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Stochastic frontier analysis of catfish (*Clarias gariepinus*) aquaculture agribusiness for sustainable fisheries development: Evidence from Nigeria

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ABSTRACT

Technical efficiency assessment and enhancement is critical to sustainable fisheries development in Nigeria. This study examines stochastic frontier of catfish aquaculture agribusiness for sustainable fisheries development. Purposive sampling technique was employed to select 110 catfish farmers in areas with high density catfish farms. Primary data were collected directly from catfish farmers using structured questionnaire. The analytical tools used were descriptive statistics, net farm income, stochastic frontier production function (SPF) and t-statistics. The result shows that most of the catfish farmers were young people within the productive age of 40-49 years. Catfish farmers had obtained various levels of formal education. Finding shows that feeds cost was the highest variable cost (72.75%). Feed had a positive and significant relationship ($P<0.05$) with catfish output. Mean technical efficiency is 53.49%. The estimated variance ($\delta 2s=0.2125$) is statistically significant ($P<0.05$), indicating that profit inefficiency is highly significant among catfish farmers. Estimated Gamma (γ) value of 0.26 implies that 26% of the total variation in catfish profit efficiency is due to the joint effect of technical inefficiency factors. The most significant efficiency factors are fish feed and pond size. The age and educational status of farmers are the most important determining factors of inefficiency in catfish production system. Lack of finance was the most serious constraint faced by catfish farmers. The study recommended that catfish farmers should form cooperative unions to facilitate their access to cooperative funding.

Keywords: Stochastic frontier analysis, Catfish aquaculture, Technical efficiency, Sustainable fisheries development

Introduction

The increasing cases of global undernourishment and starvation among human population, particularly in the developing countries had been reported at various development debate fora. This is a world-wide issue of major concern that calls for scaling up of food production. There aquaculture and fisheries are two of the three important sources (agriculture, aquaculture and fisheries) of food production. As it stands, world's natural stock of fish has finite supply limits. Most of natural water bodies have attained maximum fishing limit. Aquaculture holds the potential for sustainable aggregate fish supply to satisfy fish increasing global fish demand (Okechi, 2004).

Aquaculture refers to the cultivation of aquatic organisms under controlled or semi-controlled conditions for economic and social benefits (Fourier, 2006). Catfish farming is a subset of aquaculture which involves the rearing of catfish under controlled conditions for economic and social benefits. According to Adewunmi and Olaleye (2011), the favoured catfish for culture include *Clarias gariepinus, Heterobranchus bidorsalis, Clarias heterobranchus* hybrid (*heteroclarias*), with *C. gariepinus* and *H. bidorsalis* being the most cultured fish in Nigeria. *Clarias gariepinus* is regarded as an excellent aquaculture species because it grows fast and feeds on a variety of agricultural by-products. It is hardy and can tolerate extreme temperature, easy to produce in captivity with high annual production and good feed conversion rate. Globally, fish provides about 3 billion people with almost 20percent of their intake of animal protein, and 4.3 billion people with about 15 percent of such protein (FAO, 2012). Increasing demand for fish products has resulted in the growth of fish farms to meet a substantial part of the world's food requirement (Olasukanmi, 2012).

The current shortfall in fish supply compared to local demand is putting pressure on the price of fish and its products. This can make fish unaffordable for many households in Nigeria and further decreasing the per capita fish consumption rate (FAO, 2010). However, there is significant interest in sustainable development of the catfish industry in Nigeria. A sure means of substantially solving the demand-supply gap is by embarking on widespread homestead/ small scale fish production. Also, considerable efforts have been directed at examining productive efficiency of fish farmers in Nigeria that is exclusively focused on technical efficiency of fish farmers in general and profitability of fish farming (Kudi et al., 2008). Consequent upon the increment in awareness of catfish farming and a substantial percentage of small scale catfish farmers in Nigeria, it has prompted the interest of researchers in this field. However, most of the past studies in Nigeria focused on large scale fish farming (Obasi, 2002).

The catfish aquaculture sector is yet to record sustainable development in terms of fish output to further close demand-supply gap that is evident in the sector in Nigeria (Olujimi, 2002). Squires et al. (2003) and Achoja et al. (2020), reported that fishers' age and educational attainment have considerable impacts on the technical efficiency fish aquaculture. Young and educated fishers have potential for sustainable development of catfish aquaculture on the basis of their youthfulness and technical skills (Revilla-Molina et al. 2009; Oyinbo et al. 2016; Achoja et al. 2020).

New technologies from research and development initiatives generate sustainable development if they are made available by extension officers to catfish producers for efficient application. As it stands, the relatively high inefficiencies in the catfish aquaculture can be eroded with sustainable fisheries development policies (Baruwa and Omodara, 2019).

There is dearth of empirical information on technical efficiency of catfish aquaculture agribusiness in Nigeria. Catfish business in Nigeria has low and sub-optimal technical efficiency. This low efficiency is attributable to the poor management of some factors of production (Goksel 2008; Onoja and Achike 2011; Oyinbo et al. 2016; Baruwa and Omodara, 2019).

Stochastic frontier analysis of catfish (*Clarias gariepinus*) aquaculture agribusiness for sustainable fisheries development is a research puzzle that is worthy of investigation. The broad objective of the study is to determine the technical efficiencies of catfish aquaculture using stochastic frontier approach.

The specific objectives are to:

1. Determine the socioeconomic characteristics of catfish producers in the study area;
2. Estimate the cost and return of catfish production;
3. Estimate the technical efficiencies of catfish production;
4. Identify the production constraints faced by catfish aquaculture farmers in the study area.

Material and Methods

The study was conducted in Delta State, Nigeria. Delta State is located in the Niger delta Zone of Nigeria. It lies between latitude $5^0 00^1$ and $6^0 45^1$. The state is located in the Niger Delta Region of Nigeria and lies within mangrove swamp, fresh water swamp forest and derived savannah vegetation belts. The state is well irrigated naturally by many rivers, rivulets and streams. It has two prominent seasons, the wet season which, last from March to October and the dry season which last from November to February. The state is shared into three agricultural zones The major occupation of the people is farming and fishing. Their cropping systems are mainly mixed cropping, intercropping as well as sole cropping and the main crops cultivated in the area are cassava, yam, okra, garden egg, cocoyam, rice maize and sweet potato.

Purposive sampling technique was used to sample 110 catfish farmers selected from areas with high density catfish farms. Data were collected from primary sources. Primary data were collected using structured questionnaire which was administered on the respondents. Data collected was on the socioeconomic characteristics such as age, gender, household size, farm size, farming experience, income and level of education and data on catfish production like cost and returns, constraints to catfish production, income and expenditure of the household was also collected. The analytical tools that were employed to achieve the objectives and hypothesis of the study include descriptive statistics, net farm income, stochastic frontier production function analysis, food insecurity line and Z-statistic. Descriptive statistics such as frequency distribution, mean, percentage, minimum and maximum values was also used to achieve objectives I, and vi of the study.

Budgeting technique was used to achieve objective ii. The indicators that were used include Net Farm Income (NFI) and profitability index. NFI is expressed as:

$NFI = \Sigma P_{Yi} Yi - \Sigma P_{Xj} - \Sigma F_K$ ……………………….(1)

Where:

NFI= Net Farm Income (\$)/production cycle;

P_{Yi}=Unit price of the output of Catfish (\$)

Yi= Total output of catfish (Kg);

Pxj= Unit price of variable inputs (\$)

Xj=Quantity of variable inputs (where j=1,2,3,…,n)

F_k= Depreciated Cost of fixed inputs (\$) (where k=1,2,3,…,n)

Σ =Summation sign

The stochastic frontier function used by Onu et al. (2000) as derived from the error model of Aigner et al. (1977) was employed to achieve objectives iii and iv. The Cobb-Douglas production function was fitted to the frontier model of catfish production. The result was estimated using the maximum likelihood method. The stochastic frontier production function is written as:

$Yi = f(X:\beta) + e$ ………………….(2)

$e_i = Vi - Ui$ ……………………..…(3)

Where Yi=Output of the ith farm

Xi=Vector of inputs used by the ith farm

B=Vector of the parameters estimated

ei=Composite error term

Vi=Random error outside farmers control

Ui=technical inefficiency effects

The empirical Stochastic frontier model that will be employed is specified as follows:

$\ln Y_1 = \beta 0 + \beta_1 \ln X_1 i + \beta_2 \ln X_2 i + \beta_3 \ln X_3 i + \beta_4 \ln X_4 i + \beta_5 \ln X_5 i + \beta_6 \ln X_6 i + Vi - Ui$………(4)

Subscripts ij refer to the jth observation of ith farmer

ln= Logarithm to base e

Y=Output of catfish(kg)

β_0=Constant

β_1 -β_6=Parameters estimated

X_1= Number of Fingerlings

X_2= Fish feed (kg)

X_3=Labour (Man-days)

X_4=Drugs(\$)

X_5=Fuel (Litres)

X_6=Pond size (m^2)

V_i=Random noise (white noise)

U_i=Inefficiency effects which are non-negative with half normal distribution.

It is assumed that inefficiency effects are independently distributed and U_{ij} arises by truncation (at zero) of the normal distribution with mean U_{ij} and variance δU^2

Where U_i is specified as:

$U_i = \delta_0 + \delta_1 \ln Z1i + \delta_2 \ln Z_{2i} + \delta_3 \ln Z_{3i} + \delta_4 \ln Z_{4i} + \delta_5 \ln Z_{5i} + \delta 6 \ln Z_{6i}$

Where:

U_i= Inefficiency effect of catfish production

δ_0= Constant

δ_1 - δ_6 = Parameters to be estimated

Z_1 = Farmers age (years)

Z_2= Household size of farmer (number)

Z_3= Years of Formal education of the farmer (years)

Z_4= Years of farming experience of the farmer in catfish production (years)

Z_5= Number of years in cooperative society (years)

Z_6= Number of contacts with extension agents (measured as number of contacts in a year)

Results and Discussion

This section deals with the results and discussion of findings under the following sub-headings: Distribution of Socioeconomic Characteristics of Catfish Farmers, Cost and Return of Catfish Production, Technical Efficiencies of Catfish Production (technical efficiency factors and technical inefficiency factors), Production Constraints of Catfish farmers.

Table 1. Distribution of demographic parameters of Catfish Farmers

Parameter	Frequency/%	Mode
Age(years)		
20-29	7(8.75)	43
30-39	16(20)	
40-49	30(37.5)	
50-59	17(21.25)	
>60	10(12.5)	
Gender		
Male	55(68.75)	55
Female	25(31.25)	
Marital Status		
Married	56(70.00)	56
Unmarried	24(30.00)	
Educational Level		
No formal education	0(0.00)	61
Primary	6(7.50)	
Secondary	13(16.25)	
Tertiary	61(76.25)	
Farming Experience		
1 -5 years	49(61.25)	1-5 years
6 -10 years	22(27.50)	
11 -15 years	7(8.75)	
Above 16 years	2(2.50)	
Membership of cooperative		
Non member	52(65.00)	52
Member	28(35.00)	
Extension Contact		
No	50(62.5)	50
Yes	30(37.5)	

Socioeconomic Characteristics of Catfish Farmers

Age

The frequency distribution of respondents according to socio economic characteristics is shown in Table 1. The table shows that majority (37.5%) of the catfish farmers fell within the productive age range of 40-49years. The average age of the catfish farmers was estimated to be 43years which means that catfish farmers are in their prime and active age of production. They are likely to be productive in the next decade and catfish production in the country will likely increase. According to Sikiru, et al. (2009), this age bracket is a productive age which predicts better future for catfish production.

Gender

Table 1 showed that both men and women were actively involved in catfish production but the percentage of men were more. Men accounted for 68.5% while female were about 31.25%. The high number of males might be attributed to hard task carried out in catfish production process.

Marital status

Result from Table 1 showed that about 70% of the respondents were married. About 13.75% were single while 7.5% were divorced and 8.75% were widowed. The high number of married people in the business was to reduce labour cost as most married persons have children that constitute the labour force in catfish production.

Farming experience

Table 1 also shows the distribution of respondents by farming experience. As shown in the table there was influx of new entrants into catfish production in recent times. This could be due to the ban on importation of frozen catfish product by the federal government. This is represented by about 61.25% who had from 1-5 years of experience as the majority. This was followed by about 27.5% who had farming experience of 6-10 years, 8.75% had farming experience of 11-15 years and 2.5% had farming experience of 6-10 years, 8.75% had farming experience of 11-15 years and 2.5% had farming experience of 11-15years and 2.5% had farming experience of 6-10 years, 8.75% had farming experience of 11-15 years and 2.5% had farming experience of 16 years and above. Table 1 shows that the average farming experience of the respondents was about 5 years which means that they were still new in the business and had no experience in catfish production. This agrees with Williams et al (2012), that the ability to manage fish pond efficiently depends on the years of experience and this is directly related to the total productivity of the farm.

Educational level

The result shows that 7.5% of small scale catfish farmers had six years of formal education and 26.3% of small scale catfish farmers had 12 years of formal education while 40% of small scale catfish farmers had 10 years of formal education. With this level of education, there is tendency of the farmers being able to increase the level of technology adopted and skill acquisition. This study agrees with the findings of Ologbon

(2012) that found out that greater percentage of small scale catfish farmers in Ogun State had formal Education.

Membership of cooperative society

The result in Table 1 shows that 65% of the catfish farmers in the study area belong to a cooperative society while 35% of the respondents do not belong to any cooperative society.

Extension contact

The information in Table 1 reveals that majority of the farmers (62.5%) have access to extension service delivery while 37.5% of the catfish farmers in the study area indicated that they do not have contact with extension agents.

Cost and Return of Catfish Production

The information in table 2 shows the cost and return of catfish production in the study area. The average fixed cost incurred by the farmers in the study area amounted to the sum of $444.44. Findings indicated that cost of feed accounted for about $1,584.31 which is the greatest variable cost. This is followed by purchase of fingerlings and labour that accounted for $289.36 and $78.55 respectively. From the enterprise budget analysis for the catfish shown in the table, it could be observed that catfish production is a profitable venture in the study area. The result of the survey shows a Gross Margin of 92.32percent and average net returns was calculated to be $1,411.9. The result also revealed that rate of return was 0.575. Since the rate of return is greater than one, catfish production is considered profitable in the study area. The business is profitable with about 57.5% profit on investment. The study revealed that for every $1.00 invested in catfish production, a return of $0.57 is made. This result is consistent with the finding of Alawode (2014) who observed that fish farming is profitable. Therefore, the null hypothesis which states that catfish farming is not profitable is rejected and the alternative accepted.

Table 2. Cost and Return of catfish production

Items	Amount(₦)
Variable cost	
Feeds	570 350
Fingerlings	104 169
Medication	10 300
Labour	28 277
Water	10 804
Total variable cost	**723 900**
Fixed cost	
Pond preparation	108 000
Water pump	52 000
Total fixed cost	**160 000**
Total cost	**883 900**
Total Revenue	**1 392 217**
Gross margin	**92.32%**
Net farm income	**508 317**
Return on Investment	**0.575**

Technical Efficiency Level of Catfish Production System

Table3. Profit Efficiency and inefficiency factors of catfish production

Variables	Parameters	Coefficients	Standard error	t-value
Profit factors				
Constant	X_0	0.2116	0.1169	1.810
Fingerlings	X_1	-0.9731	0.9782	-0.9948
Fish feed	X_2	0.7128	0.2192	3.252***
Labour	X_3	-0.1581	0.1457	-1.085
Pond Size (m^2)	X_4	0.6071	0.1193	5.089***
Inefficiency Factors				
Constant	Z_0	0.7017	0.1169	6.002***
Age	Z_1	0.9397	0.1877	5.006***
Household size	Z_2	0.1434	0.0706	2.031**
Years of Education	Z_3	-0.2184	0.0526	-3.951***
Farming experience	Z_4	-0.2068	0.1648	-1.255
Number of extension contacts	Z_5	-0.3251	0.1395	-2.330**
Diagnostics statistics				
Total variance	δ^2	0.2125	0.0328	6.478***
Variance ratio	Γ	0.2574	0.1409	1.827
LR Test		0.1727		
Log-likelihood function		0.4053		

Estimated Profit Factors

The result of maximum likelihood (ML) estimates of the Cobb-Douglas stochastic frontier production function for small scale catfish farmers are presented in Table 3.

Fingerlings

The coefficient of cost of fingerlings is the a priori expected negative sign. This implies that 1% increase in the cost and quantity of fingerlings stocked beyond a threshold level will reduce the quantity of catfish output and technical efficiency of resource utilization as well as profit by 0.9731.

Fish feed

Feed has the a priori expected positive sign and significant ($p<0.05$) showing a direct relationship with output and profit efficiency. This implies that a 1% increase in of feed will increase the quantity of catfish output by 0.7128.

Pond size

Pond Size has the a priori expected positive sign and significant ($p<0.05$) showing a direct and positive relationship with output and profit efficiency. This implies that a 1% increase in of feed will increase the quantity of catfish output by 0.6071.

Technical Inefficiency Factors

Table 3 shows the result of technical inefficiency factors in catfish production system. The estimated variance δ^2 = 0.2125 is statistically significant at 1% level of probability. This value indicates that profit inefficiency is highly significant in the catfish farmers' production activities. The γ parameter shows the relative magnitude of the variance in output associated with technical efficiency. The coefficients of the variables derived from the Maximum Likelihood Estimation (MLE) are very important for discussing results of the analysis of the data. This coefficient represents percentage change in the dependent variables due to percentage change in the independent (or explanatory) variables. The value of estimated Gamma (γ) is 0.2574 and is statistically significant at ($p<0.05$) indicating that 26% of the total variation in catfish profitability is due to technical inefficiency factors.

Age

Age of catfish farmers entered the technical inefficiency model with a negative sign (-0.9397) and significant ($p<0.05$). This finding implies that increase in age (old age) of catfish farmers will increase the technical inefficiency of catfish production system in the study area. This result collaborates with the earlier report of Achoja et al. (2020) that age is an important variable in the productivity of aquaculture in Nigeria.

Household size

The result shows that house size has a positive and significant relationship ($p<0.05$) with the technical inefficiency of catfish production system. This finding implies that a 1% increase in household size will increase technical inefficiency of catfish farms by 0.1434. A catfish farmer with large household will likely divert resources meant for the fish farm to family upkeep to the detriment of the farm.

Years of education

This variable entered the technical inefficiency model with a negative coefficient (-0.2184) and significant ($p<0.05$). This finding indicates that increase in the years of education, especially with catfish orientation will reduce the technical inefficiency of catfish production system. This result implies that an educated catfish farmer will be able to adopt modern fish farming technologies and avoid wastage of farm resources, thereby reducing technical inefficiency. This result collaborates with the earlier report of Achoja et al. (2020) that education is an important human capital variable in the productivity of aquaculture in Nigeria.

Number of extension contacts

The frequency of extension contact with catfish farmer entered the technical inefficiency function with a negative coefficient (-0.3251) and it is significant ($p<0.05$). This implies that the more the number of extension contact a catfish farmer has with extension officers the lower the technical inefficiency in the catfish production system. More access to extension information on catfish production the lower the resulting technical inefficiency.

Technical Efficiency of Catfish Farms

Table 4. Analysis of Technical Efficiency of Catfish Farms

Technical Efficiency level (%)	Frequency	Percentage
<41	14	17.5
41-50	18	22.5
51-60	35	43.75
61-70	12	15
71-80	1	1.25
81-90	0	0
91-100	0	0
Total	240	100
Mean Technical efficiency	53.49%	
Minimum Technical efficiency	46.01%	
Maximum Technical efficiency	71.21%	

The Technical Efficiency shows the ability of farmers to derive maximum output from the inputs used in catfish production. Given the results of the Cobb-Douglas stochastic frontier model, the technical estimates are presented and discussed in Table 3. The Technical efficiency of the sampled households is less than 1 indicating that all the households are producing below the maximum efficiency frontier. A range of technical efficiency is observed across the sampled households where the spread is large. The best catfish household had a Technical Efficiency of 71.21%, while the worst household had a technical efficiency of 46.01%. The mean Technical efficiency was 53.49%. This implies that on the average, the respondents were able to attain approximately 53.5% of optimal technical efficiency from a given set of inputs utilization in catfish production system. This shows that catfish farmers households Technical Efficiency can be improved by 46.51% in order to raise the level of catfish technical efficiency in the study area. The finding tallies with the result obtained by Tsue et al. (2012) in their study on profit efficiency among catfish farmers in Benue State, Nigeria. Their findings showed that profit efficiency ranged from 23 percent to 99 percent with a mean efficiency of 84 percent.

Production Constraints Faced by Catfish Farmers

Some constraints were identified as hindrances to technical efficiency of catfish production system in the study area. These constraints include: lack of finance, acquisition of Land, purchase of farming inputs, technical support from government or local authorities, pollution and environmen-tal/climate change. Among the various constraints that affect the level of productivity in the study area, lack of finance was identified as the most serious challenge, followed by tech-nical support from government / local authorities. The result of this finding supports that of Tisdell (2003) who stated that the most important factor inhibiting fish farmer's productiv-ity in the study area include lack of access to financial capital and high cost of feed or other farm input.

Table 5. Production Constraints Faced by Catfish Producers

Name	Not a problem	Minor problem	Major Problem	Mean	Remarks
Lack of finance	7	26	47	2.50	Significant
Acquiring land on which to farm	7	43	30	2.29	Significant
Farming inputs(water, fingerlings, equipment and machinery)	5	59	16	2.14	Significant
Technical support from government/local authorities		44	31	2.33	significant
Pollution		37	19	1.94	Not significant
Environmental/Climate Change		43	11	1.81	Not significant

Cut off point=2.00
Mean>2.00=a problem, mean<2.00=not a problem

Conclusion

This study examines stochastic frontier of catfish aquaculture agribusiness for sustainable fisheries development in Nigeria. It was found out that catfish farming in the study area is relatively young and there is hope for an increase in level of involvement among the people in the study area. The majority of those who were involved in catfish production were able bodied men in their active age bracket, hence the potential to sustain catfish farming for many more years. A positive net farm income with increased return per naira invested indicated that catfish farming in the study area was profitable. Quantity of fingerlings and fish feed negatively and positively influencing the output of catfish respectively. The result shows that the modal class of the catfish farmers was within the productive age of 40-49 years. Catfish farmers had obtained various levels of formal education. Finding shows that feeds cost was the highest variable cost. The result also revealed that the rate of return on investment was 57.5%. The study revealed that for every $1.00 invested in catfish production, a net return of $0.57 is generated. Feed has a positive and significant relationship with catfish output. Mean technical efficiency is 53.49%. The profit inefficiency is highly significant among catfish farmers. About 26% of the total variation in catfish profitability is due to technical inefficiency factors. The most significant efficiency factors are fish feed and pond size. Age of farmer, educational attainment, lack of finance and technical support from government authorities are the most important inefficiency factors that require urgent policy attention for sustainable catfish aquaculture development.

Based on the findings of the study, the following recommendations are hereby made to promote increased catfish production in the study area.

Government should provide facilities such as incentives, subsidies and facilitate access to credit by catfish farmers in the study area by the review of the stringent lending policies of the formal lending institutions. Catfish farmers should come together to form co-operative unions to facilitate their access to credit and other inputs. Adequate trainings and seminars should be held at interval to update catfish farmers' knowledge on the art of catfish farming so that they could have access to improved methods and technologies of catfish production. Effort should be made to bring down the cost of feeds by exploring alternative sources of feed for catfish through well-funded research.

Compliance with Ethical Standard

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

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İstanbul Boğazı'ndaki ticari gemi kazalarının karar ağacı yöntemiyle analizi

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ÖZ

Bu çalışmada İstanbul Boğaz bölgesi olarak İstanbul Gemi Trafik Hizmetlerinin kapsama alanına giren Türkeli, Kandilli, Kadıköy ve Marmara sektörlerinde 2001-2016 yılları arasında meydana gelen ticari gemi kazaları incelemeye tabi tutulmuştur. Ulaştırma ve Altyapı Bakanlığı Ana Arama Kurtarma Koordinasyon Merkezi (AAKKM) veri tabanındaki kaza kayıtlarına uygulanan filtrelemeler sonucunda 500 groston üzeri ticari yük gemilerinin karıştığı 535 adet gemi kazası analiz edilmiştir. Belirtilen sektörlerde meydana gelen ticari yük gemi kazaları Ki-kare Otomatik Etkileşim Dedektörü (CHAID) karar ağacı yöntemi ile incelenmiştir. CHAID karar ağacı yöntemi sınıflandırma ve büyük veri kümelerinden anlamlı kurallar çıkarmada en yaygın kullanılan karar ağacı algoritmalarından biridir. CHAID karar ağacı yöntemi icra edilerek ticari yük gemilerinde meydana gelen kazaların tipi (çatışma/çatma, karaya oturma ve diğer) ile gemi faktörleri (gemi tipi, gemi boyu, gemi tonajı, gemi yaşı, gemi bayrağı, gemi yüklülük durumu), zaman faktörleri (kaza zamanı ve kaza mevsimi) ve diğer faktörler (kazanın meydana geldiği sektör, kaza nedeni ve gemiye pilot alınması durumu) arasındaki ilişki incelemeye alınmıştır. Kazanın meydana geldiği sektör, gemide pilot bulunması durumu, gemi tipi ve kaza zamanı kaza tipini etkileyen en önemli girdi değişkenleri olarak bulunmuştur. Veri setine uygulanan Karar Ağacı yöntemi sonucuna göre Kadıköy sektöründe meydana gelen kazaların % 86 olasılıkla çatışma/çatma, Kandilli veyahut Marmara sektörlerinde meydana gelen kazaların % 48 olasılıkla çatışma/çatma ve Türkeli sektöründe ise kazaların % 36 olasılıkla çatışma/çatma ve diğer kaza tipleri ile sonuçlandığı görülmüştür.

Anahtar Kelimeler: İstanbul Boğazı, Gemi kazaları, Kaza analizi, Karar ağacı

ABSTRACT

Analysis of merchant vessel accidents in Istanbul strait through decision tree method

In this study merchant vessel accidents which occured between 2001 and 2016 in the sectors of Türkeli, Kandilli, Kadıköy, Marmara which constitutes Istanbul Strait region under Istanbul Vessel Traffic Services scope have been examined. Data was obtained from database of Ministry of Transport and Infrastructure Main Search and Rescue Coordination Center, and after data cleansing process, 535 vessel accidents which involve merchant cargo vessels of above 500 grosston have been analyzed. Merchant cargo vessel accidents which were taken place in the specified sectors have been examined with CHAID (Chi-square Automatic Interaction Detector) Decision Tree method. CHAID Decision Tree method is one of the most common used decision tree algorithms in extracting meaningful rules from big datasets and for classification. Through conducting CHAID Decision Tree method for merchant vessel accidents relationship between accident type (collision/contact, grounding and other) and vessel factors (vessel type, Length overall (LOA), vessel gross tonnage, vessel age, flag, loading condition), time factors (accident time, season of accident) and other factors (sector where accident occured, pilot on board or not) has been analyzed. Accident occuring sector, pilot on board/not, vessel type and accident time have been found as the most important input variables. Based on the result of the Decision Tree method applied to the data set, it was observed that the accidents occurring in the Kadıköy sector were collision / contact with 86% probability, the accidents occurring in the Kandilli or Marmara sectors were collision / contact with 48% probability and in the Türkeli sector, both collision / contact and other accident types had 36% occurring probability.

Keywords: Istanbul Strait, Vessel accidents, Accident analysis, Decision tree

Giriş

Kuzeyde Anadolu Feneri ile Türkeli Feneri'nin birleştiği hattan başlayan ve güneyde ise Ahırkapı Feneri ile Kadıköy İnceburun Feneri'nin birleştiği hatta son bulan İstanbul Boğazı dünyanın en dar kanallarından birisidir ve yoğun bir deniz trafiğine maruz kalmaktadır. Boğaz coğrafi yapısı ve oşinografik özellikleri sebebiyle riskli bir suyoludur. Boğaz'ın ortalama genişliği 1500 metre iken en dar yeri 696 metredir ve en sığ yerde 19 metre derinliğe sahiptir (Özdemir, 2019). Kıvrımsı yapısından dolayı Boğaz toplam sayıları 12'yi bulan keskin dönüşe sahiptir ve buna istinaden Kandilli'de 45°'lik, Umur Bankı'nda 70°'lik ve Yeniköy'de ise 80°'lik büyük açılı rota değişikliği yapılmaktadır (Akten, 2003; Ece, 2016). Bu keskin dönüşlere ek olarak dipteki ve yüzeydeki ters akıntılar seyir emniyetini ciddi bir şekilde etkilemekte ve zaman zaman 6 ile 8 knota varan bir akıntı rejimi söz konusu olabilmektedir (Özdemir, 2019).

Karadeniz ülkelerinin ticaret hacimlerindeki ve filo hacimlerinde artış, Tuna-Ren, Tuna-Main gibi iç suyollarının devreye alınması ve son zamanlarda ölçek ekonomisinden istifade amacına binaen gemi boyutlarında görülen artışlar Boğaz'dan taşınan yük miktarında artışa sebebiyet vermiştir (Ece, 2011). Ulaştırma ve Altyapı Bakanlığı verilerine göre Boğaz'da taşınan tehlikeli yük miktarı yıldan yıla artış göstermekte ve 2010 yılında taşınan tehlikeli yük miktarı yaklaşık 359 milyon ton iken bu oran 2018 yılında yaklaşık 147 milyon tonu tankerlerce taşınmak kaydıyla yaklaşık 439 milyon tona ulaşmıştır (Deniz Ticareti İstatistikleri, 2018). Malaka Boğaz'ının ardından dünyanın en yoğun ve tehlikeli trafiğine sahne olan İstanbul Boğazı Panama Kanalından yaklaşık 3 kat, Süveyş Kanalından 2 kat ve Kiel Kanalından 1.5 kat daha yoğun deniz trafiğine sahiptir (Kiel Canal, 2018; Panama Canal, 2019; Suez Canal, 2019). Ulaştırma ve Altyapı Bakanlığı verilerine göre 8.957 adedi tanker olmak üzere 2019 yılında 41.112 gemi geçişi gerçekleşmiştir (Ulaştırma ve Altyapı Bakanlığı, 2019).

Emniyet kurallarındaki ve deniz taşımacılığındaki teknolojik gelişmelere rağmen kazalar vuku bulmaya devam etmekte, önlenememekte ve ciddi bir sorun teşkil etmeye devam etmektedir (Erol ve Başar, 2015). Boğazın hem hidrolojik, jeomorfolojik ve meteorolojik yapısı hem de yoğun deniz trafiği ve beraberinde artan gemi tonajları ile taşınan tehlikeli yükler deniz kazası riskini arttırmaktadır. Meydana gelen kazalar can/mal kaybına ve çevresel kirliliğe sebep olmaktadır. Boğaz'da meydana gelmekte olan deniz kazalarının önlenmesi ve emniyetin tesisi için evvela bu kazaların farklı yönlerden analize tabi tutulması gerekmektedir. Bu amaç doğrultusunda ele alınan bu çalışma belirtilen sıra ile organize edilmiştir. Çalışmanın ikinci kısmında İstanbul Boğazında meydana gelen gemi kazaları ile ilgili yapılan literatür çalışmalarına yer verildikten sonra üçüncü kısımda çalışmada istihdam edilen veri setinin detayları verilmiştir. Akabinde ise verilerin işlenmesinde kullanılan Ki-kare Otomatik Etkileşim Dedektörü (CHAID) karar ağacı yöntemi açıklanmıştır. Bulgular kısmında icra edilen yöntemin çıktıları verildikten sonra sonuç kısmında bazı öneriler yapılmıştır.

İstanbul Boğazında meydana gelen kazalar bağlamında literatür incelendiğinde görülmektedir ki Sezgin F. ve Kadıoğlu M. (2000) tarafından yapılan çalışma İstanbul Boğazı'nda 1982 ile 1999 yılları arasında vuku bulan 218 kaza verisini yıllara ve aylara ayırarak uygunluk analizi istatistiki yöntemi ile incelemiştir ve boğazda meydana gelen kazalar sebep, yer, meydana geldiği saat, kazaya müdahil olan geminin bayrağı, tonajı ve türü gibi değişkenler bağlamında sınıflandırılmıştır. Akten (2003) çalışmasında 1953-2002 yılları arasında İstanbul Boğazı'nda meydana gelen 461 kazayı kaza türleri ve kaza zamanına (gece-gündüz) göre ayırmıştır. Otay N. E. ve Özkan Ş. (2005) çalışmalarında İstanbul Boğazında gemilerin çarpışma, sahile vurma ve karaya oturma olasılıklarını hesaplamıştır ve elde edilen sonuçlar ışığında İstanbul Boğazı'ndaki transit gemi trafiği için kaza olasılıklarının coğrafi olarak dağılımını gösteren risk haritalarını teşkil etmişlerdir. Bayar vd. (2008) İstanbul Boğazındaki kazaları Gemi Trafik Sisteminin (VTS) etkisini görebilmek için VTS kurulumu öncesi ve sonrası dönemlerini baz alarak incelemiştir. VTS öncesi periyodu 1985 ve 2003 yılları arası baz alınarak ve VTS sonrası periyodu 2004 ve 2008 yılları arası baz alınarak incelenmiştir. Çalışmada İstanbul Boğazında meydana gelen kazalar gemi türlerine, bayrak ülkelerine ve kaza türlerine göre frekans dağılımı ile sınıflandırılmıştır ve VTS'nin kazaları azaltmada etkili olup olmadığı değerlendirilmiştir. Ece (2011) ise İstanbul Boğazı'nda Sağ Seyir Düzeni'nin icra edilmeye başlandığı 1982 yılından 2008 yılı sonuna kadar meydana gelen 341 kaza için kazaya karışan gemilerin adı, tonajı, bayrağı, kaza saat, günü, ayı, yılı, kaza türü, kaza nedeni ve kazaya karışan geminin kılavuz kaptan istihdam edip etmediği gibi bilgileri gibi değişkenleri ihtiva eden kaza veri tabanı teşkil edilmiştir. Bu değişkenlere ilişkin olarak frekans dağılım tabloları teşkil edilmiş olup değişkenler arasındaki ilişki için Ki-Kare İlişki Analizi gibi istatistiki analizler icra edilmiştir. Ayrıca Boğazdaki gemi kazalarının önlenmesine dair bazı önerilerde bulunulmuştur. Erol ve Başar (2015) çalışmalarında Karadeniz, Marmara Denizi ile Akdeniz ve Ege Denizinin bir kısmından müteşekkil olan Türkiye Arama ve Kurtarma sahasında 2001 ve 2009 yılları arasında vuku bulan 1247 deniz kazasını incelemiştir. Çalışmalarında Türkiye

Arama Kurtarma sahası Trabzon, Samsun, İstanbul, Çanakkale, İzmir, Antalya ve Mersin olarak 7 bölgeye ayrılmıştır ve elde edilen veriler frekans analizi ile sınıflandırılmış ve karar ağacı yöntemi ile analiz edilmiştir. Ece (2016) frekans dağılımları, Ki-Kare İlişki Testi ve Cramer's V Testi gibi istatistiksel analizleri yaparak İstanbul Boğaz'ında 1982 ile 2014 yılları arasında meydana gelen kazalara karışan gemiler ile bunların kılavuz kaptan almaları arasındaki ilişkiyi incelemiştir. Erol vd. (2017) veri filtreleme işlemi sonucu Boğaz'da 2001-2015 yılları arasında meydana gelen 135 deniz kazasını meteoroloji verilerini de hesaba katarak kaza şiddetine göre sınıflandırarak incelemiştir. Yılmaz ve İlhan (2018) çalışmalarında Ana Arama Kurtarma ve Koordinasyon Merkezinden elde edilen verilerle 2002 ile 2014 seneleri arasında İstanbul Boğazı'nı da içerecek şekilde Türkiye arama-kurtarma bölgesinde Türk bayraklı gemilerde veyahut Türk bayraklı gemilerin karıştığı ve ölüm, yaralanma veyahut kayıp ile sonuçlanan 182 adet deniz kazası/olayını istatistiki olarak analiz etmişlerdir. Özdemir (2019) ise 2003 ile 2013 yılları arasında Türkiye Boğazlar Sistem'inde hasıl olan deniz kazalarını frekans ve mekansal analiz yöntemiyle genel olarak ve boğazların sektörleri temelinde incelemiştir. Bu çalışmada kazalar İstanbul ve Çanakkale Gemi Trafik Hizmetleri bölgelerinde oluşan kazalar olarak iki bölümde değerlendirilmiştir ve mekansal analiz için Coğrafi Bilgi Sistemi istihdam edilmiştir. İstikbal (2020) Boğaz'da meydana gelen 3 büyük gemi kazasını 1934-1982 yılları arasında geçerli olan "Sol Seyir Düzeni" ile olan ilişkileri bağlamında değerlendirmiştir.

Materyal ve Metot

Veri Seti Detayları

Bu çalışmada, İstanbul Boğazı'nda 2001-2016 yılları arasında meydana gelen deniz kazaları konu edilmiştir. Türkiye Cumhuriyeti Ulaştırma ve Altyapı Bakanlığı Deniz ve İç Sular Genel Müdürlüğü Ana Arama Kurtarma Koordinasyon Merkezi (AAKKM) sözü geçen yıllar arasında İstanbul Boğazı'nda meydana gelen toplam 1091 deniz kazasını/olayını kayıt altına almıştır. Bu 1091 deniz kazası/olayından 535 tanesi gerekli filtrelemelerden sonra çalışmaya dâhil edilmiştir. 535 deniz kazası, 500 groston üstü ticari yük gemilerinin İstanbul Boğaz Bölgesi'ndeki Gemi Trafik Hizmetlerinin (GTH) sürdürüldüğü ve aşağıdaki Şekil 1'de de görüldüğü üzere Kadıköy, Türkeli, Marmara ve Kandilli sektörlerinde meydana gelen kazaları kapsamaktadır. Balıkçı tekneleri, yolcu gemileri, gezinti tekneleri vb. kazaları çalışma kapsamı dışında tutulmuştur. Ayrıca çalışmada sadece gemi kazaları ele alınıp gemilerde meydana gelen iş kazaları, sağlık sorunları vb. deniz olayları incelenmemiştir.

Şekil 1. İstanbul GTH alanı ve sektörleri (KEGM, 2020)

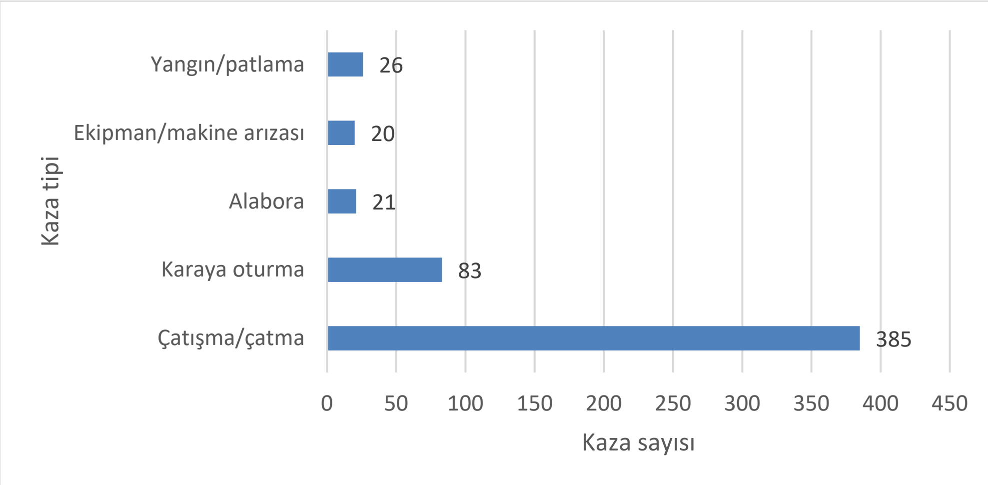
Figure 1. Istanbul VTS area and sectors (KEGM, 2020)

Tablo 1'de çalışmada kullanılan değişkenler, değişkenlerin değerleri (kategorileri) ve bunların kaza tipine göre dağılımı gösterilmiştir. Toplam 11 değişken AAKKM'nin kaza raporları incelenerek elde edilmiş olup bu çalışmada girdi (bağımsız) değişken olarak kullanılmıştır. Kaza tipi değişkeni ise çıktı diğer adıyla bağımlı değişken olarak ele alınmıştır. Şekil 2'de görüldüğü üzere çalışmada kullanılan 535 gemi kazasının 385'inin çatışma/çatma, 83'ünün karaya oturma, 26'sının yangın/patlama, 21'inin alabora ve 20'sinin makine/ekipman arızası olduğu görülmektedir. Kaza sayılarının yetersiz olması nedeniyle yangın/patlama, makine/ekipman arızası ve alabora tip gemi kazaları diğer kategorisi altında birleştirilerek çalışmada kullanılmıştır.

