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Can Plant-Associated Chironomids Be Used as an Indicator of Lake Status with the Alternative States Theory?

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Abstract: Shallow lakes according to the alternative states theory may present extremely different environmental conditions, clear water with abundant growth of macrophytes and turbid water with cyanobacterial blooms. The deterioration of water quality led to visible changes within submerged macrophytes and thus in available habitats for plant-associated biocenosis. Larvae of chironomids are the most numerous and widely distributed macroinvertebrates. Since benthic chironomids are used in the monitoring of environmental changes and in paleolimnological research, epiphytic chironomids are not well known in this regard. The larvae can be used as indicators of lake macrophyte status. The present study focuses on plant-associated chironomids of a group of ten shallow lakes of the Polesie region (eastern Poland). The lakes were classified with alternative states theory as macrophyte-dominated (MD), phytoplankton–macrophyte-dominated (PMD) and phytoplankton-dominated (PD). The domination structure of epiphytic chironomids showed significant changes between lake types, with the highest abundance of *Paratanytarsus austriacus* in MD lakes, *Endochironomus albipennis* in PMD lakes and *Cricotopus* sp. (gr. *sylvestris*) in PD lakes. The highest mean density was noted in PD lakes while the highest species diversity (values of Shannon–Wiener index) in PMD lakes. Moreover, environmental variables (macrophyte biomass, Secchi disc depth, chlorophyll-*a* and TP) differentiating lake types were used in RDA analysis to evaluate their effect on chironomid taxa distribution. Next, the variables with a significant effect on specific chironomid taxa were used in multivariate regression analysis. The results led to the creation of a model of distribution of chironomid taxa with regard to lake type.

Keywords: chironomids; bioassessment; shallow lakes; alternative states; environmental drivers

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1. Introduction

Temperate shallow lakes are often considered as an example of well-documented alternative states, clear with abundant submerged macrophytes or turbid with few submerged plants [1]. External nutrient loading from anthropogenic sources, mostly agriculture [2,3], promotes high water turbidity and phytoplankton growth. Anthropogenic eutrophication still is one of the major threats to inland waters. Potential effects of excessive inputs of phosphorus include the increase in the biomass of phytoplankton, shift to bloom-forming cyanobacteria species that might be toxic or inedible, increase in the biomass of benthic and epiphytic algae and a change in species composition of macrophytes [4,5]. These effects may be closely related to climate change. Rising nutrient inputs and increasing temperatures tend to intensify eutrophication symptoms, such as cyanobacterial dominance, predominance of floating plants and, in extreme cases, complete loss of submerged vegetation. These changes are observed as temperature increases even at lower nutrient concentrations [6,7].

The combination of all impacts will lead to severe and significant changes in the trophic structure in lakes, and thus may indirectly enhance the risk of turbid conditions [8]. The switch from clear into turbid conditions causes changes in the submerged macrophytes' structure and cover [9,10]. The deterioration of water transparency and decrease in macrophyte biomass affect habitats and the colonization area for plant-associated macroinvertebrates and further led to visible changes in their density and dominance structure.

One of the most widespread groups of macroinvertebrates in freshwater ecosystems are larvae of non-biting midges (Diptera, Chironomidae) [11–13]. Larvae can exhibit a great variety of ecological traits and, as such, chironomids are widely regarded as effective indicators of water quality and changes in environmental conditions, such as substrate type, water temperature, conductivity, salinity, lake depth, oxygen concentration and sediment organic matter content. These species–environment relationships were confirmed by research conducted, among others, by Canedo-Arguelles et al. [14], Eggermont and Heiri [15], Nandi et al. [16] and Rossaro et al. [17]. Moreover, subfossil chironomids represent an important tool for paleoclimatic reconstructions and recent changes in lake structure and function [18–20].

However, these studies were focused primarily on benthic chironomids, which are a crucial component of the bottom fauna, playing a key role in organic matter processing and recycling nutrients [21–23] as well providing a major link between producers and secondary consumers, such as small- and medium-sized fish [24].

Plant-associated chironomids were not taken into consideration in this aspect. In vegetated water bodies, such as shallow lakes, chironomids are often the most abundant group of phytophilous organisms [25–27]. Plant-associated larvae of chironomids are good colonizers and consume a wide food spectrum (algae, detritus and associated microorganisms and macrophytes) and, as such, play a key role in food–web relationships as consumers [28]. The studies of chironomid–macrophyte co-occurrence indicate the significant role of macrophytes' structure and biomass on chironomid species' richness and abundance [29,30]. Current research indicates the potential of plant-associated chironomids as bioindicators, but so far they have not been used.

The alternative states classification of shallow lakes is assumed to be based on P loadings, Secchi disc transparency and phytoplankton/macrophyte relations [31]. In our study, we intended to assess whether plant-associated chironomids could be used as a reliable indicator for tracking changes between macrophyte-dominated and phytoplankton-dominated lakes.

Assuming that (1) changes in water transparency and coverage of submerged macrophytes caused by the high loadings of P directly affect the number and dominance structure of plant-associated chironomids and that (2) the distribution of chironomids between lake types is closely related to food availability as reflected in their feeding mode structure, the specific objectives were as follows: (1) analyze the species, feeding structure and relative abundances of plant-associated chironomids with regard to lake type, (2) determine the environmental variables used in the classification of alternative states that affect plant-associated chironomids and (3) test the significance of the environment–chironomid relationships to assess their use in biomonitoring.

