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Dragonfly Functional Diversity in Dinaric Karst Tufa-Depositing Lotic Habitats in a Biodiversity Hotspot

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Abstract: Functional diversity is a key component of biodiversity that reflects various dimensions of ecosystem functioning and the roles organisms play within communities and ecosystems. It is widely used to understand how ecological processes influence biotic assemblages. With an aim to increase our knowledge about dragonfly ecological requirements in tufa-depositing karst habitats, we assessed functional diversity of their assemblages, various life history traits (e.g., stream zonation preference, substrate preference, reproduction type), and relationship between functional diversity and physico-chemical water properties in three types of karst lotic habitats (springs, streams, and tufa barriers) in a biodiversity hotspot in the western Balkan Peninsula. Dragonfly functional diversity was mainly characterized by traits typical for lotic rheophile species with medium dispersal capacity. Among the investigated habitats, tufa barriers, characterized by higher (micro)habitat heterogeneity, higher water velocity, as well as lower conductivity and concentration of nitrates, can be considered as dragonfly functional diversity hotspots. Functional diversity and most of the life history traits were comparable among different substrate types in the studied habitats, indicating higher importance of habitat type in shaping dragonfly functional diversity patterns in karst lotic habitats. Our results should be considered in the management and conservation activities of vulnerable karst freshwater ecosystems and their dragonfly assemblages.

Keywords: Odonata; assemblages; Balkan Peninsula; life history traits; environmental variables

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1. Introduction

During the past several decades, numerous ecological studies have improved our knowledge of biodiversity and its temporal and spatial changes as well as our understanding of various ecological phenomena [1–3] by investigating interactions between organisms and their environment through analysis of their functional (or life history) traits (i.e., traits highly influencing the performance of organisms [4]) and functional diversity [2,5]. Various morphological, physiological, phenological, or behavioral species characteristics (i.e., functional (and behavioral) or life history traits) [6,7] enable their survival in a certain environment [7,8]. Based on those characteristics, aquatic organisms can be placed into functional groups based on, for instance, their trophic position (e.g., grazers, shredders, collectors, predators), habitat preference (e.g., lotic, lentic, eurytopic), current preference (e.g., limnophile, rheophile), and substrate preference (e.g., phytal, lithal, fine sediments) [9]. Functional diversity (diversity of functional groups (traits) of species within an assemblage) is therefore a biodiversity component that reflects various aspects of ecosystem functioning, such as ecosystem productivity, dynamics, nutrient balance [1,10], as well as the role of organisms within the communities and ecosystems [6]. It can also be used to understand the influence of ecological processes on biotic assemblages [11].

The Dinaric Mountains, located along the western Balkan Peninsula (from northeastern Italy to Albania) are the largest continuous karst landscape in Europe, with their length of over 600 km [12]. Karst habitats are formed through the dissolution of carbonate rocks and are characterized by highly diverse morphological, hydrological, and geological characteristics [13]. One of the unique features of freshwater habitats in karst regions is tufa, a secondary deposition of calcium carbonate resulting from the interaction between the geological bed rock, physico-chemical water properties, and inhabiting biota, especially the bryophytes [14]. Hence, due to high habitat heterogeneity, Dinaric karst freshwater habitats are characterized by high biodiversity, including numerous endemic species, which is why they have long been recognized as biodiversity hotspots, e.g., [15–18].

Dragonflies (Odonata) are merolimnic insects commonly used as bioindicators of the condition and integrity of freshwater ecosystems, e.g., [19], due to many of their life history traits, e.g., [20,21]. For instance, good taxonomic knowledge allows their rather easy identification at the species level [22]. Different species have different ecological requirements, such as those for habitat type, substrate composition, and riparian and aquatic vegetation structure [23,24]. Also, due to their fast life cycles, behavioral traits, and sensitivity to habitat alteration, many dragonfly species quickly respond to changes in their habitats, including both aquatic and surrounding terrestrial ones [7,25]. As predators, they are highly important in both aquatic and terrestrial food webs, having one of the most important roles in controlling the population densities of other insects, such as mosquitoes [22]. Moreover, they play a crucial role in transferring biomass and energy from aquatic ecosystems to terrestrial food webs [26].

In ecological research, the understanding of the processes shaping biotic assemblages is of fundamental importance [27], which can be achieved through analysis of assemblages' functional traits. In a previous study [23], only the taxonomic aspect was studied (i.e., dragonfly taxonomic assemblage metrics were analyzed) in the Dinaric karst tufa-depositing lotic habitats in the western Balkan Peninsula. Hence, the main goals of this study were to increase our knowledge about dragonfly ecological requirements in such unique habitats by assessing differences in their functional diversity and life history traits (e.g., body shape, dispersal capacity, stream zonation preference, reproduction) among the three habitat and four main substrate types in the Dinaric karst tufa-depositing lotic habitats in the western Balkan Peninsula. Additionally, we wanted to determine the main physico-chemical water parameters shaping functional diversity of dragonfly assemblages in the selected habitats.

2. Materials and Methods

2.1. Study Area

The study was conducted in a biodiversity hotspot [28,29], specifically in the Plitvice Lakes National Park (NP), located in Croatia's Dinaric karst region (Supplementary Table S1; for a map of the study area, see Vilenica et al. [30]). The Plitvice hydrosystem comprises 16 lakes connected by tufa barriers, along with several small rivers and streams that act as the primary surface water sources for the lakes [31].

The climate in the area of the Plitvice Lakes NP is humid with warm summers (CfB, Koppen climate classification) [32]. The mean annual temperature is 8 °C, while the mean annual precipitation is 1500 mm [33]. During the study period (2007), the mean annual air temperature was 11.4 °C and the mean annual rainfall was 1664.1 mm (see also in Vilenica [23]).

The study sites encompassed three types of tufa-depositing lotic karst habitats: springs, streams (also including small mountainous rivers) and tufa barriers (for details see, e.g., Vilenica [23], Vilenica et al. [30]) (Figure 1).

