BIOLOŠKE I EKOLOŠKE ZNAČAJKE PORODICE CHIRONOMIDAE (INSECTA: DIPTERA) NA PODRUČJU DINARIDSKE EKOREGIJE HRVATSKE

Dorić, Valentina

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Valentina Dorić

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Valentina Dorić

BIOLOGICAL AND ECOLOGICAL FEATURES OF THE FAMILY CHIRONOMIDAE (INSECTA: DIPTERA) IN THE DINARIC ECOREGION OF CROATIA

DOCTORAL THESIS

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Ovaj doktorski rad izrađen je u Zoologijskom zavodu Biološkog odsjeka Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu, pod vodstvom izv. prof. dr. sc. Marije Ivković, u sklopu Sveučilišnog poslijediplomskog doktorskog studija Biologije pri Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu.

Životopis mentorice

IVKOVIĆ, MARIJA (Zagreb, 20. srpnja 1982.), izvanredna profesorica, 286320

Osnovnu školu i VII. gimnaziju završila je u Zagrebu gdje je 2000. godine upisala smjer ekologija na Biološkom odsjeku PMF-a, a diplomirala 2005. godine. Zaposlena je u svibnju 2006. godine na Zoologijskom zavodu gdje je i doktorirala, u srpnju 2010. godine, a trenutačno je izvanredni profesor. Tijekom 18 godina bila je na više usavršavanja, na Prirodoslovnom muzeju u Ljubljani (Slovenija, 2006.), zatim 2009. godine od siječnja do srpnja provela je na usavršavanju iz polja slatkovodne ekologije na Sveučilištima u Gironi (Španjolska), Potsdamu (Njemačka), Veszpremu (Mađarska) i Uppsali (Švedska), na Sveučilištu Komenského u Bratislavi (Slovačka, 2010.), na Nacionalnom muzeju Walesa u Cardiffu (UK, 2012.). Provela je tri mjeseca na postdoktorskom usavršavanju na Sveučilištu u Granadi (Španjolska, 2013.), a 2016. godine je dobila Synthesys projekt i bila na Prirodoslovnom muzeju u Stockholmu (Švedska), na Nord University (Norveška, 2022.) te na Sveučilištu u Novom Sadu (Srbija, 2023.). Voditelj je ili sudjeluje u vođenju nastave iz kolegija Ekologija kopnenih voda, Zdravstvena i veterinarska entomologija, Biologija onečišćenih voda, Opća Ekologija, Ekologija životinja, Filogenija i sistematika životinja. Do sada je bila neposredni voditelj šest diplomskih radova, voditelj sedam diplomskih radova, pet završnih radova i voditelj nagrađenog Rektorskog rada. Područje znanstvenog interesa Marije Ivković je slatkovodna ekologija i entomologija s posebnim osvrtom na ekologiju, taksonomiju, filogeniju i biogeografiju vodenih dvokrilaca (Diptera), osobito muha plesačica (Diptera, Empididae) i mušica svrbljivica (Diptera, Simuliidae), ali i ostalih porodica reda dvokrilaca, poput Muscidae, Dixidae, Psychodidae, Limoniidae, Pediciidae te drugih vodenih kukaca. Sudjelovala je na šest znanstvenih projekata i preko 20 stručnih projekata. Objavila je 64 znanstvena, tri stručna, tri popularno-znanstvena rada, 57 kongresnih priopćenja te je koautor Sveučilišnog udžbenika i urednik znanstvene knjige Plitvice Lakes, a obje knjige su dobile Godišnju nagradu Hrvatskih voda. Od 2014. godine je Subject Editor u znanstvenom časopisu ZooKeys, a od 2020. je Associate Editor u znanstvenom časopisu Ecological Entomology. Jedna je od osnivača Hrvatskog udruženja slatkovodnih ekologa (HUSEk) čija je trenutačna predsjednica. Do sada je bila u organizaciji nekoliko važnih kongresa iz područja slatkovodne ekologije, 11. Symposium for European Freshwater Sciences i 1., 2., 3. i 4. Simpozija o biologiji slatkih voda (SOBS).

