages used for statistical analyses. In addition, water and sediment samples to determine the concentrations of heavy metals (Cu, Fe, Zn) were taken from the same stations at seasonal intervals. Location knowledge and features of the sampling stations (coordinate details and explanatory information on selected localities) are presented at *Table 1* and the map of the studied area is shown in Figure 1.

At each station, the water samples were taken by Ruttner sampler and put into polyethylene bottles (2 L) and transported to the laboratory to analyse Ca, Mg, Cl, NO_3-N, NO_2-N, SO_4, PO_4 without delay. The analyses were carried out using classical titrimetric or spectrophotometric methods as proposed by Egemen & Sunlu (1999).

Also, for heavy metal analysis, water samples taken by Ruttner sampler and sediment samples taken by Ekman Grab (15x15 cm^2) were put into sterile polyethylene bottles (100 cc) and transported to the laboratory with the addition of 0.1 N.HNO_3 to reduce pH levels to below 2. The sediment samples were dried at 105°C (24 hours) in the laboratory before analysis. The water and sediment samples for heavy metal analysis were prepared using the techniques from different literatures to analyse by flame atomic absorption spectrophotometer (Perkin-Elmer A-Analyst 800) in laboratorial conditions (Van Loon, 1980; Welz & Sperling 1999; Karataş et al., 2007).

All obtained results were transformed by LogBase10 in Microsoft Office Excel 2003 and SPSS 9.0 for Windows to use statistical techniques (Krebs, 1999). The heavy metal and the other chemical contents of the sampling locations selected in Meriç River were then compared in the programme BioDiversity Pro 2.0 using Bray-Curtis Cluster Analysis (McAleece et al., 1997).

Results and Discussion

In this study, some chemical analyses were carried out to determine the concentrations of some micro-macro elements and their derivates at different locations in Meriç River located in the Thrace region of Turkey.

Although the water samples were taken at monthly intervals from the selected eight stations to analyze the concentrations of calcium, magnesium, chloride, nitrate nitrogen, nitrite nitrogen, sulfate, and phosphate, the seasonal averages of them were evaluated due to their close monthly rates (Table 2). The analyses results of heavy metal concentrations (Cu, Fe, Zn) in the samples of water and sediment taken from the same localities at seasonal intervals are also presented in Table 2.

Hardness is expressed as the total of the concentrations of Ca and Mg ions called macro-elements (Durhasan, 2006). Calcium in freshwater environments can originates from the dissociation of salts such as calcium chloride or calcium sulphate. Most calcium in surface waters comes from streams flowing over limestone, $CaCO_3$, gypsum, $CaSO_4 \cdot 2H_2O$ and other calcium-containing rocks and minerals (APHA, 2005). In this study the average calcium values in water samples ranged between 39-78 mg/L. The highest calcium rates in the water samples were generally observed at the 8th station. Magnesium in fresh water is typically present at concentrations ranging from 10-50 mg/L (Hem, 1992). In this study, it was found that the average magnesium values in water samples ranged between 5.9-38.5 mg/L. In previous studies performed in Meriç River, Kalebaşı (1994) reported between 3.006-5.771 mg/L of calcium, Özkan & Çamur-Elipek (2006) reported min.49 - max.69 mg/L calcium and min.16-max.26 mg/L magnesium.

Although chloride ion is a chemical component that can be found in all natural waters, it is generally observed at low concentrations (Hem, 1992). In this study, it was found that the average chloride values in the water samples ranged between 13.6-139.6 mg/L. The chloride values at the 8th station were observed higher than the other stations in all seasonal averages of the water samples (*Table 1*). In previous studies performed in Meriç River, Kalebaşı (1994) reported min.32 - max.128 mg/L of chloride; Özkan & Çamur-Elipek (2006) reported min.36 - max.57 mg/L of chloride; Altınoluk-Mimiroğlu & Çamur-Elipek (2017) reported min. 9.8 - max.1752.7 mg/L of chloride and Tokatlı (2019a) reported 125 mg/L of chloride. When chloride values increase in freshwater, it is called contaminated water (Klee, 1990). According to the Control Regulation of Water Pollution of Turkey (Anonymous, 2016), it was determined that the chloride values in seasonal averages of water samples exceeded fourth water quality level at the 8th station while it was observed at the second quality level in all other sampling stations. The reason for high chloride amounts found at the 8th station where Ergene River joint the Meriç may be that the water comes from Ergene River located on an area of intense industrial activities. Ergene River was reported as one of the rare river ecosystems that is contaminated from the source area (Tokatlı, 2019b). The increase in chloride values in contaminated waters is also supported by the literature (Klee, 1990).

Table 2. The distribution of micro-macro elements and their derivates in water and sediment samples in the sampling stations.

		1st	2nd	3rd	4th	5th	6th	7th	8th
Spring Season									
Other chemicals	Ca (mg/L)	55.30	53.16	55.57	47.02	53.43	50.76	49.69	60.11
	Mg (mg/L)	5.96	12.74	17.59	15	10.81	11.94	10.97	10.16
	Cl (mg/L)	16.65	17.65	17.65	15.32	25.32	22.99	27.32	91.63
	NO_2-N (mg/L)	0.017	0.017	0.018	0.002	0.023	0.015	0.011	0.150
	NO_3-N (mg/L)	7.678	6.903	8.634	6.350	7.27	5.90	6.756	6.347
	PO_4 (mg/L)	0.068	0.075	0.074	0.045	0.064	0.065	0.050	0.062
	SO_4 (mg/L)	1.511	1.586	1.518	1.389	1.617	1.6	1.41	1.831
Heavy metals	Cu (µg/L)	UAL	UAL	UAL	UAL	UAL	UAL	UAL	UAL
	Fe (µg/L)	152	145	387	95	200	157	77	55
	Zn (µg/L)	122	142	107	125	87	85	100	95
	*Cu (ppm)	UAL	0.46	0.66	1.34	0.14	0.04	2.3	18.2
	*Fe (ppm)	129.54	28.58	56.74	103.52	40.64	34.86	138.74	164.06
	*Zn (ppm)	8.88	3.58	6.84	15.04	5.42	3.78	15.34	7.44
Summer Season									
Other chemicals	Ca (mg/L)	58.24	58.24	56.91	52.63	49.16	48.89	49.69	45.95
	Mg (mg/L)	16.46	19.04	15.32	17.59	22.27	19.37	15.81	24.37
	Cl (mg/L)	23.32	22.32	21.99	18.32	23.99	27.99	27.32	123.8
	NO_2-N (mg/L)	0.016	0.016	0.008	0.0001	0.0001	0.0001	0.0001	0.280
	NO_3-N (mg/L)	3.769	4.587	2.992	1.849	1.886	1.593	0.742	0.897
	PO_4 (mg/L)	0.068	0.064	0.041	0.025	0.036	0.02	0.029	0.058
	SO_4 (mg/L)	1.931	2.007	2.027	1.808	1.965	1.983	2.114	2.401
Heavy metals	Cu (µg/L)	UAL	UAL	UAL	UAL	UAL	UAL	UAL	UAL
	Fe (µg/L)	70	UAL	52	65	147	162	52	42
	Zn (µg/L)	132	30	47	75	90	145	87	112
	*Cu (ppm)	0.5	UAL	UAL	UAL	0.26	UAL	1.62	UAL
	*Fe (ppm)	103.5	51.0	51.1	87.5	115.8	104.2	155.1	155.2
	*Zn (ppm)	6.3	4.26	5.72	11.58	11.4	9.3	13.48	3.1
Autumn Season									
Other chemicals	Ca (mg/L)	70.27	68.93	62.52	57.71	59.58	63.59	55.57	78.28
	Mg (mg/L)	19.69	21.31	14.52	22.91	22.43	38.57	24.53	21.62
	Cl(mg/L)	21.99	24.98	18.99	19.99	26.99	62.31	24.99	139.6
	NO_2-N(mg/L)	0.0001	0.0001	0.0001	0.0001	0.003	0.137	0.005	0.204
	NO_3-N(mg/L)	7.455	4.467	4.801	2.883	3.547	13.35	1.33	3.25
	PO_4 (mg/L)	0.09	0.08	0.06	0.047	0.064	0.059	0.047	0.088
	SO_4 (mg/L)	2.657	2.919	2.122	1.965	2.084	2.086	2.099	3.064
Heavy metals	Cu (µg/L)	UAL	UAL	UAL	UAL	UAL	UAL	UAL	UAL
	Fe (µg/L)	30	UAL	40	UAL	41	3	UAL	60
	Zn (µg/L)	117	135	100	140	115	152	202	127
	*Cu (ppm)	UAL	UAL	UAL	UAL	UAL	UAL	UAL	UAL
	*Fe (ppm)	60.38	26	27.32	58.88	36.96	95.78	41.94	146.54
	*Zn (ppm)	5.92	3.76	3.46	6.14	5.02	8.7	3.12	2.54
Winter Season									
Other chemicals	Ca (mg/L)	54.50	47.55	46.49	39	46.75	48.8	54.77	54.7
	Mg (mg/L)	15.49	17.58	9.03	8.84	11.77	12.90	12.75	15.49
	Cl (mg/L)	22.96	20.62	23.62	13.62	23.29	22.96	29.27	100.28
	NO_2-N(mg/L)	0.017	0.017	0.11	0.0001	0.01	0.003	0.009	0.107
	NO_3-N(mg/L)	10.12	10.18	6.49	2.44	5.76	7.86	19.95	9.04
	PO_4 (mg/L)	0.106	0.10	0.13	0.12	0.12	0.061	0.09	0.08
	SO_4 (mg/L)	1.435	1.499	1.468	0.905	1.371	1.430	1.455	1.647
Heavy metals	Cu (µg/L)	85	47	67	30	92	40	125	150
	Fe (µg/L)	972	692	610	405	625	500	467	675
	Zn (µg/L)	152	215	102	160	460	162	165	152
	*Cu (ppm)	2.28	1.62	0.6	7.52	6.02	1.44	0.88	1.96
	*Fe (ppm)	231.4	106.72	57.02	418.8	321	111.32	103.58	190.44
	*Zn (ppm)	13.12	17.44	5.58	21.1	14.06	9.8	4.48	4.24

