ORIGINAL ARTICLE

Odonata assemblages in anthropogenically impacted lotic habitats

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ABSTRACT

Increasing human pressures have a negative impact on freshwater habitats and their biota worldwide. To protect habitats and the species contained within them, ecological assessments over a gradient of near natural to degraded freshwater habitats are essential. Odonata assemblages were investigated at 46 study sites in Croatia encompassing slightly to heavily modified lowland rivers and streams. Nymphs were sampled between April and September 2016 using a benthos hand net. A total of 19 species was recorded, and *Ischnura elegans* (Vander Linden, 1820) and *Platycnemis pennipes* (Pallas, 1771) were most frequently recorded. RDA analysis indicated that water pollution (i.e. levels of chemical oxygen demand and total organic carbon), water temperature and oxygen concentration had the highest influence in the formation of Odonata assemblages at a specific habitat, reflecting their widely recognized bioindicator properties. This study showed that degraded lowland rivers can provide habitat for a relatively low number of species with broad ecological tolerance, while rare and specialist species are generally not able to reproduce there. These results contribute to our knowledge of Odonata occurrence in anthropogenically impacted habitats, and their relationships with such degraded environment.

INTRODUCTION

Odonata (dragonflies and damselflies), as amphibiotic insects, play an important role in transferring materials and energy from aquatic to terrestrial ecosystems (Tsui *et al*., 2012; Williams *et al*., 2017; May, 2019). Nymphs inhabit a wide range of freshwater habitats in relation to the biotic and abiotic characteristics, such as predation, food resources, aquatic and riparian vegetation, water temperature, oxygen concentration and pH (Johansson and Brodin, 2003; Vilenica, 2017; Vilenica *et al*., 2020). Adult habitat selection primarily relies on the structure of aquatic and riparian vegetation and shading (Steytler and Samways, 1995; Corbet and Brooks, 2008). Both life stages are generalized predators feeding on various small invertebrates, making them important in the regulation of population abundances of other insects, such as mosquitoes (Corbet and Brooks, 2008; May, 2019). Odonata have been widely used as ecological indicators of environmental quality in aquatic ecosystems (Butler and deMaynadier, 2008; Chovanec and Waringer, 2001) as they inhabit a wide range of freshwater habitats, are strongly affected by anthropogenic changes in freshwater environments, and clearly respond to habitat modification (Samways and Steytler, 1996; Silva *et al.,* 2010; Bried and Samways, 2015). Moreover, Odonata are relatively easily recognized in their adult stages and are popular among professional and amateur entomologists due to their distinctive appearance and behaviour (Boudot and Kalkman, 2015; May, 2019).

The growing human population has resulted in modifications and regulations of various freshwater habitats worldwide (McKinney, 2006), negatively affecting the maintenance of their native biota (Carchini and Rota, 1985; Growns and Growns, 2001; Ferreras-Romero *et al.,* 2009; Golfieri *et al.*, 2018; Kalyoncu and Salur, 2018; Vilenica *et al.,* 2016; 2019; 2020). Freshwater habitats are modified by land use regulating river and streamflow, resulting in changes to hydromorphology, substrate composition, hydrological regime, and water chemistry, which in turn leads to a significant loss of habitat and microhabitat diversity (Strayer and Dudgeon, 2010; Carpenter *et al*., 2011; Wen *et al.,* 2011). Moreover, due to the extensive urbanization, agriculture and industry, large quantities of pesticides, pharmaceuticals, industrial and domestic waste are discharged daily into freshwaters (Zhang *et al.*, 2004; Arimoro *et al.,* 2008). For effective conservation and management of freshwater habitats, ecological assessments are essential (Hughes *et al.,* 1986; Stoddard *et al.,* 2008), including the collection of detailed data on aquatic communities in both natural and impacted

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habitats, and their relations to environmental factors (Moog, 2002; Hering *et al.,* 2006a, b). With the aim of contributing to such environmental quality assessments, the main goals of this study were to determine Odonata assemblages and their relationships with environmental factors at 46 lowland lotic habitats, along a habitat gradient from slightly to heavily modified.

