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RESEARCH PAPER

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Assessing the Trophic Level of a Mediterranean Stream (Nif Stream, İzmir) Using Benthic Macro-Invertebrates and Environmental Variables

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Abstract

The study was conducted in Nif Stream (İzmir) and the aims of the study are (a) to determine the benthic macro-invertebrate composition of the stream, (b) to determine environmental features of the stream, (c) to investigate the relations between the determined species and the environmental variables and (d) to assess the trophic level of the stream which was under the pressure of industry, agriculture and settlement. Samplings of macrobenthic invertebrates were performed seasonally at eight stations between October 2013 and September 2014 in addition to measuring environmental variables of the localities. 11571 specimens were investigated and totally 77 taxa were determined. Chironomidae and Oligochaeta were the higher groups in terms of species richness with 25 and 17 species, respectively. Multivariate analyses indicate that pH, Ca^{2+} and NO_3-N were the most effective variables and explained 48.6% of the variance in the distribution and abundance of benthic macroinvertebrates in Nif Stream. Along the measured physico-chemical characteristics and scores of biological indices, the upper branches of the stream have good quality water while the middle and lower parts are heavily polluted.

Introduction

Pollution and its negative effects on aquatic habitats have increased with human civilization proportionally. Today, both lotic and lentic habitats are under the pressure of human being in various parts of the world. The degree of disturbance is higher in under developed or undeveloped countries. Ecosystem perspective and ecosystem based management approaches gain importance in order to find a solution for increased pollution and pressure on aquatic habitats, especially in streams and rivers. In running waters, just measuring physico-chemical variables for assessment and management of running water quality is not sufficient (Kazancı, Dügel & Girgin, 2008). Because, these efforts give instant signs of water quality in field, and in a running water habitat these results are not enough to determine the water quality properly. Determination of the structure and dynamics of benthic community is a key for understanding the state of a lotic habitat (Reice & Wohlenberg, 1993). Benthic macro-invertebrates are important components of running waters. They act as a vital link in the food chain of aquatic biota in addition to have importance in assessing trophic level and water quality of streams and rivers (Wetzel, 2001; Lazaridou-Dimitriadou 2002). In general, the environmental variables (physical or/and chemical) of the habitats have direct effects on macro invertebrate composition and distribution (Weatherhead & James, 2001). The benthic macro-invertebrate communities broadly reflect environmental conditions and can be used as bio-indicators in determining the level of pollution (Reice & Wohlenberg, 1993). Relationships between benthic macroinvertebrate communities and environmental variables have studied in various studies in Turkey and the first study on the subject was reported by Kazancı, İzbırak, Çağlar & Gökçe (1992).

Although there are a few studies on the pollution and its negative effects on aquatic test organisms (*Daphnia magna*) in Nif Stream, there is no study in literature on determining the water quality levels of the studied localities using both environmental variables and biological data (Boyacıoğlu, Parlak, Oral & Çakal, 2004; Parlak, Çakal-Arslan, Boyacıoğlu & Karaaslan, 2010).

The present study was conducted in Nif Stream (İzmir) and the aims of the study are (a) to determine the macro-invertebrate composition of the stream, (b) to determine environmental features of the stream, (c) to investigate the relationships between the determined species and the environmental variables and (d) to assess the trophic level of the stream which was under the pressure of industry, agriculture and settlement.

Materials and Methods

Study Area

The Nif stream has about 65 km length and is located in the western part of Anatolia, Turkey (Figure 1). It arises from the eastern slopes of Yamanlar Mountain, nearly 14 km NE of İzmir province and fed by several small creeks arising from Nif Mountain. The stream firstly flows along the Kemalpaşa plain towards the Turgutlu town and then turns its route to northwest to Manisa province. After passing several villages, the Nif Stream joins to Gediz River just before the Güzelköy village.

Sampling Procedures

Samplings were performed seasonally at eight stations between October 2013 and September 2014 (Figure 1, Table 1). Macrobenthic samples were collected by kicking benthic material with a 500 µm mesh sized kick-net. Each sampling took 3 minutes. The samples were fixed in 4% formalin solution in field and then sieved under the tap water in laboratory. All the macro-invertebrate samples were firstly sorted in groups and then identified to genera-species level where possible. They were studied both qualitatively and quantitatively. These quantitative samples have been analyzed.

Six environmental variables, namely temperature (T., °C), dissolved oxygen (DO, mg/l), oxygen saturation (%), pH, Electrical Conductivity (EC, µs/cm) and salinity (S, mg/l) were measured in situ by using a WTW pH-meter (model 330), a WTW oxygen-meter (model 330) and a YSI 30 model SCT-meter. Other variables (PO_4^{-3}- P, HCO_3^-, NO_2-N, NO_3-N, NH_4-N, Total N, Hardness, Ca^{2+},

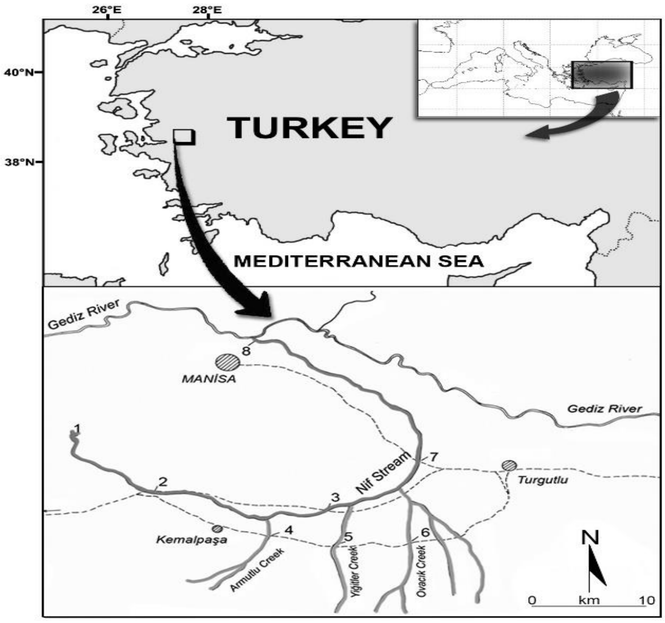

Figure 1. Nif Stream and the locations of sampling stations.

