

## Article

# Interactions between Fish and Invertebrates in the Lowland Area of the Sava River following Excessive Change in Hydrological Regime

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**Abstract:** The littoral zones of freshwaters are highly susceptible to extreme hydrological fluctuations caused by climate-induced changes in the water cycle. Disturbances in the hydrology could affect fish assemblages and their trophic interactions with invertebrates, which constitute a large part of fish diets. In 2014 and 2015, the littoral zone of the Sava River (Croatia) was studied to determine the influence of hydrological extremes on (1) fluctuations in environmental drivers and biocoenoses, and (2) the trophic relationships between fish, macroinvertebrates and zooseston, in an attempt to reveal their trophic interactions. Biocoenotic components showed different tolerance to extreme discharge, resulting in remarkable reductions in fish abundance, diversity, biomass, size and, presumably due to dilution, the abundance of zooseston, which is an important food for fish larvae. By contrast, benthic macroinvertebrates did not show significant fluctuations in abundance, but the share of benthic groups of organisms was shifted during high discharge. Gastropods and amphipods were found to be important food sources for fish. The present study helps to highlight the consequences of hydrological disturbances caused by climate change: the enhancement of stressors in riverine littoral habitats and inhabited communities.

**Keywords:** Sava River; precipitations; macrozoobenthos; zooseston; food web interactions



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## 1. Introduction

Riverine ecosystems exhibit diverse and varied hydrological, geological and limnological conditions along with longitudinal and transverse profiles [1,2], where floods and droughts, two opposite natural components of streamflow regimes, are key drivers of environmental conditions and biocoenoses [3]. Globally, climate change affects the water cycle and, thus, precipitation patterns, resulting in heavy rainfall with intense flooding and the transport of matter from terrestrial into aquatic systems, simultaneously leaching fertilizer and soil from the field into the river [4–7].

This study attempted to consider the impact of extremely high rainfall precipitation on the hydrological regime of lowland riverine littoral habitats. During highwater level many benthic and planktonic organisms are displaced may be transported to unfavorable environments. It also harms them by damaging them physically, diluting their food resources, and hindering reproduction [8–11]. Expectedly, numerous studies in lotic habitats refer to fish [12–15] and benthic macroinvertebrates, i.e., insect larvae [16,17], bivalves [18,19] and gastropods [20,21], while riverine microfaunal components have been less studied [22]. Macroinvertebrates constitute the major, most extensively used food source for numerous fishes and are the most abundant in lotic ecosystems [23,24]. Zooseston, an understudied

faunistic component of rivers, consists in benthic (bed or periphyton) and planktonic organisms (from upstream lentic sections and lakes) [25], and these small organisms are important food resources for downstream benthic invertebrates and fish [4,5,26,27]. Additionally, drift concomitantly participates in organisms' dispersion [28].

The littoral zone is often a refuge for invertebrates, fish fry and juveniles, as well as an important spawning zone for numerous fish species [15,29,30]. Due to the diversity of habitats (the presence of macrophytes, and different sizes and types of sediments), the littoral zone remarkably contributes to species diversity [31–34]. Most previous studies have focused on the size structure of zooplankton and fish, and have shown that fish can cause the loss of large zooplankton, especially cladocerans, through size-selective predation [35] and also shift the balance in favor of smaller species [36]. Changes in the hydrologic regime have a significant impact on fish communities [37]. In one study, researchers analyzed the annual variation in juvenile fish abundance as a function of habitat availability and flow extremes in a river system in the southeastern United States. The results demonstrated the importance of temporal habitat stability independently of habitat availability. They found that juvenile fish abundance was strongly correlated with flow and instream habitat variables and changes in the hydrological regime on the population of the indigenous cyprinid fish *Spinibarbus hollandi* in the Lijiang River in China [38]. Even minor changes in the hydrological regime have been found to cause changes in environmental parameters, especially evidenced in the observation of lotic stretches of the Plitvice Lakes (Croatia), where it was observed that different hydrological regimes affected various organisms. The authors concluded that benthic bdelloid rotifers, as microfilter feeders, were strongly and positively affected by the combined interaction of flow velocity and the concentration of suspended particulate organic matter (POM fractions) [39].

Most of Croatia's inland waterways belong to the Sava River basin, as a part of the Black Sea watershed, and have significant environmental and socioeconomic impacts, providing many ecosystem services, for example recreational and commercial fishing. All these ecosystems may be disturbed by huge oscillations of the hydrological regime. Thus, the purpose of the present investigation was to assess the influence of hydrological extremes on environmental parameters, biocoenosis and trophic interactions in the riverine littoral zone of the Sava River. These changes in the riverine hydrological regime may cause profound and accumulative impacts on the conservation of native riverine biota, as was confirmed for Lijiang River in China [38]. Previous studies in the Sava River have mainly studied invertebrate and fish species compositions, and environmental conditions [14,15,40]. Trophic relationships among fish, macroinvertebrates and zoosetion are however an important indicator of the functioning of an ecosystem [41–43]. The main aim of this study was to reveal the effects of hydrological extremes on littoral riverine biocoenoses, through the assessment of the environmental conditions, nutrients, consumers and predators.

For this purpose, we have measured the variations in environmental parameters and biota, and attempted to detect whether these greatly affect community structures and trophic relationships.

## 2. Materials and Methods

### 2.1. Study Area and Hydrological Features

The Sava River is the major drainage basin in Southeastern Europe and the largest tributary of the Danube River. It is 945 km long, with a large catchment area of 97,713 km<sup>2</sup>. It rises in Slovenia, flows through Croatia, Bosnia and Herzegovina and inflows into the Danube River in Belgrade (Serbia). In Slovenia, the Sava is an alpine river that, at the Slovenian–Croatian border, turns into a typical lowland river with fine-grained sediment covering the riverbed. The Sava lowlands are characterized by wide floodplains and numerous tributaries. There are mostly flat areas and areas with low hills, characterized by lower gradients and flow velocities, and smaller and meandering streams. It is characterized by the prevailing temperate climate of the Northern Hemisphere.