2. Materials and Methods

2.1. Study Area and Sampling

Ten shallow, polymictic lakes situated in the area of Polesie Lubelskie (eastern Poland) were sampled in spring (May), summer (July) and autumn (October) during the years 2015–2018 (Table 1). The lakes were classified according to alternative states theory as macrophyte-dominated, MD (clear water lakes); phytoplankton-dominated, PD (turbid water lakes); and phytoplankton–macrophyte-dominated, PMD (intermediate lakes) (Table 2). All the studied lakes have an agricultural catchment, with the share of arable land and pastures varying and ranging from 54 to 58% in MD lakes, through 78–82% in PMD lakes, up to 88–90% in PD lakes. The emergent vegetation of the studied ecosystems is well developed with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) as a dominant species in all the lakes. Moreover, in MD lakes, there is a significant share of narrow-leaved (*Typha angustifolia* L.) and broad-leaved (*Typha latifolia* L.) cattails, Spike rush (*Eleocharis palustris* (L. Roem. & Schult.)) and bulrush (*Schonoplectus lacustris* (L.) Palla); in PMD lakes, *T. latifolia* and *S. lacustris* occur in a high density; and in MD lakes, the group of accompanying species included *S. lacustris*, *T. latifolia*, sweet flag (*Acorus calamus* L.) and soft rush (*Juncus effusus* L.).

Table 1. Geographical and limnological characteristics of the studied lakes.

Lake	Coordinates	Surface Area (ha)	Max Depth (m)	Lake Code
Kleszczów	51°31′ N; 22°53′ E	53.9	2.3	MD1
Rotcze	51°22′ N; 23°06′ E	42.7	4.3	MD2
Skomielno	51°29′ N; 23°00′ E	75.0	5.5	MD3
Białe Sosnowickie	51°32′ N; 23°02′ E	144.8	2.7	PMD1
Domaszne	51°28′ N; 23°00′ E	95.0	3.1	PMD2
Głębokie Uścimowskie	51°28′ N; 22°55′ E	20.5	7.1	PMD3
Sumin	51°22′ N; 23°08′ E	91.5	6.5	PMD4
Dratów	51°20′ N; 22°56′ E	167.9	3.0	PD1
Krzczeń	51°23′ N; 22°56′ E	160.7	5.2	PD2
Syczyńskie	51°17′ N; 23°14′ E	5.6	2.9	PD3

Table 2. Environmental characteristics of the studied lakes. Variables are presented as mean values ± SD.

Lake	Secchi Disc Depth (m)	Total P (mg dm^{-3})	Chlorophyll-a (mg dm^{-3})	Macrophyte Biomass (g DW m^{-2})	Macrophyte Cover (%)
MD1	1.2 ± 0.2	0.022 ± 0.007	9.64 ± 4.5	4354 ± 200	67
MD2	1.1 ± 015	0.053 ± 0.007	8.73 ± 4.3	3270 ± 131	60
MD3	2.2 ± 0.6	0.073 ± 0.05	7.52 ± 5.8	4650 ± 244	74
PMD1	0.8 ± 0.16	0.149 ± 0.089	52.26 ± 7.5	2450 ± 282	29
PMD2	0.7 ± 0.05	0.158 ± 0.053	38.32 ± 5.3	2116 ± 145	37
PMD3	0.7 ± 0.15	0.127 ± 0.013	41.51 ± 2.1	2164 ± 178	26
PMD4	0.8 ± 0.03	0.136 ± 0.009	39.87 ± 3.1	2284 ± 231	21
PD1	0.4 ± 0.1	0.256 ± 0.013	50.87 ± 5.2	404 ± 59	12
PD2	0.5 ± 0.05	0.193 ± 0.024	48.23 ± 4.7	649 ± 85	10
PD3	0.3 ± 0.1	0.348 ± 0.028	51.18 ± 6.2	760 ± 78	15

Notes: Lake types are MD—macrophyte-dominated, PMD—phytoplankton–macrophyte-dominated, PD—phytoplankton.

Samples for the chemical analysis of water were collected at each lake and sampling date in triplicate. Water transparency and Secchi disc visibility (SD) were measured in situ. Total phosphorus (TP) and chlorophyll-*a* (Chl-a) were analyzed spectrophotometrically (spectrophotometer Specord 40, Analytik Jena, Denmark). Concentrations of TP were determined using the method with ammonium molybdate according to PN-C-04576 [32]. Prior to Chl-a analysis in lake water, the samples passed through GF/C Whatman filters were extracted for 5 min in a 90% ethanol in a water bath at 75 °C according to PN-ISO 10260 [33].

The submerged macrophyte cover (%) for lake classification was estimated by mapping from the boat in July. Macrophyte distribution was estimated along horizontal transects using a viewer and a rake. The number of transects depended on the size of the lake, and the width of a single transect amounted to 30 m. The results from each transect were used to calculate the macrophyte coverage of the bottom surface of the lake. The biomass of submerged macrophytes was evaluated by collecting plants at sediment level at an area of 0.16 m^2 at nine randomly chosen sites. Each macrophyte sample was put into a separate plastic bag. After being transported to the laboratory, collected plants were washed and dried at 105 °C for 10 h to obtain the dry weight and converted to g DWm^{-2}.

Plant-associated chironomids were collected using a perspex cylindrical apparatus (length 32 cm, diameter 13 cm and weight 800 g), with openings covered by 250 µm mesh nets and a floristic anchor [34]. The open cylinder was placed between the submerged macrophytes and a random plant fragment was moved very gently inside the apparatus with a small floristic fork. Next, the sampler was closed very slowly to avoid water movements and raised above the water surface. All the plant fragments protruding above the surface of the cylinder were cut off. Next, the water was poured from the cylinder, the apparatus was opened and the plant material was put into a plastic bag. Next, the apparatus was placed vertically and rinsed with water outside to move macroinvertebrates remaining on the net to the bottom of the cylinder to place them in a plastic bag together with the macrophytes. For each date and lake, samples of plant-associated chironomid larvae were collected from three randomly chosen sites in triplicate. Each sample was taken and packed separately.

Field samples were transported to the laboratory, where the larvae of midges were removed from macrophyte tissues and preserved in 4% formaldehyde solution. Larvae were counted and identified under an inverted microscope. The nomenclature of Chironomidae larvae followed Wiederholm [35]. The density of larvae was calculated per 100 g DW of plants.

