TÜBİTAK

Turkish Journal of Zoology

Volume 47 | Number 5 Article 5

9-13-2023

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## Recommended Citation

BAKIR, RECEP; AKYILDIZ, GÜRÇAY KIVANÇ; and DURAN, MUSTAFA (2023) "The effects of various lake typologies on the distribution of Chironomus spp. (Diptera), and arguments on optional factors of Water Framework Directive in Turkiye," *Turkish Journal of Zoology*: Vol. 47: No. 5, Article 5. https://doi.org/10.55730/1300-0179.3143
Available at: https://journals.tubitak.gov.tr/zoology/vol47/iss5/5

Turkish Journal of Zoology
http://journals.tubitak.gov.tr/zoology/
Research Article

Turk J Zool
(2023) 47: 299-314
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doi:10.55730/1300-0179.3143

# The effects of various lake typologies on the distribution of *Chironomus* spp. (Diptera), and arguments on optional factors of Water Framework Directive in Türkiye

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**Received:** 18.05.2023 • **Accepted/Published Online:** 07.09.2023 • **Final Version:** 13.09.2023

**Abstract:** Countries with heterogeneous climatic and geographic characteristics may find it challenging and limiting to fulfill the requirements of the Water Framework Directive (WFD), such as defining ecoregion and water body types. To overcome this limitation, they implement optional factors in addition to the obligatory factors defined in the WFD. As a candidate country, Türkiye is required to comply with the WFD requirements and must determine its own optional and obligatory factors. In this context, we addressed the relevance of *Chironomus* spp. in determining these factors. We used twenty distinct national lake typologies for Türkiye and identified a total of 24 *Chironomus* species. The *Chironomus* genus was found to be effective in determining the optional factors. Statistical analyses revealed that fetch, altitude, salinity, water temperature, pH, saturated and dissolved oxygen, conductivity, orthophosphate, ferrous ion, chloride, and nitrogen compounds were significant in discriminating the regions. The canonical correspondence analysis (CCA) explained 56.62% of the variance. We also statistically analyzed the effectiveness of the class boundaries of the obligatory factors used in Türkiye for regionalization. Among the official obligatory factors, only the altitude boundaries gave a significant result and explained a total of 62% variance. We recommend revising the class boundaries of obligatory factors and using fetch data as a significant genuine parameter for lake typology as an optional factor.

**Key words:** Lake typology, water framework directive, Chironomidae, ordination analysis.

## 1. Introduction

In recent years, assessing the ecological integrity of freshwaters came into prominence because of the rapid climatic changes and urbanization. Therefore, much legislation has been developed worldwide (e.g., Water Framework Directive in EU, The Water Act in the Czech Republic, Clean Water Act in the USA, National Water Act in South-Africa) (Birk et al., 2012; Reyjol et al., 2014; Poikane et al., 2016). One of them is the EU Water Framework Directive 2000/60/EC (WFD 2000) which also concerns Türkiye. The EU Water Framework Directive 2000/60/EC (WFD 2000) defines the ecological status of water bodies by characterizing each type of water body (typology) and determining conditions for quality elements with the aim of reaching a good ecological status of water. Therefore, the WFD mandates that all European water bodies, including candidate countries that are already members of the directive, are assigned to one of five ecological classes from high to bad quality, mostly based on biological indicators (Bund and Solimini, 2007; Simboura et al., 2005). The WFD sets out a timetable for the implementation of requirements for EU member and candidate countries. As a candidate country negotiating with the EU on becoming a member state, Türkiye will be obliged to abide by WFD rules during the process of membership. Türkiye declared the date of harmonization with the WFD rules as 2027 (with a possible extension to 2033) (Sumer, 2016).

The WFD recommends characterizing water body types and creating specific hydromorphological and physicochemical reference conditions for each water body before improving the ecological status. In order to achieve these goals, there are several preliminary tasks with a general-to-specific approach. It is necessary to determine the regionalization based on the basins and reveal the water body typologies as the first step. Thorp et al. (2006) stated that a water body can be expressed as a group of lakes or rivers that share natural ecological conditions in terms of biological, physicochemical, hydrological and geomorphological aspects. Typology is the generalization of the complex structure of aquatic ecosystems in simplified units, making aquatic ecosystems accessible for extensive

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analysis and comparisons. The WFD proposes two systems as obligatory and optional factors with different criteria for determining the typologies of rivers, lakes, transitional waters, and coastal waters which are called 'System A' and 'System B'. Optional factors in System B can only be used in addition to the obligatory factors given in System A. However, the useful part is that the optional factors used in System B can be region-specific. It is very reasonable to use bioindicator organisms in determining the region-specific factors to be used in System B (Simboura et al., 2005; Solimini et al., 2006). System A is insufficient in countries with rich ecological conditions such as Mediterranean countries and requires the use of System B (Solimini et al. 2006). Therefore, the WFD allows the use of System B as an alternative characterization of surface water body types with 6 obligatory (altitude, latitude, longitude, depth, geology, and size) and an additional 15 optional factors (mean water depth, lake shape, residence time, mean air temperature, air temperature range, mixing pattern, acid-neutralizing capacity, background nutrient status, mean substratum composition, water level fluctuation). The point here is that member states are able to choose suitable optional factors for their water body types and set their own class boundaries (Sandin and Verdonschot, 2006). At this point, biological and environmental parameters might be used in determining the optional factors to use in the characterization of the surface water body types (Solimini et al., 2006). As a statement, the factors used in the typology should be permanent and should not reflect human impacts.

Benthic macroinvertebrates constitute a useful proxy that integrates e.g., climatic, hydrological, and geological variables (Nyman and Korhola, 2005). The abundance of macroinvertebrates in the benthos varies according to many factors such as the distance from the littoral zone, depth, oxygenation and water quality, predation by certain groups, sediment composition, altitude of the lake, and the organism's life history (Margalef, 1984; Payne, 1986). For this reason, they are mostly preferred to use in typological studies (Lenat and Resh, 2001; Verdonschot and Nijboer, 2004; Moog et al., 2004). Particularly, the chironomids (Diptera) are the most abundant and species-rich group in fluvial and lacustrine systems (Armitage et al., 1995; Punti et al., 2009). Among them, the genus *Chironomus* can be found in lakes from the tropics to the temperate and the arctic regions except for Antarctica (Hare and Carter, 1986; Jónasson, 1972; Butler, 1982). Chironomus play crucial roles in the functioning of freshwater ecosystems as both consumers and decomposers. They also serve as a food source for other organisms such as fish, amphibians, and birds. Because of their high abundance, *Chironomus* are often used as bioindicators to assess the ecological health of freshwater systems.

The main objective of this study is to elaborate on the significant typological factors by revealing the relationship between environmental variables, abiotic factors and *Chironomus* spp. collected from natural lakes within different typologies. This research aims to address several fundamental issues regarding the use of *Chironomus* spp. in regionalization and typological studies. Specifically, we seek to answer the following questions: (1) To what extent can *Chironomus* spp. be effectively used in regionalization studies, which form the initial stage of typological studies? (2) Are there significant differences in the distribution of *Chironomus* spp. across the existing local-official lake typologies in Türkiye? (3) How can *Chironomus* spp. be utilized to determine acceptable parameters for optional factors in typological studies?