Table 1. Sampling stations and their features

Station No	Feature	Water Flow	Geographical Location
1	Creek. Upper part of the stream. Stony, gravelly habitat.	not continuous	38°29’39”N-27°20’19”E
2	Creek. Upper part of the stream. Pebbly, sandy habitat with rich aquatic vegetation.	continuous	38°27'59"N-27°20'30"E
3	Middle part of the stream. Sandy, muddy habitat with rich vegetation.	continuous	38°26’43”N-27°31’03”E
4	Upper part of the stream. Pebbly habitat with rich riparian vegetation.	continuous	38°23’36”N-27°31’58”E
5	Upper part of the stream. Stony, pebbly habitat with rich riparian vegetation.	continuous	38°23’57”N-27°36’36”E
6	Upper part of the stream. Stony, pebbly habitat with rich riparian vegetation.	continuous	38°25’05”N-27°39’54”E
7	Middle part of the stream. Muddy habitat.	continuous	38°34’43”N-27°34’30”E
8	Lower part of the stream. Muddy habitat.	continuous	38°37’52”N-27°28’42”E

Mg^{2+}, SO_4^{--}, PO_4^{--}, Alkalinity) were measured in the laboratory after following the standard methods of the American Public Health Association (APHA, 2005).

Data Analyses

ASTERICS 3.3.1 (AQEM/STAR Ecological River Classification System; AQEM Consortium 2002) software was used to analyze the results of the biological data. Saprobic Index, BMWP score (Biological Monitoring Working Party) (Paisley, Trigg & Walley, 2014), ASPT (Average Score Per Taxon) (Armitage, Moss, Wrigth & Furse, 1983), Belgian Biotic Index (BBI), diversity indices (Simpson, Shannon-Wiener and Margalef indices) were used to assess the water quality of the studied locations.

Dominance index (Bellan-Santini, 1969) of the determined taxa was calculated by the following formula;

$$Di = Ni/Nt \times 100$$

where Ni = number of individuals of species i; and Nt = total number of macrobenthic invertebrate specimens.

Frequency values of the determined taxa were calculated by Soyer's Frequency Index by the following formula;

$$F = m/M \times 100$$

where m = number of stations where the species was found and M = total number of stations × total number of seasons.

Canonical ordination techniques (CCA) were used to explain the variation in taxa along the gradients using the environmental variables. Multivariate analyses were carried out with the CANOCO 4.5 package (ter Braak & Smilauer, 2002).

Percent similarity of the studied localities (UPGMA, Unweighted Pair Group Average) was analyzed based on the quantitative data of the macroinvertebrate taxa throughout the Multi-Variate Statistical Package (MVSP) program version 3.1 (Kovach, 1998).

Results of the physico-chemical measurements and analysis were evaluated according to National Surface Water Quality Regulations (2008) in order to classify the water quality levels of the stations.

Results

Environmental Variables

During the study, 17 physico-chemical parameters were measured. The seasonal variations in water temperature, pH, dissolved oxygen and salinity values at the studied localities were shown in Figure 2 and the minimum and maximum values of the measured environmental variables were listed in Table 2. According to the results, an obvious depletion in dissolved oxygen values was observed at the 7th and 8th stations in autumn and spring period. Evidences of domestic pollution were observed during the study and the authors think that increased organic pollution can be the main reason of the mentioned oxygen depletion at the stations. Values of the dissolved oxygen were fluctuated between 0.3 mg/l (8th stat.) and 14.15 mg/l

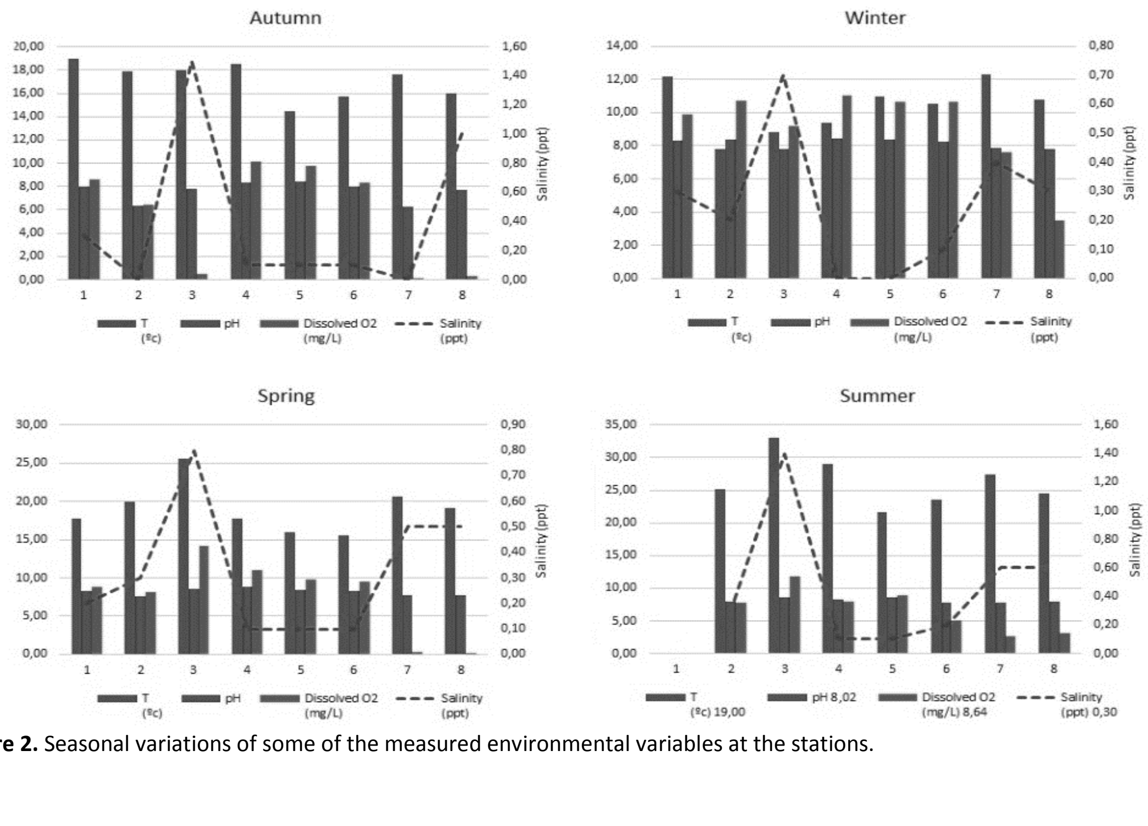

Figure 2. Seasonal variations of some of the measured environmental variables at the stations.

