

## Article

# Odonata Assemblages in Anthropogenically Impacted Habitats in the Drava River—A Long-Term Study

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**Abstract:** Lotic freshwater ecosystems are among the most threatened ecosystems worldwide due to the effects of multiple stressors, such as intensive land use in their catchments, morphological alterations, flow regulation, pollution, and climate change. Odonata are often used as valuable indicators of ecological integrity and anthropogenic disturbance of freshwater habitats. Here, we present the results of a study on Odonata assemblages in anthropogenically impacted habitats (hydropower plant reservoirs, tailrace canals, drainage ditches, and old river channels) conducted over a nine-year period. The negative impacts of anthropogenic activities on inhabiting biota were confirmed—with only 11 species recorded, the Odonata assemblages were species-poor and had low population densities. Although most species recorded were generalists, some species of national conservation concern were detected. Among the physico-chemical water parameters, the concentrations of ammonium, orthophosphates, nitrates, and mineral oils in the water were found to be the most important determinants of Odonata assemblages. The preservation of near-natural sites in the vicinity of anthropogenically impacted and man-made habitats is important for maintaining the local Odonata fauna and for the preservation of rare species. Our results highlight the importance of long-term data for determining the occurrence of Odonata species and monitoring their population dynamics.

**Keywords:** dragonflies; damselflies; species richness; human pressures; man-made habitats; environmental variables; monitoring; generalist species; rare species



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## 1. Introduction

Freshwater ecosystems cover only about 0.8% of the land's surface and account for no more than 0.01% of the world's water volume [1]. However, they are of enormous importance for life on Earth and are characterized by impressive biodiversity [2,3], harboring more than 100,000 species [1]. They also provide various ecosystem services to humans, i.e., they are used as a source of drinking water, for irrigation in agriculture, for industrial purposes, for energy production, for recreation, etc. [4,5]. Nevertheless, freshwater ecosystems are among the most degraded natural systems in the world [6,7]. They are threatened by many anthropogenic stressors, such as pollution, hydromorphological alterations, habitat fragmentation, dam construction, flow regime alterations, introduction of invasive species, urbanization, and the overexploitation of freshwater resources due to population growth [1,8–10]. In addition, the impacts of climate change on freshwater ecosystems are increasing significantly, and due to rapid changes in air temperatures and precipitation patterns, they have already heavily modified the hydrological regimes of rivers worldwide [8,11]. Some regions are affected by severe droughts, while others are facing heavy floods [12]. Such stressors negatively impact aquatic communities, leading to their homogenization and shifts in taxonomic and functional species composition, resulting in a dramatic loss of freshwater biodiversity [13–21].

Many interacting factors influence subtle ecological processes that occur over a relatively long period of time. Therefore, these processes, such as population dynamics, life cycles, and reproductive patterns, as well as the influences of various disturbances on communities, require long-term studies to be understood [22]. Long-term data could also explain the limiting factors for different species, leading to a better interpretation of their past, present, and future distributions and understanding species' resilience and responses to environmental stressors [23]. Therefore, long-term studies could serve as a useful tool to assess the impact of global change on biota [14]. Although the number of long-term studies on European benthic macroinvertebrates has increased in the 21st century, the availability of long-term data is still quite limited, including those on Odonata [14,24].

Odonata is an order of aquatic insects with an amphibious life cycle that includes an aquatic nymph phase and a terrestrial adult phase. Due to their predatory feeding habits, both nymphs and adults are at high levels in the trophic web [25]. In addition, the life history traits of different Odonata species vary widely, such as their dispersal ability, habitat and microhabitat preferences, and tolerance to water pollution and habitat degradation [26]. For these reasons, they are widely used as valuable indicators of ecological integrity and the anthropogenic disturbance of freshwater habitats [20,21,27,28]. The bottom substrate and submerged aquatic vegetation composition, as well as water clarity, are the most important determinants of Odonata nymph occurrence in a given habitat, while adults select habitats primarily based on structural heterogeneity, i.e., aquatic and riparian vegetation structure and shading [25,29,30]. The main objectives of this study were: (i) to determine Odonata species richness and their spatial and temporal distributions, and (ii) to determine the main environmental factors affecting Odonata occurrence in anthropogenically altered and man-made habitats in the Drava River system over a nine-year period. Species-rich Odonata assemblages were expected to be found in less anthropogenically impacted habitats.