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Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

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BIOLOŠKE I EKOLOŠKE ZNAČAJKE PORODICE CHIRONOMIDAE (INSECTA: DIPTERA) NA PODRUČJU DINARIDSKE EKOREGIJE HRVATSKE

Valentina Dorić
Prirodoslovno-matematički fakultet, Biološki odsjek

Trzalci (Diptera, Chironomidae) su jedna od najrasprostranjenijih i često najbrojnijih skupina vodenih kukaca svih vodnih tijela, a koriste se u analizama procjene kvalitete vode. Detaljnije istraživanje njihove ekologije i taksonomije je prijeko potrebno s obzirom na njihovu indikatorsku važnost. Nacionalni park Plitvička jezera je odabran kao primarno područje istraživanja, jer je moguće provesti sveobuhvatno i detaljno istraživanje biologije i ekologije trzalaca. Na području Plitvičkih jezera ukupno je zabilježeno 116 vrsta trzalaca čime je ukupan broj vrsta u Hrvatskoj povećan na 258. Utvrđeno je produljenje faze leta trzalaca te promjene u sastavu zajednice kao rezultat zatopljavanja i ekstremno visokih protoka koji postaju učestaliji zbog globalnih klimatskih promjena. Osim toga, kroz izradu novog indeksa kao metrike za procjenu stanja litorala dokazana je važnost trzalaca kao indikatorske skupine. Time se ukazalo na važnost taksonomske rezolucije u zaštiti i upravljanju stajaćicama, čijom primjenom se mogu poboljšati postojeći modeli procjene ekološkog stanja ekosustava.

(120 stranica, 2 slike, 110 literaturnih navoda, jezik izvornika hrvatski)
Ključne riječi: trzalci, klimatske promjene, fenologija, nitrati, protok vode
Mentor: izv. prof. dr. sc. Marija Ivković
Ocjenjivači: prof. dr. sc. Jasna Lajtner
doc. dr. sc. Vlatka Mičetić Stanković
prof. dr. sc. Đurađ Milošević

University of Zagreb
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BIOLOGICAL AND ECOLOGICAL FEATURES OF THE FAMILY CHIRONOMIDE (INSECTA: DIPTERA) IN THE DINARIC ECOREGION OF CROATIA

Valentina Dorić
Department of Biology, Faculty of Science

Chironomids (Diptera, Chironomidae) are widely distributed and often among the most abundant aquatic insects used in water quality analysis. Knowledge of their ecology and taxonomy should be studied in detail due to their significance as indicators in aquatic ecosystems. Plitvice Lakes National Park was chosen as a primary study area due to minimal anthropogenic influence and it is possible to have a comprehensive and detailed study of chironomid biology and ecology. At the end of this study a total of 116 chironomid species were recorded in the Plitvice Lakes raising total abundance of chironomid species in Croatia to 258. It was also detected that chironomid flight period extended due to higher water temperatures. A change in the chironomid community composition was also observed and atributed to extreme discharges facilitated by climate driven changes. In the end, Lake Chironomid Index was developed in order to address the importance of high taxonomic resolution in freshwater bioassessment which has the potential of improving already existing bioassessment models.

(120 pages, 2 figures, 110 references, original in Croatian)

Keywords: midges, climate change, phenology, nitrates, water discharge

Supervisor:	Associate Professor Marija Ivković, PhD
Reviewers:	Professor Jasna Lajtner, PhD
	Assistant Professor Vlatka Mičetić Stanković, PhD
	Professor Đurađ Milošević, PhD

SADRŽAJ

1. UVOD

1.1. Biologija trzalaca (Chironomidae)

Porodica Chironomidae pripada razredu Insecta, redu Diptera, podredu Nematocera. Dijeli se na 11 potporodica i 22 plemena (Ferrington, 2008). U svijetu postoji više od 8000 opisanih vrsta trzalaca (Marshall, 2012), ali svakim danom se opisuju nove vrste. Prema popisu vrsta za Europu, opisano je 1258 vrsta unutar osam potporodica (Buchonomyiinae, Chironominae, Diamesinae, Orthocladiinae, Podonominae, Prodiamesinae, Tanypodinae i Telmatogeninae). U Hrvatskoj je dosad zabilježeno 239 vrsta trzalaca (Čerba i sur., 2020), ali zbog nedostatnih novijih kontinuiranih istraživanja standardiziranim metodama nije moguće govoriti o konačnoj brojnosti vrsta na našem području.