The littoral zone is described in detail for the seven sampling sites along the Sava River (Figure 1).

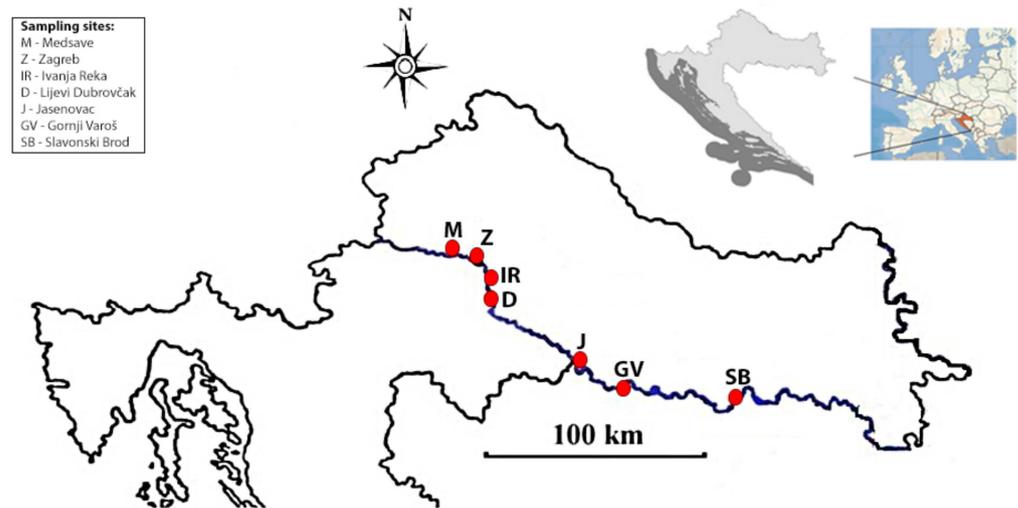


Figure 1. Sampling sites along the Sava River in Croatia (Produced by Daniel Matulić).

The sampling sites were selected considering the sample accessibility and representativeness in terms of different anthropogenic sources of pollution (e.g., industry, traffic, agricultural and urban activities). Sampling was conducted in the spring (May/June) of 2014 and 2015, along the longitudinal profile across Croatia (Table 1). Data related to the water discharge ( $Q$ ) and precipitation at seven sites of the Sava River were obtained from the Croatian Meteorological and Hydrological Service, Hydrology Department. The precipitation (mm) in the continental region of Croatia is expressed as an average value for the spring season at the seven studied sites in 2014 and 2015. The discharge in 2014 was marked as a lower discharge and in 2015 as a high discharge.

Table 1. Main morphometric features of the sampled sites (Aničić et al., 2014).

Sampling Sites (Abbreviations)	Medsave (M)	Zagreb (Z)	Ivanja Reka (IR)	Lijevi Dubrovčak (D)	Jasenovac (J)	Gornji Varoš (GV)	Slavonski Brod (SB)
Coordinates	45°50'03.77" N, 15°46'29.01" E	45°47'14.24" N, 15°59'25.46" E	45°46'48.62" N, 16°08'13.94" E	45°38'49.62" N, 16°20'24.44" E	45°16'05.68" N, 16°54'49.40" E	45°08'51.27" N, 17°14'10.53" E	45°08'58.62" N, 18°00'22.21" E
River (km)	708	682	668	653	516	476	370
Width <sub>max</sub> (m)	80	85	85	86	112	164	245
Depth (m)	0.3–1.1	0.4–1.4	0.4–1.5	0.3–1.0	0.4–1.0	0.2–1.0	0.5–1.3
Flow velocity (ms <sup>-1</sup> )	7.00	1.50	1.50	0.6	2.70	1.00	3.00
Tributary (upstream)	Gradna	Krapina			Trebež, Struga, Una	Lonja	Orljava
Habitat specification	Lotic	Lotic	Lotic	Lentic	Lentic	Lotic	Lentic
Anthropogenic impact	wastewater plant inflow	urbanisation, facilitate transport and industry	inflow of treated municipal water of Zagreb city	field leaching	field leaching	field leaching	industrial inflow
Bottom granulometry	rip-rap, rocks, gravel	rip-rap, sand	boulders, mud, debris	mud, sand	sand, mud, gravel	mud, rocks, boulders	mud, rocks
Macrophyte coverage (%)	None	None	None	None	None	20%	40%

## 2.2. Measurement of Environmental Parameters

At each sampling site, field measurements and water samples were collected for laboratory analyses once per site in May of 2014 and 2015. A range of instruments were used on site to determine the temperature, oxygen concentration, pH (Hach HQ30d, Hach Company, Loveland, CO, USA), conductivity (Hach sensION5, Hach Company) and flow velocity (Fv) (flowmeter Global Water Flow Probe, Gloal Water, Houston, TX, USA). All the nitrogen ions (ammonium, nitrites and nitrates, i.e., dissolved inorganic nitrogen, DIN) and orthophosphate (i.e., soluble reactive phosphorous, SRP) were measured using an ion chromatograph (Dionex ICS-3000, Diones, Poway, CA, USA). The phytoplankton and concentration of suspended particulate organic matter (POM) were considered as food resources in seston. The algal biomass (indicated as the chlorophyll *a*, Chl *a*, content) was determined using an ethanol extraction method [44]. The POM values (measured as the ash-free dry mass, AFDM) were obtained after drying each sample at 104 °C and ashing at 600 °C for 6 h [32].