## 2. Materials and Methods

### 2.1. Study Area

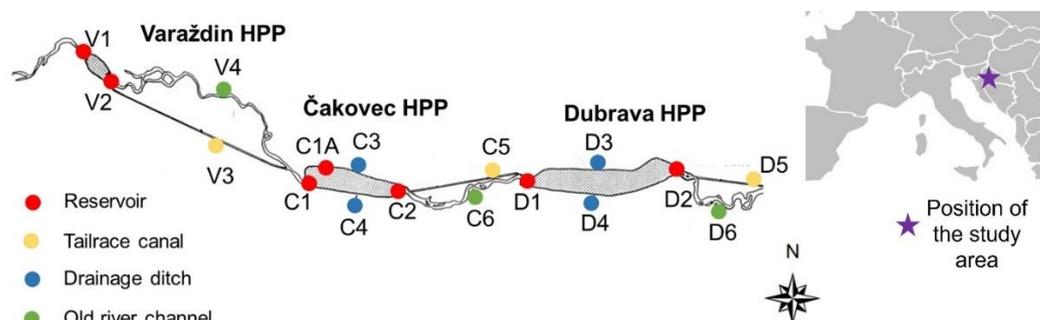
With a length of 719 km, the Drava River is the fourth-largest and longest tributary of the Danube. The river originates in Italy and flows through Austria, Slovenia, Hungary, and Croatia, encompassing a wide range of natural habitats along its middle and lower reaches that harbor unique animal and plant assemblages [31–33]. For these reasons, the Drava River has been a part of the Transboundary Biosphere Reserve Mura–Drava–Danube since 2012 [34]. However, the river has been heavily modified along its entire length by the construction of hydropower plants (HPPs), the last three of which, namely Varaždin, Čakovec, and Dubrava, are located in the north-western part of Croatia. After the construction of the HPPs, new man-made habitats were created (e.g., reservoirs, tailrace canals, and drainage ditches) that strongly influenced the water regime of the river [35].

This study was conducted at 17 sites along the entire hydropower system (Figure 1). Over a length of approximately 60 km, these sites belong to all of the major habitat types of the system (reservoir, tailrace canal, drainage ditch, and old river channel). Four sites belong to the Varaždin HPP, seven to the Čakovec HPP, and six to the Dubrava HPP (Figure 1).

### 2.2. Odonata Sampling

Odonata nymphs were sampled seasonally in April, July, October, and December over a nine-year period (2012–2020), together with other macroinvertebrates. Reservoirs (sites V1, V2, C1, C1A, C2, D1, and D2) were sampled using an Ekman grab with a sample area of 225 cm<sup>2</sup>, with three subsamples collected. The sampled macroinvertebrates were sorted out from the sediment by sieving with a benthos hand net (mesh size of 475 µm). At other sites, which included tailrace canals (sites V3, C5, and D5), drainage ditches (sites C3, C4, D3, and D4), and old river channels (sites V4, C6, and D6), macroinvertebrates were sampled using a benthos hand net (25 cm × 25 cm; mesh size = 500 µm). Ten subsamples were

collected at each site and at each sampling event, and pooled into one composite sample. Odonata nymph abundance is presented as the number of individuals per square meter.



**Figure 1.** Map of the study area located along the Drava River in Croatia.

Nymphs were identified using the relevant identification keys [36–38]. Very young or damaged individuals were identified to the family level. All voucher specimens were deposited at the Department of Biology, Faculty of Science, Zagreb, Croatia.

### 2.3. Environmental Variables

At each study site, the following environmental parameters were measured at the time of macroinvertebrate sampling: water temperature, pH (using a WTW pH 330 pH meter), dissolved oxygen concentration and oxygen saturation (using a WTW Oxi 330/SET oximeter), and conductivity (with a WTW LF 330 conductivity meter). The rest of the environmental parameters (biological oxygen demand, chemical oxygen demand, ammonium, nitrite, nitrate, total nitrogen, total phosphorous, orthophosphate, pesticide, phenol, and mineral oil concentrations in water, as well as the number of fecal coliform bacteria in 1000 mL) were analyzed according to the standard methods [39] (Table 1).

**Table 1.** Mean values (and SD, standard deviation) of environmental parameters in anthropogenically impacted habitats in the Drava River measured between 2012 and 2020. Higher values of environmental parameters are in bold.