## 2. Materials and methods

### 2.1. Study area

System A, which is proposed in the Water Framework Directive (WFD), is utilized to classify lakes into different types based on four abiotic factors: altitude, surface area, geology, and mean depth. These factors are also used to determine lake water body typologies (LWBT) in Türkiye. However, the national class boundary values for these factors differ from the ranges specified for EU ecoregions in Annex II of WFD 2000/60/EC (see Table 1). In Türkiye, lacustrine systems are classified into 23 unique LWBT, two of which represent dam (artificial) lakes while the others represent natural lakes. For this study, 20 natural lake typologies were selected and evaluated (see Figure 1). The selected lakes vary in altitude from sea level to approximately 2300 m, in surface area from 10 ha to 1600 ha, and in depth from 0.8 m to 40 m. In addition to the obligatory factors, fetch (a measure of the length-to-width ratio of a lake) and lake shape data were also included as abiotic factors. The shape of the lake basins was evaluated using Reeves' method (1968) and five different shapes (subcircular, subrectangular, elliptical, irregular, and triangular) were considered. Table 2 provides the locations of the lakes and the descriptive variables used in the study.

### 2.2. Sampling

Sampling was carried out for each lake between May and September 2018 to cover the spring and autumn periods. Guideline ISO 10870:2012 (en) was taken into consideration in order to collect chironomids from the lakes. At least 2 points in lakes smaller than 500 ha, and at least 3 points in lakes of 500 ha and above were selected to represent all the habitats in the lake. The geographic coordinates of sampling sites are given in Table 2. Three repetitive samplings were made at each pelagic and littoral point determined in the lake. The chironomids were collected by using standard hand sieves and a hand net (500 µm mesh size) from the shoreline. Ekman grab was

**Table 1.** Obligatory factors, ranges, and the codes of national class boundaries for the lake water body typologies in Türkiye. F and Pr (>F) statistics are the ANOVA outputs for the significance of obligatory factors based on the results obtained from this study.

| **Obligatory factors** | **Class boundaries** | **Code** | **F**** | **Pr (>F)**** |
|---|---|---|---|---|
| Altitude | < 800 m | R1 | 1.46 | 0.045* |
| | 800–1600 m | R2 | | |
| | > 1600 m | R3 | | |
| Depth | < 5 m | D1 | 0.78 | 0.727 |
| | > 5 m | D2 | | |
| Area | < 500 ha | A1 | 0.99 | 0.465 |
| | > 500 ha | A2 | | |
| Geology | High mineralization | J1 | 0.89 | 0.594 |
| | Low mineralization | J2 | | |

** These outputs are mentioned in the relevant part of the Results section.
* Statistically significant ($p < 0.05$).

**Figure 1.** Map of lakes showing 20 lakes (L1–L20) sampled in the twenty different typologies. For the lake water body typologies, see Table 2.

used for sampling the bottom zone of the lakes. Collected samples were taken into containers filled with 70% EtOH and brought to the laboratory for sorting and identification. There are several identification keys (Vallenduuk 2017, Vallenduuk and Moller Pillot, 2002; Webb and Scholl, 1985; Webb et al., 1985; Webb and Scholl, 1990) available on *Chironomus* spp. However, these guides may also have discrepancies with some characters among themselves at the species level. In order to avoid confusion in *Chironomus* identification, Vallenduuk (2017) and Vallenduuk and Moller Pillot (2002) were used as the main identification guide in this study. Identifications have been confirmed from other cited keys mentioned above as well. Numbers were given to the species (e.g., *C.* sp1) found suspected in terms of morphological characters and identification. Morphometric measurements and evaluations were done by using ImageJ software (Schneider, 2012) on taken photos of diagnostic parts of the *Chironomus* spp.

Water temperature (pH/Cond WTW 330i), dissolved oxygen ($dO_2$) and oxygen saturation ($sO_2$) (YSI 550A),

**Table 2.** The lake localities and the descriptive variables according to national classification system. The last four columns indicate the national typological codes for each lake (e.g., Balık Lake: R3D2A2J2). See Table 1 for the ranges of the class boundaries.

| Lake | Map code | Surface area (km²) | Latitude (N) | Longitude (E) | Shape | Depth (m) | Altitude (m, asl) | Fetch | Altitude | Depth | Area | Geology |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Balık | L1 | 34 | 39°48'19.81"<br>39°46'9.15"<br>39°44'47.18" | 43°33'10.59"<br>43°34'17.19"<br>43°34'0.20" | Subrectangular | 40.00 | 2250 | 2.43 | R3 | D2 | A2 | J2 |
| Sarıot | L2 | 68.35 | 37° 6'10.09" | 32°7'23.96" | Irregular | 0.80 | 1713 | 2.02 | R3 | D1 | A1 | J1 |
| Uzungöl | L3 | 0.2 | 40°37'11.66"<br>40°37'8.14" | 40°17'44.84"<br>40°17'30.08" | Subrectangular | 15.00 | 1090 | 2.76 | R3 | D2 | A1 | J2 |
| Golcük | L4 | 0.94 | 38°18'49.19"<br>38°19'12.49" | 28°1'41.26"<br>28°1'35.80" | Subrectangular | 7.00 | 1050 | 3.25 | R2 | D2 | A1 | J2 |
| Avlan | L5 | 0.1 | 36°34'53.53"<br>36°34'50.37" | 29°56'49.44"<br>29°55'58.56" | Subrectangular | 1.00 | 1030 | 1.11 | R3 | D1 | A2 | J1 |
| Karamık | L6 | 40 | 38°25'49.03"<br>38°22'55.91" | 30°50'5.86"<br>30°45'13.55" | Subrectangular | 3.00 | 1010 | 3.22 | R2 | D1 | A1 | J1 |
| Yarışlı | L7 | 16 | 37°34'12.83"<br>37°33'4.92" | 29°58'9.75"<br>29°57'19.40" | Subcircular | 4.00 | 920 | 1.19 | R2 | D1 | A2 | J2 |
| Kovada | L8 | 7.9 | 37°37'56.73"<br>37°37'9.17" | 30°52'58.72"<br>30°53'2.86" | Subrectangular | 7.00 | 900 | 3.74 | R2 | D2 | A2 | J1 |
| Gölbaşı | L9 | 2.19 | 37°47'58.53"<br>37°47'48.29" | 37°38'43.91"<br>37°38'9.92" | Elliptical | 37.00 | 890 | 1.87 | R2 | D2 | A1 | J1 |
| Ladik | L10 | 0.87 | 40°54'23.97"<br>40°54'44.51" | 36°0'32.92"<br>35°59'26.64" | Subrectangular | 3.00 | 869 | 2.98 | R2 | D1 | A1 | J2 |
| Işıklı | L11 | 73 | 38°13'12.68"<br>38°14'59.38"<br>38°13'55.89" | 29°54'38.94"<br>29°55'36.36"<br>29°52'34.04" | Subcircular | 6.00 | 821 | 3.7 | R2 | D2 | A2 | J2 |
| Balıkdamı | L12 | 1.47 | 39°11'43.05"<br>39°11'48.91" | 31°38'17.64"<br>31°37'58.58" | Irregular | 4.00 | 802 | 4.11 | R2 | D1 | A2 | J1 |
| Manyas | L13 | 160 | 40°11'56.01"<br>40°12'29.63"<br>40°9'50.24" | 27°57'1.84"<br>28°1'53.44"<br>27°53'59.92" | Elliptical | 4.00 | 14 | 1.9 | R1 | D2 | A2 | J1 |
| Taşkısığı | L14 | 0.9 | 40°52'15.83"<br>40°52'21.84" | 30°24'7.08"<br>30°23'53.69" | Subcircular | 5.00 | 12 | 1.22 | R1 | D1 | A1 | J1 |
| Bafa | L15 | 67.08 | 37°30'6.10"<br>37°29'51.77"<br>37°29'46.28" | 27°26'25.81"<br>27°30'41.77"<br>27°23'39.57" | Elliptical | 21.00 | 10 | 2.17 | R1 | D2 | A2 | J2 |
| AkgölSa | L16 | 1.9 | 41° 3'5.29"<br>41° 2'22.84" | 30°34'1.28"<br>30°33'40.93" | Irregular | 4.00 | 10 | 3.43 | R1 | D1 | A1 | J2 |
| Gala | L17 | 5.6 | 40°46'5.51"<br>40°46'50.89"<br>40°45'26.04" | 26°11'10.18"<br>26°12'6.35"<br>26°10'18.26" | Subrectangular | 2.20 | 2 | 4.17 | R1 | D1 | A2 | J2 |
| Mert | L18 | 0.4 | 41°51'58.14"<br>41°51'45.77" | 27°58'28.60"<br>27°58'18.39" | Triangular | 3.00 | 1 | 5.15 | R1 | D2 | A1 | J2 |
| Gebekirse | L19 | 0.75 | 37°59'10.85"<br>37°58'57.21" | 27°18'15.93"<br>27°18'9.48" | Subrectangular | 5.00 | ~0 | 2.7 | R1 | D2 | A1 | J1 |
| AkgölMe | L20 | 7.9 | 36°17'45.99"<br>36°17'52.35" | 33°57'16.16"<br>33°58'20.60" | Triangular | 1.50 | ~0 | 1.71 | R1 | D1 | A2 | J1 |