## 2.3. Biocenotical Analysis

Fish were collected by the electrofishing method. Electrofishing (Hans Grassl EL 63 II; 220/440 V; 17.8/8.9 A; Hans Grassl, Schönau am Königssee, Germany) was undertaken during daylight hours on various types of substrates and depths ranging from 0.2 to 1.5 m with a 50 × 50 cm round stainless-steel anode, and a 10 mm sized mesh netting was used. In order to minimize the operator bias, the surveys were performed using the same two-person sampling team each time [45]. The total length (TL, 1 mm) and weight (W, 0.01 g) of each fish collected were immediately measured. Fish identification was performed in accordance with the literature [46,47]. The average yearly catch-per-unit-effort (CPUE = the number of fish per 100 m of shoreline [48]) values were calculated for each species using only the data from the sites observed.

Zooseton was collected by filtering 30 L of water through a plankton net (26 µm mesh) and were fixed with 4% formalin. In those samples, rotifers, cladocerans and copepods were analyzed, and they also consisted of a considerable share of meiofaunal specimens (organisms that can pass through a 1000 µm sieve but are retained on a 44 µm one, e.g., nematodes, gastrotrichs and, oligochaetes). Zooseton was separated based on the higher taxonomic levels and counted in three subsamples under an Opton Axiovert 35 inverted microscope (125×; 400×).

Benthic macroinvertebrates were sampled using a 25 × 25 cm Surber sampler (mesh size: 300 µm). The samples were preserved in 75% ethanol and were analyzed under an Olympus SZ61 stereomicroscope (10× to 40×) by the higher taxonomic groups. In total, 42 samples were collected (7 sampling sites, 2 sampling dates, triplicate samples). Specimens were identified to the lowest possible taxonomic level.

## 2.4. Data Analysis

Basic statistics were applied for the analyses of the physicochemical parameters of the water and food resources recorded at each sampling site and are summarized as the mean values, standard deviations (SDs), minima (MINs) and maxima (MAXs). The differences in environmental parameters between low discharge and high discharge were tested by the multivariate analysis of similarities (ANOSIM) by employing the analytical package PRIMER v6 [49]. ANOSIM generates an *r*-value (and related *p*-value) ranging between −1 and +1, with a value of 0 indicating no difference among a set of samples; >0.75, as well separated; >0.5, as overlapping but clearly different; and <0.25, as barely separable [50]. The data for the measured physicochemical water parameters and food resources as well as biotic components were not normally distributed and could not be normalized by common transformations; thus, nonparametric tests were used. The Kruskal–Wallis test was used for testing differences among sampling sites, and the Mann–Whitney U test was applied for testing differences between two hydrologically different years (Statistica 9.1, StatSoft, 2010, Tulsa, OK, USA). A *p* value of 0.05 was taken to indicate statistical significance in all

tests. F value is a result of the statistical F test and explains interactions between variables. Lambda presents percentage of explained variance.

Canonical correspondence analysis (CCA), using the Canoco 4.2 program [51], was conducted for analyses of the interactions between (1) the abundance of the dominant fish species of bleak, *Alburnus alburnus* (Linnaeus, 1758); common bream, *Abramis brama* (Linnaeus, 1758); Prussian carp, *Carassius gibelio* (Bloch, 1782); pike, *Esox lucius* (Linnaeus, 1758); common roach, *Rutilus rutilus* (Linnaeus, 1758); and chub, *Squalius cephalus* (Linnaeus, 1758), and (2) the measured environmental parameters of Q (water discharge), O<sub>2</sub> (dissolved oxygen), DIN (dissolved inorganic nitrogen), SRP (soluble reactive phosphorous), POM (concentration of suspended particulate organic matter) and Chl *a* (concentration of chlorophyll *a*), and (3) the potential prey as the abundance of invertebrates in the benthos: GastrB (Gastropoda), OligB (Oligochaeta), AmphB (Amphipoda) and DiptB (Diptera larvae), and seston: BdellS (Bdelloidea), MonoS (Monogononta), ClaS (Cladocera) and CopS (Copepoda). The statistical significance of the analyzed correlations was tested with the Monte Carlo permutation test (499 permutations).

### 3. Results

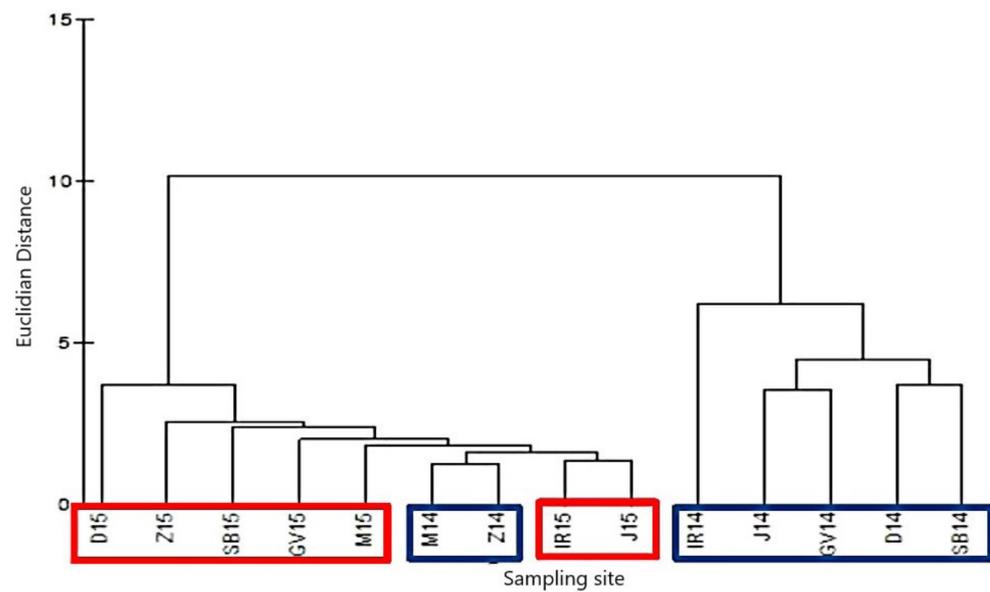
#### 3.1. Environmental Conditions

In each year, 2014 and 2015, there were no significant differences between the sampling sites for 13 measured environmental parameters (Kruskal–Wallis,  $p > 0.05$ ); hence their variations are shown as annual differences (Table 2).