Tablo 1. Çalışmada kullanılan değişkenlerin kaza tipine göre dağılımı

Table 1. Distribution of input variables according to accident type

Değişkenler	Değerler	Toplam	Kaza tipi		
			Çatışma/ Çatma	Karaya oturma	Diğer
Gemi tipi	Genel yük gemisi	359 (%67.1)	261 (%72.7)	54 (%15.0)	44 (%12.3)
	Dökme kuruyük gemisi	77 (%14.4)	59 (%76.6)	13 (%16.9)	5 (%6.5)
	Petrol tankeri	15 (%2.8)	6 (%40.0)	4 (%26.7)	5 (%33.3)
	Kimyasal tanker	25 (%4.7)	18 (%72.0)	3 (%12.0)	4 (%16.0)
	Konteyner	16 (%3.0)	10 (%62.5)	4 (%25.0)	2 (%12.5)
	Diğer	43 (%8.0)	31 (%72.1)	5 (%11.6)	7 (%16.3)
Gemi yaşı	[0-4]	32 (%6.0)	22(%68.6)	6 (%18.8)	4 (%12.5)
	[5-14]	46 (%8.6)	33 (%71.7)	8 (%17.4)	5 (10.9)
	[15-24]	94 (%17.6)	67 (%71.3)	15 (%16.0)	12 (%12.8)
	25+	363 (%67.9)	263 (%72.4)	54 (%14.9)	46 (%12.7)
Gemi grosston	[500-10.000]	431 (%80.6)	312 (%72.4)	62 (%14.4)	57 (%13.2)
	10.000+	104 (%19.4)	73 (%70.2)	21 (%20.2)	10 (%9.6)
Gemi tam boyu (m)	[0-100]	216 (%40.4)	143 (%66.2)	45 (%20.8)	28 (%13.0)
	100+	319 (%59.6)	242 (%75.9)	38 (%11.9)	39 (%12.2)
Gemi yüklülük durumu	Dolu	175 (%32.7)	124 (%70.9)	27 (%15.4)	24 (%13.7)
	Boş	360 (%62.3)	261 (%72.5)	56 (%15.6)	43 (%11.9)
Gemi bayrağı	T.C.	133 (%24.9)	94 (%70.7)	22 (%16.5)	17 (%12.8)
	Kolay bayrak (FOC)	330 (%61.7)	241 (%73.0)	49 (%14.8)	40 (%12.1)
	Diğer	72 (%13.5)	50 (%69.4)	12 (%14.5)	10 (%13.9)
Gemide pilot durumu	Var	213 (%39.8)	122 (%57.3)	48 (%22.5)	43 (%20.2)
	Yok	322 (%60.2)	263 (%81.7)	35 (%10.9)	24 (%7.5)
Kaza zamanı	Gündüz	216 (%40.4)	147 (%68.1)	30 (%13.9)	39 (%18.1)
	Gece	319 (%59.6)	238 (%74.6)	53 (%16.6)	28 (%8.8)
Kaza mevsimi	İlkbahar	105 (%19.6)	72 (%68.6)	19 (%18.1)	14 (%13.3)
	Yaz	86 (%16.1)	58 (%67.4)	16 (%18.6)	12 (%14.0)
	Sonbahar	142 (%26.5)	98 (%69.0)	26 (%18.3)	18 (%12.7)
	Kış	202 (%37.8)	157 (%77.7)	22 (%10.9)	23 (%11.4)
Kaza sektörü	Kadıköy	357 (%66.7)	307 (%86.0)	29 (%8.1)	21 (%5.9)
	Kandilli	61 (%11.4)	29 (%47.5)	20 (%32.8)	12 (%19.7)
	Marmara	31 (%5.8)	16 (%51.6)	9 (%29.0)	6 (%19.4)
	Türkeli	86 (%16.1)	33 (%38.4)	25 (%29.1)	28 (%32.6)
Kaza nedeni	İnsan hatası	147 (%27.5)	118 (%80.3)	17 (%11.6)	12 (%8.2)
	Makine/ekipman arızası	52 (%9.7)	20 (%38.5)	18 (%34.6)	14 (%26.9)
	Çevresel faktörler	136 (%25.4)	100 (%73.5)	23 (%16.9)	13 (%9.6)
	Bilinmiyor	200 (%37.4)	147 (%73.5)	25 (%12.5)	28 (%14.0)



Şekil 2. İstanbul Boğazı'nda Meydana Gelen Kazaların Kaza Tipine Göre Dağılımı

Figure 2. Distribution of vessel accidents occurred in Istanbul Strait according to accident type

Karar Ağaçları ve Ki-kare Otomatik Etkileşim Dedektörü (CHAID) Algoritması

Bu çalışmada, 2001-2016 yılları arasında İstanbul Boğazı Bölgesi'nde meydana gelen ticari yük gemisi kazaları Ki-kare Otomatik Etkileşim Dedektörü (CHAID) karar ağacı yöntemi ile IBM SPSS Modeler 18.0 kullanılarak analiz edilmiştir. Veri seti % 65'i eğitim ve % 35'i test amacıyla kullanılmak üzere ikiye bölünmüştür. Eğitim veri seti, model parametrelerini tahmin etmek ve modeli oluşturmak için kullanılırken, test veri seti modeli bağımsız verilere uygulanabilirliğini ve modelin genelleme yeteneğini test etmek için kullanılmaktadır. CHAID karar ağacı yöntemi kullanılarak ticari yük gemilerinde meydana gelen kazaların tipi (çatışma/çatma, karaya oturma ve diğer) ile gemi faktörleri (gemi tipi, gemi boyu, gemi büyüklüğü, gemi yaşı, gemi bayrağı, gemi yüklülük durumu), zaman faktörleri (kaza zamanı ve kaza mevsimi) ve diğer faktörler (kazanın meydana geldiği sektör, kaza nedeni ve gemide pilot durumu) arasındaki ilişki incelenmiştir.

Karar ağacı, hem sınıflandırıcıları hem de regresyon modellerini göstermek amacıyla kullanılabilen bir tahminleme yöntemidir (Rokach ve Maimon, 2008). Bu yöntemin temel amacı; büyük bir veri kümesinden anlamlı ve kullanılabilir bilgiler çıkarabilmektir (De Oña ve diğerleri, 2013). Karar ağacı, her iç düğümün (yapraksız düğüm) bir öznitelik üzerinde bir testi temsil ettiği, her dalın testin bir sonucunu temsil ettiği ve her bir yaprak düğümünün (veya terminal düğümünün) bir sınıf etiketi içerdiği akış şeması benzeri bir ağaç yapısıdır. Bir ağaçtaki en üstteki düğüm kök düğümdür (Han ve Pei, 2012). Karar ağaçları finans, pazarlama, mühendislik ve tıp gibi uygulamalı alanlarda sıklıkla kullanılmaktadır (Rokach ve Maimon, 2008). Sınıflandırma ve büyük veri kümelerinden anlamlı kurallar çıkarmak amacıyla kullanılan C5.0, CART, CHAID, QUEST vb. birçok karar ağacı algoritması mevcuttur. CHAID bu algoritmalar içinde en yaygın kullanılanlardan biridir (Lin ve Fan, 2019).

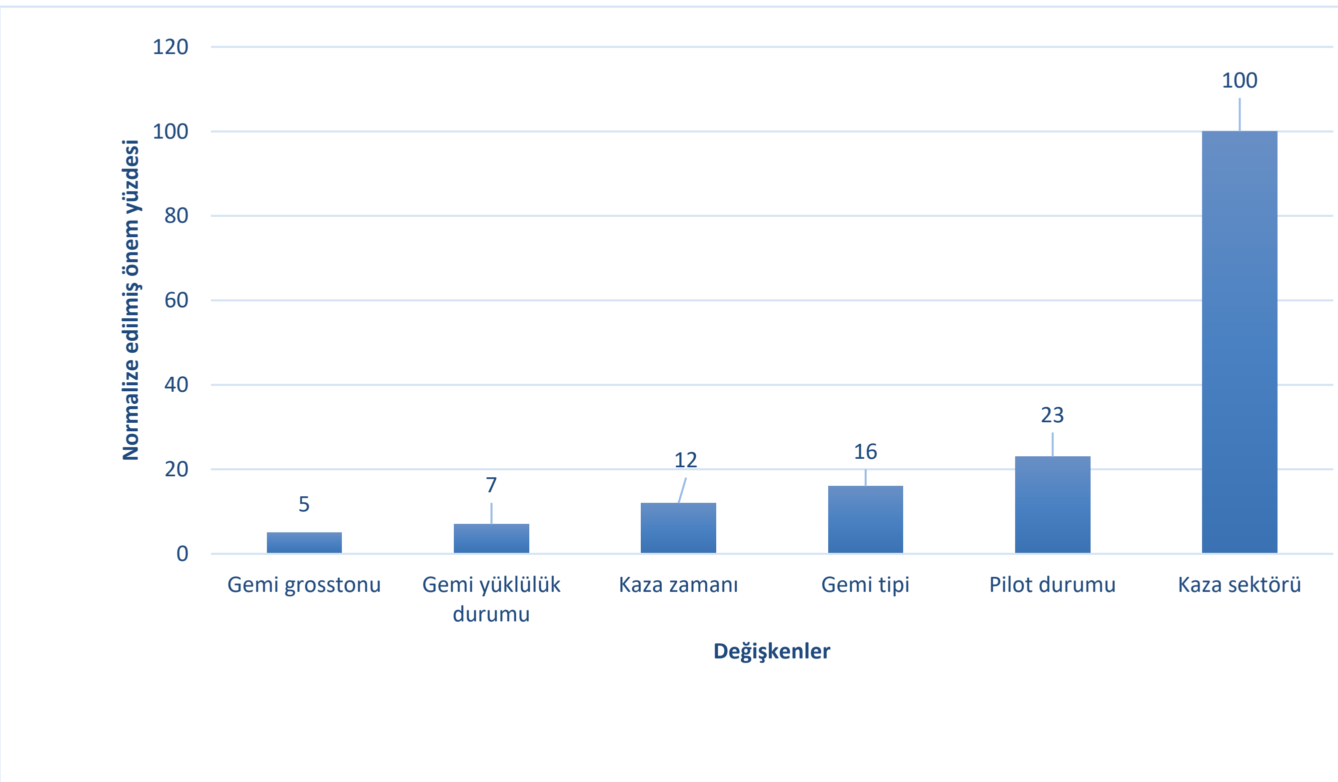
Kass (1980) tarafından ortaya çıkarılan CHAID, karar ağacında ikiden çok bölünmeler oluşturulmasını sağlayan bir tür makine öğrenme algoritmasıdır. CHAID, bir veri kümesini girdi değişkenleri (bağımsız değişken) ile çıktı değişkenleri (bağımlı değişken) arasındaki ilişkiler temelinde alt gruplara ayırır (Prati ve diğerleri, 2017). Algoritma temel olarak diğer Karar Ağaçları algoritmaları ile benzer özelliklere sahiptir, ancak ki-kare bağımsızlık testi kullanılarak ağacı bölmelere ayırır (Mistikoglu ve diğerleri, 2015; Han ve Pei, 2012). Girdi değişkenlerinin her biri ile çıktı değişkeni arasındaki çapraz tablolar, ki-kare testi kullanılarak incelenir ve CHAID en önemli girdi değişkenini seçer. Bir girdi değişkeninin ikiden fazla kategorisi varsa, CHAID karşılaştırma kategorileri karşılaştırır ve sonuçta hiçbir farkı olmayanlar bir araya getirilir (Prati ve diğerleri, 2017). Nihai CHAID karar ağacı, girdi değişkenleri ile çıktı değişkenleri arasında anlamlı bir ilişki kalmayınca sonlandırılır.

CHAID, her bir düğümde ikiden fazla dalı olan ağaçları inşa edebilmesi açısından diğer sınıflandırma yöntemlerine göre çeşitli avantajlar sağlar (Luna veMéndez, 2006; SPSS, 1998). Diğer karar ağacı algoritmalarının aksine, bölünme sonucu ortaya çıkan dal sayısının ikiden fazla olması, ağaç yapısının daha kolay anlaşılır ve yorumlanabilir olmasına olanak verir (Virens, 2001). Bununla birlikte hem kategorik hem de sürekli değişkenlerle çalışabilmesi CHAID algoritmasına kullanımı açısından esneklik sağlamaktadır (Althuwaynee ve diğerleri, 2016). Ayrıca, girdi ve çıktı değişkenleri arasındaki ilişkiyi incelemek için parametrik teste ihtiyaç duyulmaması ve verinin normal dağılımı şartının aranmaması CHAID algoritmasının diğer önemli avantajlarıdır (Díaz-Pérez ve Bethencourt-Cejas, 2016; Althuwaynee ve diğerleri, 2016).

Bulgular ve Tartışma

Daha önce belirtildiği gibi, CHAID algoritması, karar ağacı oluşturmak için kullanılan bir sınıflandırma yöntemidir. Bir karar ağacı, girdi değişkenleri (gemi tipi, kaza zamanı, kaza sektörü, vb.) ve çıktı değişkeni (kaza tipi) arasındaki ilişkiler temelinde bir veri kümesini alt gruplara ayırır. Her ağaç düğümünde veriler, bir girdi değişkeninin değerleri ile iki veya daha fazla farklı gruba özyineli olarak bölünür ve sonuç olarak bu bölünme işlemi karar ağacında başka bir anlamlı bölünme ortaya çıkmayacak duruma gelene kadar devam eder. Bu sayede CHAID, bir dizi eğer-ise -değilse kuralını (if – then – else rules) kullanarak ticari yük gemi kazalarının çatışma/çatma, karaya oturma ve diğer olarak sonuçlanma durumu hakkında oluşturduğu karar ağacı yapısı ile detaylı bilgiler sağlar.

Şekil 3'de girdi değişkenlerinin modeldeki göreceli önem derecesi diğer adıyla öngörü değeri normalize edilmiş haliyle verilmiştir. Her bir girdi değişkeninin önem derecesi, sonuç (bağımlı) değişkeninin varyansındaki azalmanın duyarlılık analizi yoluyla hesaplanması sonucu elde edilir. Kazanın meydana geldiği sektör (100), gemide pilot bulunma durumu (23), gemi tipi (16) ve kaza zamanı (12), kaza tipinin belirlenmesinde en önemli girdi değişkenleri olarak bulunmuştur. Bununla birlikte, girdi değişkenlerinin önem değeri, tek başına yordama güçlerinin altındaki mantığı anlamak için yeterli değildir. Bu yüzden, CHAID'in tahminleri hakkında daha derin bir fikir edinmek için karar ağacı yapısının ve ortaya çıkarılan kuralların incelenmesi gerekir.



Şekil 3. Değişkenlerin Normalize Edilmiş Önem Yüzdesi

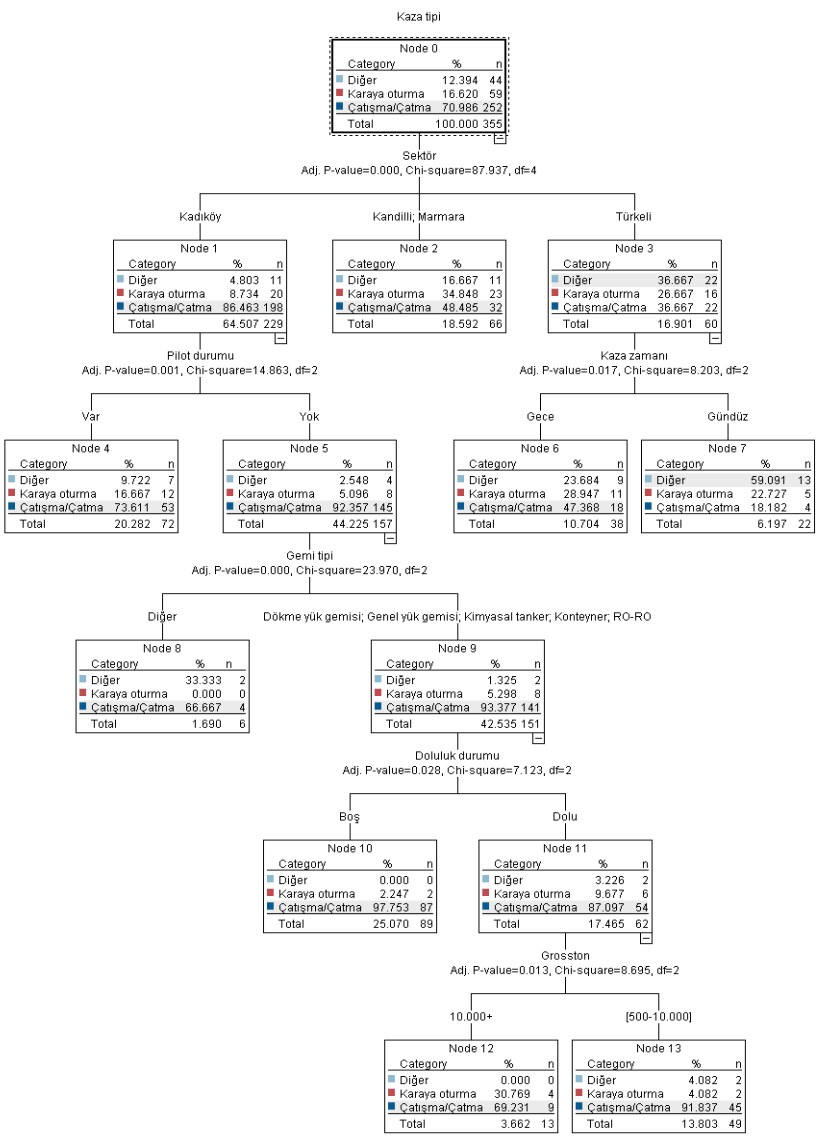
Figure 3. Normalized importance of input variables

Şekil 4'de ticari yük gemi kazalarının hangi tür kaza tipi ile sonuçlandığını belirten son ağaç yapısı görülmektedir. Karar ağacı yapısı kaza sektörü, pilot durumu, kaza zamanı, gemi tipi, gemi doluluk durumu ve gemi grostonu olmak üzere toplam altı bölünmeye neden olan değişken içermektedir. 0 düğümündeki ilk bölünme, ticari yük gemisi kazalarını üç gruba ayıran kaza sektörü ile meydana gelmiştir. Bu bölünmeye göre, kazanın meydana geldiği yer Kadıköy sektörü olduğunda kazalar %86 olasılıkla çatışma/çatma ile kazanın meydana geldiği yer Kandilli veya Marmara sektörleri olduğunda kazalar %48 olasılıkla çatışma/çatma ile ve kazanın meydana geldiği yer Türkeli sektörü olduğunda ise kazalar %36 olasılıkla çatışma/çatma ya da diğer kaza tipleri ile sonuçlanmıştır. Burada çatışma/çatma olasılığının Kadıköy sektöründe yüksek olması bir nebze buranın Boğaz bölgesindeki en yoğun deniz trafiğine sahip bölge olması ile izah edilebilir. Zira Kadıköy sektörü Boğaz'ın güney girişini temsil etmektedir ve bölgede yer alan Kartal ve Ahırkapı demir sahalarında demirleme yapan çok sayıda gemi bulunmaktadır (Özdemir, 2019).

Karar ağacının ikinci seviyesinde, Kadıköy sektöründe meydana gelen ticari gemi kazaları, gemide pilot olma durumuna göre ikiye bölünmüştür. Eğer Kadıköy sektöründe kaza yapan gemilerde pilot mevcut ise çatışma/çatma kazası meydana gelme olasılığı %73 iken, gemide pilot olmaması durumunda ise çatışma/çatışma kazası olasılığı %92'ye yükselmiştir. Boğaz geçişlerinde kılavuz kaptan istihdam etmeyen gemilerin kazalara karışma oranının artması Ece (2016) çalışmasında da teyit edilmiştir. Zira gemi kazaları çok yüksek bir oranda insan hatasından kaynaklanmaktadır ve Boğaz'ı iyi bilen, yüksek tecrübe, eğitim seviyesi ve uzmanlığa sahip kılavuz kaptanların alınması personel hatalarından kaynaklanan kazaları minimize etmektedir. Ağacın üçüncü seviyesinde, Kadıköy sektöründe gemiye pilot almadan seyir yapan ticari gemilerin yapmış olduğu kazalar gemi tipine göre diğer gemiler ve genel yük; dökme kuru yük; kimyasal tanker; petrol tankeri; Ro-Ro; konteyner gemileri olmak üzere iki alt gruba ayrılmıştır. Diğer gemilerin yaptığı altı kazadan dördü çatışma/çatma diğer ikisi ise diğer kaza tipleri ile sonuçlanmıştır. Dökme kuru yük; kimyasal tanker; petrol tankeri; Ro-Ro veya konteyner gemilerinin yaptığı kazaların ise %93 olasılıkla çatışma/çatma olduğu tahmin edilmiştir. Ağacın dördüncü seviyesinde, diğer gemi tipleri sınıfı haricindeki gemilerin yapmış olduğu kazalar geminin doluluk/yüklülük durumuna göre boş gemi ve dolu gemi olmak üzere iki alt gruba ayrılmıştır. Dolu gemiler için %87 olan çatışma/çatma kazası olasılığının, boş gemiler için %97'ye çıktığı görülmüştür. Kargo taşımayan gemilerin yüklü gemilere kıyasen daha fazla çatışma/çatmaya müdahil olması rüzgar etkisine daha açık olması, pervanesinin daha az batık olması, fribordunun (geminin su üzerinde kalan kısmı) çok olması, dümen dinlemesinin iyi olmaması, boğazdaki akıntıdan daha fazla etkilenme ve geminin yapısal durumlarının (makine, boy, dizayn, tip vs.) balastlı gemide aleyhe işlemesi gibi unsurlarla izah edilebilir. Ayrıca dolu gemilerin yapmış olduğu 62 kazadan 6'sı karaya oturma iken, boş gemiler için 89 kazadan sadece 2'sinin karaya oturma olduğu tespit edilmiştir. Son olarak ağacın beşinci seviyesinde, yüklü gemilerin yapmış olduğu kazalar, gemi grostonuna göre 10.000 groston üstü gemiler ve [500-10.000] groston arasındaki gemiler olmak üzere iki terminal düğüme ayrılmıştır. 10.000 groston üstü gemilerin geçirmiş olduğu 13 kazanın 9'u çatışma/çatma, 4'ü ise karaya oturma kazası olarak bulunmuştur. Yüksek kapasiteli gemilerde çatışma/çatma oranının yüksek olması bu gemilerin dönüş çapının artması ile ilintilendirilebilir. Diğer tarafta ise [500-10.000] groston arasındaki gemilerin yapmış olduğu kazaların yaklaşık %92'sinin çatışma/çatma ile sonuçlandığı görülmüştür. Bu bağlamda belirtilebilecek önemli bir husus şudur ki düşük tonajlı gemi zabitlerinin ehliyetinin yakın yol ehliyeti olma oranı yüksektir ve burada personel kalifikasyonunun yetersiz olmasının negatif bir rol oynadığı düşünülmektedir.

CHAID Karar Ağacı yapısının ikinci seviyesinde Türkeli sektöründe meydana gelen kazalar, kaza zamanı değişkenine göre gece ve gündüz olmak üzere iki alt gruba ayrılmıştır. Bu bölünmeye göre, Türkeli sektöründe gece meydana gelen kazalar %47 olasılıkla çatışma/çatma, %29 olasılıkla karaya oturma ve %23 olasılıkla diğer kaza tipi olduğu görülmüştür. Aynı sektörde gündüz meydana gelen kazaların %59'u diğer, %22'si karaya oturma ve %18'inin çatışma/çatma olduğu tespit edilmiştir. Türkeli sektöründe çatışma/çatma tipi ticari gemi kazalarının geceleri meydana gelme olasılığının gündüz vakitlerine kıyasla daha yüksek olduğu görülmektedir. Bu durum vardiya tutan personelde durumsal farkındalık eksikliği yani algıların azalması ile izah edilebilir. Zira personelin yorgunluğu ile beraber Boğaz manzarası ve insan popülasyonu bu bağlamda vardiya zabitinin dikkatini dağıtıcı bir unsur olarak negatif katkıda bulunduğu düşünülmektedir. Buna ek olarak köprü üstünde görevli olmayan gemi personelinin fotoğraf veya telefon görüşmeleri yapmak için köprü üstü civarında bulunarak dikkat dağıtıcı hareketlerde bulunmaları da bu minvalde belirtilebilir. Ayrıca Boğaz trafiğine dâhil olan balıkçı gemisi, yat veya tur motoru kaptanlarının ticari gemilerin geçiş üstünlüğüne engel olacak şekilde manevra yapmaları veya çatışmayı önleme kurallarına aykırı manevraları ile beraber eksik iletişim de bu durumu bir nebze izah etmektedir. Bu bağlamda son husus olarak gece özellikle Boğaz kıyısındaki sahil ışıklarının vardiya zabiti/kaptanın görüşünü olumsuz etkilediği belirtilebilir.

Bu çalışmada dikkate alınan değişkenler yukarıda belirtilenler ile sınırlıdır. Öte yandan belirtilmelidir ki karar ağacında değişkene ait yeterli bölünme sağlanabilmesi için her bir değişkene ait olan değer sayısının yeterli frekansa sahip olması gerekmektedir. Bu çalışmada, Kadıköy sektöründe meydana gelen kaza sayısı 357 iken, Kandilli/Marmara sektöründe toplam kaza sayısı 92 ve Türkeli sektöründeki mevcut kaza sayısının 86 olması ağaç yapısının bu şekilde oluşmasına neden olmuştur. Ayrıca karar ağacında meydana gelen bölünmeler sonucu elde edilen kurallardaki vaka sayısının az olması, bu kuralın genel kabul edilebilirliğini olumsuz etkilemektedir. Bu yüzden, CHAID karar ağacı uygulaması öncesinde her bir sonuç düğümündeki frekans sayısının asgari 5 olarak belirlenmesi Kandilli/Marmara ve Türkeli sektöründe daha fazla dal oluşmasına engel teşkil etmiştir. Buna istinaden, çalışmada daha ziyade Kadıköy sektörü üzerine yoğunlaşılmıştır. Bir sonraki çalışmada Uluslararası Denizcilik Örgütü'nün deniz kazalarının şiddeti-seviyesi hususunda yaptığı çok ciddi deniz kazası, ciddi deniz kazası ve deniz olayı gibi bir sınıflandırmayı temel alarak ve kazaya sebebiyet veren unsurların da AAKKM'den kaza bazlı derlenip hesaba katılması ile bu çalışmanın daha bütüncül bir analiz yapmaya daha elverişli hale geleceği düşünülmektedir.



Şekil 4. CHAID Karar Ağacı Yapısı

Figure 4. CHAID decision tree structure

Sonuç

İstanbul Boğaz'ı uluslararası taşımacılık bağlamında stratejik öneme sahip alternatifsiz bir suyoludur. Özellikle Karadeniz bölge ülkelerinin denizyolu çıkış kapısıdır ve bu boğazda meydana gelebilecek kazalar Boğaz gemi trafiğini ciddi manada sekteye uğratabilir. Bu nihayetinde bölge ülkelerinin ekonomilerine ciddi bir zarar verebilme potansiyeli ihtiva etmekle beraber can kayıplarına ve çevre felaketlerine de yol açabilir. Bu bağlamda, kazaların önlenmesi hususunda politika yapıcıların alacağı önlemlerin daha efektif olabilmesi için kazaların etkili ve kapsamlı bir şekilde analiz edilmesi ve değerlendirilmesi gerekmektedir. Bu kapsamda icra edilen çalışmada AAKKM'den elde edilen verilerle dört sektörden teşekkül olan İstanbul Boğaz Bölgesi'nde 2001-2016 yılları arasında 500 groston üzeri ticari yük gemilerinin karıştığı 535 adet gemi kazası (CHAID) karar ağacı yöntemi ile incelenmiştir. CHAID Karar Ağacı vesilesi ile ticari yük gemileri için çıktı değişkeni olan kaza tipi ile gemi tipi, kaza zamanı, kaza sektörü vs. gibi girdi değişkenleri arasındaki ilişkiler incelenmiştir ve en önemli girdi değişkenleri olarak kazanın olduğu sektör, gemiye pilot alınması durumu, gemi tipi ve kaza zamanı tespit edilmiştir. Bunu takiben görülmektedir ki en yüksek çatışma/çatma olasılığı Kadıköy sektöründe iken bu olasılık Kandilli/Marmara sektörlerinde düşüş göstermekte ve Türkeli sektöründe en düşük orana sahip olmaktadır. Kadıköy sektöründe pilot istihdam etmeden ve yüklü olarak seyir yapan ticari gemilerden genel yük, dökme kuru yük, kimyasal tanker, petrol tankeri, Ro-Ro ve konteyner gemilerinde çatışma/çatma kazası olasılığı %87 iken bu oran boş gemiler için % 97'ye çıkmaktadır. Ayrıca, Türkeli sektöründe gece oluşan kazalarda çatışma/çatma olasılığı gündüz meydana gelen kazalara kıyasen daha yüksek olduğu görülmekte iken karaya oturma olasılığı çok düşük bir oranla gece daha yüksek çıkmaktadır. Diğer taraftan gemiye pilot almadan Boğaz geçişi yapan ticari yük gemilerinin pilot alarak seyir yapan gemilere kıyasla yaklaşık iki kat daha fazla kazaya karışmış olduğu görülmüştür. Bu bağlamda, Boğaz'daki seyir emniyetinin iyileştirilmesi ve kaza riskinin azaltılması amacına binaen İdare'nin gemilerin pilot almasını teşvik edici bir rol oynaması gerekmektedir. Bu çalışmada ulaşılan sonuçların boğaz geçişini sık kullanan armatörlerin yanında tekne & makine ve emtea sigortacıları ile donatanın üçüncü partilere karşı mesuliyetini teminat altına alan Koruma ve Tazminat (P&I Club) Kulüplerinin risk değerlendirmesinde ve adil bir prim seviyesinin tespitinde dikkate alması öngörülmektedir. Bir sonraki çalışmada kazaya sebebiyet veren unsurlar olarak insan hatası, arızalar ve kazanın meydana geldiği andaki hava durumu gibi verilerin ve kazanın şiddeti bağlamında IMO sınıflandırmasına dayalı verilerin de çalışmada dikkate alınması daha bütüncül bir değerlendirme yapmak için önem arz etmektedir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışmasının olmadığını beyan eder.

Etik kurul izni: Bu çalışma için etik kurul iznine gerek yoktur.

Finansal destek: -

Teşekkür: -

Açıklama: -

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Community structure of mayflies (Insecta: Ephemeroptera) in tropical streams of Western Ghats of Southern India

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ABSTRACT

The main objective of this study was to evaluate the community structure of the order Ephemeroptera in the Southern Western Ghats ecoregion along with Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) from 2017 to 2018. Ecological parameters estimated at each collecting site were pH, dissolved oxygen, biological oxygen demand, hardness and alkalinity. This research investigation was carried out in 30 streams of Palni and Cardamom hills in Western Ghats of Southern India. With PCA examination, the sites like Dhobikana, Fern hill and Poomparai of Palni hills are plotted far apart and are not supported by the ecological parameters like in the other sites. Dhobikana of Palni hills is exceptionally contaminated in light of the fact that dhobis are associated with washing garments so cleanser contamination is prevalent around there. In cardamom hills, Santhamparai near bridge and Nayamakkadu are left far apart indicating they are not supported by physico-chemical parameters, mainly due to pollution. From to the CCA results, it is discovered that *Baetis* species favor β-mesosaprobic habitat and *Indialis badia* inclines toward high altitudinal region. From the outcomes, it is presumed that pH, dissolved oxygen, biological oxygen demand, hardness and alkalinity were the essential components administering the mayfly community and structure.

Keywords: PCA, Dhobikana, Dissolved oxygen, pH, *Baetis, Ephemera nadinae*

Introduction

Ephemeroptera also called as mayflies and they are cosmopolitan in distribution (Barber-James *et al.,* 2008). They do secondary production activity in freshwater habitat. Most families of mayflies are sensitive to pollution and they inhabit only in freshwater environment, so they serve as bio indicators of water quality.

Streams and rivers are an example of an important habitat and source of water for all living organism and human being. Pollution either by anthropogenic activity or by nature can unfavorably influence any biological ecosystem. The pollution in the freshwater ecosystem affects the mayfly's richness and diversity. Streams become contaminated by water entering from the farming area or modern destinations, and the nature of the water will be reflected by the kinds of the animals that can endure such as mayflies.

The ecological attributes like pH, turbidity, dissolved oxygen, air temperature, water temperature, alkalinity, total dissolved solids and various pollutants legitimately and by implication influence the mayfly populations. Deforestation is one of the primary threats to mayfly biodiversity and conservation in the tropics (Benstead and Pringle, 2004) whereas pollution (Rosenberg and Resh, 1993) or habitat fragmentation (Zwick, 1992) are the major causes in the temperate areas.

Numerous investigations have been made in recent years on the effect of climate change on mayflies. Clearly, climate changes are affecting the behavior and ultimately the ecology of some mayflies, for example, small increases in temperature (3°C) over the short term cause early emergence of mayflies (McKee and Atkinson, 2000). Climate changes alter precipitation pattern, leading to greater flood magnitude and frequency in certain rivers. This results in changes in ecological structure and function, and loss of diversity through too frequent scouring. With the continuing trend of temperature increase, the proportion of glacial melt and snow melt waters will change and lead to drastic changes in macroinvertebrate communities, including mayflies.

Ephemeroptera have been extensively used as bioindicators in aquatic biomonitoring programs (Srinivasan *et al.,* 2019), in biomarker studies and in ecotoxicological studies. During the last two decades, EPT concept has successfully emphasized the significance of Ephemeroptera, Plecoptera, and Trichoptera in describing environmental conditions (Lenat and Barbour, 1994). In the current investigation, we utilize multivariate examination strategy Canonical Correspondence Analysis (CCA) to establish the community structure of mayflies and Principal Component Analysis (PCA) to find the relationship between stations and environmental parameters. Multivariate investigation strategies have just been utilized to consider the connection between benthic macroinvertebrate community and ecological factors in all around the globe from the previous studies (Kazanci *et al.,* 2017; Duran and Akyildiz, 2011). As Palni and Cardamom hills belongs to the southern part of the Western Ghats, which is one of the eight hottest hotspots in the globe (Myers *et al.,* 2000) and no work had to be done in the population dynamics and community structure of mayflies in this part of Western Ghats, so this work aims to assess the relationship between mayfly community and environmental factors in Palni and Cardamom hills of Western Ghats.

Material and Methods

Study Area

Mayfly nymphs were collected from 2017- 2018 in thirty sites (16 from Palni hills and 14 from Cardamom hills) using 1m wide Kick-net (Burton and Sivaramakrishnan, 1993) with mesh size of about 1mm and they were identified with the help of various taxonomical literatures. Table 1 shows the descriptions of these sites. List of mayfly taxa of Palni and Cardamom hills is given in the Table 2 and 3 respectively.

Measuring Physicochemical Parameters

Water samples were collected from sampling sites and various physicochemical parameters like pH, dissolved oxygen, air temperature, water temperature, water current, width of the stream, hardness, alkalinity, total dissolved solids, conductivity and Biological oxygen demand were analyzed using APHA guidelines (2005).

Statistical Analysis

To view the trend of distribution of the stations based on environmental parameters the PCA (Principal Components Analysis) was used. PCA was calculated by using PAST software 4.02 (Hammer *et al.,* 2001). Mathematically, PCA consists of Eigen-analysis of a covariance or correlation matrix calculated on the original measurement data. To investigate the relationships between ecological factors and various stations, PCA was used. Canonical Correspondence Analysis (CCA) was made to found the community structure of mayflies in relationship to environmental variables.

Table 1. Details of the 30 study sites

S. No	Name of the study site	Abbreviation in PCA	Abbreviation in CCA	Stream order	Altitude (m)	Latitude (N°)	Longitude (E°)
1	Oothu	Ooth	P-1	3	1300	10º12'	77º26'
2	Perumalmalai	Peru	P-2	2	1400	10º18'	77º33'
3	Kurusadai	Kuru	P-3	2	1700	10º20'	77º28'
4	Ghandhi nagar	Gand	P-4	2	1600	10º18'	77º27'
5	Silver cascade	Silv	P-5	2	1700	10º12'	77º28'
6	Vattakanal	Vatt	P-6	2	1000	10º11'	77º25'
7	Fairy falls	Fair	P-7	3	290	10º13'	77º27'
8	Bear Shola falls	Bear	P-8	2	300	10º14'	77º27'
9	Fern hill falls	Fern	P-9	3	122	10º12'	77º20'
10	Pillar rock	Pill	P-10	1	2250	10º17'	77º28'
11	Near pillar rock	Near pill	P-11	1	2255	10º12'	77º30'
12	Pambar stream	Pamb	P-12	2	2248	10º13'	77º28'
13	Dhobikana	Dhob	P-13	3	2075	10º24'	77º24'
14	GundarFalls	Gund	P-14	2	2200	10º14'	77º26'
15	Poomparai	Poom	P-15	2	2133	10º13'	78º16'
16	Kounchi	Kouc	P-16	2	2360	10º29'	77º30'
17	Kurangani up	Kura-up	C-1	2	2410	11º00'	77º50'
18	Kurangani down	Kura-down	C-2	2	2345	11º00'	77º45'
19	B. L. Rave	B.L.Rave	C-3	1	1250	10º11'	77º25'
20	Poonthampanai	Poon	C-4	1	1300	11º12'	77º26'
21	Santhamparai near bridge	San-bridge	C-5	2	1350	11º00'	77º52'
22	Santhamparai near SDA school	San-SDA	C-6	4	1400	11º13'	77º28'
23	Mattupetty Dam stream	Matt	C-7	2	1700	11º16'	77º29'
24	Anayirankal stream	Anai	C-8	2	1950	11º18'	77º31'
25	Aranmanaiparai	Aran	C-9	3	2050	11º21'	77º33'
26	Popparai	Popp	C-10	4	1785	11º44'	77º26'
27	Bodimettu	Bodi	C-11	2	1500	11º15'	77º24'
28	Thoovanam falls	Thuv	C-12	3	1550	11º15'	77º15'
29	Nayamakkadu falls	Naya	C-13	2	2197	11º20'	77º10'
30	Chinnakanal falls	Chin	C-14	1	1800	11º27'	77º30'

Table 2. Species of Mayflies in Palni hills

ORDER	FAMILY	GENUS AND SPECIES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			Ooth	**Peru**	**Kuru**	**Gand**	**Silv**	**Vatt**	**Fair**	**Bear**	**Fern**	**Pill**	**Near pill**	**Pamb**	**Dhob**	**Gund**	**Poom**	**Koun**
	Baetidae	*Baetis acceptus*	11	12	8	31	11	19	22	19	27	17	11	17	17	24	30	17
		Baetis conservatus	8	17	11	11	8	21	18	11	17	20	23	22	18	26	19	32
		Tenuibaetis frequentus	12	21	22	8	17	43	52	29	29	33	42	32	20	42	17	42
		Baetis ordinates	8	11	5	0	0	0	14	5	10	5	10	19	10	27	42	12
		Labiobaetis geminatus	5	7	11	11	17	45	29	28	17	7	5	8	0	42	29	32
Ephemeroptera		*Centroptella similis*	12	11	20	21	17	46	23	17	12	11	0	21	0	0	17	14
		Centroptella ceylonensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Acentrella vera	0	0	0	0	0	0	0	0	0	0	0	0	12	11	0	11
	Heptageniidae	*Afronurus sp.*	26	0	0	22	0	0	0	0	0	0	0	0	34	41	0	0
		Afronurus kumbakkaraiensis	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
		Epeorus petersi	0	0	0	0	0	0	0	0	0	10	0	0	0	26	0	0
		Thalerosphyrus flowersi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0
	Leptophlebiidae	*Choroterpes alagarensis*	22	18	0	47	41	39	52	37	23	47	32	38	65	27	0	35
		Edmundsula lotica	0	0	17	10	0	33	29	27	15	53	62	31	24	17	17	33
		Indialis badia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	13
		Isca purpurea	24	19	21	25	41	43	21	26	37	27	18	23	36	28	0	39
		Nathanella indica	10	5	17	0	0	0	19	22	27	0	5	11	0	0	0	0
		Notophlebia jobi	5	0	11	0	23	16	6	17	17	7	0	23	0	0	21	0
		Petersula courtallensis	15	0	0	20	17	18	14	10	18	11	11	10	17	14	17	0
	Neoephemeridae	*Potamanthellus ganges*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
		Thraulus gopalani	0	0	6	4	0	8	5	9	0	0	13	21	0	0	21	0
	Teloganodidae	*Teloganodes dentata*	11	8	17	14	17	11	8	21	14	24	19	12	19	0	32	32
		Teloganodes kodai	17	14	8	27	14	21	22	23	33	16	62	42	0	0	27	28
		Teloganodes insignis	13	6	15	8	19	0	0	0	0	14	5	45	52	38	54	0
	Ephemeridae	*Ephemera nadinae*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52
	Caenidae	*Caenis* sp.	32	14	8	10	27	12	21	32	29	13	18	7	8	12	29	36

Table 3. Species of Mayflies in Cardamom hills

ORDER	FAMILY	GENUS AND SPECIES	17	18	19	20	21	22	23	24	25	26	27	28	29	30
			Kura-up	**Kura- down**	**B.L.Rave**	**Poon**	**San-bridge**	**San-SDA**	**Matt**	**Anai**	**Aran**	**Popp**	**Bodi**	**Thuv**	**Naya**	**Chin**
	Baetidae	*Baetis acceptus*	0	0	17	17	0	0	0	42	0	0	22	0	41	12
		Baetis conservatus	0	0	11	15	0	0	0	31	0	19	0	12	29	32
		Tenuibaetis frequentus	42	21	29	42	13	21	32	31	17	23	33	27	17	27
		Baetis ordinatus	0	0	33	27	31	17	17	21	22	17	22	18	23	30
		Labiobaetis geminatus	32	17	21	41	0	39	32	42	17	10	17	27	37	41
Ephemeroptera		*Centroptella similis*	37	21	17	31	34	13	33	27	0	15	16	22	0	30
		Centroptella ceylonensis	0	0	16	34	5	25	29	0	11	0	0	0	17	0
		Acentrella vera	0	0	25	0	0	30	31	0	0	21	32	0	0	0
	Heptageniidae	*Afronurus sp.*	0	0	0	32	0	0	33	24	29	42	0	33	32	25
		Afronurus kumbakkaraiensis	37	17	42	42	15	19	47	37	33	32	21	37	29	33
		Epeorus petersi	31	20	33	27	23	22	31	33	12	42	32	33	37	12
		Thalerosphyrus flowersi	47	28	0	0	0	0	0	0	0	33	14	0	0	0
	Leptophlebiidae	*Choroterpes alagarensis*	62	32	52	21	33	41	21	35	27	55	32	41	24	32
		Edmundsula lotica	0	0	0	0	16	17	31	0	31	32	37	33	23	27
		Indialis badia	23	32	0	0	0	0	0	29	0	0	0	0	0	0
		Isca purpurea	5	0	15	33	21	55	36	44	32	17	13	31	21	30
		Nathanella indica	32	17	0	0	0	28	0	0	0	0	0	0	0	21
		Notophlebia jobi	13	11	27	13	14	10	19	0	35	27	20	27	30	21
		Petersula courtallensis	10	15	0	0	0	0	23	27	34	32	15	13	11	0
	Neoephimeridae	*Potamanthellus ganges*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Thraulus gopalani	17	11	0	0	0	0	0	0	25	0	0	0	5	0
	Teloganodidae	*Teloganodes dentata*	0	0	0	27	21	18	0	16	20	19	27	0	27	23
		Teloganodes kodai	0	0	0	0	23	21	43	0	34	0	14	17	10	0
		Teloganodes insignis	0	0	0	0	17	17	0	0	25	0	0	0	0	0
	Ephemeridae	*Ephemera nadinae*	98	5	0	0	0	0	0	0	0	0	0	0	0	0
	Caenidae	*Caenis* sp.	27	42	42	33	17	29	21	32	19	31	0	23	39	31

Results and Discussion

PCA Analysis of Palni Hills

Based on the scree plot results, three components were chosen in both Palni and Cardamom hills. PCA of Palni hills identified three principle components of water quality measures that explained 80.05% of the variation (Table 4) in water quality between streams. Figure 1 shows the PCA of Palni hills. PCA analysis of Palni hills categorize the sites into three groups which are referred as components namely component 1, component 2 and component 3.

Component 1 is positively correlated with the parameters like water temperature, air temperature and dissolved oxygen (DO), velocity and width of the stream (Figure 2), pH and hardness are not supporting these sites. The sites included in this component are Oothu, Fairy falls, Vattakanal, Perumalmalai, Kurusadai and Bear chola. In this component Perumalmalai is distantly placed.