The species diversity of plant-associated chironomids was evaluated using the Shannon–Wiener index, calculated according to the following formula:

$$H' = \sum_{i=1}^{s} (p_i)(log_2 p_i)$$

where s—number of species and p_i—proportion of individuals belonging to the i species to the total number of individuals.

Each identified chironomid taxon was assigned to a functional feeding group (FFG) according to the classification of Mandaville [36] and Merritt et al. [37]. Four feeding groups were selected in the data, collector-gatherers (CGs), collector-filterers (CFs), scrapers (SCRs) and predators (PRDs). The relative abundance (%) of each FFG of the total assemblages was calculated for each study lake. A detailed list of taxa and their grouping in FFGs is presented in Table S1.

2.2. Data Analysis

One-way ANOVA was used to test the effect of lake type on water parameters (Secchi disc depth, TP and chlorophyll-*a*) and total chironomid density. Tukey's multiple range test (at $p < 0.05$) was used to compare means when significant differences were found.

Pearson's correlation coefficients were calculated to recognize the relationships between relative abundances of FFGs and environmental parameters (Secchi disc visibility, TP, chlorophyll-*a* and submerged macrophyte biomass).

Detrended Correspondence Analysis (DCA) was performed to explore the distribution patterns of plant-associated chironomids. As the variability gradient of chironomids did not exceed 3 SD, principal component analysis (PCA) was used to classify the lake types with respect to chironomid structure. Next, redundancy analysis (RDA) was used to explore the relationships between the chironomid taxa and environmental variables [38]. Automatic forward selection (Monte Carlo permutation test) was used to determine the significant environmental variables. Significant variables ($p < 0.05$) were presented as bold arrows on the diagram. Significant environmental variables selected by the permutation test were used in a multiple linear regression model to test their effect on dominant chironomid taxa. ANOVA, Pearson's correlations and regression analyses were processed with STATISTICA 13.3. TIBCO Software Inc., and ordination analysis was conducted with CANOCO 5.0 for Windows.

3. Results

3.1. Environmental Variables

Values of physical and chemical parameters used for lake classification showed clear variability between lake types (Table 2). Significantly, the highest water transparency (1.0–2.8 m) was noted in MD clear-state lakes; the lowest (0.3–0.5 m) was in PD turbid-state lakes (ANOVA, $F = 23.18$, $p < 0.001$). In the remaining lakes, Secchi disc visibility ranged from 0.7 to 0.8 m.

TP and chlorophyll-*a* showed an increasing trend from MD to PD lakes (Table 2). In MD lakes, concentrations of TP (0.022–0.073 mg dm^{-3}) (ANOVA, $F = 4.79$, $p = 0.017$) and chlorophyll-*a* (7.52–9.64 mg dm^{-3}) (ANOVA, $F = 8.06$, $p = 0.002$) were significantly the lowest. In PD lakes, the values of both these parameters were high (TP and chlorophyll-*a*). In PMD lakes, concentrations of TP ranged from 0.127 mg dm^{-3} to 0.158 mg dm^{-3} and chlorophyll-*a* ranged from 38.32 mg dm^{-3} to 52.26 mg dm^{-3}.

The biomass of submerged macrophytes varied significantly (ANOVA, $F = 347.31$, $p < 0.001$) between lake types and ranged from 3270 to 4650 g DW m^{-2} in MD lakes and from 404 to 760 g DW m^{-2} in PD lakes (Table 2). Macrophyte cover ranged from 67 to 84% in MD lakes and did not exceed 15% in PD lakes. In PMD lakes, the mean DW of macrophytes ranged from 2116 g m^{-2} to 2450 g m^{-2} and macrophyte cover ranged from 21 to 37%. The species composition of macrophytes depended visibly on the lake type. In MD lakes, the most common submerged macrophyte species were stoneworts (*Chara fragilis* L. and *Chara aculeolata* Kütz. in Rchb.) as well as water soldier (*Stratiotes aloides* L.). In PMD lakes, pondweeds (*Potamogeton crispus* L., *P. lucens* L. and *P. praelongus* Wulfen) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) were present. In PD lakes, mainly rigid hornwort (*Ceratophyllum demersum* L.) and sago pondweed (*Potamogeton pectinatus* L.) were observed.

3.2. Chironomid Community

Lake type (submerged macrophytes cover) significantly affected the abundance of chironomids (ANOVA, $F = 105.62$, $p < 0.001$). The lowest densities, from 39 to 53 ind. 100 g DW^{-1}, were observed in PMD lakes, and the highest were observed in PD lakes (range 242 to 399 ind. 100 g DW^{-1}) (Figure 1). A total of 19 taxa of plant-associated chironomids were identified in the lakes. The number of taxa varied between 6 (lake PD3) and 16 (lake MD3).