Habitats/Environmental Parameters	Reservoirs		Drainage Ditches		Tailrace Canals		Old River Channels	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Water temperature (°C)	14.98	2.09	13.40	0.98	14.83	1.24	<b>15.31</b>	1.55
pH	<b>8.12</b>	0.16	7.98	0.17	<b>8.12</b>	0.15	8.09	0.16
Conductivity (µS/cm)	283	25	<b>339</b>	19	284	21	307	29
Dissolved oxygen concentration (O <sub>2</sub> mg/L)	<b>9.84</b>	0.62	6.47	0.71	9.64	0.49	9.39	0.90
Oxygen saturation (%)	<b>101.56</b>	5.93	64.90	7.27	97.59	3.84	95.91	9.49
Biological oxygen demand (O <sub>2</sub> mg/L)	<b>1.59</b>	0.86	1.09	0.91	1.42	0.84	1.48	0.90
Chemical oxygen demand (O <sub>2</sub> mg/L)	<b>6.33</b>	5.90	5.87	5.30	5.60	5.49	5.50	5.27
Ammonium concentration (N mg/L)	0.028	0.021	0.046	0.057	0.020	0.008	<b>0.085</b>	0.120
Nitrite concentration (N mg/L)	0.038	0.142	<b>0.046</b>	0.147	0.045	0.170	0.044	0.138
Nitrate concentration (N mg/L)	<b>0.793</b>	0.192	0.595	0.178	0.769	0.175	0.753	0.213
Total nitrogen concentration (N mg/L)	<b>1.217</b>	0.422	0.829	0.447	1.105	0.273	1.114	0.352
Total phosphorous concentration (P mg/L)	<b>0.062</b>	0.050	0.044	0.048	0.046	0.032	0.054	0.040
Orthophosphate concentration (P mg/L)	0.017	0.007	0.020	0.016	0.015	0.003	<b>0.025</b>	0.024
Phenol concentration (mg/L)	0.0061	0.0045	0.0053	0.0030	0.0073	0.0062	<b>0.0082</b>	0.0104
Pesticide concentration (µg/L)	<b>0.0033</b>	0.0024	0.0031	0.0024	0.0031	0.0025	0.0032	0.0026
Mineral oil concentration (mg/L)	0.016	0.019	0.017	0.023	<b>0.020</b>	0.029	0.016	0.024
Faecal coliform bacteria (nr/1000 mL)	4610	5922	<b>20,728</b>	116,604	2975	4459	14,449	37,149

## 2.4. Data Analyses

Cluster analysis was performed to reveal similarities in the Odonata assemblages among the study sites. The analysis was based on the Bray–Curtis similarity index. Study sites without Odonata records were excluded from the analysis.

To determine the relationship between Odonata assemblages and environmental variables, a redundancy analysis (RDA) with the Monte Carlo permutation test for significance (with 499 permutations) was performed. This analysis was conducted using data for 13 taxa, 153 sampling points, and 15 environmental variables (water temperature, pH, conductivity, dissolved oxygen concentration, biological oxygen demand, chemical oxygen demand, ammonium, nitrate, nitrite, total nitrogen, orthophosphate, total phosphorus concentrations, concentrations of pesticides, mineral oils, and counts of fecal coliform bacteria in water).

Bray–Curtis similarity index and cluster analyses were performed using the Primer 6 software package [40], and RDA analysis was performed using CANOCO for Windows (ver. 4.02) [41]. Species data were  $\log(x + 1)$ -transformed prior to analyses.

## 3. Results

### 3.1. Environmental Variables

The mean values of environmental parameters slightly varied between habitat types (Table 1). The mean water temperature, ammonium, orthophosphate, and phenol concentrations were slightly higher in the old river channels than in the other habitat types. The mean dissolved oxygen concentration, oxygen saturation, biological and chemical oxygen demand, nitrate, total nitrogen, total phosphorous, and pesticide concentrations were slightly higher in the reservoirs than in the other habitat types. The mean conductivity, nitrite concentration, and fecal coliform bacteria counts in water were higher in the drainage ditches, while the mineral oil concentrations were higher in the tailrace canals than in the other habitat types. The mean pH was higher in the reservoirs and tailrace canals than in the other two habitat types.