*: sediment samples; UAL: Under Analysed Limit

According to the seasonal averages of NO_2-N, NO_3-N, PO_4 and SO_4 ratios in water samples, all sampling stations have changing values, but the concentrations of these parameters have always been high at the 8th station. In this study, it was determined that SO_4, PO_4, NO_3-N values of water have first quality level compared with the values in Anonymous (2016), while NO_2-N values were determined in second and third quality level. In a previous study by Kendirli et al. (2005), nitrogen and phosphorus were reported to be the most important components affecting the water quality of the Ergene and Meriç Rivers. Sulfate in freshwater ecosystems usually comes from rock or soil containing gypsum and other minerals containing sulphate (APHA, 2005). In addition, sulphate can enter aquatic ecosystems by the wastewater discharges of industrial plants and agricultural activities (APHA, 2005). Phosphate compounds can also naturally enter aquatic environments containing rocks. However, anthropogenic contaminants (such as fertilizers, pesticides, detergents and industrial wastes) cause phosphate to enter the running waters (Spellman, 2014). In this study, seasonal average values of SO_4 and PO_4 in the water samples were determined at first class quality level according to the regulation of Anonymous (2016). In previous studies performed in Meriç River, Tokatlı (2015) reported SO_4 between 64.1-120 mg/L; Altınoluk-Mimiroğlu & Çamur-Elipek (2017) reported between 23.68-422.75 mg/L, and Tokatlı (2019a) reported 86 mg/L. In a previous study performed in Meriç River by Tokatlı (2019a) the nitrate values are reported at first quality level while nitrite values are reported at second quality and phosphate values at third quality level. High nitrogen and phosphorus amounts can lead to eutrophication process in aquatic ecosystems and indirectly to change of the other physicochemical features in the water.

Some elements such as Fe, Cu, Zn, Cl, Mn, Mo, B, Ni, called micro elements, are necessary for growth of aquatic plants. Althought Fe and Cu elements are necessary for physiological activities in plant cells, high Cu concentrations are toxic to algae and secondary aquatic plants (Yruela, 2005). In this study, some heavy metal concentrations (Cu, Fe, Zn) in Meriç River were analysed in both the water samples and sediment samples. According to this:

In this study, Fe and Zn in spring season in the Meriç River, Zn in summer and autumn seasons, Cu, Fe and Zn in winter season were determined in the water of all sampling stations (*Table 2*). Fe and Zn values of the water samples were found to have first class quality level (Anonymous, 2016). However, the Cu concentrations in the water samples were observed at winter season only. While Cu values of water samples exceeded second quality level in almost all other sampling stations, it was observed that the rate reached the third water quality level in the 8th station. In the study conducted by Tokatlı (2019a), it was reported that the Cu values in the water samples taken from the Meriç River were found to have first class quality and the Zn values were found to have second class quality. While Fe concentrations in water samples were often observed at first quality level in almost all sampling stations and seasons, it was observed that the rate in the stations exceeded this level at winter season. While Zn concentrations in water samples were often observed at first quality level in almost all sampling stations and seasons, it was observed that the rate in the 5th station reached second level at winter season.

In the sediment samples, Fe and Zn were determined in spring, summer and autumn seasons and Cu, Fe and Zn were determined in winter seasons (*Table 2*). In this study, the concentrations of these heavy metals in the sediment samples were determined at first quality level (MacDonald et al., 2000). Although the Cu concentrations were observed generally the under analyzed limit, they ranged between 0.04 ppm.-18.2 ppm. The observed concentrations of Fe ranged between 26 - 418.8 ppm; the Zn concentrations were observed between min. 2.54 - max. 17.44 ppm. In a previous study performed in the Ergene River Basin including Meriç River, although the concentrations of Cu and Zn were also measured, chromium and cadmium were reported as the most risky element in sediment of the Ergene River Basin (Tokatlı, 2019b). Also, chromium and nickel elements were found to be the most concerning in terms of biological risk within the region (Tokatlı & Baştatlı, 2016; Tokatlı, 2019b).

Because some heavy metals were found at under analysed limit in a lot of locations, the multivariance analysis was applied to the data of heavy metals and the other chemicals, separately. According to the statistical analysis results for the parameters (except heavy metals) in Table 2, the 8th station was determined to be different from the other sampling stations (Figure 2). There was no statistically significant difference among other sampling stations.

Also, the multivariance analysis was used to analize the heavy metal findings (*Figure 3*). It was observed that upper and lower river areas were partially different from each other.

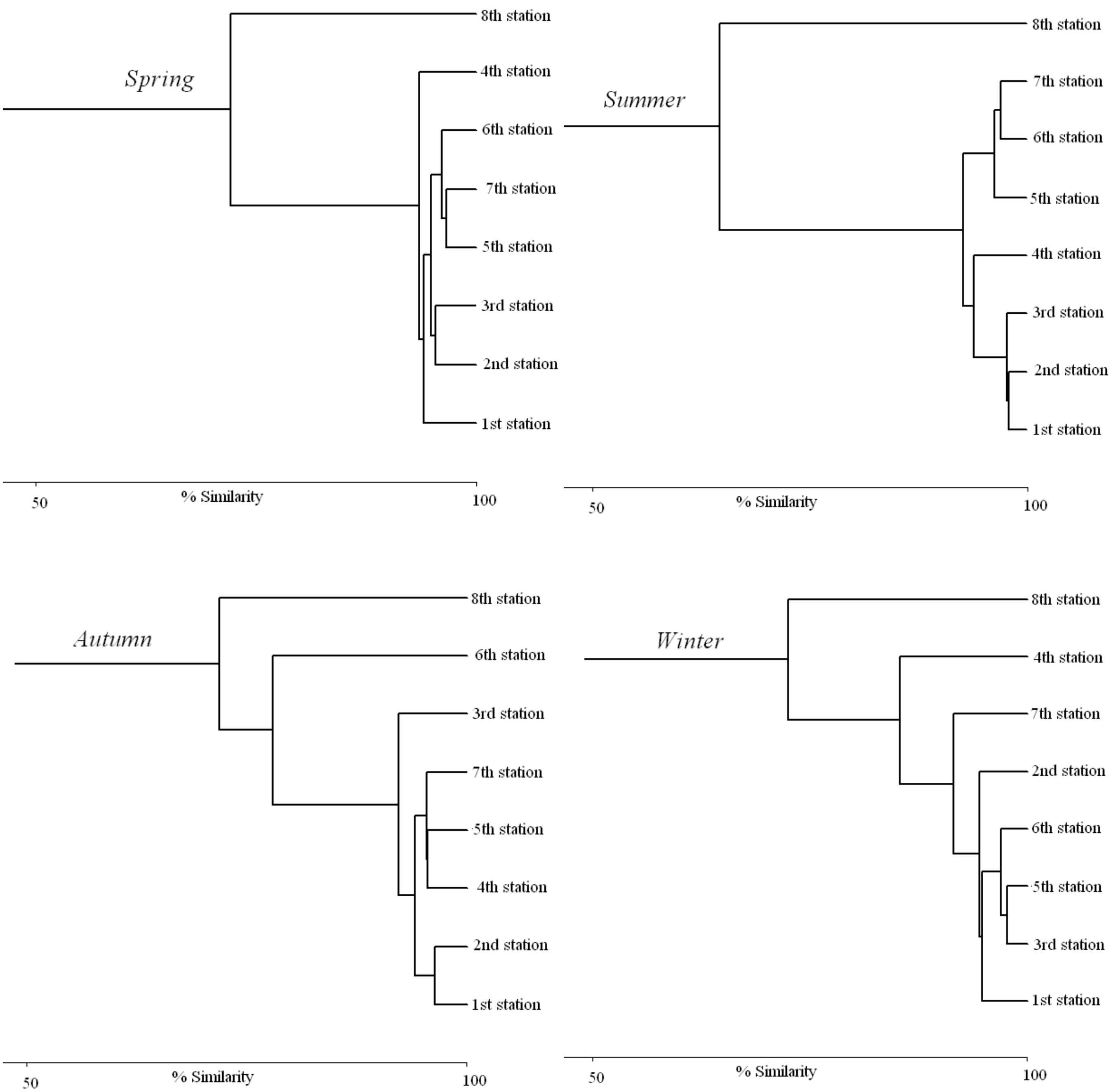

Figure 2. Bray-Curtis Cluster Analyses results of the chemical parameters in water samples**.**