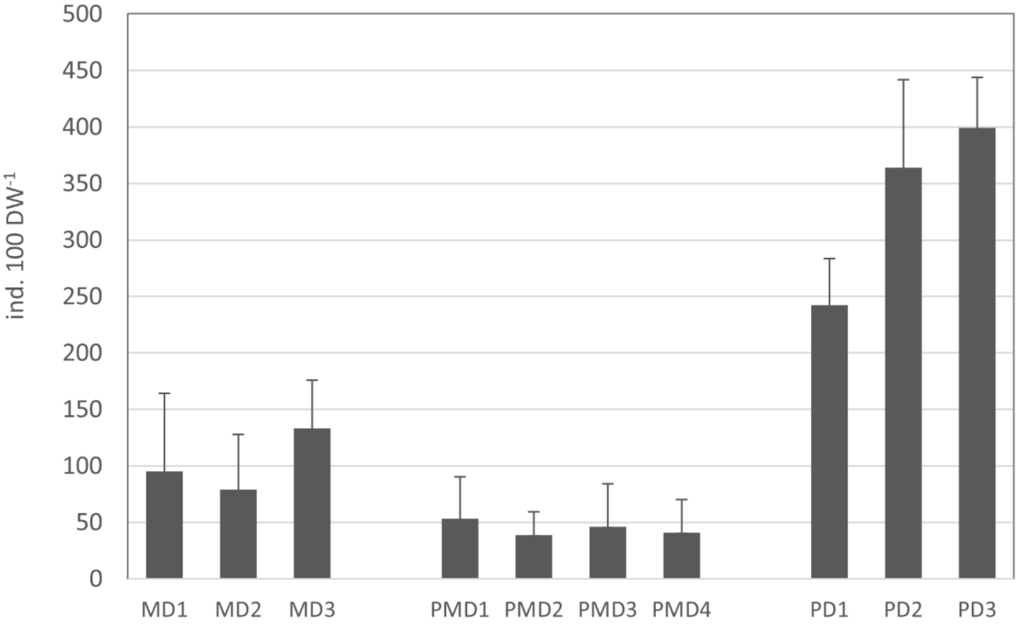

Figure 1. Mean density (±SD) of plant associated chironomids with regard to lake type.

Analysis of the dominance structure of plant-associated chironomids allowed for the identification of a dominant taxon for each type of lake (Figure 2). In MD lakes, the highest relative abundance (mean 40%, range 36–44%) showed larvae of *Paratanytarsus austriacus*. Moreover, the larvae of *Psectrocladius* sp. (gr. *sordidellus*) (mean 15%, range 12–20%) and *Dicrotendipes* sp. (mean 13%, range 8–17%) constituted an important share in the total number of chironomids. In PMD lakes, the larvae of *Endochironomus albipennis* (mean 25%, range 23–27%) dominated. The relative abundance of remaining taxa did not exceed 10% of the total density of plant-associated larvae. In PD lakes, the larvae of *Cricotopus* sp. (gr. *sylvestris*) reached the highest relative abundance between 28 and 41% (mean 34%). In addition, a higher percentage of two other taxa was noted, *Glyptotendipes* sp. (mean 11%, range 10–12%) and *Polypedilum sordens* (mean 11%, range 9–13%).

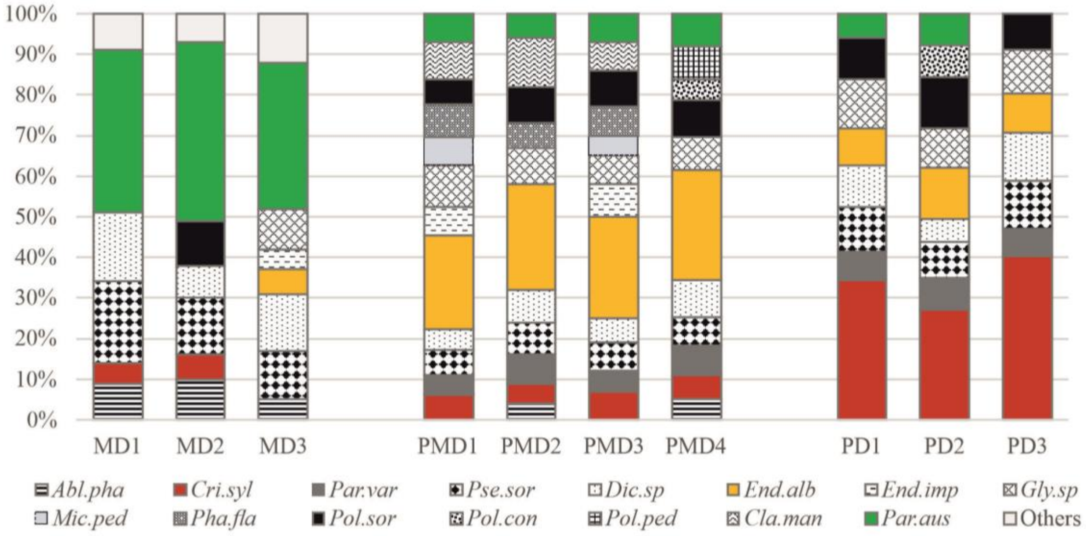

Figure 2. Relative abundances of plant associated chironomid taxa in studied lakes. Taxa codes: Abl.pha—*Ablabesmyia phatta*, Cla.lat—*Cladopelma lateralis*, Cla.man—*Cladotanytarsus mancus*, Cri.syl—*Cricotopus sylvestris*, Dic.sp—*Dicrotendipes* sp., End.alb—*Endochironomus albipennis*, End.imp—*Endochironomus impar*, Gly.sp—*Glyptotendipes* sp., Par.aus—*Paratanytarsus austriacus*, Par.var—*Parachironomus varus*, Pol.sor—*Polypedilum sordens*, Pse.sor—*Psectrocladius sordidellus*. Taxa classified as others (relative abundance < 5%): *Allochironomus* sp., *Diplocladius cultiger, Microtendipes pedellus, Phaenopsectra flavipes, Polypedilum convictum, P. nubeculosum, P. pedestre*.

The species diversity of plant-associated chironomids, as values of the Shannon–Wiener index, differed between the lake types and ranged from 2.2 to 3.4 (Figure 3). The highest values of the H′ index, H′ = 3.0–3.4, were observed in PMD lakes. In the remaining lakes, Shannon–Wiener index was lower and ranged from 2.45 to 2.85 in MD lakes and from 2.67 to 2.87 in PD lakes.