m: meter, asl: above sea level.

salinity (Cond WTW 330i/set), conductivity (Cond WTW 330i/set), pH (pH WTW 330i), oxidation reduction potential (ORP) (pH WTW 330i) and total dissolved solids (TDS) (Cond WTW 330i) were all measured during the field work. Water samples for the chemical analysis were taken from the top 30 cm of the water column at the pelagic zone and transferred directly into the polyethylene bottles (1 L) and kept in the cold storage cabinet till transported to the laboratory for processing. The water samples taken to the laboratory were filtered and analysis of the inorganic compounds ($NO^{-3}$–N, $NO^{-2}$–N, $PO_4$–P, $Fe^{+3}$, $NH^{+4}$–N, Mg, $Ca^{2+}$, Cl) was done using the spectrophotometric method (Hach–Lange DR 2800).

### 2.3 Data analysis

Two main data sets were prepared for this study, one including species and the other including environmental variables. The numerical expressions of environmental variables were standardized using the scale function in R (R Core Team, 2013) to avoid the influence of various quantitative units. To reveal the biodiversity of *Chironomus* spp. among lakes, abundance data were analyzed using Shannon-Weaver (H') diversity (1949) and Pielou's (J) (1966) evenness indices. The quantitative outputs yielded from these indices (H` and J) were included in the environmental data set for further analysis such as correlation and multiple regression. To calculate relative species abundance, the number of species in a sampled lake was divided by the cumulative number of species from all sampled lakes and stored as a percentage expression in the dataset.

The relationship between environmental variables was revealed using a Pearson parametric correlation test. The correlation matrix was visualized using the corrplot package (Wei and Simko, 2021) in R. Multiple regression analysis (MRA) was used to explore the impacts of lake morphometry (surface area, depth, fetch, altitude, and lake shape), physicochemical parameters on species diversity.

The forward and backward algorithms, which are known as stepwise modelling and are formula-based models, were used to select significant factors at a 95% confidence interval in the regression analysis, based on the Akaike information criterion (AIC). As a prerequisite, assumptions of regression analysis were checked for normality and homogeneity of variance. Variation inflation factor (VIF) was also checked for possible collinearity among the variables. The vegan library (Oksanen et al., 2020) was used for the ordination analyses in R. Stress (S) which is a statistic of the goodness of fit was used as a pretest to see ordination distances against the community dissimilarities. Subsequently, the correlation obtained from the S was then used to get the nonmetric and linear fittings.

Due to the limited number of species and genus studied, rare species could potentially cause a significant gradient in the ordination analysis. As a result, detrended correspondence analysis was initially applied to account for this possibility before using constrained methods, with CCA being the preferred method to reveal the relationship between species, sampling sites, and environmental variables. To reduce the number of constraints in CCA, the stepwise method and VIF of the environmental variables were used, and the AIC was employed with permutation tests at each step. Then, a constrained ordination plot was generated by using selected parameters. The study aimed to determine if *Chironomus* could be used in regionalization studies; therefore, the K-means clustering algorithm was applied to site scores (weighted averages of species scores) of CCA1 and CCA2 to identify groups in the data space. The broken stick model determined the number of groups, which were then plotted on the CCA ordination plot.

It is common knowledge that certain factors, including altitude, surface area, geology, and mean depth of a lake, are used to determine the typology and boundaries of different lake water bodies, as outlined in Table 1. In order to understand how grouping lakes according to their known typology affects them, separate canonical correspondence analyses (CCA) were conducted for each obligatory factor. The outputs from the multiple regression analysis and ordination analysis were subject to analysis of variance (ANOVA), and the resulting F statistical values were tested with a 95% confidence interval.

## 3. Results

### 3.1 Biodiversity

In this study, efforts were made to identify *Chironomus* spp. in all sampled lakes. A total of 24 different *Chironomus* taxa were identified among 1128 individuals, and their taxon-specific metrics and presence-absence data can be found in Table 3. The relative abundance of species varied across lakes, with *Chironomus muratensis*, *C. balatonicus*, and *C. salinarius* being the most abundant and prevalent species, accounting for 19.24%, 17.20%, and 15.96% of total individuals, respectively. Some of the lakes, such as Sarıot L. (L2), Gölcük L. (L4), Manyas L. (L13), Taşkısığı L. (L14), Bafa L. (L15), and Akgöl L. (L20), were represented by only a single *Chironomus* taxon. Certain *Chironomus* species were found in only one lake, including *C. annularius*, *C. cingulatus*, and *C. vallenduuki* from Mert L. (L18); *C. melanescens*, *C.* sp1, and *C.* sp3 from Balık L. (L1); *C. nippodorsalis* from Sarıot L. (L2); and *C. riihikimiensis* from Karamık L. (L6). The highest richness was observed in Balık L. (L1), where seven *Chironomus* species (*C.* sp1, *C.* sp3, *C. melanescens*, *C. bernensis*, *C. alpestris*, *C. riparius*, *C. luridus*) were found. Mert L. (L18) had the highest value (H'= 1.75) for the Shannon-Wiener diversity index, while Gölbaşı L. (L9) had the highest value (J = 1.00) for the Peilou's evenness index. Notably, *Chironomus* larvae have not been reported in Yarışlı L. (L7), which has brackish water (18‰) characteristics.