The study sites in component 2 are Dhobikana, Poomparai and Pambar are highly correlated with Hardness, alkalinity, water temperature, air temperature and width of the stream (Figure 3). pH, conductivity, DO and BOD are negatively correlated with the component 2 sites. In this component, Dhobikana and Poomparai are distantly placed. Component 3 (Figure 4) includes Silvercascade, Kounchi, Pillar rock, near pillar rock, Fern hill, Gundar and Ghandhinagar. Here pH, water temperature, air temperature, conductivity, width and TDS are highly positively correlated whereas DO, velocity, hardness, alkalinity and BOD are negatively correlated. Fern hill is placed away from other sites.

Table 4. Variance explained and the eigen values for the physico-chemical variables of Palni hills

Axis	Eigen value	% Variance
1	**2.94849**	**26.804**
2	**2.5278**	**22.98**
3	**1.86774**	**16.979**
4	**1.23612**	**11.237**
5	0.904433	8.2221
6	0.722309	6.5664
7	0.422977	3.8452
8	0.210023	1.9093
9	0.088112	0.80101
10	0.069933	0.63575
11	0.002067	0.018794

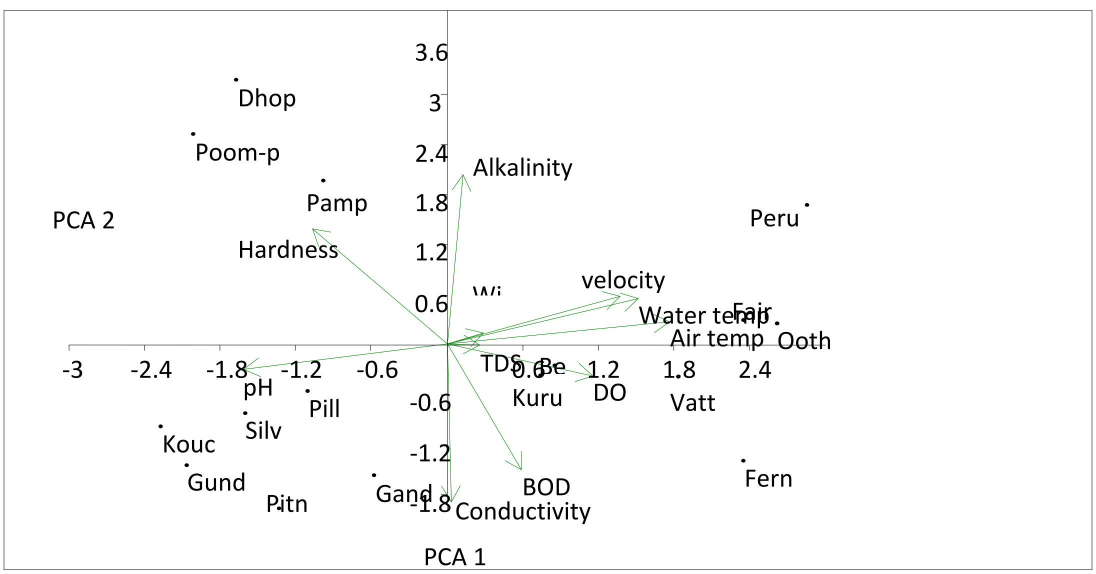


Figure 1. Principal component Analysis for Palni hills

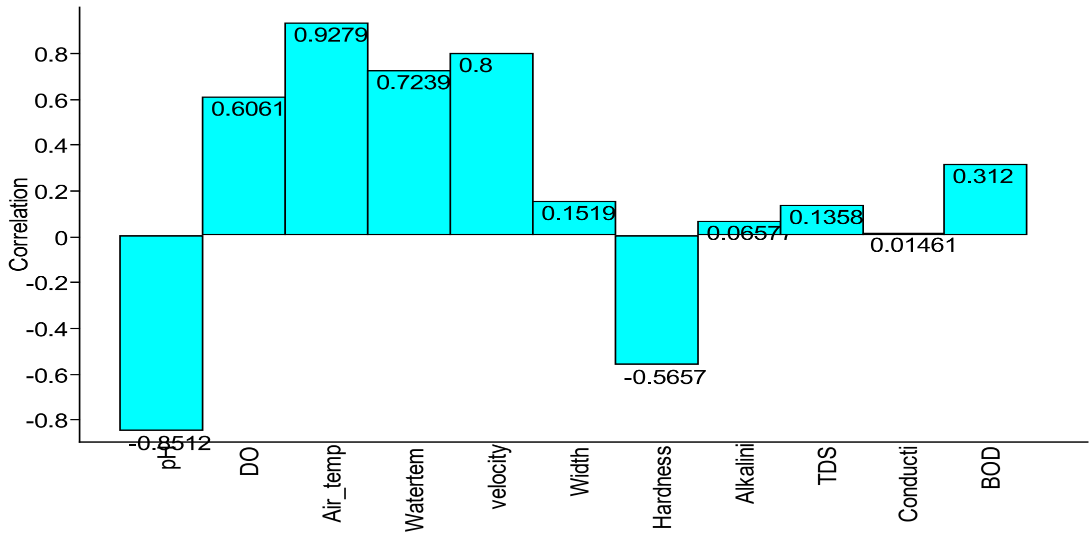


Figure 2. Component 1 of PCA in Palni hills

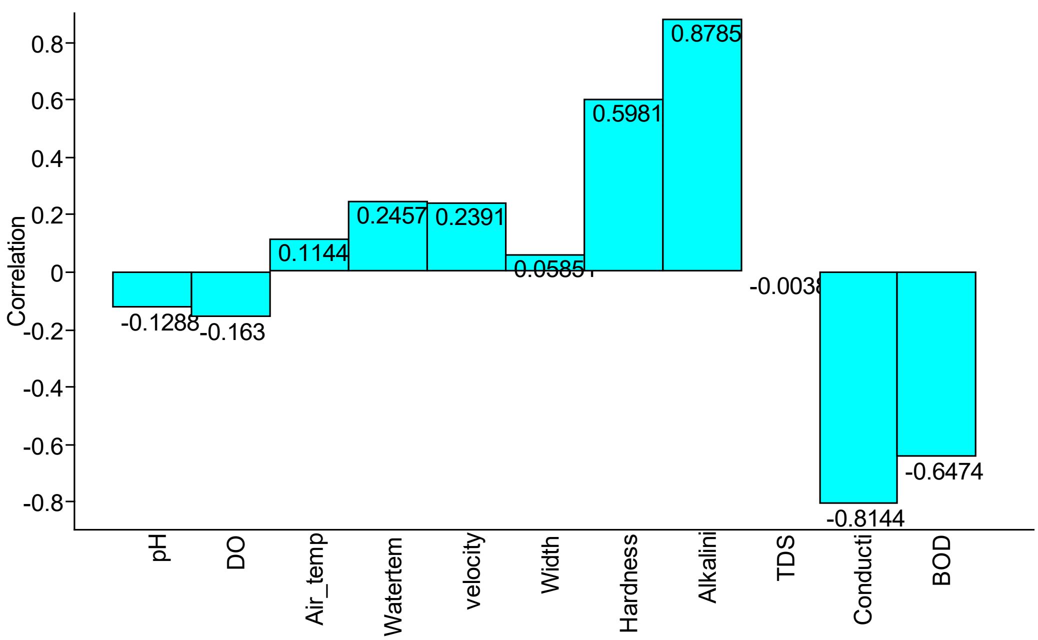


Figure 3. Component 2 of PCA in Palni hills

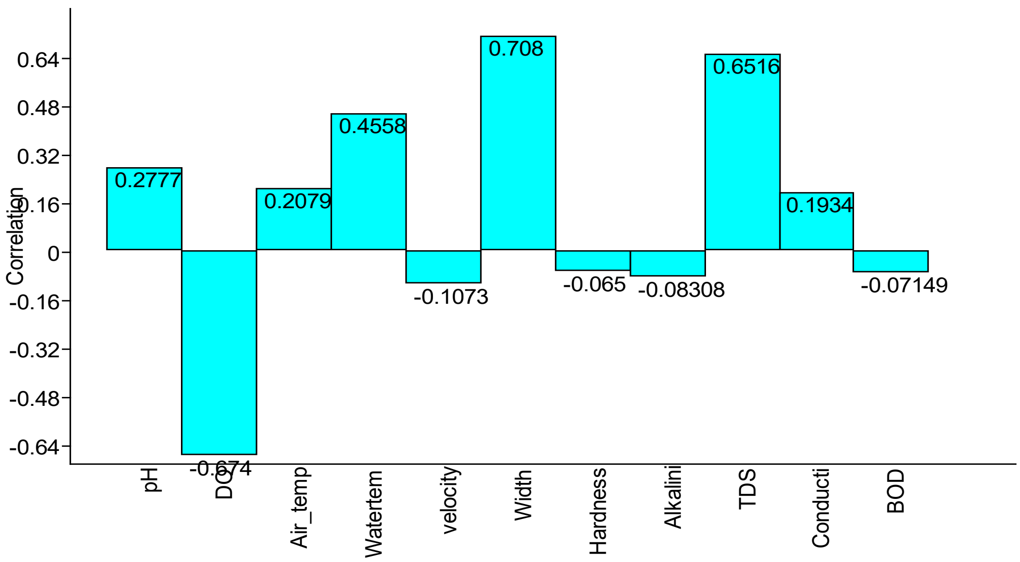


Figure 4. Component 3 of PCA in Palni hills

PCA Analysis of Cardamom Hills

PCA for Cardamom hill is shown in Figure 5. The first three components of the PCA for physicochemical parameters at the sites collectively explained 77% of the variability at these sites (Table 5).

In component 1 (Figure 6) DO, air temperature, water temperature, hardness, alkalinity, TDS, conductivity and BOD are positively correlated with the sites Popparai, Poonthampanai, Bodimettu, Anayirangal, Chinnakanal, Aranmanaiparai and Mattupatti. But these sites are negatively correlated with pH, velocity and width of the stream. Supporting factors of component 2 (Figure 7) are pH, velocity, width of the stream, hardness, BOD and alkalinity. Air temperature, water temperature, DO, TDS and conductivity are negatively correlated to the site Nayamakkadu. Nayamakkadu alone is placed under component 2.

Figure 8 representing component 3 shows that all the parameters are highly positively correlated with the sites. The sites included in this component are Kurangani up, Kurangani down, Thoovanam, B. L. Rave, Santhamparai SDA and Santhamparai near bridge. The site Santhamparai near bridge is plotted far away in the third components.

Table 5. Variance explained and the eigenvalues for the physico-chemical variables of Cardamom hills

Axis	Eigen value	% Variance
1	**3.58035**	**32.549**
2	**2.38903**	**21.718**
3	**1.77327**	**16.121**
4	**1.06369**	**9.6699**
5	0.880061	8.0006
6	0.468153	4.2559
7	0.416393	3.7854
8	0.222051	2.0186
9	0.118548	1.0777
10	0.062119	0.56472
11	0.026342	0.23947

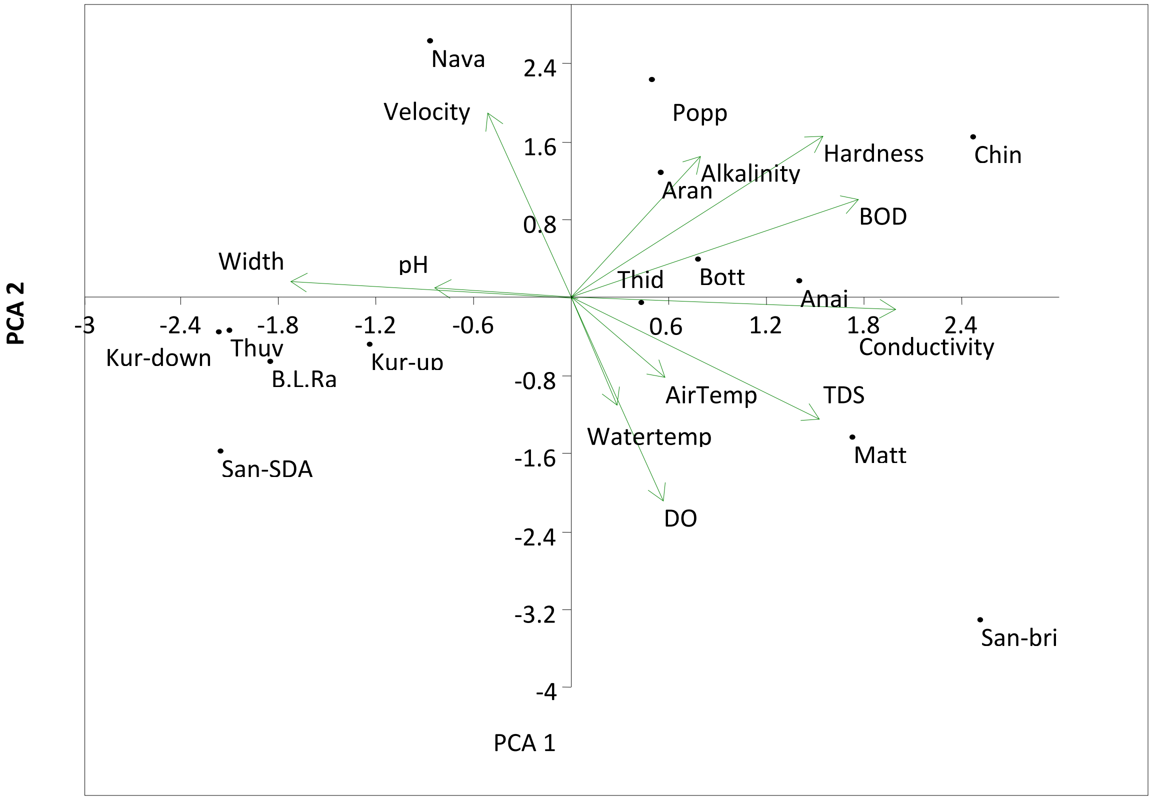


Figure 5. Principal component Analysis for Cardamom hills

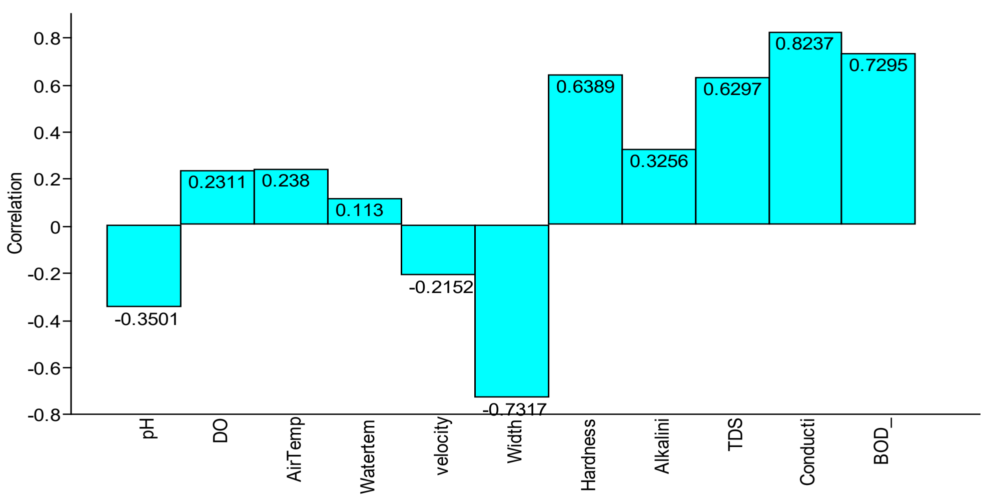


Figure 6. Component 1 of PCA in Cardamom hills

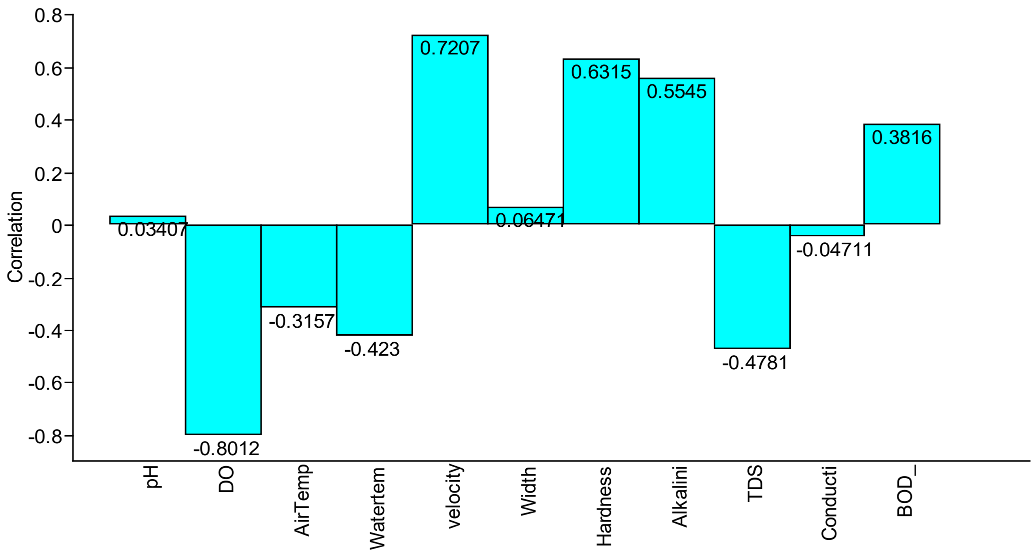


Figure 7. Component 2 of PCA in Cardamom hills

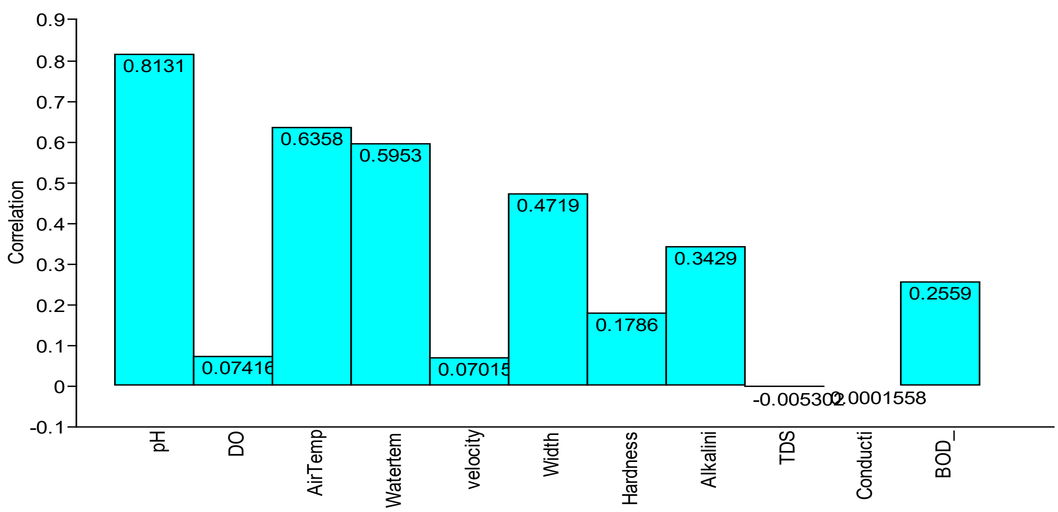


Figure 8. Component 3 of PCA in Cardamom hills

pH

Mayfly nymphs are sensitive to low pH. In Palni hills components 1 and 2 are negatively correlated with pH whereas, component three is positively correlated. The pH of the sites ranges between 6.3-7.52 (Table 6). Due to anthropogenic impacts, the pH is slightly acidic in certain sites like Vattakanal. Streams typically have a slightly basic pH value ranging from 7 to 8. Most organisms have optimal pH ranges in which they live that fall between 6 and 8 (Campbell and Wildberger, 2001). Even slight changes in the normal pH can have considerable effects on mayflies. In cardamom hills except component 1 the other two components are negatively correlated with pH. In this hill the pH ranges 6.0 to 7.4 (Table 7).

Dissolved Oxygen

The standard levels indicate that Dissolved Oxygen (DO) 4 – 7 mg/l is good for mayflies (Payne, 1986). DO plays a vital role in supporting aquatic life and is susceptible to slight environment changes and hence DO have been extensively used as a parameter delineating water quality and to evaluate the degree of freshness of a river (Fakayode, 2005). DO is positively correlated with component 1 of Palni and Components 1 and 3 are positively correlated in Cardamom hills. The DO in the sites was found within the range (4 -7.7 mg/L) (Table 6).

Water Temperature and Air Temperature

Based on the earlier works it is understood that the positive correlation with the water temperature and air temperature plays a major role in maintaining the number of organisms. The PCA results of both the hills show positive correlation for Water temperature and air temperature except component 2 in cardamom hills.

Alkalinity

Total alkalinity of water is due to presence of mineral salt present in it. It is primarily caused by the carbonate and bicarbonate ions. Levels of alkalinity between 100 and 200 mg/L provide ideal buffering within a stream. But it has the permissible limit up to 600mg/L. In the present study the alkalinity falls under the permissible limit (345-367 mg/L). Among both the hills alkalinity show positive relation with all components except Component 3 of Palni hills.

Total Dissolved Solids (TDS)

Total Dissolved Solid is a measurement of inorganic salts, organic matter and other dissolved materials in water, TDS above 1340 mg/l may adversely affect mayflies (Good fellow *et al.,* 2000; SETAC, 2004). TDS and the associated elevated conductivity seem to be particularly toxic and stressor to mayfly community in the streams (Pond *et al.,* 2008). In the present study TDS is highly correlated with all the components.

Hardness

Total hardness is used to describe the effect of dissolved minerals (mostly Ca and Mg). Optimal values of hardness of aquatic life range from 100 to 200 mg/L. At levels above 250 mg/L, calcium carbonate will begin to precipitate. According to the results, hardness is negatively correlated with components 1 and 3 in Palni hills, and in Cardamom hills it is positively correlated with all the sites. The high value of total hardness may be due to discharge of sewage from nearby places, use of soaps and detergents by laundries, washing, bathing by people.

Biological Oxygen Demand (BOD)

Healthy streams which have BOD reading of less than 2 mg/l, whereas polluted streams is of 10 mg/L. In components 2 and 3 in Palni hills, the BOD is negatively correlated and the other components are positively correlated.

Conductivity

Most streams ranges between 50 to 1500 µS/cm. Ideal levels of conductivity for the mayfly richness ranges 150 to 500 µS/cm. In the study sites, components 2 of both the hills are negatively correlated.

Velocity and Stream Width

Velocity is correlated positively except component 3 for Palni hills and component 1 of Cardamom hills. Stream width is positively correlated except component 1 of Palni hills.

CCA Analysis

The relationship between the mayfly's communities and the environmental parameters was depicted by CCA (Canonical Correspondence Analysis). For CCA analysis, eleven environmental variables were used. Figure 9 shows the result of ordination of sites and Ephemeroptera with respect to environmental variables in Palni hills. From the outcomes, the destinations P-1, P-4, P-11, P-13, P-14 and P-16 were found away from the referenced ecological factors and were influenced by anthropogenic action and slightly polluted. The sites P-2, P-3, P-5, P-6 P-7, P-8, P-9 and P-10 were decidedly connected with factors like air temperature, water temperature, DO, BOD, TDS, conductivity and current speed whereas it is it is contrarily related with pH, altitude, hardness and alkalinity. Sites include P-12 and P-15 were positively correlated with pH, altitude, hardness and alkalinity and adversely related with air temperature, water temperature, DO, BOD, TDS, current speed and conductivity.

Table 6. Physico-chemical features, water quality parameters of the sampling sites in Palni hills

S. No	Site	pH	D.O (mg/L)	Air temp (ºC)	Water temp (ºC)	Current speed (sec m^{-1})	Width (m)	Hardness (mg/L)	Alkalinity (mg/L)	Total dissolved solids (ppt)	Conductivity (µs/cm)	BOD (mg/L)
1	Ooth	6.8	4.6	18.7	15	11	6 – 7	73	342	0.132	108	2.3
2	Peru	6.3	4.7	18.3	15.4	11	2 – 3	87	342	0.099	79	2.45
3	Kuru	7.3	4.2	17.7	16	6	2.5 – 3	83	312	0.089	87	3.88
4	Gand	7.35	4.3	16	14	5	2 – 3	90	288	0.093	112	2.65
5	Silv	7.36	4.2	15.7	13.4	3	5 – 10	98	299	0.084	103	2.34
6	Vatt	6.3	4.9	18.3	14	5	1 – 2	85	321	0.079	102	2.53
7	Fair	6.5	4.4	19.7	16.1	9	1 – 2	94	314	0.083	97	2.78
8	Bear	7.1	4	18.9	15	6	2 – 3	85	323	0.769	110	2.57
9	Fern	6.5	4.8	19	14.6	8	1 – 2	102	312	0.094	125	4.12
10	Pill	7.47	4.4	15	11.4	6	0.5 – 1	90	343	0.122	105	3.23
11	Near pill	7.4	4.5	15.4	10	6	1 – 2	92	288	0.093	112	2.65
12	Pamb	6.9	4.3	15.8	13.2	5	2 – 3	112	345	0.098	93	1.3
13	Dhob	7.52	4	16.3	14.1	6	1 – 2	156	367	0.085	76	2.32
14	Gund	7.45	4	14.5	13	4	4 – 6	107	304	0.097	126	2.87
15	Poom	7.25	4.3	15.4	12	5	2 – 3	143	353	0.112	88	1.4
16	Kouc	7.3	4.4	14	10.5	4	0.25–0 .5	94	314	0.083	97	2.78

Table 7. Physico-chemical features, water quality parameters of the sampling sites in Cardamom hills

S. No	Site	Ph	D.O (mg/L)	Air Temp (ºC)	Water temp (ºC)	Current speed (sec m^{-1})	Width (m)	Hardness (mg/L)	Alkalinity (mg/L)	Total dissolved solids (ppt)	Conductivity (µs/cm)	BOD (mg/L)
1	Kura-up	6	6.7	21	18.5	3 – 4	10	72	312	0.077	80	1.74
2	Kura-down	7.3	6.4	22	18.4	4 – 5	11	82	267	0.089	79	1.32
3	B.L.Rave	7.2	6.3	23	19.1	4 – 6	8	48	333	0.082	92	0.89
4	Poon	7.4	6	22	20.2	3 – 4	10	94	384	0.132	108	2.3
5	San-bridge	6.5	7.7	23	18.3	2 – 3	5	87	267	0.688	124	1.65
7	San-SDA	7.4	6.8	23	20.2	4 – 5	11	55	237	0.073	86	1.54
6	Matt	6.4	7.4	24	19.1	3 – 4	4	89	306	0.129	105	2.65
8	Anai	7.1	7.4	24	20.3	3 – 4	8	173	435	0.1	89	3.46
9	Aran	6.5	6	23	16.5	4 – 6	7	102	354	0.093	106	2.32
10	Popp	7.5	5.2	23	18.4	5 – 6	12	156	345	0.098	105	4.32
11	Bodi	6.4	6.5	20	19.4	4 – 5	4	98	299	0.084	103	2.76
12	Thuv	6.4	6.8	22	17.1	3 – 4	10	61	321	0.043	79	1.32
13	Naya	6.8	4.1	21	16.4	4 – 5	8	143	342	0.098	93	1.3
14	Chin	6	6.3	22	18.3	5 – 6	3	156	345	0.096	106	4.35

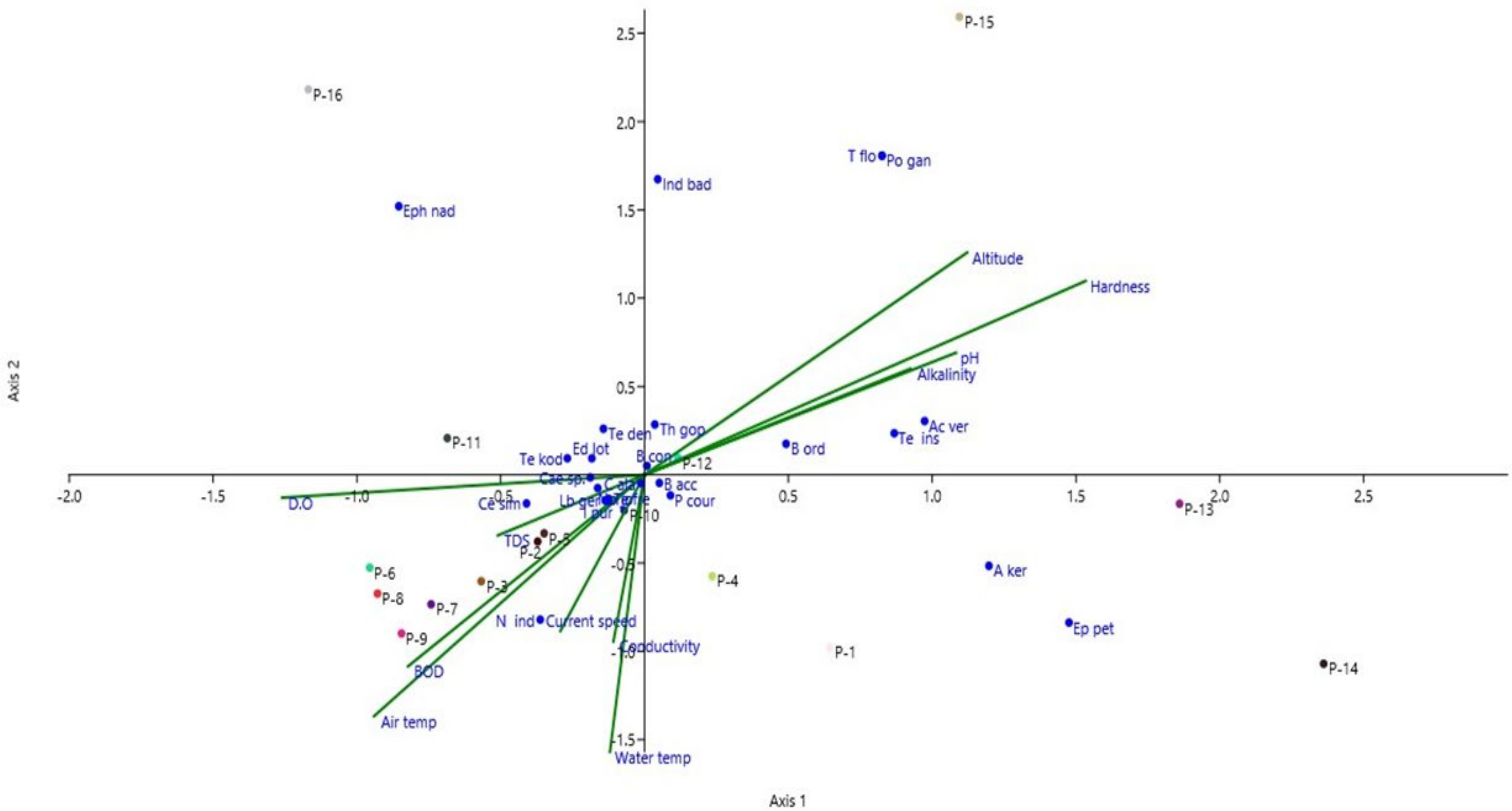
The taxa like B con- *Baetis conservatus,* B ord- *Baetis ordinatus*, Ac ver- *Acentrella vera,* T flo- *Thalerosphyrus flowersi,* C ala- *Choroterpes alagarensis,* Ed lot- *Edmundsula lotica,* Ind bad- *Indialis badia*, Po gan- *Potamanthellus ganges*, Th gop- *Thraulus gopalani,* Te ins- *Teloganodes insignis* gets upheld by environmental attributes like pH, altitude, hardness and alkalinity and negatively correlated with air temperature, water temperature, DO, BOD, TDS, conductivity and current speed. If riparian vegetation is high, there is an increase of pH in natural water (Wetzel, 2001). So the outcomes appears, the above taxa lean toward denser riparian environment for their source of living..Te fre- *Tenuibaetis frequentus*, Lb ger- *Labiobaetis geminatus,* Ce sim- *Centroptella similis,* Ce cey- *Centroptella ceylonensis,* A kum- *Afronurus kumbakkaraiensis,* C ala- *Choroterpes alagarensis,* I pur- *Isca purpurea,* N ind- *Nathanella indica,* No jo- *Notophlebia jobi,* Cae sp- *Caenis* sp gets enriched by the attributes like air temperature, water temperature, DO, BOD, TDS, conductivity and current speed and diminished by the factors pH, altitude, hardness and alkalinity. They don't lean toward low temperature and low dissolved oxygen, so above reference taxa just incline toward cool condition and they are the markers of natural contamination since they are profoundly tolerant to the acidic and basic situations. B acc- *Baetis acceptus*, A ker- *Afronurus* sp., Ep pet- *Epeorus petersi,* P cour- *Petersula courtallensis,* Te den- *Teloganodes dentata,* Te kod- *Teloganodes kodai* and Eph nad- *Ephemera nadinae* shows no relation to any of the above 11 environmental variables.

The ordination diagram of CCA (Figure 10) displays the sites of Cardamom hills with species of Ephemeroptera and environmental variables. They display variation in species composition over the sites. From the results, sites C-3, C-4, C-8, C-10, C-13 and C-14 shows positive correlation with BOD, pH, alkalinity and hardness and shows negative correlation with DO. Sites C-5, C-6, C-7, C-9, C-11 and C-12 shows positive correlation with TDS, conductivity and air temperature and negatively correlated with water temperature, current speed and altitude. Site 2 shows negative correlation with ecological attributes like TDS, conductivity and air temperature and shows positive correlation with water temperature, altitude and current speed.

Ce sim- *Centroptella similis,* T flo- *Thalerosphyrus flowersi,* N ind- *Nathanella indica,* P cour- *Petersula courtallensis* and Th gop- *Thraulus gopalani* were sensitive to low amount of DO and delicate to high discharge of BOD, pH, alkalinity and hardness. It proves the above reference organisms are good indictaors of water quality because they are highly sensitive to acidic, alkaline environment. B acc- *Baetis acceptus*, B con- *Baetis conservatus,* B ord- *Baetis ordinatus*, Lb ger- *Labiobaetis geminatus,* A ker- *Afronurus* sp., A kum- *Afronurus kumbakkaraiensis*, Ep pet- *Epeorus petersi,* P cour- *Petersula courtallensis,* Po gan- *Potamanthellus ganges* and Te den- *Teloganodes dentata* were sensitive to low BOD, pH, alkalinity and hardness and sensitive to high DO. It shows that *Baetis* genera are tolerant to acidic and alkaline environment. Taxa such as Ce cey- *Centroptella ceylonensis*, Ac ver- *Acentrella vera,* Ed lot- *Edmundsula lotica,* I pur- *Isca purpurea,* No jo- *Notophlebia jobi,* Te kod- *Teloganodes kodai* and Te ins- *Teloganodes insignis* shows sensitivity to low levels of TDS, conductivity and air temperature and shows sensitivity to high levels of water temperature, current speed and altitude. Te fre- *Tenuibaetis frequentus* and Cae sp- *Caenis* sp shows sensitivity to high levels of TDS, conductivity and air temperature and shows less sensitivity to high levels of water temperature, whereas Ind bad- *Indialis badia* shows less sensitivity to altitude and current speed

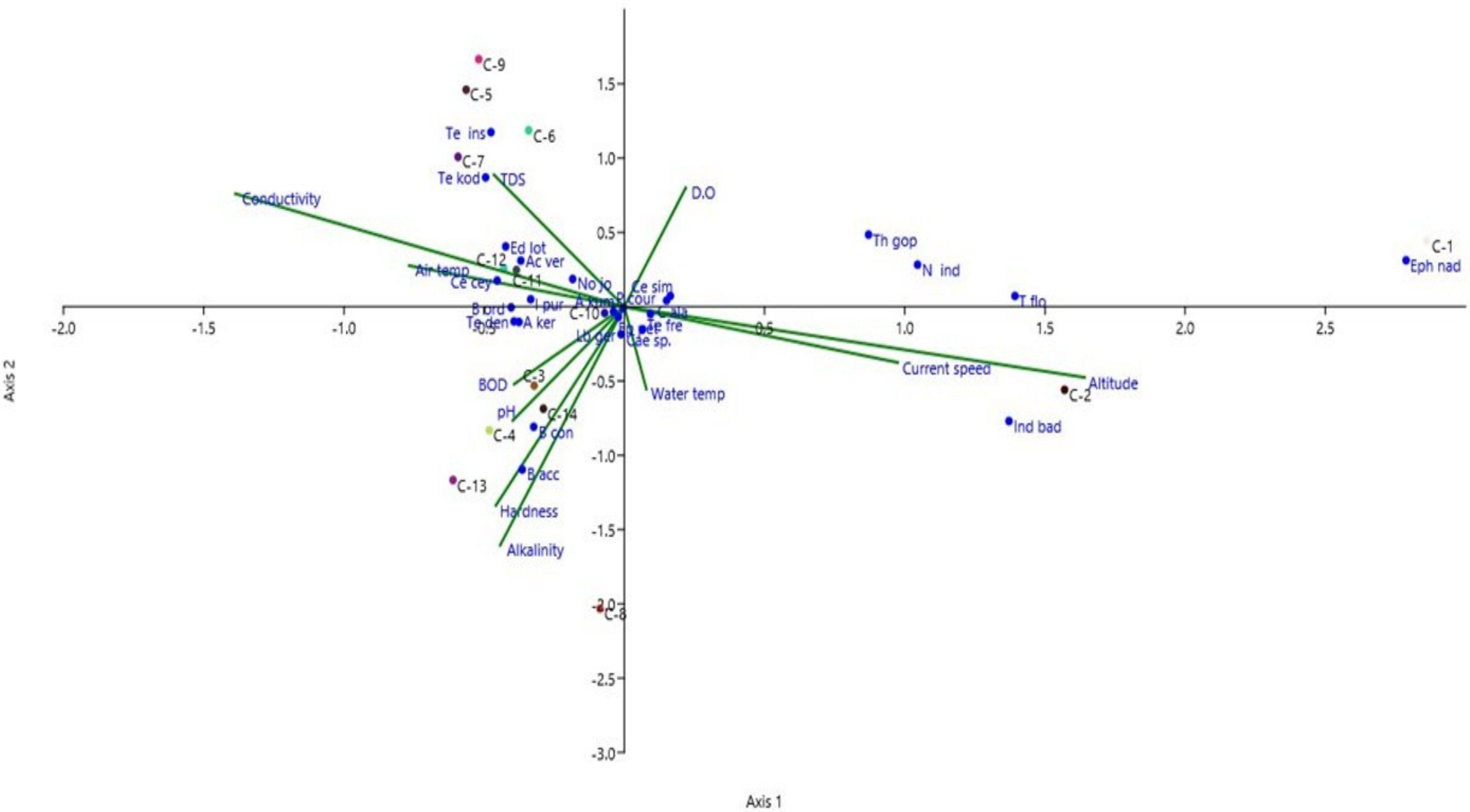
Site C-1, which is not delicate to any of the eleven ecological factors, and *Ephemera nadinae*, which additionally shows no relationship or availability with any of the variable. The CCA results of both Palni and Cardamom hills substantiates that the distribution and community structure of *Ephemera nadinae* is mysterious and still needs further more investigation of overwhelming metals and other environmental properties. *Baetis* and *Tenuibaetis* both shows distinctive community structure pattern, though they both originates from a single ancestor. Gencer Turkmen and Ozkan (2011) and Gencer Turkmen and Nilgun Kazanci (2020) suggests that *Baetis milani* is β-mesosaprobic, our results also substantiates with that as *Baetis* species are sensitive to high DO and it prefers β-mesosaprobic habitat. According *Caenis* sp. in both Palni and Cardamom hills prefers different habitats and it proves that more *Caenis* species complex were present in Palni and Cardamom hills. The outcome likewise demonstrates *Indialis badia* diversity and distribution were more in high altitudinal area. From the results, it is apparent that *Centroptella similis* prefer oligosaprobic environment.

It was noticed that locales Poomparai, Dhobikana, Kounchi of Palni hills and Kurangani up and down of Cardamom hills are plotted away from all the variables and the only reason that can be suggested is human impedance (i.e. Anthropogenic impacts).



(B acc- *Baetis acceptus*, B con- *Baetis conservatus,* Te fre- *Tenuibaetis frequentus*, B ord- *Baetis ordinatus*, Lb ger- *Labiobaetis geminatus,* Ce sim- *Centroptella similis,* Ce cey- *Centroptella ceylonensis*, Ac ver- *Acentrella vera,* A ker- *Afronurus* sp, A kum-*Afronurus kumbakkaraiensis* , Ep pet- *Epeorus petersi,* T flo- *Thalerosphyrus flowersi,* C ala- *Choroterpes alagarensis,* Ed lot- *Edmundsula lotica,* Ind bad- *Indialis badia*, I pur- *Isca purpurea,* N ind- *Nathanella indica,* No jo- *Notophlebia jobi,* P cour- *Petersula courtallensis,* Po gan- *Potamanthellus ganges*, Th gop- *Thraulus gopalani,* Te den- *Teloganodes dentata,* Te kod- *Teloganodes kodai,* Te ins- *Teloganodes insignis*, Eph nad- *Ephemera nadinae,* Cae sp- *Caenis* sp.)

Figure 9. Canonical Correlation Analysis of Palni hills



(B acc- *Baetis acceptus*, B con- *Baetis conservatus,* Te fre- *Tenuibaetis frequentus*, B ord- *Baetis ordinatus*, Lb ger- *Labiobaetis geminatus,* Ce sim- *Centroptella similis,* Ce cey- *Centroptella ceylonensis*, Ac ver- *Acentrella vera,* A ker- *Afronurus* sp., A kum- *Afronurus kumbakkaraiensis* , Ep pet- *Epeorus petersi,* T flo- *Thalerosphyrus flowersi,* C ala- *Choroterpes alagarensis,* Ed lot- *Edmundsula lotica,* Ind bad- *Indialis badia*, I pur- *Isca purpurea,* N ind- *Nathanella indica,* No jo- *Notophlebia jobi,* P cour- *Petersula courtallensis,* Po gan- *Potamanthellus ganges*, Th gop- *Thraulus gopalani,* Te den- *Teloganodes dentata,* Te kod- *Teloganodes kodai,* Te ins- *Teloganodes insignis*, Eph nad- *Ephemera nadinae,* Cae sp- *Caenis* sp.)

Figure 10. Canonical Correlation Analysis of cardamom hills

Conclusions

Based on PCA results, it tends to be reasoned that the sites in Palni hills, which are plotted far apart like Dhobikana, Fern hill and Poomparai, are not supported by the physico-chemical parameters like the other sites in Palni hills. This is absolutely a direct result of anthropogenic activity, because Fern hill and Poomparai are excursion spots where human obstruction is more in these streams. Whereas Dhobikana is a place where dhobis are involved in washing clothes so detergent pollution is more there. In cardamom, hills Santhamparai near bridge and Nayamakkadu are left far apart indicating they are not supported by physicochemical parameters, mainly due to pollution. However, Ephemeroptera were sensitive to water quality changes specially to DO, pH, conductivity and hardness their number is less in these stations compare with other stations. From the CCA results, it is found that *Baetis* species lean towards β-mesosaprobic habitat and *Indialis badia* favors high altitudinal area. Sites like Poomparai, Dhobikana, Kounchi of Palni hills and Kurangani up and down of Cardamom hills are plotted away from all the environmental variables.