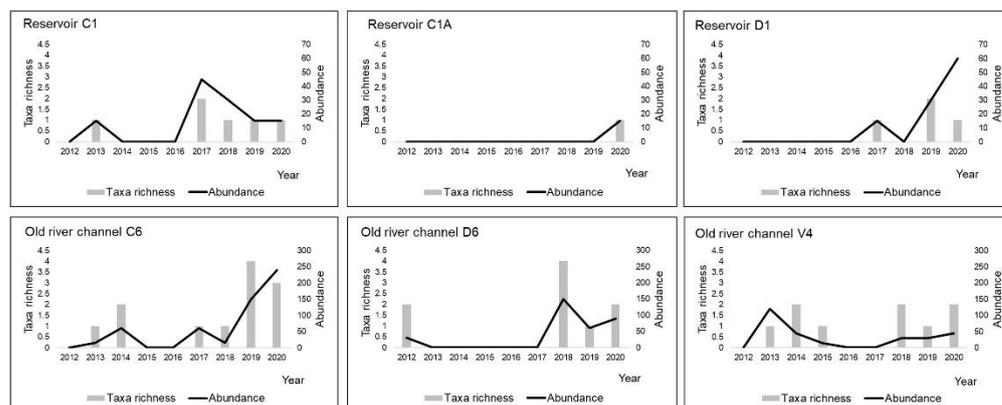
### 3.2. Odonata Assemblages

During the study period, a total of 12 taxa of Odonata (11 species) was recorded (Table 2). *Platycnemis pennipes* and *Onychogomphus forcipatus* were the most widespread species, detected at eight and six sites, respectively (Table 2). Old river channels had the highest Odonata abundance (number of individuals per m<sup>2</sup>) and species richness (number of species) (sites C6, D6, and V4). No Odonata species were detected at several sites, including four reservoirs (C2, D2, V1, and V2) and two drainage ditches (C3 and C4) (Table 2).

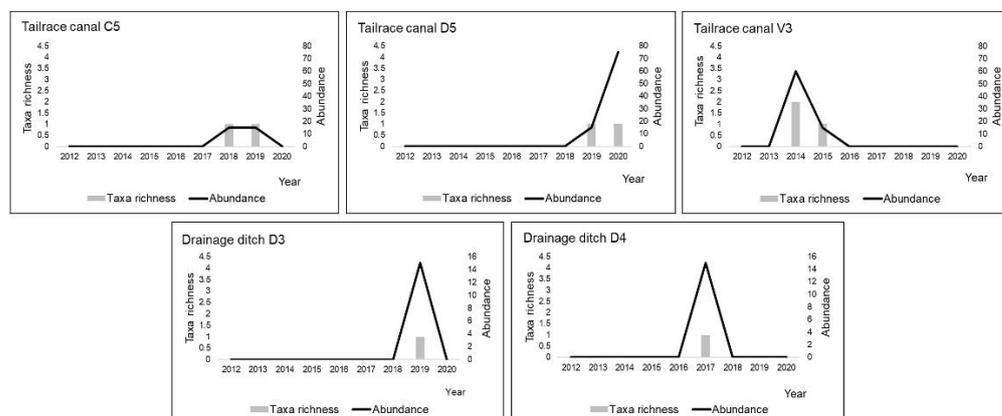
**Table 2.** Odonata taxa recorded in anthropogenically impacted habitats in the Drava River between 2012 and 2020. Abundance is shown as the number of individuals per m<sup>2</sup>.

Habitat Type	Reservoirs						Drainage Ditches				Tailrace Canals			Old River Channels				
	Site	C1	C1A	C2	D1	D2	V1	V2	C3	C4	D3	D4	C5	D5	V3	C6	D6	V4
<i>Platycnemis pennipes</i> (Pallas, 1771)	6.7	1.7		3.3									3.3	6.7		30.0	15.6	1.7
<i>Calopteryx splendens</i> (Harris, 1782)											1.7							
<i>Calopteryx virgo</i> (Linnaeus, 1758)	1.7														3.3			
<i>Coenagrionidae</i> non det.				6.7						1.7								
<i>Ischnura elegans</i> Vander Linden, 1820																	2.8	1.7
<i>Erythromma najas</i> Hansemann, 1823																	1.7	
<i>Erythromma lindenii</i> (Selys, 1840)																11.7		
<i>Enallagma cyathigerum</i> (Charpentier, 1840)																11.7		
<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)																		1.7
<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)	5.0			1.7											5.0	6.7	10.0	15.0
<i>Orthetrum brunneum</i> (Fonscolombe, 1837)																		1.7
<i>Orthetrum cancellatum</i> (Linnaeus, 1758)																		6.7
Taxa richness	3	1	0	3	0	0	0	0	0	1	1	1	1	1	2	4	5	5
Abundance	13.4	1.7	0.0	11.7	0.0	0.0	0.0	0.0	0.0	1.7	1.7	3.3	6.7	8.3	60.1	36.8	21.8	

The Odonata species richness and abundance varied between study years and habitat types (Figures 2 and 3). Very low Odonata occurrence was observed at reservoirs, tailrace canals, and drainage ditches, where Odonata were detected in low abundances and only in some years, especially in the second half of the study period (after 2017) (Figures 2 and 3). On the other hand, at the old river channels, Odonata were detected during almost the entire study period. The exception was the old river channel next to the Dubrava HPP (D6), where no Odonata were detected between 2013 and 2017 (Figure 2).



