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Antenna and mouthpart defect in *Chironomus (Camptochironomus) tentans* larvae (Chironomidae) and their relevance with habitat characteristics

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Abstract

A shallow, eutroph and metal contaminated lake (one of the Ramsar sites in Turkey, Lake Uluabat) was investigated from August 2004 to July 2005 to determine the defects at some mouthparts (mentum, mandible and epipharyngis) and antenna of *Chironomus (Camptochrinomus) tentans* larvae. Although a total of 1800 chironomid larvae belonging twelve taxa were found in Lake Uluabat, it was dominated by *Chironomus* (*Camptochironomus*) *tentans* Fabricius, 1805. A total of 327 *C.* (*C.*) *tentans* were examined, 55.04% of which possessed defects. A total of 12 stations were sampled but all samples of *C.* (*C.*) *tentans*, collected from two stations where water circulation is reduced, demonstrated the highest incidence of deformities during the study. Defects were found in all mouthparts and antenna in *C.* (*C.*) *tentans* but mentum and epipharyngis defects were the most frequent.

In each sample station, dissolved oxygen, temperature, pH and depth of the lake water were measured as in situ. In addition, concentrations of eight metals (Pb, Zn, Cd, Cu, Ag, Cr, Fe and Ni) were measured monthly in lake water (for all samples at each sampling station, n = 9) and sediment (n) monthly. = 9). Analyses of water and sediment from Lake Uluabat showed the presence of metal pollutants such as zinc, nickel, lead and copper.

The relationship between defect and water-sediment toxicity relationship was analyzed by Correspondence Analysis. According to the results, the total rate of defect were largely correlated with high amounts of Ni, Zn, Pb and Cd in sediment and PO_4, pH in water. In mouthpart; the mentum defects positively correlated with Ag and Zn in sediment, SO_4 and COD in water and negatively correlated with Cd in water and Cu in sediment; epipharyngis defect positively correlated with Zn in water and with Ag in sediment. Antenna defects showed a statistically correlation with Cr in sediment and COD, NO_3–N, SO_4 in water.

Key words: Defects in Chironomidae larvae, Lake Uluabat

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Chironomus (Camptochironomus) tentans (Chironomidae) larvaları anten ve ağız parçalarındaki şekil bozuklukları ve habitat özellikleri ile ilişkileri

Özet

Türkiye'deki önemli Ramsar alanlarından biri olan Uluabat Gölü sığ, ötrof ve metal ile kontamine olmuş bir göldür. *Chironomus (Camptochrinomus) tentans* larvalarının anten ve ağız parçalarındaki (mentum, anten, mandibul ve epifarinks) şekil bozukluklarını incelemek amacıyla Ağustos 2004-Temmuz 2005 tarihleri arasında örnekler toplanmıştır. Araştırmada toplam 1800 chironomid larvası incelenmiş, Uluabat Gölü'nün 12 Chironomid taxonu içerdiği ve *Chironomus* (*Camptochironomus*) *tentans* Fabricius, 1805 türünün baskın olduğu tespit edilmiştir. Toplamda 327 *C.* (*C.*) *tentans* incelenerek bunların %55.04'ünde anten ve ağız parçalarında şekil bozukluklarının olduğu saptanmıştır. Su sirkülâsyonunun azaldığı iki istasyondan toplanan tüm *C.* (*C.*) *tentans* örnekleri çalışma süresince en yüksek oranda şekil bozukluğunun tespit edildiği örneklerdir. *C.* (*C.*) *tentans* larvaları tüm ağız parçalarında şekil bozuklukları tespit edilmiş olup, bu normalden sapmaların mentum ve epifarinkste daha yaygın olduğu belirlenmiştir.

Her bir örnekleme noktasında, su ölçümleri (derinlik, pH, çözünmüş oksijen ve sıcaklık) *in situ* olarak yapılmıştır. Ek olarak, sekiz metalin (Cd, Cr, Pb, Cu, Ni, Fe, Zn ve Ag) göl suyunda (her bir istasyonda bütün örnekler için, n=9) ve sedimentteki (n=9) konsantrasyonları aylık olarak analiz edilmiştir. Uluabat Gölü'nden sediment ve su analizleri nikel, çinko, bakır ve kurşun gibi metal kirleticilerin varlığına işaret etmektedir.

Anten ve ağız parçalarındaki şekil bozuklukları ile su-sediment toksisitesi arasındaki ilişki Correspondence Analysis (Uyum Analizi) ile incelenmiştir. Sonuçlarımıza göre anten ve ağız parçalarındaki toplam şekil bozukluğu oranı

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sedimentte nikel, çinko, kadmiyum ve kurşun, suda ise pH ve fosforun yüksek içerikleri ile pozitif korelasyon göstermektedir. Mentumdaki şekil bzuklukları ise sedimentteki Ag ve Zn, suda SO_4 ve COD ile pozitif korelasyon gösterirken, sudaki Cd ve sedimentteki Cu ile negatif korelasyon göstermektedir. Anten şekil bozuklukları sedimentte krom, suda kimyasal oksijen ihtiyacı, nitrat, sülfat ile pozitif korelasyon göstermektedir. Epifarinks şekil bozuklukları ise suda çinko, sedimentte gümüş ile pozitif korelasyon göstermektedir. Epifarinksteki şekil bozulmaları sudaki Zn ve sedimentteki Ag ile pozitif korelasyon gösterirken, anten yapısındaki bozulmaların sedimentteki Cr ve sudaki COD, NO_3–N, SO_4 içeriği ile pozitif korelasyon gösterdiği tespit edilmiştir.

Anahtar kelimeler: Uluabat Gölü, Chironomidae larvalarında şekil bozuklukları

1. Introduction

Chironomidae larvae are approved as bioassay organisms by reason of spending most of their lifetime in the surface of sediments where these organisms are subject of different toxicants. Given the ecological importance of the Chironomidae in the dynamics of aquatic ecosystems, they were often used as bioindicators in studies monitoring water quality (Vermeulen, 1995; Warwick, 1985). The existence of deformed individuals among chironomid larvae shows toxic stress (Janssens de Bisthoven, 1999). Therefore, their inclusion in community assessment adds inferring power to assess ecosystem health (Diggins & Stewart, 1998). Chironomidae larvae, as *apneustic aquatic insects*, often represent an important part of the benthic fauna in polluted aquatic systems. However, difficulties in identification often force the researcher to examine them at the tribe, subfamily or family level (Armitage & Blackburn, 1985).