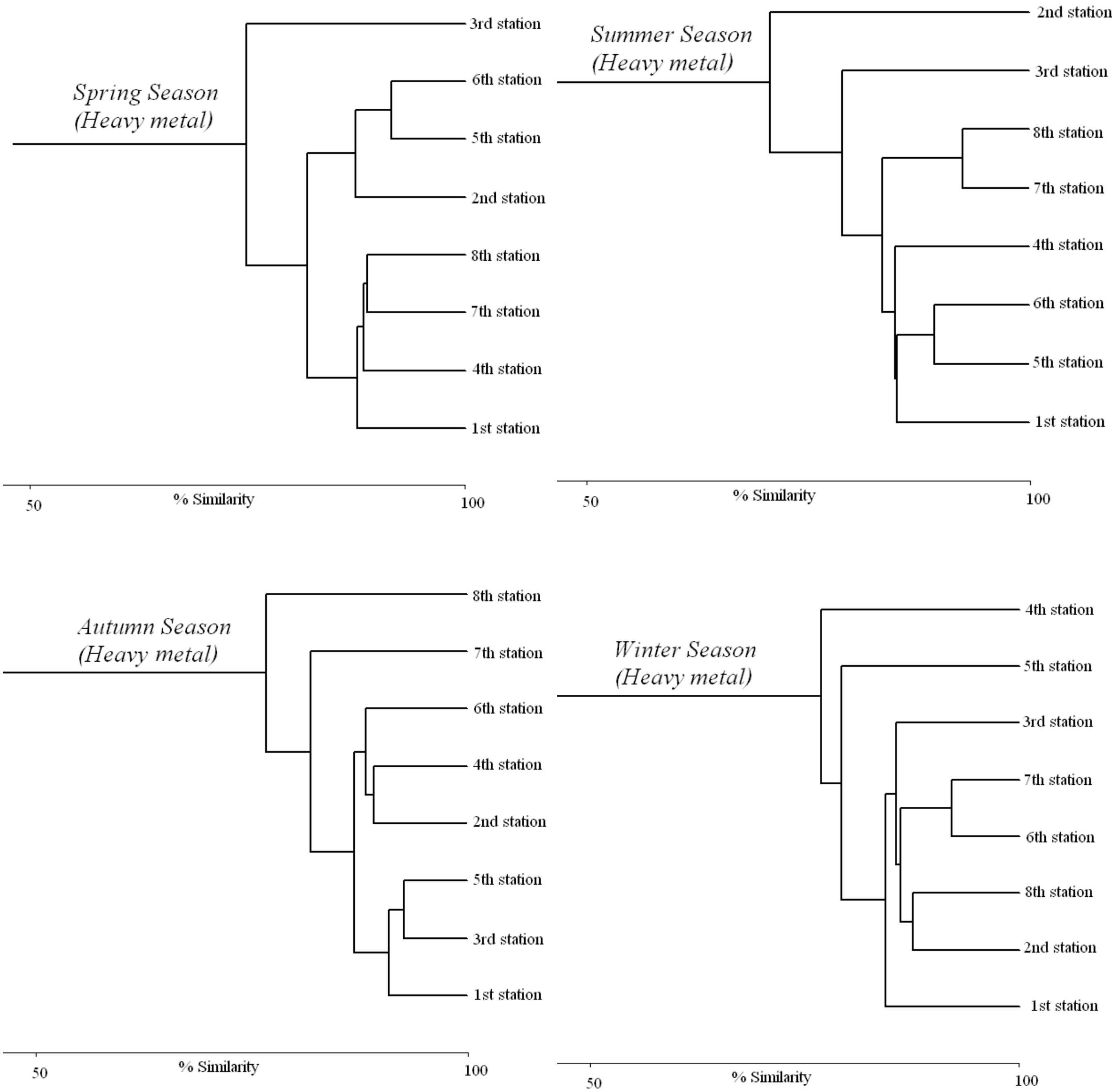

Figure 3. Bray-Curtis Cluster analyses results of the heavy metals in water and sediment samples.

In this study, it was observed that some heavy metal concentrations decreased in summer season. Depending on the chemical structure of the metal, the decrease in the levels of some metals can be caused by the compound formed with other chemicals due to the temperature (Kalyoncu et al., 2016). Also, in this study, the Fe concentrations were determined the most accumulated metal in sediment of the sampling stations. Usero et al. (2003) explained that Fe is abundant in the sediment of aquatic ecosystems because of it being the most abundant metal in the earth's crust. In the summer season, copper was not found in water at some stations (1^{st}, 5^{th} and 7^{th} stations), while it was detected in sediment. While some metals do not detect in water, they can be present in the sediment. This may be related to the sediment particles absorbing the metals in the water and the precipitation of high molecular weight metals to the bottom (Kır et al., 2007). Furthermore, the concentration of heavy metals accumulated in the sediment varies according to the ratio of sediment particles at the bottom, the size of the particles and the presence of organic substances in the sediment (Kır et al., 2007). Our findings in this study support the literature.

Another important risk related to heavy metals is that these substances accumulate in the soil in the long term. One of the most important sources of heavy metal pollution in rivers is soil (sediment) and organic substances mixed with water as a result of soil erosion. Sediment, organic and inorganic substances mixed in the water play an important role in heavy metal amount especially in rainy months (Dökmen, 2000). The reason for the high rate of heavy metal at the 8^{th} station in autumn season can be considered as the mixing of heavy metals in the soil into the Meriç River through the Ergene River.

According to DSI data, while the flow rate of the Ergene river was $2m^3$/sec until 1995, it has been flowing at an average of $8m^3$/sec since 1995 due to the increasing groundwaters of industrial or urban usage (Arabacı et al., 2015). Tokatlı (2015) reports that the water quality of Meriç River has decreased significantly after merging with Ergene River. Altınoluk-Mimiroğlu & Çamur-Elipek (2017) pointed out that Meriç River water quality has fallen to the second quality level after it is merged with Ergene River having fourth quality. It was also reported that Meriç Basin is exposed to intensive inorganic pollution and is under the effect of industrial applications sourced from the Ergene Basin (Tokatlı & Baştatlı, 2016).

Transport, dissolution, precipitation, complex formation, adsorption and bioaccumulation mechanisms of heavy metals in aquatic environments are quite complex processes and are affected by the physicochemical properties of water (Metin-Dereli et al., 2017). In this study, the high amount of heavy metal in some sampling stations and low in the other stations can be explained by this situation.

Conclusion

Rivers are dynamic ecosystems whose physical, chemical and biotic characteristics are greatly affected by anthropogenic activities in drainage basins (Moyaka et al., 2004). Lotic ecosystems often react to external and internal variables (Gecheva & Yurukova, 2013).

In this study, it was found that the upper and lower parts of Meriç River are different from each other in terms of some chemical contents. Although the micro-macro elements and their derivates are necessary for plant growth, high values of them lead to eutrophication in freshwater ecosystems and they have toxic effects to the organisms. In this study, some macro elements and their derivates were found at high concentrations. Therefore, some precautions should be taken for the sustainable use of the river. Consequently, some suggestions are offered:

- the wastewater should not discharged the river without treatment
- fertilizers should be used at appropriate doses in agricultural areas around the river
- basin management should be provided to manage the river ecosystem
- cross-border cooperations should be provided for the management of the Meriç River Basin and be supported by projects
- water analyses in the basin should be done regularly and changes in water quality should be monitored

Compliance with Ethical Standard

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

Ethics committee approval: All authors declare that this study does not include any experiments with human or animal subjects.

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Macroinvertebrates in a high Andean wetland (Chalhuanca) of southern Peru during the dry and wet season

César R. Luque-Fernández[1], G. Anthony Pauca[1], Luis N. Villegas Paredes [1,2], Johana del Pilar Quispe Turpo[3], J. Francisco Villasante Benavides [1,2]

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1 Universidad Nacional de San Agustín de Arequipa, Instituto de Investigación de Ciencia y Gestión Ambiental (ICIGA-UNSA), Calle San Agustín 108, Arequipa, AR, Perú

2 Universidad Nacional de San Agustín de Arequipa, Departamento Académico de Biología, Av. Alcides Carrión s/n, Arequipa, AR, Perú

3 Universidad Nacional de San Agustín de Arequipa, Escuela Profesional de Biología, Av. Alcides Carrión s/n, Arequipa, AR, Perú

ORCID IDs of the author(s):
C.R.L-F. 0000-0001-8050-461X
G.A.P. 0000-0001-6367-5345
L.N.V.P. 0000-0002-7605-4326
J.d.P.Q.T. 0000-0002-6428-7229
J.F.V.B 0000-0002-6577-3122

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Correspondence:
César R. LUQUE-FERNÁNDEZ
E-mail: cluquef@unsa.edu.pe

ABSTRACT

Macroinvertebrates of the Chalhuanca high Andean wetland (bofedal) is presented, which presents two aquatic environments, river and water pools within bofedal vegetation. This wetland is located in the district of Yanque (Caylloma, Arequipa) at 4300 meters, in southern Peru. Aquatic macroinvertebrates in wetlands such as these have been sparsely studied in Peru and other localities, especially in the southern region, which is added to their taxonomic complexity for identification. For this bofedal 32 families were identified, distributed in 21 orders and 12 classes. The richest groups were Diptera, Coleoptera, Trichoptera and Anomopoda. The other groups presented only one family. By type of environment, 25 families were registered for bofedal and 26 for the river, where exclusive families were presented for found environments 05 exclusive families of bofedal (Chydoridae, Coenagrionidae, Ilyocryptidae, Lumbriculidae, Dytiscidae), and 05 exclusive families of river (Gripopterygidae, Hydrobiosidae, Hydrophilidae, Leptoceridae, Saldidae).

Keywords: Bofedal, Benthonic, Aquatic, Peatland, Surber net, Insects

Introduction

High Andean wetlands are fragile ecosystems due to their high vulnerability to climate change and anthropic disturbances (Walker *et al*., 2012). Despite this, through particular and dynamics mechanisms, try to adapt to preserve their functions, structures and interactions, and their socio-ecological attributes, such as climatic, geomorphological, hydrological, biotic and social, which determine their functionality (Walker *et al*., 2012; Andrade *et al*., 2012). The degradation and overexploitation of wetlands implies the loss of their different attributes, and with it, the sustainability of the ecosystem and the ecosystem services they provide (Vidal *et al*., 2013). The monitoring of these arises as a necessity to know their status, within these the monitoring of their waters through the use of aquatic macroinvertebrates are presented as an interesting proposal, since these have been recognized and long used as indicators of the quality of the water (Helawell, 1986; Rosenberg & Resh, 1993; Resh *et al*., 1995, Bunn & Davies, 2000; Allan, 2004) which is widespread throughout the world, however, the composition and knowledge of these may vary and be specific to each site, ecosystems and characteristics associated with them. Thus it is important to have base information on the macroinvertebrates that inhabit this environments, as well as their presence related to a seasonal change, especially in wetlands like these, that in many contribute to the main water basins of the rivers of southern Peru. Therefore, the present study aims to present a checklist of the families of aquatic macroinvertebrates present in the high Andean wetland of Chalhuanca in southern Peru, during the dry and wet season of 2018 as well as a physicochemical description that puts in context our results.