### 3.2 Abiotic factors and environmental variables

At broad scales, lake biota is influenced by a range of abiotic factors and environmental variables. Table 4 presents descriptive statistics for the environmental parameters of the lakes, which vary widely in surface area,

**Table 3.** Taxa specific metrics and presence-absence data. The index values of the lakes represented with a single taxon are given as N/A.

| **Lakes** | **Balık L.** | **Sarıot L.** | **Uzungöl L.** | **Gölcük L.** | **Avlan L.** | **Karamık L.** | **Yarışlı L.** | **Kovada L.** | **Gölbaşı L.** | **Ladik L.** | **Işıklı L.** | **Balıkdamı L.** | **Manyas L.** | **Taşkısığı L.** | **Bafa L.** | **Akgöl (Sakarya) L.** | **Gala L.** | **Mert L.** | **Gebekirse L.** | **Akgöl (Mersin) L.** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MapCode** | **L1** | **L2** | **L3** | **L4** | **L5** | **L6** | **L7** | **L8** | **L9** | **L10** | **L11** | **L12** | **L13** | **L14** | **L15** | **L16** | **L17** | **L18** | **L19** | **L20** | **Abundance** |
| *C. annularius* Kieffer, 1926 | | | | | | | | | | | | | | | | | | + | | | **0.89** |
| *C. aprilinus* Meigen, 1830 | | | | | | | | | | | | + | | | | | | | + | | **6.21** |
| *C. balatonicus Devai, Wulker and Scholl, 1983* | | | + | | + | + | | + | + | + | + | | | + | | + | | | | | **17.20** |
| *C. bernensis* Wülker and Klöztli, 1973 | + | | + | | | | | | | | | | | | | | | | | | **1.15** |
| *C. cingulatus* Meigen, 1830 | | | | | | | | | | | | | | | | | | + | | | **1.15** |
| *C. acerbiphilus* Tokunaga, 1939 | | | | | + | | | | | | + | + | | | | | | | | | **0.98** |
| *C. alpestris* Goetghebuer, 1934 | + | | | | | | | | | + | | | | | | | | | | | **2.39** |
| *C. luridus* Strenzke, 1959 | + | | + | | | + | | + | | | | + | | | | + | + | + | | | **7.18** |
| *C. melanescens* Keyl, 1962 | + | | | | | | | | | | | | | | | | | | | | **1.15** |
| *C. muratensis* Ryser and Scholl, 1983 | | | | + | + | | | | + | + | | | + | | | + | + | | | | **19.24** |
| *C. cf nippodorsalis* Sasa, 1979 | | + | | | | | | | | | | | | | | | | | | | **2.22** |
| *C. nuditarsis* Keyl, 1961 | | | | | + | | | | | | + | + | | | | + | | | | | **6.83** |
| *C. plumosus* Linnaeus, 1758 | | | + | | | | | | | | | | | | | + | | | | | **1.86** |
| *C. pseudothummi* Strenzke, 1959 | | | | | | | | | | | | + | | | | | + | + | | | **4.52** |
| *C. riihimakiensis* Wülker, 1973 | | | | | | + | | | | | | | | | | | | | | | **0.62** |
| *C. riparius* Meigen, 1804 | + | | + | | | + | | | | + | | | | | | + | | | | | **4.70** |

**Table 4.** Descriptive statistics of the environmental variables. Relevant lake codes are given in parentheses.

| Parameters | Min–Lake | Max–Lake | Mean | SD |
|---|---|---|---|---|
| **Surface area (km$^2$)** | 0.1 (L5) | 160 (L13) | 24.48 | ±40.59 |
| **Fetch** | 1.11 (L5) | 5.15 (L18) | 2.74 | ±1.11 |
| **Depth (m)** | 0.8 (L2) | 40 (L1) | 8.68 | ±11.27 |
| **Altitude (m)** | Sea level (L19, L20) | 2250 (L1) | 669.70 | ±645.63 |
| **Salinity (‰)** | 0.00 (L1-L6, L8-L11, L13, L14, L16) | 18.5 (L7) | 2.62 | ±5.30 |
| **Water temperature (°C)** | 7.5 (L6) | 33.3 (L15) | 23.56 | ±8.13 |
| **$dO_2$ (mg/L)** | 1.51 (L16) | 9.98 (L13) | 6.92 | ±2.00 |
| **Conductivity (μS/cm)** | 30 (L2, L7) | 8450 (L19) | 1284.76 | ±2331.01 |
| **pH** | 6.61 (L3) | 10.59 (L13) | 8.57 | ±0.98 |
| **$sO_2$ (%)** | 19.4 (L16) | 131 (L13) | 83.90 | ±26.83 |
| **TDS (mg/L)** | 110 (L3) | >2000 (L2, L7, L15, L17-L20) | 1995.53 | ±2270.69 |
| **ORP (mV)** | –224 (L13) | 40 (L6) | –98.60 | ±64.66 |
| **$PO_4$ (mg/L)** | 0.08 (L12) | 3.84 (L4) | 1.04 | ±1.25 |
| **Fe (mg/L)** | <0.02 (L6) | 0.55 (L13) | 0.09 | ±0.12 |
| **$NO_3$ - N (mg/L)** | 0.1 (L7) | 3.7 (L13) | 0.89 | ±0.77 |
| **$NO_2$ - N (mg/L)** | <0.002 (L6, L7, L11, L12, L17, L18) | 0.04 (L16) | 0.01 | ±0.01 |
| **Mg (mg/L)** | <0.05 (L11, L12, L17) | 3.46 (L5) | 1.66 | ±1.24 |
| **Ca (mg/L)** | <0.05 (L2, L3, L5, L9, L12, L13, L20) | 9.87 (L17) | 1.03 | ± 2.43 |
| **$NH_4$ - N (mg/L)** | <0.02 (L12) | 2.68 (L15) | 0.45 | ± 0.68 |
| **Cl (mg/L)** | <0.1 (L3, L9) | 455.3 (L19) | 80.89 | ± 135.85 |

Min: minimum value, Max: maximum value, SD: standard deviation, $sO_2$: saturated oxygen, $dO_2$: dissolved oxygen, ORP: oxidation and reduction potential, TDS: total dissolved solids.

fetch, depth, and altitude. These basin-related parameters are critical, as they affect the physicochemical properties of water and biodiversity, irrespective of the water's chemical properties. The smallest lake in terms of surface area was L5, which measured 0.1 km$^2$, while the largest was L13, with a surface area of 160 km$^2$. L5 had the smallest fetch value of 1.11, while L18 had the highest fetch value of 5.15. The shallowest lake was L2, with a depth of 0.8 m, and the deepest was L1, with a depth of 40 m. Two lakes, L19 and L20, were at sea level, while the lake at the highest altitude was L1, at 2250 m. To account for the correlation between environmental parameters, the dataset included results for the Shannon-Weaver and evenness indices (H', J). A correlation plot of the Pearson analysis was provided in Figure 2.