METHODS

Study area

The study was conducted in the Pannonian lowland ecoregion (ER11) in Croatia (Illies, 1978), between April and September 2016. The area is characterized by a temperate humid climate with warm summers (Cfb, Köppen classification) where the average temperature of the warmest month is below 22°C (Šegota and Filipčić, 2003). The average annual air temperature is around 12°C and the average annual rainfall is between 800 and 1100 mm (Zaninović *et al.,* 2008).

The study was conducted at 46 slow-flowing, small to mid-sized lowland streams and rivers (Supplementary Tabs. 1 and 2, Fig. 1). The sites are characterized by a certain level of anthropogenic disturbance, *i.e.* channelization and/or modification of the water flow or riverbed, removal of the riparian vegetation, and pollution, as most sites are situated in the vicinity of urban areas, cattle farms, or agricultural fields (detailed description presented in Supplementary Tabs. 1 and 2). The main hydromorphological impacts on these rivers are interventions (past and present) to the river channel, altering the river morphology, though not hydropower related alterations. Channelization of a river or stream not only alters the morphological attributes of the riverbanks and riverbed, but also greatly alters the flow regime (Poff *et al*., 1997). The magnitude of discharges is generally greater and time to peak discharge is much quicker in channelized streams than in natural streams (King *et al*., 2009).

Sampling protocol

Odonata nymphs were collected together with other macroinvertebrates (AQEM protocol- AQEM expert consortium, 2002) between April and September 2016. At each site, 20 subsamples were collected proportionally according to the available microhabitat presence, using a benthos handnet (25 × 25 cm; mesh size = 500 μm) and pooled into one composite sample. Substrates were composed mostly of fine sediment (sand, silt, mud), lithal (stones, gravel) and aquatic vegetation (detailed description presented in Supplementary Tab. 1). Samples were stored in 96% alcohol and analysed in the lab. Nymph identification was after Gerken and Sternberg (1999), Askew (2004), and Brochard *et al.* (2012). Macrophytes were assessed using the Reference Index Croatia (RI-HR), which evaluates the difference between a reference community and the actual aquatic vegetation, depending on river type. At most study sites, reference macrophyte communities were absent with no macrophytes or with the presence of degradation indicators such as *Cladophora* sp., *Potamogeton pectinatus*, *P. crispus*, *P. pusillus*, *P. berchtoldii*, *Ceratophyllum demersum*, *Lemna minor*, *Lemna* sp., *Spirodela polyrhiza*, *Sparganium erectum* agg., *Typha latifolia*, *Glyceria fluitans* agg., *Glyceria maxima*. Detailed data on the macrophyte assemblage composition is presented in Kerovec and Ternjej (2017).

Environmental factors

At each study site, the following environmental parameters were measured at the time of benthic macroinvertebrate sampling: water temperature, dissolved oxygen concentration and saturation (using the oximeter WTW Oxi 330/SET), conductivity (with the conductivity meter WTW LF 330), pH (using the pH-meter WTW ph 330), water width and depth (using a hand meter/measuring tape). The remaining environmental parameters are presented as the mean value of 12 composite monthly samples collected over a one-year period (January–December 2016). Water chemistry analyses were carried out according to standard methods (APHA, 1992). Variables describing intensive agricultural land use in the catchment area of each site were calculated with GIS tools, using the CORINE Land Cover classification (CORINE Land Cover Hrvatska, 2013). A relative measure of hydromorphological (HYMO) alternation was given by calculating the River fauna index (RFI) using macroinvertebrate species sensitivity scores. A version of the RFI adapted for Croatian rivers and streams following Urbanič (2014) gives a score of hydromorphological (HYMO) alternation based on the response of macroinvertebrate assemblages. Although this index contains some Odonata indicator taxa as well, a prescreening test showed that the calculations do not differ significantly between RFI scores with and without the Odonata species (RFI groups – explained later in data analysis, based on this index are completely equal). The scores are then normalized with regard to reference states in the form of the WFD (Water Framework Directive) recommended EQRs (ecological quality ratios), ranging from 0 (the worst HYMO conditions) to 1 (reflecting reference states). The hydromorphological evaluation of rivers was performed based on the European Standards EN 14614 and EN 15843. Type specific River fauna index (RFI) was used as a relative measure of HYMO alternation since hydromorphological evaluations were not available for all of the investigated rivers.