Material and Methods

Study Site

The town of Chalhuanca belongs to the district of Yanque (Caylloma, Arequipa, Peru) located over 4300 meters (15°43'4.12"S; 71°19'13.41"W), corresponding the Andean region, in southern Peru, and is part of the National Reserve of Salinas and Aguada Blanca (SERNANP). In this location the high Andean wetlands can be found, which are locally known as bofedales (onwards), which are a typical form of vegetation of these areas and altitudes, presenting small plants which are prostrate in the soil, mostly leathery and cushion forming, that highly depend on the water regime, the characteristic species of this bofedal are *Distichia muscoides*, *Aciachne pulvinata* and *Phylloscirpus deserticola.* These bofedales cover an approximate extension of 880 ha (Pauca *et al*. in preparation), where it is located one of the most important water dams that are part of the sub-basin of the Chili River. In this bofedal we find a river with the same name of the town, which crosses the bofedal throughout its route and has a variable width. In this ecosystem, two seasons can be distinguished, wet and dry, where the first one occurs between December to March, with long periods of precipitation reaching between 200 and 590 mm (Coaguila *et al*., 2010), and the latter occurs between April and November, presenting the lowest temperatures during the year (around -9°C) (Ramos, 2018), and there may also be rain or snowfall events, as well as the presence of frost events, generally in the months of June and July.

Data Collection

The sampling of aquatic macroinvertebrates was carried out during the dry and wet season of 2018, selecting the two aquatic environments present, bofedales formed by water pools in the middle of the vegetation, and the river. Four monitoring stations were established in each environment, distributing two in the southern region and two in the northern region of the evaluated bofedal (Figure 1). The samples at the water pools stations were obtained through the use of a D-net (MINAM, 2014), with a 500 µm mesh size, with which a sweep of the coastal area ($1m^2$) and center of the water body was performed. And for the river stations, a Surber net (30 x 30cm, 500 µm mesh size) was used, which was placed on the river shore, where the area covered by the net was cleaned by hand (MINAM, 2014), collecting the samples in a 500 bottle ml. For both, water pools and river, triplicate samples were taken at the sampling stations. The specimens were preserved in 5% formalin.

In the laboratory, the samples were processed by separating the large material (vegetation) and subsequently washed and processed by means of a series of sieves (2.3, 1.4, 0.7 and 0.3 mm), and finally preserved in 70% ethanol. The identification was carried out by microstereoscope or microscope according to need, the identification was carried out to the family level, for which the guidelines of: Roldan (1996), Heckman (2006, 2008), Merrit *et al*. (2008), Borkent and Spinelli (2007), Domínguez and Fernández (2009), Huamantinco and Ortiz (2010), Noreña *et al*. (2015), and the nomenclature was followed The World Register of Marine Species (WORMS, 2019). Finally, for the analysis of the composition of families between sampling stations and seasons, a cluster analysis of similarity based on presence and absence was used through the Jaccard index with PAST 3.25 software.

Additionally, at each station, physicochemical parameters of the water were measured during the evaluation season, considering temperature, pH, conductivity, dissolved oxygen, oxygen saturation and total dissolved solids (TDS). These were recorded with a portable multiparameter (Hanna HI 9829).

Figure 1. Location map of water pools within the Chalhuanca bofedal and river (Arequipa, Peru) selected for this study.

Results and Discussion

In total, 32 families were identified in the Chalhuanca River and water pools within the bofedal (supplementary material), distributed in 21 orders and 12 classes (Table 1). The most diverse orders correspond to *Diptera*, *Coleoptera*, *Trichoptera* and *Anomopoda*, where the remaining orders were represented by a single family only. In general, the diversity of aquatic macroinvertebrates in high-Andean ecosystems, such as wetlands, has been poorly studied (Molina et al., 2008; Nieto et al., 2016; Gomez, 2016; Oyague & Maldonado, 2014), the southern Peruvian wetlands being the least researched. The reason for this could be the difficulty in the taxonomic determination of macroinvertebrates (Jacobsen et al., 2008). However, it is necessary to know these inventories as it would help to understand the diversity associated with these ecosystems. On the other hand, compared against other similar researched wetlands (Oyague & Maldonado, 2014; Canchapoma et al., 2016), these results showed a low richness at the family-level assessment in the Chalhuanca bofedal. Likewise, among the theories of diversity distribution, in the case of macroinvertebrates, it is stated that diversity usually decreased at higher altitudes. (Jacobsen et al., 2008; Molina et al., 2008).

Table 1. Macroinvertebrate families present in the bofedal and Chalhuanca River (Arequipa, Peru), during the dry and wet season of 2018

Order	Family	Wet								Dry						
		Water pools				River				Water pools			River			
		S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S4	S1	S2	S3	S4
Oribatida	Limnozetidae	x	x	x	x			x	x			x				
Trombidiformes	Limnesidae	x	x	x	x					x	x	x	x	x	x	x
Sphaeriida	Sphaeriidae	x	x	x	x	x	x	x				x				
Anomopoda	Chydoridae	x	x	x	x					x	x	x				
	Daphniidae	x	x						x							
	Ilyocryptidae	x	x	x	x							x				
Lumbriculida	Lumbriculidae	x	x	x	x											
Rhynchobdellida	Glossiphoniidae	x	x	x	x	x		x			x	x	x			
Dorylaimida	Longidoridae	x	x	x	x		x	x		x	x	x	x	x	x	x
Basommatophora	Planorbidae	x	x			x	x	x		x		x	x	x		x
Cyclopoida	Cyclopidae	x	x	x	x					x		x	x			
Anthoathecata	Hydridae						x			x						
Coleoptera	Dytiscidae									x		x				
	Elmidae	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Hydrophilidae							x	x							
Diptera	Ceratopogonidae											x	x	x	x	x
	Chironomidae	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Ephydridae											x				x
	Simuliidae							x	x			x			x	x
Ephemeroptera	Baetidae	x	x	x	x	x	x	x	x			x		x	x	x
Hemiptera	Corixidae	x	x	x	x	x	x	x	x	x		x	x		x	
	Saldidae					x										
Odonata	Coenagrionidae											x				
Plecoptera	Gripopterygidae					x	x	x	x						x	
Trichoptera	Hydrobiosidae													x		
	Hydroptilidae	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Leptoceridae					x		x	x							
	Limnephilidae				x				x			x				
Amphipoda	Hyalellidae	x	x	x	x	x	x	x	x	x		x	x	x		x
Podocopida	Cyprididae	x	x	x	x	x	x	x	x	x		x	x		x	
Tricladida	Dugesidae									x		x	x			x
Oligochaeta	Aelosomatidae					x	x	x	x	x		x	x	x	x	x

Water Pools VS River

Regarding the assessed environments, a total of 25 families were recorded for the water pools and 26 for the river. Out of them, five families were bofedal-exclusive (*Chydoridae*, *Coenagrionidae*, *Ilyocryptidae*, *Lumbriculidae*, *Dytiscidae*) and five were river-exclusive (*Gripopterygidae*, *Hydrobiosidae*, *Hydrophilidae*, *Leptoceridae*, *Saldidae*).

In terms of the richness of the families by seasons, similarities were found both in the dry and the wet seasons (25 families), but only five exclusive families were found during the wet season (*Daphniidae*, *Hydrophilidae*, *Leptoceridae*, *Lumbriculidae*, *Saldidae*) and five exclusive families during the dry season (*Ceratopogonidae*, *Coenagrionidae*, *Saldidae*, *Ephydridae*, *Hydrobiosidae*).

As for the similarity in the sampling stations (Figure 2), there was a greater similarity between the dry and wet seasons than between the assessed environments, where the bofedal stations assessed during the wet season were the most similar to each other (> 0,85). On the other hand, the DB2 station was different from all the others, as it presented the lowest richness among families (7) compared to the other stations, which presented an average of 14 families.

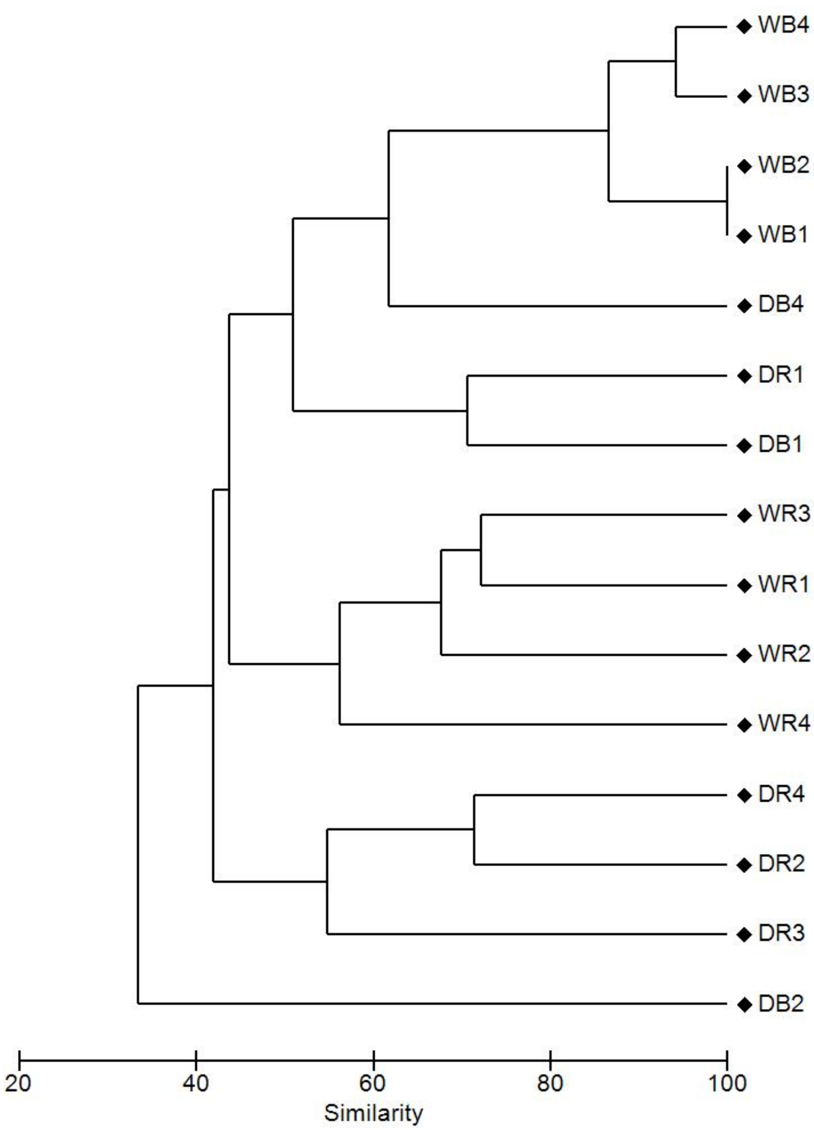


Figure 2. Similarity cluster based on the Jaccard Index for sampling stations and seasons, where the first letter corresponds to the season (W = wet, D = dry) and the second letter to the assessed habitat (B = bofedal, R = river).