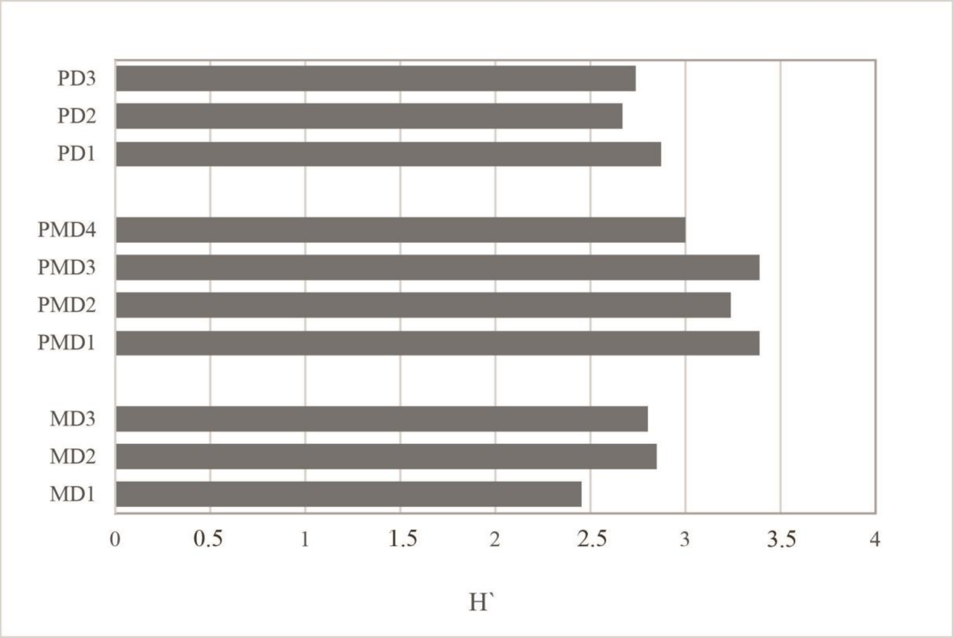

Figure 3. Values of Shannon-Wiener (H′) index for plant-associated chironomids with regard to lake type.

According to the results, the studied lakes were classified into three groups, macrophyte-dominated lakes as the *Paratanytarsus austriacus*-type, phytoplankton-dominated lakes as the *Endochironomus albipennis*-type and phytoplankton-dominated lakes as the *Cricotopus sylvestris*-type.

3.3. Functional Feeding Groups

Relative abundances of FFGs changed visibly between lake types and showed significant relationships with environmental parameters (Figure 4). The abundances of collector-filterers varied between 54% (lake MD2) and 6% (lake PD1) and were highly positively correlated with macrophyte biomass (r = 0.97, $p < 0.001$) and Secchi disc depth (r = 0.85, $p < 0.001$). We also observed significant negative correlations between CF and TP (r = −0.69, $p < 0.001$) and chlorophyll-*a* concentrations (r = −0.55, $p = 0.002$). Relative abundances of scrapers ranged from 10% (lake MD1) up to 67% (lake PD2) and were significantly negatively correlated with macrophyte biomass (r = −0.93, $p < 0.001$) and Secchi disc visibility (r = −0.73, $p < 0.001$) and positively correlated with TP (r = 0.76, $p < 0.001$) and chlorophyll-*a* (r = 0.47, $p < 0.001$). The percentage of collector-gatherers varied from 15% (lake PMD3) to 39% (lake MD1) and was negatively correlated with TP (r = −0.38, $p = 0.038$). Relative abundances of predators varied from 5% (lake MD3) to 15% (lake PMD4) and were not significantly related to any of the environmental parameters.

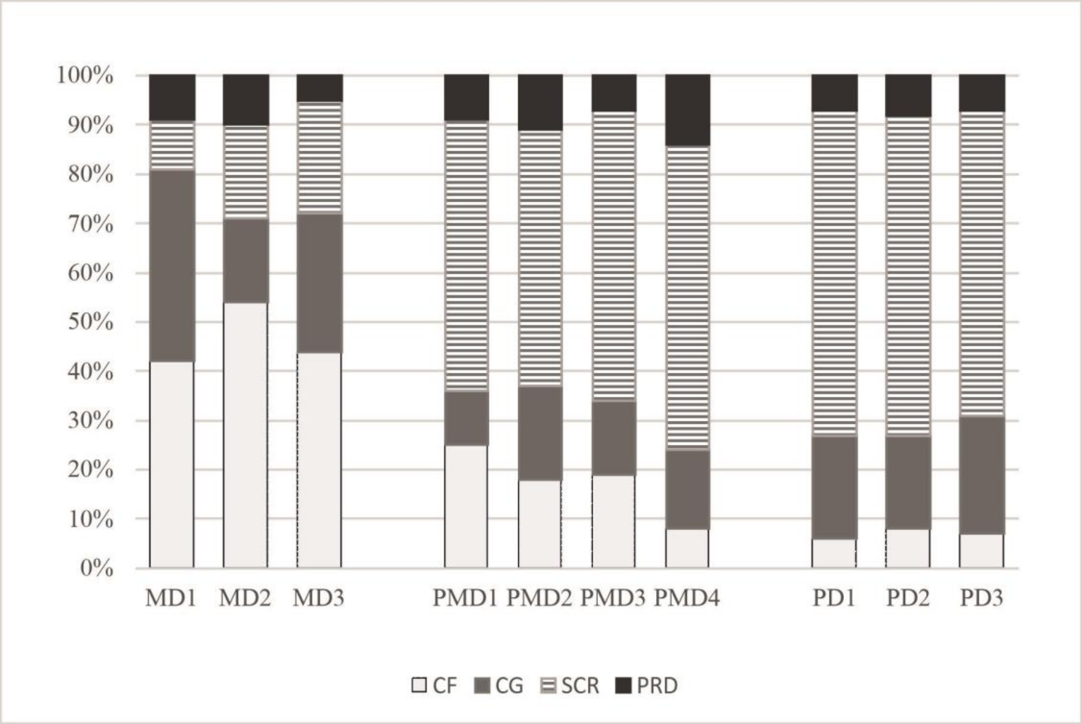

Figure 4. Structure of FFGs of plant-associated chironomids with regard to lake type (CF—collector filterers, CG—collector gatherers, SCR—scrapers, PRD—predators).