Trzalci (Chironomidae) su holometabolni kukci koji pripadaju redu dvokrilaca (Diptera). U uvjetima vrlo niskih koncentracija kisika trzalci će biti jedina skupina vodenih kukaca prisutna u sedimentu što ih čini jednom od najuspješnijih porodica vodenih kukaca. Nastanjuju sve tipove slatkovodnih, kao i kopnena, morska pa čak i podzemna staništa (Armitage i sur., 1995; Andersen i sur., 2016). Trzalci su osvojili razna ekstremna staništa pa ih je moguće pronaći na ledenjacima Himalaje gdje su aktivni i pri temperaturi od −16 °C (Kohshima, 1984), u Bajkalskom jezeru na dubini od 1580 m (Papoucheva i sur., 2003), na Antartici gdje su jedini nativni kukci upravo dvije vrste trzalaca (Richard i sur., 1994) te valja spomenuti vrstu *Polypedilum vanderplanki* Hinton, 1951 koja nastanjuje povremene bazene u bazaltnim stijenama sjeverne Nigerije, a tijekom dijapauze izgubi i do 97 % vode u tijelu (Knutelski i sur., 2022). Jedno od najneobičnijih staništa na koje su se trzalci adaptirali je svježi kravlji izmet, koji čini jedino stanište ličinki vrste *Camptocladius stercorarius* (DeGeer, 1776). Trzalci pružaju brojne usluge ekosustava uključujući povezivanje vodenih i kopnenih ekosustava, bioturbaciju, čišćenje vode i pohranjivanje ugljika (Armitage i sur., 1995; Baranov i sur., 2016a; 2016b).

Ženke trzalaca obično liježu jaja samo jednom i to kao pakete jaja koji se nalaze unutar galerte (Slika 1). Kod manjih vrsta izliježe se 20 do 30 jaja u paketu, a u većih i do 2000 jaja u paketu. Ženke većine vrsta trzalaca polažu jaja na čvrst supstrat (kamenje, listinac ili vodena vegetacija) u blizini ruba vodnog tijela. U rijeđim slučajevima jaja se polažu izravno u vodu, a ako je riječ o tekućici jaja se polažu u mirnijem djelu toka (Nolte, 1993). Jaja se nalaze zaštićena unutar galerte koja se u dodiru s vodom raširi i potone na dno stajaćice ili se zalijepi za prvu čvrstu stvar u vodi (npr. vodena vegetacija). Polaganje jaja je pod utjecajem promjene dnevnog svjetla pa se u većini slučajeva jaja polažu u zoru ili sumrak (Armitage i sur., 1995). Najčešći grabežljivci jaja trzalaca su vodengrinje koje uz to što se hrane jajima polažu i svoja jaja unutar njih. Brzina kojom se jedinke razvijaju iz jaja ovisi o temperaturi vode. Ukoliko je temperatura vode viša, utoliko će razvoj ličinke biti brži (Armitage i sur., 1995; Vallenduuk i Moller Pillot, 2007).

Slika 1: Jaja trzalca potporodice Chironominae. (Preuzeto i prilagođeno prema https://bugguide.net/node/view/641162).

Ličinački razvoj većine vrsta trzalaca sastoji se od četiri ličinačka stadija. Ličinka (lat. *larva*) prvog stadija razvoja je pokretljivija od starijih ličinačkih stadija, a u stajaćicama prvih par dana živi planktonskim načinom života. Nakon tri do četiri dana ličinka prvog stadija razvoja se polako spušta na dno gdje započinje bentoski način života. Ličinke drugog, trećeg i četvrtog stadija razvoja (Slika 2a) razlikuju se u veličini, stupnju sklerotizacije glave i tijela te boji. Duljina trajanja pojedinog stadija razvoja ovisi o temperaturi vode, koncentraciji kisika, razini vode i količini dostupne hrane, a u nepovoljnim uvjetima bilo koji od njih može započeti s dijapauzom. Osim preživljavanja nepovoljnih uvjeta, dijapauza trzalcima služi i za postizanje sinkronizacije emergencije čime se povećava uspješnost parenja (Vallenduuk i Moller Pillot, 2007).

Ličinke trzalaca pripadaju različitim funkcionalnim hranidbenim skupinama (Moog i Hartmann, 2017). Organizmi se u funkcionalne hranidbene skupine (eng. *functional feeding groups*) svrstavaju na temelju načina hranjenja i vrste hrane koju konzumiraju. Prema načinu na koji se hrane podijeljeni su na: usitnjivače (eng. *shredders*) koji žvaču ili drobe hranu, a najčešće alge, makrofite i listinac; strugače (eng. *grazers*) koji mogu sastrugati hranu s kamena, drveta ili drugih podloga, a najčešće se hrane algama ili mikroorganizmima; filtratore (eng. *filtering collectors*) koji filtriraju usitnjenu organsku tvar u vodenom stupcu; sakupljače detritusa (eng. *detritus feeders*), a hrane se vrlo sitnom organskom tvari koja je nataložena na dnu; bušače (eng. *miners*) koji se ubušuju te hrane lišćem i stabljikama makrofita; ksilofage (eng. *xylophagous*) koji se hrane drvenim materijalom; grabežljivce (eng. *predators*) koji napadaju i ubijaju svoj plijen te parazite (eng. *parasites*) koji pronađu odgovarajućeg domaćina i koriste njegove resurse što je duže moguće (Moog i Hartmann, 2017). Ličinke trzalaca najpoznatije su po tome što mogu preživjeti uvjete vrlo niskih koncentracija kisika što im omogućuje hemoglobin kao pigment njihove hemolimfe. Osim što im služi za prijenos kisika po tijelu, kisik se može „pospremiti" u hemoglobin i iskoristiti u nepovoljnim uvijetima (Lancaster i Downes, 2013). Neke vrste trzalaca nepovoljne uvijete preživljavaju tako da koriste kisik pohranjen u rupama „U" oblika koje kopaju u sedimentu, a mogu poslužiti služe kao džepovi kisika (Charbonneau i Hare 1998; Baranov i sur., 2016b).