On the absence of differences in the number of families found in the research stations and environments, this is due to the great capacity of these organisms to adapt to the conditions presented, which has been widely documented (Molina et al., 2008; Domínguez & Fernández 2009; Oyague & Maldonado, 2014). On the other hand, the bofedal environment would be expected to present the greatest aquatic macroinvertebrate richness because it provides greater places of refuge, both in the substrate and in the vegetation that compose it for macroinvertebrates. This could also be supported by the identification at a more specific taxonomic level, where these differences could have been shown (Moya et al., 2009; Nieto et al., 2016) compared to the Chalhuanca River environment.

On the presence of the families found in this study, many of them (*Baetidae*, *Elmidae*, *Simuliidae*, *Chironomidae*, *Gripopterygidae*, *Hyalellidae*) correspond to what was found in other high-Andean water ecosystems studied, which is mentioned by Nieto et al. (2016), who studied the patterns of aquatic macroinvertebrate communities in the Argentine *puna*.

As for the exclusivity of some families found in the water pools environment, this would be correlated with the heterogeneity of the bofedal and the physicochemical conditions of the water bodies forming within them, as well as the associated vegetation (Oyague & Maldonado, 2014). However, several of the families found exclusively in this environment can also be found in lotic environments (Roldan, 1996; Dominguez & Fernandez, 2009), except for individuals of the *Dysticidae* family that are more associated with slow environments with fallen leaves and vegetation (Dominguez & Fernandez, 2009).

In the case of the river environment, the *Plecoptera* and *Trichoptera* were exclusive groups, which, according to bibliography (Roldan, 1996), are usually more associated with areas of cold, fast and well-oxygenated waters, with special relevance in rivers with rocky bottoms located along 2000 meters above sea level. In addition, despite there are records of individuals from the *Hydrophilidae*, *Saldidae* and *Dytiscidae* families, little information is available about the species that make them up, especially for South America (Roldan, 1996), where even the *Dytiscidae* taxa have been considered of interest due to their rarity (Ansaloni et al., 2016).

Physical-Chemical Characterization

The physicochemical data are shown in Table 2, where the temperature of the water bodies varies between 6°C and 23°C, and the temperature within the bofedal is higher than that recorded in the river. The pH presented neutral values that varied between 7.3 - 8.4. During the dry season, the pH values in the bofedal were lower than in the river, while during the wet season they were higher, with the exception of the first station (S1). The conductivity varied between 40 and 70 $\mu S\ cm^{-1}$, presenting a constant between seasons (wet and dry) and the assessed sampling stations, as well as TDS values. As to dissolved oxygen, the values were between 3 ppm and 7 ppm, where the dry season showed higher values, similar to those present in oxygen saturation that occurred between 60% and 90%.

Table 2. Physicochemical parameters in the bofedal and Chalhuanca River (Arequipa, Peru), during the dry and wet season of 2018

Parameter	wet								dry						
	bofedal				river				bofedal			river			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S4	S1	S2	S3	S4
Temperature (°C)	14.07	18.32	18.25	22.81	9.95	14.39	14.47	16.52	9.97	13.04	11.74	5.68	12.29	13.58	13.08
pH	7.65	8.45	7.79	7.89	7.98	7.55	7.33	7.35	7.57	7.50	7.58	7.38	7.90	7.87	7.48
Conductivity(µS/cm)	65.27	44.44	48.89	64.25	51.67	48.86	49.00	44.14	55.50	47.00	19.50	62.50	46.50	50.00	50.00
Dissolved oxygen (ppm)	3.60	4.07	4.15	2.92	3.70	3.83	3.29	3.24	6.63	5.08	4.14	5.43	5.10	4.67	4.46
Dissolved oxygen saturation (%)	67.73	80.54	83.92	66.23	63.18	67.33	62.50	64.43	72.05	79.05	62.25	73.60	80.00	70.80	68.50
TDS (ppm)	32.55	22.22	24.67	32.00	25.83	24.43	24.60	22.14	28.00	23.50	10.50	31.50	21.00	25.00	25.00

Based on the values of the obtained physicochemical parameters, these exhibit acceptable ranges for water, according to with the Water Quality Standards of the Peruvian Law (ECA, as per its initials in Spanish). Likewise, the obtained values are similar to the values of other assessed wetlands in Peru (Oyague & Maldonado, 2014; Sulca et al., 2017) and Bolivia (Coronel et al., 2009; Molina et al., 2008; Loza et al., 2015), except for the dissolved oxygen values that were lower for the Chalhuanca wetlands, especially during the wet season.

Furthermore, as already known, macroinvertebrates have been used as water-quality bioindicators (Hellawell, 1986; Metcalfe-Smith, 1994; Bonada et al., 2006; Roldán-Perez, 2016), where some of the families found in this study (*Gripopterygidae*, *Hidrobiosidae*, *Limnephilidae*, *Leptoceridae*) would characterize the waters of the studied environments, from acceptable to regular conditions, compared to the scales of the Biological Monitoring Working Group (BMWP) and the Andean Biotic Index (ABI) (Armitage et al., 1983; Acosta et al., 2009; Ríos-Touma et al., 2014), for this study, the highest accumulated scores of macroinvertebrates were present in the river, this occurred for both seasons (47.88 ±13.16) in comparison to the wetland pools (36.71 ±13.03), As for the average score per taxon, there were no differences for the environments and seasons where the values were around 4.62 ±0.55.

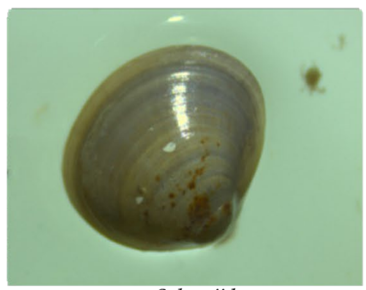
Conclusion

In conclusion, this study found 32 families of macroinvertebrates, which evaluated together with the physicochemical and biological parameters would qualify this bofedal as in acceptable conditions. It is noted that even assessing at the family level, the knowledge about biodiversity in the aquatic macroinvertebrate community in high-Andean systems is quite significant, regarding their fragility, even more so when they are part of a protected natural area like National Reserve of Salinas and Aguada Blanca in Peru. Moreover, since they serve as indicators of water condition or the impacts that water bodies would be suffering, whether they are natural changes or disturbances caused by human intervention, the data presented here could serve as a baseline for future monitoring of water quality changes across time.

Supplementary material: Showing the individuals belonging to the macroinvertebrates families present in the high Andean wetland of Chalhuanca (Arequipa, Peru)

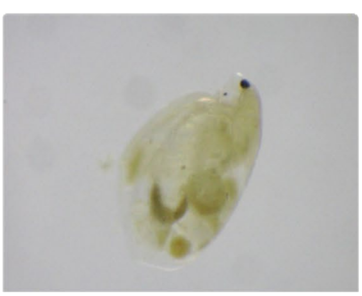
01 *Limnozetidae* ORIBATIDA ARACHNIDA

02 *Limnesiidae* TROMBIDIFORMES AR ACHNIDA

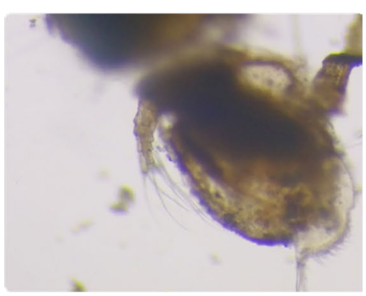


03 *Sphaeriidae* SPHAERIIDA BIVALVIA

04 *Chydoridae* ANOMOPODA BRANCHIOPODA



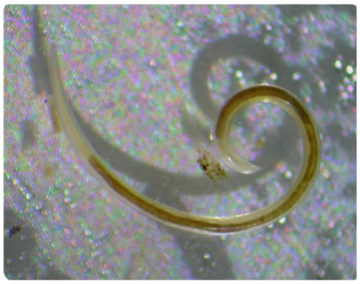
04 *Daphniidae* ANOMOPODA BRANCHIOPODA



06 *Ilyocryptidae* ANOMOPODA BRANCHIOPODA

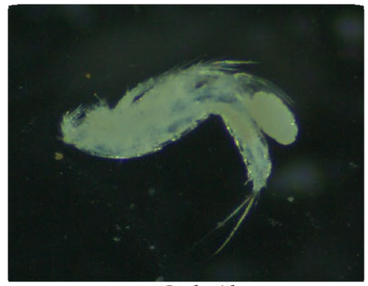
07 *Lumbriculidae* LUMBRICULIDA CLITELLATA