Data analysis

In order to show similarities in the composition of Odonata assemblages among study sites with different RFI values, we applied a Cluster analysis using the zero-adjusted Bray-Curtis similarity index (the zero-adjusted version was used in order to consider the sites with no Odonata). SIMPER analysis was performed to determine how Odonata assemblages differ among sites with varying degrees of RFI values in terms of their species composition and abundance contribution. Odonata assemblages from sites classified as high and good by the RFI EQR (EQR>0.6) represented Group 1, from moderate sites (0.4>EQR<0.6) represented Group 2 and from sites classified as poor and bad (EQR<0.4) represented Group 3 in the Cluster analysis and SIMPER of the (zero-adjusted Bray-Curtis) similarity between Odonata assemblages.

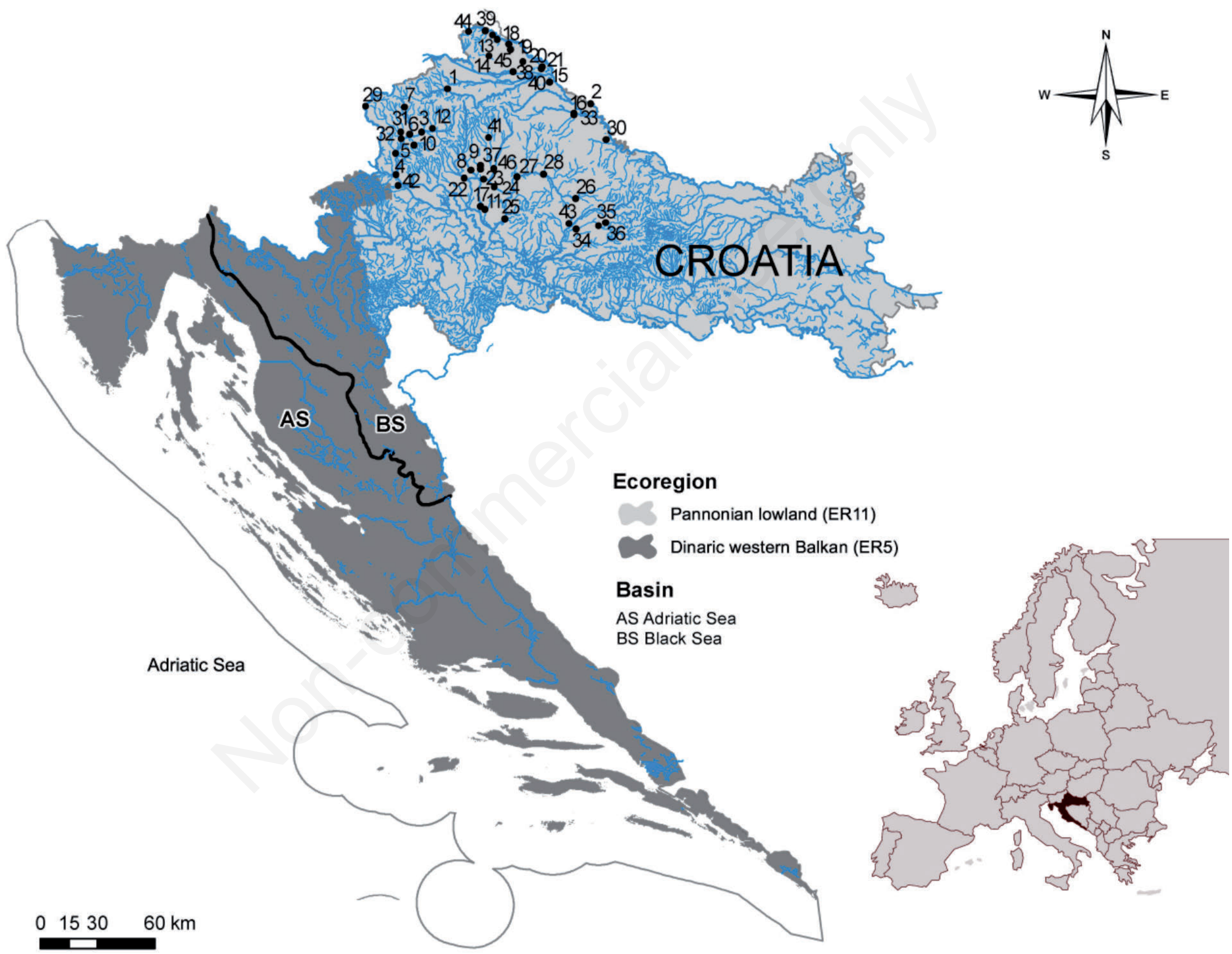

Fig. 1. Map of the 46 anthropogenically impacted study sites located in the Pannonian lowland ecoregion in Croatia.
Study sites: 1 - Bednja, Stažnjevec village; 2 - Ždalica, Ždala village; 3 - Krapina, Bedekovčina village; 4 - Krapina, Zaprešić town; 5 - Krapina, Kupljenovo village; 6 - Krapinica, Zabok town; 7 - Krapinica, Krapina town; 8 - Rajna, between Vrbovec town and Lonjica village; 9 - Zlenin, Vrbovec village; 10 - Vukšinac, Stubice village; 11 - Deanovac lateral canal, near Ivanić Grad town; 12 - Reka, Lovrečan village, 13 - Brodec, Peklenica village; 14 - Lateral canal Mihovljan, Čakovec town; 15 - Poloj, between Legrad and Đelekovec villages; 16 - Zdelja, Molve village; 17 - Lonja, near Ivanić Grad town; 18 - Jalšovnica, Ferketinec village; 19 - Bošćak, Domašinec village; 