In Europe and America, many studies have showed the existence of defect (or deformities) in the larvae of several chironomid genera (e.g. *Procladius*, *Chironomus* and *Cryptochironomus* spp.), and results strongly indicate that abnormalities are associated with polluted sediments (Vermeulen, 1995). In Turkey, great efforts have been realized in the last three decades to testing pollution in many lakes and rivers (including Lake Uluabat) (Dalkıran et al., 2006; Kökmen et al., 2007; Filik-İşçen et al., 2008; Emiroğlu et al., 2010; Çamur-Elipek et al., 2010; Arslan et al., 2010a; Arslan et al., 2010b). However, much less relevance is gave to the using of aquatic organisms for environmental bioassessments. Although determination of trace metals in benthic invertebrates started several decades ago in Turkey, our knowledge of mouthpart and antenna defects in chironomidae species is still limited.

The objectives of this paper are: (1) to elucidate and illustrate mouthpart and antenna defects (epipharyngis, antennae, mentum, and mandibles) in larval *C.* (*C.*) *tentans* for the first time in Turkey, (2) to compare deformation severity in *C.* (*C.*) *tentans* collected at different sites in the contaminated Lake Uluabat, and (3) to evaluate the effects of pollution on mouthpart defects.

2. Materials and methods

Cd, Cr, Pb, Cu, Ni, Zn, Fe and Ag concentrations were examined monthly in lake water (for all samples at each sampling station, (n=9), sediment (n=9)) between August 2004 and July 2005 from 12 sites in Lake Uluabat.

2.1. Study Area

Lake Uluabat (Lake Apolyont) is respected as one of the most important Bird Areas (IBA), both of in Turkey and in the Palearctic region (Magnin and Yarar, 1997). It is situated between 62º 00' and 65º 00' E longitude and 44º 40' and 44º 60' N latitude in Bursa, Turkey, to the south of the Marmara Sea (Figure 1). The lake is currently evaluated as showing a typical eutrophication character (Magnin and Yarar, 1997) and protected by the Ramsar Convention, 1998. The Uluabat Lake is a shallow (maximally 3 m deep), but large freshwater lake, which encompass an area of between 135 and 160 km^2, depending on the water level. An expanding and large delta has been constituted by silt deposition around the mouth of Mustafakemalpaşa River in the southwest part and its single outlet is in the northwest part where it drains into the Kocaçay River. Heavy metal concentrations and water quality in Uluabat Lake were investigated in different studies and levels of metal accumulation were reported (Turgut, 2005; Kazancı et al, 2010).

2.2. Sampling

C. (*C.*) *tentans* larvae were collected monthly (except during the winter season because of extreme weather conditions) from twelve stations in Lake Uluabat. A variety of defects on the head capsule were observed and the same scoring system as Lenat, 1993 was used. At each sampling site some parameters of water, dissolved oxygen, pH, depth and temperature were measured *in situ* with a DOK-TAO mark portable water quality checker (WQC-22A). At the same time, 1 L in volume of water samples were taken into plastic bottles at each sampling point and pH was adjusted to 2 by adding HNO_3, kept cool for metal analysis in the laboratory. Sample bottles were washing with detergent and then keeping them in 50% HCl for 24 hours before sampling. At the end, the bottles were washed with distilled water. Bottles were soaking in 1% nitric acid before their use. In addition, at each sampling site sediment was collected from three random sites and mixed. The upper layers of sediment were collected for metal analysis at all stations by using an Ekman-dredge (surface area 225 cm^2), taking small parts from the center of the dredge by using a polyethylene spoon to beware contamination by metallic parts of the dredge.

Maximum, minimum and average values of the environmental parameters determined in the lake water and sediment are given in Table 1. At each sampling site, Chironomid larvae were collected by using an Ekman-dredge and sieving *in situ* using a 200-µm mesh size. They were fixed in 4% formalin in the field. All collected samples were identified to species level.

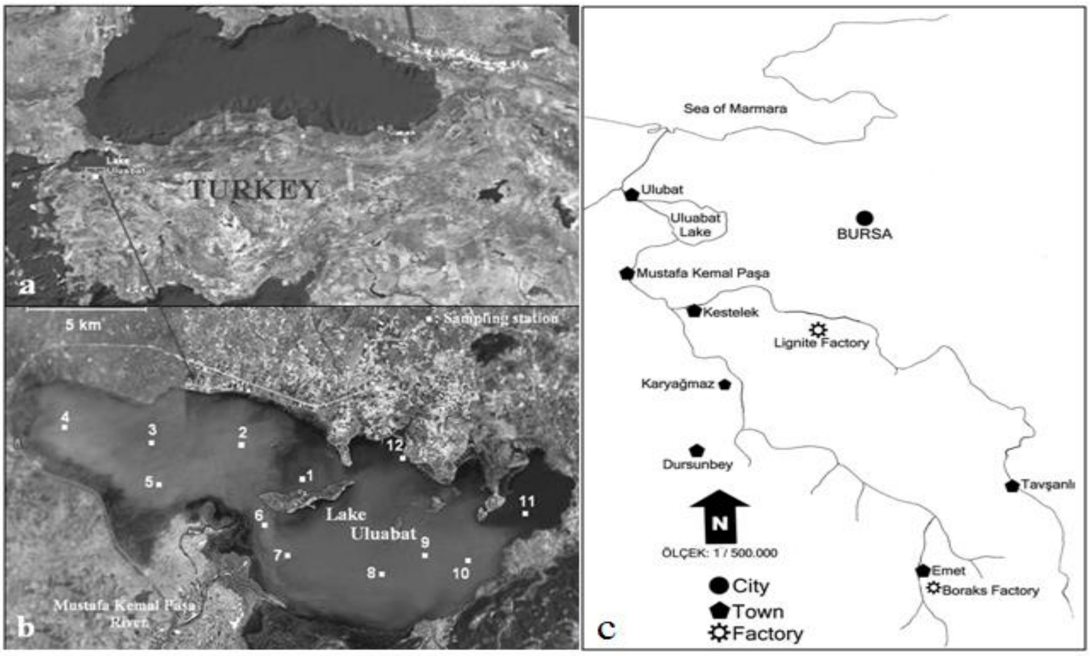

Figure 1. a- Geographical situation of Lake Uluabat; b- Sampling stations; c- Lake Uluabat basin