A negative correlation was observed between several inorganic parameters, including $PO_4$, salinity, $NH_4$–N, pH, and water temperature, and the expected decrease in biodiversity. Furthermore, a strong negative correlation was discovered between pH and ORP. Similarly, a strong negative correlation was observed between the altitude parameter and $NH_4$–N, salinity, TDS, conductivity, chloride, and water temperature. Positive correlations were found between salinity, TDS, and chloride, as expected. The multiple regression model identified fetch, altitude, salinity, water temperature, dissolved oxygen, conductivity, oxygen saturation, $PO_4$, Fe, and $NH_4$–N as significant parameters ($p < 0.05$) in explaining the species diversity of *Chironomus* spp. (refer to Table 5). In the regression model, the effect of the remaining parameters on species diversity was found to be insignificant.

### 3.3. Ordination analysis

The $r^2$ values for nonmetric and linear fits, which measured the association between ordination distance and observed dissimilarity, were found to be 0.967 and 0.821, respectively. DCA calculated a gradient length of 6.31, indicating a very long gradient on the first axis due to the presence of rare species. CCA was then performed using a best-fit model

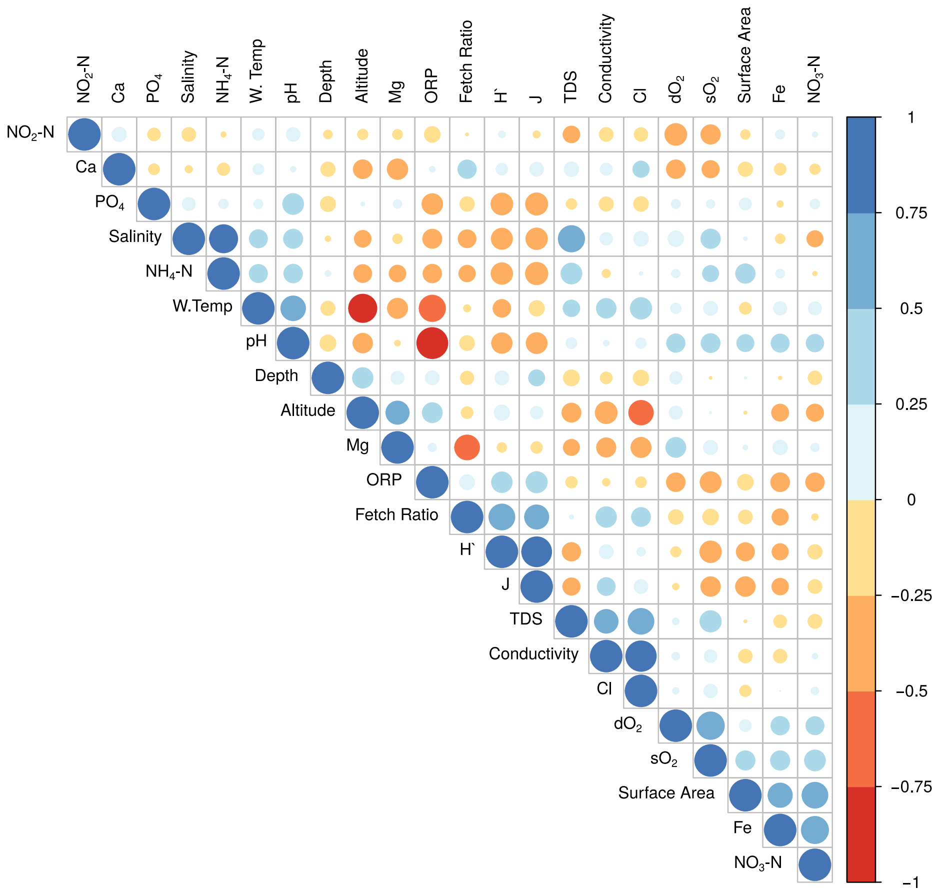

**Figure 2.** Pearson's correlation heatmap of the 20 environmental variables and 2 taxa metrics (H` and J). Color intensity and the size of the circle are proportional to the correlation coefficients: red spectrum indicates a negative correlation, and blue spectrum indicates a positive correlation.

and VIF on selected parameters (fetch, altitude, dissolved oxygen, salinity, water temperature, $PO_4$, Cl, and $NH_4$–N). With these parameters, the constrained model explained 56.62% of the total variance (axis1: 32.35%, axis2: 24.27%) as depicted in Figure 3. Significant correlations were observed between salinity, $PO_4$, and $NH_4$–N with the first axis, whereas fetch, dissolved oxygen, and altitude showed correlations with the second axis. Water temperature, on the other hand, had a positive correlation with both axes 1 and 2. The first axis highlighted the urban and anthropogenic impacts, with high levels of phosphate and ammonium salts being notable factors. The second axis, however, was mainly influenced by the basin and topographical characteristics of the lake. Among these parameters, fetch was particularly notable, as it is a topographical feature with minimal anthropogenic impact.

Therefore, it is possible to understand the distribution of certain *Chironomus* species on the ordination. For example, *C. salinarius*, which is linked to salinity, is prominent in L15, L19, and L20, but it has not been found in Mert L. (L18). Conversely, some species (*C. annularis, C. cingulatus, C. vallenduuki*) have only been observed in Mert L. (L18), where topological features such as fetch play a more significant role. The ordination analysis successfully classified the *Chironomus* assemblages into four major groups of lakes. Group–I consists of L15, L19, and L20, which are characterized by high salinity (9.40‰ ± 4.8) and $NH_4$–N (1.40 mg/L ± 1.29) concentrations. *C. salinarius* is identified as an indicator species in this group. Group–II comprises L12, L17, and L18, where a strong positive correlation was established with fetch (4.48 ± 0.58). Group–III includes L4, L6, L8, L9, L13, and L16, which are positively correlated with the altitude parameter (669.70 m ± 645.63). Group–IV consists of L1, L3, L5, L10, L11, L14, which are relatively rich in $PO_4$ (1.28 mg/L ± 1.37) and negatively correlated with salinity (0‰ ± 0.0) and $NH_4$–N (0.21 mg/L ± 0.33).