**Figure 2.** Odonata taxa richness and abundance at the studied reservoirs and old river channels in the Drava River between 2012 and 2020. Sites of the same habitat groups without Odonata records are not shown.



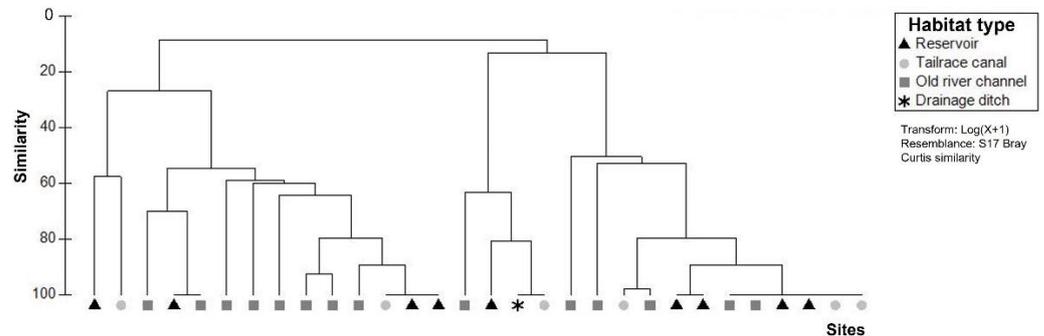
**Figure 3.** Odonata taxa richness and abundance at the studied tailrace canals and drainage ditches in the Drava River between 2012 and 2020. Sites in the same habitat groups without Odonata records are not shown.

In the cluster analysis, Odonata assemblages were mainly not grouped by habitat type—only old river channels partially clustered together (Figure 4).

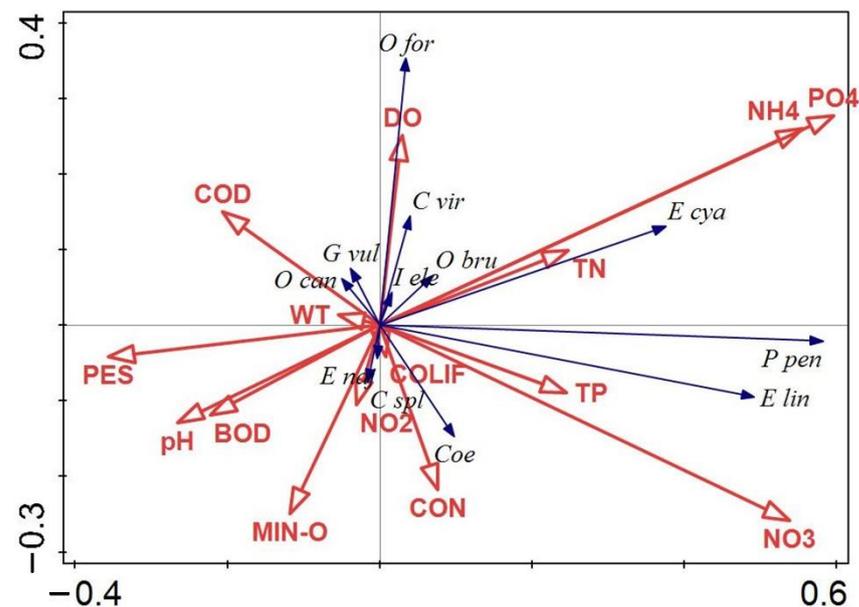
### 3.3. Odonata and Environmental Variables

The results of the ordination of species and environmental data from the RDA are shown in an  $F1 \times F2$  ordination plot (Figure 5). The distribution of Odonata assemblages was highly related to axes 1 and 2; the eigenvalues were 0.19 and 0.04, respectively, yielding a species–environment correlation of 22.5%. A Monte Carlo permutation test indicated that the species–environment ordination was significant (first axis:  $F\text{-ratio} = 31.71$ ,  $p = 0.02$ ; overall:  $\text{trace} = 0.24$ ,  $p = 0.03$ ), suggesting that taxa were significantly related to the tested set of environmental variables. Axis 1 was related to the orthophosphate ( $R = 0.368$ ) and ammonium concentrations ( $R = 0.342$ ) and axis 2 was associated with the nitrate ( $R = -0.100$ ) and mineral oil concentrations in water ( $R = -0.097$ ), suggesting that they were the most important parameters in explaining the patterns of Odonata assemblages in