08 *Glossiphoniidae* RHYNCHOBDELLIDA CLITELLATA



08 *Longidoridae*
DORYLAIMIDA
ENOPLEA

10 *Planorbidae*
BASOMMATOPHORA
GASTROPODA

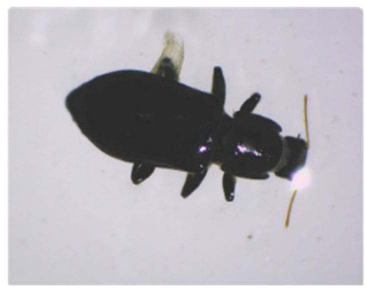


11 *Cyclopidae*
CYCLOPOIDA
HEXANAUPLIA

12 *Hydridae*
ANTHOATHECATA
HYDROZOA

12 *Dytiscidae*
COLEOPTERA
INSECTA

14 *Elmidae*
COLEOPTERA
INSECTA



15 *Elmidae*
COLEOPTERA
INSECTA

16 *Hydrophilidae*
COLEOPTERA
INSECTA

17 *Ceratopogonidae*
DIPTERA
INSECTA

18 *Chironomidae*
DIPTERA
INSECTA

19 *Ephydridae*
DIPTERA
INSECTA

20 *Simuliidae*
DIPTERA
INSECTA

21 *Baetidae*
EPHEMEROPTERA

22 *Corixidae*
HEMIPTERA

23 *Saldidae*
HEMIPTERA

24 *Coenagrionidae*
ODONATA

24 *Gripopterygidae*
PLECOPTERA

25 *Hidrobiosidae*
TRICHOPTERA

27 *Hydroptilidae*
TRICHOPTERA

28 *Hydroptilidae*
TRICHOPTERA

29 *Limnephilidae*
TRICHOPTERA

30 *Hyalellidae*
AMPHIPODA
MALACOSTRACA

31 *Cyprididae*
PODOCOPIDA
OSTRACODA

32 *Dugesiidae*
TRICLADIDA
TURBELLARIA

Chalhuanca's Bofedal, Caylloma, Arequipa-Peru

Compliance with Ethical Standard

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

Ethics committee approval: This study was conducted according to the ethics committee procedures and the evaluations were carried out under research authorization provided by the R.N. of Salinas and Aguada Blanca-SERNANP (Resolución Jefatural N°-002-2018 SERNANP-DGANP- JEF).

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Effects of intermittent feeding regimes on growth performance and economic benefits of Amur catfish (*Silurus asotus*)

Gladys Mwaka Holeh[1,2], Patrick Appenteng[1], Mary A. Opiyo[3], Jeonghwan Park[1], Christopher Lyon Brown[1]

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[1.] World Fisheries University Pilot Programme Pukyong National University (PKNU), 45 Ongso-ro, Nam-gu, Busan 48513, South Korea

[2.] Kenya Marine & Fisheries Research Institute, P.O. Box 81651-80100, Mombasa, Kenya

[3.] Kenya Marine & Fisheries Research Institute, National Aquaculture Research Development & Training Center, P.O. Box 451-10230, Sagana, Kenya

ORCID IDs of the author(s):
G.M.H. 0000-0002-7103-9485
P.A. 0000-0002-8335-2516
M.A.O. 0000-0001-9660-5888
J.P. 0000-0002-6524-3383
C.L.B. 0000-0002-4578-3593

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Correspondence:
Gladys Mwaka HOLEH
E-mail: holegladys@gmail.com

ABSTRACT

A study was conducted to determine the growth performance and economic viability of culturing Amur Catfish (*Silurus asotus*) using four different feeding regimes: Every-day feeding (EDF), Every two-days feeding (ETDF), Tertian feeding (TF) and Quartan feeding (QF) for 65 days. Twenty fingerlings of sizes between 2-3 grams each were randomly distributed in 12 glass aquaria and assigned to each of the feeding regime in triplicates. Fish were fed on commercial feed (Woosung feed) containing 50% crude protein. Highest specific growth rates (SGR) (5.15 ±0.06%) was recorded in EDF with significance differences in all treatments ($P > 0.05$). Feed conversion ratio (FCR) was significantly lower in TF ($P < 0.05$) while survival rate ranged from 83.33% to 96.67% and was not significantly different among the treatments ($P > 0.05$). The length-weight relationship (LWR) analysis indicated that the regression slope *b* values were not significantly different ($P > 0.05$) among the treatments. Partial enterprise budget analysis of *S. asotus* using different feeding regimes indicated that net returns above total costs were significantly higher in EDF ($P < 0.05$). This shows that every day feeding to satiation is the best feeding regime to be adopted for economic benefits of rearing Amur catfish.

Keywords: Amur catfish, Feeding regimes, Alternate feeding, Bio-economy, Growth

Introduction

Fish feed is among the most critically important factors influencing the ability of cultured fish to grow profitably in a fish farm (Ereiegha, 2018). Rising feed costs increase production costs, often threatening the economic viability of fish farming. According to Yusuf & Buhari (2016), some farmers have abandoned production because Catfish prices were not sufficient to offset the cost of production, which had gone up from U$ 1 to U$ 1.40 per kg due to the increase in cost of feed. Earlier studies reported that feeds play a significant role in effective fish production and its profitability, since almost 40-60% of the total cost for fish production is covered by the feed expenses (Craig and Helfrich (2002), Jamu and Ayinla (2003). Fishmeal costs are particularly volatile, and the use of fishmeal as a source of nutrients for fish farming is environmentally questionable (Hossain et al., 2019). Efforts to decrease the cost of feeds have been done by replacing fish meal with plant proteins (Ergun et al., 2008a,b; Yigit et al., 2010) using different feeding regimes and schedules (Wu et al., 1999; Patel and Yakupitiyage 2003; Davies et al., 2006; Goda et al., 2007; Abdel-Tawwab and Ahmad (2009)), optimizing feeding frequencies (Marimuthu et al., 2010) and adjustment of offset timing of supplemental feeding (Brown et al., 2000) but this papers focuses on different feeding regimes to decrease the production cost, without decreasing growth performance of fish.

Amur catfish, also known as Japanese common catfish (*Silurus asotus*), is a freshwater catfish species from the family Siluridae. It is found in Japan and continental East Asia and is considered as one of the important freshwater species in Korea. The production of Amur catfish in Korea increased from 2,745 tons in 2000 to 5,139 tons in 2017 with a direct sales value of over US$ 13.7 million (FAO, 2020). Amur catfish is usually reared in water bodies around paddy fields in peninsular and efforts have been made to establish intensive production systems, requiring careful nutritional management at an elevated production cost. According to Jobling (1982), fish fed less frequently consume larger amounts of feed at each feeding time compared to fish fed on a daily basis, contributing to improved growth performance. The rate of feeding to conversion relationship is of significant interest, since insufficient or excessive feeding contributes to decreased feed efficiency and growth, elevated production costs and deteriorating water quality (Shell 1996; Luthada 2012). Research to decrease the production costs of Amur catfish has been done by use of alternative protein-rich ingredients and the use of a fermented by-product of mushroom as a fishmeal replacer (Katya *et al*., 2014).

Managing feed to improve production and economic returns have been investigated for various fish species including Nile tilapia (*Oreochromis niloticus*; Bolivar *et al*., 2006; Opiyo et al., 2014), Gilthead sea bream (*Sparus aurata)* (Yigit et al., 2012); two band Seabream (*Diplodus vulgaris*; (Bulut et al., 2014) and African catfish (*Clarias gariepinus*) Davie et al., 2006). Feeding frequency can strongly affect the ingestion of feed and nutrient absorption hence influencing the growth performance of fish (Marimuthu et al., 2010). This study was designed to assess the effectiveness of intermittent feeding regime on growth performance and yield of Amur catfish (*Silurus asotus*) by determining the feeding regime that gives the best economic returns of *S. asotus* cultured in tanks.

Material and Methods

Experimental Design and Diet

The experiment was done in a completely randomized design in glass aquaria at the Pukyong National University, Busan in the Republic of Korea. A daily and alternate feeding program of one, two and three alternating days was used on Amur catfish fingerlings obtained from the Namsangju fish farm (Namsangju, Republic of Korea). A total of 240 fingerlings with a length of 6-7 cm and a weight ranging between 2-3 g were acclimated to experimental conditions prior to the start of the feeding trial. Afterwards, 20 fingerlings were distributed in each of 12 aquaria tanks in a semi-recirculating system of 28 litres, containing filtered fresh water with aeration. The aquaria had a constant water flow rate of 2 L min^{-1} and water temperature maintained at 25°C.

The 12 aquaria were randomly assigned treatments of different feeding regimes of every-day feeding (EDF), every two-days feeding (ETDF), tertian feeding (TF) and quartan feeding (QF) in triplicate treatments. Fish were fed on a commercial sinking catfish feed (pellet size 4mm), obtained from Woosung feed company limited, Republic of South Korea for the first two weeks (Table 1) and later changed to 6 mm floating pellets from the same company for the remainder of experimental period. The proximate composition of the diet used as prescribed by the manufacturing company is presented in Table 1. Feeding frequencies were monitored according to a feeding schedule "weekly guide" with appropriate days of feeding (Table 2). Fish were fed according to the feeding schedule except for the sampling days after every two weeks. At every feeding day, feeding was done twice a day at 1000 hrs and 1500 hrs to satiation. Feeds of every tank was weighed, broadcasted to the fish, until they stopped eating, then the remaining amount was weighed to get the actual amount of feed eaten.

Table 1 . Proximate composition of the commercial diet

Parameter	% Composition
Crude protein	50.0%
Lipid	13.0%
Calcium	2.0%
Crude fibre	3.0%
Phosphorus	2.7%
Crude Ash	17.0%

Table 2. Weekly feeding schedule

Day	Every-day feeding (control, EDF)	Every two-days feeding (ETDF)	Tertian feeding (TF)	Quartan feeding (QF)
Monday	✓	✓	✓	✓
Tuesday	✓	-	-	-
Wednesday	✓	✓	-	-
Thursday	✓	-	✓	-
Friday	✓	✓	-	✓
Saturday	✓	-	-	-
Sunday	✓	✓	✓	-
Monday	✓	-	-	-

Fish Sampling

Fish were starved for 24 hours prior to sampling. Total length to the nearest 0.1mm was measured using a 30 cm measuring board, and weight was measured using analytical balance with the precision of 0.01g. At the end of the trial, fish were counted in every tank to calculate survival. Growth parameters including weight gain, specific growth rate (SGR), Length-weight relationship, survival rate, Feed conversion ratio (FCR), Feed intake and the enterprise budget were calculated using the following formulae respectively.