In this work, it is discovered that not all physicochemical parameters are emphatically or adversely corresponded. But it is understood that pH, dissolved oxygen, BOD, hardness and alkalinity were the crucial factors. This agrees with numerous investigations that have been done previously (Steinman *et al.,* 2003). Dissolved oxygen is the key to the abundance of Ephemeroptera as oxygen is the significant component to all living creature to remain alive and slight changes on the pH likewise change the presence of Ephemeroptera.

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 in accordance with ethics committee procedures of animal experiments.

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Disclosure: -

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The fishes of the Bolaman Stream, Northern Turkey

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ABSTRACT

In this study, the fish species inhabiting the Bolaman Stream drains to the Black Sea from the Fatsa coast (Ordu Province, Turkey) was reported for the first time. The study was caught out non-periodically by sampling from seven stations in the Bolaman Stream between July 2017 and November 2018. Fish samples were captured with an electroshock device. With this study, it was determined that the fish fauna of the Bolaman Stream is represented by 10 species in five families (Acheilognothidae, Cyprinidae, Gobiidae, Leuciscidae, and Salmonidae). These species were as follows, respectively *Rhodeus amarus*, *Barbus tauricus*, *Capoeta banarescui*, *Neogobius fluviatilis*, *Ponticola turani*, *Alburnus derjugini*, *Squalius cephalus*, *Vimba vimba*, *Alburnoides fasciatus*, and *Salmo coruhensis*.

Keywords: Fish fauna, Fish taxonomy, New record, Inland waters

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Introduction

Since three-quarters of the world constitute an aquatic habitat, there is a continuous rise as a result of increasing scientific studies in the species number of fish having the fifty percentages of vertebrates. It was reported that this number has been 35672 by adding new species within the last quarter of 2020 (Fricke et al., 2020). In Turkey, the first ichthyo-faunistic study began in the first half of the nineteenth century by sending twenty marine species collected from Trabzon Province to British researcher (Abbot, 1835). In the last 15 years, it has been observed that the number of fish detected has increased from 236 (Kuru, 2004) to 384 (Çiçek et al., 2020) in Turkey. This number reached 391 with some recent additional records by Çiçek (2020), Kaya et al. (2020a; 2020b), and Kaya (2020). However, in another source, it was informed that this species number reached only 401 in freshwaters of Turkey (Froese and Pauly, 2019). Many studies in this area are still ongoing, and it is understood that Turkish inland waters have a potential for new species that have not yet been discovered. By redefining previously discovered fish species, species confusion is also being tried to be eliminated. There are many small streams of various sizes such as the Kurna, Tabakhane (Ünye), Çalış (Fatsa) Streams for, etc. in the Black Sea basin and have not been studied as ichthyofaunistic yet. The Bolaman stream in Ordu Province was one of them, also.

The Bolaman is a stream to flow into the Black Sea in the northern Turkish provinces of Ordu and Tokat. The stream was called Sidenus in antiquity. The Bolaman Stream rises in the Canik Mountains, a mountain range of the Pontic Mountains. The Bolaman stream continues its course to the north and pours into the Black Sea in the eastern of Fatsa (Anonymous, 2018).

In consider the other taxonomic studies conducted in a lake and several rivers in around Ordu province, in which have been Melet River, Ilıca and Yalıköy Streams, Gaga Lake, Turnasuyu Stream, Curi Stream, Elekçi Stream, Ilıca Stream and Tifi Brook (Turan et al., 2008; Darçın, 2014; Dönel and Yılmaz, 2016; Bostancı et al., 2015; 2016; Yılmaz, 2016; Saygun et al., 2017; Turan et al. 2017).

In this study, it was aimed to reveal actual taxonomic status of the fish species living in the Bolaman Stream.

Material and Methods

The study was carried out by sampling nonperiodically fish at seven sampling stations on the Bolaman Stream (Figure 1) between July 2017 and November 2018 as specified in the examined material section below. The Bolaman Stream initially flows along the provincial borders of Tokat and Ordu in a westerly direction. Later, it turns north and passes through the village of Zaferimilli in Ordu Province. It flows shortly afterward east past the city Aybastı. The Bolaman Stream then flows through the city Kabataş. Then the Gölköy Stream flows from the right into the stream. In Eleşi Brook meets the stream from the left (Anonymous, 2018). Detailed survey information (coordinates, altitudes, species, specimen quantity, and collection codes) of stations were listed in Table 1. At least five fish samples from each species were collected quarterly with an electroshock device (SAMUS™-725MP). After sampling, the fish specimens were firstly anesthetized with oil of cloves and after stopping breath then stored within a 4% formaldehyde solution in Fatsa Faculty of Marine Science (FFMS) of Ordu University (ODU) for species identification. Later, the meristic characters dorsal (D), pectoral (P), pelvic (V), and anal fin (A) ray numbers (spinous and branched rays) with lateral line (LL) scale counting were made. Lateral line scales were counts from the anteriormost scale (the first one to touch the shoulder girdle) to posteriormost one (at the end of the hypural joint) (Stoumboudi et al., 2006). Standard length (SL) was measured from the point of the snout to the end of the hypural joint (Stoumboudi et al., 2006). Head length (HL) was measured from the anteriormost part of the head (jaws closed) to the posteriormost point of the opercular bone, excluding spines and gills membrane (Holčík, 1989).

All the other morphometric characters changed from species to species were recorded in Microsoft Excel™ program by measuring digital caliper (Dasqua™) with 0.01 mm precision as methods reported by Holčík (1989). According to morphometric measurement results obtained from the study, the percentages of some metric characters of fish samples were calculated by proportioned of the standard length (SL%) and by the head length (HL%) for different fish families (Holčík, 1989; Bănărescu, 1999; Bănărescu and Bogutskaya, 2002; Bănărescu and Paepke, 2003; Miller, 2003; Verep et al., 2006; Turan et al., 2014; 2017).

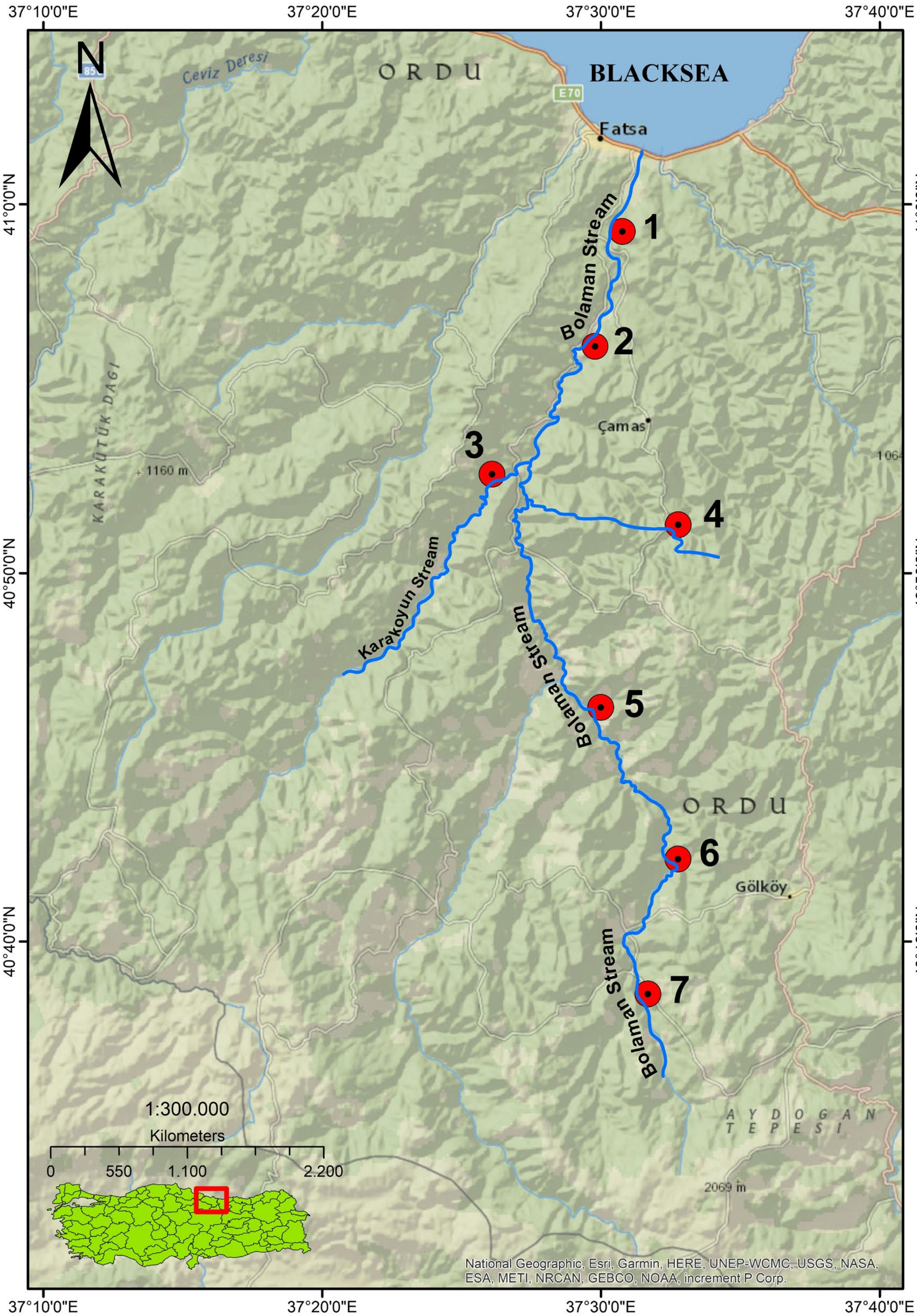


Figure 1. Fish sampling stations in the Bolaman Stream (Table 1)

Table 1. Distribution of fish species as sampling stations in Bolaman Stream, sp: sampling points.

sp	Coordinates	Altitudes (m)	Species						Sampling
			n	Cyprinoids	n	Gobioids	n	Salmonids	Date
1	40°59'15"N 37°29'55"E	24	2 17 5 9	*A. fasciatus* *B. tauricus* *C. banarescui* *S. cephalus*	2 11	*N. fluviatilis* *P. turani*		-	06.07.2017
2	40°56'23"N 37°29'32"E	55	1 11 23 10 4 20 5	*A. fasciatus* *A. derjugini* *B. tauricus* *C. banarescui* *R. amarus* *S. cephalus* *V. vimba*	2 12	*N. fluviatilis* *P. turani*		-	11.07.2017
3	40°52'28"N 37°26'01"E	163	5 11 3	*C. banarescui* *R. amarus* *S. cephalus*	4 2	*N. fluviatilis* *P. turani*		-	19.05.2018
4	40°51'14"N 37°32'47"E	753	33	*C. banarescui*		-	5	*S. coruhensis*	10.11.2018
5	40°45'21"N 37°30'38"E	526	1 10 4 2 3 9	*A. fasciatus* *A. derjugini* *B. tauricus* *C. banarescui* *R. amarus* *S. cephalus*	10	*P. turani*		-	30.06.2018
6	40°42'01"N 37°32'58"E	710	8 6 2 5 2	*A. derjugini* *B. tauricus* *C. banarescui* *R. amarus* *S. cephalus*	4	*P. turani*		-	30.07.2018
7	40°38'16"N 37°23'10"E	772				No sample			30.07.2018

Results and Discussion

As a result of the study, a total of 263 specimens of 10 species were sampled in six sampling points determined on the Bolaman Stream (Figure 2) but the seventh station because not come across to any fish species. The details of sampling stations, as well as the fish species discovered in each one of them, were presented in Table 1. There were described two species from Cyprinidae, a species from Acheilognothidae, four species Leuciscidae, two species from Gobiidae, and one species from Salmonidae in systematic order as follows. It was seen that cypriniform species were predominant as in other streams in the region and *Ponticola turani*, which is a Gobioidae species, was also observed to be dense (Figure 3).

When looked at Figure 2, the percentages of seven cypriniform species consisted of Cyprinidae, Acheilognothidae, and Leuciscidae were seen to be 81% (211 samples) of all specimens.

According to the sampling stations in the Bolaman Stream, all species, except *Salmo coruhensis*, were found in 2nd station. Although all the stations of *Capoeta banarescui* were also encountered but seventh station, which allowed no fish species. Only two species identified in the fourth station which were *C. banarescui* and *S. coruhensis* (Table 1).

The average percent data calculated according to some morphometric values of the fish species obtained in the Bolaman Stream were represented in Tables 2, 3, and 4. In these tables, the values were computed in percentages proportion to different morphometric data of standard length (SL) and head length (HL) (for all species). However, means ($\bar{x}$) of morphometric values percentage accounted standard deviations (±) of unclassified samples and distribution range (*m-M*) for each sample were given in the tables.

Table 2. Mean ($\bar{x}$) percentage values of some morphometric characteristics of Cypriniform species obtained from the Bolaman Stream according to standard length (SL) and head length (HL). ± Standard deviation, *m*-M minimum-maximum values.

	***Barbus tauricus* (n=50)**			***Capoeta banarescui* (n=57)**		
	$\bar{x}$	±	***m*-M**	$\bar{x}$	±	***m*-M**
Standard Length (mm)	106.50	16.31	72.61-139,62	97.70	21.02	59.39-142.62
In percent of standard length						
Maximum body height	20.13	1.76	17.31-24.39	20.58	1.43	16.42-22.56
Minimum body height	9.71	0.54	8.70-11.44	10.25	0.62	8.79-11.76
Caudal peduncle height	11.19	0.83	9.47-14.44	11.78	0.80	10.08-13.30
Predorsal distance	50.22	3.13	46.53-66.59	48.72	1.58	45.59-52.11
Postdorsal distance	37.46	2.86	34.04-52.73	37.19	1.73	32.86-40.17
Prepelvic distance	53.37	2.82	48.38-69.10	53.61	1.55	51.18-58.02
Preanal distance	74.20	2.23	64.74-77.86	73.94	2.28	68.90-80.10
Length of caudal peduncle	18.95	1.86	13.93-26.68	19.05	1.66	14.61-21.58
Length of dorsal fin	13.44	1.34	11.83-20.01	12.80	1.25	10.02-14.78
Dorsal fin height	19.50	1.51	16.45-25.73	20.08	1.53	17.45-24.13
Length of anal finbase	7.72	0.92	4.94-10.87	8.71	1.40	6.99-12.62
Depth of anal fin	18.09	1.32	15.51-23.23	17.71	1.40	12.46-19.54
Length of pectoral fin	18.52	1.30	15.83-23.81	18.36	1.02	15.99-19.64
Length of ventral (pelvic) fin	16.17	1.79	13.58-23.61	15.77	1.38	13.74-19.25
Distance between pectoral and pelvic fins	28.18	4.31	24.14-51.51	30.88	2.13	25.67-35.67
Distance between pelvic and anal fins	22.54	2.40	19.59-33.82	21.96	3.08	10.07-30.12
Body width	14.61	1.33	12.29-17.77	14.49	0.99	12.60-16.20
Caudal peduncle width	5.05	0.64	3.37-6.19	5.33	0.70	4.23-6.65
Head length	25.99	1.45	23.53-33.47	23.11	1.50	20.71-27.06
In percent of head length						
Preorbital distance (snout length)	41.25	2.13	37.33-48.13	31.95	2.98	25.36-37.95
Horizontal diameter of eye	17.68	2.31	14.39-23.54	18.05	2.50	13.18-22.96
Postorbital distance	43.67	2.48	37.09-47.57	48.09	2.62	39.69-53.62
Head depth (at nape)	58.13	3.36	50.98-66.98	65.23	6.13	59.19-94.45
Head depth (at center of eye)	44.39	3.61	39.02-53.04	48.37	2.73	42.56-55.51
Head width	51.96	4.37	43.10-63.47	55.64	5.49	38.87-68.00
Interorbital distance	30.18	2.20	25.07-34.84	36.18	3.37	23.50-42.75
Distance between nostrils	17.42	1.85	13.69-24.23	22.76	2.99	16.66-28-94
Length of anterior barbel	18.59	1.99	12.72-22.45	16.21	3.26	10.66-24.46
Length of posterior barbel	22.70	2.29	17.60-27.23	20.16	3.56	13.10-27.96

continuation of Table 2

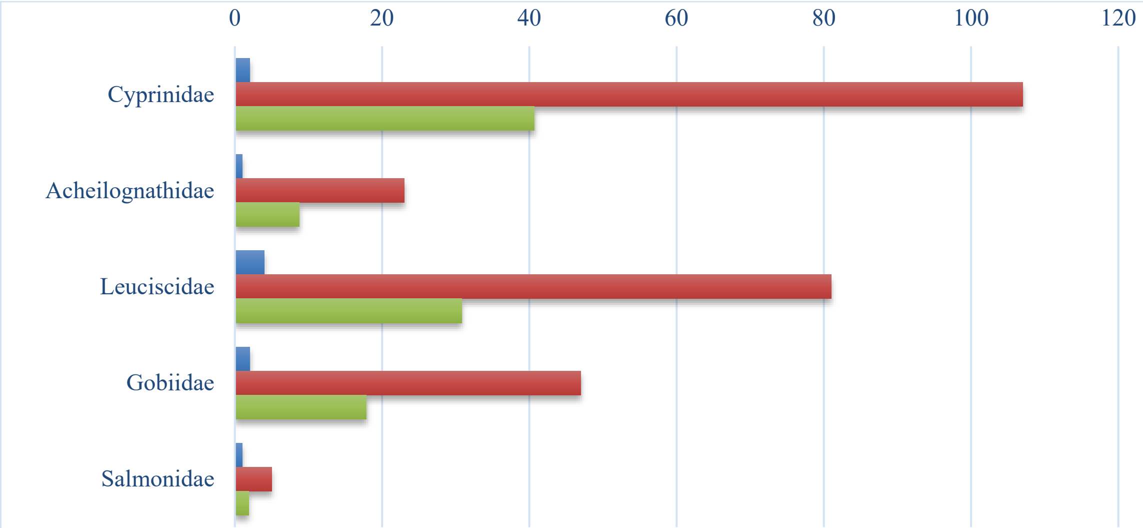
	Rhodeus amarus (n=23)		
	$\bar{x}$	±	*m*-M
Standard Length (mm)	106.50	16.31	72.61-139.62
In percent of standard length			
Maximum body height	21.73	1.21	19.04-23.69
Minimum body height	10.82	0.63	9.68-12.09
Caudal peduncle height	12.51	0.82	10.85-13.96
Predorsal distance	52.01	1.82	49.77-56.35
Postdorsal distance	36.77	2.07	31.50-39.74
Prepelvic distance	49.08	0.96	47.64-50.97
Preanal distance	70.64	4.40	67.08-87.50
Length of caudal peduncle	21.20	1.51	18.51-23.51
Length of dorsal fin	10.90	1.08	9.34-13.58
Dorsal fin height	18.30	1.46	15.74-20.98
Length of anal finbase	10.43	0.72	9.06-11.56
Depth of anal fin	15.90	0.85	13.80-17.11
Length of pectoral fin	17.04	1.16	14.38-19.18
Length of ventral (pelvic) fin	13.75	0.76	12.69-15.74
Distance between pectoral and pelvic fins	25.46	1.60	22.56-28.70
Distance between pelvic and anal fins	20.80	0.79	19.54-22.23
Body width	13.87	1.34	11.09-15.88
Caudal peduncle width	5.31	0.83	3.66-6.66
Head length	24.32	0.93	22.78-26.42
In percent of head length			
Preorbital distance (snout length)	27.87	2.63	23.60-32.68
Horizontal diameter of eye	21.26	1.87	17.85-25.38
Postorbital distance	52.71	3.11	47.32-61.55
Head depth (at nape)	66.29	4.18	54.33-72.77
Head depth (at center of eye)	48.77	2.61	42.98-54.57
Head width	55.14	4.83	45.17-64.33
Interorbital distance	36.93	3.22	31.57-42.90
Distance between nostrils	21.45	2.56	16.24-27.68

continuation of Table 2

	Alburnus derjugini (n=29)			*Squalius cephalus* (n=43)		
	$\bar{x}$	±	*m*-M	$\bar{x}$	±	*m*-M
Standard Length (mm)	102.79	15.84	62.24-133.19	97.49	25.98	57.74-150.22
In percent of standard length						
Maximum body height	19.98	0.95	17.43-21.77	26.61	1.12	25.88-28.83
Minimum body height	8.08	0.42	7.30-8.97	9.70	0.08	9.59-9.80
Caudal peduncle height	10.37	0.75	9.24-12.63	11.52	0.72	10.51-12.40
Predorsal distance	52.63	6.73	20.40-56.88	51.74	0.53	50.84-52.45
Postdorsal distance	35.34	1.75	32.86-40.30	38.70	1.66	35.80-40.18
Prepelvic distance	46.12	1.31	43.09-48.43	48.22	1.31	46.14-49.71
Preanal distance	64.82	2.01	60.86-70.23	66.89	1.91	64.57-70.16
Length of caudal peduncle	21.21	1.47	18.06-23.72	18.20	1.66	16.03-20.49
Length of dorsal fin	11.05	1.06	9.16-12.97	12.22	0.53	11.36-12.91
Dorsal fin height	16.52	1.54	13.73-19.99	21.34	2.32	18.08-24.16
Length of anal finbase	15.69	1.17	13.44-17.64	18.44	0.99	16.53-19.18
Depth of anal fin	12.36	1.32	10.13-15.24	13.46	1.05	12.04-14.77
Length of pectoral fin	18.02	1.40	15.21-20.44	18.07	0.98	16.80-19.36
Length of ventral (pelvic) fin	13.57	1.53	11.11-19.49	16.24	0.66	15.10-16.85
Distance between pectoral and pelvic fins	24.77	1.33	21.69-27.43	21.52	0.91	19.77-22.42
Distance between pelvic and anal fins	18.94	1.13	16.79-21.67	21.11	0.91	19.55-22.07
Body width	12.21	1.46	10.00-16.05	12.86	0.88	11.56-13.86
Caudal peduncle width	5.06	0.54	4.08-6.13	4.36	0.21	4.13-4.61
Head length	23.13	1.98	20.91-31.97	24.94	0.61	24.07-25.74
In percent of head length						
Preorbital distance (snout length)	29.78	3.13	20.32-34.63	31.47	2.75	28.79-35.39
Horizontal diameter of eye	25.55	3.35	18.43-31.75	21.98	1.11	20.16-23.55
Postorbital distance	43.65	4.44	30.11-49.70	46.43	2.42	42.85-49.61
Head depth (at nape)	63.73	5.24	44.66-73.64	71.46	3.69	67.69-78.12
Head depth (at center of eye)	47.65	4.08	35.50-54.81	53.06	2.65	50.58-56.89
Head width	45.61	3.56	31.75-49.05	48.34	1.08	46.49-49.39
Interorbital distance	28.78	3.59	20.94-40.58	31.86	2.33	29.46-35.77
Distance between nostrils	14.48	2.51	8.69-19.94	17.25	1.09	15.72-18.25

continuation of Table 2

	Vimba vimba (n=5)			*Alburnoides fasciatus* (n=4)		
	$\bar{x}$	±	*m*-M	$\bar{x}$	±	*m*-M
Standard Length (mm)	115.86	5.26	105.75-120.23	67.39	1.77	64.41-69.06
In percent of standard length						
Maximum body height	26.61	1.12	19.04-23.69	20.47	0.88	19.15-21.47
Minimum body height	9.70	0.08	9.68-12.09	8.25	0.26	7.90-8.57
Caudal peduncle height	11.52	0.72	10.85-13.96	9.77	0.37	9.18-10.11
Predorsal distance	51.74	0.53	49.77-56.35	42.67	2.07	41.28-46.24
Postdorsal distance	38.70	1.66	31.50-39.74	29.88	1.29	28.19-31.73
Prepelvic distance	48.22	1.31	47.64-50.97	39.20	2.03	36.80-42.41
Preanal distance	66.89	1.91	67.08-87.50	17.43	0.76	16.17-18.10
Length of caudal peduncle	18.20	1.66	18.51-23.51	16.05	0.49	15.65-16.89
Length of dorsal fin	12.22	0.53	9.34-13.58	11.93	1.17	10.53-13.76
Dorsal fin height	21.34	2.32	15.74-20.98	17.11	3.28	11.96-21.04
Length of anal finbase	18.44	0.99	9.06-11.56	14.98	2.08	11.78-17.13
Depth of anal fin	13.46	1.05	13.80-17.11	14.38	1.41	12.45-16.08
Length of pectoral fin	18.07	0.98	14.38-19.18	17.43	0.76	16.17-18.10
Length of ventral (pelvic) fin	16.24	0.66	12.69-15.74	14.63	0.59	13.65-15.21
Distance between pectoral and pelvic fins	21.52	0.91	22.56-28.70	20.11	0.84	18.79-21.07
Distance between pelvic and anal fins	21.11	0.91	19.54-22.23	16.99	2.26	14.39-20.58
Body width	12.86	0.88	11.09-15.88	10.31	0.37	9.90-10.86
Caudal peduncle width	4.36	0.21	3.66-6.66	4.59	0.37	4.08-5.11
Head length	24.94	0.61	22.78-26.42	20.06	0.17	19.89-20.34
In percent of head length						
Preorbital distance (snout length)	31.47	2.75	23.60-32.68	26.95	2.66	23.13-29.56
Horizontal diameter of eye	21.98	1.11	17.85-25.38	27.49	3.05	22.35-30.04
Postorbital distance	46.43	2.42	47.32-61.55	46.32	2.16	42.63-48.12
Head depth (at nape)	71.46	3.69	54.33-72.77	73.54	1.72	71.88-76.38
Head depth (at center of eye)	53.06	2.65	42.98-54.57	55.40	3.80	51.39-61.54
Head width	48.34	1.08	45.17-64.33	47.35	3.28	44.98-52.94
Interorbital distance	31.86	2.33	31.57-42.90	34.03	3.46	31.14-39.93
Distance between nostrils	17.25	1.09	16.24-27.68	15.92	2.42	13.09-19.78



	Salmonidae	Gobiidae	Leuciscidae	Acheilognathidae	Cyprinidae
Species Quantity	1	2	4	1	2
n (Sample)	5	47	81	23	107
n %	1,90	17,87	30,80	8,75	40,68

Figure 2. Families' distribution of Bolaman Stream according to specimen quantity

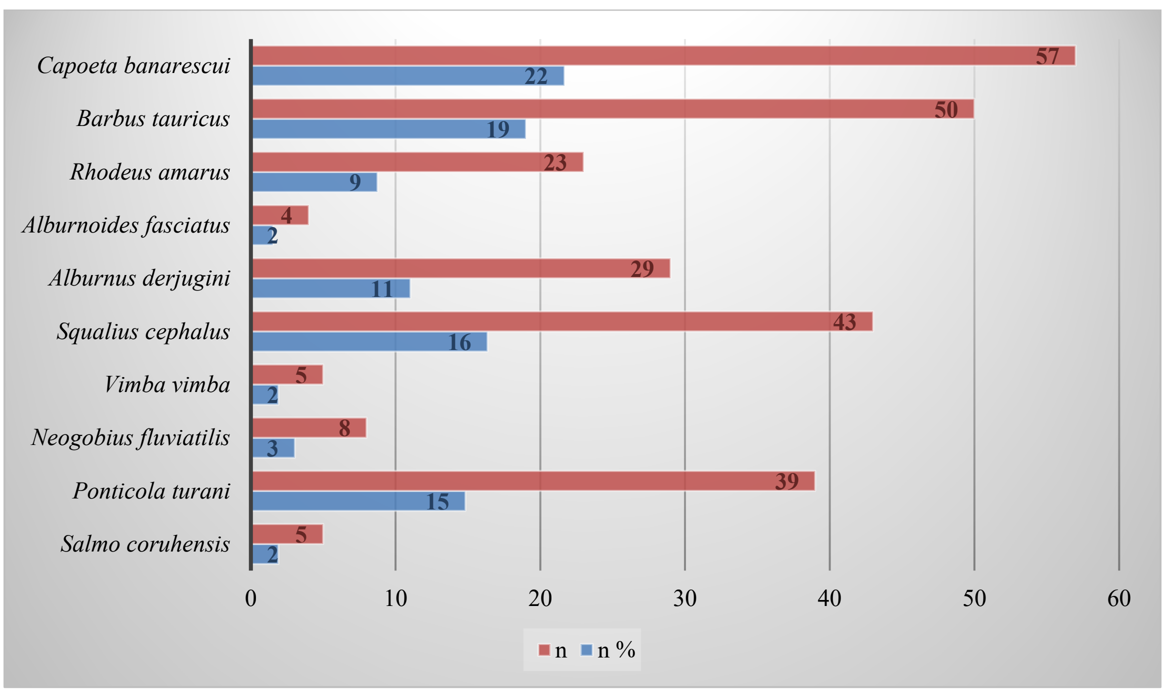


Figure 3. Number of samples (n) and percentage ratios (n %) of fish species obtained from Bolaman Stream

Family: Cyprinidae

***Capoeta banarescui* Turan, Kottelat, Ekmekçi & Imamoğlu, 2006** (Figure 4)

Examined Material. ODUFFMS 52410-06040, 5, 59.39-142.62 mm SL; Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52410-06041, 10, 83.52-123.74 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52420-06042, 5, 98.09-122.40 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018. – ODUFFMS 52430-06043, 33, 70.82-100.98 mm SL; Kestaneyokuşu, Çamaş/Ordu: Bolaman Stream; S. Saygun, 10 Nov 2018. – ODUFFMS 52600-06044, 2, 115.65-116.52 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018. – ODUFFMS 52600-06045, 2, 81.37-122.43 mm SL; Çetilli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jul 2018.

Figure 4. *Capoeta banarescui*, ODUFFMS 52410-06040, 142.62 mm SL; Turkey: Bolaman Stream

Capoeta banarescui including in Cyprinidae is known as one of the widely resident species in Turkish freshwaters through The Middle and East Blacksea Regions. Their distributions are only accepted from northeast Turkey from the Çoruh River system, which drains through Georgia and the Black Sea. Turan et al. (2006) reported that it was a different species from *Capoeta tinca*. In the study, *C. banarescui* was obtained at every stations except two sampling points as the second most common Cyprinoid species in the Bolaman Stream. The meristic characters of this barbel fish were designated as D I/7-8, A I/12-14, P I/14-15, V I/8 and LL 65-69. Morphometric ratios percent were shown in Table 2.

***Barbus tauricus* Kessler, 1877** (Figure 5)

Examined Material. ODUFFMS 52410-06030, 17, 72.61-139.62 mm SL; Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52410-06031, 23, 83.85-129.15 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52600-06032, 4, 76.92-127.27 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 10 Nov 2018. – ODUFFMS 52600-06033, 6, 88.39-125.70 mm SL; Çetilli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018.

Barbus tauricus is a barbel fish and the second most common species of cyprinoids in the Bolaman Stream. Results of morphometric measurement ratios percent procured from forty specimens of Crimean barbell were presented in Table 2. As the numerical counts of meristic characters were fixed to be D I/9, P I/8-9, V I/7-8, A I/5 and LL 50-58.

Figure 5. *Barbus tauricus*, ODUFFMS 52410-06030, 95.67 mm SL; Turkey: Bolaman Stream

Family: Acheilognothidae

***Rhodeus amarus* (Bloch, 1872)** (Figure 6)

Examined Material. ODUFFMS 52410-06070, 4, 47.30-57.06 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52430-06071, 11, 40.39-61.28 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018. – ODUFFMS 52600-06072, 3, 42.48-46.05 SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018. – ODUFFMS 52600-06073, 1, 36.22-60.16 mm SL; Çetilli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jul 2018.

Figure 6. *Rhodeus amarus*, ODUFFMS 52410-06070, 55.28 mm SL; Turkey: Bolaman Stream

The European bitterling (*Rhodeus amarus*) originates in Europe, ranging from the Rhone River basin in France to the Neva River in Russia. It was originally described as *Cyprinus amarus* by Marcus Elieser Bloch in 1782 and has been referred to in scientific literature as *Rhodeus sericeus amarus* (Kottelat & Freyhof, 2007). However, while it was previously estimated to found a subspecies of *Rhodeus sericeus* in Turkey's freshwaters, *R. amarus* was determined to be only one species belonging to Acheilognothidae (Tan and Armbruster, 2018) family in Turkey (Bektaş et al., 2013).

In this study, 23 European Bitterling specimens were caught at four stations (2nd, 3rd, 5th, and 6th stations) at the Bolaman Stream (Table 1). Results of the morphometric percent ratios of these specimens were represented in Table 2. Looking at meristic specialties being the other characters of bitterling, were counted that D I/8-9, A I/8-9, P I/6-8, V I/6 and LL 35-38 in the research.

Family: Leuciscidae

***Alburnoides fasciatus* (Nordmann, 1840)** (Figure 7)

Examined Material. ODUFFMS 52410-06010, 2, 52.48-56.11 mm SL, Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52420-06011, 1, 57.66 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52600-06012, 1, 56.07 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018.

Figure 7. *Alburnoides fasciatus*, ODUFFMS 52410-06010, 56.11 mm SL; Turkey: Bolaman Stream

In the study, the Transcaucasian Sprilin, *Alburnoides fasciatus* was obtained the least number of the sample with four specimens from the Bolaman Stream. Morphometric percent ratios of *A. fasciatus* samples was shown in Table 2. The meristic characters of this species were determined D I/8-9, A I/12-14, P I/11-12, V I/7 and LL 42-45.

***Alburnus derjugini* Berg, 1923** (Figure 8)

Examined Material. ODUFFMS 52410-06020, 11, 91.12-101.54 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52420-06021, 8, 95.85-128.94 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018. – ODUFFMS 52600-06022, 10, 62.24-133.19 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018.

Figure 8. *Alburnus derjugini*, ODUFFMS 52410-06020, 98.05 mm SL; Turkey: Bolaman Stream

The Georgian shemaya (*Alburnus derjugini*), a species of Cyprinoid fish in the genus *Alburnus* and collected also in the Bolaman Stream, distributed in eastern Black Sea tributaries, from south of the Caucasus in Russia and Georgia, to the south the Çoruh River in eastern Anatolia and to the west the Sakarya River (Freyhof, 2014). According to the latest published molecular phylogenetic study (Bektaş et al., 2020), *A. derjugini* was determined that synonymized species of *A. istanbulensis*, *A. carinatus* and *A. schischkovi*.

A. derjugini was one of the common cyprinoid species in the Bolaman Stream and had an 11% ratio into total fish samples in a rank of fourth (Figure 3). The countable characters of Georgian Shemaya were defined being D I/8, A I/13-14, P I/12-13, V I/8 and LL 57-60. Percent ratios as to metric measurements of 27 samples from the Bolaman Stream were represented in Table 2.

***Squalius cephalus* (Linneaus, 1758)** (Figure 9)

Examined Material. ODUFFMS 52410-06090, 9, 57.74-150.22 mm SL; Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52410-06091, 20, 65.65-138.14 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52420-06092, 3, 113.97-121.49 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018. – ODUFFMS 52600-06093, 9, 74.02-118.80 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018. – ODUFFMS 52600-06094, 2, 79.02-118.65 mm SL; Çetilli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jul 2018.

Figure 9. *Squalius cephalus*, ODUFFMS 52410-06090, 150.22 mm SL; Turkey: Bolaman Stream

The *Squalius* living from the rivers of the European and eastern Black Sea are usually identified as *S. cephalus* (Kottelat and Freyhof, 2007). *S. orientalis* is available for the 'Eastern' lineage while they temporarily use *S. cephalus* for the 'Western' lineage (Özuluğ and Freyhof, 2011). *Squalius* sampled in our study indicated that it belongs to the Western lineages. *Squalius cephalus* (Chub) sample shown in Figure 9 was one of the 43 samples procured from the Bolaman Stream. In the Table 2, morphometric ratios percent of this species were shown. The meristic results were detected D I/8, A I/8, P I/12-13, V I/7-8 and LL 42-44.

***Vimba vimba* (Linnaeus, 1758)** (Figure 10)

Examined Material. ODUFFMS 52410-06100, 5, 105.75-120.23 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017.

Figure 10. *Vimba vimba*, ODUFFMS 52410-06100, 120.13 mm SL; Turkey: Bolaman Stream

Vimba vimba species was one of the two species that collected the minimum number in the Bolaman Stream. Five specimens captured from the only the second station was measured and counted some metric characters. The counting characters were found D I/8, A I/13-14, P I/13-14, V I/8 and LL 50-54. Looking at the Table 2, the percentages of morphometric measurements ratios were given in this species.

Family: Gobiidae

***Neogobius fluviatilis* (Pallas, 1814)** (Figure 11)

Examined Material. ODUFFMS 52450-06040, 2, 82.55-94.24 mm SL; Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52410-06051, 2, 88.96-106.72 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52420-06052, 4, 59.56-105.93 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018.

Figure 11. *Neogobius fluviatilis,* ODUFFMS 52410-06050, 94.24 mm SL; Turkey: Bolaman Stream

Neogobius fluviatilis shown a specimen in Figure 11 were sampled eight specimens in the Bolaman Stream in this study. It was counted and measured their meristic and metric characters of Monkey goby specimens. The meristic characters were indicated to be D1 V, D2 14-18, A I/13-14, LL 66-69. In the Table 3, morphometric percent ratios of this species were shown.

***Ponticola turani* (Kovačić & Engin, 2008)** (Figure 12)

Examined Material. ODUFFMS 52410-06060, 11, 79.32-111.09 mm SL; Turkey: Karataş, Fatsa/Ordu: Bolaman Stream; S. Saygun, 06 Jul 2017. – ODUFFMS 52410-06061, 12, 84.35-108.18 mm SL; Örencik, Fatsa/Ordu: Bolaman Stream; S. Saygun, 11 Jul 2017. – ODUFFMS 52420-06062, 2, 67.67-91.32 mm SL; Dere, Çatalpınar/Ordu: Karakoyun Stream; S. Saygun, 19 May 2018. – ODUFFMS 52600-06063, 10, 77.00-110.98 mm SL; Direkli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jun 2018. – ODUFFMS 52600-06064, 4, 67.94-91.89 mm SL; Çetilli, Gölköy/Ordu: Bolaman Stream; S. Saygun, 30 Jul 2018.

Figure 12. *Ponticola turani*, ODUFFMS 52410-06060, 111.09 mm SL; Turkey: Bolaman Stream

Aksu goby, one of the endemic species of Turkey, was the fourth most caught species in the Bolaman Stream (Figure 3). Percent ratios as to metric measurements of specimens from the Bolaman Stream were represented in Table 3. Countable characters were found to be D1 VI, D2 15-16, A I/11-14, LL 60-65.

Family: Salmonidae

***Salmo coruhensis* Turan, Kottelat & Engin, 2010** (Figure 13)

Examined Material. ODUFFMS 52430-06080, 5, 116.94-230.50 mm SL; Kestaneyokuşu, Çamaş/Ordu: Bolaman Stream; S. Saygun, 10 Nov 2018.

The Çoruh trout, one of the endemic species in North inland waters of Turkey, was observed in this study, too. *Salmo coruhensis*, which is living commonly in cold streams of Eastern Black Sea in Turkey and which is described as a new endemic species by Turan et al. (2009), is still accepted as a valid species in taxonomic literature but, in a molecular study performed by Kalaycı et al. (2018) it was reported that this species and similar salmonid species are from the Danube lineage of brown trout (*Salmo trutta*). *S. coruhensis,* which naturally lives in higher places compared to other species, has also been found at approx. 753m altitude (Table 1) of the stream in this study. In the seventh station, the highest sampling point, it was unbelievable not to come across neither salmonids nor any fish species. The meristic data were counted D I/10-12, A I/9-10, P I/12, V I/8-9 and LL 87-90.

Figure 13. *Salmo coruhensis*, ODUFFMS 52430-06080, 230.50 mm SL; Turkey: Bolaman Stream

Some morphometric characters of *S. coruhensis* specimens were accounted for as a percentage ratio according to standard length (SL) and head length (HL). Morphometric percentage ratios of this species were shown in Table 4.

Conclusion

In this study, the number of species in the Bolaman Stream was also the highest in the Cyprinoid species with about 80% (211 samples), but the *Barbus* and *Capoeta* species were approx. 41% of the total sample number (Figure 2). According to the distribution of fish species in the stream, the least intense sampled species were about 2% of *Alburnoides fasciatus*, *Salmo coruhensis* and *Vimba vimba*. The *Ponticola turani* (approx. 15%) was the most common forth species after *C. banarecui*, *B. tauricus* and *S. cephalus*. As a single species, *Salmo coruhensis* from Salmonidae family and *Rhodeus amarus* species from Acheilognothidae family were obtained. However, when the distribution in stations of the samples obtained in the study was examined, the second station (nine species) has the highest number of species compared to other sampling points.

Squalius cephalus and *S. orientalis* are two species that are similar to each other and have been difficulty distinguished. Berg (1949) had identified *S. cephalus* from *S. orientalis* (as subspecies of *S. cephalus*) by the number of branched anal-fin rays (usually $8^{1/2}$ in *S. cephalus* vs. usually $9^{1/2}$ in *S. orientalis*) and body shape (body more elongate in *S. orientalis*) (Özuluğ and Freyhof, 2011). 95% of the 43 samples (ODUFFMS 52410-06090) obtained in this study had the specified feature which are quite elongate and have all $8^{1/2}$ branched anal-fin rays.

As the conclusion of this study, for the first time, it was identified ten species in five different genera belonging to five families (Acheilognothidae, Cyprinidae, Gobiidae, Leuciscidae, and Salmonidae) in the Bolaman Stream. During the sampling performed, it was seen that there is pollution in sections, which also is less the water than the main riverbed and even in the high parts of the stream. Moreover, it was determined that there are few or no species at some stations, where environmental conditions threaten the habitats of the fish in the research. According to data obtained from samplings conducted in the study during the summer months, the Hydroelectric Power Plants founded on the Bolaman Stream have been observed that been threaten enough water regime for the survival of fish. Contrary to what I expect in this study, it is possible to say that environmental conditions threaten the habitat of fishes along the stream as a result of taking more fish samples of different species from small streams that flow into the Bolaman Stream and are relatively cleaner than the stream. The fact that no samples of any species were not obtained from a station (7^{th} st) on one of the highest elevations can be also an indication of this. Monitoring of changes threatening the future of fauna and flora in the stream and more detailed physicochemical and taxonomic studies are needed within or after a decade.