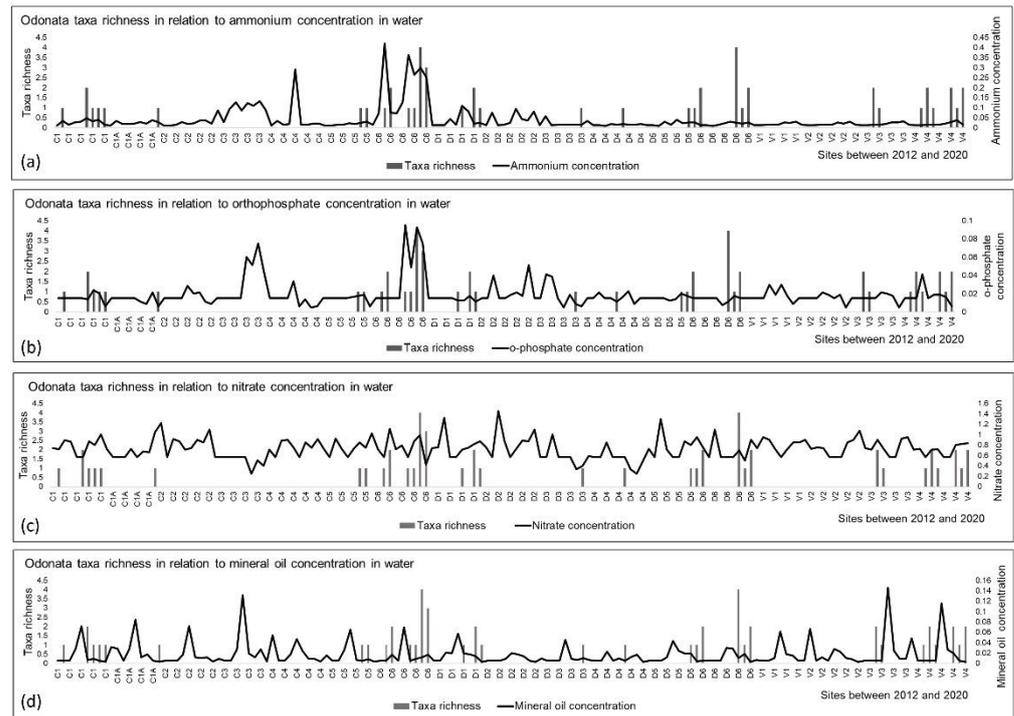
the habitats studied. Odonata taxa richness and/or abundance were higher at sites and in years with higher concentrations of ammonium and orthophosphates in water and lower concentrations of nitrates and mineral oils (Figures 6 and 7).



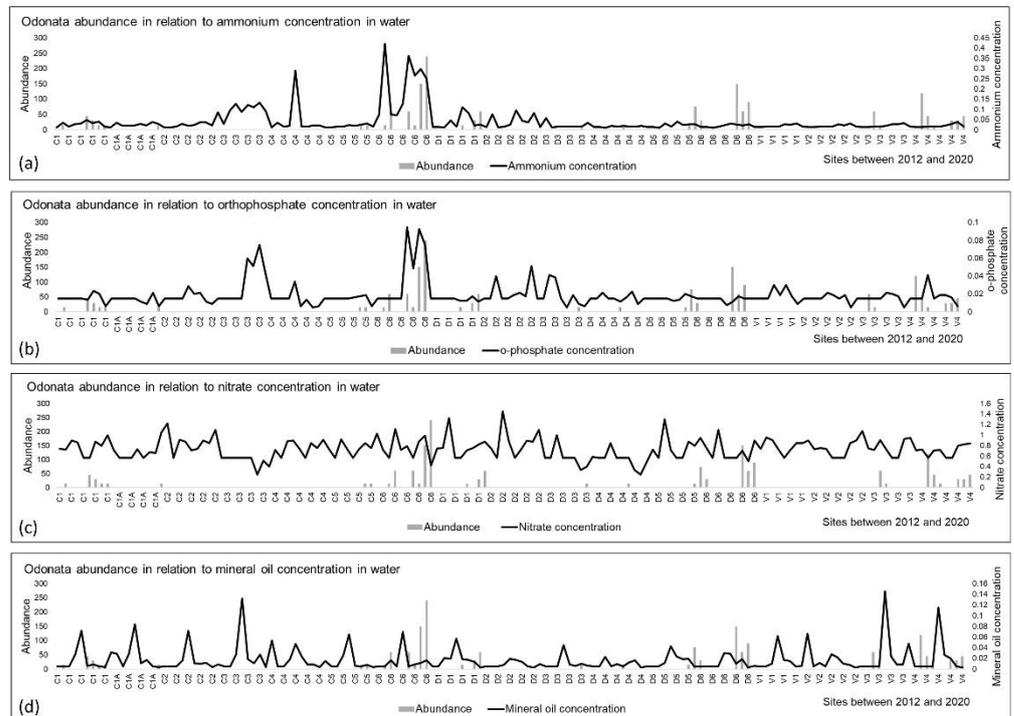
**Figure 4.** Cluster analysis of Odonata assemblages based on the Bray–Curtis similarity coefficient (group average linking) and their log(x + 1)-transformed abundances at anthropogenically impacted habitats in the Drava River between 2012 and 2020. Sites without Odonata records were excluded from the analysis.