Stadij kukuljice (lat. *pupa*) (Slika 2b) je relativno kratak i predstavlja najosjetljiviji razvojni stadij trzalaca jer predstavlja vrlo važan izvor hrane mnogim vrstama riba i ptica (Vallenduuk i Moller Pillot, 2007). Tijekom izlijetanja vanjska površina kukuljice puca, iz unutrašnjosti izlazi odrasla jedinka, a iza nje ostaje svlak (lat. *exuvium*). Kukuljice većine vrsta trzalaca imaju tri glavne uloge: presvlačenje ličinačke kutikule, prijenos kisika i prijenos jedinke s dna na površinu vode za emergenciju (Armitage i sur., 1995).

Slika 2: Razvojni stadiji trzalca roda *Chironomus*: A) ličinka, B) kukuljica, C) odrasla jedinka, ♂ (slike su preuzete i prilagođene prema: A) https://bugguide.net/node/view/268448; B) https://swittersb.com/2008/12/28/chironomid-midge-gnat-klaus-peter-brodersens-amazing-images; C) https://www.marylandbiodiversity.com/view/15498).

Emergencija je proces tijekom kojeg vodeni kukac izlazi iz vode i pretvara se u odraslu jedinku (Slika 2c) (Lancaster i Downes, 2013). Ovim procesom se povezuju vodeno i susjedna kopnena staništa prijenosom energije i hranjivih tvari među njima (Nakano i Murakami, 2001). Odrasli vodeni kukci su vrlo važan izvor hrane kopnenim grabežljivcima poput pauka, trčaka, ptica i šišmiša (Paetzold i Tockner, 2005; Dreyer i sur., 2015). Temperatura vode i fotoperiod (duljina trajanja dana) se smatraju ključnim okolišnim čimbenicima koji utječu na početak i duljinu trajanja emergencije (Ivković i sur., 2013a).

U sjevernom umjerenom pojasu većina vrsta trzalaca emergira od svibnja do listopada u stajaćicama i tekućicama, a u sastavu vrsta dominiraju potporodice Tanypodinae i Chironominae. S vremenom je uočen jasan obrazac emergencije između potporodica trzalaca pa tako potporodice Orthocladiinae, Diamesinae i Podonominae imaju proljetnu emergenciju, a potporodice Tanypodinae i Chironominae ljetnu (Armitage i sur., 1995; Milošević i sur., 2013).

Temperatura vode i fotoperiod su glavni ekološki čimbenici koji utječu na emergenciju trzalaca (Armitage i sur., 1995; Doria i sur., 2021). U istraživanju Nordlie i Arthur (1981) su eksperimentalno podigli temperaturu vode za 10 °C što je ubrzalo vrhunac emergencije za jedan do četiri tjedna. Duljina trajanja dana, količina dostupne hrane u ličinačkom stadiju, kompeticija i predacija nad ličinkama također utječu na emergenciju (Armitage i sur., 1995). Osim sezonskih obrazaca emergencije uočen je i dnevni ritam emergencije trzalaca. Rijetke vrste emergiraju tijekom cijeloga dana (u periodu od 24 sata) dok većina vrsta emergira u zoru ili u sumrak. U isto vrijeme formiraju se i rojevi trzalaca koji se mogu sastojati od svega nekoliko jedinki do ogromnih rojeva sa stotinama milijuna jedinki kao u vrste *Lymnophyes minimus* (Meigen, 1818). Omjer spolova u roju varira, ali najčešće dominiraju mužjaci s manjim brojem ženki. Smatra se da takav sastav roja omogućuje ženkama da povećaju vjerojatnost osjemenjivanja i da odaberu najboljeg mužjaka za parenje (veličina jedinke te brzina i spretnost leta utječu na odabir). Partenogeneza je zabilježena samo kod nekih vrsta potporodice Orthocladiinae i plemena Tanytarsini (Armitage i sur., 1995; Vallenduuk i Moller Pillot, 2007).