Table 2. The minimum and maximum values of the measured physico-chemical variables

	Station No	T (C°)	pH	Dissolved O_2 (mg/L)	O_2 Saturation (%)	Salinity (ppt)	Conductivity (µS/cm)	Alkalinity	Nitrite N (mg/L)	Nitrate N (mg/L)	Ammonium N (mg/L)	Total N (mg/L)	Orthophosphate (mg/L)	Total P (mg/L)	Sulphate (mg/L)	Ca^{++} (mg/L)	Mg^{++} (mg/L)	Hardness (d°H)
Minimum	1	12.2	8.02	8.64	94.70	0.2	662.00	4	0.02	0.80	0.02	6.91	0.16	0.08	0.23	87.6	13.6	15.5
	2	7.8	6.34	6.46	67.20	0.0	0.1	4.5	0.01	1.92	0.02	3.68	0.10	0.09	16.20	101	20.6	19.1
	3	8.8	7.76	0.50	5.10	0.7	2.7	6.2	0.01	0.52	0.02	4.17	0.72	0.59	13	139	25.5	25.6
	4	9.4	8.23	8.00	98.00	0.0	272	2	0.01	0.09	0.01	0.49	0.09	0.07	2.91	44.9	2.86	6.95
	5	11	8.38	8.88	98.80	0.0	268	2.2	0.01	0.77	0.01	1.32	0.05	0.07	1.8	55.4	0.64	8.62
	6	10.5	7.82	5.00	60.00	0.1	316	2.7	0.01	0.85	0.01	1.60	0.08	0.07	3.11	9.77	0.23	1.37
	7	12.3	6.25	0.11	1.40	0.0	0.2	3.7	0.01	0.47	0.02	2.49	0.36	0.33	25.5	84.5	9.58	17.4
	8	10.8	7.70	0.03	0.40	0.3	2.05	3.6	0.02	0.31	0.02	4.60	0.12	0.21	20.6	84.3	13.2	14.9
Maximum	1	19	8.33	9.92	96.80	0.3	714	5.6	0.04	7.02	0.08	7.58	0.52	0.21	137	117	22.6	21.6
	2	25.2	8.40	10.74	97.20	0.3	770	6.4	0.03	4.30	0.02	7.69	1.41	1.42	229	155	27.3	28
	3	33.1	8.63	14.15	177.20	1.5	1695	12.1	0.36	2.89	0.92	6.28	2.47	2.39	125	144	153	55.5
	4	29	8.77	11.04	118.70	0.1	377	3.4	0.02	1.76	0.04	6.32	0.49	0.15	12.9	63	5.54	10.1
	5	21.7	8.59	10.67	103.90	0.1	355	3.4	0.03	1.22	0.02	8.07	0.51	0.14	12.6	70	3.71	9.96
	6	23.5	8.31	10.68	98.50	0.2	479	8.7	0.03	3.59	0.02	7.84	0.55	0.57	6.88	77.4	3.96	11.6
	7	27.4	7.87	7.64	71.50	0.6	1334	8	0.16	7.04	0.18	12.90	0.81	0.82	136	113	32	22.9
	8	24.5	8.03	3.50	39.30	1.0	1289	6	0.35	3.28	8.20	10.90	1.00	1.05	130	130	36	24.3

(3rd stat.) during the sampling period. In the summer period, environmental variables couldn't be measured at the first station because of drought. Water temperature values have the richest points during this period with a peak (>33 °C) at the 3rd station. Water temperature values of the sampling localities were changed between 7.8 °C (2nd stat.) and 33.1 °C (3rd stat.) throughout the year. Additionally, salinity values usually were higher at the 3rd station than the remaining ones during the study period. pH values fluctuated from 6.25 to 8.77 throughout the study. Total nitrogen values were changed between 0.49 mg/l and 12.90 mg/l and the two highest values were observed at 7th (12.90 mg/l) and 8th (10.90 mg/l) stations. When the total phosphorus values take into account, the highest values were observed at the third (2.39 mg/l) and second (1.42 mg/l) stations, respectively. Similarly, the highest magnesium value (153.00 mg/l) was observed at the third station while the values at the remaining stations were changed between 3.71 mg/l and 36.00 mg/l.

Water quality classes of the studied stations in Nif Stream have been determined according to Surface Water Quality Regulations (SWQR) (Table 3). In this procedure, the quality levels are categorized in four classes and water quality decreases by increasing the number of class: "1" indicates the best and "4" indicates the worst one. As a result of the classification, the fourth and fifth stations can be classified as "second class" because of total phosphorus levels. Oppositely, the last two stations were labeled as "fourth class".

Benthic Macroinvertebrate Data

At the end of the study, 11571 specimens were investigated and totally 77 taxa were determined. Chironomidae and Oligochaeta were the higher groups in terms of species richness with 25 and 17 species, respectively (Table 4). Additionally, Diptera has the highest total dominance value with 48% and was followed by Oligochaeta with 40%. The two Chironomid larvae, *C. anthracinus* and *C. riparius*, have the highest total dominance values among the determined taxa and were followed by *L. udekemianus* (Figure 3).

C. riparius has the highest mean dominancy value with 2.548 throughout the study and was followed by another *Chironomus* species, *C. anthracinus*, with 2.538 and by *L. udekemianus* with 2.470.

Each of the three Oligochaeta species, *T. tubifex, L. hoffmeisteri* and *L. udekemianus*, had the highest frequency value (87%) during the study and was followed by a Chironomidean larvae, *C. anthracinus*, with a frequency value of 75% (Table 4). Oligochaeta species were sampled from all the studied localities except the first station.

When macrobenthic invertebrate groups of the studied localities were carried out, a distinct dominancy value of Chironomidean larvae is observed at the first six stations. On the contrary, Oligochaeta has great dominancy values at last two stations which are bigger than 90% (Figure 4).

The third station is the richest one in terms of individual numbers. 4700 specimens were sampled from third station and more than half of total numbers of the determined specimens belong to Chironomidae. The sixth station followed the third one as the second richest station with 2841 specimens and the Chironomidean larvae consisted of the greatest group at this station (Figure 4). The first and fourth stations were the weakest ones in terms of individual numbers with 287 and 293 specimens, respectively.

Multivariate Analyses

Multivariate analyses indicate that pH, Ca^{2+} and NO_3-N were the most effective variables and explained 48.6% of the variance in the distribution and abundance of macroinvertebrates in Nif Stream (Figure 5). According to the CCA biplot diagram, higher pH values negatively affect the distribution of *C. bernensis* in the present study. Similarly, *Conchapelopia* sp., *C. intersectus*, *G. guttipennis*, *Cleon dipterum* and *Procladius* sp. preferred more polluted habitats where nitrate values were high. *C. anthracinus*, *H. ventriculosa* and *T. blanchardi* were placed near the central point of the biplot (Figure 5).

Biological Indices

As a result of the BMWP scores, the 6th station has the highest value (82) while the 7th and 8th ones have the lowest. Similarly, the 6th station has a high ASPT value with 6.30 but it is not the highest one because the 5th station has 6.36. According to BMWP scores, there is no locality in "*best quality*" in Nif Stream but when the results of the ASPT scores take into consideration, the 5th and 6th stations can be classified as "clean water" and the 4th one as "*slightly polluted water*". It is obvious that, the last two stations were in "*heavily polluted*" class. The results of the Diversity Index (Margalef Index) suggest that, the 4th and 5th stations have the highest diversity values while the last two stations have the lowest (Table 5).