20 - Bistrec, Rakovnica I; 21 - Bistrec, Rakovnica II; 22 - Zelina, Božjakovina village; 23 - Connecting canal Zelina-Lonja-Glogovnica-Česma, Poljanski lug village; 24 - Glogovnica, before mouth to Česma; 25 - Česma, Obedišće village; 26 - Česma, Pavlovac village; 27 - Česma, Sišćani village; 28 - Česma, Narta village; 29 - Sutla, Luke Poljanske village; 30 - Rogostrug, Podravske Sesvete village; 31 - Kosteljina, Jalšje village; 32 - Horvatska, Veliko Trgovišće village; 33 - Bistra Koprivnička, Molve village; 34 - Toplica, Sokolovac village; 35 - Toplica, downstream from Daruvar town; 36 - Toplica, upstream from Daruvar town; 37 - Luka, Vrbovec town; 38 - Sewage collector, Prelog town; 39 - Gornji potok, between Selnica and Praporčan villages; 40 - Kotoribski kanal, Kotoriba village; 41 - Črnec, Gornji Dubovec vilage; 42 - Gostiraj, Ježdovec village; 43 - Tomašica, Tomašica village; 44 - Jalšovec, between Bukovje and Štrigova villages; 45 - Murščak, between Domašinec and Stara Straža villages; 46 - Glogovnica, Koritna village.

Redundancy analysis (RDA) was used to ordinate Odonata occurrence with respect to environmental variables. The analysis was performed using data for 25 taxa (rare taxa were downweighed) and 11 selected environmental variables. The Monte Carlo permutation test with 499 permutations was used to test the statistical significance of the relationship between all taxa and all variables.

Odonata taxa abundances were correlated against agricultural land cover data, using the Spearman coefficient to determine if, and to what extent, the type of land cover in the catchment area influences specific taxa occurrence.