Broj generacija godišnje (voltinizam) varira od jedne generacije svakih sedam godina do pet ili više generacija godišnje (Vallenduuk i Moller Pillot, 2007). Voltinizam trzalaca određen je proučavanjem ličinačkih stadija i bilježenjem emergencije odraslih. Vrste su većinom univoltne (33 %) ili bivoltne (44 %), rijeđe multivoltne (18 %). Najveći broj generacija, njih sedam, su zabilježili Learner i Edwards (1966) kod vrste *Chironomus riparius* (Meigen, 1804) iz onečišćene engleske rijeke. Emergencija univoltnih vrsta najčešće se dešava u ljeto, bivoltnih u proljeće i kasno ljeto ili ranu jesen, a multivoltnih vrsta od proljeća do kasne jeseni s više izraženih maksimuma emergencije (Armitage i sur., 1995). Za bivoltne kao i multivoltne vrste karakteristično je da je prva emergencija u rano proljeće najobilnija dok su ostale slabije, a jedinke koje tada emergiraju su veće (Armitage i sur., 1995; Shipley i sur., 2022).

Nakon emergencije odrasle jedinke većine vrsta trzalaca se ne hrane, a žive kraće od jednoga dana. Ženke polažu jaja samo jednom, ali je u nekih vrsta koje se hrane moguće polaganje druge mase jaja nakon hranjenja. Trzalci se rasprostranjuju pasivno nošeni vjetrom ili strujom vode (Vallenduuk i Moller Pillot, 2007).

Trzalci se određuju temeljem svih životnih stadija, ali najčešće temeljem ličinke što često dovodi do nepotpunih podataka o sastavu vrsta, jer u nekim slučajevima nije moguće pouzdano odrediti vrstu. S druge strane, uzorkovanje ličinki omogućuje procjenu veličine populacije što nije moguće uzorkovanjem niti jednog drugog životnog stadija. Određivanjem svlakova i odraslih jedinki pouzdana je determinacija do razine vrste, a temeljem odraslih jedinki moguć je i opis novih vrsta (Vallenduuk i Moller Pillot, 2007; Andersen i sur., 2013).

1.2. Povijest istraživanja trzalaca

Rana istraživanja trzalaca u Europi počinju u prvoj polovici 20. stoljeća, a naglasak je na taksonomskim istraživanjima i upoznavanju životnih ciklusa različitih vrsta. Kieffer, Edwards i Goetghebuer jedni su od najznačajnijih znanstvenika koji su postavili temelje za daljnja istraživanja trzalaca u Europi (Kieffer, 1915; Goetghebuer, 1919; Edwards, 1929). Sredinom 20. stoljeća razvojem mikroskopije i ekologije šire se i istraživanja trzalaca pa se tada znanstvenici više okreću ekološkim funkcijama trzalaca u okolišu (Brundin, 1949; Davies, 1975). Tijekom druge polovice 20. stoljeća trzalci se počinju koristiti kao bioindikatori kvalitete vodenih staništa (Saether, 1979).

Danas je naglasak na multidisciplinarnom pristupu istraživanja trzalaca. Razvija se njihova upotreba kao indikatora u paleobiologiji (Luoto, 2009; Bitušík i sur., 2017), istražuje se utjecaj klimatskih promjena na raznolikost i rasprostranjenost trzalaca (Hamerlík i Bitušik, 2009; Engels i sur., 2019) te se razvijaju nove metode za poboljšanje i olakšavanje njihove upotrebe kao već postojećih bioindikatora stanja slatkovodnih staništa (Serra i sur., 2016; Milošević i sur., 2020).

Istraživanja trzalaca Dinaridske ekoregije su malobrojna i usputna, a poznata je kao jedan od centara bioraznolikosti vodenih dvokrilaca (Ivković i Plant, 2015). U njoj je opisana i prva poznata vrsta letećeg troglobionta, a radi se o trzalcu vrste *Troglocladius hajdi* (Andersen i sur., 2016). Dinaridi su najveći neprekinuti krški krajolik Europe (Mihevc i sur., 2010) s vrlo kompleksnom hidrologijom (Bonacci i sur., 2013), a zbog jedinstvene slatkovodne faune Illies (1978) ih svrstava u zasebnu Dinaridsku ekoregiju (*Western Dinaric Balkan ecoregion,* ER 5).