**Figure 5.** F1 × F2 plane of redundancy analysis for species–environment relationships for Odonata assemblages in anthropogenically impacted habitats in the Drava River between 2012 and 2020. Taxa abbreviations (blue arrow symbols): *P pen* = *Platynemesis pennipes*, *Cal spl* = *Calopteryx splendens*, *Cal vir* = *Calopteryx virgo*, *Coe* = *Coenagrionidae*, *I ele* = *Ischnura elegans*, *E naj* = *Erythromma najas*, *E lin* = *Erythromma lindenii*, *E cya* = *Enallagma cyathigerum*. *G vul* = *Gomphus vulgatissimus*, *O for* = *Onychogomphus forcipatus*, *O bru* = *Orthetrum brunneum*, and *O can* = *Orthetrum cancellatum*. Environmental factors (red arrow symbols): WT = water temperature (°C), pH = pH value, CON = conductivity (µS/cm), DO = dissolved oxygen (%), BOD = biological oxygen demand (O<sub>2</sub> mg/L), COD = chemical oxygen demand (O<sub>2</sub> mg/L), NH<sub>4</sub> = ammonium concentration (N mg/L), NO<sub>2</sub> = nitrite concentration (N mg/L), NO<sub>3</sub> = nitrate concentration (N mg/L), TN = total nitrogen concentration (N mg/L), TP = total phosphorous concentration (P mg/L), PO<sub>4</sub> = orthophosphate concentration (P mg/L), PES = pesticide concentration (µg/L), MIN-O = mineral oil concentration (mg/L), and COLIF = fecal coliform bacteria (nr/1000 mL).



**Figure 6.** Odonata taxa richness in relation to the (a) ammonium, (b) orthophosphate, (c) nitrate, and (d) mineral oil concentrations in water in anthropogenically impacted habitats in the Drava River between 2012 and 2020.



**Figure 7.** Odonata abundance in relation to the (a) ammonium, (b) orthophosphate, (c) nitrate, and (d) mineral oil concentrations in water in anthropogenically impacted habitats in the Drava River between 2012 and 2020.

#### 4. Discussion

With a total of 11 Odonata species recorded, representing 16% of the Croatian and 8% of the European Odonata fauna [42,43], our results indicate low dragonfly species richness in anthropogenically impacted and man-made habitats in the Drava River system, confirming the results of previous studies [20,21,44]. Although a rather low species richness was expected, one should consider the limitations of the sampling method used, which was not designed as a model for the study of Odonata. Sampling Odonata along with other benthic macroinvertebrates could result in the under-collection of their nymphs because only a relatively small area of habitat is sampled with a benthos hand net [45,46]. Odonata nymphs are relatively large compared with most other benthic macroinvertebrates, and their abundance in benthos samples tends to be low [20,21,44]. In addition, many authors have already emphasized the need to sample all stages of the Odonata life cycle, i.e., including adults and exuviae, in order to obtain a complete list of species at a particular site [45,47–49]. The absence of Odonata at several sites throughout the study period could be explained by the combination of the sampling method used here and the low ecological quality of these habitats. Most likely, these sites were not suitable for Odonata nymph development due to the poor water quality, habitat homogenization, i.e., lack of suitable microhabitats (such as aquatic vegetation), and intense water level or discharge fluctuations [20,21,50,51]. Because Odonata nymphs were not collected throughout the study period, our results confirm the importance of long-term data collection to determine Odonata species richness and population dynamics. Previous long-term studies have already emphasized the disadvantages of short-term studies for capturing the true assemblage composition, pointing out that conclusions from short-term ecological studies can be misleading and reduce the relevance of management activities [52]. In the study by Donly et al. [53], the authors observed different qualitative (species richness and specialization) and quantitative (abundance and dominance) results in long-term studies and long-term monitoring. To cover 95% of the species richness of Odonata, they needed 16 years. Based on their results, a sampling period of at least 10 years is suggested as the ideal period to identify more than 80% of the species.