2.3. Evaluation of metal concentrations

Cr, Cd, Pb, Cu, Ni, Zn, Ag and Fe were analyzed in sediments and the lake water. Bottom sediment which are air-dried samples were dried for 3 hours at 105ºC to use in metal analysis. After whole samples had been sieved by a nylon (0.5 mm), 0.5 g of each sample was sited in pyrex reactors of a CEM Star 2 microwave digestion unit. $HClO_4$:HNO_3 acids of 1:3 proportions for samples were placed in the reactors. Samples were mineralized for 30 minutes at 200°C later. The samples were filtered by means of making their volumes up to 100 ml with ultrapure distilled water. Metals were identified by the flame atomic absorption spectrophotometric (Varian Spectra A 250 Plus model) method (EPA, 1998; APHA, 1992; ASTM, 1985; EPA, 2001). The metal analyses in bottom sediment and water were recorded as means triplicate measurements. In the FAAS analysis, the following wavelength lines were used: Cr 357.9 nm, Pb 217.0/283.3 nm, Cu 324.8 nm, Ni 232.0 nm, Cd 228.8 nm and Zn 213.9 nm. The analytical quality phase was also controlled by standard reference material NIST-SRM 1573a and certified reference biological material of NCS DC73350.

2.4. Evaluation of defects in the larvae

The preserved larvae were moved to a petri dish including 10% KOH solution and kept in the solution to digest the larval muscles for 24-48 hours. After then, the permanent slide mounts of the larvae were prepared following the method of Epler (2001). The slide mounted larvae were defined to species level using available taxonomic keys (Şahin, 1991; Epler, 2001) and some mouthparts (epipharyngis, mentum, mandibles) and antennae were examined for defect existence. The percentage of defect was calculated with the following formula: % defect= (number of deformed larvae/total number of larvae examined) x100. The severity of deformities was used as a standard to estimate the influences of pollution and was scored using the TSI of Janssens de Bisthoven et al. (1998); Class 1 (CL.1–individuals without any morphological deformity); Class 2 (CL.2–individuals with weak deformity): one or two round teeth, one additional or missing tooth, two joined teeth, one bifid tooth, weak asymmetry,; Class 3 (CL. 3–individuals with strong deformity). Toxic Score = No.of Class I + 2. (No.of Class II) + 3.(No.of Class III) * 100/ Total Number of Larvae.

2.5. Statistical analyses

The relationship between defect and water-sediment toxicity was analyzed by Correspondence (Greenacre and Blasiusi, 1994; Özdamar, 1999; Yıldız, 2004). Measurements were performed on samples obtained from Lake Uluabat on which deformation on the some mouthpart and antenna of *C.* (*C.*) *tentans* was observed. Similarly, the analysis of metals in sediment and water and the analysis of environmental parameters of water were also carried out. All these variables were normalized by taking the average station based values; and then, by labeling non-deformed larvae as 1, deformed larvae as 2, and for the other data, lower than average values as 1 and higher values as 2, they were categorized. The correspondence analysis was performed with categorical variables. Later, the levels of the variables' locations were given in a coordinate system. The relationship between the levels of the variables was interpreted by using the cosine value (Cos(α)) of the angle between the lines that are drawn from the levels to origin (Uzgören and Uzgören, 2007).

3. Results

3.1. Elemental Analyses

Environmental parameters measured monthly at the sampling sites from August 2004 to July 2005 are given in Table 1. According to the results, the concentrations of copper, lead, zinc and nickel in the water samples were observed higher than the limits of Regulation on Management of Surface Water Quality (2015). In addition, metals in the sediment of Lake Uluabat were found at generally 100–200 times higher than in lake water (Table 1). Zinc was found in the highest concentrations in the lake water at all sampling sites (except sites 5, 7, 8 and 9), while lead was measured in the highest concentrations at sites 5, 7, and 8 (Fig. 2). These two metal concentrations in lake water were higher than the criteria maximum concentration (CMC) limits given by the EPA (2006) (0.120 and 0.065 mg L^{-1}, respectively) (US EPA, 2006). According to average values, the metal levels were as follows for the water in Lake Uluabat: Zn > Ag > Cu > Fe > Pb > Ni > Cr > Cd.

The metal concentrations were measured in the upper layer of the sediments of the 12 sampling sites varied significantly. Except the essential metals (such as nickel and zinc), lead and copper were found in the highest concentrations at 3, 5 and 11 sampling sites. In addition, all the concentrations amounts of the eight investigated metals of the lake's sediment were found to be higher than those of lake water.

Table 1: Environmental parameters of Lake Uluabat in the investigated period from August 2004 to July 2005 (WT: water temperature; BOD: Biological oxygen demand; DO: dissolved oxygen; COD: Chemical oxygen demand; DW; dry weight).

Limnological Parameters									
Months	**DO** (mg L^{-1})	**BOD** (mg L^{-1})	**COD** (mg L^{-1})	**WT** (ºC)	**pH**	**NO_2 – N** (mg L^{-1})	**NO_3-N** (mg L^{-1})	**NH_3-N** (mg L^{-1})	**PO_4^{-3}** (mg L^{-1})
Aug.04	6.5	16.3	41.2	23.9	8.5	0.051	0.298	0.623	0.951
Sep. 04	5.4	13	39.1	23.1	8.2	0.075	0.788	0.153	0.493
Oct. 04	7.4	5.5	27.4	18.3	8.3	0.018	1.838	0.026	0.477
Nov. 04	9.3	3.5	27.7	12	8	0.041	1.533	0.138	0.466
Mar.05	6.4	10.8	45.6	13.4	8.8	0.036	1.147	0.128	0.323
Apr. 05	7.5	6.6	73.4	19.1	8.3	0.048	0.833	0.114	0.331
May. 05	7	7.1	70.8	22.5	8.3	0.028	0.708	0.083	0.148
Jun. 05	7.5	7.6	78.3	23	8.4	0.045	1.167	0.285	0.331
Jul. 05	6.3	18.2	66.4	25.7	8.8	0.044	0.617	0.463	0.302
Stations									
1st	8.5	15.4	62.3	20.4	8.5	0.036	0.531	0.400	0.369
2nd	8.6	7.6	48.5	20	8.5	0.046	0.762	0.174	0.302
3rd	9.3	7.4	47.8	19.5	8.4	0.068	1.077	0.157	0.570
4th	7.7	7.7	60.6	19.5	8.4	0.027	1.111	0.239	0.378
5th	9.2	7.8	56.3	19.7	8.5	0.052	1.012	0.128	0.281
6th	8.3	7.6	34.6	19.6	8.3	0.053	0.944	0.182	0.527
7th	7.5	8.1	51.6	20	8.3	0.036	1.103	0.202	0.314
8th	7.4	7.1	51.9	19.8	8.4	0.038	1.238	0.302	0.355
9th	7.7	14.6	41.6	21	8.4	0.051	0.749	0.222	0.532
10th	7.4	11.9	63	20.4	8.4	0.039	1.199	0.201	0.475
11nd	6.1	15.2	47.2	21.5	8.5	0.040	1.139	0.311	0.574
12th	5.4	6	73.6	18.8	8.4	0.021	1.074	0.114	0.306
Average concentration of metals (min.-max value±SD) in lake water and sediment									
	Cd	**Cr**	**Pb**	**Cu**	**Ni**	**Zn**	**Fe**	**Ag**	
Water (mg L^{-1})	0.003 (n.d.-0.018)	0.017 (n.d.-0.132)	0.105 (n.d.-0.401)	0.118 (0.007-0.337)	0.056 (n.d.-0.245)	0.284 (0.363-3.85)	0.107 (0.09-1.56)	0.1375 (0.048-0.059)	
Sediment (DW,mgkg^{-1})	0.699 (n.d.-7.4)	57.9 (n.d.-132.7)	110.7 (n.d.-372.5)	119.226 (38.6-289.8)	209.4 (75.6-303.7)	171 (17.2-395.73)	65.10 (58.6±154.89)	21.6 (7.35±78.13)	