3.4. Statistical Analysis

The PCA revealed the formation of three groups of lakes consistent with alternative states classification (Figure 5A). The first and second axes of the PCA explained 46.7% and 15.3% of the variability of the data, respectively. These two axes showed that chironomid abundances are closely related to lake type.

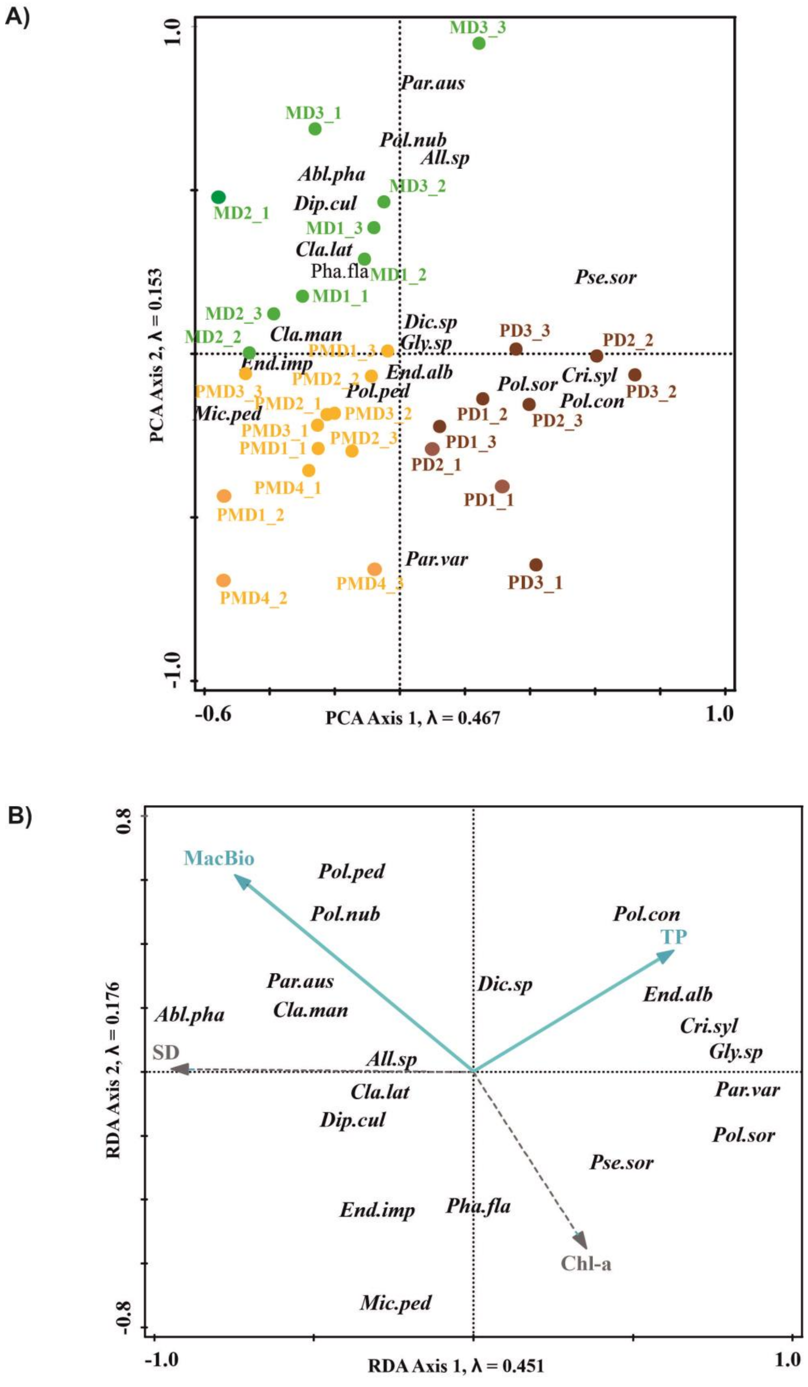

Figure 5. Biplots of ordination analyses (**A**) PCA for axis 1 and 2 showing samples (lakes) and plant-associated chironomids. Lake codes: green cycles—macrophyte-dominated lakes, yellow cycles—phytoplankton-dominated lakes, brown cycles—phytoplankton-dominated lakes. Axes are derived from the variation in the taxonomic data-matrix and (**B**) RDA for plant associated chironomid taxa and environmental variables. Solid blue arrows indicate significant variables based on permutation test ($p < 0.05$). SD—Secchi disc visibility; Chl-a—chlorophyll-a, TP—total phosphorous; MacBio—macrophytes biomass. Taxa codes: All.sp—*Allochironomus* sp., Abl.pha—*Ablabesmyia phatta*, Cla.lat—*Cladopelma lateralis*, Cla.man—*Cladotanytarsus mancus*, Cri.syl—*Cricotopus sylvestris*, Dic.sp—*Dicrotendipes* sp., Dip.cul—*Diplocladius cultiger*, End.alb—*Endochironomus albipennis*, End.imp—*Endochironomus impar*, Gly.sp—*Glyptotendipes* sp., Mic.ped—*Microtendipes pedellus*, Par.aus—*Paratanytarsus austriacus*, Par.var—*Parachironomus varus*, Pha.fla—*Phaenopsectra flavipes*, Pol.nub—*Polypedilum nubeculosum*, Pol.ped—*Polypedilum pedestre*, Pol.sor—*Polypedilum sordens*, Pse.sor—*Psectrocladius sordidellus*.