**Table 2.** Range, mean and SD values ( $n = 14$ ) of analyzed environmental parameters at seven sampling sites in the littoral zone along the Sava River. The abbreviations for the physicochemical variables are LQ—low discharge; HQ—high discharge; Chl *a*—chlorophyll *a* concentration; POM—particulate organic matter concentration; PIM—particulate inorganic matter.

Year	LQ 2014		HQ 2015	
Variable	Mean ± SD	Min–Max	Mean ± SD	Min–Max
Water discharge (m <sup>3</sup> s <sup>-1</sup> )	341.87 ± 225.59	133.00–669.10	1217.27 ± 623.16	443.20–2166.00
Flow velocity (m s <sup>-1</sup> )	0.25 ± 0.25	0.06–0.79	2.41 ± 2.17	0.60–7.00
Temperature (°C)	21.07 ± 1.00	19.90–22.60	20.42 ± 3.19	16.40–23.30
O <sub>2</sub> (mg L <sup>-1</sup> )	8.69 ± 3.33	5.75–13.42	6.47 ± 0.90	5.42–7.78
pH	7.94 ± 0.58	6.75–8.51	7.82 ± 1.03	5.50–8.38
Conductivity (µS cm <sup>-1</sup> )	467.71 ± 116.67	385.00–724.00	411.14 ± 31.07	366.00–465.00
N–NH <sub>3</sub> (mg L <sup>-1</sup> )	0.477 ± 0.642	0.040–1.850	0.136 ± 0.098	0.000–0.260
N–NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.162 ± 0.259	0.010–0.730	0.174 ± 0.202	0.070–0.630
N–NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	1.265 ± 1.137	0.310–3.540	5.889 ± 6.976	0.010–19.600
PO <sub>4</sub> <sup>3-</sup> (mg L <sup>-1</sup> )	0.260 ± 0.264	0.030–0.810	2.889 ± 5.227	0.280–14.600
Chl <i>a</i> (µg L <sup>-1</sup> )	7.442 ± 3.129	4.736–14.208	5.032 ± 2.257	2.101–7.696
POM (mg AFDM L <sup>-1</sup> )	153.428 ± 35.518	125.333–229.333	140.000 ± 44.127	100.00–229.333
PIM (mg DM L <sup>-1</sup> )	35.714 ± 9.946	26.000–56.666	77.714 ± 86.727	32.666–271.333

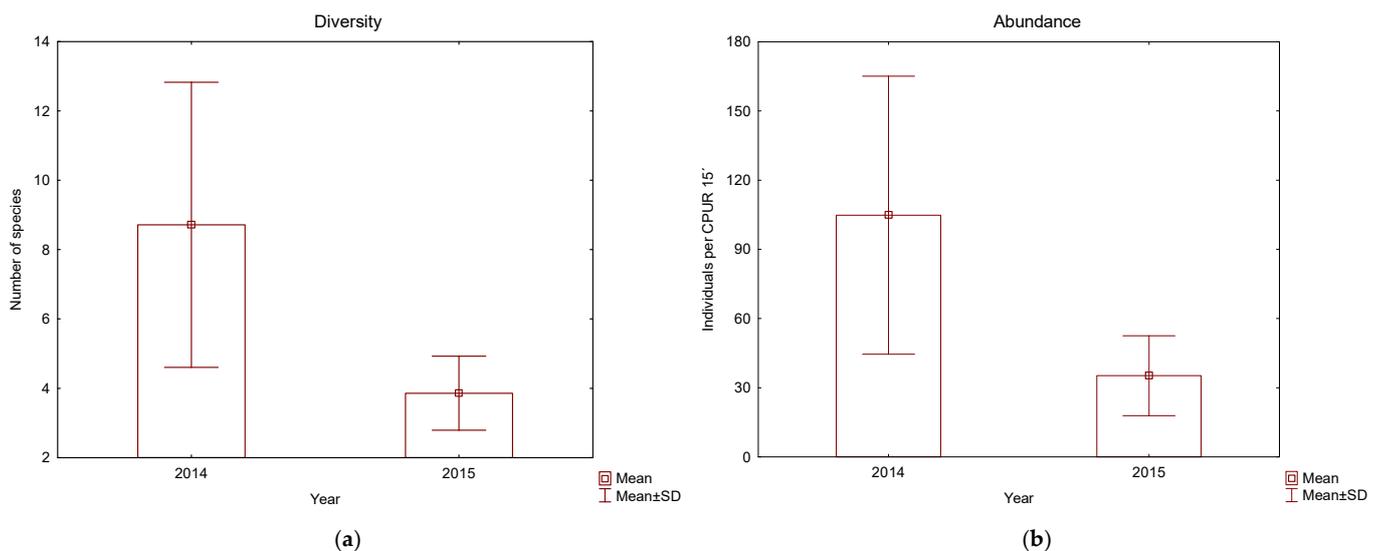
In the two years of sampling, the amount of precipitation was significantly higher in 2015 (Mann–Whitney,  $Z = -2.556$ ,  $p < 0.01$ ), being almost twice as that of 2014. Significantly higher values of discharge and flow velocity (Mann–Whitney,  $Z_Q = -2.556$ ,  $p < 0.01$ ;  $Z_{Fv} = -2.939$ ,  $p < 0.003$ ) across all sampling sites were measured in 2015 ( $Q = 1217 \pm 623 \text{ m}^3\text{s}^{-1}$ ;  $Fv = 2.471 \pm 2.175 \text{ ms}^{-1}$ ), compared to 2014 ( $Q = 342 \pm 226 \text{ m}^3\text{s}^{-1}$ ;  $Fv = 0.254 \pm 0.255 \text{ ms}^{-1}$ ). The same between-year trend was observed for the ortho-phosphate concentrations ( $\text{PO}_4^{3-}_{2015} = 2.889 \pm 5.227 \text{ mg L}^{-1}$ ,  $\text{PO}_4^{3-}_{2014} = 0.260 \pm 0.264 \text{ mg L}^{-1}$ ; Mann–Whitney,  $Z = -2.300$ ,  $p < 0.02$ ). The environmental conditions indicated overlap; however, the between-year fluctuations along the Sava River littoral zone were clearly different (ANOSIM,  $r = 0.5$ ,  $p < 0.004$ ; Figure 2).