2.2. Environmental Variables

Every month over a one-year period, at every study site, we measured the following physico-chemical water properties above each microhabitat that was sampled: oxygen concentration, oxygen saturation, water temperature (using the oximeter WTW Oxi 330/SET), pH (using the pH meter WTW pH 330), conductivity (using the conductivity meter WTW LF 330), alkalinity (by titration with 0.1 M HCl), water velocity (using the P-670-M velocimeter), and nutrients (ammonium by HRN ISO 70-3:1998 method and nitrates by HRN ISO 7890-3:2001 method) (see more in Vilenica [23]).

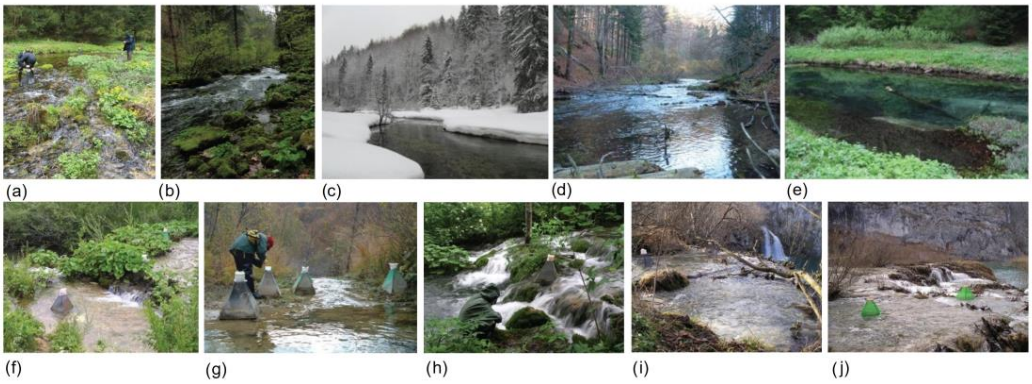

Figure 1. Photo examples of study sites included in the study: springs: (**a**) Bijela rijeka spring, (**b**) Crna rijeka spring; streams (and small mountainous rivers): (**c**) Bijela rijeka middle reaches, (**d**) Crna rijeka middle reaches, (**e**) Crna rijeka lower reaches, (**f**) Plitvica, (**g**) Korana; tufa barriers: (**h**) Labudovac, (**i**) Kozjak–Milanovac, (**j**) Novakovića Brod.

2.3. Dragonfly Sampling

Macrozoobenthos sampling (including dragonfly nymphs) was conducted every month between February 2007 and February 2008 at ten study sites belonging to the three above-mentioned Dinaric karst tufa-depositing lotic habitats (Figure 1, Supplementary Table S1) in the Plitvice Lakes NP, Croatia.

At each site and each sampling event, samples were taken in all dominant substrate types (those with a share of at least 5% coverage (bryophytes (mosses and liverworts), cobbles, sand, and silt with leaf litter), defined according to Wentworth [34]).

Samples were collected following the standard macrozoobenthos sampling methodology as described in Vilenica [23], i.e., using Surber samplers (mesh size: 0.5 mm; surface area: 14 × 14 cm on bryophytes and 25 × 25 cm on all other microhabitats). Microhabitats at the lower reaches of Crna rijeka were due to the greater water depth, sampled using a D-frame hand net (mesh size: 0.5 mm; surface area: 25 × 25 cm). At each study site, 36 macroinvertebrate samples were collected over a one-year period (i.e., 360 samples in total). Dragonfly abundance was calculated as number of individuals per m^2. Species were identified using relevant identification keys [35–37]. The voucher specimens are at the Department of Biology, Faculty of Science, Zagreb, Croatia.

2.4. Data Analysis

In a previous study [23], a total of eight dragonfly species were recorded (Table 1, Supplementary Table S2). Prior to the analyses, all quantitative data were tested for normality using the Shapiro–Wilk test in Statistica, version 10.0 [38].

The functional diversity of dragonfly assemblages was quantified using a total of 36 functional traits from seven groups of functional (life history) traits (Table 2) (taken from Schmidt-Kloiber and Hering [9]; Dijkstra et al. [39]). The assignment of a species to a particular functional trait category within each functional trait group used is based on a single category assignment system (as in the case of body type and dispersal capacity trait groups) or a 10-point assignment system (as in the case of the rest of the functional traits used) (see in Dijkstra et al. [38]).

The Rao quadratic diversity (RaoQ) coefficient is a measure of functional diversity that considers both the differences between species (in terms of their functional traits) and their relative abundances, and it was used to measure the functional diversity of dragonflies in the studied habitats. This coefficient reflects patterns of trait convergence or divergence relative to what would be expected by chance. To assess shifts in mean trait values within dragonfly assemblages, community weighted mean (CWM) values were calculated (combining species-specific functional traits with the relative abundance of each species within the assemblage) for each functional trait, with an aim to capture the effects of environmental selection on specific functional trait categories [40]. Both RaoQ and CWM values were calculated using the CANOCO software package, version 5.15 [41].

Table 1. Dragonfly species recorded at three lotic habitat types in the Plitvice Lakes NP, Croatia (for details see Vilenica [23]).

Dragonfly Species/Habitat Type	Springs	Streams	Tufa Barriers
Gomphus vulgatissimus (Linnaeus, 1758)			X
Onychogompus forcipatus (Linnaeus, 1758)		X	X
Cordulegaster bidentata (Selys, 1843)		X	X
Orthetrum coerulescens (Fabricius, 1798)			X
Crocothemis erythraea (Brullé, 1832)			X
Platycnemis pennipes (Pallas, 1771)		X	X
Calopteryx virgo (Linnaeus, 1758)			X
Coenagrion puella (Linnaeus, 1758)			X
Number of species (S)	0	3	8

Table 2. Dragonfly functional traits used for quantifying dragonfly functional diversity at three lotic habitat types in the Plitvice Lakes NP, Croatia.