The redundancy analysis processed for the chironomid and environmental data showed that the eigenvalues for RDA axis 1 (0.451) and axis 2 (0.176) accounted for 62.7% of the variance in chironomid taxa. The forward selection procedure (Monte Carlo permutation test) indicated the significant effect ($p \leq 0.05$) of two environmental variables, macrophyte biomass ($\lambda = 27.9$; $F = 10.9$; $p = 0.004$) and TP ($\lambda = 23.5$; $F = 8.6$; $p = 0.01$), on plant-associated chironomids. The distribution of chironomids on the RDA biplot indicate that the presence of *P. austriacus*, *Ablabesmyia phatta*, *Allochironomus* sp., *Cladotanytarsus mancus*, *Polypedilum pedestre* and *Polypedilum nubeculosum* is related to macrophyte biomass, while the occurrence of taxa such as *C. sylvestris*, *Dicrotendipes* sp., *E. albipennis*, *Glyptotendipes* sp. and *Polypedilum convictum* is related to TP concentrations (Figure 5B).

The regression analysis of the responses of dominant chironomids for TP and macrophyte biomass showed that the model was significant for all analyzed taxa (Figure 6). For *P. austriacus*, both environmental predictors explained a total of 90% of its variation ($F_{(2.27)} = 118.05$, $p < 0.001$, $R^2 = 0.894$), and macrophyte biomass was significantly important. The variability of *E. albipennis* was explained as 64% ($F_{(2.27)} = 24.45$, $p < 0.001$, $R^2 = 0.644$) with a significant effect of both predictor variables. The analyzed model explained 78% of the variability of the third dominant larvae of *C. sylvestris* ($F_{(2.27)} = 47.31$, $p < 0.001$, $R^2 = 0.777$), with a significant effect of TP (Table 3).

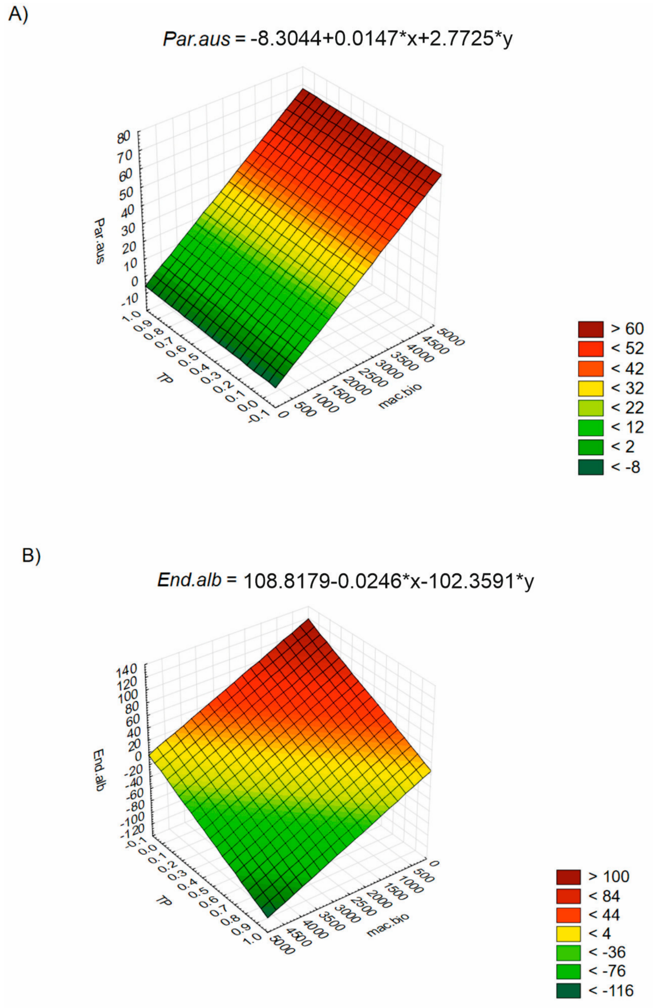

Figure 6. *Cont.*

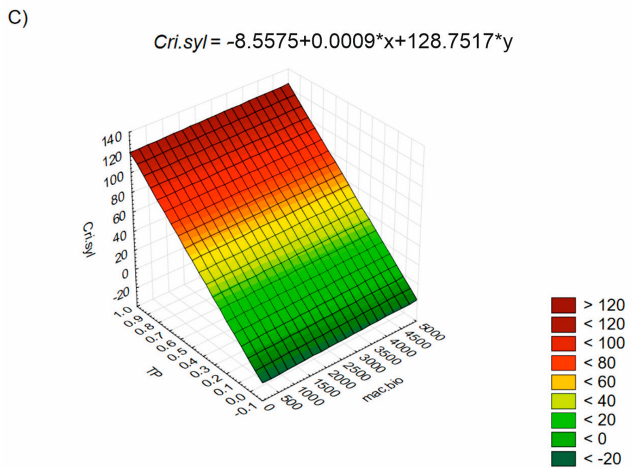

Figure 6. Regression surface plots for macrophyte biomass and TP vs. dominant chironomid taxa (**A**) *Paratantrarsus austriacus*, (**B**) *Endochironomus albipennis*, (**C**) *Cricotopus sylvestris*.

Table 3. Results of multiple regression analyses for the relationships between chironomid dominants and environmental variables (mac.bio—macrophyte biomass, TP—total phosphorous, Par.aus—*Paratanytarsus austriacus*, End.alb—*Endochironomus albipennis*, Cri.syl—*Cricotopus sylvestris*; significant variables are marked with bold and asterisks).