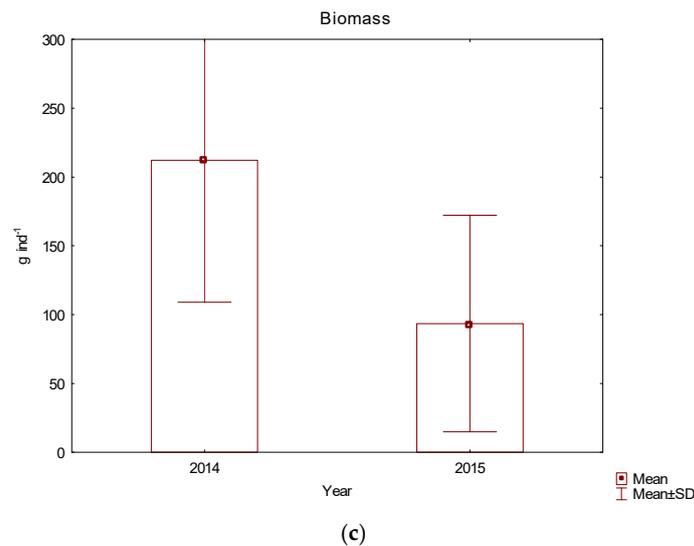
**Figure 2.** Clustering of sampling sites in relation to the environmental parameters at low discharge in 2014 (marked blue) and high discharge (marked red) (produced by Maria Špoljar).

### 3.2. The Fish Community at the Studied Sites of the Sava River

During this research, 27 fish species, mostly from the family Cyprinidae (17 species), were caught at the sampling sites on the Sava River, 22 of which were native and 5 of which were alien (Table S1, Supplementary Materials). In 2014, the highest abundance was determined at the sampling sites GV and J. In the same year, the fish taxa were the most diverse at the SB site where 14 different species were identified (Table S1). Fish diversity, abundance and biomass were diverse and reduced at high discharge in comparison to low discharge in 2015 in comparison to 2014, respectively (Figure 3). These differences were exhibited in a significant decrease in fish diversity (Mann–Whitney,  $Z = 2.044$ ,  $p < 0.04$ ) and mean abundance (Mann–Whitney,  $Z = 2.29$ ,  $p < 0.02$ ).



**Figure 3.** Cont.

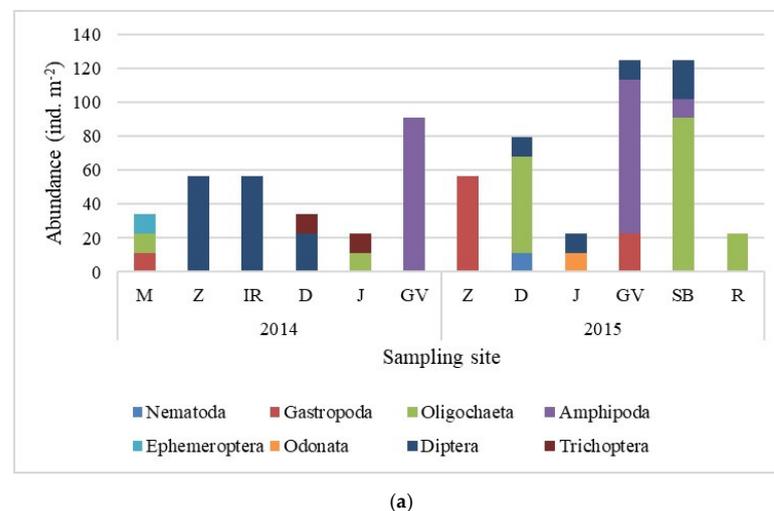


**Figure 3.** Differences in the fish diversity and abundance in 2014, with low discharge, and 2015, with high discharge (produced by Maria Špoljar). (a) Mean ( $\pm$ SE) number of species and diversity; (b) Mean ( $\pm$ SE) individuals per CPUE and abundance; and (c) Mean ( $\pm$ SE) g/individuals and biomass.

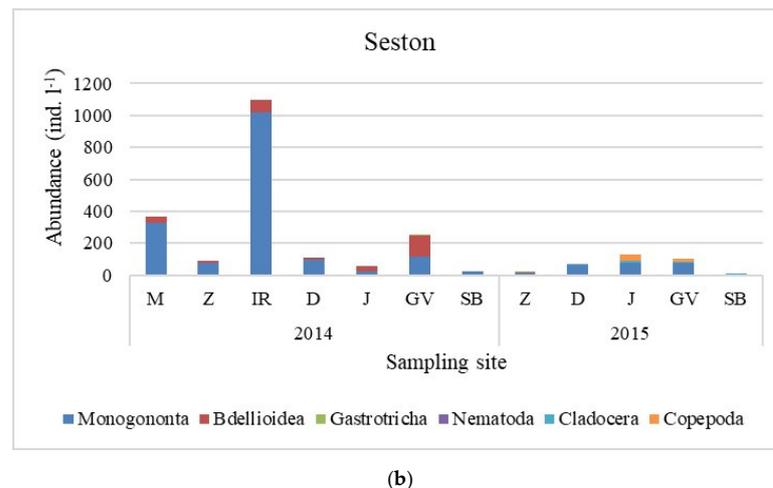
Higher frequency of occurrence during low discharge in 2014 was recorded for AA bleak (present at all the sites), SC chub (present at 86% sites) and CG Prussian carp (present at 71% sites). A different species composition appeared during high discharge in 2015, where the species were present at fewer sites along the littoral zone of the Sava River, e.g., bleak, 86%; chub, 71%; and CE Balkan loach *Cobitis elongata* (Heckel & Kner, 1858), 57%, in frequency (Table S1).