Prvo istraživanje na području Nacionalnog parka Plitvička jezera, a ujedno i prvo istraživanje u Hrvatskoj s upoznavanjem faune trzalaca kao glavnim ciljem proveli su Kostić-Brnek i Brnek-Kostić (1971). Tada je zabilježeno ukupno 50 vrsta trzalaca iz dvaju najvećih jezera, Prošća i Kozjaka. Njih 45 određeno je prema ličinačkom stadiju, a njih pet na temelju odraslih jedinki (Kostić-Brnek i Brnek-Kostić, 1971). Do tada su trzalci bili istraživani u sklopu biocenoloških istraživanja cijelog makrozoobentosa te su uglavnom bili određivani do razine porodice ili eventualno potporodice (Matoničkin i Pavletić, 1965). Noviji podaci o sastavu vrsta temeljeni na fauni odraslih trzalaca dostupni su za izvore Crne i Bijele rijeke, zabilježeno je 40-ak vrsta (Ivković i sur., 2015), a od toga jedna je opisana kao nova vrsta za znanost (Giłka i sur., 2013). Na samim sedrenim barijerama zabilježene su tek četiri vrste trzalaca: *Parametriocnemus stylatus* (Spärck, 1923), *Psectrocladius barbimanus* (Edwards, 1929), *Stempellina bausei* (Kieffer, 1911) i

Prodiamesa olivacea (Meigen, 1818) (Matoničkin i sur., 1971). Osim na Plitvičkim jezerima, zajednice trzalaca i njihove prilagodbe istraživane su u hiporeiku Drave (Meštrov i sur., 1976), na različitim područjima duž toka rijeke Save, u Sloveniji i Hrvatskoj (Tavčar, 1984), u tekućicama na području Psunja i Papuka (Tavčar, 1988) te bentosu Butonige i njenih pritoka (Tavčar, 1993). Tijekom posljednih 20-ak godina znanstveno zanimanje za trzalce u Hrvatskoj se pojačalo pa su provedena istraživanja na zajednicama trzalaca u jezerima Visovac (Mihaljević i sur., 2000) i Njivice (Mihaljević i sur., 2004), fitofilne zajednice na području Parka prirode Kopački rit (Čerba i sur., 2010; 2022), pa čak i u fontanama Osijeka i Varaždina (Čerba i Hamerlík, 2021).

Zahvaljujući naporima Čerbe i sur. (2020) koji su objedinili svo znanje do tada, znamo da na prostoru Hrvatske živi barem 239 vrsta trzalaca koje pripadaju u pet potporodica: Chironominae (125 vrsta), Orthocladiinae (83 vrste), Tanypodinae (23 vrste), Diamesinae (šest vrsta) i Prodiamesinae (dvije vrste).

1.3. Utjecaj klimatskih promjena na slatkovodne ekosustave

Klimatske promjene su promjene u stanju klime koje se mogu statističkim metodama utvrditi kao promjene u srednjim vrijednostima ili varijanci svojstava klime, a koje traju dulje vremensko razdoblje, obično desetljeće i duže. Do klimatskih promjena može doći uslijed prirodnih procesa ili ustrajnih promjena u sastavu atmosfere ili korištenju Zemljine površine uzrokovanih antropogenim utjecajem (Solomon i sur., 2007).

Ekstremne pojave, poput požara ili poplava, su prirodan i važan dio mnogih ekosustava, ali uslijed klimatskih promjena dolazi do promjena u režimu i trajanju tih pojava. U mnogim dijelovima svijeta su već uočene pojave produljene suše, toplinskih valova, požara te ekstremnih količina oborina koje rezultiraju poplavama (Douville i sur., 2021).

Prema IPCC (*Intergovernmental Panel on Climate Change*) izvješću iz 2022. godine opažene promjene u rasponu rasprostranjenosti, fenologiji, fiziologiji i morfologiji kopnenih i slatkovodnih vrsta mogu se pripisati klimatskim promjenama na regionalnoj i globalnoj razini, a posebice povećanoj učestalosti i jačini ekstremnih pojava (Parmesan i sur., 2022).