Functional Trait Group	Functional Trait	Explanation
Body type	Anisoptera Zygoptera	
Dispersal capacity	High Medium	
Stream zonation preference	Metarhithral Hyporhithral Epipotamal Metapotamal Hypopotamal Littoral	lower trout region grayling region barbel region bream region brackish water region lentic habitats
Lateral connectivity preference	Eupotamon Parapotamon Plesiopotamon (including lakes) Palaeopotamon (including pools, ponds) Temporary waterbodies	lotic habitats lentic habitats
Current preference	Limnophile	preferring lentic habitats, rarely also occur in slow-flowing lotic habitats
	Limno- to rheophile	preferring lentic habitats, but often also in slowly flowing lotic habitats
	Rheo- to limnophile	preferring slow-flowing lotic habitats and their lentic zones, can also be found in lentic habitats
	Rheophile	occurring in lotic habitats, preferably with moderate and fast water velocity

Table 2. *Cont.*

Functional Trait Group	Functional Trait	Explanation
Substrate type preference	Argyllal	silt, loam, clay
	Pelal	mud
	Psammal	sand
	Akal	fine- to medium-sized gravel
	Lithal	coarse gravel, stones, cobbles, boulders, bedrock
	Phytal	algae, bryophytes, macrophytes
	POM	particulate organic matter
Reproduction	Reproduction mode and the form and location of oviposit clutches	eggs laid attached to substrate
		eggs laid into the substrate
		eggs laid not attached to/in substrate
		eggs laid into open water
		eggs laid inside plant tissue
		eggs laid onto plant material
		eggs laid on exposed soil or rock

The differences in physico-chemical water parameters among the three habitat types (springs, streams, and tufa barriers) as well as functional metrics among the three habitat types and among the substrate types (cobbles, bryophytes, sand, silt with leaf litter) were tested using the Kruskal–Wallis H test, followed by a multiple comparisons *post hoc* test to determine which groups differ from each other. Those analyses were performed in Statistica, version 10.0 [38].

To assess the impact of physico-chemical water parameters on the spatial distribution of CWM values for functional traits in dragonfly assemblages, a redundancy analysis (RDA) was conducted. Before performing the RDA, dragonfly abundances were centered and standardized based on average functional traits. The analysis included CWM data for eight dragonfly species and six physico-chemical water parameters that showed significant differences among habitat types. The statistical significance of the relationship between dragonfly functional traits and physico-chemical water parameters was assessed using a Monte Carlo permutation test with 499 permutations. Both the RDA and Monte Carlo tests were conducted using the CANOCO package, version 5.15 [41].

3. Results

3.1. Environmental Variables

Concentration of nitrates (Kruskal–Wallis H test, N = 118, DF = 2; H = 19.66, $p < 0.001$), pH (H = 34.06, $p < 0.001$), oxygen saturation (H = 13.19, $p < 0.01$), water velocity (H = 10.97, $p < 0.01$), conductivity (H = 40.43, $p < 0.001$), and alkalinity (H = 32.66, $p < 0.001$) significantly differed among the three habitat types (Figure 2).

The multiple comparisons *post hoc* test showed that springs had lower oxygen saturation ($p < 0.01$) and lower pH ($p < 0.001$) compared to streams and tufa barriers. Water velocity was higher in tufa barriers compared to springs ($p < 0.01$), and conductivity, alkalinity, and concentration of nitrates were lower in tufa barriers compared to springs and streams ($p < 0.001$) (Figure 2).

Water temperature (H = 5.12, $p > 0.05$), oxygen (H = 4.83, $p > 0.05$), and ammonia concentrations (H = 1.78, $p > 0.05$) were comparable among the three habitat types (Figure 3).

3.2. Dragonfly Life History Traits and Functional Diversity at Different Karst Lotic Habitats

Dragonfly functional diversity (RaoQ index) was significantly higher at tufa barriers (Kruskal–Wallis H test, N = 30, DF = 2; H =16.46, $p < 0.001$) compared to springs ($p < 0.001$) and streams ($p < 0.05$) (Supplementary Table S3, Figure 4).