### 3.3. Macrozoobenthos and Zoosetion of the Riverine Littoral Zone

The macrozoobenthos abundance did not show a significant difference between low discharge and high discharge (Mann–Whitney test,  $p < 0.05$ ); however, a higher abundance was recorded at high discharge ( $71 \pm 40$  ind.  $m^{-2}$ ) in comparison to low discharge ( $60 \pm 36$  ind.  $m^{-2}$ ; Figure 4a). During both hydrological extremes, amphipods inhabited the littoral zone in a similar share; 31% at low discharge in 2014, and 25% at high discharge in 2015. Dipteran larvae were less abundant, with 46% at low discharge and 14% at high discharge, and, oligochaetes and gastropods were less abundant, with 36% to 19% at high and low discharge, respectively.



**Figure 4.** Cont.



**Figure 4.** Macrozoobenthos abundance in sediment (a) and zoosetion comprising micro-/meiofauna (b) at sampling sites during two sampling seasons in the littoral zone of the Sava River (produced by: Tea Tomljanović). Sammlit sites: M–Medsave; Z–Zagreb; IR–Ivanja Reka; D–Lijevi Dubrovčak; J–Jasenovac; GV–Gornji Varoš; SB–Slavonski Brod.

The abundance of zoosetion specimens was four times higher at low discharge ( $286 \pm 377 \text{ ind. L}^{-1}$ ) than at high discharge ( $68 \pm 52 \text{ ind. L}^{-1}$ ). Cladocerans showed a different trend, with higher abundance at high discharge (Mann–Whitney U test,  $Z = -2.160$ ,  $p = 0.03$ ). Monogonont rotifers prevailed in the zoosetion along the littoral zone of the Sava River during each hydrologically different year, at both low discharge and high discharge (Figure 4b).

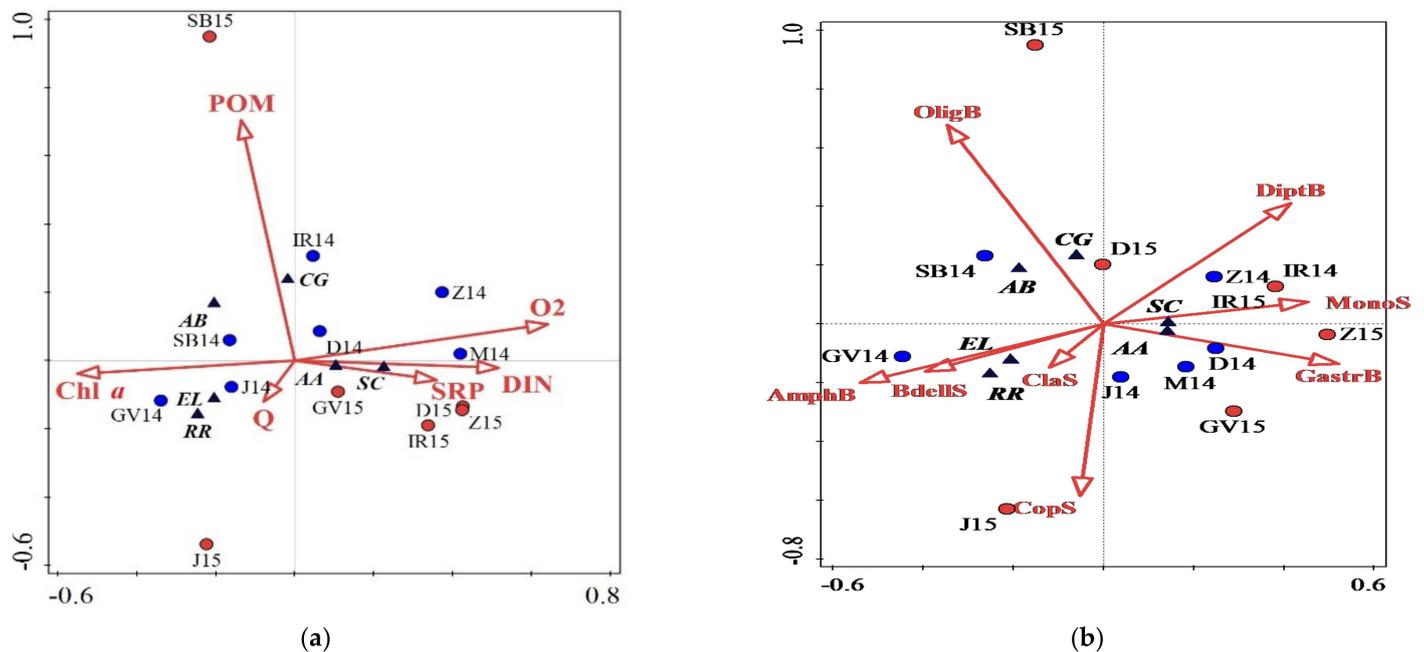
All the abiotic-biotic interactions were tested by determining the Spearman correlations ( $p < 0.05$ ), and significant interactions are shown in Table 3. Nutrients (nitrate and phosphate) affected rotifers and positively influenced the abundance of cladocerans, while nitrates negatively affected the abundance of both groups of rotifers; monogonont and bdelloids.

**Table 3.** Spearman correlations ( $p < 0.05$ ) between water nutrient content, richness of fish and invertebrates in the benthos and seston. The abbreviations for the fishes: CG—Prussian carp; CE—Balkan loach; AB—common bream; EL—pike.