3.2. Defects in *Chironomus (Camptochironomus) tentans*

A total of 1800 chironomid larvae were examined from the twelve sampling sites. Chironomid larvae were abundant in the sediments of Lake Uluabat, representing on average 12.3% of the benthic fauna. *C.* (*C.*) *tentans* was the dominant chironomid species consisting 66.2% of the total chironomid limnofauna. Among the identified chironomid species the highest frequencies of defects were found in *C.* (*C.*) *tentans.* A total of 327 *C.* (*C.*) *tentans* were examined, 55.04% of which possessed defects (Table 2). 4th instar *C.* (*C.*) *tentans* larvae which were deformed for some mouthparts (mentum, mandibles, epipharyngis) and antenna are illustrated in Figure 2. Although defects were found in the mentum, mandibles, epipharyngis and antennae in *C.* (*C.*) *tentans*, mentum and epipharyngis defects were the most frequent (Table 2 and Figure 2).

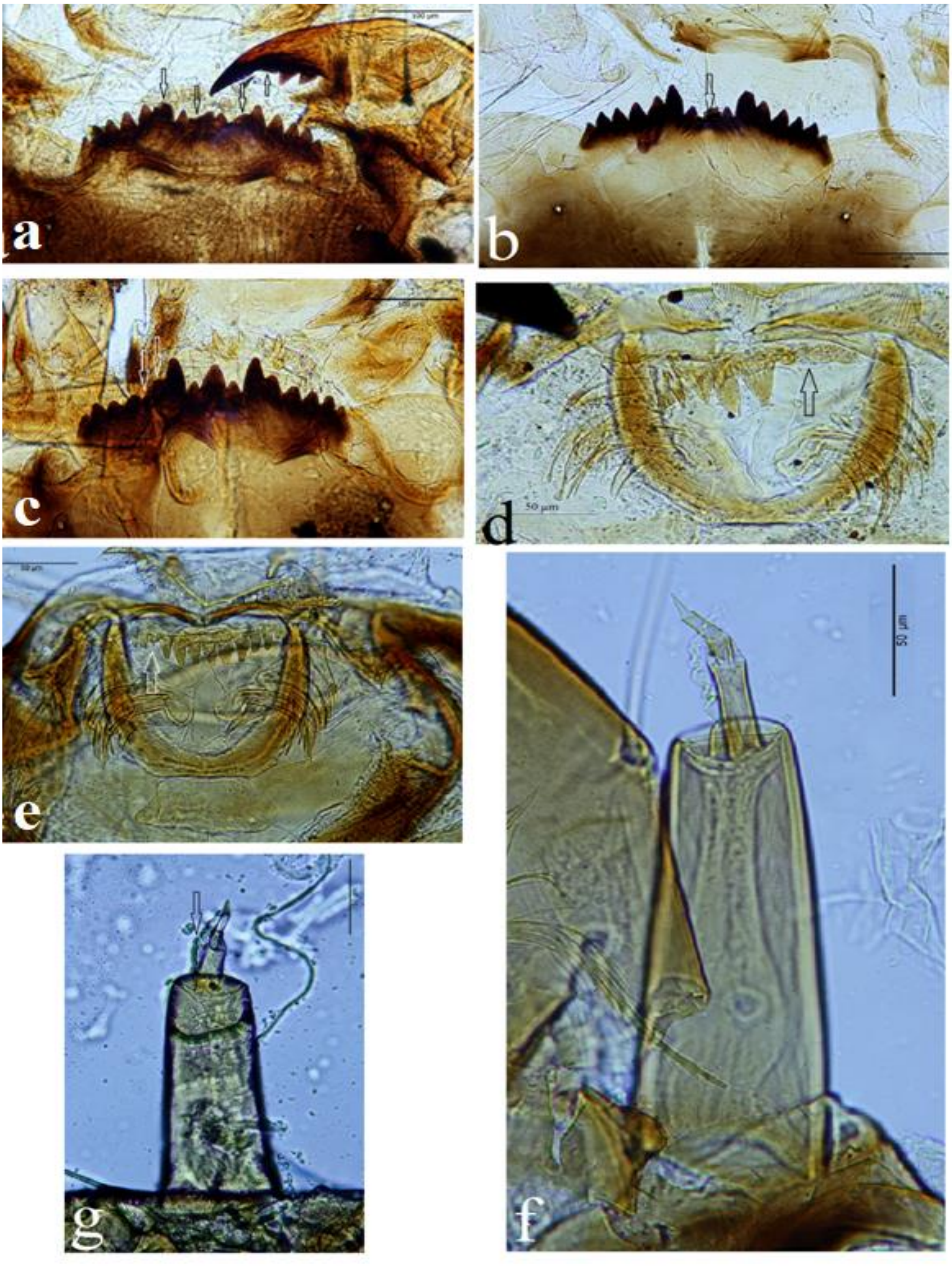

Figure 2. Defects in *Chironomus* (*Camptochironomus*) *tentans* larvae collected from Lake Uluabat: Mouthpart deformities; a-c: mentum deformities, d-e: epipharyngis deformities; and antenna deformities f-g: antenna

3.3. Statistical analyses

The most common type of mentum defect observed was the loss or strong reduction of median teeth, and asymmetry in the number of lateral teeth and gaps in the mentum were very common. Epipharyngis defects were characterized into two general types, ranging from the absence of the lateral teeth and asymmetry in the number of lateral teeth as demonstrated in Figure 2a-c. Existence of extra lateral teeth was another deformity but it was very rare.