The zero-adjusted Bray-Curtis similarity index, Cluster and SIMPER analysis were conducted in Primer 6 (Clarke and Gorley, 2006). The RDA analysis was performed using CANOCO 5.00 (ter Braak and Šmilauer, 2012). The Spearman coefficient was calculated using Statistica 13.0 (TIBCO Software Inc., 2017). Species data were log-transformed prior to analyses to give a more balanced ordination of abundance data and so the data could better reveal the structure of our community giving biological relevance not only to the few dominant species (Májeková *et al*., 2016). All figures were processed with Adobe Illustrator CS6.

RESULTS

Odonata assemblages

A total of 19 species (25 taxa, of which 6 belonged to juvenile and/or damaged individuals) was recorded, with *Ischnura elegans* and *Platycnemis pennipes* most frequently recorded (at 13 study sites) (Supplementary Tab. 3). Study site 28 (Česma River at Narta) had the highest species richness with five recorded species. Twelve sites had no Odonata records (Supplementary Tab. 3). Only one endangered/protected species was recorded: *Onychogomphus cecilia* (VU, Red List of Croatian Odonata Fauna, Belančić *et al*., 2008), found at the Sutla River (site 29). Cluster analysis (Fig. 2) did not show any specific grouping of study sites based on RFI scoring.

Environmental variables

Among the study sites, water temperature ranged between 9 and 25°C, oxygen concentration between 1.53 and 10.70 mg/L, conductivity between 207 and 982 µS/cm, pH between 5.68 and 9.20, ammonium concentration between 0.014 and 5.007 mgN/L, nitrates between 0.100 and 6.541 mgN/L, total nitrogen between 0.466 and 14.023 mgN/L, orthophosphates between 0.010 and 6.545 mgP/L, total organic carbon between 1.000 and 27.671 mgC/L, biological oxygen demand between 0.729 and 22.856 mgO_2/L, and chemical oxygen demand between 0.936 and 18.933 mgO_2/L (Supplementary Tab. 1).

Environmental variables and Odonata

The results of the ordination of species and environmental data of the RDA analysis are presented on the F1 × F2 ordination plot. The eigenvalues for the first two RDA axes were 0.15 and 0.05 and together explained 61.2% of the species-environment relations. The Monte Carlo permutation test showed that the species-environment ordination was significant (first axis: F-ratio = 5.82, p=0.03; overall: trace = 0.31, F=1.44, p=0.04) indicating that Odonata assemblages were significantly related to the tested set of environmental variables. Axis 1 was related to chemical oxygen demand (R=0.41) and total organic carbon (R=0.27) and axis 2 to water temperature (R=0.35)