Variable	$\text{NO}_3^-$	$\text{PO}_4^{3-}$	CG	CE	Amphipoda
AB					0.561
EL					0.606
<b>Seston (ind. <math>\text{L}^{-1}</math>)</b>					
Bdelloidea	−0.727			−0.573	
Monogononta	−0.641				
Cladocera	0.542	0.696			
<b>Benthos (ind. <math>\text{m}^{-2}</math>)</b>					
Gastropoda				0.561	
Total invertebrates			0.552		

According to the CCA results, the abundance of dominant fish was strongly correlated with several environmental parameters ( $\text{O}_2$ , DIN, POM, Chl *a* and Q) and biotic interactions, presumably through food-web links with amphipods and gastropods (Figure 5, Table 4). The CCA explained 73% of the variance in the relationships between the abundance of dominant fish and environmental conditions (Figure 5a). The sampling sites were mainly grouped according to the low discharge or high discharge extremes. At high discharge, the

nutrient concentrations were DIN and SRP. The results showed smaller amounts of POM and Chl *a* during the high discharge year.



**Figure 5.** CCA-triplet illustrating the differences between (a) sampling sites, physicochemical variables and abundances of dominant fish species; (b) sampling sites and co-occurrence between the abundance of fish and invertebrates in the benthos and seston. Sampling sites in 2014 at low discharge are marked by blue circles and in 2015 at high discharge by red circles. The abbreviations for the physicochemical variables: POM—particulate organic matter concentration; Chl—chlorophyll *a* concentration, Q—discharge, SRP—soluble reactive phosphorous, DIN—dissolved inorganic nitrogen, O<sub>2</sub>—dissolved oxygen concentration. The abbreviations for the invertebrates: AmphB—Ampipoda in benthos; BdellS—Bdelloidea in seston; ClaS—Cladocera in seston; CopS—Copepoda in seston; DiptB—Diptera in benthos; GastrB—Gastropoda in benthos; OligB—Oligochaeta in benthos; MonoS—Monogononta in seston. The abbreviations for the fishes: AA—bleak; AB—common bream; CG—Prussian carp; EL—pike; RR—common roach; SC—chub.

**Table 4.** Results of the Monte Carlo permutation test (% of variance,  $F$ ,  $p < 0.05$ ) for CCA based on relationships between fish abundance and physicochemical environmental conditions (Env) and biotic interactions with invertebrate abundance in the benthos. Environmental parameter and size structure abbreviations are given in Materials and Methods.

Interactions	Axis 1 (%)	Axis 2 (%)	Variable	%	F	<i>p</i>
Fish abundance vs. Env	52	21	O <sub>2</sub>	23	3.3	0.03
			DIN	19	3.2	0.012
			Chl <i>a</i>	18	2.4	0.05
			POM	12	2.3	0.1
			Q	9	2	0.12
Fish abundance vs. Invertebrates	42	20	AmphB	18	2.7	0.05
			GastrB	15	1.9	0.09

Additionally, the results indicated that the majority of the fish species occurred only during lower discharge. Food-web co-occurrence between fish, as predators, and benthic macroinvertebrates and zoosetion, as prey, explained 62% of the interactions by CCA (Figure 5b). Thus, the results show that monogonot rotifers (MonoS) were a significant part

of the diet of chub (SC) and bleak (AA), whilst bdelloids (BdellS), copepods (CopS) and cladocerans (ClaS) were potential prey for the common roach (RR) and pike (EL).

#### 4. Discussion

Extreme precipitations cause hydrological disturbances and mainly trigger huge alterations in the biocoenoses of the littoral zones of standing [52–54] and running waters [55,56]. The precipitations in the continental region of Croatia were five to ten times higher than the average of 700 to 1200 mm, common in temperate climates.

The majority of the environmental parameters did not indicate a significant difference between low discharge and high discharge years, probably due to the riverine littoral zone being more protected from the water pulses than the main watercourse. However, the high discharge in 2015 caused sediment resuspension, concomitantly with the release of sediment-bound phosphorous [57,58]. Higher phosphorus concentrations, probably due to the dilution effect, did not increase productivity, as indicated by the lower amount of suspended POM. The lentic sampling sites (J and SB) were the exceptions, where a higher phosphorus concentration increased phytoplankton growth, indicated by the higher chlorophyll *a* concentration.

The zooseston in the littoral zone of the Sava River, in our study, consisted of monogonont and bdelloid rotifers, but the abundance of seston was four times higher at low discharge in comparison to high discharge. Those small-sized organisms were negatively affected, and presumably drifted during high discharge as they were attached to particles in the loose sediment, as with those in the sand or mud in this research, and therefore zooseston abundance decreased at high discharge. Otherwise, bdelloid rotifers could resist the high discharge if they had a stable, attached surface (i.e., tufa sediment or mosses) [39]. For instance, on a more compact moss-covered tufa barrier on the Plitvice Lakes, bdelloids dominated at a high discharge, enhanced by feeding with drifted particles [59]. There was an exception at the sampling site IR, where, as there was an inflow of treated municipal wastewater from the City of Zagreb, semiplanktonic monogonont rotifers prevailed during low discharge and developed abundant populations of more than 1000 ind. L<sup>-1</sup>. Presumably, higher concentrations of suspended organic matter and bacteria, their main food sources, enhanced their growth [60].

In our study, hydrological factors were identified as the main driver for cladoceran populations, where high discharge contributed to a higher water depth in the lentic riverine zone, providing a suitable environment for the development of a higher abundance of planktonic cladocerans. Additionally, a higher phosphorous concentration at high discharge facilitated phytoplankton growth in the lentic stations of the riverine littoral and could concomitantly have a positive effect on the abundance of algivorous cladocerans [11,61]. In the study in the Parana River watershed (Brazil) [34], differences in the abundance and diversity of cladocerans in the littoral zones of lakes and rivers affected by variations in macrophyte cover were compared. They concluded that the cladocerans in rivers are more determined by hydrological factors, and those in lakes, by habitat structure. Accordingly, in this study, cladocerans increased in abundance at the sampling site GV, moderately covered by macrophytes, indicating a transitional habitat from lotic toward lentic.