Similarity of the Stations

According to determined macroinvertebrate data, the last two stations seem to be similar to each other with about 60% similarity ratio. Both of the stations have very similar ecological conditions which are low water flow low, high turbidity, silty-muddy bottom, low slope and highly polluted habitats. The similarity values of the remaining stations were much less than 50% and the first station was the least similar to the other stations. The first station constitutes the outgroup which is least similar to the other stations (Figure 6).

Table 3. Water Quality Classes of the studied stations in Nif Stream (According to Surface Water Quality Regulations)

Stream :		Nif Stream					Water quality classes (According to SWQR)				
Locality :						Station 1					
Season	Unit	Autumn	Winter	Spring	Summer	Mean	Autumn	Winter	Spring	Summer	Result
Temperature	°C	19.00	12.20	17.80	--	16.30	1	1	1	--	1
pH	---	8.02	8.33	8.28	--	8.21	1	1	1	--	1
Conductivity	µs/cm	675	714	662	--	683.67	2	2	2	--	2
Dissolved Oxygen	mg/L	8.64	9.92	8.80	--	9.12	1	1	1	--	1
Ammonium N ($NH4^{+}$-N)	mg/L	0.051	0.022	0.077	--	0.05	1	1	1	--	1
Nitrate N ($NO3^{-}$-N)	mg/L	6.70	7.02	0.80	--	4.84	2	2	1	--	2
Total Phosphorus	mg/L	0.143	0.212	0.084	--	0.146	2	3	2	--	3
Locality :						Station 2					
Temperature	°C	17.90	7.80	19.90	25.20	17.70	1	1	1	3	3
pH	---	6.342	8.4	7.566	7.909	7.55425	1	1	1	1	1
Conductivity	µs/cm	100	572	770	767	527.275	1	2	2	2	2
Dissolved Oxygen	mg/L	6.46	10.74	8.13	7.84	8.2925	2	1	1	2	2
Ammonium N ($NH4^{+}$-N)	mg/L	0.02	0.021	0.015	0.015	0.01775	1	1	1	1	1
Nitrate N ($NO3^{-}$-N)	mg/L	1.92	2.64	3.31	4.3	3.0425	1	1	1	1	1
Total Phosphorus	mg/L	1.42	0.296	0.088	0.149	0.48825	4	3	2	2	4
Locality :						Station 3					
Temperature	°C	18	8.8	25.6	33.1	21.375	1	1	3	4	4
pH	---	7.76	7.82	8.539	8.625	8.186	1	1	3	3	3
Conductivity	µs/cm	301	1421	1695	2700	780.4275	1	3	3	3	3
Dissolved Oxygen	mg/L	0.5	9.2	14.15	11.75	8.9	4	1	1	1	4
Ammonium N ($NH4^{+}$-N)	mg/L	0.923	0.028	0.022	0.021	0.2485	2	1	1	1	2
Nitrate N ($NO3^{-}$-N)	mg/L	0.515	2.43	2.89	2.5	2.08375	1	1	1	1	1
Total Phosphorus	mg/L	2.05	0.801	0.588	2.39	1.45725	4	4	3	4	4
Locality :						Station 4					
Temperature	°C	18.5	9.4	17.7	29	18.65	1	1	1	3	3
pH	---	8.34	8.44	8.774	8.23	8.446	1	1	1	1	1
Conductivity	µs/cm	369	272	297	377	328.75	1	1	1	1	1
Dissolved Oxygen	mg/L	10.16	11.04	11.02	8	10.055	1	1	1	1	1
Ammonium N ($NH4^{+}$-N)	mg/L	0.043	0.018	0.021	0.006	0.022	1	1	1	1	1
Nitrate N ($NO3^{-}$-N)	mg/L	0.023	0.022	0.019	0.009	0.01825	1	1	1	1	1
Total Phosphorus	mg/L	0.152	0.115	0.071	0.128	0.1165	2	2	2	2	2
Locality :						Station 5					
Temperature	°C	14.5	11	15.9	21.7	15.775	1	1	1	1	1
pH	---	8.46	8.38	8.402	8.59	8.458	1	1	1	1	1
Conductivity	µs/cm	353	268	300	355	319	1	1	1	1	1
Dissolved Oxygen	mg/L	9.82	10.67	9.81	8.88	9.795	1	1	1	1	1
Ammonium N ($NH4^{+}$-N)	mg/L	0.024	0.019	0.022	0.006	0.01775	1	1	1	1	1
Nitrate N ($NO3^{-}$-N)	mg/L	1.01	1.22	0.772	0.832	0.9585	1	1	1	1	1
Total Phosphorus	mg/L	0.139	0.075	0.065	0.1	0.09475	2	2	2	2	2
Locality :						Station 6					
Temperature	°C	15.7	10.5	15.5	23.5	16.3	1	1	1	1	1
pH	---	7.98	8.24	8.309	7.82	8.08725	1	1	1	1	1
Conductivity	µs/cm	441	316	332	479	392	2	1	1	2	2
Dissolved Oxygen	mg/L	8.3	10.68	9.48	5	8.365	1	1	1	1	1
Ammonium N ($NH4^{+}$-N)	mg/L	0.02	0.019	0.018	0.012	0.01725	1	1	1	1	1
Nitrate N ($NO3^{-}$-N)	mg/L	1.21	1.9	0.847	3.59	1.88675	1	1	1	1	1
Total Phosphorus	mg/L	0.139	0.066	0.094	0.568	0.21675	2	2	2	3	3
Locality :						Station 7					
Temperature	°C	17.6	12.3	20.7	27.4	19.5	1	1	1	3	3
pH	---	6.25	7.86	7.72	7.87	7.425	1	1	1	1	1
Conductivity	µs/cm	200	949	1203	1334	871.55	1	2	3	3	3
Dissolved Oxygen	mg/L	0.11	7.64	0.26	2.62	2.6575	4	2	4	4	4
Ammonium N ($NH4^{+}$-N)	mg/L	0.18	0.024	0.184	0.015	0.10075	1	1	1	1	1
Nitrate N ($NO3^{-}$-N)	mg/L	7.04	3.24	0.469	1.71	3.11475	2	1	1	1	2
Total Phosphorus	mg/L	0.414	0.819	0.329	0.534	0.524	3	4	3	3	4
Locality :						Station 8					
Temperature	°C	16	10.8	19.1	24.5	17.6	1	1	1	1	1
pH	---	7.74	7.83	7.7	8.033	7.82575	1	1	1	1	1
Conductivity	µs/cm	205	738	1115	1289	786.0125	1	2	3	3	3
Dissolved Oxygen	mg/L	0.3	3.5	0.03	3.12	1.7375	4	3	4	3	4
Ammonium N ($NH4^{+}$-N)	mg/L	8.2	0.023	0.083	0.019	2.08125	4	1	2	1	4
Nitrate N ($NO3^{-}$-N)	mg/L	0.31	3.28	0.442	2.19	1.5555	1	1	1	1	1
Total Phosphorus	mg/L	1.05	0.212	0.35	0.289	0.47525	4	3	3	3	4