Specific Growth Rate (SGR) and length-weight relationship were calculated as described by Novoa et al., (1990).

Specific Growth Rate (SGR)

$$= \frac{\text{Log}_e(\text{final weight}) - \log_e(\text{initial weight})}{\text{Culture Days}} \times 100\% \quad \textit{Equation.1}$$

A table showing ‘a' and ‘b' values of the length-weight relationship was determined as per the Le Cren law of 1951: $W= aL^b$, Where; W= Weight of fish in grams, L= the observed total length in (cm), a= the regression intercept, b= regression slope

% survival rate (%SR)

$$= \frac{\text{Final number of fish}}{\text{The initial number of fish}} \times 100\% \quad \textit{Equation. 2}$$

Feed Conversion Ratio (FCR)

$$= \frac{\text{Weight of dry feed fed (g)}}{\text{Live weight gains of fish (g)}} \quad \textit{Equation. 3}$$

Weight Gain (g)

= Final mean weight (g) – Initial mean Weight (g) *Equation. 4*

Feed intake (FI)

= Amount fed – wasted feed *Equation. 5*

Net fish yield

= total weight of fish at harvest–total weight of fish at stocking

Equation 6

Water Quality Management

Solid wastes were removed in a sedimentation tank at least twice a week for maintenance of water quality. Physico-chemical parameters, including temperature and pH were measured using a pH meter (YSI model: JA-100), Dissolved Oxygen (DO) was measured two times a day at 10.00 AM and 4.00 PM using a dissolved Oxygen meter, (model No: PDO-519) . Total Ammonia Nitrogen (TAN), and Nitrite-Nitrogen were measured once a week at 9AM using standard laboratory water quality analysis methods according to Boyd & Tucker (1998).

Profitability Analysis (Economic Feasibility)

A partial enterprise budget was used to assess the economic performance of the different feeding regimes. The costs considered for the enterprise budget were the Investment costs (Capital), Variable costs (VC) and Fixed costs (FC) according to (Bailey et al 1992). Daily fed fish were considered as the reference point for feeding frequency. The budget was restricted to cost and revenue items influenced by proposed variations in feeding frequency and in determining the possible variations in profit at different feeding frequencies (Opiyo et al., 2014). The cost benefit analysis of the current enterprise was done for each treatment. In the current study, the cost of feeds, fingerlings and other items were estimated as per the existing market prices. The variables included in the enterprise budget were as follows:-

Gross Returns

This income was generated from the sale of the Amur catfish.

Variable Cost (VC)

These are costs that vary with production. They are also called operational costs. They are the cost of fingerlings and feeds that were used in every tank during the culturing period.

Fixed Cost (FC)

These are costs that were incurred regardless of the level of production of the enterprise. They are the depreciation, interest on the investment, water analysis kits cost, culture facility, permits and licenses, taxes, insurance etc. and any other cost that are not related to the actual enterprise production.

Total Cost (TC)

It was obtained by adding the Variable costs (VC) and Fixed costs (FC).

Net Return Above Total Costs

It was obtained by subtracting Fixed costs (FC) from Returns above variable cost.

Yield

This was the total biomass (kg) obtained from every feeding regime at the end of the culturing period.

Unit Selling Price

It is the price of selling 1kg of the products from every feeding regime

Break-Even Price (BEP) Above Total Cost

It was obtained by dividing the Total costs over the yield in kg of every tank/system

Break Even Price Above Variable Cost

This was calculated by dividing the Variable cost (VC) by total production (Yield). It determines the production cost and the market price that is required to recover variable and fixed cost.

Break Even Yield

It was obtained by dividing the Total Costs by the Unit selling price.

The Input Expenditure

- Cost of Amur catfish fingerlings @ US 0.05 $
- Cost of 50% crude protein feed @ US 0.5 $ per kg
- Cost of fish harvested per tank @ US 3 $ per kg
- Other miscellaneous @ 5 US $ per tank

Data Analysis

Data were expressed as means ± SE. All the trial data were analysed using one-way analysis of variance (ANOVA) to determine difference among groups.. Comparison of means between groups was done by Tukey's HSD test. Statistical differences were considered at (P <0.05). Data were analysed by SPSS version 20 statistical software (Version 20 for windows).

Results and Discussion

The overall calculated variables of all results including initial-final length and weight, weight gained, SGR, feed intake, FCR, condition factor and survival rate are summarized in Table 3.

Final mean weight was higher in fish in the EDF regime (171.74 ±10.09 g) and lowest in QF. The highest weight gain was observed in the EDF (165.70 ±9.98 g) followed by ETDF (136.46 ±4.92 g). SGR over the culture period decreased with an increase in feeding frequency. The highest was on EDF (5.15 ±0.06 % day $^{-1}$) while the lowest SGR was in QF with a value of 4.24 ±0.12 % day $^{-1}$. There were significant differences among the treatments for final body weight, weight gain and SGR (P < 0.05). With regard to feed intake, EDF consumed the highest amount of feed (2813g) for the sixty-five days while QF consumed the least amount of feeds (656g). On the other hand, FCR increased with the decrease in feeding frequencies except at TF where the FCR value was 0.65. The lowest FCR was observed in QF and the highest FCR was observed in EDF. With regard to feed utilization, there was a significant difference (P < 0.05) in the FCR of the different feeding regimes. Due to feeding sinking pellets and changing to floating pellets in the third week of experiment, it was observed that less amount of feed was consumed during the first two days of the third week after changing sinking to floating pellets to the fish. Additionally, fish were using more effort to reach feeds, which was unusual compared to feeding on sinking pellets in the first two weeks.

In this study, the highest survival rate was observed in ETDF (96.67 ±3.33 %) followed by EDF having 95.00 ±2.87 %. The survival rates among the treatments were not significantly different (P > 0.05). Length-weight relationship of the fish indicated highest b-value for QF (2.76 ±0.29) followed by EDF. The (b) values were however not significantly different in all treatments (P > 0.05). The condition factors of fish in all the feeding regimes ranged from 0.70 to 0.73 and were not affected by the feeding regime (P>0.05).

Water Quality Parameters

Water temperature was kept at 25°C in the entire culture period in all the treatments. Higher dissolved oxygen was observed in QF and was increasing with the decrease in feeding frequency (Table 4). Dissolved Oxygen values were significantly affected by the feeding regimes (P>0.05). High Total ammonia nitrogen (TAN) and Nitrite-Nitrogen (NO_2-N) levels were observed in EDF which was receiving feed daily and were at 0.86 ±0.22 mg L^{-1} and 0.73 ±0.22 mg L^{-1} respectively. However, the TAN levels were not significantly different (P>0.05) in all the feeding regimes.

Table 3. Growth performance of amur catfish (*Silurus asotus*) on different feeding regimes for 65 days

Variables	Treatments				
	Every-day feeding (EDF)	Every two-day Feeding (ETDF)	Tertian feeding (TF)	Quartan feeding (QF)	P-value
Initial Length (cm)	8.10 ±0.00^a	7.97 ±0.14^a	8.02 ±0.22^a	7.67 ±0.12^a	0.23
Final Length (cm)	28.75 ±0.45^a	27.05 ±0.39^a	24.60 ±0.69^b	23.14 ±0.43^b	0.00
Initial Weight (g)	6.04 ±0.11^a	5.28 ±0.32^a	5.46 ±0.66^a	5.55 ±0.31^a	0.61
Total Initial Weight (TIW) (g)	120.75 ±2.19^a	105.65 ±6.40^a	109.13 ±13.15^a	111.07 ±6.11^a	0.60
Final Weight (g)	171.74 ±10.09^a	141.75 ±10.09^b	104.50 ±10.09^c	87.65 ±5.62^d	0.00
Total Final Weight (TFW) (g)	3307.30 ±84.50^a	2688.30 ±41.3^b	1789.70 ±77.40^c	1369.70 ±60.7^d	0.00
WG (%)	2740.70 ±1.16^a	2611.50 ±2.38^a	1834.10 ±1.15^b	1486.20 ±1.25^b	0.00
SGR	5.15 ±0.06^a	5.07 ±0.14^a	4.55 ±0.09^b	4.24 ±0.12^b	0.00
Feed Intake (g)	2813.30 ±52.07^a	1551.50 ±139.55^b	1099.90 ±32.97^c	656.51 ±9.07^d	0.00
FCR	0.88 ±0.01^a	0.60 ±0.01^b	0.65 ±0.01^{bc}	0.52 ±0.03^a	0.00
Survival Rate (%)	95.00 ±2.87^a	96.67 ±3.33^a	86.67 ±6.01^a	83.33 ±6.01^a	0.22
***b*-value**	2.65 ±0.06^a	2.62 ±0.20^a	2.64 ±0.15^a	2.76 ±0.29^a	0.96
Condition factor (K)	0.72 ±0.01^a	0.73 ±0.02^a	0.70 ±0.01^a	0.70 ±0.01^a	0.21

The values are articulated as Mean ± Standard Error (SE). Values in the same row with same superscript letters are not significantly different (P > 0.05)

Table 4. Water quality parameters of Amur catfish (*Silurus asotus*) on different feeding regimes for 65 days

Variable	Treatments				
	Every-day feeding Control (EDF)	Every two-day feeding (ETDF)	Tertian feeding (TF)	Quartan feeding (QF)	P-Value
Temperature (°C)	24.00 ±1.06^a	24.00 ±1.06^a	24.00 ±1.06^a	24.00 ±1.06^a	0.07
Dissolved Oxygen (mg L^{-1})	5.86 ±0.18^b	6.14 ±0.17^{ab}	6.40 ±0.18^{ab}	6.60 ±0.18^a	0.04
pH	6.87 ±0.17^a	6.86 ±0.20^a	6.45 ±0.11^a	6.36 ±0.18^a	0.07
NO_2-N (mg L^{-1})	0.73 ±0.22^a	0.53 ±0.15^a	0.48 ±0.14^a	0.46 ±0.14^a	0.63
TAN (mg L^{-1})	0.86 ±0.25^a	0.73 ±0.22^a	0.65 ±0.22^a	0.64 ±0.38^a	0.87

Enterprise Budget Analysis of Amur Catfish

The economic performance of the different feeding schedules is summarized in table 5 below. There was a significant difference between the net returns above variable cost (VC) for all treatments (P<0.05) and the returns were decreasing with increasing feeding frequency. The cost of feed increased with an increase in feed frequency. The cost of feed was significantly higher in EDF with an equivalent of 1.11 U$D. For all treatments, the returns above variable costs and net returns above total costs were significantly higher in EDF (P < 0.05) in comparison to the rest of the treatments. However, none of the treatments had a negative net return on total costs and the break-even price above total variable costs was below the unit-selling price of each fish in all the treatments.