Temperatura vode u rijekama se unutar jednog desetljeća povisila i do 1 °C, a u jezerima za 0,45 °C (Parmesan i sur., 2022). Ono što je poznato jest da s povišenjem temperature vode dolazi do smanjenja koncentracije kiska u vodi i povećanja stope primarne produkcije (Hyndman, 2014). Istovremeno se smanjio opseg snježnog pokrivača i za do 25 %, a njegovo trajanje je skraćeno i za više od 2 tjedna (Parmesan i sur., 2022). Promjene u protoku vode dovode do smanjenja povezanosti riječnih staništa (Poff i sur., 1997). Indirektne promjene uključuju promjene u riječnoj morfologiji, sastavu supstrata, koncentraciji kisika i stratifikaciji (Hyndman, 2014; Parmesan i sur., 2022).

Kao odgovor na klimatske promjene, vrste se mogu prilagoditi novonastalim uvjetima ili promijeniti svoje područje rasprostranjenosti. Uslijed povišene temperature vode uočeno je povlačenje obalčara vrste *Zapada glacier* (Baumann & Gaufin, 1970), koja je endemska vrsta alpskih potoka Montane (SAD), na uzvodna staništa koja se nalaze na višoj nadmorskoj visini i hladnija su (Giersch i sur., 2015). U njemačkom potoku Breitenbach uočeno je produljeno trajanje emergencije vodenih vrsta kukaca i to za 15 dana (Baranov i sur., 2020). Povišena temperatura vode bi mogla izravno utjecati na rast i razvoj ličinki trzalaca što bi moglo rezultirati ubrzavanjem njihovog životnog ciklusa. To može dovesti do ranije emergencije trzalaca i ranijeg vrhunca emergencije kao što je opisano u Baranov i sur. (2020) čime je moguć nastanak problema za vrste čiji životni ciklusi ovise o njima kao izvoru hrane (Shipley i sur., 2022). Osim toga moguće su promjene u rasprostranjenosti vrsta. Neke vrste bi se mogle proširiti u nove areale dok bi neke mogle izumrijeti u svojim dosadašnjim staništima. Oba scenarija mogu dovesti do značajnih promjena unutar lokalnih ekosustava i hranidbenih mreža (Parmesan i sur., 2022).

Promjene u režimu oborina i povećanoj učestalosti ekstremnih vremenskih prilika povezanih s klimatskim promjenama mogu dovesti do promjena u slatkovodnim staništima ili, kao u slučaju Nacionalnog parka Plitvička jezera, nestajanja pojedinih tipova staništa uslijed pucanja sedrenih barijera (Radišić i sur., 2021; Vurnek i sur., 2021), što na kraju može imati negativan utjecaj na sastav i strukturu zajednica.

1.4. Monitoring slatkovodnih ekosustava

Slatkovodni ekosustavi su esencijalni za održavanje ljudske populacije što se vidi i iz stoljećima dugog naseljavanja uz putove rijeka. Kao posljedica stalnog iskorištavanja vodenih ekosustava pojavila se potreba za zaštitom istih. Danas je poznato da su izmjene u vodotocima, gubitak staništa i opadanje njegove kvalitete, zagađenje vode i uvođenje stranih vrsta glavni uzroci opadanja broja vrsta i degradacije ekosustava (Malmqvist i Rundle, 2002). Naravno, niti jedan od navedenih pritisaka ne djeluje sam, odvojen od ostalih, a kad se još dodaju i klimatske promjene zaista je teško u realnom vremenu pratiti sve promjene koje se zbivaju.

Prema Okvirnoj direktivi o vodama (*Water Framework Directive*, WFD) „voda nije kao drugi komercijalni proizvodi, već je naslijeđe koje treba čuvati, zaštititi i postupati s njome kao takvom“. Glavni cilj Okvirne direktive o vodama je postizanje dobre kvalitete svih voda na području Europe kroz monitoring i upravljanje vodama (Directive 2000/60/EC, 2000). Uvjeti za postizanje dobrog ekološkog stanja su sljedeći:

1) Svi biološki elementi kakvoće (makrozoobentos, ribe, fitobentos, fitoplankton i makrofita) moraju postići „dobar ekološki status“,
2) Koncentracije svih onečišćivala koja su specifična za slivno područje ne smiju prelaziti granične koncentracije,
3) Vrijednosti ostalih elemenata kakvoće i hidromorfologije se moraju nalaziti unutar granica koje omogućuju dobro funkcioniranje ekosustava.

Ekološki monitoring i procjena stanja su osnova uspješnog upravljanja ekosustavima. „Stanje površinske vode” je općeniti izraz koji označava stanje nekog tijela površinske vode, određeno njegovim ekološkim ili kemijskim stanjem, ovisno o tome koje je lošije (Park i Hwang, 2016). Prema Zakonu o vodama (Narodne novine, br. 153/09, 63/11, 130/11, 56/13 i 14/14) jedan od ciljeva upravljanja vodenim ekosustavima je postizanje i očuvanje dobrog stanja voda, što uključuje postizanje dobrog ekološkog stanja svih površinskih vodnih tijela.