Table 4. List of the determined taxa and their occurrence, dominancy and frequency values at the stations

TAXON	D%								D% (mean)	F%
	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8		
Mollusca										
Melanopsis preamorsa (Linnaeus, 1758)	0	0	0	0.026	1.685	0	0	0	0.214	25.0
Physella acuta (Draparnaud, 1805)	0	0	2.256	0	0	0	0	0.009	0.283	25.0
Radix labiata (Müller, 1774)	0.009	0	0	0	0	0	0	0	0.001	12.5
Gyraulus albus (Muller, 1774)	0	1.746	0	0	0	0	0	0	0.218	12.5
Ancylus fluviatilis Müller, 1774	0	0	0	0.026	0.017	0.086	0	0	0.016	37.5
Valvata piscinalis (Müller, 1774)	0	0	0.017	0	0	0	0	0	0.002	12.5
Pisidium casertanum (Poli, 1791)	0	0.009	0	0	0	0	0	0	0.001	12.5
Ephemeroptera										
Baetis scambus (Eaton, 1870)	0	0	0.009	0	0	0	0	0	0.001	12.5
Baetis rhodani Leach, 1815	0	0	0	0.199	0.536	2.256	0	0	0.374	37.5
Baetis vernus Curtis, 1834	0.043	0	0	0	0	0	0	0	0.005	12.5
Cloeon dipterum (Linnaeus, 1761)	0.795	0.017	0	0.043	0	0	0	0	0.107	37.5
Serratella ignita (Poda, 1761)	0	0	0.009	0.346	0.104	0.017	0	0	0.059	50.0
Caenis luctuosa (Stephens, 1835)	0	0	0	0.035	0.06	0.017	0	0	0.014	37.5
Torleja major (Klapek, 1905)	0	0	0	0.069	0	0	0	0	0.009	12.5
Ecdyonurus macani Thomas and Sowa, 1970	0	0	0	0	0.035	0	0	0	0.004	12.5
Ecdyonurus submontanus Landa, 1969	0	0	0	0	0	0.095	0	0	0.012	12.5
Diptera										
Chironomusriparius Meigen, 1804	0	0.475	16.03	0	0	3.552	0.242	0.086	2.548	62.5
Chironomus anthracinus Zettersted, 1860	0.009	0	5.220	0.043	0.389	14.380	0.259	0	2.538	75.0
Brillia modesta (Meigen, 1830)	0	0	0	0.035	0.147	0.130	0	0	0.039	37.5
Polypedilum convictum (Walker, 1856)	0.311	0	0	0	0.277	0.026	0	0	0.077	37.5
Macropelopia nebulosa (Meigen, 1804)	0	0	0	0	0	0.095	0	0	0.012	12.5
Prodiamesa olivacea (Meigen, 1818)	0	0	0	0	0	0.026	0	0	0.003	12.5
Microtendipes pedellus (de Geer, 1776)	0	0.043	0.026	0	0.173	0	0	0	0.030	37.5
Ablabesmyia longistyla Fittkau, 1962	0	0	0	0.164	0.026	0	0	0	0.024	25.0
Apsectrotanypus sp.	0	0	0	0	0.009	0	0	0	0.001	12.5
Polypedilum pedestre (Meigen, 1830)	0	0	0	0.060	0.078	0	0	0	0.017	25.0
Polypedilum laetum (Meigen, 1818)	0	0	0	0	0.121	0	0	0	0.015	12.5
Endochironomus albipennis (Meigen, 1830)	0	0	0	0.544	0	0	0	0	0.068	12.5
Procladius sp.	0.035	0.406	0	0.233	0	0	0	0	0.084	37.5
Cricotopus vierriensis Goetghebuer, 1935	0	0	0	0.052	0	0	0	0	0.006	12.5
Conchapelopia sp.	0.233	0	0	0.017	0	0	0	0	0.031	25.0
Tanypus kraatzi (Kieffer, 1912)	0	0.994	0	0	0	0	0	0	0.124	12.5
Cricotopus tremulus (Linneaus, 1758)	0	0.130	0	0	0	0	0	0	0.016	12.5
Chironomus commutatus Keyl, 1960	0	0.302	0	0	0	0	0	0	0.038	12.5
Endochironomus lepidus (Meigen, 1830)	0	0.017	0	0	0	0	0	0	0.002	12.5
Phaenopsectra sp.	0	0.026	0	0	0	0	0	0	0.003	12.5
Guttipelopia guttipennis (van der Wulp, 1861)	0.795	0	0	0	0	0	0	0	0.099	12.5
Cricotopus intersectus (Staeger, 1839)	0.242	0	0	0	0	0	0	0	0.030	12.5
Chironomus bernensis Kloetzli, 1973	0	0	0	0.311	0	0.579	0	0	0.111	25.0
Synendotendipes sp.	0	0	0	0	0	0.009	0	0	0.001	12.5
Thienemannimyia sp.	0	0	0	0	0	0.009	0	0	0.001	12.5
Tipula sp.	0	0.052	0.009	0	0	0	0	0	0.008	25.0
Syrphus sp.	0	0	0.060	0	0	0	0	0	0.008	12.5
Tabanus sp.	0	0.009	0.009	0.043	0.199	0.449	0	0	0.089	62.5
Limonia sp.	0	0	0	0	0	0.017	0	0	0.002	12.5
Simulium sp.	0	0	0	0	0	0.164	0	0	0.021	12.5
Atherix sp.	0	0	0	0	0.009	0	0	0	0.001	12.5
Oligochaeta										
Tubifex tubifex(Muller, 1774)	0	1.374	0.596	0.060	0.026	0.458	0.095	0.294	0.363	87.5
*Tubifex blanchardi*Vejdovsky, 1891	0	0.017	1.227	0	0	0.268	0	0.009	0.190	50.0
Tubifex newaensis(Michaelsen, 1903)	0	0.009	6.162	0	0.017	0	0.017	0.147	0.794	62.5
Ophidonais serpentina(Muller, 1774)	0	0	0	0.043	0	0	0	0	0.005	12.5
*Limnodrilus hoffmeisteri*Claparede, 1862	0	0.13	0.207	0.017	0.259	0.302	0.121	0.268	0.163	87.5
*Limnodrilus udekemianus*Claparede, 1862	0	0.665	2.904	0.026	0.337	0.562	4.727	10.530	2.470	87.5
*Limnodrilus claparedianus*Ratzel, 1869	0	0	0	0.009	0	0	0	0	0.001	12.5
Potamothrix bavaricus(Oschmann, 1913)	0	0.095	0	0	0	0	0	0	0.012	12.5
Potamothrix hammoniensis(Michaelsen, 1901)	0	0.017	0	0	0	0	0	0	0.002	12.5
Psammoryctides albicola(Michaelsen, 1901)	0	0.009	0	0	0	0	0	0	0.001	12.5
Psammoryctides deserticola(Grimm, 1876)	0	0.009	0	0	0.026	0	0	0	0.004	25.0
Mesenchytraeus armatus(Levinsen, 1884)	0	0.009	0.484	0.026	0.026	0.804	0	0	0.169	62.5
Cognettia glandulosa(Michaelsen, 1888)	0	0.009	1.962	0	0	0.052	0	0	0.253	37.5
Cognettia sphagnetorum(Vejdovsky, 1878)	0	0	3.094	0.017	0	0.009	0	0	0.390	37.5
*Spirosperma ferox*Eisen, 1879	0	0	0	0	0.009	0.009	0	0	0.002	25.0
Henlea ventriculosa(d'Udekem, 1854)	0	0	0.337	0	0	0.009	0	0	0.043	25.0
*Henlea perpusilla*Friend, 1911	0	0	0	0	0.501	0	0	0	0.063	12.5