Records of macroinvertebrates in benthos were also considered in the ecological view, especially related to the hydrological stressors. In our study, the abundance of benthic macroinvertebrates did not show significant fluctuations. The replacement of the Diptera and Amphipoda at low discharge with the Oligochaeta and Gastropoda at high discharge suggested a shift in the composition of macrozoobenthos in the sediment in both hydrological events. The higher abundance of the Oligochaeta could be explained by their tiny and elongated body shape. Namely, their tubular body allows them to become entangled in plants or sediment particles and seek shelter therein. In the Baía and Ivinhema rivers (Brazil), the results confirmed that the intensity and amplitude of the photomorphosis positively affected the density, richness and composition of the Oligochaeta, since many species were transported by high water current velocities and/or died due to the low

oxygen levels that are characteristic of this phase [62]. Gastropods were more present at high discharge; they are more resistant to high discharge due to the possibility of a firmer radula grip on the substrate.

The results of sampling in 2014 at low discharge, compared to that in 2015 at high discharge, showed that the floods, as short-term stressors, significantly affected entire littoral fish communities, which may lead to long-lasting stress events [63]. The fish populations in the littoral zone of the Sava River were impoverished in all the structural traits at high discharge. In our study, twelve fish species were absent after a high discharge event (2015), some of which were abundant at low discharge (2014), such as the pumpkinseed, *Lepomis gibbosus*; gudgeon, *Gobio obtusirostris*; and ide, *Leuciscus idus*, resulting in significantly lower fish diversity. In both hydrologically different years, the same species dominated, but in lower abundance at high discharge: bleak, common bream and chub, which are typical species for lowland rivers. The diversity of the fish from this study is consistent with previous research in this area [64,65]. In the littoral zone, which is an important habitat for food sources, mostly juvenile fish specimens were observed, presumably seeking shelter from predators and high flow velocity or discharge [66–68].

Biocoenotic components in the littoral zone of the Sava River showed different tolerances to the hydrological extremes, leading to a remarkable decrease in the abundance, diversity, biomass and sizes of fish and, probably due to dilution, a decrease in zoosetion abundance, which serves as an important food for fish larval stages. Benthivorous fish (common bream, pumpkinseed and Balkan loach) played an important role in the flux of matter in aquatic ecosystems. It is indicated that the presence of fish species, e.g., Prussian carp, particularly affects large-bodied zooplankton. Fish predation over macrocrustaceans was size selective, with larger macroinvertebrate taxa generally more vulnerable and affected [69]. Additionally, as potential prey for fish, Oligochaeta, Diptera and Amphipoda were the most abundant groups [70,71]. Our results also suggest that, in addition to changes in hydrology, fish abundance is a strong predictor of macroinvertebrate abundance. This is in contrast to research by [72], who reported that the macroinvertebrate community composition was more dependent on fish food preferences than on fish abundance. In addition, several studies have shown that increased habitat complexity can also strongly influence fish–prey interactions in several ways, primarily by increasing the diversity of habitats and food resources for macroinvertebrates and reducing their vulnerability to fish predation [73].

In this scenario, the abundance of benthic macroinvertebrates did not show significant fluctuations, but the proportion of benthic groups of organisms was shifted during high discharge in favor of Oligochaeta and Gastropoda. For most of the dominant fish, gastropods were not the dominant food source, so this had the effect of increasing the population of gastropods. This is particularly evident at lotic sites, where gastropods were reported during low and high discharge, at M, GV, Z and GV, respectively.

In our study, the results suggest that common bream had a significant effect on occurrence of oligochaetes. Common bream's benthivorous and/or planktivorous feeding habits are frequently a cause of bioturbation, which can increase nutrient release from the reservoir bottom [74]. Macrozoobenthos is known to constitute a large part of its diet, as described in shallow Lake Balaton (Hungary) [75], Pierzchaly Reservoir (Poland) [76] and a Baltic Sea lagoon [77]. The diet of common bream in the Włocławek Reservoir also mainly consisted of benthic cladocerans and insect Chironomidae larvae [78].

We noticed a great influence of pike to Amphipods occurrence. As in many freshwaters of the northern temperate zone, pike is an important top–down controller of food webs due to its piscivorous diet [79] and is often considered a key species for the functioning of ecosystems [80]. Pike exhibits a wide range of prey, including salmonids, percids and cyprinids [81]. In our study, with low discharge in 2014, pike reached high abundance, presumably simultaneously with an increased abundance of juvenile cactus roach *Rutilus virgo* and common bream. A positive correlation between the abundance of pike and the abundance of juvenile fish, which are the main food for predatory fish such as pike [82], has

already been detected in various hydrosystems, e.g., the Arizona Reservoir in Brazil [83], Ladik Lake in Turkey [84] and the rivers Rena and Sena in France [85].

## 5. Conclusions

This study contributes to the existing research upon the stressor effects of extreme hydrological events on environmental conditions and communities, reflected in a reduction in fish diversity and abundance. The hydrological extremes in precipitation and the riverine regime also altered macrozoobenthos and zooseston components due to the changes in water and sediment habitat conditions. In the food-web network among fish, macrozoobenthos and zooseston hydrological stressors caused remarkable changes in food resources and functional feeding traits. The resilience and recovery of the ecosystem greatly depended on biodiversity, which enhanced shorter recovery period of unbalanced ecosystem. Ongoing climate change, on top of the baseline of water cycle alteration, creates a demanding task for the future of ecosystem and fisheries management. The present study helps to highlight the consequences of hydrological disturbances caused by climate change: the enhancement of stressors in riverine littoral habitats and inhabited communities. Accordingly, a study of the effects of zooseston and macroinvertebrate ingestion on the fish populations under varying hydrological conditions is planned.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/hydrobiology1020015/s1>, Table S1: Common and scientific names (\* alien fish species), families and abbreviations of all sampled fish species by occurrence sites (+).

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