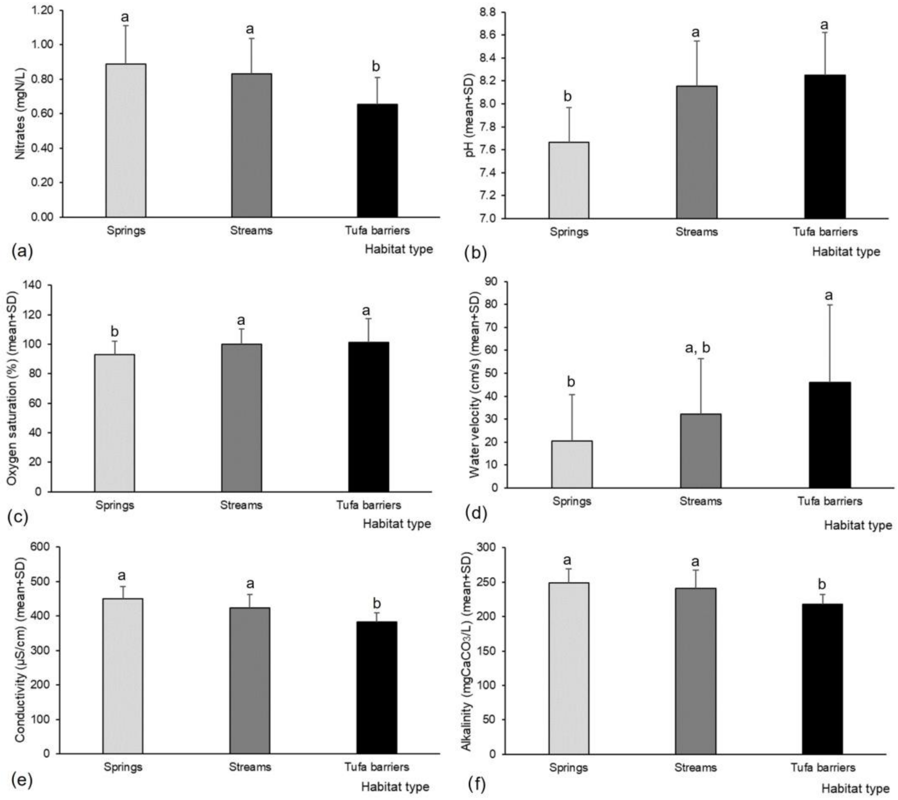

Figure 2. Environmental variables at three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia (shown as mean annual values with standard deviation, SD): (**a**) nitrate concentration, (**b**) pH, (**c**) oxygen saturation, (**d**) water velocity, (**e**) conductivity, and (**f**) alkalinity. Significant differences among the habitat types are indicated by different letters (Kruskal–Wallis H test with multiple comparisons *post hoc* test, $p < 0.05$).

Significant differences among the habitat types were recorded for the following life history traits: the share of Zygoptera (Kruskal–Wallis H test, N = 30, DF = 2; H = 14.00, $p < 0.01$), Anisoptera body types (H = 9.86, $p < 0.01$), species with medium dispersal capacity (H = 12.03, $p < 0.01$), species preferring metarhithral (H = 17.94, $p < 0.001$), hyporhithral (H = 14.88, $p < 0.001$), and epipotamal (H = 10.69, $p < 0.01$) stream sections, littoral-preferring species (H = 12.83, $p < 0.01$), eupotamon (H = 11.12, $p < 0.01$) and parapotamon (H = 13.80, $p < 0.01$) species, species preferring microhabitats with psammal (H = 10.32, $p < 0.01$), akal (H = 10.97, $p < 0.01$), lithal (H = 12.84, $p < 0.01$), phytal (H = 14.00, $p < 0.01$), and particulate organic matter (H = 11.42, $p < 0.01$) substrates, rheophile species (H = 10.72, $p < 0.01$), species laying the eggs into the substrate (H = 10.82, $p < 0.01$), not attached to or in the substrate (H = 10.97, $p < 0.01$), into open water (H = 10.97, $p < 0.01$), and inside plant tissue (H = 14.00, $p < 0.01$) (Supplementary Table S3, Figure 5).

The multiple comparisons *post hoc* test showed that tufa barriers had a higher share of Zygoptera compared to springs ($p < 0.05$) and streams ($p < 0.05$), and tufa barriers had a higher share of Anisoptera compared to springs ($p < 0.05$). A higher share of species preferring metarhithral and hyporhithral stream sections were recorded at tufa barriers compared to the other two habitats ($p < 0.01$), and a higher share of epipotamal and littoral species were recorded at tufa barriers compared to springs ($p < 0.001$). Higher shares of eupotamon ($p < 0.01$) and parapotamon ($p < 0.01$) species were recorded at tufa barriers compared to springs. Higher shares of species preferring microhabitats with psammal, akal, lithal, and particulate organic matter substrates ($p < 0.01$) were recorded in tufa barriers compared to springs, while tufa barriers also had a higher share of species preferring phytal compared to springs and streams ($p < 0.05$). A higher share of rheophile species was recorded at tufa barriers compared to springs ($p < 0.01$), as well as of species laying their eggs into the substrate ($p < 0.01$), not attached to or in the substrate ($p < 0.05$), and into open water ($p < 0.01$), while tufa barriers also had a higher share of species laying their eggs inside plant tissue compared to springs and streams ($p < 0.05$) (Figure 5).

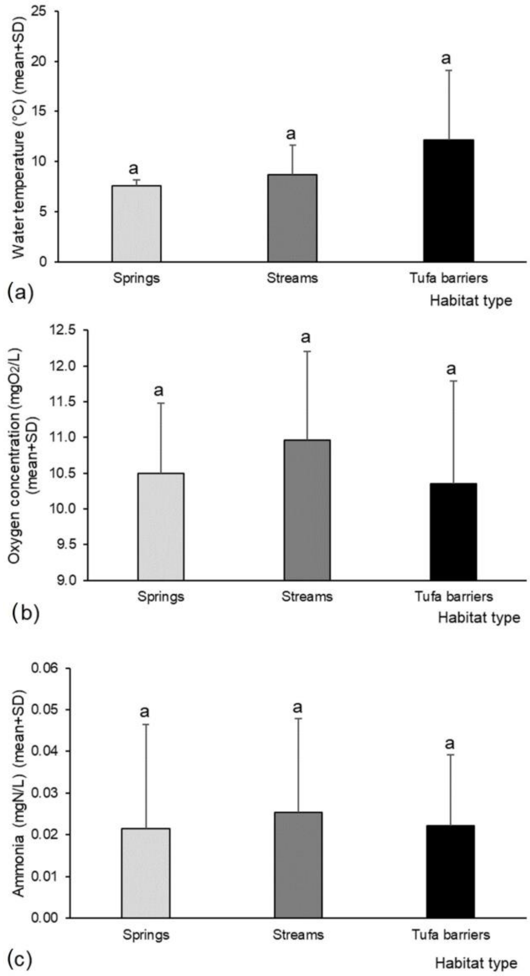

Figure 3. Environmental variables at three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia (shown as mean annual values with standard deviation, SD): (**a**) water temperature, (**b**) oxygen concentration, and (**c**) ammonia concentration. Non-significant differences among the habitat types are indicated by the letter a (Kruskal–Wallis H test with multiple comparisons *post hoc* test, $p > 0.05$).