Table 3. Mean ($\bar{x}$) percentage ratios of some morphometric characters of Gobioid species obtained from the Bolaman Stream according to standard length and head length. ± Standard deviation, *m*-M minimum-maximum values

	***Neogobius fluviatilis* (n=8)**			***Ponticola turani* (n=39)**		
	$\bar{x}$	±	***m*-M**	$\bar{x}$	±	***m*-M**
Standard Length (mm)	83.46	17.68	59.56-106.72	93.35	11.33	67.67-111.09
In percent of standard length						
Length of head	26.84	0.82	25.78-28.39	27.50	1.66	22.01-30.14
Head depth (at nape)	15.27	1.36	13.48-17.13	18.16	2.13	13.61-24.59
Predorsal distance 1	31.84	1.46	29.67-34.13	32.71	3.09	27.53-48.80
Predorsal distance 2	46.34	2.83	39.83-49.50	47.37	1.68	43.88-50.93
Pength of dorsal fin 2	40.30	1.10	37.73-41.73	46.25	2.50	38.36-51.72
Dorsal fin heigth2	13.76	1.57	11.48-17.06	29.40	3.60	22.50-41.84
Length of pectoral fin	23.14	2.39	19.17-27.37	21.82	2.87	11.55-29.16
Length of pelvic fin	19.20	1.82	16.19-21.90	16.45	1.18	14.17-18.79
Length of anal finbase	30.95	2.45	26.82-35.63	27.42	1.76	23.04-31.03
Length of caudal peduncle	18.23	1.62	15.81-20.69	17.78	2.33	12.90-22.74
Minimum body height	7.02	0.26	6.53-7.37	9.56	0.56	8.40-10.60
Maximum body height	17.06	1.40	14.52-19.11	19.98	1.33	16.19-22.52
Head width	17.19	2.08	15.21-22.07	21.58	1.73	18.24-25.44
In percent of head length						
Preorbital distance	35.21	1.56	33.90-39.13	32.05	5.03	22.88-48.68
Horizontal diameter of eye	17.74	1.75	14.84-21.32	19.31	2.15	15.23-24.01
Postorbital distance	50.18	2.82	46.17-54.67	52.18	6.16	41.65-74.13
Head depth (at nape)	56.98	5.88	48.30-65.35	66.19	8.08	53.04-89.67
Head width	64.08	7.78	55.04-81.01	78.74	7.74	64.01-98.03
Interorbital distance	15.99	3.43	9.84-21.77	13.44	2.56	8.64-21.18

Table 4. Mean ($\bar{x}$) percentage ratios of some morphometric characters of *Salmo coruhensis* from the Bolaman Stream to standard length and head length, ± Standard deviation, *m*-M minimum-maximum values

	***Salmo coruhensis* (n=5)**		
	$\bar{x}$	±	***m*-M**
Standard Length (mm)	163.49	50.69	116.94-230.50
In percent of standard length			
head length	25.98	2.01	24.11-29.88
Maximum body height	21.96	1.20	20.59-23.90
Minimum body height	9.28	0.73	8.24-10.28
Predorsal distance	45.06	1.26	43.64-46.76
Postdorsal distance	41.73	2.08	39.63-45.55
Length of adipose finbase	4.00	0.49	3.20-4.58
Distance between adipose and caudal finbases	16.94	1.07	15.35-18.56
Prepelvic distance	54.19	1.84	51.97-56.82
Preanal distance	70.91	1.01	69.55-72.21
Distance between pectoral and pelvic fins	30.00	0.86	29.05-31.32
Dist. between pelvic and anal fins	19.62	0.61	18.91-20.34
Length of caudal peduncle	19.09	1.54	16.79-21.15
Length of dorsal fin	13.89	0.89	12.77-15.07
Dorsal fin heigth	18.47	1.62	15.86-20.16
Length of anal finbase	10.84	1.14	9.76-12.81
Depth of anal fin	15.44	1.41	13.21-16.73
Length of pectoral fin	18.13	1.07	17.28-20.23
Length of pelvic fin	14.22	0.69	13.11-15.16
In percent of head length			
Head depth (at nape)	61.82	5.17	54.11-69.50
Head depth (at center of eye)	47.87	3.53	42.68-52.10
Preorbital distance	27.65	1.17	25.97-29.02
Horizontal diameter of eye	20.41	1.25	18.97-22.70
Interorbital distance	29.24	1.46	27.52-31.43
Postorbital distance	50.57	2.85	47.34-54.91
Depth of upper jaw	12.39	0.71	11.11-13.27
Upper jaw length	48.63	2.14	46.07-51.13
Lower jaw length	68.38	10.00	59.09-83.96

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 approved with Document Number and Date of 82678388 / 27.01.2016 given by Ordu University Animal Experiments Local Ethics Committee Approval Document.

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Short Communication

A review on maximum length of the greater weever *Trachinus draco* Linnaeus 1758 (Perciformes: Trachinidae) with a new maximum length from Oran Bay (Western Algeria)

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ABSTRACT

On the 15th April 2017, one female specimen of the greater weever, *Trachinus draco* measuring 44.69 cm in total length and weighting 885 g was captured by trammel net in Oran Bay (Cape Rousseau) at 120 m depth. Up to date, this length is a new record of maximum length reached for this trachinidae for Algerian waters and the second maximum length recorded in Mediterranean basin according to Fischer *et al.*, 1987 observation noted at 45 cm.

Keywords: The greater weever, *Trachinus draco*, Maximum size, Oran Bay, Mediterranean Sea

Introduction

The greater weever is a trachinidae found in Eastern Atlantic; Norway to Morocco, Madeira and Canary Islands, including the Mediterranean and the Black Sea (Fischer *et al*., 1987; FishBase: Froese and Pauly, 2020). *Trachinus draco* Linnaeus 1758 inhabits sandy, muddy or gravelly bottoms, from a few meters to about 150 m. Rest on the bottom, often buried with eyes and tip of first dorsal fin exposed (Frimodt, 1995). The first dorsal fin rays, as well as the spine on the pre-operculum contains venomous spines protecting the species from predators. During night, the greater weever leaves the burrow to feed on small invertebrates and fishes (Carpenter *et al*., 2015). At night, it also swims around freely, even pelagically (Muus and Nielsen 1999). *T. draco* is oviparous, eggs and larval stages are pelagic (Tortonese, 1986). There are dark markings along the scales; the anterior dorsal fin is black and contains venomous spines. Its length is very common between 10 and 30 cm with a maximum of 45 cm in the Mediterranean and common between 15 to 20 cm with a maximum of 36 cm in the Black Sea (Fischer *et al*., 1987).

Available bibliography for *T. draco* is diversified dealing with reproduction (Bagge, 2004; Ak and Genç, 2013), Parasites (Azizi *et al*., 2016; Kayiş and Er, 2016), lipid content (Loukas *et al*., 2010), feeding habits (Santic *et al*., 2016), population structure and dynamics (Quigley, 1994; Portillo Strempel *et al*., 2008; Buz and Basusta, 2015; Carpenter *et al*., 2015; Custovic *et al*., 2014) but most of them focused on envenomation and toxin properties (Muir evans 1907; Skeie, 1962; Chahl and Kirk, 1975; Perriere and Michel, 1986; Halstead and Vinci, 1987; Chhatwal and Dreyer, 1992; Bouree and Lançon, 2002; Church and Hodgson, 2002; Acciaro *et al*., 2003; Verdiglione *et al*., 2003; Berger and Caumes, 2004; Russell and Emery, 2006; Lopacinski *et al*., 2009; Benlier *et al*., 2010; Portillo Strempel and Ceballos, 2012) and mainly on weight length relationship (Dorel 1986; Coull *et al*., 1989; Gonçalves *et al*., 1997; Merella *et al*., 1997; Moutopoulos and Stergiou, 2002; Mendes *et al*., 2004; Mendes *et al*., 2006; Ozaydin *et al*., 2007; Karakulak *et al*., 2006; lkyaz *et al*., 2008; Mata *et al*., 2008; Ak *et al*., 2009; Giacalone *et al*., 2010; Benmessaoud *et al*., 2015; Öztekin *et al*., 2016; Özdemir *et al*., 2017; Hamed *et al*., 2016).

In fisheries science maximum length and maximum age are important theoretical parameters found as entry data in majority of the models used in stock assessments (Allen, 1971; Pauly, 1980; Welcomme, 1999; Froese and Binohlan, 2000). In this context, updating the maximum size of a species harvested for commercial or recreational purposes is gaining more importance (Borges, 2001; Dulčić and Soldo, 2005; Akyol and Şen, 2008). The maximum observed length is a useful tool for a rapid evaluation of growth rates in the absence of basic data (Legendre and Albaret, 1991, Froese and Binohlan, 2000). To date, for Algerian waters no such studies were leaded on this trachinidae.

Material and Methods

On the 15th April 2017, one female specimen of the greater weever, *Trachinus draco* measuring 44.69 cm in total length and weighting 885 g was captured by captured by trammel net operating in Oran Bay (Cape Rousseau: 35°48'45.0"N 0°36'46.8"W) on sandy/rocky bottom at 120 m depth (Fig. 1).

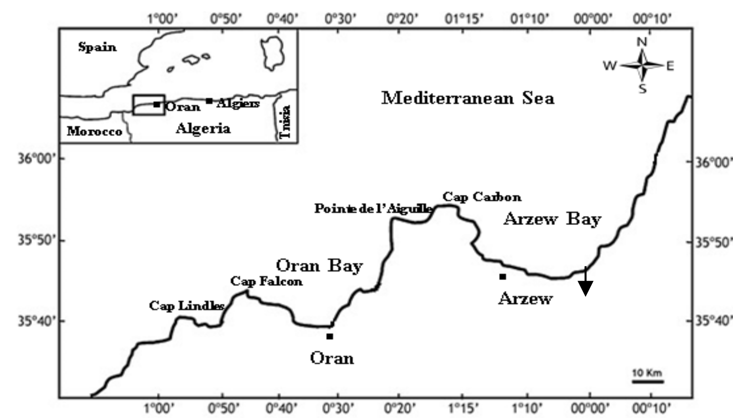
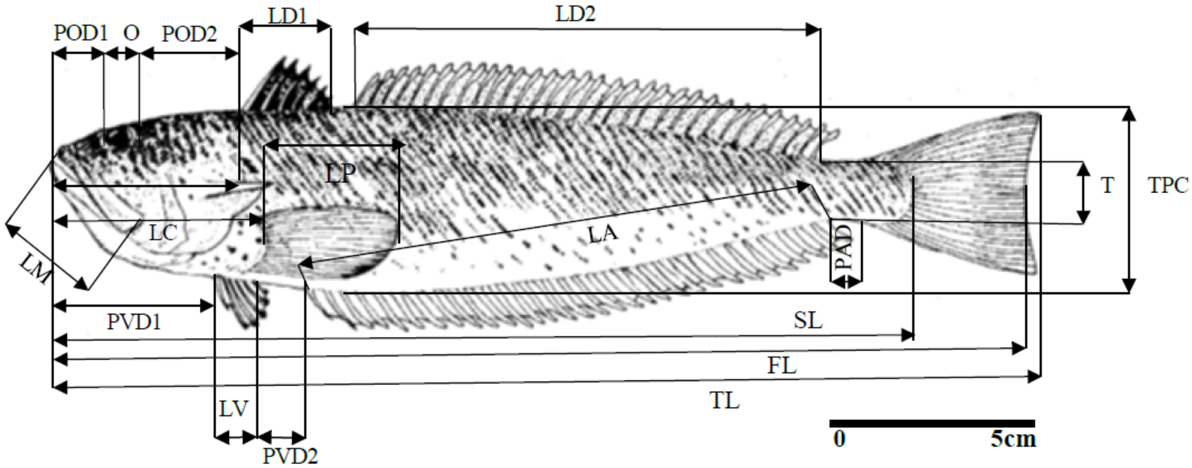


Figure 1. Sampling location of greater weever (*Trachinus draco*) specimen.

The specimen was measured with an electronic caliper to 0.1 mm precision and weighted to the nearest 0.1 g then photographed. Eighteen morphometric characteristics were measured (Figure 2): Total length (TL), Fork length (FL), Standard length (SL), Pectoral fin length (LP), Ventral fin length (LV), 1st dorsal fin length (LD1), 2nd dorsal fin length (LD2), Cephalic length (LC), Maxillary length (LM), Post-orbital distance (POD1), Eye diameter (O), Post-orbital distance (POD2), Pre-ventral fin distance (PVD1), Post-ventral fin distance (PVD2), Anal fin length (LA), Post-anal fin distance (PAD), Caudal peduncle minimal depth (T), Maximum body height (TPC), Total weight (TW). Description, measurements and percentage of each body part are reported to total length are given in (Table 1).



Total length (TL), Fork length (FL), Standard length (SL), Pectoral fin length (LP), Ventral fin length (LV), Cephalic length (LC), Maxillary length (LM), Pre-orbital distance (POD1), 1st Dorsal fin length (LD1), 2nd Dorsal fin length (LD2), Eye diameter (O), Post-orbital distance (POD2), Anal fin length (LA), Caudal peduncle minimal depth (T), Maximum body height (TPC), Pre-ventral fin distance (PVD1), Post-anal fin distance (PAD), Post ventral fin distance (PVD2).

Figure 2. Morphometric measurements of the greater weever *Trachinus draco* adapted from Fischer *et al*. (1987).

Results and Discussion

On the 15th April 2017, one female specimen of the greater weever, *Trachinus draco* measuring 44.69 cm in total length and weighting 885 g was captured by trawler operating in Oran Bay at 120 m depth. Species identification sheets (Fischer *et al*., 1987; Djabali *et al*., 1993) were used to identify the specimen of *T. draco* (Fig.3) where the body appear elongated and compressed. Small eyes located near the dorsal profile of the head; width of the interorbital space roughly equal to half the diameter of the eye; large oblique mouth, the maxillary extending beyond the posterior edge of the eye when the mouth is closed with villiform teeth.

According to Fischer *et al*., (1987) *T. draco* has a strong venomous spine on the operculum, 2 spines on the anterodorsal edge of the orbit and another above the upper lip, in front of the eye. Two dorsal fins, the first short counting 5 to 7 spines, the second, long counting 29 to 32 soft rays; anal with 2 spines and 29 to 32 soft rays. Generally, the greater weever is greenish brown back with dark spots on the head, yellowish-white flanks according to the oblique rows of scales, of brown, blue, yellow lines; second dorsal fin yellowish, anal mauve.

Figure 3. *Trachinus draco* (44.69 cm TL ♀) caught in Oran Bay, (Photographed by: ADDA NEGGAZ Hichem).

Measurements, meristic characteristics, weight and percentage of each body part of the greater weever caught in Oran Bay reported to total length are given in (Table 1).

Table 1. Morphometric measurements as percentage of total length (% TL) of *Trachinus draco* caught in Oran Bay (W. Mediterranean Sea).

Morphometric characteristics	Measurement (cm)	Proportion (%)
Total length (TL)	44.69	100.00
Fork length (FL)	43.44	97.20
Standard length (SL)	39.30	87.94
Pectoral fin length (LP)	5.76	12.90
Ventral fin length (LV)	3.28	7.360
1st dorsal fin length (LD1)	2.74	6.140
2nd dorsal fin length (LD2)	19.76	44.22
Cephalic length (LC)	7.67	17.16
Maxillary length (LM)	1.41	3.160
Post-orbital distance (POD1)	2.96	6.620
Eye diameter (O)	0.77	1.720
Post-orbital distance (POD2)	5.68	12.72
Pre-ventral fin distance (PVD1)	6.15	13.78
Post-ventral fin distance (PVD2)	2.70	6.060
Anal fin length (LA)	25.70	57.51
Post-anal fin distance (PAD)	2.66	5.950
Maximum body height (TPC)	2.57	5.750
Caudal peduncle minimal depth (T)	8.40	18.80
Total weight (TW)	0.88	-
Meristic characteristics		
Operculum spines	2*	
Short eye spines	2	
1st dorsal fin spines	7 (2*+5)	
2nd dorsal fin	32	
Pelvic fin	6	
Pectoral fins	15	
Anal fin	31	
Caudal fin	16	

*: venomous

According to Portillo Strempel *et al*., 2008 *T. draco* showed a seasonal migratory behavior, with a preference for shallower waters, up to 75 m depth during autumn and for deeper waters up to 160 m depth, during spring in the northern Alboran Sea (SW Mediterranean) which is the case of our specimen captured in April 2017.

The maximum length ever recorded of *T. draco* belongs to IGFA 2001 in the Atlantic Ocean (Canary island, 56cm) and by Otel (2007) in Danube Delta (53cm) followed by Fischer *et al*., 1987 (45cm) in the Mediterranean, all successive records are shown in Table 2.

Greater Weever is caught as bycatch in the majority of fisheries and landings are declared from the following FAO regions: Northeast Atlantic, Mediterranean and Black Sea. The overall trend in landings is one of dramatic fluctuations with a general increase in landings over time (Carpenter *et al*., 2015). As stated previously little is known on its ecobiology, population trends and most of studies focused on its toxins.

In the Mediterranean Sea, the maximum length of *T. draco* were reported as 45 cm TL (n=1124); 36 cm from Black Sea (Fischer *et al*.,1987); If we consider to maximum length recorded during our study so this length represents the maximum length for both Algerian and Western Mediterranean Sea. The aim of this paper is to present a compilation of maximum length for *T. draco* with a new record for the greater weever caught in Western Mediterranean Sea (Oran Bay).

Wootton 1990; 1999 in Helfman *et al*. (2009) stated that factors such as temperature, food availability, nutrient availability, light regime, oxygen, salinity, pollutants, current speed, predator density, intraspecific social interactions, and genetics often working in combination, creating large variations in size of fishes of the same and different ages, also populations exposed to high fishing mortality/pressure will respond by reproducing at smaller average sizes and ages. Generally, in the Mediterranean and Black Sea where fishing activity is intensive, the maximum length was relatively low (Table 2) 32 cm in Tunisian waters, 38cm in French waters, 32.9 in Greek waters, 23 cm in Egyptian waters cm TL.

Contrarily, in oceanic and northern seawaters individuals doesn't face the same fishing pressure, maximum length appears more important with a maximal length recorded in Canary Islands reaching 56 cm (IGFA, 2001). In this context, frequenting the eastern part of oranian shoreline an area undergoing a less fishing pressure than the western area (Oran Bay) (*Pers.obs*) we can explain that our specimen may have reached this maximum length observed. Also, we can add the fact that there is no predator known for *T. draco* at the top of the trophic chain.

Table 2. Maximum length records of *Trachinus draco* given by several authors.

Location		Depth (m)	TL (cm)	TW (g)	References
Turkey	Aegean Sea	<30	35.2	235.82*	Karakulak *et al*., (2006)
	Saros Bay	28-370	37.0	427.00	Ismen *et al*., (2007)
	Izmir Bay	-	34.1	288.99*	Ozaydin *et al*., (2007)
	N. Eastern Mediterranean	5-100	20.0	53.18	Sangun *et al*., (2007)
	Aegean Sea	30-70	36.6	365.42*	Ilkyaz *et al*., (2008)
	Aegean Sea	-	36.6	401.43	Kınacigil *et al*., (2008)
	Eastern Black Sea	60	35.0	549.20	Ak *et al*., (2009)
	İskenderun Bay	18-19m	20.6	55.84	Gökçe *et al*., (2010)
	Eastern Black Sea	60	25.8	131.76	Ak and Genç, (2013)
	Iskenderun Bay	-	28.7 M 32.0 F	145.21 237.48	Buz and Basusta, (2015)
	Aegean Sea	0-400	36.4	294.00	Öztekin *et al*., (2016)
Romania	Black Sea	-	40	-	Bănărescu, (1964)
	Agigea Eforie Nord Area	9.3-12.5	16.5	18-27	Roşca *et al*., (2010)
	Danube Delta	-	53.0	-	Otel, (2007)
Tunisia	Gulf of Tunis	-	32.0	236.30	Hamed and Chakroun-Marzouk, (2016)
France	Gulf of Gascogne	-	38.5	317.97*	Dorel, (1986)
	Catalan coast	1-80	38.5	375.00	Crec'hriou *et al*., (2012)
Greece	Cyclades, Aegean Sea	4-90	32.5		Erzini *et al*., (1999)
	Greece, Aegean Sea	-	32.0	219.03*	Moutopoulos and Stergiou, (2002)
	Greece, Thermaikos Gulf	-	30.5	189.37*	Karachle and Stergiou, (2008)
	Greece North Aegean Sea	15-800	28.8	149.40	Torres *et al*., (2012)
	Korinthiakos Gulf	50-300	32.9	206.13*	Moutopoulos *et al*., (2013)
Spain	Balearic Islands	40-80	24.2	83.90*	Merella *et al*., (1997)
		0.5-1713	26.5	125.00	Morey *et al*., (2003)
		-	34.0	259.64*	Garmon, (2005)
	Eastern Atlantic	<20	29.5	167.94*	Mata *et al*., (2008)
	Alboan Sea	50-164	39.0	-	Portillo Strempel *et al*., (2008)
Portugal	Eastern Atlantic Ocean	13-55	34.0	502.06*	Gonçalves *et al*., (1997)
	Algarve coast	-	39.6	554.10	Santos *et al*., (2002)
	Eastern Atlantic	30-350	39.0	460.00	Mendes *et al*., (2004)
Egypt	Alexandria Bay	30-200	23.0	200.52*	Abdallah *et al*., (2002)
Croatia	Eastern Adriatic	-	26.8	330.00	Dulčić and Kraljević, (1996)
Italy	Sicily	10-200	29.5	169.26*	Giacalone *et al*., (2010)
Ireland	Schull Bay	-	42.0	510.00	Went, (1973)
	Keem Bay (Co Mayo)	-	35.4	311.00	Quigley *et al*., (1990)
	Ballycotton Bay (Co Mayo)	-	38.7	-	Quigley *et al*., (1994)
-	North East Atlantic		35.0	344.00	Coull *et al*., (1989)
	North Sea/North-East Atlantic	-	36.5	352.78*	Wilhelms, (2013)
	Western Atlantic, Canary island	-	56.0	1740.00	IGFA, (2001)
	Northern/Central Adriatic	-	32.8	-	Custovic *et al*., (2014)
Denmark	Kattegat	9.9-27.1	32.5 M 37.6 F	221.40 350.20	Bagge, (2004)
-	Mediterranean Sea Black Sea	-	45.0 36.0	-	Fischer *et al*., (1987)
Algeria	Oran Bay	120	44.69	885.00	Present study

*Weight calculated from LWR (length weight relationship).

Conclusion

As conclusion, more efforts and means must be deployed to explore Oran Bay biodiversity deeply, target large specimens and try to study fish population's dynamics and their interaction with different biotopes present in the area (sandy, muddy, rocky, gravelly).

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: Ethics committee approval is not required for this study.

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Short Communication

Diagnosis of *Aeromonas sobria* and *Saprolegnia* sp. co-infection in rainbow trout fry (*Oncorhynchus mykiss*)

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ABSTRACT

The aim of the study was to determine a considerable level of mortalities occurred in rainbow trout fry (*Oncorhynchus mykiss*) cultured in a research hatchery located in Marmara Region/Turkey. Totally 18 individuals (1-8 g) were investigated by using bacteriological and mycological methods. The affected fish showed skin darkening, prolapses and fungal mats at the base of the fins. According to morphological and biochemical characteristics of the isolates, all of them were identified as *Aeromonas sobria*. *Saprolegnia* sp. was also observed in squash preparations of the skin. All isolates were determined to be sensitive against florfenicol, enrofloxacin and ciprofloxacin. Histopathologically, vacuolar degeneration, haemorrhage and hyperaemia in the liver, tubular necrosis, periglomerular edema, and multifocal melanomacrophage deposits in the kidney and depletion of white pulp in the spleen were determined. Distal type epithelial cells hyperplasia was also observed in the gills. In diseased fish, motile Aeromonad septicaemia was described and *Saprolegnia* sp. was also detected in fins and skin lesions but it was considered to be a secondary infection.

Keywords: Rainbow trout fry, *Oncorhynchus mykiss*, *Aeromonas sobria, Saprolegnia* sp.

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Introduction

Aquaculture activities have begun in Turkey in 1970s with rainbow trout *(Oncorhynchus mykiss)*. Production of rainbow trout reached to 103.192 t in 2018 in Turkey. In this period of time, by the increase of the number of fish farms and the production, disease problems has begun and until recently, these epizootics have increased rapidly parallel to the epizootics in other countries.

Motile *Aeromonas* septicaemia (MAS, Bacterial hemorrhagic septicemia, Aeromonad septicemia or Red Pest) caused by motile members of the genus *Aeromonas* is a ubiquitous disease affecting freshwater and marine fish all over the world. Fish pathogenic motile Aeromonads have often been associated with *A. hydrophila* (Austin and Austin, 2007). However, other members of the genus including *A. sobria* (Toranzo et al., 1989), *A. allosaccharophila* (Martinez-Murcia et al., 1992), *A. caviae* (Candan et al., 1995), *A. jandaei* (Esteve et al., 1995), *A. veronii* biovar *sobria* (Rahman et al., 2002) and *A. schubertii* (Alvarez et al., 2004) have also been detected as fish pathogens. These bacteria are known to cause various infections, especially gastroenteritis, by passing from animals to humans through food (Plumb and Hanson, 2011).

Aeromonas sobria has been recognized as a fish pathogen since 1987 and it was first reported in wild gizzard shad (*Dorosoma cepedianum*) in USA (Toranzo et al., 1989). In Turkey, *A. sobria* was first reported in intestinal tracts of healthy Atlantic salmons (*Salmo salar*) in Black Sea (Karatas, 1996). This pathogen was also detected in rainbow trout (*Oncorhynchus mykiss*) (Kayis et al., 2009; Ozer et al., 2009; Durmaz and Turk, 2009; Onuk et al., 2017), gold fish (*Carassius auratus*) (Korun and Toprak, 2007), green terror (*Andinoacara rivulatus*) (Şahin et al., 2019), gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Avsever et al., 2012) in Turkey. Wahli et al. (2005) declared that *A. sobria* is a causative agent of ulcerative skin diseases in farmed perch (*Perca fluviatilis* L.) with high mortality in Switzerland. *A. sobria* caused mass mortality of *Garra rufa* in a hatchery in Slovakia (Majtan et al., 2012).

Motile *Aeromonas* septicaemia are usually associated with stressors (Shoemaker et al., 2015). Poor feeding response, lethargy, pale gills, exophthalmia, and haemorhagic eyes are the most common clinical findings of MAS. Scale loss, frayed fins and necrotic lesions on the skin and friable organ are also observed (Toranzo et al., 1989; Karatas, 1996; Yardımcı and Aydın, 2011; Shoemaker et al., 2015).

Infection of eggs, fry and larger fish by water mould is another problem in cultured rainbow trout. Fungal infections can occur as a secondary infection alongside bacterial, viral or parasitic infections (Roberts, 2012). The present study aimed to determine the cause of mortalities in rainbow trout fry (*Oncorhynchus mykiss*) cultured in a research hatchery of Marmara Region in Turkey.

Material and Methods

Bacteriological and Fungal Examination

Totally 18 moribund individuals were investigated by using basic bacteriological and mycological methods. Samples were taken from internal organs and streaked onto Tryptic Soy Agar and Sabouraud %2 Dextrose Agar and incubated at 22°C for 48-72h. After incubation, the isolated bacterial pure cultures were examined using standard protocols according to Austin and Austin (2007) and Buller (2004). Also rapid diagnosis kit API 20E was used in all isolates. Before necropsy, scrapings taken from fungal mats at the base of fins were stained with lacto-phenol cotton blue and examined under light microscope.

Antimicrobial Susceptibility Testing

All bacterial isolates were tested for antimicrobial susceptibility by using Kirby-Bauer disc diffusion method and performed using multidiscs (chloramphenicol, florfenicol, erythromycin, oxytetracycline, kanamycin, streptomycin, ampicillin, enrofloxacin, ciprofloxacin and flumequine). Isolates were streaked onto Mueller-Hinton agar (Oxoid), incubated at 22°C for 48h and interpreted according to Clinical and Laboratory Standards Institute (CLSI) data (CLSI, 2013).

Histological Examination

Histopathological tissue samples from the internal organs were taken and fixed in 10% buffered formalin, processed with routine methods and embedded in paraffin blocks. Sections (5µm) were stained with haematoxylin-eosin (HE) (Culling, 1963) and examined under light microscope using the image analysis system NIS-Elements BR Microscope Imaging Software (Nikon Instruments).

Results and Discussion

The moribund fish samples externally showed loss of color or darkening of the skin (Figure 1A, B), eroded fins and fungal mats at the base of fins (Figure 1B), slight exophthalmia (Figure 1C) and prolapse (Figure 1D). Internally, pale liver and visceral fat and accumulation of a mucoid yellowish fluid in the intestines were observed (Figure 1E). Petechial haemorrhage in the liver and splenomegaly were detected in a few sample (Figure 1F). These clinical findings are similar to the typical MAS findings reported by many researchers (Snieszko and Bullock, 1965; Toranzo et al., 1989; Karatas, 1996; Yardımcı and Aydın, 2011; Shoemaker et al., 2015). In MAS, outbreaks are associated with poor management and environmental status and this stress-related situation causes mortality (Plumb and Hanson, 2011). In this study, a considerable level of mortalities occurred in the hatchery fish stocks.

Total of twenty Gram-negative motile isolates were recovered from kidney, spleen, liver and blood of ten moribund fish samples. These motile Gram-negative short rods produced white-cream convex colonies on Tryptic Soy Agar at 22°C after 48h. All isolates showed the same biochemical, morphological characteristics and API 20E results. The bacterium produces arginine dihydrolase, catalase, β-galactosidase, indole, lysine decarboxylase and oxidase but does not hydrolyse esculin. The API 20E profile is so similar with previous *A. hydrophila* reports (Buller, 2004; Austin and Austin, 2007). They were characterized by haemolytic colonies on sheep blood agar when grown at 25°C and 30°C and all of them were identified as *Aeromonas sobria* (Table 1).

Table 1. Some phenotypic and morphological characteristics of *Aeromonas sobria* isolates

Characteristics	A. *sobria* n=20
Colony colour	Cream
Morphology	Rods
Gram Staining	-
Motility	+
Cytochrome Oxidase	+
Catalase	+
O/F Test	+/+
Inositol	-
Arabinose	-
Sorbitol	-
Esculin	-
Production of H_2S	-
Citrate	+
ONPG	+
0/129 (150µg)	R
Indole	+
Methyl Red	V
Voges-Proskauer Test	+
Arginine	+
Lysine	-
Ornithine	-
API 20E results	7 2 4 7 1 3 7 5 7

+: positive reaction, -: negative reaction, R: resistant, V: variable

Oomycetes of the genus *Saprolegnia*, especially *S. parasitica*, are economically important pathogens of fish. *Saprolegnia* species also affect to decline of the amphibians and crustaceans. In Japan, *S. parasitica* and *S. diclina* caused high mortality of cultured rainbow, coho salmon (*Oncorhynchus kisutch*) and ayu (*Plecoglossus altivelis*) (Yuasa et al., 1977; Yuasa and Hatai, 1995). Grey-white patches on fish skin having cotton-like appearance underwater were also observed in some rainbow trout fries. When fungal mats that stained with lacto-phenol cotton blue were examined under light microscope, *Saprolegni*a sp. observed in fins and skin lesions. No growth was detected on Sabouraud %2 Dextrose Agar after the incubation at 22°C after 72h. For this reason, *Saprolegnia* infection was considered as a secondary, opportunistic infection.

Durmaz and Türk (2009) reported that *Aeromonas sobria* isolates were highly resistant to oxytetracycline, streptomycin and carbenicillin in their study. Onuk et. al (2017) reported that *A. sobria* strains, that were isolated from rainbow trout, from mullet and in water resources from different geographic regions of Turkey, were gentamicin-sensitive but resistant to beta-lactam antibiotics and ampicillin. In this study, all isolates were found to be susceptible to florfenicol, enrofloxacin and ciprofloxacin, while they were semi-sensitive to chloramphenicol and flumequin, and resistant to erythromycin, oxytetracycline, kanamycin, streptomycin, ampicillin and vibriostatic agent O/129. Similar sensitivity to ciprofloxacin and enrofloxacin were reported by other researchers (Guz and Kozinska, 2004; Durmaz and Turk, 2009; Onuk et al., 2017; Şahin et al., 2019). We found that the highest resistance was against ampicillin and oxytetracycline. While ampicillin is used for the treatment of human diseases, oxytetracycline is used in the treatment of bacterial diseases in freshwater fish farming in Turkey. The possible cause of resistance to these antibiotics is thought to be due to the heavy use of these antibiotics.

Histopathologically, vacuolar degeneration and necrosis in the parenchyma cells of liver, hyperaemia and haemorrhage in the liver (Figure 2A, B), depletion of the spleen (Figure 2C), myopathy (Figure 2D), tubular necrosis, periglomerular edema and multifocal melanomacrophage centers in the kidney (Figure 2E), hyperplasia of gill lamellae and disruption of gill epithelium (Figure 2F) and depletion of white pulp in the spleen were determined. Our histopathological results are similar with previously reported histopathological findings of Motile Aeromonad infections (Yardımcı and Aydın, 2011; Laith and Najiah 2013; Saharia et al., 2018).

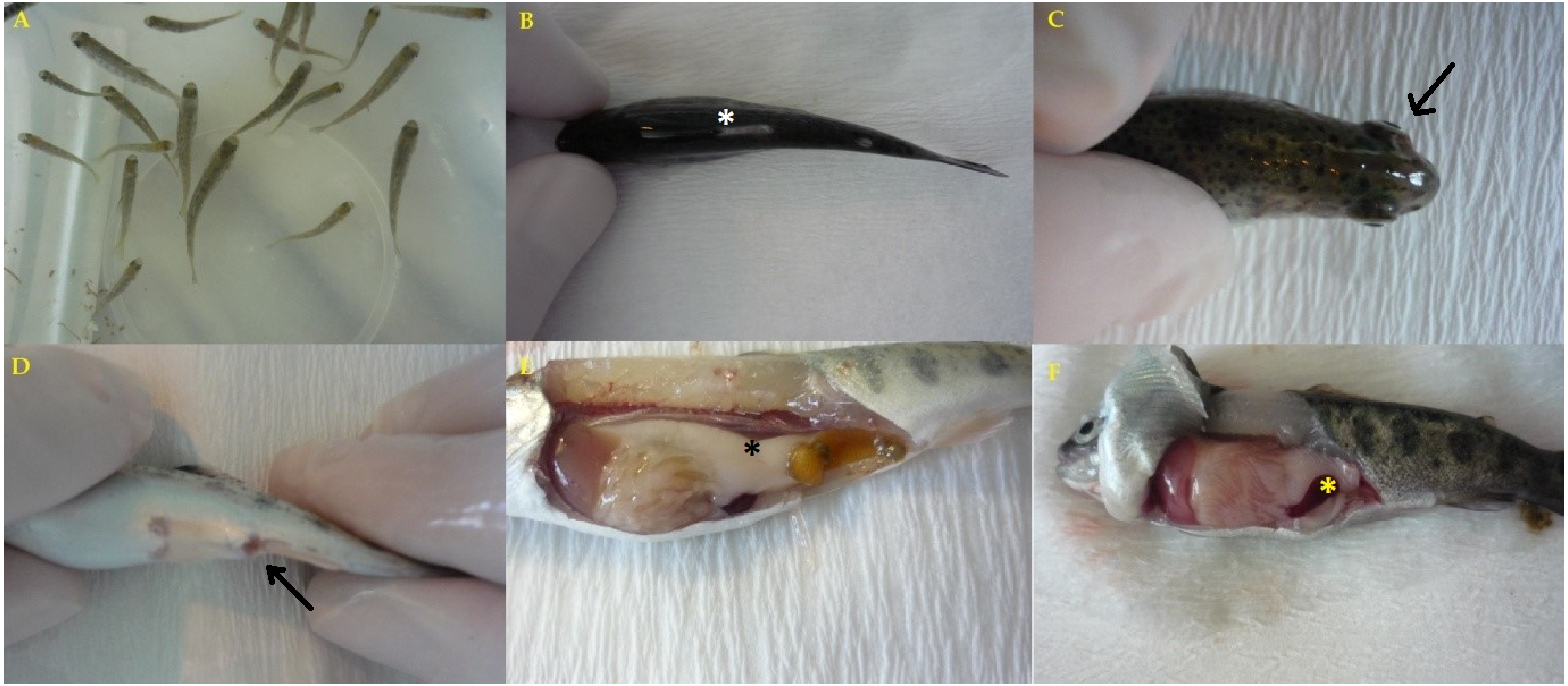

Figure 1. Moribund fish showed (A) depigmentation of the skin, (B) eroded fins and fungal mats at the base of fins (*), (C) slight exophthalmia (arrowed), (D) haemorrhage and prolapse (arrowed), (E) visceral fat (*) and (F) splenomegaly(*).

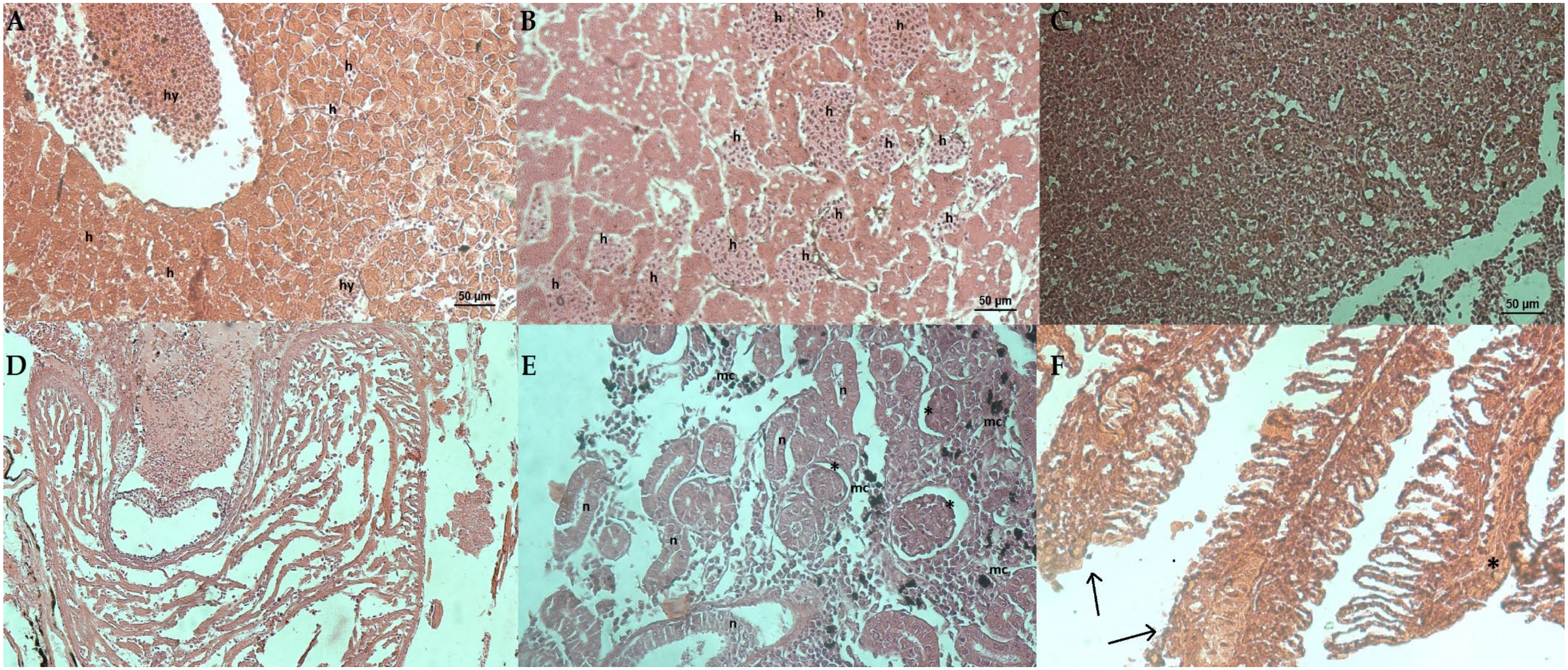

Figure 2. Photomicrographs of the tissue sections of moribund rainbow trout fries. (A) Vacuolar degeneration and necrosis in the parenchyma cells of liver, hyperaemia and haemorrhage in the liver H&E X20, (B) necrosis in the parenchyma cells of liver and haemorrhage H&E X20, (C) depletion of white pulp in the spleen H&E X20, (D) myopathy H&E X10, (E) tubular necrosis (n), periglomerular edema (*) and multifocal melanomacrophage centers (mc) in the kidney H&E X20, (F) hyperplasia of the gill lamellae (arrowed) and disruption of the gill epithelium (*) H&E X20.

A co-infection caused by *Saprolegnia parasitica* and *Aeromonas hydrophila* were described previously in sea bass by Dinçtürk et al. (2018) in a recirculating aquaculture system and they suggested that *S. parasitica* could be dominant in the outbreak, but in our case we found that *Aeromonas sobria* was the primary cause.

Conclusion

In aquaculture, increased production, pressures on faster growth, high stock density could create conditions conducive to outbreak of bacterial infectious diseases. In conclusion, motile Aeromonad septicaemia was described in this study. Saprophytic fungus *Saprolegnia* sp. played a role as a secondary pathogen in this infection. In addition, according to the data obtained from this study, enrofloxacin was successful with a feed dose of 20 g / 10 kg / 7-8 days in the treatment of diseased fish.

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 approved by Istanbul University Local Committee on Animal Research Ethics (Decision year 2013/ number 70) and conducted in accordance with ethics committee procedures of animal examination.

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Disclosure: -

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Gıda güvenliği açısından su ürünlerinde mikroplastik riski ve araştırma yöntemleri

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ÖZ

Son yıllarda artan plastik kullanımı ve yanlış geri dönüşüm politikaları ekosistemde plastik atıkların birikmesine neden olmuştur. Sucul ekosistemdeki canlılar üzerindeki etkilerinin görülmesiyle birlikte plastik kirliliği küresel bir sorun haline gelmiştir. Ortamda farklı fiziksel, kimyasal ve biyolojik etkenlerden dolayı mikroplastiklere (MP'lere) ve nanoplastiklere (NP'lere) parçalanan plastikler, besin zincirine girerek insan sağlığını tehdit etmektedir. Yaygın plastik kirliliğinin bir sonucu olarak, mikroplastikler ve nanoplastikler zooplanktonlardan, balıklara, kabuklu su ürünlerinden deniz memelilerine kadar birçok farklı canlı tarafından yutulmaktadır. Su ürünlerinin bünyelerine giren mikroplastikler canlı dokuda sindirilip, doku ve organlar arasında yer değiştirebilmektedir. Bununla birlikte su ürünleri işleme teknolojilerinde yer alan bazı aşamalar da mikroplastik kontaminasyon kaynağı olabilmektedir. Mikroplastiklerin neden olduğu fiziksel, kimyasal ve biyolojik toksisite etkileri henüz tam olarak bilinmemektedir. İleride yapılacak olan çalışmalarda, tüketici sağlığı açısından işlenmiş su ürünlerindeki mikroplastiklerin kaynağının ve bulaşma yollarının incelenip belirlenmesi önem arz etmektedir. Bu derlemede, sucul ekosistemlerden besin zincirine giren mikroplastiklerin gıda güvenliği açısından işlenmiş ürünlerdeki riskleri tartışılıp, bu araştırma alanındaki mikroplastiklerin identifikasyonu ve sayımı için analitik yöntemler incelenmiştir.