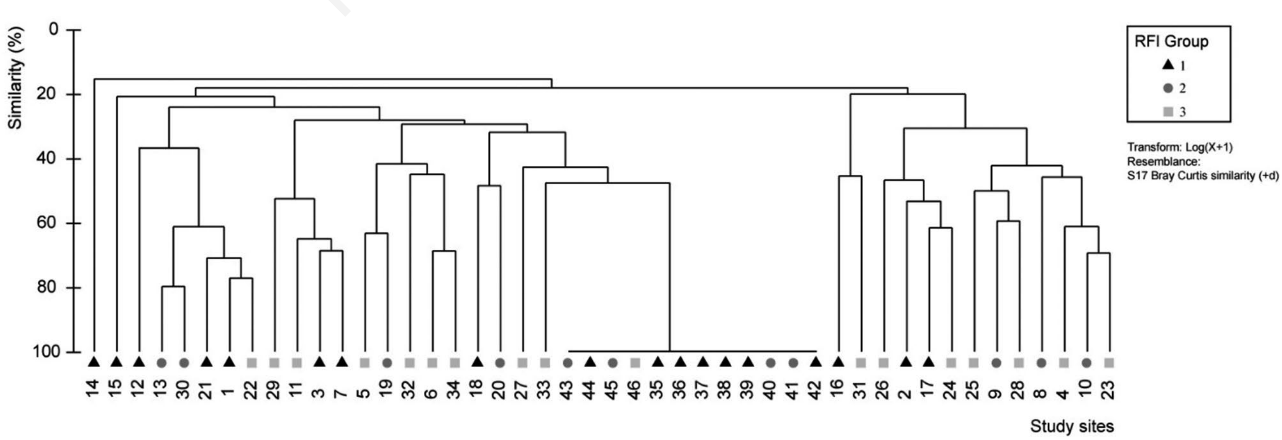

Fig. 2. Cluster analysis of Odonata assemblages based on the zero-adjusted Bray-Curtis similarity coefficient (group average linking) and their log-transformed abundances at 46 anthropogenically impacted habitats in Croatia ("zero dummy" added for sites with no Odonata records). Abbreviations of the study sites are presented in Fig. 1. RFI - Groups are as in Supplementary Tab. 1.

and dissolved oxygen concentration (R=0.35), indicating that these were the most important parameters in explaining patterns of Odonata assemblages (Fig. 3).

The SIMPER group similarity analysis (Tab. 1) showed that all groups of sites (Group 1 - good and high EQR, Group 2 - moderate EQR, and Group 3 - poor and bad EQR based on RFI) were associated with the ubiquitous taxa *Platycnemis pennipes* and *Ischnura elegans*. *Onychogomphus forcipatus* individuals were frequently found in both the poor and bad Group 3 and the good and high Group 1 sites. *Gomphus vulgatissimus* and *Somatochlora meridionalis* were predominantly associated with sites having higher RFI values (Group 1), whereas *Calopteryx splendens* was usually associated with more degraded sites (sites with lower RFI EQR values, Group 3).

Abundance of *Calopteryx splendens* ($r = 0.323$; $p=0.029$) and *Aeshna cyanea* ($r = 0.301$; $p=0.042$) significantly increased with increasing ratios of intensive agriculture in the catchment area, whereas other Odonata species did not show significant correlations to intensive agriculture in the catchment.

DISCUSSION

The results indicate a moderately high species richness of anthropogenically impacted lotic habitats, with the presence of 28% of the total Croatian Odonata fauna (Belančić *et al*., 2008; Boudot and Kalkman, 2015). Nevertheless, with an average of two or three species per site, the local species richness is far from high. These results

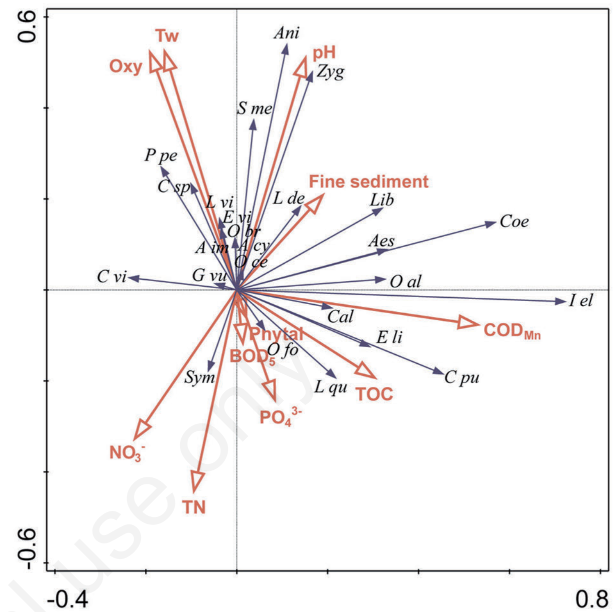

Fig. 3. F1×F2 plane of RDA analysis for 25 Odonata taxa and 11 environmental variables. Legend: Values and abbreviations of environmental parameters (red arrows) are presented in Supplementary Tab. 1 and taxa codes (blue arrows) are presented in Supplementary Tab. 3.

Tab. 1. Results of the SIMPER analysis based on Odonata assemblages from sites of different River fauna index (RFI) values. RFI - Groups are as in Supplementary Tab. 1.