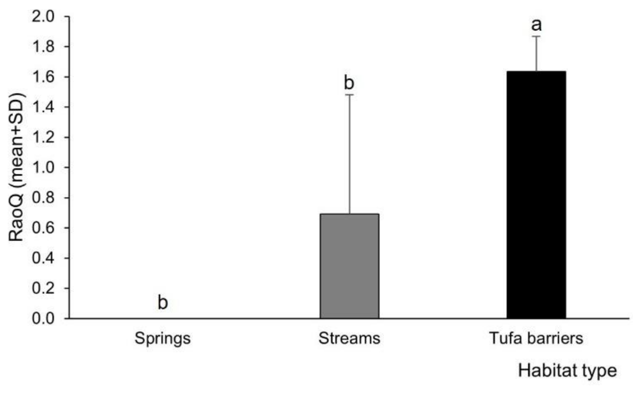

Figure 4. Dragonfly functional diversity (RaoQ index) at three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia (shown as mean with standard deviation, SD). Significant differences among the habitat types are indicated by different letters (Kruskal–Wallis H test with multiple comparisons *post hoc* test, $p < 0.05$).

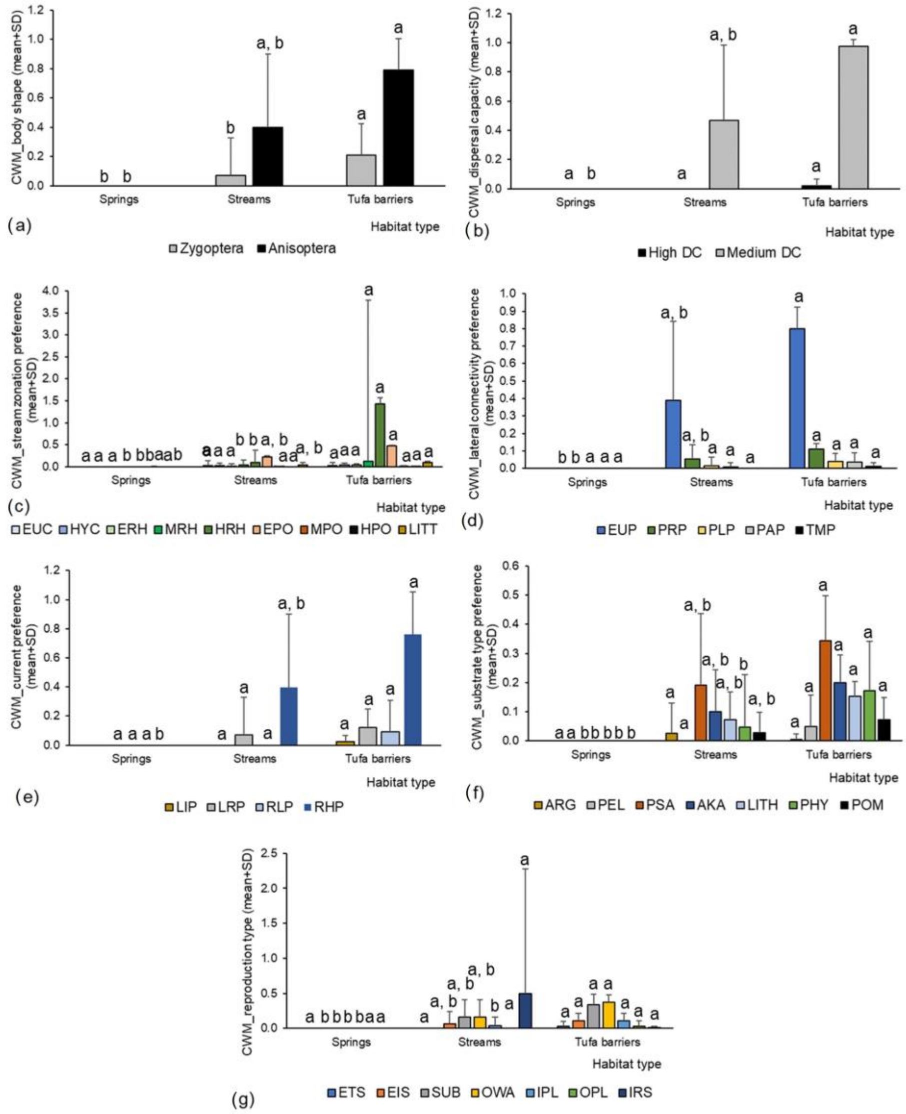

Figure 5. Dragonfly functional traits at three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia (shown as mean with standard deviation, SD): (**a**) body shape, (**b**) dispersal capacity, (**c**) stream zonation preference, (**d**) lateral connectivity preference, (**e**) current preference, (**f**) substrate type preference, and (**g**) reproduction type. Significant differences among the habitat types are indicated by different letters (Kruskal–Wallis H test with multiple comparisons *post hoc* test, $p < 0.05$). Legend: DC = dispersal capacity; EUC = eucrenal, HYC = hypocrenal, ERH = epirhithral, MRH = metarhithral, HRH = hyporhithral, EPO = epipotamal, MPO = metapotamal, HPO = hypopotamal, LITT = littoral; EUP = eupotamon, PRP = parapotamon, PLP = plesiopotamon, PAP = palaeopotamon, TMP = temporary water bodies; LIP = limnophil, LRP = limno- to rheophil, RLP = rheo- to limnophil, RPH = rheophil; ARG = argyllal, PEL = pelal, PSA = psammal, AKA = akal, LITH = lithal, PHY = phytal, POM = particulate organic matter; ETS = eggs laid attached to substrate, EIS = eggs laid in substrate, SUB = eggs laid not attached to or in substrate, OWA = eggs laid in open water, IPL = eggs laid inside plant tissue, OPL = eggs laid onto plant material, IRS = eggs laid into submerged soil or onto submerged rock.

Other dragonfly life history traits were comparable among the three habitat types ($p > 0.05$), or significance was marginal (i.e., a multiple comparisons *post hoc* test did not determine differences between the habitat pairs) (Supplementary Table S3, Figure 5).