Anahtar Kelimeleri: Plastik kirliliği, Nanoplastik, Kontaminasyon, İşleme teknolojileri, FTIR, Raman

ABSTRACT

Microplastic risks in the seafood in terms of food safety and their research methods

Plastic waste has accumulated in the aquatic ecosystem as a result of the increasing use of plastic in recent years and their wrong recycling policies. Plastic pollution has become a global problem with its effects on aquatic organisms. Plastics that break down into microplastics (MPs) and nanoplastics (NPs) due to different physical, chemical and biological factors in the environment enter the food chain and directly threaten human health. As a result of widespread plastic pollution, microplastics and nanoplastics are ingested by many different species, from zooplankton, fish, shellfish to marine mammals. Microplastics that enter into marine organisms can move within living tissue and move between tissue and organ. However, some stages in seafood processing technologies can also be a source of microplastic contamination. Physical, chemical and biological toxicity effects caused by microplastics are not fully known yet. In future studies, it is important to examine and determine the source and transmission routes of microplastics in seafood for consumer health. In this review, the risks of microplastics entering the food chain from aquatic ecosystems in seafood products in terms of food safety are discussed, and analytical methods for the identification and extraction of microplastics in this research area are examined.

Keywords: Plastic pollution, Nanoplastics, Contamination, Seafood processing technologies, FTIR, Raman

Giriş

Günümüz toplumunda plastik, düşük yoğunlukları, çok geniş bir sıcaklık aralığında kullanılabilirlikleri, kimyasallara ve ışığa karşı dirençli olmaları, kolayca işlenebilir özellikte olmaları ve nispeten düşük maliyetleriyle günlük yaşamın vazgeçilmez bir parçası haline gelmiştir (Ryan, 2015). Ondokuzuncu yüzyılın başında termoplastiklerin gelişimiyle birlikte kullanım alanı oldukça geniş olan doğal ve sentetik polimerler üzerine araştırmalar başlamıştır. Plastiğin modern gelişimi ise yirminci yüzyılların başında en az 15 yeni polimer sınıfının sentezlenmesiyle genişlemeye başlamıştır (Andrady ve Neal, 2009). Toplu plastik üretiminin başladığı 1930-1940'lardan bu yana, plastik üretim hacmi istikrarlı bir şekilde artmaktadır (Ryan, 2015). Günümüzde küresel plastik üretimi yıllık 359 milyon tona ulaşmıştır (PlasticsEurope, 2019) ve öngörülebilir gelecekte sürekli hızlı bir büyüme ile artması beklenmektedir (Ryan, 2015).

Katlanarak artan plastik üretim hacmi, uygun olmayan şekilde taşınan atık plastik miktarlarıyla birleşince tüm dünya ekosistemini tehdit eden küresel bir sorun ortaya çıkmıştır (Barnes ve diğ., 2009; Ryan, 2015). Özellikle 1950'lerden bu yana sanayinin gelişimiyle birlikte plastik malzemeler ekosistemdeki tüm ortamlarda kirlilik oluşturmakta ve içerisindeki canlıları etkilemektedir (Cózar ve diğ., 2014). Plastik ve neden olduğu etkiler tüm dünyada yüzey suları (Cincinelli ve diğ., 2019; Lorenz ve diğ., 2019), derin deniz sedimentleri (Van Cauwenberghe ve diğ, 2013; Zhang ve diğ., 2020), amfipodlar (Jamieson ve diğ., 2019), balıklar (Zhu ve diğ., 2019), çift kabuklular (Moreschi ve diğ., 2020); yumuşakçalar (Oliveira ve diğ., 2020), buzullar (Obbard ve diğ., 2014; Bergmann ve diğ., 2019), toprak (Scheurer ve Bigalke, 2018), hava ortamı (Dris ve diğ., 2017), deniz kuşları (Amélineau ve diğ., 2016) ve sofra tuzları (Gündoğdu, 2018) gibi farklı madde ve ortamlarda tespit edilmiştir.

Plastikler, altısı "büyük altı" olarak da adlandırılan yirmiden fazla polimer ailesini içerir: polipropilen (PP), yüksek ve düşük yoğunluklu polietilen (HDPE ve LDPE), polivinil klorür (PVC), poliüretan (PUR), polietilen tereftalat (PET), polistiren (PS) ve bu polimerler Avrupa'daki plastik üretiminin % 80'ine karşılık gelmektedir (Dehaut ve diğ., 2016; Andrady ve Rajapakse, 2019; PlasticsEurope, 2019). Şu anda üretilen plastiklerin çoğu fosil yakıt bazlı malzemelerdir ve küresel olarak mevcut fosil yakıtın %4 kadarı ham plastik üretimi için kullanılmaktadır (Geyer ve diğ., 2017; Ašmonaitė, 2019).

Günümüzde büyük bir sorun haline gelen aşırı plastik kullanımı ve bunların oluşturduğu atıklar sonucu özellikle sucul ekosistemlere yoğun bir plastik partikül deşarjı söz konusudur (Galgani ve diğ., 2015). Okyanus gibi büyük su kitlelerine girdikten sonra plastik materyaller, mekanik ve biyolojik işlemler sonucu mikroplastiklere parçalanmaktadırlar. Parçalanan bu materyaller rüzgar ve akıntı yardımıyla uzun mesafelere taşınıp, kökenlerinden çok uzaklardaki ortamlarda birikebilmektedirler (Barnes ve diğ., 2009).

Mikroplastikler (MP'ler) genelde boyutu 5 mm'nin altındaki polimerik partiküller olarak tanımlanmaktadır (GESAMP, 2015). EFSA (European Food Safety Authority: Avrupa Gıda Güvenliği Otoritesi) (2016)'da yayınladığı raporda MP'leri 5mm-100 nm arası olarak tanımlarken, 100 nm'den küçük polimerik parçaları nanoplastikler (NP'ler) olarak tanımlanmıştır. Bir başka tanımlamada ise 2,5 cm'den büyük parçalar makroplastik (Galgani ve diğ., 2015), 500 µm–5 mm arası mezoplastik, 50–500 µm arası mikroplastik ve 50 µm'dan küçük parçalar ise nanoplastik olarak tanımlanmıştır (Ryan, 2015). Literatürde çok sayıda farklı boyut temelli tanımı yapılmış olan bu maddelerin henüz yaygın, standartlaştırılmış bir tanımı bulunmamaktadır (GESAMP, 2015; EFSA, 2016; Ašmonaitė, 2019).

Mikroplastik Kaynakları

Deniz kıyılarında, yüzeyinde ve tabanında biriken atıkların önemli bir kısmını plastik maddeler oluşturmaktadır. Plastik torbalar, balıkçılık malzemeleri, gıda ambalajları gibi sahillerde en yaygın bulunan öğeler, bulunan atıkların %80'inden fazlasını oluşturmaktadır (Galgani ve diğ., 2015). Bunun yanı sıra MP'ler, büyük plastik ürünlerin üretiminde kullanılan peletler, granüller, lifler ve tozlar, kişisel bakım ürünlerindeki mikro aşındırıcı partiküller, ilaçlar ve sentetik giysilerin yıkanması sonucunda da doğrudan çevreye deşarj olabilmektedirler (Browne, 2015).

Geniş coğrafi ölçekleri aşarak kutuplardan tropik ve ılıman bölgelere kadar tüm ekosistemlerde görülen mikroplastik kirliliğinin kaynakları, doğrudan kullanım için öğütülerek veya ekstrüzyon ile parçalanan plastik partiküllerin oluşturduğu "birincil kaynaklar" ve daha büyük plastik materyalin çevrede giderek daha küçük parçalara parçalanmasıyla oluşturduğu "ikincil kaynaklar" şeklinde ikiye ayrılmaktadır. Birincil kaynaklı MP'ler, lastiklerin aşınması veya yıkama sırasında sentetik tekstillerin aşınması gibi üretim, kullanım veya bakım sırasında büyük plastik nesnelerin aşınmasından da kaynaklanabilirler (Browne, 2015; Boucher ve Friot, 2017; Ašmonaitė, 2019). Deniz ortamındaki mikroplastiklerin %69-81'i ikincil kaynaklı MP'lerden oluşmakta ve doğaya atılan tek kullanımlık plastiklerden, hayalet ağlara kadar geniş bir kapsamı bulunmaktadır (Boucher ve Friot, 2017; EU, 2017).

Düşünülenin aksine plastik kirliliğine neden olan etmenlerin önemli ölçüde bir kısmı da içerisindeki materyalin özelliğine çok da dikkat edilmeyen ürünlerinden kaynaklanmaktadır; yıkama esnasında sentetik fiber salınımı yapan kumaşlı giysiler, yollarda aşınan araba lastikleri, suya veya toprağa aşınım yapan gemi ve otoyol boya maddeleri ve mikroeksfoliyant madde içeren kişisel kozmetik ürünleri bunlara örnektir (Boucher ve Friot, 2017). Örneğin, yapılan deneysel bir çalışmada, ev tipi çamaşır makinelerinden atık suya boşaltılan elyaf sayısını incelenmiştir ve makinenin yıkama başına 1900 adet elyaf suya salınım yaptığı sonucuna ulaşılmıştır. Bu çalışma sonucunda deniz habitatlarında bulunan mikroplastik liflerin büyük bir kısmının, giysilerin yıkanmasının bir sonucu olarak suya bulaşabileceği düşünülmektedir (Browne, 2015). Rochman ve diğ. (2015), insan tüketimine sunulan balık ve midyelerde tekstil kökenli MP'lerin varlığını bildirmişlerdir.

MP'ler, okyanuslara girdikten sonra yüzme veya batma eğilimindedir ve bir kısmı da okyanus akıntılarından kaynaklanan girdaplarda birikmektedirler. Bu MP'lerin 93,000 ila 268,000 tonunun (93-268 kilotonunun) şu anda okyanuslarda yüzdüğü tahmin edilmektedir (Boucher ve Friot, 2017). Yoğunluklarına göre, örneğin polipropilen gibi deniz suyundan daha hafif MP'ler okyanuslarda yüzerek geniş bir alana dağılım gösterirken, akrilik gibi deniz suyundan daha yoğun MP'ler büyük olasılıkla okyanus tabanında birikerek nihayetinde besin zincirlerine (Seltenrich, 2015) giriş yapmaktadırlar (Boucher ve Friot, 2017).

Mikroplastiklerin Sınıflandırılması

MP'ler tek tip mikrosferlerden, düzensiz şekilli plastik parçalara, peletlere, mikroskobik liflere, köpüklere, filmlere ve filamanlara kadar farklı şekillerde bulunmaktadır (Hidalgo-Ruz ve diğ., 2012). Genellikle birincil kaynaklı MP'ler, ikincil kaynaklı MP'lere göre daha düzenli ve tutarlı bir morfolojiye sahiptirler (Boucher ve Friot, 2017). Kullanım amacına göre farklılık içerebildiği için çok farklı renklerde üretilen plastikler, siyah, şeffaf, kırmızı, mavi, beyaz, pembe, sarı, mor, turuncu, yeşil, kahverengi veya çok renkli şeklinde doğada bulunabilmektedir (Hidalgo-Ruz ve diğ., 2012).

Plastiklerin Mikroplastiklere Bozunması

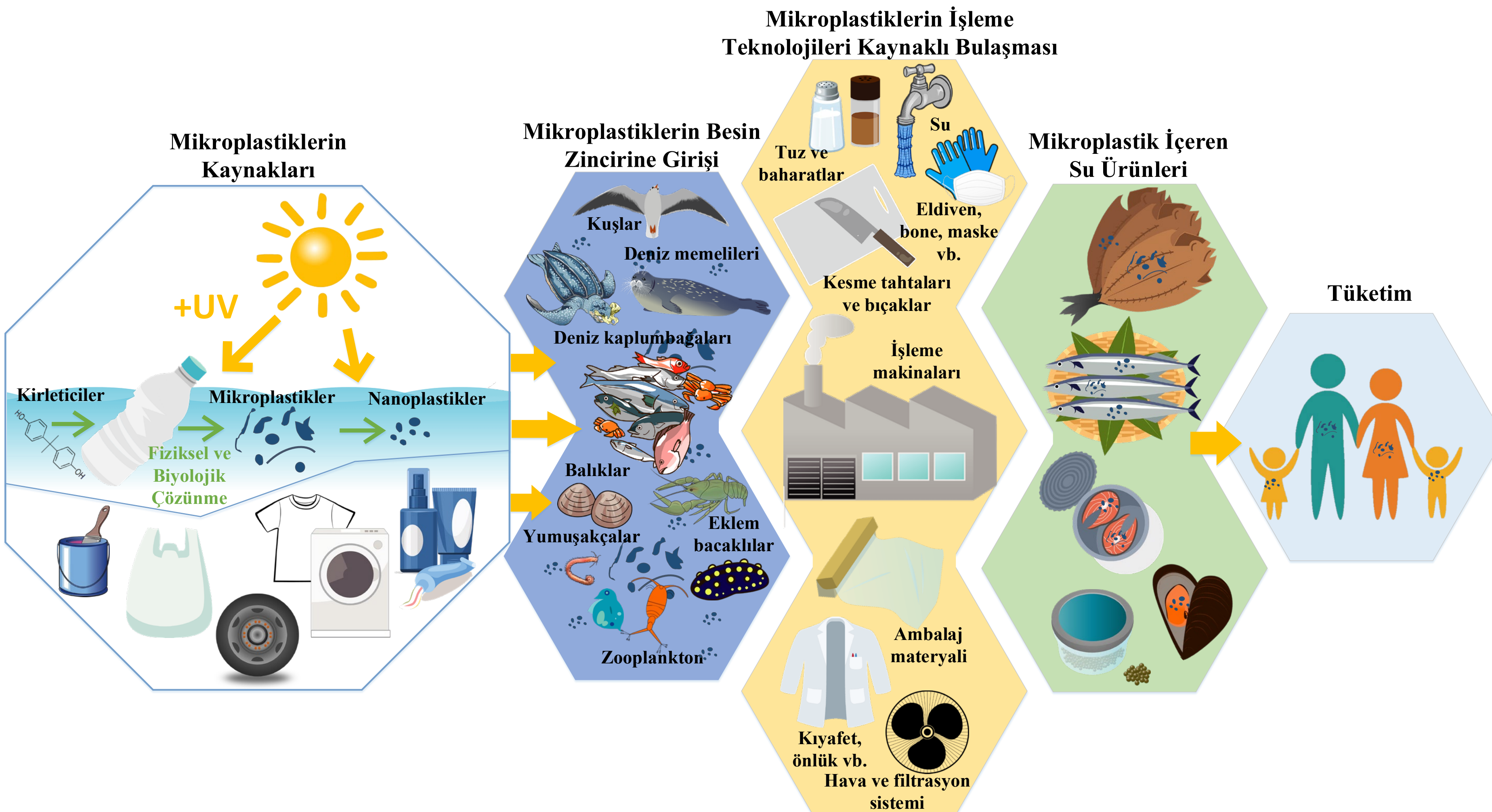
Doğaya bırakılan plastik parçacıklar zamanla farklı etmenler sonucu daha küçük parçacıklara parçalanmaya ve bozulmaya devam etmektedir. Bozulma sonucunda yapıları değişen polimerlerde, renk bozulması, yüzey çatlaması ve parçalanma gibi etkiler gözlemlenebilmektedir (UNEP, 2015). Bu parçalanma biyolojik bozunma (mikroorganizmalar), kimyasal ayrışma (UV ışınlar yardımıyla) veya fiziksel etkenler (dalga hareketi, rüzgar, aşındırıcı kum veya çökelti yardımıyla) nedeniyle oluşmaktadır (Barnes ve diğ., 2009; Hidalgo-Ruz ve diğ., 2012; Browne, 2015). MP'lerin bozunma süreçleri beş farklı şekilde kategorize edilmiştir; fotodegradasyon (UV ışık gibi ışık veya fotonların etkisiyle), termal bozunma (yüksek sıcaklıkla) termo-oksidatif bozunma (yavaş oksidatif bozunma veya orta sıcaklıklarda gerçekleşen moleküler bozulma), hidroliz (su ile reaksiyon sonucu) ve biyolojik bozunma (organik materyallerin mikroorganizmalar tarafından ayrıştırılması). Bu bozunma, polimer türü ve polimer yaşıyla birlikte güneş ışığı, sıcaklık, yağmur, nem, ışınlama, pH, kirleticiler, termal döngüler ve oksijen içeriği gibi çevresel koşullar dahil olmak üzere birçok farklı faktöre bağlıdır (Veerasingam ve diğ., 2020).

Özellikle sahillerde UV'ye ve oksidasyona maruz kalan plastiklerde bu süreç, daha yüksek sıcaklıktaki bölgelerde veya fiziksel aşınmanın meydana geldiği yerlerde daha hızlı olmaktadır. Plastik materyal tortu, toprağa gömüldüğünde veya su sütunu içerisinde parçalanmanın hızı önemli ölçüde azalmaktadır (UNEP, 2015). Su, ışığın neden olduğu oksidatif bozunmayı baskıladığından, sudaki plastiğin bozunma hızı havadakinden veya kumsallardakinden çok daha yavaştır (Veerasingam ve diğ., 2020).

MP'lerin boyutu çok küçük olduğundan, zooplankton, omurgasızlar ve küçük balıklar tarafından yanlışlıkla yiyecek olarak yutulabilmektedir (Veerasingam ve diğ., 2020) ve bu şekilde besin zincirine girmektedirler (GESAMP, 2015). Plastikler genelde yapılarında stabilizatörler, plastikleştiriciler, alev geciktiriciler ve suya salınabilen pigmentler gibi bazı katkı maddelerini de içermektedir (EU, 2017). İnsan sağlığı açısından en büyük endişelerden biri de gıda zincirinde biriken MP'lerin içerisinde ortamdan emilen bazı toksik maddelerin taşınmasıdır (Ericksen ve diğ., 2014). Bu kimyasallar (katkı maddeleri) biyolojik dokulara sızarak, organizmalarda ve gıda zincirinde biyolojik birikim yapabilmektedirler. Farklı su ve canlı ortamlarındaki MP'lerin polimerik bileşimini belirlemek için birçok analitik yöntem kullanılmaktadır (Veerasingam ve diğ., 2020).

Besin Zincirine Mikro ve Nanoplastiklerin Girişi

Mikroplastikle kontamine olmuş gıda maddesi dışında, gıdanın işlenmesi sırasında kullanılan teknik ekipman, katkı maddeleri, işleme prosedürü sırasında kullanılan yardımcı ekipmanlar, kullanılan ambalaj ve diğer dış kaynaklar ile kontamine olan gıdanın tüketimi besin zincirine mikro ve nanoplastiklerin girişini açıklamaktadır.



Şekil 1. Sucul canlılardan ve işleme proseslerinden kaynaklı insan tüketimine bulaşan mikroplastik ve nanoplastik riski

Figure 1. Microplastic and nanoplastic contamination risks of human consumption due to aquatic species and seafood processing technologies

Besin zincirine giren plastikler ve potansiyel zararlı etkileri son yıllarda hem su ürünleri hem de onları tüketen insanlar için tehlike oluşturmaktadır (Şekil 1). Plastikler sucul canlıların hareket etmesine, nefes almasına ve beslenmesine engel olacak şekilde vücutlarına dolanabilir veya takılabilir. Diğer bir yol ise plastiklerin canlılar tarafından yiyecek zannedilip yutulması yoluyla gerçekleşmektedir (Kühn ve diğ., 2015).

Mikroplastiklerin besin zincirine girişi, kasıtlı veya yanlışlıkla yutulması sonucu ve ikincil kaynaklı sindirimi sonucu gerçekleşmektedir. MP'ler renkleri sebebiyle sucul canlılar tarafından gıda zannedilip kasıtlı olarak tüketilmektedirler (Kühn ve diğ., 2015).

Kabuklu deniz canlıları, balıklar ve balinalar gibi bazı deniz memelileri suyu filtre ederek beslendiklerinden dolayı sudaki MP'leri de yutmaya yatkındırlar. İkincil kaynaklı sindirim ise, canlıların, bünyesinde MP bulunduran diğer canlılar ile beslendiğinde oluşan durumdur. Sudan zooplankterlere ve daha sonra da balıklara kadar olan bu geçiş sonucu mikro-nano plastikler (MNP'ler) yüksek trofik seviyelerde birikime yol açabilmektedirler. Besin zincirinde ilerleyen MP'ler, su ürünleri tüketimi sonucu insanlara geçmektedir (GESAMP, 2015; Hantoro ve diğ., 2019). Bu durum insanlar için özellikle bütün olarak tüketilen kabuklu su ürünlerini ve hamsi, sardalya gibi küçük balıklar açısından risk oluşturmaktadır (Hantoro ve diğ., 2019). Balıklarda yapılan MP çalışmaları incelendiğinde, bulunan mikroplastik parçacıkları çoğunlukla mide ve sindirim sistemlerinde bulunmakta ve bunlar genellikle temizleme sırasında atılmaktadır. İç organların temizlenmesi işlemi mikroplastiklerle teması doğrudan en aza indirebilen bir yöntemdir (Hantoro ve diğ., 2019; Zhu ve diğ., 2019). Mikroplastikler, sucul canlıların sindirim sistemlerinde yer değiştirerek başka organ ve dokulara geçebilmektedir (Browne ve diğ., 2008; Zeytin ve diğ., 2020). Levrek yeminde bulunan MP'lerin (1-5 µm), çok düşük seviyelerde de olsa, kas dokusuna geçebildiği tespit edilmiştir. Bu geçişin nasıl gerçekleştiği tam olarak bilinmemekle birlikte, mevcut çalışmada bağırsak yoluyla geçtiği tahmin edilmektedir (Zeytin ve diğ., 2020). Benzer bir MP çalışmasında da (Karami ve diğ., 2017), solungaç-iç organlarda ve solungaç-iç organları çıkarılmış bütün (tuzlanmış-kurutulmuş) balıkta yapılan incelemeler sonucu temizlenmiş balık etinde, iç organ içeriğinden çok daha fazla sayıda MP tespit edilmiştir. Bulunan bu plastik partiküllerinin sindirim sisteminden ete geçtiği düşünülmektedir (Karami ve diğ., 2017). Bu nedenle, gıda güvenliği açısından ileride yapılacak olan çalışmalarda MP'lerin canlı vücudu içerisindeki yerinin (translokasyonunun) ve yenilebilir dokuya geçişinin incelenmesi önem arz etmektedir (Zeytin ve diğ., 2020).

Küresel su ürünleri tüketimi 2020 yılı itibariyle, tüketilen tüm proteinin %7'sini ve hayvansal protein tüketiminin yaklaşık %17'sini temsil etmektedir. Tüketilen su ürünlerinin büyük bir kısmı avcılık yoluyla (96,4 milyon ton), diğer kısmı ise su ürünleri yetiştiriciliği (82,1 milyon ton) yoluyla sofralara gelmektedir (FAO, 2020). Su ürünleri yetiştiriciliğindeki canlıları, havuzlarda, tanklarda veya seçilmiş su kütlelerinde yetiştirerek çevresel koşullarını kontrol etmek mümkündür. Yetiştiriciliği yapılan canlıların yaşam süresi genellikle doğadan avlanan canlı türlerine kıyasla daha kısa olduğundan zamanla MP birikimi açısından bunların tüketiminin daha az tehlikeli olduğu düşünülmektedir (Smith ve diğ., 2018). Literatürde yetiştiricilik ve avcılıktan elde edilen su ürünlerinin mikroplastik içeriklerindeki farklılıklar ve kaynakları hakkında belirsizlik bulunmaktadır. Van Cauwenberghe ve Janssen (2014), çiftlik midyelerinin, doğadan yakalanan midyelerden (126 adet) önemli ölçüde daha yüksek miktarlarda MP içeriğine (178 adet) sahip olduğunu bulmuşlardır. Ek olarak, Rochman ve diğ. (2015) tarafından Endonezya ve ABD'deki pazarlarda ticari olarak satılan balık ve midyelerde mikroplastiklerin (>500 µm) varlığı bildirilmiştir (Rochman ve diğ., 2015).

Bununla birlikte su ürünleri sektörünün yan ürünü olarak üretilen bazı ürünlerde de sucul kaynaklardan geçen MP izlerine rastlamak mümkündür. İnsan tüketimi için kullanılmayan su ürünlerinden yapılan ticari bir ürün olan balık ununda, polistren (PS) ve polietilen tereftalat (PET) maddeleri tespit edilmiştir (Castelvetro ve diğ., 2020). Yapılan çalışmalarda, mikroplastik içeren balık unu ile beslenen yetiştiricilik balıklarında MP geçişi olduğu gözlemlenmiştir (Hantoro ve diğ, 2019; Hanachi ve diğ., 2019).

Gıda güvenliği açısından diğer bir önemli konu da, gıda maddelerinin işlenmesi sırasında potansiyel kaynaklarla MP ile kontamine olmasıdır. Su ürünlerinin, işleme teknolojileri, üründe kullanılan katkı maddeleri, işleme prosesinde kullanılan yardımcı elemanlar, ambalaj veya dış etkenler kaynaklı MP ile kontamine olabildiği düşünülmektedir (Şekil 1). İnsan tüketimine sunulan balık, midye, ve deniz algi (nori) gibi canlılarda ve birçok farklı işlenmiş su ürününde MP varlığına ilişkin çok sayıda kaynak bulunmaktadır (Van Cauwenberghe ve Janssen, 2014; Rochman ve diğ., 2015; Karami ve diğ., 2017; Karami ve diğ., 2018; Zhu ve diğ., 2019; Akhbarizadeh ve diğ., 2020; Gündoğdu ve diğ., 2020; Li ve diğ., 2020).

Gıda maddelerine, işleme sırasında kullanılan katkı malzemeleri kaynaklı MP bulaşması gerçekleşebilmektedir (Liebezeit ve Liebezeit, 2014; Smith ve diğ., 2018). İşleme sırasında kullanılan su (Koelmans ve diğ., 2019), tuz (Gündoğdu, 2018), bal ve şeker (Liebezeit ve Liebezeit, 2013) gibi bazı gıda maddelerinde MNP kirliliğine rastlanmıştır. Piyasadaki balık (sardalya ve çaça balığı) konservelerinde yapılan araştırmada 13 ülkeden alınan 20 farklı markada, düşük seviyelerde de olsa, MP izine rastlanmıştır (Karami ve diğ., 2017). Bir diğer çalışmada da incelenen 7 farklı marka balık (ton ve uskumru) konservelerinin %80'inde en az 1 adet MP bulunmuştur. Konserve balıklarda, balıkların temizlenmesi ve/veya konservelenmesi aşamasında kullanılan katkı maddeleri potansiyel MP kaynakları olarak bildirilmiştir (Akhbarizadeh ve diğ., 2020). Ülkemizde 5 farklı şehirde 40 farklı satıcıda tüketime sunulan midye dolmalardaki (n=317) MP seviyelerini inceleyen Gündoğdu ve diğ. (2020), toplamda 204 adet partikül tespit edilmiştir. Paketlenmemiş olan bu midye dolmalarda bulunan MP'lerin, işleme sırasında dış kaynaklardan dolayı mı bulaştığı yoksa canlının içerisinde var olan miktar mı olduğu bilinmemektedir (Gündoğdu ve diğ., 2020). Benzeri işlenmiş su ürünleri için bu durum tüketici sağlığı açısından risk oluşturmaktadır. Mikroplastik konsantrasyonu en aza indirmek için tuzlama, kurutma, dumanlama, paketleme vb. işleme teknolojileri sırasında oluşabilecek MP kontaminasyon kaynakları (Şekil 1) ivedilikle incelenmelidir.

Çapraz kontaminasyonu önlemek amacıyla kolay temizlenebilen ve maliyeti az olan plastik malzemeler işletmelerde tercih edilmektedir. Bununla birlikte bazı işlemede yardımcı makinalar ve ekipmanlar da içerisinde plastik parçalar bulundurabilmektedirler. İşleme akışındaki bu kaynaklardan gıdalara plastik ve MP girişi olabilir (Şekil 1). Bu kaynaklardan bazıları; plastik kesme tahtaları, bıçaklar, straforlar, işleme makinaları, çalışanların giydiği eldiven, önlükler, yüz maskeleri vb. şeklinde sıralanabilmektedir. İşlenmiş ürün içerisinde bulunan plastik vb. yabancı maddeler sonucu gıda ürünleri ile ilgili büyük ürün geri çağırmaları meydana gelmektedir (Wallace ve diğ., 2010; Fadare ve diğ., 2020; Fadare ve Okoffo, 2020). Hem ekonomik açıdan hem de insan sağlığı açısından plastik maddelerin kontaminasyonunun önlenmesi önemlidir.

İşlenmiş su ürünlerinin paketlenmesinde farklı yapıda ambalaj malzemeleri kullanılmaktadır. Sektörde bu amaçla en çok kullanılan materyal plastik olup, ambalaj içerisinde yer alan kalıntı monomerlerinin ve katkı maddelerinin son ürüne taşınarak paketlenmiş ürünün kalitesini bozabildiği bilinmektedir. Ambalaj malzemelerinden ve bileşenlerinden onlarla temas eden gıda maddelerine geçen tehlikeli maddeler tüketicinin sağlığını ve güvenliğini etkilemektedir (Şekil 1) (Alasalvar ve diğ., 2010; Du ve diğ., 2020; Fadare ve diğ., 2020). Önceleri cam kaplarda ambalajlı olarak sunulan birçok gıda ürünü günümüzde artık plastik kaplarda paketlenmektedir (Wallace ve diğ., 2010). Su ürünleri işleme endüstrisinde, plastik ve plastik kaynaklı malzemeler, fabrikalarda hammaddelerin ve işlenmiş ürünlerin depolanmasında veya yarı sert ve diğer esnek biçimlerde ince ambalajlar şeklinde yaygın olarak kullanılmaktadır (Alasalvar ve diğ., 2010).

Su ürünlerinin paketlemesinde yardımcı, yaygın olarak kullanılan plastiklerden bazıları, polipropilen (PP), polistren (PS), düşük yoğunluklu polietilen (LDPE), yüksek yoğunluklu polietilen (HDPE), lineer alçak yoğunluk polietilen (LLDPE), yüksek moleküler yüksek yoğunluklu polietilen (HMHDPE), polyester, naylon, etilen akrilik asit (EAA) ve poliakrilonitril (PAN) şeklinde sıralanabilmektedir (Alasalvar ve diğ., 2010).

Gıdaya bulaşan MP'lerin kaynağı olarak görülen diğer risk etkenleri ise ortamdaki hava ve çalışanlardır (Şekil 1). MP'lerin atmosferik emisyon ile iç ve dış ortamlardaki havada bulunabildiği tespit edilmiştir (Dris ve diğ., 2017). İşleme tesislerinde yer alan havalandırma sistemleri ve filtreler bu açıdan birer kontaminasyon kaynağı olup, bunları kontrolü sağlanmalıdır. Bunun yanı sıra çalışanların üzerinde giydiği kıyafetlerde (önlük, bone, yüz maskesi) MP fiberler içerebilmektedir ve yapılan çalışmalarda ortamdaki havada bulunan MP'lerin kaynağı oldukları tespit edilmiştir (Dris ve diğ., 2017; Du ve diğ., 2020; Fadare ve Okoffo, 2020). İşlenmiş su ürünlerinde ve işleme tesislerinde yapılacak olan inceleme ve araştırmalar sonucu bu MP'lerin kökeni ve bulaşma kaynakları incelenerek gıda güvenliği ile ilgili uygun yönergeler ve limitler belirlenmelidir.

Mikroplastikler ve İnsan Sağlığına Etkileri

Genel olarak 150 μm veya daha büyük plastik partiküllerin bağırsak sistemi tarafından emilemediği bildirilmiştir. Yenilebilir kas dokusunda ve farklı organlarda bulunan MP'lerin ise mideye alınan <20 μm MP'ler olduğu varsayılmaktadır (EFSA, 2016; Lusher ve diğ., 2017). 0,1–10 μm aralığındaki MP'lerin ise organlara, hücre zarlarına, kan-beyin bariyerlerine ve plasentaya nüfuz edebildiği belirlenmiştir (Browne ve diğ, 2008; EFSA, 2016; Lusher ve diğ., 2017). Benzer olarak Zeytin ve diğ. (2020), levrek balığında bulunan MP'lerin (1-5 μm) bağırsak sisteminden kan hücreleri yardımıyla kas dokusuna geçtiğini varsaymaktadır. İnsanlar tarafından yutulan mikro ve nanoplastiklerin %90'ından fazlasının insan vücudunun boşaltım sistemi yoluyla atıldığı düşünülmektedir (Smith ve diğ., 2018). İnsan hücreleri ile yapılan *in vitro* çalışma sonuçlarına göre, MP (10 μm) ve NPlerin (40-250 nm) insan hücreleri üzerindeki potansiyel sitotoksik etkileri gösterilmiştir (Schirinzi ve diğ., 2017).

Bununla birlikte mikro ve nanoplastikler, yapılarındaki patojenik ve patojenik olmayan bakteriler, kimyasallar ve katkı maddeleri kaynaklı da insan sağlığı açısından tehlike yaratmaktadırlar (Smith ve diğ., 2018; Dehaut ve diğ., 2019). Yapılan çalışmalarda sahillerde örneklenen plastiklerde poliklorlu bifeniller gibi kalıcı organik kirleticiler tespit edilmiştir. Polisiklik aromatik hidrokarbonlar, bisfenol-A, ftalatlar gibi katkı maddelerinin salınımı insan vücudunda meydana gelebilmektedir (Rochman, 2015; Akhbarizadeh ve diğ., 2020). Birden fazla MP kaynağına uzun süreli maruz kalındığında vücuttaki kümülatif etki sağlık sorunlarına neden olabildiği görüşü bulunmaktadır (Karami ve diğ., 2018).

İnsan vücudundaki MP'lerin dokuda tutulma ve doku dışına atılım oranı, şekil, boyut, polimer tipi, yüzey kimyası ve içerdikleri kimyasal maddeler gibi çeşitli faktörlerden etkilenmektedir (Smith ve diğ, 2018). MP'lerin hem insanlar hem de su ürünleri üzerindeki uzun vadeli etkileri tam olarak bilinmemektedir. MP'lerin insan sağlığı üzerine etkileri, tüketilen konsantrasyon seviyesine bağlıdır ve günümüzde MP tüketim miktarına ilişkin yasal sınır değerler henüz düzenlenmemiştir (Smith ve diğ., 2018; Zeytin ve diğ., 2020). Yapılan çalışma sonuçlarından midye tüketim oranı ve maruz kalınan MP sayısı hesaplandığında, Avrupa'nın yüksek miktarda midye tüketicilerinin kişi başı yılda 11.000 adete kadar MP yuttuğu sonucuna varılmıştır (Van Cauwenberghe ve Janssen, 2014). İnsanların gıda yoluyla maruz kalabileceği gerçek mikroplastik miktarını değerlendirmek için yeterli bilgi bulunmamaktadır ve bu konuda yapılacak olan araştırmalara ihtiyaç duyulmaktadır.

Mikroplastiklerin ve Nanoplastiklerin İdentifikasyonu ve Sayımı

Mikroplastikleri ve Nanoplastikleri Ortamdan Ayırma Teknikleri

Mikro ve nanoplastiklerin (MNP) doğru ve kolay tanımlanması için analizi yapılacak karmaşık yapılı örneklerden plastik partiküllerini izole etmek gerekmektedir (Nguyen ve diğ., 2019). Bu partikülleri izole etme teknikleri; elle manuel olarak ayırma veya partiküllerin fiziksel ve kimyasal özelliklerinden yararlanılarak ayırma şeklinde uygulanmaktadır. Diseksiyon, fiksasyon ve kriyoseksiyon yöntemleri manuel olarak partiküllerin ayrımında kullanılan ucuz yöntemlerdir (Dehaut ve diğ., 2016; Nguyen ve diğ., 2019). Diseksiyon işlemi, balık, balina, midye vb. canlıların gastrointestinal sistemlerinde >500 µm mikroplastiklerin görsel olarak tanımlanması için kullanılan bir yöntemdir. Bununla birlikte, daha küçük mikroplastikler diğer dokulara ve organlarda yer değiştirebilmektedir ve fiksasyon ve kriyoseksiyon yöntemleri ise bu tip örneklerde, farklı MP translokasyonlarını (Karaciğer dokusu gibi) incelemede yararlı olabilmektedir (Nguyen ve diğ., 2019). Bu yöntemler karmaşık biyolojik materyallerde yetersiz olabilmektedirler.

Kimyasal ayırma işleminde yaygın olarak asitler (HNO_3, HCl vb.), bazlar (KOH, NaOH vb.), okside edici ürünler (H_2O_2, NaClO, Fenton ayıracı vb.) ve enzimler (Tripsin, Proteinaz K vb.) kullanılmaktadır (Dehaut ve diğ., 2019; Nguyen ve diğ., 2019). Biyolojik matrislerin yok edilmesinde asitler oldukça faydalıdır; fakat sıcaklığa göre farklılaşmakla birlikte çok güçlü asit veya alkali ortamlar, plastik polimerlerinin bozulmasına neden olup çalışmalarda analitik hatalara neden olabilmektedir (Barbosa ve diğ., 2020). Kimyasal ayırma işleminde kullanılan maddeler, plastiğe etkisi ve analiz açısından etkinliği hakkında literatürde metodoloji çalışmaları da yapılmaktadır (Dehaut ve diğ., 2016; Karami ve diğ., 2017).

Plastikler, bulundukları ıslak çevresel ortamdan daha az yoğun ve daha hidrofobik olma eğiliminde olduklarından dolayı yoğunluk farkı ile de yüzdürülerek kolayca ayrılıp ardından boyut ayırma yoluyla filtrelenirler (Silva ve diğ., 2018; Nguyen ve diğ., 2019). MNP'lerin ayrılması için yoğunluğa dayalı ayırmada NaCl, NaI, KI, $ZnCl_2$, $ZnBr_2$ gibi doymuş tuz çözeltileri kullanılmaktadır (Barbosa ve diğ., 2020). Plastik polimerlerin yoğunluğu 0,8-1,7 g cm^{-3} arasında değişmektedir (Löder ve Gerdts, 2015).

Literatürde önerildiği üzere, yoğunluk ayrımları rutin olarak santrifüjleme yoluyla gerçekleştirilmektedir (Nguyen ve diğ., 2019). MP'lerin yoğunluk farkı veya santrifüj yoluyla balık dokusundan ayrımı başarılı olarak literatürde uygulanmıştır (Karami ve diğ., 2017; Gündoğdu ve diğ., 2020).

Ayrıca bulundukları ortamın katı içeriği düşük olduğunda, plastikler boyut bazlı filtreleme ile kolayca ayırt edilebilirler (Nguyen ve diğ., 2019). Bu yöntemlerin dezavantajı küçük boyutlu partiküller için dışarıdan da kontaminasyon olabileceği için riskli olmasıdır.

Fiziksel ayırma işleminde ise filtrasyon, ultrasonik ve yerçekimi kaynaklı ayırma teknikleri kullanılmaktadır. Filtrasyon işleminde filtre tıkanmadan olabildiğince küçük partikülleri yakalayıp ayırması temel alınmalıdır. Çalışmalarda giderek daha küçük gözenek boyutlarının kullanıldığı sıralı filtreleme, filtre tıkanmasını en aza indirebilen bir yöntem olarak uygulanabilir (Dehaut ve diğ., 2016; Nguyen ve diğ., 2019). Filtrelemeden sonra, örneklerdeki partiküllerin kalitatif (renk, şekil ve bileşen tanımlama) analiz ve niceliksel analiz (sayısı ve boyut dağılımı/aralığı) için filtrede tutulması gerekmektedir (Hidalgo-Ruz ve diğ, 2012).

Doğru Filtre Seçiminin Önemi

Kullanılan filtrenin gözenek boyutu ve türü yapılan çalışmaların hassasiyeti açısından oldukça önemlidir (Dehaut ve diğ., 2019; Toussaint ve diğ., 2019; Cai ve diğ., 2020). Filtrelerin yapı tipleri ve gözenek boyutları MP'lerin miktar tayini üzerinde etkili olup yanlış filtre kullanımı örnekteki MP sayısının eksik hesaplanarak yanlış sonuca neden olabilmektedir. Gözenek boyutu azaldıkça bulunan plastik parçaların sayısı da artmaktadır (Toussaint ve diğ, 2019; Cai ve diğ, 2020; Akhbarizadeh ve diğ., 2020).

MP çalışmalarında, örnekleme yöntemini ve özellikle kullanılacak ağ/membran filtre türlerini açıklayan tek tip bir protokol henüz bulunmamaktadır. Su numunelerinde MP analizi yapılan bazı araştırmalarda, örnekler Manta-trol ve nöston vb. ağlarla toplu olarak filtrelenmektedir (GESAMP, 2015). Örneklemeden sonra, MP'leri ayırmak için naylon, nitroselüloz, cam elyaf, polikarbonat veya paslanmaz çelikten yapılmış çeşitli filtreler kullanılmaktadır (Cincinelli ve diğ., 2017; Güven ve diğ., 2017; Dehaut ve diğ., 2019; Toussaint ve diğ., 2019; Cai ve diğ., 2020).

Partiküllerin tutulma şekillerine bağlı olarak membran filtreler, gözenek derinliği ve gözenek genişliğine göre iki tipte sınıflandırılır. Gözenek derinliğine sahip filtreler, paslanmaz çelik ağ, naylon, cam elyaf/pamuk elyaf ve nitroselüloz/karışık selüloz filtrelerdir. Yapı olarak birbirinden farklı olan bu filtre türlerinin gözenekleri derin ve kıvrımlı olup boyutları ortalama bir değerdir (Yu ve diğ., 2010). Polikarbonat membran filtreler gibi gözenek genişliğine göre sınıflandırılan filtrelerde ise ölçülen dairesel gözenekler filtrenin gerçek boyutu olup, kanalları ise sığ ve düzdür (Cai ve diğ., 2020).

Çalışmalarda seçilen filtre ve etkinliğini inceleyen Cai ve diğ. (2020), filtre türü ve gözenek boyutunun yapılacak olacak çalışma koşullarına uygun olarak minumum zaman alacak şekilde seçilmesi gerektiğini önermiştir. Filtrenin seçilen gözenek boyutu, MP analizinin daha sonraki aşaması olan mikroskopla gözlem, seçim ve tanımlama aşamalarıyla da tutarlı olmalıdır.

Laboratuvar Ortamında Mikroplastik Kontaminasyonunun Kontrolü

Mikroplastiklerin analizinin yapıldığı ortam ve kişilerden kaynaklı bulaşma söz konusu olup bu durum analiz sonuçlarının olduğundan fazla hesaplanmasına neden olabilmektedir (Dehaut ve diğ., 2019). Laboratuvar ortamında çalışan kişilerin pamuk yerine sentetik yapılı kumaşlardan yapılma önlük kullanımı sonucu MP bulaşması söz konusu olabilmektedir (Van Cauwenberghe ve diğ, 2013; Dehaut ve diğ., 2019). Bunun yanı sıra çalışanların ellerinde de tekstil kaynaklı MP fiberleri bulunabilir. Bunun önlenmesi adına filtrelenmiş su/alkol solüsyonları veya basınçlı hava kullanılması önerilmektedir. Analiz yapılan alan temiz filtreye sahip olduğundan emin olunan laminer hava akışlı tezgah veya davlumbaz olmalıdır. Kullanılan çözeltilerin hepsi önceden düşük mikron boyutlarında filtreden geçirilmelidir (Dehaut ve diğ., 2019). Standartlaştırılmış bir MP analizi gerçekleştirmek için bu adımlar önemlidir.