Species	Average abundance per site (ind/m^2)	Similarity contribution within group (%)
Group 1 - good and high EQR based on RFI (EQR > 0.6) Average similarity: 8.98		
Gomphus vulgatissimus	0.62	47.23
Onychogomphus forcipatus	0.35	12.78
Platycnemis pennipes	0.38	12.62
Ischnura elegans	0.28	9.27
Somatochlora meridionalis	0.34	6.95
Calopteryx virgo	0.36	6.77
Group 2 - moderate EQR based on RFI (0.4 > EQR < 0.6) Average similarity: 6.71		
Calopteryx virgo	0.55	31.43
Platycnemis pennipes	0.77	22.80
Ischnura elegans	1.27	21.42
Coenagrionidae juv.	1.20	19.90
Group 3 - poor and bad EQR based on RFI (EQR < 0.4) Average similarity: 15.24		
Ischnura elegans	1.36	33.02
Platycnemis pennipes	1.48	31.03
Calopteryx splendens	0.88	13.20
Coenagrionidae juv.	0.73	7.61
Onychogomphus forcipatus	0.46	6.24

corroborate those presented by Vilenica *et al.* (2020) referring to lentic waterbodies. However, it is important to emphasize that the sampling protocol (AQEM methodology) used enables the assessment of ecological quality in European streams based on benthic macroinvertebrates, following the same unified evaluation scheme (AQEM consortium, 2002). As such, it was not designed as a model for studying Odonata, which in our case, probably resulted in incomplete species list at a particular site. Furthermore, different Odonata life stages are faced with different ecological limitations and mobility (aquatic *vs.* aerial), and the different sampling methods employed likely lead to the incomparability of results (Giugliano *et al*., 2012). In order to more accurately understand species richness and its relationship with a particular habitat, all phases of the Odonata life cycle must be considered, i.e. nymphs, exuviae and adults (Horning and Pollard, 1978; Samways *et al*., 2009; Raebel *et al*., 2010; Golfieri *et al*., 2016). Some studies have shown that nymphal diversity in polluted streams may be considerably lower than that of adults (as seen in May 2019). Site sampling should also be conducted on multiple occasions to ensure the collection of both spring and summer species (Askew, 2004; Corbet and Brooks, 2008; Dijkstra and Lewington, 2006). Therefore, the absence of Odonata from a rather high number of sites and their general low abundance could be related to the poor ecological quality of studied sites, but could also have been partly influenced by the sampling methodology here implemented, which should be inspected with more Odonata-focused future studies. Previous studies have shown that near-natural lowland rivers are characterized by higher Odonata diversity due to the heterogeneous habitat structure (Raab, 1998; Buczyński, 2012, Golfieri *et al*., 2016.), where *e.g.* various *Cordulegaster* species can occur in the rhithron, while species such as *Coenagrion ornatum* (Selys, 1850), *Orthetrum brunneum* (Fonscolombe, 1837) and *Gomphus flavipes* (Charpentier, 1825) could be found in the potamon (Dijkstra and Lewington, 2006; Chovanec *et al.*, 2015).

The majority of the species reported here are characteristic both for lotic and lentic habitats (such as *Erythromma lindenii*, *Onychogomphus forcipatus*), though predominantly lotic (such as *Calopteryx virgo*) and lentic species (such as *Coenagrion puella, Aeshna cyanea*) were also recorded (Janecek *et al.,* 1995; Schmedtje and Colling, 1996; in AQEM expert consortium, 2002). The Cluster analysis did not reveal similarities between the study sites based on the degree of hydromorphological alteration, likely since the majority of species are generalists with a broad distribution and ecological tolerance for habitat conditions (*e.g.*, *Coenagrion puella, Ischnura elegans, Libellula depressa*), corroborating previous studies (Samways and Steytler, 1995; Vilenica *et al.,* 2020). Therefore, it is not surprising that the eurytopic *Ischnura elegans* together with *Platycnemis pennipes*, a species that inhabits a wide range of lotic habitats (Dijkstra and Lewington, 2006), were also the most widespread in this study. Many authors have stated that the dominance of *Ischnura elegans* might indicate heavily contaminated running water systems (Rehfeldt, 1983; Solimini *et al*., 1997). Moreover, Heidemann and Seidenbusch (1993) extended this observation to a range of abiotic factors (contamination, salinity, oxygen saturation), and concluded that this species can survive under conditions which no other (European) dragonfly species can tolerate. Several riverine species that can also be present in lentic habitats (*Onychogomphus focipatus, Gomphus vulgatissimus, Somatochlora meridionalis, Calopteryx splendens*) showed various incidences at habitats of different degree of hydromorphological alteration, likely related to their higher preference for oxygenated habitats than to habitats of a specific morphology (Janecek *et al*., 1995; Schmedtje and Colling, 1996; Kalkman *et al.,* 2018).