Table 2. Defect frequencies (%) in larvae (n=327) of *C.* (*C.*) *tentans* collected from Lake Uluabat (Ss: Sampling stations; TS: Toxic score)

Ss	Total larvae	*C.* (*C.*) *tentans*	Class I	Class II	Class III	Non-defect Larvae	Defect Rate (%)	Mentum defect rates (%)	TS (%)
1	172	54	26	27	1	26	16	39.3	48.2
2	202	50	21	27	2	21	14	37.9	40
3	132	42	24	18	-	24	14	55.6	45.4
4	75	3	-	3	-	-	4	33.3	8
5	84	2	1	1	-	1	1	0	3,6
6	110	6	2	3	1	2	4	25	10
7	133	7	2	5	-	2	4	20	9,02
8	384	75	32	41	2	32	11	20.9	31.2
9	265	49	22	24	3	22	10	48.1	30
10	168	36	17	18	1	17	11	26.3	33.3
11	14	3	-	3	-	-	21	66.7	43
12	61	-	-	-	-	-	-	0	0

Correspondence analysis showed significant correlations between the deformed mouthpart (mentum, epipharyngis) and antennae of *C.* (*C.*) *tentans* larvae and the environmental parameters of water and sediment. Mouthpart defect incidence did not equally correspond to the environmental parameters of the water and sediment (Figures 3, 4 and 5). According to our results, the deformity rate showed a significant relation with high contents of Ni, Pb, Cd and Zn in sediment and pH, PO_4 in water.

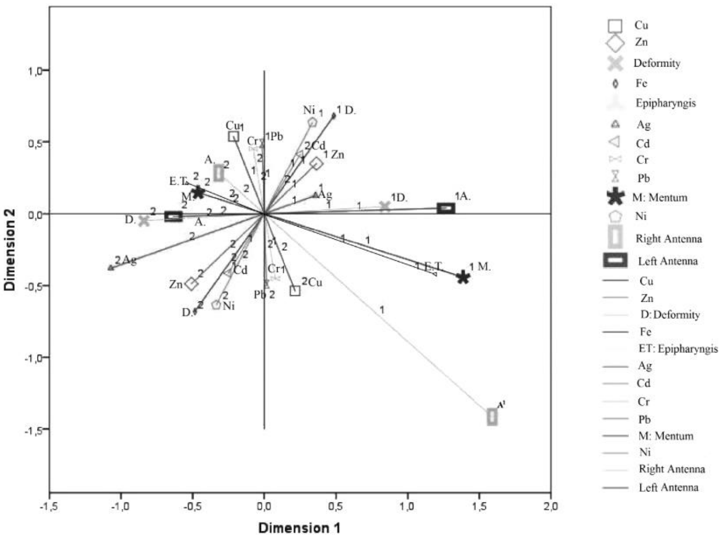

Figure 3. Correlation coefficients between mouthpart defects and sediment metal concentrations

With regard to Correspondence analysis, Zn in water, Ag in sediment showed a significant positive relationship with epipharyngis deformities, whereas Cu in sediment showed a significant negative relationship. In addition, it was found that the relation between NO_3–N, PO_4, temperature, SO_4 and epipharyngis deformities were significantly directly proportional. The mentum deformities positively correlated with Ag and Zn in sediment, SO_4 and COD in water and negatively correlated with Cd in water and Cu in sediment. Antenna deformities positively correlated with Cr in sediment and COD, NO_3–N, SO_4 in water.

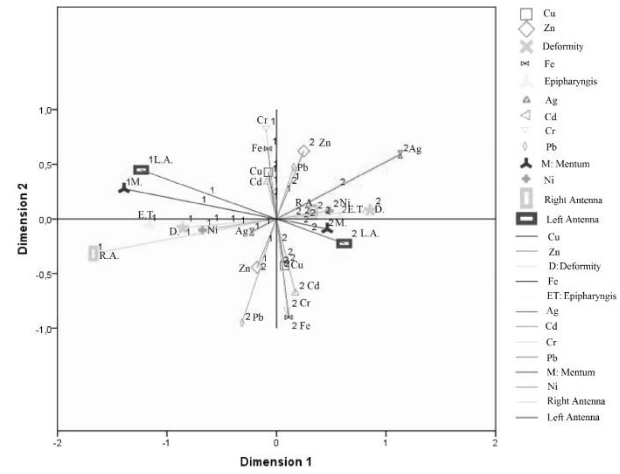

Figure 4. Correlation coefficients between mouthpart defects and water metal concentrations

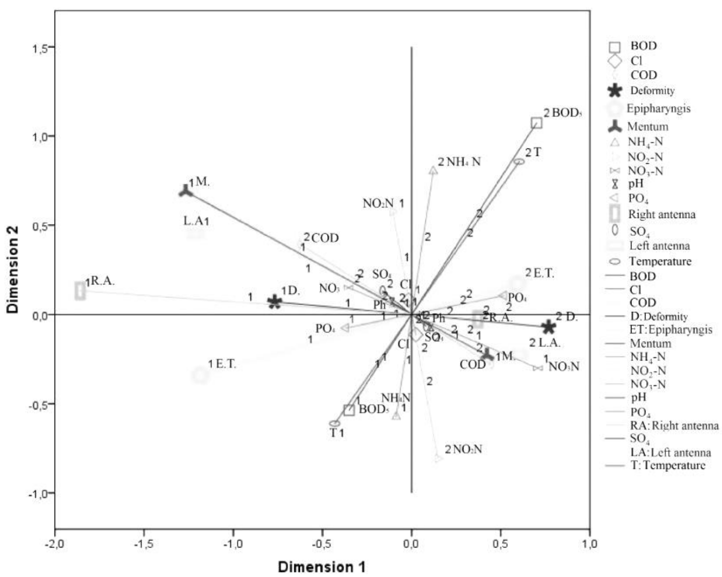

Figure 5. Correlation coefficients between water parameters and mouthpart defects