Mikroplastikler her alanda bulunduğu için analizden önce ve analiz sırasında numune kontaminasyonu kaçınılmazdır (Barbosa ve diğ., 2019). Çalışmalarda kontaminasyon seviyesi kontrol yapılarak sürekli her aşamada belirlenmelidir. Bunun için ortama temiz bir cam petride filtre kâğıdı bırakılır ve herhangi bir aşamada MP kontaminasyonu gerçekleştiğinde sayısı belirlenir (Dehaut ve diğ., 2019).

Mikroplastiklerin ve Nanoplastiklerin Kompozisyonlarının Tespitinde, Tanımlanmasında ve Miktarlarının Belirlenmesinde Kullanılan Teknikler

Mikro ve nanoplastiklerin karakterizasyon yöntemlerinde kullanılan cihaz ve metotların avantaj ve dezavantajları Tablo 1'de özetlenmiştir.

Görsel Analiz Metotları

Yapılan çalışmalarda; parçacığın boyutlarının ve morfolojisinin belirlenmesi ve polimer miktarının belirlenip ve ölçülmesi elzemdir. Beş yüz µm'ye kadar olan partiküllerin görsel gözlemi basit olarak, çıplak gözle veya bir diseksiyon mikroskobu kullanılarak yapılabilmektedir (Renner ve diğ., 2018). Daha ayrıntılı incelemeler için farklı cihazlar kullanılmaktadır. Partiküllerin boyut ve morfolojik açıdan ayrımında optik, elektron ve tarama özelliklerine dayanan polarize ışık mikroskobu, stereoskop veya floresan mikroskobu kullanılmaktadır (Barbosa ve diğ., 2020). Bu yöntemler hızlı, ucuz ve kolay metotlar olmasına rağmen plastik polimerlerinin kimyasal yapılarını tanımlamada sınırlı potansiyele sahiptirler ve insan hatasına da oldukça açık metotlardır (Silva ve diğ., 2018). Taramalı Elektron Mikroskobu (SEM), Transmisyon Elektron Mikroskobu (TEM), Taramalı Tünelleme Mikroskobu (STM) gibi elektron ve taramalı prob mikroskopları, MP'lerin ayrımını görüntü tabanlı tekniklerle inceleyen yaygın tekniklerdir; fakat aynı zamanda büyük ölçekli analizlerde oldukça zaman almaktadırlar (Nguyen ve diğ., 2019).

Tablo 1. Mikro ve nanoplastiklerin karakterizasyon yöntemlerinin avantaj ve dezavantajları (Shim ve diğ., 2017; Silva ve diğ., 2018; Nguyen ve diğ., 2019 ve Toussaint ve diğ., 2019' dan modifiye)

Table 1. Advantages and disadvantages of characterization methods of microplastics and nanoplastics (Modified from Shim et al., 2017; Silva et al., 2018; Nguyen et al., 2019 and Toussaint et al., 2019)

Tespit/Tanımlama/Miktar Belirleme Yöntemleri	Cihaz	Avantajları	Dezavantajları
Görsel Analiz	Diseksiyon Mikroskobu Polarize Işık Mikroskobu Floresan Mikroskobu Taramalı Elektron Mikroskobu (SEM) Transmisyon Elektron Mikroskobu (TEM) Taramalı Tünelleme Mikroskobu (STM)	Basit, hızlı ve kolay analiz imkanı; Diğer metotlara göre daha ucuz	Kimyasal doğrulama yok; Yüksek oranda yanlış tanımlama olasılığı; Küçük ve şeffaf plastik partiküllerin eksik olma olasılığı yüksektir; Polimer bileşimi tanımlanamaz; Büyük ölçekli analizlerde zaman alıcı
Optik Titreşim Spektroskopisi	**FTIR spektroskopisi** Mikro-FTIR Spektroskopisi (μ-FTIR)	Polimeri tanımlar; Seçici ve tekrarlanabilir analiz; Küçük numune miktarları gerektirir; Örneğin ön işlem hazırlığı azdır; Örneği tahrip etmez; Canlı organizmalardaki kalıntıları lokalize edebilir	Polimer yapısı zarar görmüş örneklerde etkili değildir; FTIR-mikroskop kombinasyonu pahalıdır; Geniş alan görüntüleme için zaman alıcı (20 saate kadar süren)
	Azaltılmış Toplam Yansımalı-Fourier Dönüşüm Kızılötesi Titreşimli Spektroskopisi (ATR-FTIR)	Polimeri tanımlar; Örneğin ön işlem hazırlığı azdır; Hızlı ve ucuz analiz	Büyük partikülleri tanımlayabilir; Örneği tahrip edebilir
	Mikro-Raman Spektroskopisi (μ-Raman)	Polimeri tanımlar; Seçici ve tekrarlanabilir analiz; Küçük numune miktarları gerektirir; Örneğin ön işlem hazırlığı azdır; Kısmen tahribatsız analiz eder; Raman mikroskobu, tek tek mikron altı parçacıkları ve türlerini tanımlayabilir; Canlı organizmalardaki kalıntıları lokalize edebilir	Örneklerin otomatik floresansı, Raman sinyalini maskeleyebilir; Polimer yapısı zarar görmüş örneklerde etkili değildir; Örnekler lazerle zarar görebilir; Geniş alan görüntüleme için zaman alıcı (38 saate kadar); Raman mikroskop aletleri pahalıdır
X-ışını Spektroskopisi	Enerji Dağılımlı X-ışını Spektroskopisi (EDS, EDX, EDXS veya XEDS)	Polimeri tanımlar; Karbon baskın plastiklerin inorganik parçacıklardan ayrımında etkilidir (SEM ile kombine edildiğinde).	Sadece belirli polimerleri tanımlayabilir (SEM ile kombine edildiğinde); Kombine EDS cihazları pahalıdır; Uzun zaman ve çaba gerektiren ön işlem gerektirir
İkincil İyon Kütle Spektrometresi	Uçuş Zamanlı İkincil İyon Kütle Spektrometresi (TOF-SIMS)	Polimerleri tanımlar; Yüksek uzaysal çözünürlüklü görüntüleme mümkündür; Örneği tahrip etmez; Parçacık karışımları için uygundur; Düşük μm aralığında parçacıkları tespit edebilir (ppm ile ppb arasını dahil analiz edebilir); Potansiyel olarak inorganik ve organik kimyasal kirleticiler (ağır metaller) hakkında bilgi sağlayabilir	Kompleks data içeriğinden dolayı uzman bir operatöre ihtiyaç vardır; Pahalıdır; Hava koşullarına veya yüzey kirleticilerine göre tanımlamada yanlışlık olabilmektedir; Numune, vakum uyumlu olmalıdır
Termal Analizler	Diferansiyel Tarama Kalorimetre (DSC)	Hızlı, basit; Referans malzemeleri kullanarak polimerleri tanımlar	Polimer karışımlarına sınırlı uygulama
	Piroliz-Gaz Kromatografisi-Kütle Spektrometrisi (Pyr-GC/MS)	Polimerleri ve organik katkı maddelerini tanımlar; Kütle konsantrasyonundaki küçük miktarları belirler (<0.5 mg); Örneğin ön işlem hazırlığı azdır; Biyolojik matrisler ve çevresel örnek taraması için uygundur	Zaman alıcı; Örneği tahrip eder; Partikül numarası, boyutu ve şekli hakkında sonuç vermez; Kompleks data içeriğinden dolayı uzman bir operatöre ihtiyaç vardır; Daha düşük partikül boyutunu mm' nin kesirleri ile sınırlayan numunenin manuel manipülasyonu gerekli
	Termal Ekstraksiyon ve Desorpsiyon-Gaz Kromatografisi/Kütle Spektrometrisi (TDS-GC-MS)	Polimerleri ve organik katkı maddelerini tanımlar; Örnek hazırlama aşaması kolaydır, Örneğin ön işlem hazırlığı azdır; Yüksek kütleli (100 mg' a kadar) örneklerdeki miktarları belirler	Örneği tahrip eder; Zaman alıcı; Kalitatif analizlerde sınırlı ölçüm

Optik Titreşimli Spektroskopi

MP polimer parçacıklarını tanımlama ve sayımını aynı anda yapabilen Raman ve Fourier Dönüşüm Kızılötesi (FT-IR) titreşimli spektroskopileri de kullanılmaktadır. Bu spektroskopik yöntemler örneği tahrip etmeden analiz etmeyi sağlayan nispeten yavaş, fakat kesin ve doğru sonuç veren tekniklerdir. FTIR mikroskopları 5 µm'ye, Raman ise 1 µm'ye kadar açısal (uzaysal) çözünürlüğe sahiptirler (Elert ve diğ., 2017; Nguyen ve diğ., 2019). Genellikle, daha büyük MP'ler ATR-FTIR (Azaltılmış Toplam Yansımalı-Fourier Dönüşüm Kızılötesi Titreşimli Spektroskopisi) ile tanımlanırken, 20 µm'ye kadar olan MP'ler µ-FTIR ile ve ≤20 µm olan MP'ler ise µ-Raman ile karakterize edilir (Käppler ve diğ., 2016; Veerasingam ve diğ., 2020). İki yüz–300 µm'den büyük boyutlu tek partiküller ise, FTIR analizinden önce görsel inceleme yapılarak analiz edilmektedir. ATR-FTIR analizi maliyet etkin bir yöntem olduğundan ve örnek hazırlama veya karmaşık matematiksel işlem gerektirmediğinden ötürü olmadığından tercih edilmektedir; fakat analiz sonucunda MP örneği kristal ile ezildiğinden ötürü tahrip görebilmektedir (Veerasingam ve diğ., 2020). µ-FTIR ise, dedektör yardımıyla daha geniş filtre alanlarının yüksek çözünürlüklü kimyasal görüntülemesine izin veren bir cihazdır (Cincinelli ve diğ., 2017). µ-FTIR ve Raman spektroskopisini karşılaştıran Käppler ve diğ. (2016), özellikle ≤20 µm parçacıklar için daha hassas ölçüm yapabilen Raman spektroskopisini önermiştir; fakat bu yöntem µ-FTIR spektroskopisine (yaklaşık 20 saate kadar) göre daha çok zaman aldığı için (yaklaşık 38 saate kadar) metot tercihinde sıkıntı oluşturmaktadır (Tablo 1). Ayrıca Raman teknolojisinin diğer dezavantajları arasında, fiberleri veya pigment içeren parçacıkları kolayca tanımlayamaması ve lazerinin yüksek enerji yoğunluğu nedeniyle plastik numune parçacıklarını tahrip etme özelliği bulunmaktadır (Dehaut ve diğ., 2019). FTIR cihazının ise, nem içeriğine karşı duyarlı olması ve siyah partikülleri tanımlamadaki yetersizliği dezavantajları arasında sıralanabilir (Käppler ve diğ., 2016). Yapılan çalışmalardan özetle, FTIR Raman'a göre küçük partikülleri (özellikle <20 µm, analiz etmede daha yetersiz olduğundan %35 daha az plastik tespit edebilmektedir (Käppler ve diğ., 2016).

FTIR ve Raman cihazlarına ek olarak, SEM, TEM vb. elektron mikroskobu ile görüntülenen MP'ler, Enerji dağılımlı X-ışını spektroskopisi (EDS) ile analiz edilerek temel bileşimleri identifiye edilebilmektedir. Kombine SEM-EDS cihazı maliyet açısından pahalıdır ve örnek hazırlama ve örnek inceleme için önemli ölçüde zaman ve çaba gerektirir, bu da analiz edilebilecek örnek sayısını sınırlamaktadır. MP'lerin renkleri SEM cihazında tanımlayıcı olarak kullanılamaz, bu nedenle bu cihaz sadece plastik parçacıkların yüzey karakterizasyonu ve temel bileşim analizi için önerilmektedir (Shim ve diğ., 2017; Silva ve diğ., 2018). Buna ek olarak, bir diğer benzer yöntem olan Uçuş Zamanlı İkincil İyon Kütle Spektrometresi (TOF-SIMS) cihazı ile de, SEM-EDS cihazı ile analizi mümkün olmayacak bazı organik malzemelerden ve doku bölümlerinden polietilen gibi MP'lerin karakterizasyonu yapılabilmektedir (Jungnickel ve diğ., 2016; Toussaint ve diğ., 2019).

Termal Analiz Metotları

Termal analiz, belirli polimer türlerinin kimyasal tanımlanması için spektroskopiye dayalı yöntemlere alternatiftir. Bu yöntemlerin en önemli dezavantajı, cihaza verildikten sonra MP numunelerini tahrip ederek analiz etmeleridir (Shim ve diğ., 2017).

Diferansiyel tarama kalorimetrisi (DSC), polimerik malzemelerin termal özelliklerini incelemek için yararlı bir yöntemdir. Yöntem, polimer türlerini tanımlamak için referans malzemeleri gerektirir, çünkü her plastik ürün, DSC'de farklı özelliklere sahiptir. DSC ile analiz nispeten basit ve hızlıdır; ancak çevresel numunelerde karışık polimer ürünlerden MP'lerin tanımlanmasında sınırlıdır (Shim ve diğ., 2017; Toussaint ve diğ ., 2019).

Bununla birlikte plastik partiküllerin kimyasal bileşimleri (polimer türü) ise, spektroskopi veya kütle spektrometrisine dayalı teknikler kullanılarak incelenebilmektedirler. Bu tekniklerden polimerlerin hızlı tanımlanmasında etkili ancak boyut dağılımlarını belirleyemeyen Pyr-GC/MS (Piroliz-gaz kromatografisi-kütle spektrometrisi) veya TDS-GC-MS (Termal Ekstraksiyon ve Desorpsiyon-Gaz Kromatografisi/Kütle Spektrometrisi) gibi örnek tahrip edici termoanalitik yöntemler en yaygınlarıdır (Zeytin ve diğ., 2020).

Pyr-GC/MS yüksek sıcaklıklarda ayrıştırılan numunelerin gaz kromatografisi ile ayrılması ve kütle spektrometrisi ile analizini içeren bir cihazdır (Nguyen ve diğ., 2019). Piroliz gaz kromatografisi-kütle spektrometrisi tekniğinin avantajı, katı numune doğrudan cihaza verilebildiği için ön işlem ihtiyacını ortadan kalkmış olur ve örnek miktarı oldukça azdır (5-200 µg). Sadece numune başına polimer kütlesini ölçen Pyr-GC-MS'nin mikroplastiklerin sayısını, türünü veya morfolojisini belirlemede yararlı değildir, bu nedenle MP'lerin analizinde optik tekniklerle birlikte kullanılmalıdır (Dehaut ve diğ., 2016; Silva ve diğ., 2018).

TDS-GC-MS, numunenin 1000°C'ye kadar olan sıcaklıklara ısıtılmasıyla kütlesinin termogravimetrik olarak ölçülmesi prensibine dayanır (Nguyen ve diğ., 2019). Nispeten yüksek kütleli (100 mg'a kadar) numuneler için uygun bir tekniktir; ancak kalitatif analizlerde sınırlıdır (Duemichen ve diğ., 2015; Nguyen ve diğ., 2019). TDS-GC/MS ile karşılaştırıldığında, Pyr-GC/MS yaklaşık 50 µg' a kadar olan nanoplastiklerin tanımı ve ölçümünde daha hassastır (Nguyen ve diğ., 2019).

Sonuç

Günümüzde oldukça popüler bir çalışma alanı haline gelen su ürünlerinde mikroplastik kirliliği insan sağlığı açısından araştırılması gereken önemli bir konudur. İnsanların su ürünleri kaynaklı mikroplastik tükettiği bilinmektedir. Bu tüketim hesaplanarak, insanlar için günlük limit alım değerlerinin belirlenmesi elzemdir. İnsan tüketimine sunulan işlenmiş su ürünlerinde bulunan MP'lerin, sucul kirlilik kaynaklı mı yoksa işleme prosesleri sırasında mı bulaştığıyla ilgili kaynağı tespit etmeye yönelik henüz bir çalışma bulunmamaktadır. Gıda güvenliği açısından MP'lerin analizi, insan sağlığı açısından riskleri ve tüketilen gıdalara MP'lerin bulaşma kaynakları gibi çalışma konularının uygun metot ve araştırma yöntemleri kullanılarak araştırılması gerekmektedir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışmasının olmadığını beyan eder.

Etik kurul izni: Yazarlar, bu çalışmanın etik izin gerektirmediğini beyan etmişlerdir.

Finansal destek: -

Teşekkür: -

Açıklama: -

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Checklist of marine diatoms from the Turkish coastal waters with updated nomenclature

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ABSTRACT

Marine diatom research in the coastal waters of Turkey started nearly two centuries ago. In the last decades, increasing numbers of contributions extended the knowledge of the marine phytoplankton. While several studies dedicated to planktonic forms and the checklists published concerning on the phytoplankton, relatively low numbers of benthic diatom studies were performed. Therefore, this is the first detailed list of the marine diatoms including both planktonic and benthic forms in Turkish coasts. This paper brings up the checklist of the past research referring to the authors in the last two centuries within the scope of the latest nomenclature. A total of 767 taxa (species, varieties and forms) belonging to 183 genera were listed. This study focussed into the study areas according to the reviewed literature and showed that many areas are yet to be investigated.

Keywords: Biogeography, Checklist, Epilithic, Epipelic, Epiphytic, Marine diatoms, Phytoplankton, Turkey

Introduction

The knowledge of biodiversity and biogeography of diatoms showed remarkable progress in the last two centuries. In marine diatoms, drawings contributed into systematics and biogeography of the diatoms in mid-late 19th century and continued within the early 20th century with many atlas and monographs (Smith, 1853; Schmidt, 1874-1959; Cleve, 1894-1895; Peragallo and Peragallo, 1897-1908), which are still fundamental sources for marine diatom researchers. In the last decades with the aid of light microscopy (LM) and scanning electron microscopy (SEM), many species were discovered in the marine coastal waters, which extended the knowledge of diatom assemblages from different coasts. The recent monographs revealed many endemic species and increased the data of widespread taxa and some location-specific taxa with introductions to new locations; e.g., Baltic Sea, Snoeijs (1993), Snoeijs and Vilbaste (1994), Snoeijs and Potapova (1995), Snoeijs and Kasperovičienè (1996), and Snoeijs and Balashova (1988), the Bahamas Hein et al. (2008), the Mediterranean, Blanco and Blanco (2014), Madagascar Metzeltin and Lange-Bertalot (2002), Kryk et al., (2020), the Pacific, Lobban et al., (2012), Stidolph et al., (2012). Witkowski et al. (2000) published a monograph of the taxa from different locations of the marine coasts around the world. These monographs and checklists (Hendey, 1974; López-Fuerte and Siqueiros-Beltrones, 2016) provided complete biogeographical dispersal of the benthic diatoms and led further studies in family, genus or even taxon-specific levels (molecular and phylogeny).

The first study in Turkish coastal waters conducted in mid 19th century; Ehrenberg (1844), described species from the Bosphorus in the Sea of Marmara, after some time, Hustedt also described species with drawings in Bosphorus and the Golden Horn in the Sea of Marmara (Hustedt, 1930-1966). Simonsen (1987) illustrated these taxa from Hustedt collection later. However, the first studies were conducted quite a while ago in Turkey; still today, there is very little data in terms of benthic diatoms. The past papers published on the phytoplankton of Turkish coastal waters included several species with ≥ 30 µm diameter which also observed in the benthos. Therefore, some diatom taxa living in benthos with relatively bigger cell size or showing centric morphology which allow cells to suspend in the water column easily (e.g., *Pleurosigma elongatum* W. Smith, *Striatella unipunctata* (Lyngbye) C. Agardh, *Navicula directa* (W. Smith) Brébisson, *Coscinodiscus* spp.) were reported from the surface waters or open waters in Turkish seas. Several authors published checklists regarding phytoplankton composition in the last decades. Koray (2001) reported the phytoplankton from Turkish seas, while Balkıs (2004) published a checklist from the Sea of Marmara, and Taş and Okuş (2016) from the Black Sea. A checklist of freshwater algae, which includes freshwater diatoms, by Aysel (2005), also covered many benthic diatoms; however, any lists comprising exclusively marine benthic diatoms has yet not published. Even though research on marine phytobenthos is increasing, the biodiversity in the marine coasts and the biogeography of the diatoms in Turkey remained unclear. The low number of studies conducted in the coastlines and the outdated nomenclature used in the previous papers prevented to produce a collective list of the benthic diatoms, even though, Turkey surrounded by seas; Black Sea, Mediterranean Sea, the Aegean Sea, and has an inland sea; the Sea of Marmara. However, several benthic studies were already performed, or checklists were published in the adjacent countries, e.g., Romania (Caraus, 2002, 2012, 2017); Russia (Nevrova, 2016); Greece (Foged, 1986; Louvrou, 2007).

Furthermore, the lack of drawings and micrographs or morphological features allowed most of the taxa remained as doubtful in the past studies (Koray 2001). There were recently increasing research, including morphological details providing comparable data for further studies. However, there was still a need for marine diatom lists to understand and enhance taxonomy and biogeography of diatoms in Turkish coasts.

This paper aimed to create a list of reported marine diatom species with updated nomenclature in the coasts of Turkey with an emphasis on doubtful and illustrated taxa within the results of major marine diatom studies performed so far. The list was created to gather all the scattered previous data to provide a comparable key particularly relying on the illustrated taxa for the future research in Turkey.

Methodology

This diatom checklist was created using information from the publications between 1844-2020. The literature search revealed 56 references included planktonic diatoms and marine benthic diatoms in the coasts, coastal lake and lagoons of Turkey. Habitat information and the study areas were presented for each species (Table 1). The references included articles, reviews, checklists, PhD and MSc thesis. Freshwater species reported in some studies were not excluded due to riverine pressure and salinity changes in different locations from the Mediterranean to the Black Sea. However, there were several marine diatom species found in inland waters, according to Maraşlıoğlu and Gönülol (2020), these taxa were not taken into account due to possible misidentifications.

Some species reported with different names from the latest taxonomical order. An effort was put to classify the species with the updates to their current names, according to Guiry and Guiry (2020) and Kociolek et al. (2020). Systematic classification followed Round et al. (1990). Taxa cited in species-level with "confer" and "sp." were not evaluated in the list due to a need of confirmation of these species.

Many species lack images and even reported for the first time, there were no illustrations or morphological features (e.g., dimensions, striae or fibulae counts). Therefore, references used in the list with illustrations were shown with an asterisk in Table 1.

The Current Status of Marine Diatoms in Turkey

The list contained a total of 767 taxa (species, varieties and forms) belonged to 183 genera. Amongst all, 70 taxa belonged to class Coscinodiscophyceae, 130 taxa to Mediophyceae and 567 taxa to Bacillariophyceae (See Checklist). Current nomenclature of the formerly reported taxa was listed (See Updated Nomenclature). According to the literature, the most cited genera with highest numbers of species were *Navicula* Bory (57), *Nitzschia* Hassal (55), *Chaetoceros* Ehrenberg (53), *Mastogloia* Thwaites (31), *Cocconeis* Ehrenberg (23), *Amphora* Ehrenberg ex Kützing (18), *Halamphora* (Cleve) Mereschkowsky (16). The most common six genera in terms of species numbers consisted of 33.1% of all taxa found in the literature. The most common species were *Cylindrotheca closterium* (Ehrenberg) Reinmann & Lewin which was reported from 21 studies and followed by *Nitzschia longissima* (Brébisson ex Kützing) Grunow (cited 19), *Coscinodiscus radiatus* Ehrenberg, *Thalassionema nitzschioides* (Grunow) Mereschkowsky, *Pleurosigma normanii* Ralfs (cited 17), *Licmophora abbreviata* Agardh, *Achnanthes brevipes* Agardh (cited 15) (Table 2).

The reviewed literature revealed that studies in the coastal waters of Turkey were low in numbers. The Sea of Marmara and the Aegean Sea was the most intensively studied areas with 20 and 19 studies, respectively. Studies in the Mediterranean and the Black Sea were rather scarce with 11 and 14 research in terms of both benthic and planktonic species composition and biogeographical dispersal.

The current paper represents the first exclusively detailed marine diatom list in the Turkish coastal waters. Since the studies started in 1844 with Ehrenberg, several authors showed a significant contribution to the diatom composition throughout the years. Several checklists published in the past by various authors (Koray, 2001; Balkıs, 2004; Taş and Okuş, 2016), however, the latest and progressing systematics of diatoms resulted in many synonyms and transferred taxa into a new species of a genus. This paper brings the data altogether with the latest nomenclature with an emphasis on the current names of the cited taxa. An essential part of the studies performed dedicated to mostly planktonic forms of diatoms; the papers here reviewed generally had both benthic and planktonic diatoms. The number of the taxa seems to be high in the checklist; however, diatom species number from the coasts of Turkey are yet far from similar checklists (Hendey, 1974; López-Fuerte and Siqueiros-Beltrones, 2016; Caraus, 2017). The number of only benthic species determined in the Adriatic Sea was 518 (Viličić et al., 2002). In Turkey, the reason for a low number of taxa might be related to the low number of benthic studies performed in these coasts. Most of the studies reviewed in this paper used a similar sampling technique (plankton net), which aimed to take phytoplankton; and in some of these studies, benthic diatoms were also observed. Therefore, the most cited taxa found to be relatively higher in cell size, which observed through the plankton net. However, the recent findings showed (Kaleli et al., 2020) that the number of taxa could increase with the forthcoming studies, which especially aim to reveal benthic species.

The checklist revealed that a high number of freshwater taxa observed in these studies (*Cymbella* spp., *Gomphonema* spp., *Navicula* spp.) mostly in the Black Sea coasts (Soylu et al., 2011; Baytut et al., 2016). It might be related to the freshwater inputs e.g., two major rivers; Kızılırmak and Yeşilırmak, where relatively lower salinity might be a factor to detect the freshwater diatoms in the Black Sea and yielded favourable conditions for the taxa to survive; however, it was observed that marine taxa could dominate in these coastal waters (Kaleli et al., 2017) as well as in the northern Black Sea coasts (Nevrova and Petrov, 2019). Many taxa reported in the list have no documentation or sufficient data on geographical distribution. It is difficult to evaluate the local distribution of the species in comparison to the papers reviewed; however, the list gives an insight into the underlying distribution in the seas of Turkey. The studies used in this checklist revealed that many species lack morphological features and documented as the first record in Turkey. However, in newly reported taxa, LM, SEM and molecular techniques should be given in detail for comparison with the species found in the coasts of Turkey, other coastal areas and beyond. Therefore, this paper revealed the diatom composition in the Turkish coastal waters with the latest nomenclature and could be used as a fundamental list for the latter studies.

Table 1. Literature with all species or partially reported with illustrations marked with an asterisk. Black Sea (B), Sea of Marmara (S), Aegean Sea (A), Mediterranean Sea (M).

Reference	Habitat	Location
1. Ehrenberg (1843)	Epizoic	S
2. Hustedt (1930-1966)*	Benthos	S
3. Egemen et al. (1999)	Planktonic	A
4. Witkowski et al. (2000)*	Benthos	M
5. Aktan (2001)*	Epilithic, Epipelic	S
6. Koray (2001)	Planktonic	B,S,A,M
7. Polat & Işık (2002)	Planktonic	M
8. Türkoğlu & Koray (2002)	Planktonic	B
9. Balkıs (2003)	Planktonic	S
10. De Stefano & Marino (2003)*	Epiphytic	A
11. Balkıs (2004)	Planktonic	S
12. Aktan & Aykulu (2005)*	Epipelic	S
13. Balkıs (2005)*	Benthos	A
14. Baytut et al. (2005)*	Benthos	B
15. Aka & Polat (2006)*	Planktonic	M
16. De Stefano et al. (2006)*	Epiphytic	A
17. Çevik et al. (2008)	Benthos	M
18. Çolak Sabancı (2008)*	Benthos	A
19. De Stefano et al. (2008)*	Epiphytic	A
20. Sıvacı et al. (2008)	Benthos	B
21. Deniz & Taş (2009)	Planktonic	S
22. Gönülol et al. (2009)	Epipelic	B
23. Çolak Sabancı (2010)*	Benthos	A
24. Çolak Sabancı & Koray (2010)*	Benthos	A
25. Polge et al. (2010)	Epipelic	S
26. Altuğ et al. (2011)	Planktonic	S,A
27. Soylu et al. (2011)	Epiphytic	B
28. Çolak Sabancı (2012)*	Benthos	A
29. Özman-Say & Balkıs (2012)	Planktonic	M
30. Çolak Sabancı (2013)*	Benthos	A
31. Ağlaç & Balkıs (2014)	Planktonic	A
32. Aktan et al. (2014)	Epipelic	S
33. Balkıs & Toklu-Alıçlı (2014)	Planktonic	S
34. Blanco & Blanco (2014)*	Benthos	A
35. Pailles et al. (2014)*	Fossil	S
36. Taş (2014)	Planktonic	A
37. Baytut et al. (2016)	Planktonic	B
38. Kısa & Pabuççu (2016)	Benthos, Planktonic	B
39. Pennesi (2016)*	Epiphytic	A

Table 1. Continue

40. Yıldız (2018)*	Benthos	S
41. Kaleli et al. (2017)*	Epilithic	B
42. Kaleli et al. (2018)*	Epilithic	B
43. Li et al. (2018)*	Benthos	A,M
44. Kaleli et al. (2019)*	Epilithic, Epipsammic	M
45. Kaleli et al. (2020)*	Benthos	M
46. Tüfekçi et al. (2008)	Planktonic	S
47. Topçu (2011)	Planktonic	A
48. Taşkın et al. (2019)	Planktonic, Benthic	B,S,A,M
49. Baytut et al. (2013)*	Planktonic	B
50. Taş & Okuş (2006)	Planktonic	B
51. Balkıs & Taş (2016)	Planktonic	S
52. Taş (2017)*	Planktonic	S
53. Taş & Becerril (2017)*	Planktonic	S
54. Ayaz et al. (2018)	Benthic	M
55. Eker-Develi & Kıdeyş (2003)	Planktonic	B
56. Feyzioğlu & Seyhan (2007)	Planktonic	B

Table 2. Most common species in the Turkish coastal waters.

Taxon	Citations	Taxon	Citations
Cylindrotheca closterium	21	***Thalassionema fraunfeldii***	15
Nitzschia longissima	19	***Striatella unipunctata***	15
Coscinodiscus radiatus	17	***Ditylum brightwelli***	14
Thalassionema nitzschioides	17	***Cerataulina pelagica***	13
Pleurosigma normanii	17	***Chaetoceros affinis***	13
Licmophora abbreviata	16	***Chaetoceros lorenzianus***	13
Pseudosolenia calcar-avis	16	***Hemiaulus hauckii***	13
Achnanthes brevipes	15	***Pseudo-nitzschia pungens***	13
Grammatophora marina	15	***Skeletonema costatum***	13
Melosira moniliformis	15	***Proboscia alata***	12

Conclusion

Investigation of the marine diatoms started nearly two centuries ago in Turkish coasts, however, still, a lot of geographical spots were not investigated, and their diatom composition remains unknown. The list comprised many benthic diatoms as well as the previously expressed planktonic forms, nevertheless, showed that total taxa in the Turkish coasts were not entirely determined yet. The future studies should focus on the benthic diatom composition with the help of LM and SEM to provide additional data for further implications. Morphological details of the benthic diatoms would enhance the knowledge not only on the taxonomy but also the biodiversity and their geographical dispersal in the coastal waters like estuarine areas, coastal lakes and lagoons. This paper brought marine planktonic and benthic studies together and comprised a dataset which could be comparative for the future studies in Turkey and other regions. Although there were checklists on marine plankton, this study provided a list of diatoms including both forms from the coasts of Turkey. However, many of the species were not illustrated, future studies could extend the knowledge with accurate identification with the aid of the illustrated studies in the region. It should be noted that there have been no changes in the taxonomy of the genera and species, the nomenclature was updated in several taxa which were transferred or currently not in use anymore, therefore, more research should be carried out to clarify the systematic problems of diatoms in Turkish coastal waters.

Etik Standart ile Uyumluluk

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Updated Nomenclature

List of updated species nomenclature (Taxa) cited in the previous studies (Cited Name), and their synonyms.

Current Name	Cited Name
***Achnanthes brevipes* var. *intermedia* (Kützing) Cleve**	*Achnanthes intermedia* Kützing
***Achnanthes parvula* Kützing**	*Achnanthes brevipes* var. *parvula* (Kützing) Cleve
***Achnanthes wellsiae* (Reimer) Witkowski & Lange-Bertalot**	*Astartiella welsiae* (Reimer) Witkowski & Lange-Bertalot
***Actinocyclus octonarius* var. *ralfsii* (Smith) Hendey**	*Actinocyclus ralfsii* (Smith) Ralfs
***Amphicocconeis disculoides* (Hustedt) Stefano & Marino**	*Cocconeis disculoides* Hustedt
***Amphitetras antediluviana* Ehrenberg**	*Triceratium antediluvianum* (Ehrenberg) Grunow
***Aneumastus tuscula* (Ehrenberg) Mann & Stickle**	*Navicula tuscula* (Ehrenberg) Grunow
***Ardissonea formosa* (Hantzsch) Grunow**	*Synedra formosa* Hantzsch
***Asterionellopsis glacialis* (Castracane) Round**	*Asterionella japonica* Cleve
***Azpeitia nodulifera* (Schmidt) Fryxell & Sims**	*Coscinodiscus nodifer* Schmidt
***Bacillaria socialis* (Gregory) Ralfs**	*Bacillaria paradoxa* Gmelin *Nitzschia paradoxa* (Gmelin) Grunow
***Berkeleya scopulorum* (Brébisson ex Kützing) Cox**	*Navicula scopulorum* Brébisson ex Kützing var. *scopularum*
***Biddulphia biddulphiana* (Smith) Boyer**	*Biddulphia pulchella* Gray
***Brebissonia lanceolata* (Agardh) Mahoney & Reimer**	*Navicula lanceolata* (Agardh) Kützing
***Caloneis amphisbaena* var. *subsalina* (Donkin) Cleve**	*Caloneis subsalina* (Donkin) Hendey
***Catacombas gaillonii* (Bory) D.M.Williams & Round**	*Synedra gaillonii* (Bory) Ehrenberg
***Chaetoceros atlanticus* var. *neopolitanus* (Schröder) Hustedt**	*Chaetoceros neapolitanus* Schröder
***Chaetoceros neogracilis* Van Landingham**	*Chaetoceros gracilis* Schütt
***Chaetoceros protuberans* Lauder**	*Chaetoceros didymus* var. *protuberans* (Lauder) Gran & Yendo
***Chaetoceros willei* Gran**	*Chaetoceros affinis* var. *willei* (Gran) Hustedt
***Cocconeis distans* Gregory**	*Cocconeis granulifera* Greville
***Conticribra weissflogii* (Grunow) Stachura-Suchoples & Williams**	*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle
***Coronia decora* (Brébisson) Ruck & Guiry**	*Campylodiscus decorus* Brébisson

***Coscinodiscopsis jonesiana* (Greville) Sar & Sunesen**	*Coscinodiscus jonesianus* (Greville) Ostenfeld
***Coscinodiscus pavillardii* Forti**	*Coscinodiscus perforatus* var. *pavillardi* (Forti) Hustedt
***Craticula cuspidata* (Kützing) Mann**	*Navicula cuspidata* (Kützing) Kützing
***Craticula cuspidata* var. *heribaudii* (Peragallo) J.Y.Li & Y.Z.Qi**	*Navicula cuspidata* var. *heribaduii* (Peragallo) Cleve
***Craticula halophila* (Grunow) Mann**	*Navicula cuspidata* var. *halophila* Grunow
***Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round**	*Synedra pulchella* (Ralfs) Kützing
***Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin**	*Nitzschia closterium* (Ehrenberg) Smith
***Cymbopleura inaequalis* (Ehrenberg) Krammer**	*Cymbella inaequalis* (Ehrenberg) Rabenhorst
***Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer**	*Cymbella naviculiformis* (Auerswald) Cleve
***Dactyliosolen fragilissimus* (Bergon) Hasle**	*Rhizosolenia fragilissima* Bergon
***Dactyliosolen mediterraneus* (H. Peragallo) H.Peragallo**	*Leptocylindrus mediterraneus* (H.Peragallo) Hasle
***Delphineis australis* Watanabe, Tanaka, Reid, Kumada & Nagudo**	*Rhaphoneis surirella* var. *australis* (Petit) Grunow
***Delphineis surirella* (Ehrenberg) Andrews**	*Rhaphoneis surirella* (Ehrenberg) Grunow
***Didymosphenia geminata* (Lyngbye) Mart.Schmidt**	*Gomphonema geminatum* (Lyngbye) Agardh
***Diploneis crabro* (Ehrenberg) Ehrenberg**	*Navicula crabro* (Ehrenberg) Kützing
***Ellerbeckia arenaria* (D.Moore ex Ralfs) R.M.Crawford**	*Melosira arenaria* Moore ex Ralfs
***Encyonema leiblenii* (Agardh) Silva, Jahn, Veiga Ludwig & Menezes**	*Encyonema prostratum* (Berkeley) Kützing
***Entomoneis alata* (Ehrenberg) Ehrenberg**	*Amhiprora alata* (Ehrenberg) Kützing
***Entomoneis costata* (Hustedt) Reimer**	*Amphiprora costata* Hustedt
***Entomoneis pulchra* (Bailey) Reimer**	*Amphiprora pulchra* Bailey
***Eupyxidicula turris* (Greville) S.Blanco & C.E.Wetzel**	*Stephanopyxis turris* (Greville) Ralfs
***Fallacia forcipata* (Greville) Stickle & Mann**	*Navicula forcipata* Grevillei
***Fallacia forcipata* var. *densestriata* (A.W.F.Schmidt) Gogorev**	*Navicula forcipata* Grevillei var. *densestriata* A.Schmidt
***Fogedia finmarchica* (Cleve & Grunow) Witkowski, Metzeltin & Lange-Bertalot**	*Navicula finmarchica* (Cleve & Grunow) Cleve
***Fragilaria tabulata* var. *truncata* (Greville) Lange-Bertalot**	*Synedra fasciculata* Ehrenberg var. *truncata* (Greville) Patrick
***Fragilariopsis rhombica* (O'Meara) Hustedt**	*Nitzschia angulata* Hasle
***Grammatophora oceanica* Ehrenberg**	*Grammatophora oceanica* (Smith) Hustedt
***Guinardia delicatula* (Cleve) Hasle**	*Rhizosolenia delicatula* Cleve
***Guinardia striata* (Stolterfoth) Hasle**	*Rhizosolenia stolterfothii* H. Peragallo
***Gyrosigma acuminatum* (Kützing) Rabenhorst**	*Gyrosigma spenceri* (Quekett) Griffith & Henfrey
***Gyrosigma macrum* (W.Smith) J.W.Griffith & Henfrey**	*Pleurosigma macrum* Smith
***Gyrosigma reversum* (Gregory) Hendey**	*Pleurosigma reversum* Gregory
***Gyrosigma robustum* (Grunow) Cleve**	*Pleurosigma robustum* Grunow
***Halamphora coffeiformis* (C.Agardh) Levkov**	*Amphora coffeaeformis* (Agardh) Kützing
***Halamphora costata* (W.Smith) Levkov**	*Amphora costata* Smith
***Halamphora exigua* (Gregory) Levkov**	*Amphora exigua* Gregory
***Halamphora holsatica* (Hustedt) Levkov**	*Amphora holsatica* Hustedt
***Halamphora normanii* (Rabenhorst) Levkov**	*Amphora normannii* Rabenhorst
***Halamphora subholsatica* (Krammer) Levkov**	*Amphora subholsatica* Krammer
***Halamphora veneta* (Kützing) Levkov**	*Amphora veneta* Kützing
***Halamphora wisei* (Salah) Álvarez-Blanco & Blanco**	*Amphora turgida* var. *wisei* Salah
***Helicotheca thamesis* (Shrubsole) Ricard**	*Streptotheca tamesis* Shrubsole
***Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski**	*Navicula capitata* Ehrenberg
***Iconella biseriata* (Brébisson) Ruck & Nakov**	*Surirella biseriata* Brébisson
***Karayevia clevei* (Grunow) Round & Bukhtiyarova**	*Achnanthes clevei* Grunow
***Karayevia rostrata* (Hustedt) Bukhtiyarova**	*Achnanthes clevei* Grunow var. *rostrata* Hustedt
***Lyrella abrupta* (Gregory) Mann**	*Navicula abrupta* (Gregory) Donkin
***Lyrella atlantica* (Schmidt) Mann**	*Navicula abrupta* var. *atlantica* (Schmidt) Peragallo & Peragallo
***Lyrella lyra* (Ehrenberg) Karajeva**	*Navicula lyra* Ehrenberg
***Lyrella lyroides* (Hendey) Mann**	*Navicula lyroides* Hendey
***Mastogloia albertii* A.Pavlov, E.Jovanovska, C.E.Wetzel, L.Ector & Z.Levkov**	*Mastogloia smithii* var. *amphicephala* Grunow
***Mastogloia lacustris* (Grunow) Grunow**	*Mastogloia smithii* var. *lacustris* (Grunow) M.Voigt