Table 5. Partial enterprise budget of Amur catfish (*Silurus asotus*) on different feeding regimes (US$)

Variable	**Treatment**			
	Every-day feeding (EDF)	**Every two-day feeding (ETDF)**	**Tertian feeding (TF)**	**Quartan feeding (QF)**
Gross Revenue	9.92 ±0.25^{a}	8.07 ±0.12^{b}	5.37 ±0.23^{c}	4.11 ±0.18^{d}
Variable Cost (VC)	2.11 ±0.03^{a}	1.78 ±0.02^{b}	1.55 ±0.02^{c}	1.33 ±0.00^{d}
Returns above VC	7.81 ±0.23^{a}	6.29 ±0.10^{b}	3.82 ±0.21^{c}	2.78 ±0.18^{d}
Fixed Cost (FC)	0.30 ±0.00^{a}	0.30 ±0.00^{a}	0.30 ±0.00^{a}	0.30 ±0.00^{a}
Total Cost (TC)	2.41 ±0.03^{a}	2.08 ±0.02^{b}	1.85 ±0.02^{c}	1.63 ±0.00^{d}
Net returns above TC	7.51 ±0.23^{a}	5.99 ±0.10^{b}	3.52 ±0.21^{c}	2.48 ±0.18^{d}
Yield (Kg/pond)	3.31 ±0.08^{a}	2.69 ±0.04^{b}	1.79 ±0.08^{c}	1.37 ±0.06^{d}
Unit selling price	3.00 ±0.0^{a}	3.00 ±0.00^{a}	3.00 ±0.00^{a}	3.00 ±0.00^{a}
Amount of feed consumed (kg)	2.22 ±0.05^{a}	1.55 ±0.04^{b}	1.11 ±0.03^{c}	0.65 ±0.01^{d}
Cost of Feeds	1.11 ±0.03	0.78 ±0.02	0.55 ±0.02	0.33 ±0.01
Break Even Price of total cost	0.73 ±0.01^{c}	0.77 ±0.01^{c}	1.04 ±0.04^{b}	1.19 ±0.05^{a}
Break Even Price of Variable Cost	0.64 ±0.01^{b}	0.66 ±0.00^{b}	0.87 ±0.03^{a}	0.97 ±0.04^{a}
Break Even Yield (kg) of TC	0.80 ±0.01^{a}	0.69 ±0.01^{b}	0.62 ±0.01^{c}	0.54 ±0.00^{d}

*Values are articulated as mean ± SE. Values in the same row with the same superscript letters are not significantly different (P > 0.05).

Growth Parameters

This study indicates that feeding frequency has a substantial impact on fish growth. Fish that were fed daily to satiation had a high weight gain and high SGR. The high growth performance acquired by the fish fed daily was because of the high amount of feed consumed daily by the fish and were efficiently converted to flesh (Verreth & Eding, 1993). Similarly, better growth rate and food efficiency have been reported for African catfish (*C. gariepinus*) fed twice per day, (Marimuthu et al., 2010) and catfish (*Heterobranchus longifilis*) fed twice per day (Davies et al., 2006) which exhibited higher weight gain, SGR and average final weight in comparison to fish fed once per day. Ali and Jouncey (2004) also observed high growth rate on *C. gariepinus* fed daily (P<0.05).

Other studies have reported no significant difference in SGR of fish fed daily and those fed alternatively (Schnaittacher et al., 2005; Cho et al., 2007). The results of this study are similar to the findings of Biswal et al., (2006) and Opiyo et al. (2014) which reported high growth rate in Indian major carps (*Catla catla*) and Nile tilapia (*Oreochromis niloticus*) fed daily, respectively. In a different study, no significant difference were observed in the mean weight gain, daily weight gain, survival rates except on the amount of feeds used in Nile tilapia (Bolivar et al., 2006), although the consumption of pond plankton was an uncontrolled variable in that study. It is also observed that fish fed less frequently consumed a large amount of feed at once compared to the fish fed daily during each meal, resulting in high growth performance.

High FCR was recorded in fish that were daily fed (EDF) followed by tertian fed (TF) fish. This was similar to findings of Salama, (2008) whose experiment on Sea bass (*Lates calcarifer*) fed twice daily which recorded a FCR of 2.43.The results are an indication that feed consumption and feed conversion efficiency was subjective to the feeding frequency. Fish fed every two days did not utilize the feed efficiently compared to fish on quartan feeding, , indicating that fish on quartan feeding had better feed conversion to flesh with an FCR of 0.52. However, Opiyo et al. (2014) observed a low

FCR of 1.04 in fish that were fed after two-days which was contradicting the results of the present study whose quartan feeding regime had the lowest FCR. The relationship between the feeding frequency and the rate of conversion is very important in fish culture. When fish are insufficiently fed or excessively fed, their feed utilization efficiency may decrease, causing an increase in production cost and water quality deterioration (Luthada, 2012). For these and possibly other reasons, studies comparing various feeding schedules often lead to the recommendation that moderate feeding is commercially optimal (Brown et al., 2004).

In this study, feeding frequency affected the survival rate of *Silurus asotus*. Alternate every two-day feeding treatment had the highest survival rate percentage followed by the daily feeding treatment. This observation contradicts that of Opiyo et al., (2014) which showed a significantly high survival rate of daily fed fish but in line with the study of Bolivar, Jimenez & Brown, (2006) which recorded a mean survival rate of 63.42 and 55.35 for alternatively fed samples and daily fed samples respectively.In the current study, the lowest survival rate for the whole period was 83% in fish on quartan feeding and could be attributed to high cannibalistic nature of *Silurus asotus* when left unfed for more than one day.

A decrease in growth performance was observed with a decrease in feeding frequency. Highest decrease was however observed in fish fed daily. According to Shell (1996), fish fed excessively results in poor growth due to detrimental effects of uneaten food decaying in water leading to poor water quality (Abou-Zied, 2015). Additionaly, regular fish feeding in controlled conditions increases stress in fish due to the vigorous movement of fish, which leads to loss of energy (Anderson et al., 1996).

The mean b value of the quartan feeding was higher (2.76) compared to the other regimes with no significant difference. This could be an indication of fish having more girth or more fat as it grows longer, and spends extra energy for growth and reproduction (Anderson et al., 1996). The quartan feeding treatment had fish considered healthier because of b-value of 2.76 - almost 3, the standard exponential b-value of most fish species; a parameter, which could also be used to deduce the health condition of fish (Froese 2006). The low b value of the daily fed fish could be because of high stocking density, which might have led to stressful environment in the culture unit. The b-values recorded in this study (2.62 - 2.76) are similar to that of Skipjack tuna (*Katsuwonus pelamis*) observed by Jin et al. (2015), who further suggested that the b values should mainly be used for assessing growth rates due to their high rates of increase during the growth stage.

All condition factors in the present study were less than 1, the standard figure for condition factor. When K is 1, the fish is considered to be healthy but less than that shows an unhealthy condition (Ighwela et al., 2011). For this reason, all the feeding regimes depicted an unhealthy condition of fish. This could be as a result of the high stocking density which could possibly led to increasing stress levels of the fish in all the feeding regimes.

Enterprise Budget

In the present study, holding tanks, water and power were kept constant and were not considered in the enterprise budget analysis. Positive net returns were recorded in all the feeding regimes.. This was in line with the result of Opiyo et al., (2014) who observed the profitability of Nile tilapia on alternate feeding regimes in fertilized earthen ponds.. Positive returns above variable costs indicate that it is profitable to operate the enterprise in the short run (Engle and Neira, 2005) and negative net returns indicate that the enterprise is not profitable for the long term (Stone et al., 2008). Engle (1977) observed a negative net return in every acre of land on Arkansas catfish production budget and this was because of high cost of labour in paying the overall manager and supervisors.

The break-even price above the variable cost of the present was 0.64 US $ per kg for the fish fed on a daily basis. This indicates that Amur catfish production can be profitable as long as the price is above 0.6 US $. Feeding fish daily was the utmost profitable compared to other feeding frequency due to the fast and high growth performance of the fish because of high feed consumption leading to a high weight gain? The cost of feed for the daily fed fish was higher (1.11 US $) compared to the other feeding regimes. The high cost of feed observed in fish fed daily could be because of the high consumption of feed during the culture period, unlike the other feeding regimes.

Conclusion

This study built on the fish growth performance and the utilization of feed for Amur catfish (*S. asotus*) to be fed to satiation every day for optimum growth, survival, and better economic returns. Feeding fish to satiation twice every day was more profitable than other feeding regimes though quartan feeding had reduced labour and production costs.

Compliance with Ethical Standard

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

Ethics committee approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors during the study.

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An eBook	Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015)
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Preview article in a journal with DOI	Gaudio, J.L. & Snowdon, C.T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
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Photograph (from book, magazine or webpage)	Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015).
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