4. Conclusions and discussion

It is known that Lake Uluabat is dominated by pollution-tolerant invertebrate taxa, like Nematoda, Oligochaeta, Chironomidae spp. (Kökmen et al., 2007; Arslan et al., 2010a). Lake Uluabat is most likely contaminated with domestic and waste agricultural chemicals and it also receives industrial discharges from surrounding areas (Filik-İşçen et al., 2008). The larvae of the subfamily Chironomini are considered as filter or sediment feeders (Armitage and Blackburn, 1985). *C.* (*C.*) *tentans* belonging to the subfamily Chironominae was the most abundant chironomid in Lake Uluabat, showing a higher rate of mouthpart defects. All samples of *C.* (*C.*) *tentans*, collected from stations 4th and 11th where water circulation is reduced (Figure 1a) demonstrated the highest effects of defects throughout the study.

As is known, heavy metal contamination is a main cause of defects in the chironomid larvae (Martinez et al., 2004; Bhattacharyay et al., 2005; Al-Shami et al., 2010). The metal concentrations were measured in the upper layer of the sediments in the 12 sampling sites varied significantly. Except the essential metals (nickel and zinc), lead and copper were measured in the highest concentrations at 3, 5 and 11 sampling sites. In addition, all the concentrations values of the eight investigated metals of the sediment of the lake proved to be higher than those of lake water. Almost all deformed *C.* (*C.*) *tentans* samples pertain to instar IV exposed to toxic sediment substances for the longest period. This indicates that the high frequency of defects is related to high levels of sediment contamination. High lead and copper concentrations were measured in the Uluabat Lake sediments (Table 1). The relationship between sediment metals (especially Ag, Zn, Cr, Pb and Cd) and chironomid mouthpart defects was significantly positive. These results were in agreement with others (Madden et al., 1992 and 1995; Bird, 1994; van Urk et al., 1992; Thornberg, 1995) who recorded an almost linear relationship between increasing levels of heavy metals and deformity rates. In addition, our results show that the relation between deformity rates strongly correlated with pH, PO_4, SO_4, NO_3–N and COD in water. Janssens de Bisthoven et al. (1994) were reported that the relationship between pollutants and defects is not always linear and depends greatly on the pollutant involved. They found that defects in the mandibles correlated with exposure to heavy metals whereas the menta and antennae were more sensitive to organic xenobiotic exposure.

The results of the present study are different from the results of Janssens de Bisthoven et al. (1994) because we found that defects in the antennae and menta also correlated with metals Cr and Ag-Zn in sediment respectively.

As we mentioned before, zinc was found in the highest concentrations in the lake water at all sampling sites (except sites 5, 7, 8, and 9), while lead was measured in the highest concentrations at sites 5, 7, and 8. Zinc in water showed a significant positive relationship with epipharyngis defects and, in addition, mentum defects positively correlated with Zn and Ag in sediment. Furthermore, the present study found a positive correlation between Cd and Pb in deformity rates and sediments. Although zinc is known as an essential metal and cofactor of many enzyme systems among the elements analyzed, it is clear that the high concentration of this metal, as well as Pb and Cd in water and sediment (either alone or in combination), may be the agents inducing the mouthpart deformities.

Many contaminants have been recommended as causal agents for the deformities in chironomid larvae. However, overview of the literature finds out that heavy metals generally are the only common factor. However, Simkiss and Taylor (1989) reported that as with other metals, the uptake or toxicity of zinc in aquatic organisms is modified by environmental conditions such as temperature, salinity and pH. In addition, lead and cadmium are very toxic to aquatic organisms and pH, temperature, and water hardness are factors that affect its toxicity (Hellawell, 1998). In Lake Uluabat, the temperature did not diverse along the lake, and a normal seasonal change was observed (Table 2). pH values were high and slightly alkaline. The present study found a positive relationship between pH and epipharyngis deformity.

Janssens de Bisthoven et al. (1994) reported that a possible relationship between specific defect types and specific contaminants, supposing that defect type can be used as a potential indication of the responsible contaminant. He showed that Pb was most likely to be related to mentum defects while there was no certain relationship between Zn and Cu and specific defects. However, Martinez et al. (2001) found that Pb and Zn set off defects in the mentum at a higher rate than in the mandibles. They also reported that Zn was associated with higher rates of missing and fused mentum teeth. Deformities exposed to Pb in chironomids were most likely to be Kohn gaps and missing mentum teeth (Martinez et al., 2001).

In this study it was found that the percentage of deformation in the mentum is higher that of the other mouthparts. This reveals that the Zn, Cu and Pb contents in the water and sediment is of very high concentration and that they affect the mentum more than the mouthparts. However, contrastly to the results of the study by Martinez et al. (2001), the percentage of deformation in the mandibula is not very high.

Nickel, zinc, copper and lead concentrations in both sediment and water appear to set off defects in the mentum at a higher proportion than in mandibles, with Kohn gaps and missing or fused mentum teeth the most common deformity types. As can be seen in Table 2, the metal with the second highest concentration in water is silver (0.137 mg/L). The average concentration in the sediment was established to be 0.21 mg/kg and the highest concentration to be 78.35 mg/kg. It is known that concentrations of Ag in sediment from highly industrialised areas range from 1 to 150 mg/kg, whereas background concentrations in nonurban areas are usually <0.1 mg/kg (Eisler, 1996). There are many industrial plants located in Lake Uluabat's catchment area. This shows that silver was carried from the river basin to the lake and that it was accumulated in the lake water and the sediment.

According to the mean value of lake water parameters, NO_2 –N was generally determined at three-fourths quality, NO_3 –N was determined at first quality level, and NH_3 –N was determined at second quality level and phosphate was determined at third quality level (Regulation on Management of Surface Water Quality, 2015). The maximum value of ammonium concentrations were found at stations 1, 4, 6, 8 and 11. All samples of *C.* (*C.*) *tentans*, collected from stations 1st, 8th and 11th showed the highest defect rate throughout the study (16%, 11% and 21% respectively). This result shows that mouthpart deformities not only correlated with metals but also correlated with water parameters such as NO_3–N, NO_2–N, NH_3–N and PO_4.