3.3. Dragonfly Functional Traits and Environmental Variables

The RDA analysis (Figure 6) showed significant differences in the dragonfly functional traits among the three habitat types, with explanatory variables accounting for 54.80% of the variance (F ratio = 4.85, DF = 6, $p = 0.002$). The first two ordination axes (eigenvalues of 0.49 and 0.04) explained 53.26% of the variation. The first axis showed the strongest correlation with conductivity (R = −0.76), while the second axis was primarily correlated with nitrate concentration in water (R = 0.18) and water velocity (R = −0.15).

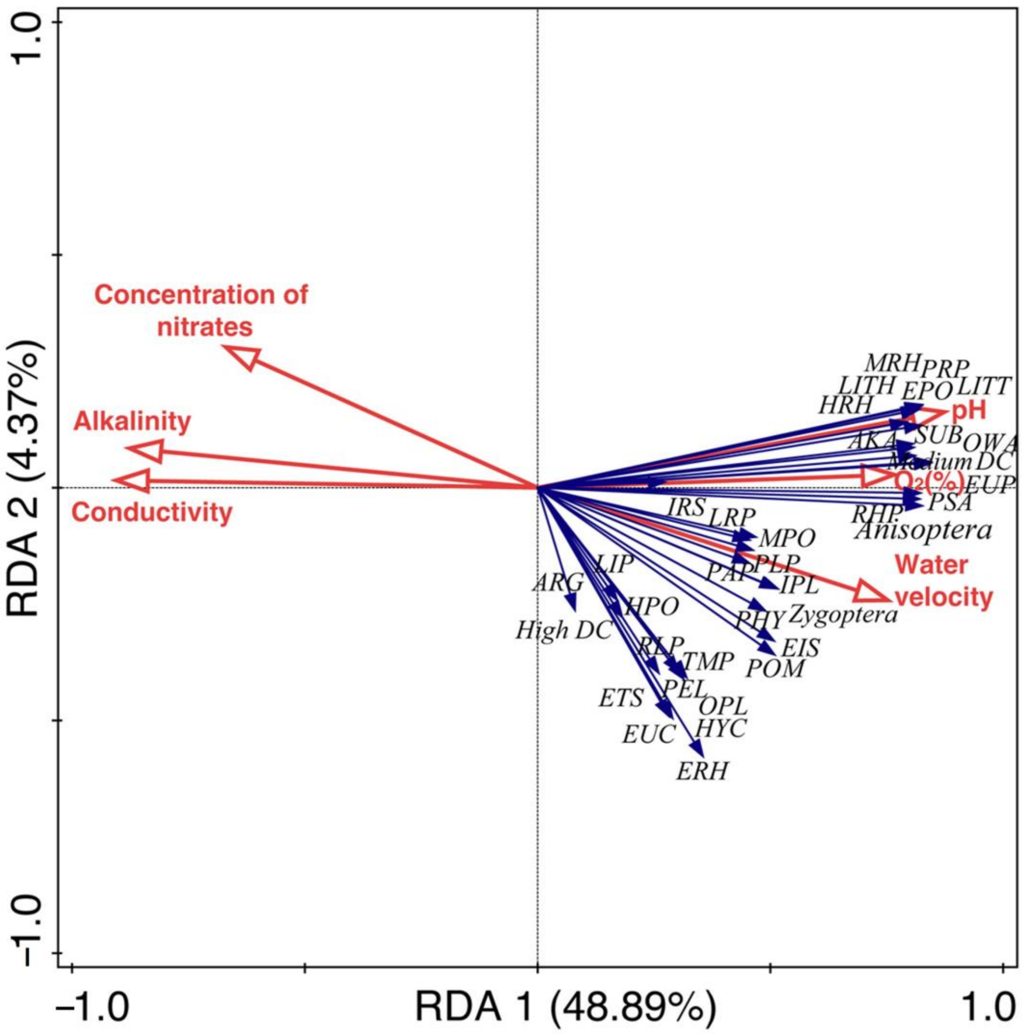

Figure 6. Redundancy analysis (RDA) ordination biplot showing the relationship between dragonfly functional traits and six significant environmental variables in Dinaric karst lotic habitats in the Plitvice Lakes NP, Croatia. Abbreviations of the functional (life history) traits are in Figure 4.

3.4. Dragonfly Life History Traits and Functional Diversity at Different Substrate Types

Dragonfly functional diversity (RaoQ index) (Figure 7) and most life history traits (Figure 8) were comparable among the four main substrate types in the studied lotic habitats, or differences were marginally significant (Kruskal–Wallis H test, N = 30, DF = 2; Supplementary Table S4). In terms of life history traits, only the share of species preferring microhabitats with particulate organic matter substrates was significantly higher (Kruskal–Wallis H test, N = 30, DF = 3; H = 11.22, $p < 0.05$) at silt with leaf litter compared to bryophytes substrates ($p < 0.05$) (Supplementary Table S4, Figure 8).

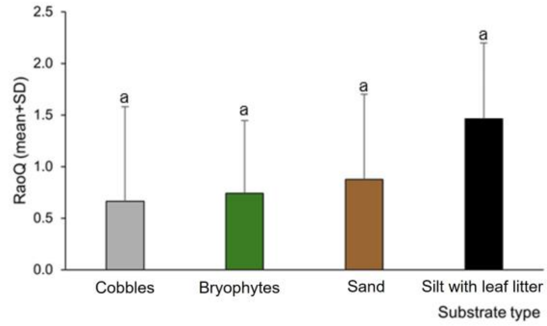

Figure 7. Dragonfly functional diversity (RaoQ index) at four main substrate types in three Dinaric karst lotic habitats in the Plitvice Lakes NP, Croatia (shown as mean with standard deviation, SD) Non-significant differences among the habitat types are indicated by the letter a (Kruskal–Wallis H test with multiple comparisons *post hoc* test, *p* > 0.05).