Responsible Variable	Predictor Variables	b	Partial Correlation	t	*p* Value
Par.aus.	**mac.bio**	**0.97**	**0.894**	**10.34**	**<0.001** *
	TP	0.03	0.063	0.36	0.722
End.alb	**mac.bio**	**−1.223**	**−0.803**	**−6.991**	**<0.001** *
	TP	**0.938**	**0.718**	**5.358**	**<0.001** *
Cri. syl	mac.bio	0.037	0.051	0.270	0.789
	TP	**0.909**	**0.784**	**6.579**	**<0.001** *

4. Discussion

Our results show that plant-associated chironomids were clearly differentiated into clear, intermediate and turbid water lakes. In the study, the species structure of chironomids was assigned to a particular type of lake, with a dominance of a single species. In MD lakes, the larvae of *P. austriacus* dominated. These larvae are known to live preferentially in aquatic vegetation [39]. The tube-building behavior of these larvae is observed on the branches of submerged macrophytes, as shown by Tarrats et al. [40]. In PMD intermediate state lakes, chironomids showed a significant decline in numbers and a new dominant appeared, *E. albipennis* larvae. These larvae are widely occurring in eutrophic lakes and are usually classified as filtrator/grazers fed on pinnate diatoms, cyanobacteria and detritus [7]. Larvae of *C. sylvestris*, dominating in PD hypertrophic lakes, are frequently associated with productive sites [41,42], and in shallow lakes, they are classified as pollution-tolerant taxa [27,43].

Observed chironomid–macrophyte relationships confirm that macrophytes' presence and abundance is one of the main drivers of chironomid assemblages. Such relations have already been reported in several studies by Rumes [44] and van Hardenbroek et al. [22], among others. These responses of chironomid diversity to environmental parameters, such as macrophyte biomass, were ambiguous and reflected mostly in their composition and abundance [45]. In the present study, we observed the highest abundance of chironomids in PD lakes and the highest species diversity in PMD lakes. As stated by Langdon et al. [46] and Williams et al. [47], it seems to be related to changes in the habitat structure and its availability. The increasing TP inputs leading to eutrophication and a change in the trophic state affect some key components of habitat structure such as submerged vegetation and food availability [48,49]. The shift from a clear into intermediate or turbid water state is connected to phytoplankton biomass fluctuations, most often dominated by cyanobacteria, thus mediating changes in food availability. We observed that the feeding groups of the dominating chironomids changed alongside the lake status, from collector-filterers (*P. austriacus*) in MD lakes to periphytic scrapers (*C. sylvestris*) in PD lakes. *C. sylvestris*, dominant in turbid lakes, is able to feed only on detritus and associated microorganisms under hypertrophic conditions [50]. There is evidence of associations between *Cricotopus* and cyanobacterial and other bacterial colonies [51].

Multiple regression confirmed that macrophyte biomass and TP are the key determinants of plant-associated chironomids. The model used explains from 64 to 90% of their variability with taxa-specific contributions of these predictors. The variation of *P. austriacus* indicates a direct relationship with macrophytes. Jones and Sayer [52] found that chironomids can use plants as substrates for living or for the growth of periphyton and utilize it as food. Van de Mutter et al. [53], Kumari et al. [7] and da Silva et al. [45] describe submerged macrophytes associated with clear waters as harboring the highest diversity of Chironomidae. We did not observe such a relation in our study. We found that the most diverse with the highest number of species were PMD intermediate state lakes.

The results of regression analysis for *E. albipennis* and *C. sylvestris* show the importance of TP as a significant predictor of their distribution. Similarly to observations of Brodersen et al. [41], it can be concluded that in temperate shallow lakes, the relationship between chironomids and TP is probably indirect, mediated through productivity and associated food availability (high biomass of periphytic algae). Both chironomid species belong to periphytic scrapers with significantly higher numbers in more productive PMD and PD lakes observed in the present study.

Changes in the chironomid community alongside the alternative states can be used for tracking changes in shallow lake ecosystems under eutrophication processes. Increased temperature and agricultural inputs which result in higher lake productivity are reflected within chironomid communities [54,55]. Our research allowed us to observe ecological change which has occurred in the chironomid structure. It was a shift from a relatively low species number and abundance of plant-associated chironomids in MD lakes, through high species diversity and lower abundance in PMD lakes, towards a relatively species-poor community, with very high density in PD lakes. Similarly to the observations of Langdon et al. [49] and Wiliams et al. [47], productivity parameters, nutrients (TP) and macrophyte biomass (habitat) have been recognized as key potential drivers responsible for the shift between *Paratanytarsus austriacus*-type (MD lakes) and *Cricotopus sylvestris*-type (PD lakes) lakes.

Many previous studies, such as those by Feio and Dolédec [56], Feio et al. [57], Saulino et al. [58], Beauchard et al. [59], Kuzmanovic et al. [60], Serra et al. [61] and Castro et al. [62], indicate that the evaluation of functional feeding groups, among the functional attributes, is used in biomonitoring programs to assess the environmental conditions (level of disturbance) of the ecosystem. Due to the fact that eutrophication is a major process of shallow lakes' degradation, an analysis of the FFGs of plant-associated chironomids can be used to evaluate the level of ecosystem disturbance. In our study, MD lakes were dominated by collector-filterer and collector-gatherer chironomids. As environments with large quantities of organic matter (high biomass of submerged macrophytes), MD lakes favor the occurrence of collector organisms [63,64]. As the eutrophication process progresses, the biomass of macrophytes decreases, and due to the increase in periphytic algae biomass, scrapers become the dominant FFG. In the studied PMD lakes, the relative abundance of chironomid scrapers increased more than three times compared to MD lakes. The results confirm the observations of Trivinho-Strixino [65], Serra et al. [61] and Gomez et al. [63] that larval chironomids have specific attributes that can be used to evaluate environmental conditions and that these macroinvertebrates are useful for differentiating ecological conditions in natural aquatic ecosystems under various levels of disturbance of anthropogenic origin.

5. Conclusions

Our research shows a different view on alternative state theory which is primarily based on macrophytes, chlorophyll-*a* and P loadings. The results indicate the high monitoring potential of plant-associated Chironomidae. The application of multiple regression analysis gives precise information about the effect of environmental drivers, macrophyte biomass and TP on the abundance of indicatory species. The use of plant-associated chironomid-based indicators may contribute to a better assessment of changes in shallow lakes as a result of anthropogenic disturbance, including the impact of climate change on shallow lake ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16141984/s1, Table S1: Classisication of plant-associated chironomid taxa to functional feeding groups and their relative abundances with regard to lake type.

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