In *C.* (*C.*) *tentans* (Table 2) defect, analysis of the mentum showed a deformity incidence of 55.04% (CL.2+CL.3; 180 of 327 specimens). CL.2 deformity affected 51.98% of specimens whereas strong deformity (CL.3) affected 3.05% of specimens. Lenat (1993) suggested that toxic score = 25 should be taken as the threshold value above which a watercourse should be considered as toxically contaminated. TS values calculated for our *C. (C.) tentans* samples are shown in Table 2. Samples in which TS is over 25 were found in sampling sites 1, 2, 3, 8, 9, 10 and 11 (TS>30). In addition, Lenat (1993) also suggests that the frequency of severe deformities (especially Class II and Class III deformities) is generally greater than 6% at toxic sites. In most of our samples, the frequency of severe deformities was higher than 6%. In addition, Diggins and Stewart (1998), search the heavily industrialized Buffalo River, reported a 15% deformity rate for the predator *Procladius* and a 22–66% deformity proportion for the deposit-feeder *Chironomus*. The current study also found that deformities were most prevalent in *C.* (*C.*) *tentans*, although our highest (21%) deformity proportion was less than their lowest deformity rate. The results of our study show that the water and sediment of Lake Uluabat, which is a Ramsar zone, are heavily contaminated. Furthermore, taking the literature data into account, the high proportions of TS, Class II and Class III deformities show that the effects of past pollution are extreme. Warwick and Tisdale (1988), and Bird (1994) reported that deformities of the mentum are a common developmental anomaly in *Chironomus* spp.

larvae, and our result supports this knowledge. In addition, *Chironomus* spp. larvae are known as organisms which are highly susceptible to morphological deformation. *Chironomus* spp. are, therefore, one of the important indicators of the effects of sediment and water bound contaminants (Vermeulen, 1995; Hudson and Ciborowski, 1996). Kosalwat and Knight (1987) and van de Guchte and Urk (1989) have reported experiments with various *Chironomus* species that show direct relationships between deformities and heavy metal levels. Our data are in agreement with the studies mentioned above. In summary, our results show that not only high metal concentrations (especially Ni, Zn, Pb, Cu and patially Ag) in water and sediment but also NO_3–N, NO_2–N, NH_3–N and PO_4 concentrations can set off deformities in chironomid larvae subjected to these metals during their lifetime. The surveyed deformity induction in the current study supports the potential for use of chironomid deformity rates for bioassay applications.

Consequantly, larvae of *C.* (*C.*) *tentans* in Lake Uluabat have a high effect of mouthpart defects. *C.* (*C.*) *tentans* is the best bioindicator because it closely reflects the condition of the sediment influenced by the hydrological regime of the lake.

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References

Al-Shami, S., Rawi, C. S. M., Nor, S. A. M., Ahmad, A. H., Ali, A. (2010). Morphological deformities in *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River System, Penang, Malaysia. Journal of Environmental Entomology, 39, 210–222.

APHA, American Public Health Association. (1992). Standard methods for the examination of water and wastewater. CSS (Complete Statistica System): Statistica. References for Statistical Procedures (DOS and Windows version), Release 3.1. StatSoft Inc., Tulsa (OK): Washington StatSoft Inc.

Armitage, P. D., Blackburn, J. H. (1985). Chironomidae in a pennine stream system receiving mine drainage and organic enrichment. Hydrobiologia, 121, 165–172.

Arslan, N., Ayık, O., Sahin, Y. (2010a). Diversity and structure of Chironomidae (Diptera) limnofauna of Lake Uluabat, a Ramsar site of Turkey, and their relation to environmental variables. Turkish Journal Fisheries and Aquatic Sciences, 10(3), 235-243.

Arslan, N., Koç, B., Cicek, A. (2010b). Metal contents of macroinvertebrates (Oligochaeta - Chironomidae), in water, and bottom sediments of Lake Uluabat, a Ramsar site of Turkey. The Scientific World Journal Environment, 10, 1269-1281.

ASTM, American Society for Testing and Materials. (1985). Preparation of biological samples for inorganic chemical analysis. 1. Annual Book of ASTM Standards, D-19: 740–747.

Bhattacharyay, G., Sadhu, A. K., Mazumdar, A., Chaudhuri, P. K. (2005). Antennal deformities of chironomid larvae and their use in biomonitoring of heavy metal pollutants in the River Damodar of West Bengal, India. Environmental Monitoring and Assessment, 108, 67–84.

Bird, G. A. (1994). Use of chironomid deformities to assess environmental degradation in the Yamaska River, Quebec. Environmental Monitoring and Assessment, 30, 163–175.

Camur Elipek, B., Arslan, N., Kırgız, T., Oterler, B., Guher, H., Ozkan, N. (2010). Analysis of benthic macroinvertebrates in relation to environmental variables of Lake Gala (Edirne / Turkey). Turkish Journal of Fisheries and Aquatic Sciences, 10, 315-322.

Dalkıran, N., Karacaoglu, D., Dere, S., Senturk, E., Torunoglu, T. (2006). Factors affecting the current status of a eutrophic shallow lake (Lake Uluabat, Turkey): Relationships between water physical and chemical variables. Chemistry and Ecology, 22(4), 279–298.

Diggins, T. P., Stewart, K. M. (1998). Chironomid deformities, benthic community composition, and trace elements in the Buffalo River (New York) area of concern. Journal of the North American Benthological Society, 17(3), 311–323.

Eisler R. (1996). Silver hazards to fish, wildlife and invertebrates: A synoptic review. Contaminant Hazard Reviews. Laurel, MD (US): Department of the Interior Patuxent Wildlife Research Center. Report No.:32.

Emiroglu, O., Cicek, A., Arslan, N., Aksan, S., Ruzgar, M. (2010). Boron concentration in water, sediment and different organisms around large borate deposits of Turkey. Bulletin of Environmental Contamination and Toxicology, 84(4), 427-431.

EPA Method 2007. (2001). Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry.

EPA Method 3051. (1998). Microwave assisted acid digestion of sediments, sludges, soils, and oils.

EPA, Environmental Protection Agency (US). (2006). National recommended water quality criteria-correction. Office of Water. 822-z-99-001.

Epler, J. H. (2001). Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the Southeastern United States, including Florida. North Carolina, Special Publication, SJ2001-SP13.

Fılık Iscen C., Emiroglu, O., Ilhan, S., Arslan, N., Yılmaz, V. (2008). Application of multivariate statistical techniques in the assessment of surface water quality in Uluabat Lake, Turkey. Environmental Monitoring and Assessment, 144, 269-276.