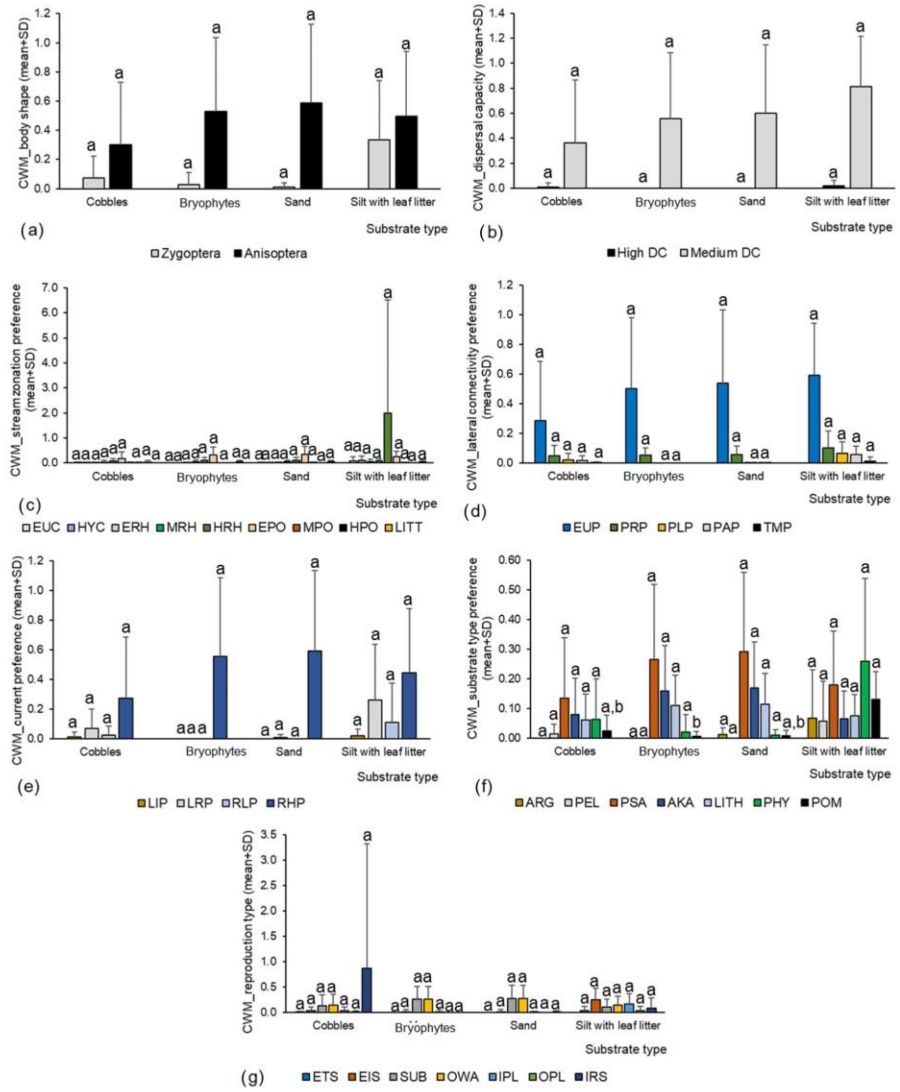

Figure 8. Dragonfly functional traits at four main substrate types in three Dinaric karst lotic habitats in the Plitvice Lakes NP, Croatia (shown as mean with standard deviation, SD): (**a**) body shape, (**b**) dispersal capacity, (**c**) stream zonation preference, (**d**) lateral connectivity preference, (**e**) current preference, (**f**) substrate type preference, and (**g**) reproduction type. Significant differences among the habitat types are indicated by different letters (Kruskal–Wallis H test with multiple comparisons post hoc test, *p* < 0.05). Abbreviations of the functional (life history) traits are in Figure 5.

4. Discussion

The Plitvice barrage-lake hydrosystem represents a rather harsh environment due to rather low water temperature and productivity [42,43], but also high conductivity and alkalinity [12,44]. Although this system is considered to be a biodiversity hotspot (e.g., [16]), for some groups of aquatic organisms, such as dragonflies, it could be challenging to cope with such conditions, in combination with the presence of alien fish species [45], which are generally known to negatively influence dragonfly abundance [46]. Most probably for these reasons, a rather low number of dragonfly species (i.e., eight) occurs in the system's lotic habitats [23]. Moreover, only a low share of European dragonflies is specialized to inhabit forest streams with cold-water and/or high-water current [39,47], especially within the studied geographical range. However, most of the recorded species were previously reported from karst habitats [48,49]. Our results showed that despite the low dragonfly taxonomic diversity, their functional diversity is rather high in Dinaric karst tufa-depositing habitats in the western Balkan peninsula [38]. Among the three studied habitat types, namely springs, streams, and tufa barriers, the diversity of dragonfly functional traits was the highest at the latter, similar to as found for taxonomic assemblage metrics [23]. In our study area, functional diversity was predominantly characterized by traits characteristic for specialists, lotic rheophile species, in accordance with habitat characteristics. Moreover, most of the recorded species were those with medium dispersal capacity, a trait typical for lotic species [50], as those can more easily disperse along their habitats compared to species that inhabit patchy lentic habitats, which then in turn have higher dispersal abilities [51].