Table 4. Continued

TAXON	D%								D% (mean)	F%
	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8		
Amphipoda										
Gammarus izmirensis Özbek, 2007	0	0	0	0	0.562	0	0	0	0.070	12.5
Trichoptera										
Hydropsyche instabilis (Curtis, 1834)	0	0	0	0	0.233	0	0	0	0.029	12.5
Glossossoma conformis (Neboiss, 1963)	0	0	0	0	0.017	0	0	0	0.002	12.5
Odonata										
*Aeshna affinis*Vander Linden, 1820	0	0	0	0.017	0.017	0.052	0	0	0.011	37.5
Calopteryx splendens(Harris, 1780)	0	0	0	0	0	0.009	0	0	0.001	12.5
*Calopteryx virgo festiva*Brullé, 1832	0	0	0	0	0	0.009	0	0	0.001	12.5
Enallagma cyathigerum(Charpentier, 1840)	0	0.389	0	0.009	0	0	0	0	0.050	25.0
Enallagma sp.	0	0.009	0	0	0	0	0	0	0.001	12.5
Epallage fatime (Charpentier, 1840)	0	0	0	0	0.060	0	0	0	0.008	12.5
*Gomphus schneldem*Selys. 1850	0	0	0	0.026	0.017	0.009	0	0	0.006	37.5
Lestes sp.	0	0	0	0	0.009	0.009	0	0	0.002	25.0
*Libellula depressa*Linnaeus, 1758	0	0.017	0	0	0	0	0	0	0.002	12.5
Onychogomphus sp.	0.009	0	0	0	0	0	0	0	0.001	12.5
*Onychogomphus forcipatus albotibialis*Schmidt, 1964	0	0.009	0	0	0.026	0	0	0	0.004	25.0
*Ophiogomphus carolus*Needham, 1897	0	0	0	0.017	0	0	0	0	0.002	12.5
*Ophiogomphus reductus*Calvert, 1898	0	0	0	0.017	0.009	0.086	0	0	0.014	37.5
Total	2.48	6.99	40.6	2.53	6.02	24.6	5.46	11.3		

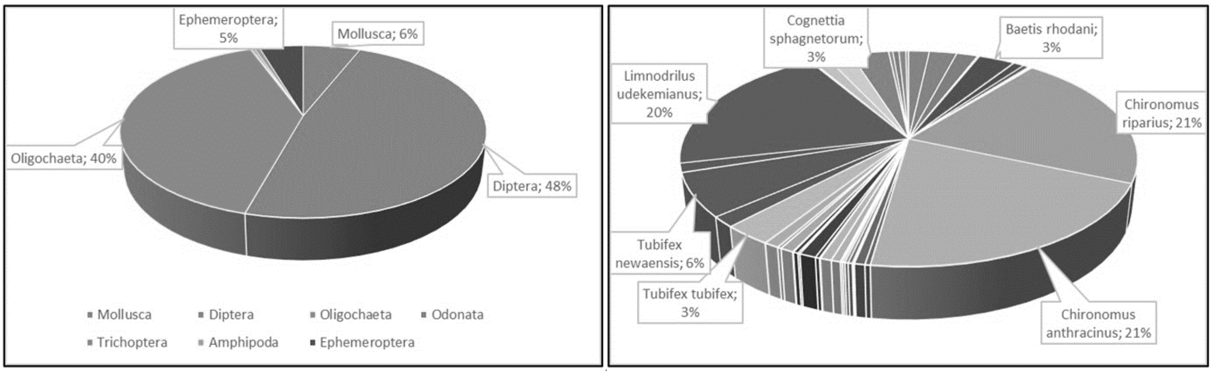

Figure 3. Total dominance values of the determined groups (left) and species (right) throughout the study.

Discussion

Inland waters or freshwaters are one of the most important natural resources, but they are also under high pressure of civilization. Threat on running waters will probably increases in the future (Malmqvist & Rundle, 2002). Pollution is one of the worst results of civilization on freshwater habitats and its negative effects are observed in undeveloped countries more heavily.

In the present study, water quality levels of the Nif Stream, which is located a few kilometers away from the third biggest city of Turkey, and effects of pollution were studied throughout environmental analysis and biological indices. Although some parts of the stream were surrounded by factories, agricultural areas and human settlements, there isn't any comprehensive study focused on the subject in the study area.

As a result of physico-chemical measurements, some peaks of nitrite values at 1st, 3rd, 7th and 8th stations were observed during the study. In general, nitrite is absent or in very low concentrations in clean waters. Having higher nitrite concentrations is not favorable condition in freshwaters because of possible toxic effects on aquatic organisms (Berenzen, Schulz & Liess, 2001). Observing alteration in nitrite values indicates industrial and domestic pollution in aquatic habitats (Girgin & Kazancı, l997). Similarly, the presence of high nitrite values at the mentioned stations can be judged as presence of industrial and domestic pollution. The authors observed visual and olfactory signs of pollution at 3rd, 7th and 8th stations especially in warm periods. During the study, nitrite and phosphate concentrations measured at 7th and 8th stations were higher than 0.05 mg/l and 0.65 mg/l, respectively and these results indicate that water quality of the mentioned localities must be 4th class which is "heavily polluted water" according to Surface Water Quality Regulations (SWQR). Similarly, the cleanest station was the 5th one, 2nd class (slightly polluted water) and was followed by 1st, 4th and 6th stations which were third class (polluted water) and the remaining ones were in forth class (heavily polluted water) according to environmental measurements and SWQR.