Melosira moniliformis **(Müller) Agardh**	*Melosira moniliformis* (Müller) Agardh var. *moniliformis*
Metacolioneis tumida **(Brébisson ex Kützing) Blanco & Wetzel**	*Scoliopleura tumida* (Brébisson ex Kützing) Rabenhorst
Microtabella interrupta **(Ehrenberg) Round**	*Striatella interrupta* (Ehrenberg) Heiberg
Navicula capitatoradiata **H.Germain**	*Navicula cryptocephala* var. *intermedia* Grunow
Navicula comoides **(Dillwyn) Peragallo & Peragallo**	*Navicula pseudocomoides* Hendey
Navicula cryptotenella **Lange-Bertalot**	*Navicula radiosa* var. *tenella* (Brébisson ex Kützing) Van Heurck
Navicula phyllepta **Kützing**	*Navicula lanceolata* var. *phyllepta* (Kützing) Van Heurck
Navicula ramosissima **(Agardh) Cleve**	*Navicula ramosissima* var. *ramosissima* (Agardh) Cleve
Navicula tripunctata **(Müller) Bory**	*Navicula gracilis* Ehrenberg
Navicula veneta **Kützing**	*Navicula cryptocephala* var. *veneta* (Kützing) Rabenhorst
Navicula viridula **var.** ***avenacea*** **(Brébisson) Van Heurck**	*Navicula avenacea* (Brébisson et Godey) Brébisson ex Grunow
Navicymbula pusilla **(Grunow) Krammer**	*Cymbella pusilla* Grunow
Neocalyptrella robusta **(Norman ex Ralfs) Hernández-Becerril & Meave del Castillo**	*Rhizosolenia robusta* G.Norman ex Ralfs
Odontidium hyemale **(Roth) Kützing**	*Diatoma hiemalis* Heibe
Olifantiella muscatinei **(Reimer & Lee) Van de Vijver, Ector & Wetzel**	*Olifantiella pseudobiremis* Riaux-Gobin
Pantocsekiella comensis **(Grunow) Kiss & Ács**	*Cyclotella comensis* Grunow
Pantocsekiella kuetzingiana **(Thwaites) Kiss & Ács**	*Cyclotella meneghiniana* var. *kutzingiana* (Thwaites) Playfair
Pantocsekiella ocellata **(Pantocsek) Kiss & Ács**	*Cyclotella ocellata* Pantocsek
Paraplaconeis placentula **(Ehrenberg) Kulikovskiy & Lange-Bertalot**	*Navicula placentula* (Ehrenberg) Kützing
Parlibellus berkeleyii (Kützing) E.J.Cox	*Navicula pseudocomoides* Hendey
Parlibellus plicatus **(Donkin) Cox**	*Navicula plicata* Donkin
Petroneis latissima **(Gregory) Stickle & Mann**	*Navicula latissima* Gregory
Pinnunavis elegans **(Smith) Okuno**	*Navicula elegans* Smith
Placoneis elginensis **(Gregory) Cox**	*Navicula elginensis* (Gregory) Ralfs
Plagiogramma minus (W.Gregory) **Chunlian Li, Ashworth & Witkowski**	*Dimerogramma minor* (Gregory) J.Ralfs
Plagiogrammopsis vanheurckii **(Grunow) Hasle, Stosch & Syvertsen**	*Plagiogramma van-heurckii* Grunow
Planothidium hauckianum **(Grunow) Bukhtiyarova**	*Achnanthes hauckiana* Grunow
Planothidium hauckianum **var.** ***rostratum*** **(Schulz ex Hustedt) Andresen, Stoermer & Kreis, Jr.**	*Achnanthes houckiana* Grunow var. *rostrata* Shultz
Planothidium lanceolatum **(Brébisson ex Kützing) Lange-Bertalot**	*Achnanthes lanceolata* (Brébisson) Grunow
Pleurosigma formosum **var.** ***dalmatica (dalmaticum)*** **Grunow**	*Pleurosigma decorum* W. Smith var. *dalmaticum* sensu Peragallo
Pleurosigma normanii **Ralfs**	*Pleurosigma affine* Grunow
Proboscia alata **(Brightwell) Sundström**	*Rhizolenia alata* Brightwell *Rhizosolenia alata* Brightwell f. *gracillima* (Cleve) Gran
Proboscia indica **(H.Peragallo) Hernández-Becerril**	*Proboscia alata* f. *indica* (H.Peragallo) Gran
Psammodictyon panduriforme **(W.Gregory) D.G.Mann**	*Nitzschia panduformis* Gregory
Psammodiscus nitidus **(Gregory) Round & Mann**	*Coscinodiscus nitidus* Gregory
Pseudo-nitzschia pungens **(Grunow ex Cleve) Hasle**	*Nitzschia pungens* Grunow ex Cleve
Pseudo-nitzschia seriata **(Cleve) H.Peragallo**	*Nitzshia seriata* Cleve
Pseudosolenia calcar-avis **(Schultze) Sundström**	*Rhizosolenia calcar-avis* M.Schultze
Rhizosolenia acuminata **(H. Peragallo) H. Peragallo**	*Rhizosolenia acuminata* (H. Peragallo) Gran
Rhizosolenia imbricata **Brightwell**	*Rhizolenia shrubsolei* Cleve *Rhizosolenia imbricata* var. *shrubsolei* (Cleve) Schröder
Rhopalodia constricta **(W.Smith) Krammer**	*Rhopalodia musculus* var. *constricta* (Brébisson ex Smith) H. & M. Peragallo
Sellaphora pupula **(Kützing) Mereschkowsky**	*Navicula pupula* Kützing
Seminavis eulensteinii **(Grunow) D.B.Danielidis, K.Ford & D.Kennett**	*Amphora angusta* var. *ventricosa* (Gregory) Cleve
Meuniera membranacea **(Cleve) P.C.Silva**	*Stauroneis membranacea* (Cleve) Hustedt
Staurophora amphioxys **(Gregory) Mann**	*Stauroneis gregorii* Ralfs
Stellarima stellaris **(Roper) Hasle & Sims**	*Coscinodiscus stellaris* Roper

***Tabularia affinis* var. *acuminata* (Grunow) Aboal**	*Synedra tabulata* var. *acuminata* (Grunow) Hustedt
***Tabularia fasciculata* (Agardh) Williams & Round**	*Synedra affinis* Kützing *Synedra fasciculata* (Agardh) Kützing *Synedra tabulata* (C. Agardh) Kützing var. *fasciculata* (Kützing) Grunow
***Tetramphora intermedia* (Cleve) Stepanek & Kociolek**	*Amphora rhombica* Kitton var. *intermedia* Cleve
***Tetramphora ostrearia* (Brébisson) Mereschkowsky**	*Amphora ostrearia* Brébisson ex Kützing
***Thalassionema fraunfeldii* (Grunow) Hallegraeff**	*Thalassiothrix frauenfeldii* (Grunow) Grunow
***Thalassiosira eccentrica* (Ehrenberg) Cleve**	*Coscinodiscus excentricus* Ehrenberg
***Thalassiosira gravida* Cleve**	*Thalassiosira rotula* Meunier
***Thalassiosira leptopus* (Grunow) Hasle & Fryxell**	*Coscinodiscus lineatus* Ehrenberg
***Toxarium hennedyanum* (Gregory) Pelletan**	*Synedra hennedyana* Gregory
***Toxarium undulatum* Bailey**	*Synedra undulata* (Bailey) Gregory
***Triceratium pelagicum* (Schröder) Sournia**	*Biddulphia pelagica* Schröder
***Trieres mobiliensis* (Bailey) Ashworth & Theriot**	*Biddulphia mobiliensis* (Bailey) Grunow & Van Heurck *Odontella mobiliensis* (Bailey) Grunow
***Trieres regia* (Schultze) Ashworth & Theriot**	*Odontella regia* (Schulze) Ostenfeld
***Tryblionella acuminata* Smith**	*Nitzschia acuminata* (W.Smith) Grunow
***Tryblionella apiculata* Gregory**	*Nitzschia apiculata* (Gregory) Grunow
***Tryblionella granulata* (Grunow) Mann**	*Nitzschia granulata* Grunow
***Tryblionella navicularis* (Brébisson) Ralfs**	*Nitzschia navicularis* (Brébisson ex Kützing) Grunow
***Tryblionella punctata* Smith**	*Nitzschia punctata* (Smith) Grunow
***Ulnaria danica* (Kützing) Compère & Bukhtiyarova**	*Synedra ulna* (Nitzsch) Ehrenberg var. *danica* (Kützing) Grunow
***Ulnaria ulna* (Nitzsch) Compère**	*Fragilaria ulna* (Nitzsch) Lange-Bertalot *Synedra ulna* (Nitzsch) Ehrenberg

Checklist

List of diatom species reported from previous 56 papers. Nomenclature updated, according to Guiry and Guiry (2020) and Kociolek et al. (2020). Systematics followed Round et al. (1990).

Coscinodiscophyceae	
Actinocyclus cuneiformis (Wallich) F.Gómez, Lu Wang & Senjie Lin	3
Actinocyclus normnanii f. *subsalus* (Juhlin-Dannfelt) Hustedt	37
Actinocyclus octonarius Ehrenberg	6,37,48
Actinocyclus octonarius var. *ralfsii* (Smith) Hendey	6
Actinocyclus subtilis (Gregory) Ralfs	5
Actinoptychus splendens (Shadbolt) Ralfs	6,48
Asterolampra grevillei (Wallich) Greville	6,7,29,31,36,48,51
Asterolampra marylandica Ehrenberg	6,7,15,29,31,36,48
Asterolampra vanheurckii Brun	6,48
Asteromphalus flabellatus (Brébisson) Greville	6,7,18,29,36,48
Asteromphalus heptactis (Brébisson) Ralfs	6,7,29,36,48
Asteromphalus hookeri Ehrenberg	6,15,29,48
Asteromphalus hyalinus Karsten	6,48
Aulacoseira granulata (Ehrenberg) Simonsen	14,25,37
Aulacoseira italica (Ehrenberg) Simonsen	25
Azpeitia nodulifera (Schmidt) Fryxell & Sims	3,6,48,56
Coscinodiscopsis jonesiana (Greville) Sar & Sunesen	6,48
Coscinodiscus asteromphalus Ehrenberg	6,48
Coscinodiscus centralis Ehrenberg	6,8,11,29,48,51
Coscinodiscus concinnus Smith	6,8,21,31,37,48,51,52
Coscinodiscus gigas Ehrenberg	6,48
Coscinodiscus granii Gough	3,6,8,9,11,48,51,52,56
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Coscinodiscus marginatus Ehrenberg	6,8,48
Coscinodiscus oculus-iridis (Ehrenberg) Ehrenberg	6,48,51
Coscinodiscus pavillardii Forti	6,8,48
Coscinodiscus perforatus Ehrenberg	7,8,9,11,15,29,31,37,51
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Dactyliosolen blavyanus (H.Peragallo) Hasle	6,48
Dactyliosolen fragilissimus (Bergon) Hasle	6,8,9,11,33,46,48,50,51,52,56
Dactyliosolen mediterraneus H.Peragallo	6,29,31,48,51
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Eupyxidicula turris (Greville) S.Blanco & C.E.Wetzel	6,11,51
Gallionella asperula Ehrenberg	1
Guinardia cylindrus (Cleve) Hasle	6,9,48,51
Guinardia delicatula (Cleve) Hasle	6,8,11,31,48,50,51,52
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Guinardia striata (Stolterfoth) Hasle	6,8,11,17,29,31,47,48,50,51,52
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Neocalyptrella robusta (Norman ex Ralfs) Hernández-Becerril & Meave del Castillo	3,6,31,48,51,52
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Proboscia alata f. *gracillima* (Brightwell) Sundström	6,50
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Biddulphia biddulphiana (Smith) Boyer	6,7,29,48
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Chaetoceros compressus Lauder	6,8,37,48,50,51,52
Chaetoceros constrictus Gran	6,8,9,11,37,48,50,51,52,53,56
Chaetoceros contortus Schütt	53
Chaetoceros costatus Pavillard	6,9,11,31,48,50,51,52,53
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Chaetoceros dadayi Pavillard	6,29,31,48
Chaetoceros danicus Cleve	6,8,9,11,29,31,48,50,51,52,53,56
Chaetoceros debilis Cleve	6,8,9,48,51,52,53
Chaetoceros decipiens Cleve	6,8,9,29,31,37,47,48,50,51,52,53,56
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Chaetoceros diadema (Ehrenberg) Gran	6,9,11,31,48,50,51,52,53
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Chaetoceros messanensis Castracane	6,8,31,48,50,51
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Chaetoceros pendulus Karsten	37
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Cyclotella caspia Grunow	56
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Cyclotella striata (Kützing) Grunow	3
Cymatosira belgica Grunow	45
Cymatosira lorenziana Grunow	41,45
Detonula confervacea (Cleve) Gran	6,37,48,50,51,52
Detonula pumila (Castracane) Gran	6,11,48,50,51
Ditylum brightwelli (T.West) Grunow	6,8,9,11,31,33,37,46,47,48,50,51,52,56
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Helicotheca tamesis (Shrubsole) Ricard	6,9,11,33,48,51
Hemiaulus hauckii Grunow	6,8,9,29,31,33,37,47,48,50,51,52,56
Hemiaulus membranaceus Cleve	6,48,51
Hemiaulus sinensis Greville	6,11,31,48,51,52,56
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Leptocylindrus danicus Cleve	6,8,9,33,37,46,47,48,50,51,52,56
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Thalassiosira allenii Takano	6,8,9,11,21,48,50,51
Thalassiosira angulata (Gregory) Hasle	6,9,11,37,48,51
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Thalassiosira decipiens (Grunow) Jörgensen	6,8,21,48,56
Thalassiosira eccentrica (Ehrenberg) Cleve	6,8,11,25,37,50,51
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Thalassiosira gravida Cleve	6,8,9,11,21,31,33,37,48,51,52,56
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Thalassiosira leptopus (Grunow) Hasle & Fryxell	6,8,11,48,51
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Achnanthes danica (Flögel) Grunow	45
Achnanthes kuwaitensis Hendey	18
Achnanthes lacunarum Hustedt	25
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Achnanthes parvula Kützing	17,18,25,28
Achnanthes pseudobrevipes Aleem	18
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Asterionellopsis glacialis (Castracane) Round	6,7,8,11,15,29,36,48,50,51,52,56
Bacillaria paxillifera (Müller) T.Marsson	3,6,7,8,11,15,17,20,26,29, 31,37,48,51
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Berkeleya micans (Lyngbye) Grunow	40,41
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Cymbella lanceolata (C.Agardh) C.Agardh	6,8,20
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Cymbopleura inaequalis (Ehrenberg) Krammer	14
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Delicata delicatula (Kützing) Krammer	37
Delphineis australis Watanabe, Tanaka, Reid, Kumada & Nagudo	18,28
Delphineis karstenii (Boden) G.Fryxell	18,44
Delphineis minutissima (Hustedt) Simonsen	41
Delphineis surirella (Ehrenberg) Andrews	45
Denticula subtilis Grunow	41
Diatoma moniliformis Kützing	37
Diatoma tenuis C.Agardh	37
Diatoma vulgaris Bory	6,27,37,38
Didymosphenia geminata (Lyngbye) Mart.Schmidt	6,56
Dimeregramma acutum Hustedt	34
Diplomenora cocconeiformis (Schmidt) Blazé	45
Diploneis aestuari Hustedt	41
Diploneis bombus (Ehrenberg) Ehrenberg	4,6,9,11,29,33,44,48,51
Diploneis chersonensis (Grunow) Cleve	17,37,41,48
Diploneis crabro (Ehrenberg) Ehrenberg	6,8,48
Diploneis decipiens Cleve-Euler	48
Diploneis interrupta (Kützing) Cleve	20
Diploneis notabilis (Greville) Cleve	18
Diploneis ovalis (Hilse) Cleve	20
Diploneis parma Cleve	20

Diploneis pseudovalis Hustedt	4,22,27
Diploneis smithii (Brébisson) Cleve	37
Diploneis stroemii Hustedt	41
Encyonema leibleinii (Agardh) Silva, Jahn, Veiga Ludwig & Menezes	22,27,37
Encyonema minutum (Hilse) Mann	22,27,37
Encyonema silesiacum (Bleisch) D.G.Mann	22
Encyonopsis cesatii (Rabenhorst) Krammer	37
Entomoneis alata (Ehrenberg) Ehrenberg	5,6,12,18,22,48,51
Entomoneis calixasini Paillès, Blanc-Valleron & Poulin	35
Entomoneis costata (Hustedt) Reimer	25,48
Entomoneis gigantea (Grunow) Nizamuddin	6,15,29,48
Entomoneis ornata (Bailey) Reimer	48
Entomoneis paludosa (W.Smith) Reimer	22,48
Entomoneis pulchra (Bailey) Reimer	3
Eolimna minima (Grunow) Lange Bertalot	37
Epithemia argus (Ehrenberg) Kützing	20
Epithemia gibba (Ehrenberg) Kützing	20
Epithemia muelleri Fricke	17,48
Epithemia sorex Kützing	20,22,27,37
Eucocconeis flexella (Kützing) Meister	20
Eunotia arcus Ehrenberg	37
Eunotia bigibba Kützing	37
Eunotia septentrioralis Østrup	37
Fallacia clepsidroides Witkowski	4
Fallacia cryptolyra (Brockmann) Stickle & D.G.Mann	25
Fallacia florinae (M.Møller) Witkowski	40
Fallacia forcipata (Greville) Stickle & Mann	18,28,37,41
Fallacia forcipata var. *densestriata* (A.W.F.Schmidt) Gogorev	18,28
Fallacia nyella Hustedt	41
Fallacia pseudony (Hustedt) Mann	41,45
Fallacia pygmaea (Kützing) Stickle & D.G.Mann	37
Fallacia schaeferae (Hustedt) Mann	44,45
Fallacia subforcipata (Hustedt) D.G.Mann	41
Fogedia finmarchica (Cleve & Grunow) Witkowski, Metzeltin & Lange-Bertalot	18
Fogedia giffeniana (Foged) Witkowski, Lange-Bertalot, Metzeltin & Bafana	41
Fragilaria amphicephaloides Lange-Bertalot	37
Fragilaria capucina Desmazières	37
Fragilaria crotonesis Kitton	6,37
Fragilaria eichornii Witkowski & Lange-Bertalot	41
Fragilaria gracillima Mayer	37
Fragilaria inflata Pantocsek	37
Fragilaria striatula Lyngbye	48
Fragilaria tabulata var. *truncata* (Greville) Lange-Bertalot	25
Fragilaria vaucheriae (Kützing) J.B.Petersen	37,38
Fragilariopsis atlantica Paasche	6,48
Fragilariopsis cylindrus (Grunow ex Cleve) Helmcke & Krieger	6,51
Fragilariopsis oceanica (Cleve) Hasle	5,12,48,51
Fragilariopsis rhombica (O'Meara) Hustedt	56
Frustulia creuzburgensis (Krasske) Hustedt	37
Frustulia rhomboides (Ehrenberg) De Toni	20
Gedaniella flavovirens (H.Takano) Chunlian Li, A.Witkowski & M.P.Ashworth	43
Gedaniella guenter-grassi (Witkowski & Lange-Bertalot) Chunlian Li, S.Sato & Witkowski	40
Gedaniella mutabilis Chunlian Li & Witkowski	40

Geisleria acceptata (Hustedt) Lange-Bertalot & Metzeltin	25
Glyphodesmis distans (W.Gregory) Grunow	41
Gomphonema acuminatum Ehrenberg	20
Gomphonema affine Kützing	37
Gomphonema angustatum (Kützing) Rabenhorst	20,25
Gomphonema capitatum Ehrenberg	22
Gomphonema helveticum Brun	27
Gomphonema minutum (C.Agardh) C.Agardh	37
Gomphonema olivaceum (Hornemann) Brébisson	20,27,37
Gomphonema parvulum (Kützing) Kützing	22,27,38
Gomphonema subtile Ehrenberg	37
Gomphonema truncatum Ehrenberg	37
Gomphonemopsis obscura (Krasske) Lange-Bertalot	40,41
Grammatophora angulosa Ehrenberg	18,28,41,44,48,51
Grammatophora angulosa var. *mediterranea* Grunow	45
Grammatophora arcuata Ehrenberg	18,34
Grammatophora macilenta W.Smith	41
Grammatophora marina (Lyngbye) Kützing	5,6,8,9,11,15,18,29,32,36,37,48,50,51,56
Grammatophora oceanica Ehrenberg	18,28,41,48
Grammatophora oceanica var. *macilenta* (Smith) Grunow	18
Grunowia solgensis (A.Cleve) Aboal	37
Gyrosigma acuminatum (Kützing) Rabenhorst	6,8,18,20,25,37,38
Gyrosigma attenuatum (Kützing) Robenhorst	6,29,48
Gyrosigma balticum (Ehrenberg) Rabenhorst	6,7,15,17,18,28,29,48
Gyrosigma eximium (Thwaites) Boyer	17,37,44
Gyrosigma fasciola (Ehrenberg) Griffith & Henfrey	5,6,9,11,12,25,32,37,48,51
Gyrosigma hippocampus Ehrenberg	3,6
Gyrosigma macrum (W.Smith) J.W.Griffith & Henfrey	6
Gyrosigma obscurum (W.Smith) Griffith & Henfrey	25,37
Gyrosigma peisone (Grunow) Hustedt	25
Gyrosigma reversum (Gregory) Hendey	13,33,48,51
Gyrosigma robustum (Grunow) Cleve	18
Gyrosigma scalproides (Rabenhorst) Cleve	25
Gyrosigma strigilis (Smith) Cleve	27
Gyrosigma tenuissimum (W.Smith) Griffith & Henfrey	6,15,17,29,41,48
Halamphora acutiuscula (Kützing) Levkov	37,44
Halamphora capitata (Hagelstein) A.Blanco & S.Blanco	40
Halamphora coffeiformis (C.Agardh) Levkov	5,12,18,20,25,37,41,51
Halamphora costata (W.Smith) Levkov	5,12,51
Halamphora exigua (Gregory) Levkov	5,12,18,28,37,51
Halamphora holsatica (Hustedt) Levkov	20,37
Halamphora hyalina (Kützing) Rimet & R.Jahn	6,48
Halamphora kolbei (Aleem) A.Blanco & S.Blanco	40
Halamphora normanii (Rabenhorst) Levkov	20,37
Halamphora pseudohyalina (Simonsen) J.G.Stepanek & Kociolek	41
Halamphora staurophora (Juhlin-Dannfelt) Alvarez Blanco & S.Blanco	41
Halamphora subholsatica (Krammer) Levkov	4,44
Halamphora tenerrima (Aleem & Hustedt) Levkov	40,44
Halamphora turgida (Gregory) Levkov	28,37,41
Halamphora veneta (Kützing) Levkov	18,37
Halamphora wisei (Salah) Álvarez-Blanco & Blanco	18,34
Hannaea arcus (Ehrenberg) Patrick	6,37,56
Hantzschia amphioxys (Ehrenberg) Grunow	5,12,18,20,28,32,37,51
Haslea britannica (Hustedt & Aleem) Witkowski, Lange-Bertalot & Metzeltin	41

Haslea nautica (Cholnoky) Giffen	41
Haslea ostrearia (Gaillon) Simonsen	48
Haslea spicula (Hickie) Bukhtiyarova	38,48
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	20
Iconella biseriata (Brébisson) Ruck & Nakov	17,22
Karayevia amoena (Hustedt) Bukhtiyarova	2
Karayevia clevei (Grunow) Round & Bukhtiyarova	18,28
Karayevia rostrata (Hustedt) Bukhtiyarova	25
Licmophora abbreviata Agardh	5,6,7,8,9,11,12,18,29,31,34,36,48,50,51,52
Licmophora dalmatica (Kützing) Grunow	40
Licmophora debilis (Kützing) Grunow ex Van Heurck	40,41
Licmophora ehrenbergii (Kützing) Grunow	6,18,28,37,48,56
Licmophora ehrenbergii f. *grunowii* (Mereschkowsky) Hustedt	18,45
Licmophora flabellata (Greville) Agardh	6,8,18,28,41,48,51
Licmophora gracilis (Ehrenberg) Grunow	6,8,11,18,28,48
Licmophora hyalina (Kützing) Grunow	18,28
Licmophora lyngbyei (Kützing) Grunow ex Van Heurck	3,17,37
Licmophora paradoxa (Lyngbye) Agardh	5,6,8,12,32,48,51
Lyrella abrupta (Gregory) Mann	18,28,37,41
Lyrella atlantica (Schmidt) Mann	18
Lyrella clavata (W.Gregory) D.G.Mann	41
Lyrella lyra (Ehrenberg) Karajeva	5,12,13,18,32,33,48,51
Lyrella lyroides (Hendey) Mann	18
Mastogloia acutiuscula Grunow	44
Mastogloia albertii A.Pavlov, E.Jovanovska, C.E.Wetzel, L.Ector & Z.Levkov	17,18
Mastogloia angulata Lewis	6,17,44,48
Mastogloia aquilegiae Grunow	18,30
Mastogloia baldjikiana Grunow	30
Mastogloia belaensis Voigt	44
Mastogloia binotata (Grunow) Cleve	18,28,30,34,44
Mastogloia braunii Grunow	18,20,28,48
Mastogloia crucicula (Grunow) Cleve	44,45
Mastogloia crucicula var. *alternans* Zanon	44
Mastogloia danseyi (Thwaites) Thwaites ex Smith	48
Mastogloia emarginata Hustedt	45
Mastogloia exigua Lewis	18,37
Mastogloia fimbriata (Brightwell) Cleve	6,39,48
Mastogloia gieskeii Cholnoky	41
Mastogloia grevillei W.Smith	48
Mastogloia grunowii Schmidt	18,30
Mastogloia ignorata Hustedt	30,41
Mastogloia labuensis Cleve	17
Mastogloia lacustris (Grunow) Grunow	18,48
Mastogloia lanceoalata Thwaites ex Smith	18,28,44
Mastogloia ovalis A.Schmidt	45
Mastogloia paradoxa Grunow	30,41
Mastogloia pumila (Grunow) Cleve	2,18,20,28,30,37,41
Mastogloia pusilla Grunow	40
Mastogloia pusilla var. *subcapitata* Hustedt	40,41
Mastogloia similis Hustedt	30,41
Mastogloia smithii Thwaites ex Smith	17,18,20,22,28,37
Mastogloia splendida (Gregory) Peragallo	6
Mastogloia urvae Witkowski	40,48
Mastogloia vasta Hustedt	30,48

Meloneis mimallis Louvrou, Danielidis & Economou-Amilli	45
Metacolioneis tumida (Brébisson ex Kützing) Mann	18
Microtabella interrupta (Ehrenberg) Round	6,8,48
Nanofrustulum shiloi (J.J.Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche	43
Navicula abrupta var. *rattrayi* (Pantocsek) Peragallo & Peragallo	18
Navicula arenaria Donkin	18,28
Navicula arenaria var. *rostellata* Lange-Bertalot	45
Navicula cancellata Donkin	6,18,48
Navicula capitatoradiata H.Germain	18
Navicula cari Ehrenberg	20,27
Navicula cincta (Ehrenberg) Ralfs	18,28,37,38
Navicula cryptocephala Kützing	5,12,18,20,25,28,32,51
Navicula cryptotenella Lange-Bertalot	18,25,28,37,44
Navicula decussata Ehrenberg	1
Navicula digitoradiata (Gregory) Ralfs	18
Navicula directa (W.Smith) Ralfs	5,12,51
Navicula distans (Smith) Ralfs	3,48
Navicula exigua W.Gregory	25
Navicula formenterae Cleve	18
Navicula germanopolonica Witkowski & Lange-Bertalot	40
Navicula globosa F.Meister	37
Navicula gregaria Donkin	27,37
Navicula grevilleana Hendey	18
Navicula inornata Grunow	18
Navicula johanrossi Giffen	28
Navicula libonensis Schoeman	37
Navicula lusoria Giffen	45
Navicula menaiana Hendey	18,48
Navicula menisculus Schumann	5,12,20,38,51
Navicula metareichardtiana Lange-Bertalot & Kusber	44
Navicula palpepralis Brébisson ex W.Smith	5,12,32,41,51
Navicula parapontica Witkowski, Kulikovskiy, Nevrova & Lange-Bertalot	40,41
Navicula pavillardii Hustedt	40
Navicula pennata Schmidt	6,8,18,37,48
Navicula pennata var. *pontica* Mereschowsky	37
Navicula phyllepta Kützing	18,37
Navicula pontica (Mereschkowksy) A.Witkowski, M.Kulikovskiy, E.Nevrova & Lange-Bertalot	41
Navicula pseudosilicula Hustedt	18
Navicula radiosa Kützing	20,22,25,27
Navicula ramosissima (Agardh) Cleve	18,25,44,48
Navicula ramosissima var. *mollis* (W. Smith) Hendey	18
Navicula ramosissima var. *mucosa* (Aleem) Hendey	5,12,18,28,32,51
Navicula resecta J.R.Carter	37
Navicula rostellata Kützing	5,12,38,51
Navicula ryhncocephala Kützing	27,37
Navicula salinarum Grunow	18,22,25,37
Navicula scabriuscula (Cleve & Grove) Mereschkowsky	28
Navicula sigma Ehrenberg	37
Navicula slesvicensis Grunow	20
Navicula subagnita Proschkina Lavrenko	40,41,44
Navicula subinflata Grunow	18
Navicula tenella Brébisson ex Kützing	5,12,25,51
Navicula transitans Cleve	29,48

Navicula tripunctata (Müller) Bory	5,12,18,22,25,27,28,51
Navicula tripunctata var. *schizonemoides* (Van Heurck) R.M.Patrick	25
Navicula trivialis Lange-Bertalot	37
Navicula veneta Kützing	18,22,37
Navicula viridula (Kützing) Ehrenberg	38
Navicula viridula var. *avenacea* (Brébisson) Van Heurck	18
Navicula zostereti Grunow	6,8,48
Navicymbula pusilla (Grunow) Krammer	18,28,38,44
Navicymbula pusilla var. *lata* Krammer	40,44
Neidiopsis levanderi (Hustedt) Lange-Bertalot & Metzeltin	37
Neidium dubium (Ehrenberg) Cleve	37
Neosynedra provincialis (Grunow) Williams & Round	40,41
Nitzschia acicularis (Kützing) Smith	3,22,37
Nitzschia aequorea Hustedt	45
Nitzschia amabilis Suzuki	41,45
Nitzschia amphibia Grunow	38
Nitzschia amplectens Hustedt	37
Nitzschia angularis Smith	18,28
Nitzschia bilobata Smith	3
Nitzschia brevissima Grunow	20
Nitzschia capitellata Hustedt	2
Nitzschia clausii Hantzsch	20,37,41
Nitzschia communata Grunow	25,38
Nitzschia compressa var. *balatonis* (Grunow) Lange-Bertalot	24
Nitzschia constricta f. *parva* Grunow	18
Nitzschia dissipata (Kützing) Rabenhorst	22,27,37,38
Nitzschia elegantula Grunow	44
Nitzschia flexa Schumann	37
Nitzschia fonticola (Grunow) Grunow	22,38
Nitzschia fontifuga Cholnoky	44
Nitzschia frustulum (Kützing) Grunow	5,12,18,28,51
Nitzschia frustulum var. *perpusilla* (Rabenhorst) Van Heurck	5,12
Nitzschia fusiformis Grunow	41
Nitzschia improvisa Simonsen	44
Nitzschia incerta (Grunow) M.Peragallo	37
Nitzschia inconspicua Grunow	44
Nitzschia insignis W.Gregory	26
Nitzschia intermedia Hantzsch	18,28
Nitzschia kuetzingiana Hilse	25
Nitzschia linearis (Agardh) Smith	18,20,28,37
Nitzschia linearis var. *subtilis* Hustedt	24
Nitzschia longissima (Brébisson ex Kützing) Grunow	3,5,6,7,8,9,11,12,15,18,21,29,36,37,48,50,51,52, 56
Nitzschia lorenziana Grunow	6,18,28,29,33,48,51
Nitzschia microcephala Grunow	24
Nitzschia nana Grunow	37
Nitzschia nanodissipata Chunlian Li & Witkowski	45
Nitzschia navis-varingica Lundholm & Moestrup	54
Nitzschia obtusa Smith	18,37
Nitzschia ovalis Arnott	37,38
Nitzschia palea (Kützing) Smith	5,12,20,27,32,37,38,51
Nitzschia prolongata Hustedt	40
Nitzschia recta Hantzsch ex Rabenhorst	37
Nitzschia rectilonga Takano	21,50,51

Nitzschia rectirobusta Lange-Bertalot	24
Nitzschia reversa W.Smith	41
Nitzschia scalpelliformis Grunow	24,28
Nitzschia sicula (Castracane) Hustedt	6,48
Nitzschia sigma (Kützing) Smith	6,7,8,17,18,20,29,33,37,50,51
Nitzschia sigmoidea (Nitzsch) Smith	3,15,17,26,29,33,37,48,51
Nitzschia socialis Gregory	18
Nitzschia socialis var. *massiliensis* Grunow	18,40
Nitzschia supralitorea Lange-Bertalot	24
Nitzschia tryblionella Hantzsch	20,37
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	22,37
Nitzschia valdestriata Aleem & Hustedt	44,45
Nitzschia vermicularis (Kützing) Lange-Bertalot	24
Nitzschia vidovichii (Grunow) Grunow	24
Odontidium hyemale (Roth) Kützing	56
Olifantiella muscatinei (Reimer & Lee) Van de Vijver, Ector & Wetzel	42
Paraplaconeis placentula (Ehrenberg) Kulikovskiy & Lange-Bertalot	20,22,32
Parlibellus bennikei Witkowski	28
Parlibellus berkeleyi (Kützing) Cox	18,40,41
Parlibellus calvus Witkowski, Metzeltin & Lange-Bertalot	40
Parlibellus delognei (Van Heurck) E.J.Cox	41
Parlibellus plicatus (Donkin) Cox	17
Petrodictyon gemma (Ehrenberg) Mann	6,18,28,29,31,48,51,52
Petroneis humerosa (Brébisson ex Smith) Stickle & Mann	5,12,20,32,48,51
Petroneis latissima (Gregory) Stickle & Mann	3
Phaeodactylum tricornutum Bohlin	6,48
Pinnularia aestuarii Cleve	37
Pinnularia bipectinalis (Schumann) Greguss	37
Pinnularia borealis Ehrenberg	37
Pinnularia claviculus (Gregory) Rabenhorst	37
Pinnularia gentilis (Donkin) Cleve	37
Pinnularia interrupta W.Smith	22
Pinnularia lundii Hustedt	37
Pinnularia microstauron (Ehrenberg) Cleve	37
Pinnunavis elegans (Smith) Okuno	3
Placoneis amphibola Cleve	25
Placoneis clementis (Grunow) Cox	20
Placoneis elginensis (Gregory) Cox	27,38
Placoneis gastrum (Ehrenberg) Mereschkowsky	38
Placoneis placentula (Ehrenberg) Mereschkowsky	20,32,51
Plagiogramma minus (Gregory) Chunlian Li, Ashworth & Witkowski	18,41
Plagiogramma minus var. *nanum* (Gregory) Chunlian Li, Ashworth & Witkowski	41,45
Plagiogramma pulchellum var. *pygmaeum* (Greville) H.Peragallo & M.Peragallo	45
Plagiogramma tenuissimum Hustedt	41,45
Plagiotropis lepidoptera (Gregory) Kuntze	37,41
Plagiotropis lepidoptera var. *minor* (Cleve) Czarnecki & J.L.Wee	41
Plagiotropis lepidoptera var. *proboscidea* (Cleve) Reimer	41
Planothidium depertidum (Giffen) Witkowski, Lange-Bertalot & Metzeltin	40,41
Planothidium hauckianum (Grunow) Bukhtiyarova	18,38
Planothidium hauckianum var. *rostratum* (Schulz ex Hustedt) Andresen, Stoermer & Kreis, Jr.	25
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	25
Planothidium lilljeborgei (Grunow) Witkowsi, Lange-Bertalot & Metzeltin	45
Planothidium rostratoholoarcticum Lange-Bertalot & Bąk	25

Pleurosigma aestuarii (Brébisson ex Kützing) W.Smith	37
Pleurosigma angulatum (Quekett) Smith	5,6,8,12,51
Pleurosigma delicatulum Smith	6,48
Pleurosigma elongatum Smith	6,7,15,17,25,29,37,38,44,48
Pleurosigma formosum Smith	6,41
Pleurosigma formosum var. *dalmatica (dalmaticum)* Grunow	18
Pleurosigma normani Ralfs	3,6,7,9,15,17,18,21,29,31,33,36,41,48,50,51,56
Pleurosigma rigidum Smith	6,18,48
Pleurosigma salinarum (Grunow) Grunow	5,12,18,25,28,51
Pleurosigma strigosum Smith	3,44
Podocystis perrinensis Ricard	6,17,29
Proschkinia bulnheimii (Grunow) Karayeva	45
Proschkinia complanata (Grunow) D.G.Mann	41
Psammodictyon panduriforme (W.Gregory) D.G.Mann	5,6,12,18,28,37,48,51
Psammodictyon panduriforme var. *continuum* (Grunow) Snoeijs	45
Psammodictyon rudum (Cholnoky) D.G.Mann	41
Psammodiscus nitidus (Gregory) Round & Mann	18,28,48
Pseudo-nitzschia calliantha Lundholm, Moestrup & Hasle	48,49,51,52
Pseudonitzschia delicatissima (Cleve) Heiden	6,8,11,31,48,50,51,56
Pseudo-nitzschia fraudulenta (Cleve) Hasle	6,11,48,50,51
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle	6,8,9,29,31,48,51,55
Pseudo-nitzschia pungens (Grunow ex Cleve) Hasle	3,6,8,9,11,31,46,47,48,50,51,52,56
Pseudo-nitzschia pungens var. *aveirensis* Lundholm, Churro, Carreira & Calado	49
Pseudo-nitzschia seriata (Cleve) H.Peragallo	11,48,51
Pseudostaurosira elliptica (Schumann) Edlund, Morales & Spaulding	44
Pseudostaurosira perminuta (Grunow) Sabbe & Wyverman	41
Pteroncola inane (Giffen) Round	5
Rhabdonema adriaticum Kützing	5,6,8,15,18,28,29,36,48
Rhabdonema arcuatum (Lyngbye) Kützing	48
Rhabdonema minutum Kützing	37
Rhaphoneis amphiceros f. *gemmifera* (Ehrenberg) Peragallo & Peragallo	18
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	20,27,37
Rhoicosphenia marina (Kützing) Schmidt	5
Rhopalodia acuminata Krammer	44
Rhopalodia constricta (W.Smith) Krammer	18
Rhopalodia gibberula (Ehrenberg) Müller	20
Rhopalodia musculus (Kützing) Müller	17,18,28
Scoliopleura peisonis Grunow	28
Sellaphora pupula (Kützing) Mereschkowsky	20,32,51
Seminavis basilica Dainelidis	41
Seminavis eulensteinii (Grunow) D.B.Danielidis, K.Ford & D.Kennett	18
Seminavis insignis A.Blanco & S.Blanco	40,41
Seminavis robusta Danielidis & Mann	23,41,48
Seminavis strigosa (Hustedt) Danielidis & Economou-Amilli	40,44
Simonsenia delognei (Grunow) Lange-Bertalot	20
Stauroneis anceps Ehrenberg	20
Stauroneis membranacea (Cleve) Hustedt	5
Stauroneis smithii Grunow	22
Staurophora amphioxys (Gregory) Mann	18,28
Staurosirella martyi (Héribaud-Joseph) E.A.Morales & K.M.Manoylov	37
Staurosirella pinnata (Ehrenberg) D.M.Williams & Round	38
Stenopterobia sigmatella (Gregory) Ross	6
Striatella delicatula Kützing Grunow ex Van Heurck	6,8,48,56

Striatella unipunctata (Lyngbye) Agardh	5,6,7,8,9,11,15,17,18,29,31,32,36,37,41,48,50,51,52
Surirella angusta Kützing	20,37
Surirella brebissonii Krammer & Lange-Bertalot	22
Surirella elegans Ehrenberg	17,37
Surirella fastuosa (Ehrenberg) Ehrenberg	3,6,7,17,18,29,48
Surirella minuta Brébisson	25,37
Surirella muelleri Hustedt	37
Surirella ovalis Brébisson	14,20,27,37
Surirella pandura Peragallo & Peragallo	6,48
Surirella robusta Ehrenberg	3
Surirella striatula Turpin	3,4,6,17,18,20,25,48
Synedra fulgens (Greville) Smith	6
Synedra gaillonii var. *macilenta* (Grunow) H.Peragallo	18,23
Tabellaria flocculosa (Roth) Kützing	6
Tabularia affinis var. *acuminata* (Grunow) Aboal	25
Tabularia fasciculata (Agardh) Williams & Round	5,12,18,25,28,37,44,51
Tabularia investiens (W.Smith) Williams & Round	37,41
Tabularia parva (Kützing) D.M.Williams & Round	5,12,18,44,51
Tabularia tabulata (C.Agardh) Snoeijs	6,25,32,44
Tetramphora intermedia (Cleve) Stepanek & Kociolek	18
Tetramphora lineolata (Ehrenberg) Mereschkowsky	41
Tetramphora rhombica (Kitton) Stepanek & Kociolek	6,48
Tetramphora sulcata (Brébisson) Stepanek & Kociolek	41
Tetramphora ostrearia (Brébisson) Mereschkowsky	45
Thalassionema fraunfeldii (Grunow) Hallegraeff	3,6,7,8,15,17,21,26,29,31,36,48,50,51,52
Thalassionema nitzschioides (Grunow) Mereschkowsky	3,6,7,8,9,11,21,29,31,36,37,48,50,51,52,55,56
Thalassiophysa hyalina (Greville) Paddock & P.A.Sims	7,29,48
Thalassiothrix longissima Cleve & Grunow	3,6,8,26,29,48,50,51
Thalassiothrix mediterranea Pavillard	6,7,8,15,29,31,36,37,48,50
Toxonidea insignis Donkin	5,12,18,51
Trachyneis aspera (Ehrenberg) Cleve	13,18,41,48
Trachysphenia acuminata Peragallo	45
Trachysphenia australis Petit	18,28,48
Trachysphenia australis var. *rostellata* Hustedt	23
Tryblionella acuminata Smith	18,20,28
Tryblionella angustata W.Smith	22,25
Tryblionella apiculata Gregory	5,12,18,27,28,41,44,51
Tryblionella circumsuta (Bailey) Ralfs	48
Tryblionella coarctata (Grunow) Mann	4,28,41
Tryblionella compressa (Bailey) Poulin	24,28
Tryblionella granulata (Grunow) Mann	18,44
Tryblionella hungarica (Grunow) Frenguelli	25
Tryblionella levidensis W.Smith	22
Tryblionella marginulata (Grunow) Mann	45
Tryblionella navicularis (Brébisson) Ralfs	18
Tryblionella pararostrata (Lange-Bertalot) Clavero & Hernández-Mariné	44
Tryblionella punctata Smith	18
Tryblionella victoriae Grunow	38
Ulnaria acus (Kützing) Aboal	22
Ulnaria danica (Kützing) Compère & Bukhtiyarova	14,37
Ulnaria ulna (Nitzsch) Compère	6,7,15,17,20,27,29,34,38,56

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References

Tables (all tables give in the main text)

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Original Articles: This is the most important type of article since it provides new information based on original research. **The main text should contain "Introduction", "Materials and Methods", "Results and Discussion" and " Conclusion" sections.**

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted in main document WORD files (in JPEG or PNG format) through the submission system. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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References

Reference System is APA 6th Edition

In-text Citation with APA

The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Note: All second and third lines in the APA Bibliography should be indented.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Table 2. Major Citations for a Reference List

Material Type	Reference List/Bibliography
A book in print	**Baxter, C. (1997).** *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	**Haybron, D.M. (2008).** Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
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 10.10.15)
An article in a print journal	**Carter, S., Dunbar-Odom, D. (2009).** The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
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
Websites - professional or personal sites	***The World Famous Hot Dog Site.* (1999, July 7).** Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015)
Websites - online government publications	**U.S. Department of Justice. (2006, September 10).** Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.2015)
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)
Artwork - from library database	**Clark, L. (c.a. 1960's).** *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
Artwork - from website	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015)