### 3.4 Typological factors

As previously mentioned, the lake water body typologies (LWBT) are expected to be characterized by four major criteria based on System A, namely altitude, surface

**Table 5.** Model outputs of linear regression model and constrained ordination to explore the significant variables on *Chironomus* spp. diversity. A negative t-value indicates a reversal in the directionality of the effect. The Pr(> \|t,F\|) acronym found in the model output relates to the probability of observing any value equal to or larger than t and F.

| | | **Multiple linear regression model** | | | | | **Constrained ordination** | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Model output** | | **ANOVA of the results** | | **Significant factors selected by AIC model in MLR** | **Model output** | | **Significant factors selected by AIC model in CCA** |
| | | **t-value** | **Pr(>\|t\|)** | **F-value** | **Pr(> \|F\|)** | | **F-value** | **Pr(> \|F\|)** | |
| **Lake morphometry** | **Surface area** | 0.102 | 0.920 | 3.340 | 0.095 | | 0.677 | 0.850 | |
| | **Shape (irregular)** | 0.957 | 0.359 | 1.057 | 0.422 | | 0.759 | 0.875 | |
| | **Shape (subcircular)** | 0.913 | 0.381 | 1.057 | 0.422 | | 0.759 | 0.875 | |
| | **Shape (Subrectangular)** | 1.08 | 0.303 | 1.057 | 0.422 | | 0.759 | 0.875 | |
| | **Shape (triangular)** | 0.953 | 0.361 | 1.057 | 0.422 | | 0.759 | 0.875 | |
| | **Fetch** | 2.809 | 0.017 | 8.783 | 0.013 | * | 2.014 | 0.010 | * |
| | **Depth** | 1.469 | 0.170 | 4.482 | 0.057 | | 1.249 | 0.290 | |
| | **Altitude** | – 0.034 | 0.974 | 0.001 | 0.973 | * | 1.986 | 0.010 | * |
| **Physicochemical parameters** | **Salinity** | – 1.287 | 0.008 | 6.54 | 0.022 | * | 2.063 | 0.020 | * |
| | **Water temperature** | – 1.514 | 0.152 | 0.894 | 0.363 | * | 1.922 | 0.010 | * |
| | **Dissolved $O_2$** | 2.168 | 0.048 | 0.002 | 0.961 | * | 0.269 | 0.980 | |
| | **Conductivity** | 2.316 | 0.036 | 3.520 | 0.085 | * | 0.423 | 0.915 | |
| | **pH** | – 1.679 | 0.121 | 0.481 | 0.5 | | 1.205 | 0.270 | |
| | **Saturated $O_2$** | – 2.925 | 0.011 | 8.554 | 0.011 | * | 0.343 | 0.965 | |
| | **TDS** | 0.755 | 0.466 | 0.001 | 0.965 | | 2.000 | 0.015 | |
| | **ORP** | – 1.601 | 0.137 | 2.564 | 0.137 | | 1.418 | 0.150 | |
| **Inorganic parameters** | **$PO_4$** | – 1.946 | 0.077 | 6.361 | 0.022 | * | 0.962 | 0.550 | |
| | **Fe** | –0.542 | 0.598 | 2.186 | 0.158 | * | 1.159 | 0.345 | |
| | **$NO_3$-N** | –0.049 | 0.961 | 0.092 | 0.766 | | 1.161 | 0.330 | |
| | **$NO_2$-N** | –0.199 | 0.845 | 0.002 | 0.969 | | 0.626 | 0.800 | |
| | **Mg** | –0.758 | 0.464 | 0.002 | 0.963 | | 1.032 | 0.370 | |
| | **Ca** | –0.289 | 0.777 | 0.048 | 0.829 | | 0.670 | 0.770 | |
| | **$NH_4$-N** | –1.919 | 0.081 | 4.525 | 0.049 | * | 2.090 | 0.035 | * |
| | **Cl** | –0.377 | 0.713 | 0.141 | 0.713 | | 1.876 | 0.045 | * |

* statistically significant ($p < 0.05$).
ORP: oxidation and reduction potential, TDS: total dissolved solids, MLR: multiple linear regression.

area, geology, and mean depth of the lake. One of the objectives of this research was to investigate whether there is a significant variation in the distribution of *Chironomus* spp. based on the class boundary values (R1: <800, R2: 800–1600, R3: >1600 m) of these typological factors in local lakes in Türkiye. Among these typological factors, only altitude class boundary data (R1, R2, R3) consistently provided a statistically significant distinction. The CCA

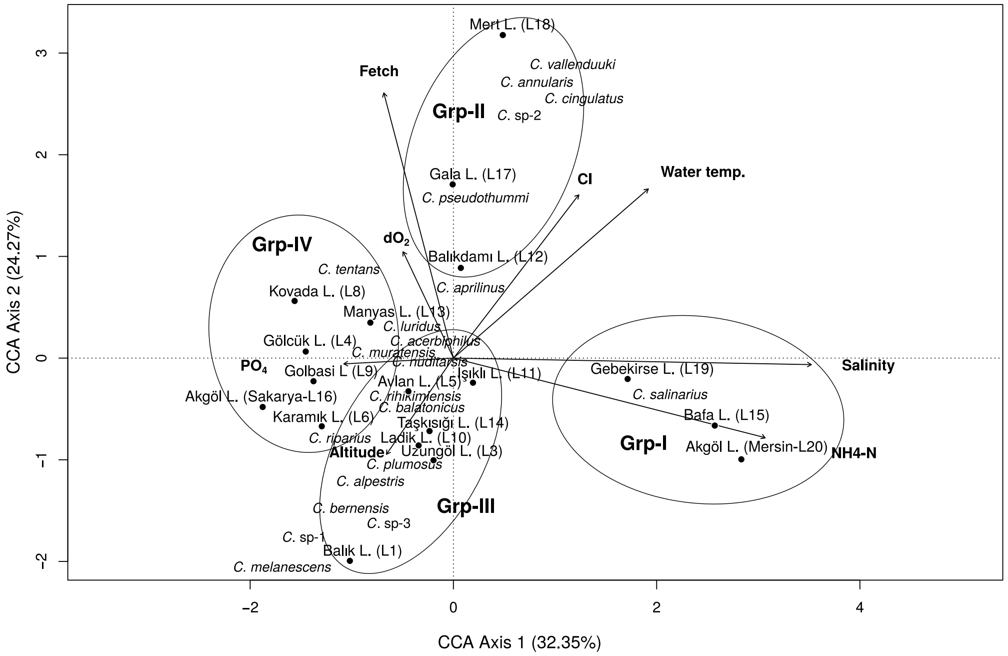

**Figure 3.** CCA ordination of the sampled lakes (L1 to L20, except L2), significant environmental variables and species. Environmental variables ranging over (VIF > 10) were omitted from the ordination. A total of 56.62% variance was expressed in the first two axes. Groups (Grp I–IV) spotted by using K-means algorithm were mapped on the biplot. ORP: oxidation and reduction potential.

ordination considering the class boundary values showed that the first two axes explained 62% of the variance (Figure 4). The significance of altitude class boundary was further confirmed by a permutation test for CCA under the constraint model, and the model was found to be significant ($p < 0.05$) in terms of altitude. However, there was no significant ordination observed for the other typological factors (surface area, geology, depth) and their class boundaries. The ANOVA results for the typological factors are provided in Table 1.

## 4. Discussion

### 4.1 Biodiversity and ordination analysis

The goodness of fit values for both the nonmetric and linear fits were high ($r^2$ values of 0.967 and 0.821, respectively), providing a good starting point for further analysis. It was important to understand the length of the gradient in order to avoid the arch effect, which was achieved using constrained correspondence analysis (CCA), as recommended by Šmilauer and Lepš (2014). The length of the gradient was found as 6.31 according to DCA. According to the recommendations of the Water Framework Directive (WFD, 2003), lakes were allocated based on physicochemical parameters and supporting biological components. The restricted model was able to explain 56.62% of the total variance.