Greenacre, M., Blasius, J. editors. (1994). Correspondence analysis in the social science. San Diego: Academic Press.

Hellawell M. J., 1998. Toxic substances in rivers and streams. Environmental Pollution, 50, 61–85.

Hudson, L. A., Ciborowski, J. J. (1996). Spatial and taxonomic variation in incidence of mouthpart deformities in midge larvae (Diptera: Chironomidae, Chironomini). Canadian Journal of Fisheries and Aquatic Sciences, 53, 297-304.

Janssens de Bisthoven, L. (1999). Biomonitoring with deformities in aquatic organisms. In: Gerhardt A editor. Biomonitoring of Polluted Water. Zürich: Transtech Publications. Environmental Science Forum, 9, 65–94.

Janssens de Bisthoven, L., Huysmans, C., Ollevier, F. (1994). The *in situ* relationships between sediment concentrations of micropollutants and morphological deformities in *Chironomus* gr. *thummi* larvae (Diptera, Chironomidae) from lowland rivers (Belgium): a spatial comparison. In: Cranston PA editor. Chironomids: from genus to ecosystems. CSIRO, Melbourne. p. 63-80.

Janssens de Bisthoven, L., Nuyts, P., Goddeeris, B., Ollevier, F. (1998). Sublethal parameters in morphologically deformed *Chironomus* larvae: clues to understanding their bioindicator value. Freshwater Biology, 39, 179-191.

Kazancı, N., Leroy, S. A. G., Öncel, S., İleri, Ö., Toprak, Ö., Costa, P., Sayılı, S., Turgut, C., Kibar, M. (2010). Wind control on the accumulation of heavy metals in sediment of Lake Uluabat, Anatolia, Turkey. Project supported by Technical and Scientific Research Council of Turkey (YDABCAG 103Y102), and by NATO (EST.CLG.978645). https://bura.brunel.ac.uk/bitstream/2438/4190/1/JOPL1528R3.pdf.

Kokmen, S., Arslan, N., Filik, C., Yılmaz, V. (2007). Zoobenthos of Lake Uluabat, a Ramsar site in Turkey, and their relationship with environmental variables. Clean, 35(3), 266-274.

Kosalwat, P., Knight, A. W. (1987). Chronic toxicity of copper to a partial life cycle of the midge *Chironomus decorus*. Archives of Environmental Contamination and Toxicology, 16, 283–290.

Lenat, D. R. (1993). Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in streams. Journal of the North American Benthological Society, 12(3), 265–269.

Madden, C. P., Austin, A. D., Suter, P. J. (1995). Pollution monitoring using chironomid larvae: what is a deformity? In: Cranston P editor. Chironomids: from genes to ecosystems. CSIRO Pub East Melbourne, Australia. p. 89–100.

Madden, C. P., Suter, P. J., Nicholas, B. C., Austin, A. D. (1992). Deformities in chironomid larvae as indicator of pollution (pesticide) stress. Netherland Journal of Aquatic Ecology, 26, 551–557.

Magnin, G., Yarar, M. (1997). Important bird breeding areas in Turkey, İstanbul.

Martinez, E. A., Moore, B. C., Schaumloffel, J., Dasgupta, N. (2001). Induction of morphological deformities in *Chironomus tentans* exposed to zinc- and lead-spiked sediments. Environmental Toxicology and Chemistry, 20(11), 2475–2481.

Martinez, E. A., Moore, B. C., Schaumloffel, J., Dasgupta, N. (2004). Teratogenic versus mutagenic abnormalities in chironomid larvae exposed to Zinc and Lead. Archives of Environmental Contamination and Toxicology, 47, 193–198.

Ozdamar, K. (1999). Paket programlarla istatistiksel veri analizi "çok değişkenli analizler". Eskişehir: Kaan Kitabevi.

Republic of Turkey Ministry of Environment and Forest. (2015). Regulation on Management of Surface Water Quality.

Sahin, Y. (1991). Türkiye Chironomidae Potamofaunası. Project No.:TBAG-869.

Simkiss, K., Taylor, M. G. (1989). Metal fluxes across the membranes of aquatic organisms. Critical Reviews in Aquatic Sciences, 1, 173–188.

Thornberg, A. E. (1995). Mouthpart deformities in chironomids from heavy metal contaminated sediments of the Coeur d' Alene region [master's thesis]. [Pullman (WA)]: Washington State University.

Turgut, C. (2005). Ulubat Gölü çökellerinde ve göl suyunda metal konsantrasyonlarının incelenmesi. Yüksek Lisans Tezi, Gebze Yuksek Teknoloji Enstitüsü, Gebze, Kocaeli, 92 p.

Uzgoren, N., Uzgoren, E. (2007). Dumlupınar Üniversitesi lisans öğrencilerinin memnuniyetini etkileyen bireysel özelliklerin istatistiksel analizi-hipotez testi, ki-kare testi ve doğrusal olasılık modeli. Dumlupınar Üniversitesi Sosyal Bilimler Dergisi, 17, 173-192.

van de Guchte, C., van Urk, G. (1989). Discrepancies in the effects of field and artificially heavy metal contaminated aquatic sediments upon midge larvae. In: Vernet JP editor Heavy metals in the environment. 7th International Conference; Geneva.

van Urk, G., Kerkum, F. C. M., Smit, H. (1992). Life cycle patterns, density and frequency of deformities in *Chironomus* larvae (Diptera: Chironomidae) over a contaminated sediment gradient. Canadian Journal of Fisheries and Aquatic Sciences, 49, 2291–2299.

Vermeulen, V. C. (1995). Elaborating chironomid deformities as bioindication of toxic sediment stress: the potential application of mixture toxicity concepts. Annales Zoologici Fennici, 32, 265-285.

Warwick, W. F. (1985). Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystem: indicating antennal deformities in *Chironomus meigen*. Canadian Journal of Fisheries and Aquatic Sciences, 42, 1881-1914.

Warwick, W. F., Tisdale, N. A. (1988). Morphological deformities in *Chironomus*, Cryptochironomus, and *Procladius* (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lakes, Saskatchewan. Canadian Journal of Fisheries and Aquatic Sciences, 45, 1123–1144.

Yıldız, Z. (2004). Eğitim düzeyi ile suç türü arasındaki ilişkinin araştırılması. Kuram ve Uygulamada Eğitim Yönetimi Dergisi, 38, 278-291.

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