Flow regulations result in a significant decline in diversity of macrozoobenthic communities, including Odonata, due to the loss of habitat and microhabitat heterogeneity (Usseglio-Polatera and Beisel, 2002; Vilenica *et al.,* 2016; Chovanec, 2018). Our results indicate that degraded habitats are not suitable for rare and endangered species. Nevertheless, at the national level, the record of the vulnerable (VU) species *Ophiogomphus cecilia* at the Sutla River (site 29) is highly important. This species has a limited distribution in continental Croatia, and its populations are threatened by modifications and regulations of lowland rivers (Belančić *et al.,* 2008). Although the site is characterized by a high level of hydromorphological alteration, the water chemistry is not poor thus enabling the species occurrence at this site. *Ophiogomphus cecilia* is a typical burrower preferring sandy substrates, while adults require the presence of surrounding vegetation for resting (Askew, 2004; Hacet and Aktaç, 2008), and these conditions were present at this site. However, given the low abundance, its presence could also be considered as accidental, and should be inspected in greater detail with future species-targeted studies along the whole river course.

Previous studies have shown that the presence of aquatic and riparian vegetation, water velocity and temperature, and substrate grain size are the most important parameters influencing Odonates in lotic habitats (Hardersen, 2008; Silva *et al*., 2010, Golfieri *et al.* 2016). In this study, Odonata assemblages were highly influenced by descriptors of the polluted aquatic environment, *i.e.* chemical oxygen demand and total organic carbon in water, likely originating from domestic sewage, surrounding agricultural fields and cattle farms (Shi *et al*., 2010). Moreover, two species with rather differing ecological demands, *Aeshna cyanea* and *Calopteryx splendens*, seemed to be rather successful in a polluted environment, as their abundances increased with increasing ratios of intensive agriculture area in the catchment area. *Calopteryx splendens* is an euryecious species, favouring unshaded streams and rivers and achieving its maximum in natural, relatively slow lowing lowland rivers open to the sun. In contrast, *Aeshna cyanea* can mostly be found in small and at least partly shaded standing waters (Askew, 2004; Dijkstra and Lewington, 2006). What they have in common is their ability to inhabit alpha- and beta-mesosaprobic waters (Janecek *et al*., 1995; Mihaljević, 2011). Furthermore, heavily polluted sites were also characterized by higher water temperature and low oxygen concentration, two additional variables that have proven to be important determinants for Odonata occurrence (Sato and Riddiford, 2007; McPeek 2008). These sites were also overgrown by dense vegetation. Excessive growth of aquatic macrophytes and algae is supported by the high level inflow of nutrients in water, consequently resulting in low oxygen concentrations in the water-body (Boeykens *et al.,* 2017), which in turn limits the Odonata presence at such habitats (Rose and Crumpton, 1996). Therefore, less polluted sites with higher oxygen levels and lower water temperatures harbour more Odonata species (Osborn, 2005).

CONCLUSIONS

This study confirms the bioindicator properties of Odonata and shows their sensitivity to water pollution. Our findings confirmed that human pressures on the lotic freshwater system result in impoverished Odonata assemblages that consist mainly of widespread, common species, while the rare and protected species are generally not able to reproduce there. These results contribute to our knowledge of Odonata occurrence in anthropogenically impacted habitats, and their relationships with such degraded environment.

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