Due to higher taxonomic diversity [23], tufa barriers consequently had a higher share of both the Anisoptera and Zygoptera body shape trait, streams were inhabited only by anisopterans *Cordulegaster bidentata* and *Onychogomphus forcipatus* and zygopteran *Platycnemis pennipes*, while no species were recorded at springs [23]. Tufa barriers had the highest share of rheophile lotic species preferring the upper reaches of lotic habitats (such as *Cordulegaster bidentata*, *Orthetrum coerulescens*, and *Calopteryx virgo*), but also some species that preferably occur in the lower reaches of running waters (*Gomphus vulgatissimus*, *Onychogomphus forcipatus*), and those typical for lentic habitats (*Crocothemis erythraea*, *Coenagrion puella*) were found there [23,39]. As explained in Vilenica [23], lentic species present at lotic tufa barriersnatural lake outlets, most probably have drifted from the upstream lakes [52]. Due to (micro)habitat heterogeneity, species with various substrate preferences were recorded at tufa barriers. Hence, a higher share of species occurring in akal (*Onychogomphus forcipatus*), psammal (e.g., *Gomphus vulgatissimus*, *Onychogompus forcipatus*, *Cordulegaster bidentata*), lithal (e.g., *Onychogomphus forcipatus*, *Calopteryx virgo*), phytal (e.g., *Crocothemis erythraea*, *Platycnemis pennipes*, *Calopteryx virgo*), and particulate organic matter (e.g., *Cordulegaster bidentata*, *Orthetrum coerulescens*) substrates was recorded there [39]. Moreover, a rather high diversity of available microhabitats resulted in tufa barriers being represented by higher ethodiversity [7], i.e., higher variability of behavioral traits related to reproduction type (oviposition), where we documented a higher share of species laying eggs into the substrate (e.g., *Cordulegaster bidentata*, *Platycnemis pennipes*), not attached to the substrate (e.g., *Gomphus vulgatissimus*, *Onychogompus forcipatus*), into open water (e.g., *Orthetrum coerulescens*, *Crocothemis erythraea*), and into plant material (*Platycnemis pennipes*, *Calopteryx virgo*, *Coenagrion puella*) [39]. Although studies focused on dragonfly functional diversity in the European karst lotic habitats are still rare, some showed similar results, where higher dragonfly functional diversity was related to higher habitat heterogeneity [24]. Moreover, studies conducted on other aquatic insects in the same study area, such as caddisflies and mayflies, showed that functional traits (functional feeding groups and stream zonation preference of species) changed along with the habitat types, characterized by differences in microhabitat composition and abiotic water parameters [53,54]. Therefore, our results confirm the importance of habitat and microhabitat heterogeneity not only for taxonomic [23] but also functional diversity of dragonfly assemblages [30,55]. In addition, tufa barriers have already been recognized as special habitats with high vulnerability and high complexity in terms of their hydrogeological, hydrological, and biological characteristics and defined as freshwater reefs where high biodiversity prevails, e.g., [56,57].

Higher dragonfly functional diversity was associated with lower conductivity and concentration of nitrates as well as higher water velocity, parameter values associated with tufa barriers. The slightly elevated nitrate concentrations and corresponding increase in conductivity could be attributed to the substantial rise in tourism pressure within the National Park area [58], which is why the water quality is recommended to be systematically monitored together with populations of freshwater communities. However, even though dragonflies are considered as good indicators of habitat and water quality, many studies have shown that their assemblages respond more strongly to habitat degradation (particularly to changes in hydro-morphology of the waterbody and in the structure of aquatic and riparian vegetation) than to water pollution [59–61]. A previous study determined water temperature amongst the most important physico-chemical water parameters influencing the occurrence of dragonfly species and their taxonomic diversity in lotic habitats of the Plitvice barrage-lakes system [23]. In line with these findings, tufa barriers, characterized by higher water temperature, diverse microhabitats, and abundant food resources, were identified as the most suitable habitats for the greatest number of dragonfly species [23,30,62]. In contrast, low water temperature in the studied karst springs was most probably one of the most important determinants of dragonfly absence from such habitats [23], similar to the findings of Cíbik et al. [63]. Therefore, instead of defining the most important physico-chemical water parameters shaping dragonfly functional diversity in the studied habitats, we suggest that the interplay of physico-chemical water parameters, microhabitat diversity, and most likely food availability and predator presence, had a synergistic impact, and resulted in tufa barriers being the most suitable habitats for the highest number of species, and consequently in the highest dragonfly functional diversity.

Functional diversity and most of the life history traits were comparable among different substrate types, even though the previous study of [23] showed that the recorded species mainly avoided microhabitats with bryophyte substrates and the highest water velocity, while higher species richness was associated with microhabitats with lithal and psammal and lower water velocities. Significant differences were only found in species preferring microhabitats with fine substrates (i.e., particulate organic matter) [39], such as *Orthetrum coerulescens*, being the most abundant at microhabitats with silt and leaf litter (see also Vilenica [23]).

5. Conclusions

Our study confirmed the high value of highly sensitive tufa barriers as local diversity hotspots. The presented results showed that functional diversity of dragonfly assemblages in Dinaric karst lotic habitats is influenced by the interplay of physico-chemical water properties, microhabitat composition, and most probably food availability and predator presence. Habitats with higher (micro)habitat heterogeneity (i.e., tufa barriers) supported higher functional diversity of dragonfly assemblages, thus acting as functional diversity hotspots within the studied karst lotic system and highlighting the valuable use of this approach in ecological research and the planning of conservation activities. Preservation of natural habitat structure, variability of microhabitats, and removal of alien fish species should be imperative to preserve vulnerable karst lotic systems and their dragonfly assemblages.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d16100645/s1: Table S1: Geographic coordinates and altitude of the study sites belonging to three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia.; Table S2: Dragonfly species and their mean abundances (number of individuals per m^2) recorded at ten study sites belonging to three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia. Legend: BRS = Bijela rijeka spring, CRS = Crna rijeka spring; BRMR = Bijela rijeka middle reaches, CRMR = Crna rijeka middle reaches, CRLR = Crna rijeka lower reaches, PL = Plitvica, KR = Korana; LB = Labudovac, KM = Kozjak-Milanovac, NOB = Novakovića Brod.; Table S3: Differences (Kruskal-Wallis H test with multiple comparisons *post hoc* test) in community weighted mean (CWM) values of drag-onfly functional traits among three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia. SP = springs, ST = streams, TB = tufa barriers. Significant results are in bold.; Table S4: Differences (Kruskal-Wallis H test with multiple comparisons *post hoc* test) in community weighted mean (CWM) values of dragonfly functional traits among three Dinaric karst lotic habitat types in the Plitvice Lakes NP, Croatia. B = bryophytes, SLL = silt with leaf litter. Significant results are in bold.

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