Gebekirse L. (L19), Akgöl L. (Mersin) (L20), and Bafa L. (L15) that was formerly a bay of the Aegean Sea, which are located in Group–I, are brackish waters due to their permeable topographic structure and are somehow connected with marine water. Due to their high mineral content and the absence of intense freshwater sources feeding the lakes, they were solely represented by the species *C. salinarius*. In recent studies, it has been reported that the ammonium values of these lakes have been increased (Minareci and Sungur, 2019; Küçüksümbül et al., 2020; Şengörür and Demirel, 2002). Nutrient flows into Group–I may be higher in muddy sediment with higher organic matter and interstitial water content (Fukuhara and Sakamoto, 1988). Mert L. (L18), which is in Group–II, is also quite close to the seashore line (5 m), and it is fed by freshwater from İğneada floodplain forests. Similarly, located near the seaside (10 km), Gala L. (L17) is fed by the drainage canal (Basamaklar Stream), Kızkapan Stream, and other small tributaries. Balıkdamı L. (L12) is located 70 km away from the Sakarya river and its main feeding source is Sakarbaşı springs. Mert L. (L18) and Gala L. (L17) lakes, due to their connections with the sea, and Balıkdamı L. (L12), due to its location in rich mineral soils (Yeniyol,

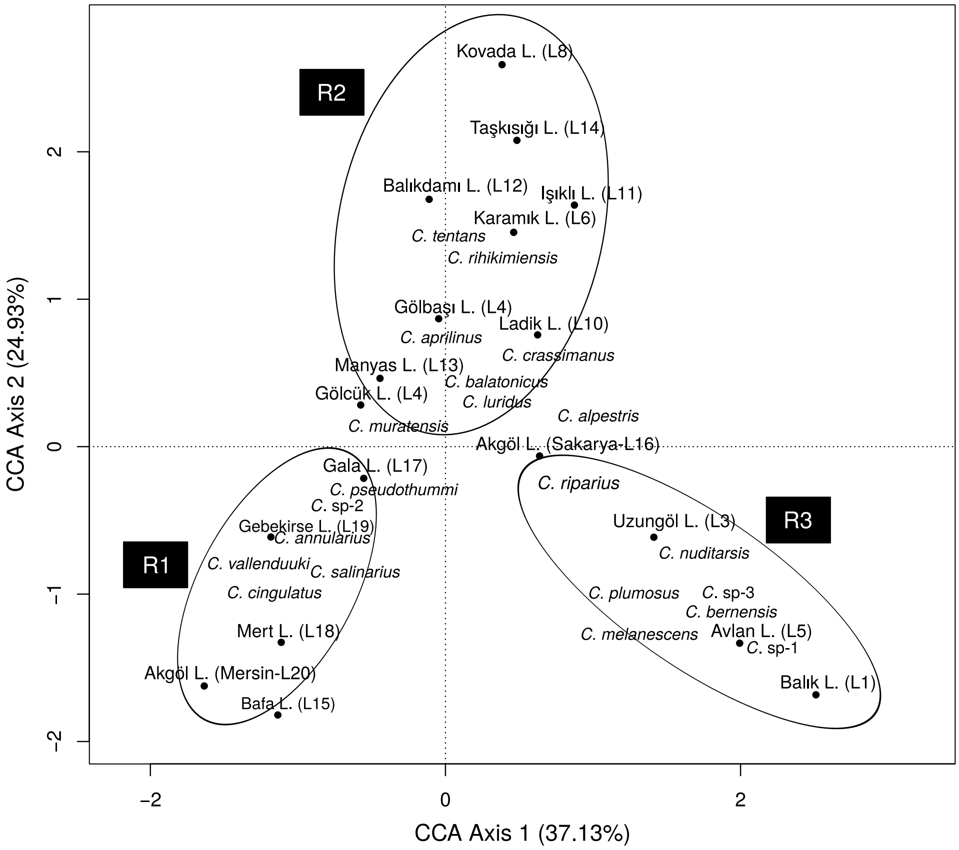

**Figure 4.** CCA ordination of the sampled lakes (L1 to L20, except L2) based on national altitude class boundaries (R1: <800 m, R2: 800–1600 m, R3: >1600 m). A total of 62.06% variance was expressed in the first two axes. The distinction of the three groups was found to be statistically significant ($p < 0.05$). For the significance levels of the typological factors, see Table 1.

1992), are slightly saline lakes. It has been determined that the lakes in Group–II have more species diversity (*C. uliginosus*, *C. annularius*, *C. cingulatus*, *C. pseudothummi*) than Group–I lakes (*C. salinarius*) due to their estuary habitat characteristics. Lakes (L1, L3, L5, L10, L11, L14) in Group–III have been found associated with ORP. The lakes (L10, L11, L14) related to high $PO_4$ concentrations and relatively close to Group–IV are known with their high trophic levels (LWAP 2017). The chronology of Avlan L. (L5) is quite unfortunate. The lake was dried between the years 1975–1980. In 2001, the lake water gates were closed, and water began to be retained. The $PO_4$ value (0.53 mg/L) of the lake, which has a very low water holding capacity, was measured relatively high. Avlan L. (L5) which is considered eutrophic can be classified in a separate group with Ladik (L10), Işıklı (L11) and Taşkısığı (L14) lakes. Balık L. (L1), which is located in a separate position in Group–III, is one of the highest lakes (2241 m) in Türkiye. This lake is the second one that has been exposed to the least $PO_4$ load in the group due to the lack of urbanization and agricultural lands around it. Uzungöl L. (L3), which is born from Haldizen Mountains and fed by spring waters, has the least $PO_4$ load in Group–III. This lake was also the only one in the group with a positive ORP value (+ 9 mV). It has been noticed that the lakes (L4, L6, L8, L9, L13, and L16) in Group–IV were related to $PO_4$ (see Figure 3). *C. muratensis*, which is speciation within the *plumosus* group (Michailova and Fischer, 1986), was found prominent in Group–IV and *plumosus* group is known to be associated with eutrophication (Saether, 1979; Brennan and McLachlan, 1979; van Hardenbroek et al., 2011; van Nes and Smit, 1993; Smit et al., 1992; Svensson, 1997). Furthermore, it was reported in the study of Smit et al. (1992) which was held in Wolderwijd Lake that the larval tubes of *C. muratensis* were fragile and their density increased in the sheltered parts of the lakes that were not exposed to the wind. This observation also emphasizes the significance of fetch. *C. muratensis* could not be detected only from Kovada L. (L8) and Karamık L. (L6) lakes in Group–IV. While Kovada L. (L8) was associated with *C. tentans*, Karamık L. (L6) was associated with *C. riparius*. *C. tentans* and *C. riparius* are often referred to as indicators of organic pollution in the literature (Paine and Gaufin, 1956). *C. muratensis*, *C. riparius*, *C. tentans*, high $PO_4$ concentration, and eutrophic and hypertrophic states can be considered as the forces that gathered all these lakes in one group.