The Odonata assemblages consisted mainly of generalists, such as *Platycnemis pennipes*, *Erythromma lindenii*, *Ischnura elegans*, and *Calopteryx splendens*, species that are relatively tolerant of the environmental conditions in their habitats [26,53,54], similar to previous studies on man-made and anthropogenically impacted habitats [20,21,44]. Nevertheless, there was an obvious difference in species richness and abundance between sites with significant anthropogenic impacts (reservoirs, tailrace canals, and drainage ditches) and sites with low human influences (i.e., near-natural old river channels). As expected, old river channels were more suitable for the development of a greater number of Odonata species, as such habitats were characterized by sections of slow-flowing water, lentic sections, and rich aquatic and riparian vegetation. Such habitat heterogeneity allows for the completion of the life cycle of both riverine species, such as *Gomphus vulgatissimus* and *Onychogomphus forcipatus*, and standing water Odonata species, such as *Enallagma cyathigerum* and *Erythromma lindenii* [54,55]. In addition, our results suggest that habitats with low anthropogenic influence, such as the old river channels associated with HPP reservoirs, may also provide adequate conditions for some species of conservation concern. There we detected *Erythromma najas*, a species characteristic of habitats with rich aquatic vegetation and standing or slow-flowing water, such as ponds, lakes, canals, and old oxbows [37,42,54]. The species is considered near-threatened in Croatia due to its low dispersal potential, introduction of invasive fish species, degradation of large lowland river floodplains, and construction of HPP reservoirs [42]. Several previous studies have shown that man-made and anthropogenically impacted habitats can harbor protected Odonata species if they are characterized by high habitat heterogeneity, i.e., have well-developed aquatic and riparian vegetation structures [20,21,44,56–58].

Many studies have already shown a close relationship between Odonata and environmental variables that characterize their habitats, such as water velocity, water tem-

perature, pH, oxygen concentration, and substrate composition [20,21,28,50,55,56]. Anthropogenic interventions in freshwater ecosystems alter the morphology, hydrology, and physico-chemical water parameters, which also affect the inhabiting biota, including dragonflies [20,21,44,59–61]. Of the measured physico-chemical water parameters in our study area, Odonata assemblages were most significantly affected by the concentrations of ammonium, orthophosphates, nitrates, and mineral oils in water. Sites and years with higher concentrations of ammonium and orthophosphates in the water had higher numbers of Odonata species or their abundance. Some studies have shown that slightly elevated concentrations of ammonium from animal waste disposal and orthophosphates from pesticides in water can promote aquatic macrophyte growth [62,63]. Aquatic macrophytes are used by Odonata nymphs for hiding from predators and lurking for prey, and by adults for resting, perching, and oviposition [25,64–67]. Higher nutrient concentrations may also have resulted in higher population densities of Odonata prey, such as mayflies, which should be investigated in future analyses. Lower Odonata species richness, abundance, or even their absence was recorded at sites and in years with slightly elevated nitrate and mineral oil concentrations. Nitrates in the Drava River system originate mainly from agricultural activities and wastewater. Sites with slightly elevated nitrate levels, such as reservoirs, were most likely highly unsuitable habitats for Odonata, as the water levels fluctuated greatly, preventing the development of aquatic vegetation. An exception is the very shallow Varaždin HPP reservoir, with its dense *Elodea canadensis* growth, but also dense populations of predatory fish, which most likely also drastically reduce Odonata populations there. Mineral oils are commonly used in agriculture to prevent pest infestations and plant diseases, and negatively affect many terrestrial and some aquatic insects, including some Odonata [67,68].

## 5. Conclusions

Lotic freshwater ecosystems are among the most threatened ecosystems worldwide, affected by multiple stressors, such as intensive land use in their catchments, morphological alterations, flow regulation, pollution, and climate change. The current study confirmed the negative effects of such stressors on inhabiting biota; more precisely, species-poor Odonata assemblages were recorded with low population densities due to the effects of morphological modifications and water pollution. On the other hand, the preservation of near-natural sites in the vicinity of anthropogenically impacted and man-made habitats is important for maintaining the local Odonata fauna, as well as for the preservation of species of conservation concern. Our results highlight the importance of long-term data for identifying the occurrence of Odonata species and monitoring their population dynamics, and can be used for planning of management and protection activities of lowland rivers and their assemblages according to the requirements of the European Water Framework Directive.

**Author Contributions:** Conceptualization, M.V. and Z.M.; methodology, M.V. and Z.M.; validation, M.V. and Z.M.; formal analysis, M.V.; investigation, M.V. and Z.M.; resources, Z.M.; data curation, M.V. and Z.M.; writing—original draft preparation, M.V.; writing—review and editing, M.V. and Z.M.; visualization, M.V.; supervision, M.V. and Z.M.; project administration, Z.M.; funding acquisition, Z.M. Both authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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