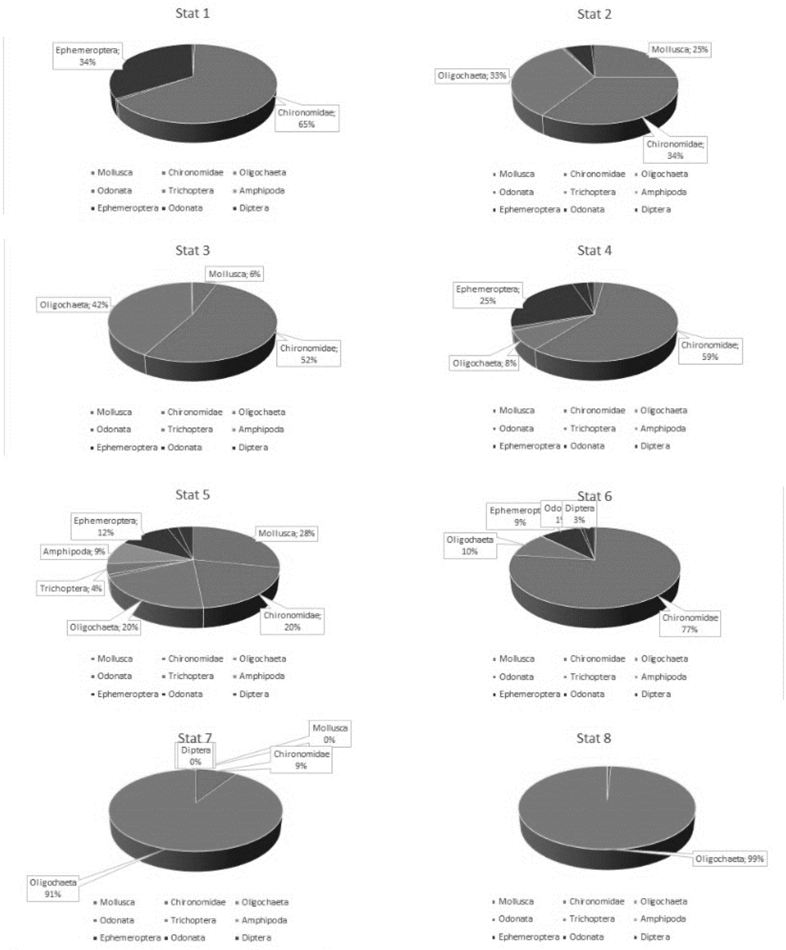

Figure 4. Benthic macro invertebrate compositions of the stations.

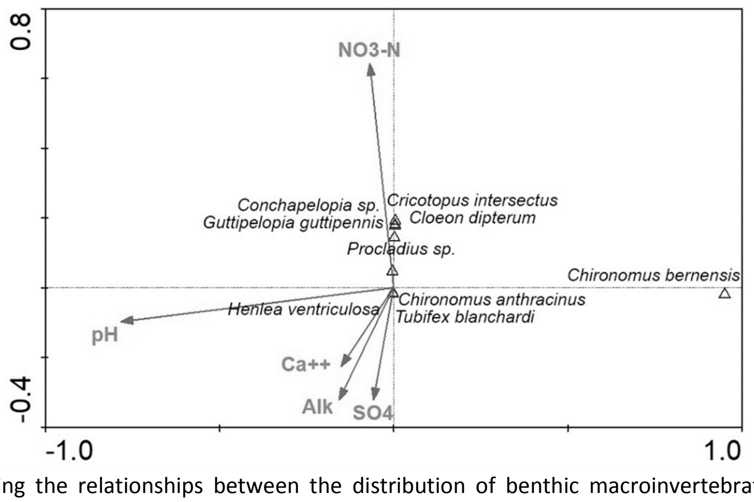

Figure 5. CCA biplot showing the relationships between the distribution of benthic macroinvertebrates and the environmental variables.

The salinity and conductivity values measured at third station were higher than the remaining ones (Figure 2) but there is no connection with sea at that locality. So, the main reason of the observed higher salinity and conductivity values can be presence of pollutants. Similarly, Souto *et al.* (2011) stated that high values of conductivity are an indirect sign of pollution. The 3rd station was surrounded by industrial factories and there are chemical pollution signs at the mentioned locality because of somehow milky color of water and irritating odor of unknown chemical compounds around the station.

Diptera is the most widely distributed and frequently the most abundant order of aquatic insects in freshwater environments (Armitage *et al.* 1983). Dominancy of Chironomid and Oligochaeta specimens in

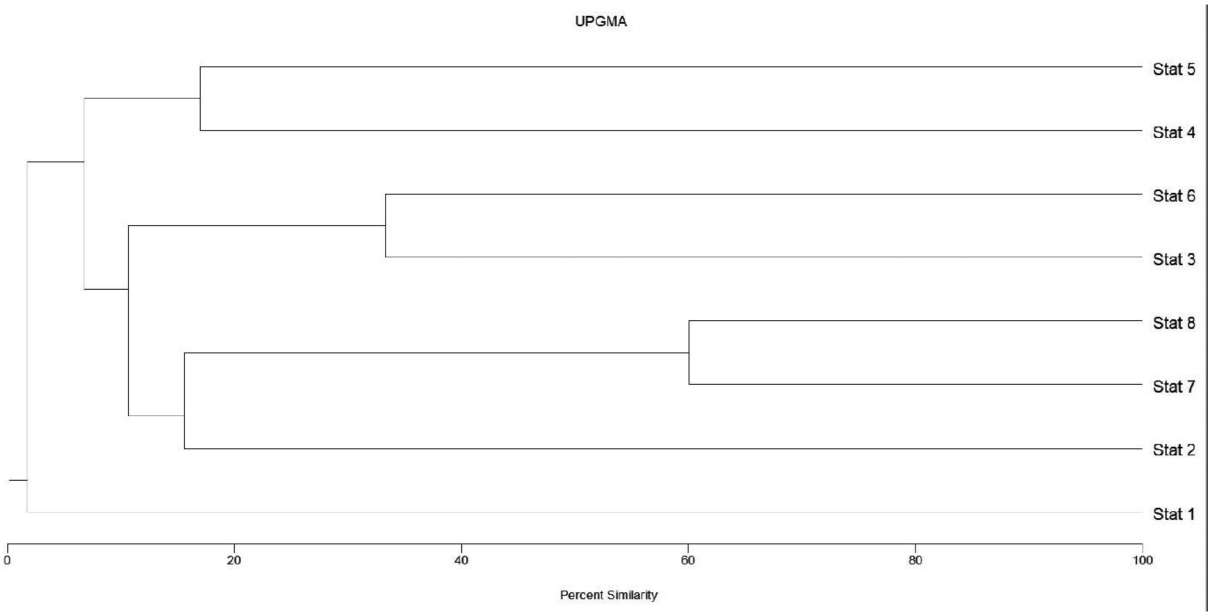

Figure 6. Similarity diagram of the studied localities based on benthic macro-invertebrate data.

an aquatic habitat can be considered presence of organic pollution. Chironomidae was the richest family in the present study in terms of species number and was the most abundant and dominant group at many localities where pollution levels were high (Table 4). These results are consistent with the findings of Arslan, Ayık & Şahin (2010) who stated that large numbers of pollution-tolerant chironomids are often indicative of poor water quality (characterized by low dissolved oxygen and high nutrient concentrations). Similarly, Dermott, Kalff, Leggett & Spence (1977) reported that the distribution of *C. anthracinus*, which was one of the dominant species in Nif Stream, is positively correlated with nutrient inputs into lakes.