### 4.2. Abiotic factors and environmental variables

An abiotic element refers to the nonliving constituents that shape the environment of an ecosystem. Along with abiotic factors, environmental variables have a great impact on the habitat preferences of benthic macroinvertebrates (Nyman et al., 2005; Rae, 2013; Everaert et al., 2014). However, analyzing numerous parameters can make interpreting results, such as ordination, challenging, and ultimately lead to the same conclusion as using no data at all (Lepš and Šmilauer, 2003). Abiotic factors and environmental parameters were assessed and categorized into three essential elements, namely climate, hydrology, and geology, as proposed by the WFD (2003, 2009). Nonetheless, the directive provides little clarity regarding which factors to include and the boundary limits for each factor (Wallin et al., 2003; Søndergaard, 2005). One of our primary objectives was to determine the factors that impact biodiversity. In the first step, we utilized the results of multiple regression modelling to identify the factors that satisfied this objective. The fetch, altitude, salinity, water temperature, dissolved oxygen, conductivity, oxygen saturation, Fe, $PO_4$, and $NH_4$–N emerged as the primary determinants of biodiversity. As a major abiotic component, altitude is already recognized to have a significant effect on benthic macroinvertebrate assemblages and vegetation changes (Jacobsen, 2003; Scheibler et al., 2014). According to the WFD (2000, Annex II), altitude is a mandatory descriptor while temperature is an optional one. High altitude lakes usually have low vegetation density due to their location on nonsoluble geological formations. Compared to lakes at lower altitudes, high altitude lakes are less prone to agricultural and wastewater contamination, as per research by Fureder et al. (2006) and Feret (2017). The fetch is an essential parameter that determines the height of waves for a given wind speed and aids water oxygenation, but there may not be a clear relationship between fetch and oxygen parameters due to various factors (Blottiere, 2015). The quality of water bodies depends on the amount of dissolved oxygen that it contains (Tamburrino and Martinez, 2017). This interaction might be suppressed by a variety of reasons. While the correlation matrix was a useful tool in this study to identify links between factors, it does not imply causation (Senthilnathan, 2019). However, a positive correlation between fetch and biodiversity was clear in the matrix. Including the Shannon and evenness indices' results in the correlation matrix and directly observing the relationship between those parameters was very unique and useful for this research.

### 4.3. Typological factors

According to the Water Framework Directive (WFD), research on lake type assessments should use both "System A" and "System B." "System A" has mandatory factors that cannot have their threshold values modified, while "System B" has optional factors with adjustable threshold values based on the basin's structure. Although both "System A" and the national classification systems in Türkiye have the same descriptors, they differ in terms of class boundaries. In Annex II of the WFD, altitude ranges are categorized as lowland (<200 m), midaltitude (200–800 m), and high (>800 m). However, in Türkiye, these class boundaries are <800 m, 800–1600 m, and >1600 m. This study found altitude to be a significant descriptor, with mean values of 2.6, 707.5, and 1095 m for the R1, R2, and R3 groups, respectively. However, lake depth, lake surface area (catchment size), and geology did not differ significantly in terms of class boundaries of the national classification system and measurements obtained in this study (Table 1). The lake depth is defined in two categories as <5 m and >5 m in the national classification system. It is given as <3 m and >3 m in the WFD. In both cases, the depth parameter is not expected to show a significant distribution. The importance of depth is actually related to stratification. However, there is no definite criterion for stratification in the national classification system. In addition to the depth ranges specified in the WFD, there are nonstratified and stratified criteria are also included. The boundaries of the lake surface area are specified as <5 $km^2$ and >5 $km^2$ in the national classification system while it is categorized as very small (0.5 $km^2$), small-large (0.5–100 $km^2$), and very large (> 100 $km^2$) according to the WFD. However, no significant difference was found in terms of lake surface area in both the national classification system, and ordination analysis, and GLM analysis applied within the scope of this study. Fetch parameter plays a key role here which is directly related to the oxygen concentration of the lake as well as the lake surface area (Blottiere, 2015; Tamburrino and Martínez, 2017). As it is known, the selected parameters can explain the natural diversity of biological components; it should consist of abiotic components that support the structure of the riparian zone, oxygen status, permeability, and nutrient status (Sharma and Rathore, 2017; Solheim et al., 2019). The geological structure of the lake is classified as high and low mineralization in the national classification system with a purely qualitative approach. In the WFD, it is seen that the geological structure is divided into several geochemical categories such as siliceous, calcareous, and organic. However, this distinction is also not very helpful in execution due to these geochemical categories in the country database covering much larger areas than lakes and it does not give the exact geochemical structure of the lake. For this reason, it is useful to use quantitative data of water chemistry to reflect the geology of the lake itself. In fact, the alkalinity value can be used to determine the geochemical structure of water (Solheim et al., 2019), and of course, the pH value can also be used as an auxiliary element in determining the alkalinity. The salinity parameter can also be used as quantitative data in terms of geochemical structure, as it may originate from lithostructures in such aquatic sources that do not show coastal or transitional water characteristics.

## 5. Conclusion

There were three questions to be answered in this research and the first concerns the regionalization of the basins. The results of the ordination analysis revealed that *Chironomus* larvae performed well in grouping the lakes on their typological characteristics in Türkiye. In this context, *Chironomus* larvae can also be used in reference site determination studies. Chironomid larvae can be found in a variety of habitats, including streams and lakes. Moreover, as a member of benthic macroinvertebrates, Chironomid larvae are a key component of the benthic ecosystem, and we sought to extend this perspective at the species level. Another aspect we touched upon in this study was how *Chironomus* larvae would respond to ranges of the current national class boundary ranges, and *Chironomus* larvae were shown to have a significant ($p < 0.05$) distribution with altitude, which is one of the four criteria utilized in the national class boundaries. However, we suggest that the class boundaries of the altitude (see Table 1) in the national class boundaries should be revised. There was no significant variation in depth, lake area and mineralization. Despite the importance of these typological factors, it has been noted that the class boundaries did not work well in the ordination analysis and should definitely be revised in future studies. In fact, *Chironomus* larvae are indicators of depth as they are well able to spread in the profundal zone. It is obvious that the 5-m threshold given in the national class boundaries was insufficient. The same notion stands true for mineralization criteria. The class boundaries of mineralization contain qualitative concepts, whereas quantitative standards would be more effective. However, considering geochemical characteristics such as siliceous, calcareous, and organic may present some issues for lake beds. These assessments are made using GIS programs; however, lake beds are blind spots in this perspective. As a result, interpolation produced by GIS applications would not present a satisfying outcome. Therefore, lake beds should be divided into transects, and physical evidence such as sediment cores should be used to reveal the geomorphological structure of the lake bed. Finally, neither the ordination analysis of the research data nor the boundaries of the lake typology yielded a significant result for the lake area. Instead, the fetch parameter was found to be far more useful and the use of fetch data in 'System B' could be quite effective.

**Acknowledgments**
This research was supported by Pamukkale University, Scientific Research Projects Coordination Unit (2018FEBE028).

**Funding**
This research was funded by Pamukkale University, Scientific Research Projects Coordination Unit (2018FEBE028).

**Availability of data and material**
The datasets generated during the current study are not publicly available due to it belongs to a PhD project (Project No: 2018FEBE028–Pamukkale University, Scientific Research Projects Coordination Unit) that is not open access yet but are available from the corresponding author on reasonable request.

**Conflict of interest**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Consent to participate**
All the participants voluntarily agreed to participate in the study on which the present manuscript is based.

**Consent for publication**
All the authors gave their consent to publish the present manuscript in Turkish Journal of Zoology.

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