T. tubifex and *L. hoffmeisteri* are the cosmopolitan Oligochaeta species and frequently observed inhabitants of organically enriched water bodies (Timm & Veldhuijzen van Zanten, 2002). *T. tubifex* can be found in several freshwater habitats and is quite tolerant against organic pollution. *L. hoffmeisteri* has similar ecological requirements with *T. tubifex* and prefers streams and rivers where lower nitrate and higher ammonia concentrations occur (Timm, Seire & Pall, 2001). Both of the species have poly-sabrobic character (Johnson, Wiederholm & Rosenberg, 1993). The Chironomidean species *C. thummi* and *T. tubifex* are the typical species of heavily polluted waters in several indices running on benthic macro invertebrates (Armitage *et al.* 1983). In the present study, the members of Oligochaeta were observed frequently and have a great dominancy especially at the last two stations where pollution level was high.

The cosmopolitan *P. casertanum* is the only bivalve species determined in the present study and was found at the second station only (Table 4). It can be found in almost all types of freshwater bodies such as streams, rivers, lakes and swamps (“IUCN,” 2017). Additionally, there are six gastropod species determined during the study and *A. fluviatilis*has the highest frequency ratio among them. *A. fluviatilis*is a beta-meso saprobic species and distributes in clean habitats where dissolved oxygen values are high unlike most other Pulmonata species which have euryoecious character (Yıldırım, 2000). In the present study, the individuals of *A. fluviatilis* were sampled from the 4th, 5th and 6th stations where dissolved oxygen values were high and pollution level is relatively low. *M. praemorsa* was another frequently observed gastropod species in the study and was one of the most common gastropod species in Turkey (Yıldırım, 1999). It also distributes in Italy, Crete, Aegean Islands, Syria and Mesopotamia (Fechter & Falkner, 1990). *P. acuta* is the typical mollusk species of the organically polluted streams and littoral parts of lakes and has an alpha-meso saprobic character (Johnson *et al.* 1993). It was sampled from the 7th and 8th stations as compatible with its ecological requirements.

The only Amphipoda species determined in this study is *G. izmirensis* which is an endemic for Turkey and was identified from Nif Stream by the first author in 2007. The species is a member of *Gammarus pulex* group and seems like in beta-meso-saprobic character (Özbek, 2007).

Two species of Trichoptera were found and both of them were sampled from the 5th station where dissolved oxygen values high and water temperature was relatively low. Additionally, the station was one of the cleanest localities with high ASPT and diversity index values (Table 5). So, the ecological characteristics of the locality fit the requirements of the determined Trichoptera species.

Ephemeroptera was represented by 9 species in the present study. *Baetis* is one of the riches and the only Ephemeropteran genus whose members can inhabit extremely cold habitats such as glacier mountain rivers or tundra's located almost 5000 m a.s.l. (Bauernfeind & Soldan, 2012). *B. rhodani* was the dominant Ephemeroptera species and sampled at three stations in this study. It is a tolerant species against higher water temperature values and can be found almost all habitat types between hipo-crenon and potamon zones of running waters. There are several records of the species from Turkey (Kazancı, 2001). Similarly, *C. dipterum* has a cosmopolitan distribution and oligo- and beta-meso saprobic character (Johnson *et al.* 1993). The members of *Ecdyonurus* genus prefer stony habitats of rivers where water flows rapidly and they have limited tolerance to organic pollution. On the contrary, *Caenis* larvae can inhabit all types of running waters, prefer gravely, sandy or muddy bottoms and have tolerance against organic pollution (Bauernfeind & Soldan, 2012).

Odonata was represented by 13 taxa in the present study. *A. affinis*, *G. schneldem* and *O. reductus* have the highest frequency values (37.5%) (Table 4). The representatives of the group were sampled from all the stations except 3rd, 7th and 8th in this study.

When the water quality classes (according to National Surface Water Quality Regulations) of the studied localities are compared with the results of ASPT scores, there is no locality in "first quality" in terms of environmental variables (according to NSWQR) but there are two localities (5th and 6th stations) according to ASPT score results. Both of the categorizations indicate that the lower part of the stream is heavily polluted and must be classified as "4th class" (Table 5). According to similarity analysis based on macro invertebrate data and results of biological indices show the similar result: the last two stations are heavily polluted and similar to each other in terms of their ecological conditions. They have very low diversity values because of negative effects of pollution.

According to canonical correspondence analysis, pH, Ca^{2+} and NO_3-N were the most effective environmental variables on the distribution of macro invertebrate species in Nif Stream. Increased nitrate concentrations limited most of the pollution sensitive species but oppositely supported the tolerant species such as *Conchapelopia* sp., *C. intersectus*, *G. guttipennis*, *Cleon dipterum* and *Procladius* sp. In general, increased nitrite and nitrate concentrations cause a decrease in species richness and diversity values (Zeybek, 2017). pH was another effective variable on benthic macroinvertebrates and maybe one of its discriminative effect was on *T. blanchardi*, which is a sympatric species with *T. tubifex*. In certain conditions, pH, salinity, mercury and hardness concentrations make stress on *T. tubifex* specimens and induce a gradual loss of hair and pectinate setae, thus generating individuals similar to "blanchi" form (Marotta, Crottini, Prada & Ferraguti, 2009). pH levels are also effective on Chironomidean larvae. Havas & Hutchinson (1982) stated that the adaptation capacity of *C. riparius* to low pH conditions is better than *Orthocladius consobrinus* and both chironomid species have better adaptation capability than the crustaceans tested.

Just measuring some environmental variables is not enough to determine water quality levels in running waters. Biological data, especially benthic macro invertebrates, and biotic indices must be taken into consideration in assessing the ecological status of streams and rivers. This study is the first attempt to determine the water quality levels of Nif Stream, which is under the pressure of industrial, agricultural and domestic pollution, together with measuring environmental variables and determining biological data. And also, the present study can be qualified as a basis to improve sustainable management efforts for the stream.

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