Ocjenjivanje ekološkog stanja voda predstavlja mjerenje promjene stanja i funkcije ekosustava u odnosu na prirodno, odnosno referentno. U odnosu na veličinu promjene, razvrstava se u jednu od od 5 kategorija ekološkog stanja (Tablica 1): 1. Vrlo dobro, 2. Dobro, 3. Umjereno, 4. Loše i 5. Vrlo loše stanje. Za ocjenu od 3 do 5 potrebno je provesti mjere koje će za posljedicu imati poboljšanje ekološkog stanja. Ekološko stanje tijela površinske vode određuje se temeljem rezultata monitoringa bioloških elemenata kakvoće, ali i hidromorfoloških, osnovnih fizikalno-kemijskih i kemijskih elemenata. Prema WFD, izmjenjena i umjetna vodna tijela se promatraju odvojeno od prirodnih i za njih se određuje ekološki potencijal (Arle i sur., 2016).

Tablica 1: Kategorije ekološkog stanja (preuzeto i prilagođeno iz: Hrvatske vode, 2016).

Kategorije ekološkog stanja	Boja
Vrlo dobro	Plava
Dobro	Zelena
Umjereno	Žuta
Loše	Narančasta
Vrlo loše	Crvena

Makrozoobentos je jedan od bioloških elemenata kakvoće koji se učestalo koristi za praćenje stanja vodenih ekosustava zbog svoje raznolikosti, sedentarnog načina života i kratkog životnog ciklusa, a među pripadnicima makrozoobentosa se osobito ističu trzalci (Bae i sur., 2014).

1.5. Indikatorska vrijednost trzalaca

Od samih početaka monitoringa slatkih voda različiti organizmi se koriste kao pokazatelji (indikatori) onečišćenja. Trzalci su imali jednu od glavnih uloga u biološkoj klasifikaciji jezera zahvaljujući njihovoj dominaciji u profundalu jezera i odgovoru na razinu trofije (Brundin, 1949). Sastav zajednice trzalaca u profundalu jezera je pod utjecajem koncentracije kisika i količine te kvalitete dostupne hrane pa tako profundal oligotrofnih jezera nastanjuju slobodnoživuće vrste trzalaca koje se mogu pokretati u potrazi za rijetko dostupnom hranom. S druge strane, profundal eutrofnih jezera je bogat hranjivim tvarima pa se vrste koje ga nastanjuju nalaze zaštićene unutar cijevi izgrađenih od detritusa, a uglavnom se hrane pasivnim filtriranjem (Armitage i sur., 1995). U redovnom monitoringu stajaćica u Hrvatskoj trzalci se određuju do razine vrste (Mihaljević i sur., 2018a; 2018b).

Klasifikacija tekućica nikada nije rezultirala uspjehom kakav su imale stajaćice. Najveći problem je u longitudinalnoj promjenjivosti tekućica što rezultira mnogobrojnim zonama unutar jedne tekućice (Giller i Malmqvist, 1998). Ono što je moguće očekivati jest da će u hladnim ledenjačkim potocima dominirati vrste iz potporodice Diamesinae, a ostatak će činiti vrste iz potporodice Orthocladiinae. Nizvodno od izvora postepeno će opadati zastupljenost vrsta iz obje potporodice dok će u donjem toku ostati svega nekoliko vrsta iz potporodice Orthocladiinae. U donjem toku tekućica dominiraju vrste plemena Chironomini, jer su odlično prilagođene za preživljavanje u uvjetima niske koncentracije kisika, velike količine otopljenih hranjivih tvari i ujednačene podloge. Zanimljivo je napomenuti da vrste potporodice Tanypodinae i plemena Tanytarsini nisu korelirane ni sa jednim dijelom toka tekućica i njihova zastupljenost u ukupnoj brojnosti vrsta varira od 15 do 20 % (Armitage i sur., 1995).

Povećana gustoća vrsta roda *Chironomus*, posebice vrsta *C. plumosus* (Linnaeus, 1758) i *C. riparius*, povezana je s onečišćenjem organskim tvarima. U posebno onečišćenim tekućicama najčešće se pronalaze vrste rodova *Chironomus* i *Procladius*. Vrsta povezanih s rutinskom ocjenom stanja posebno čistih tekućica nema, jer je određivanje tih vrsta izrazito zahtjevno (Armitage i sur., 1995).

Osim kao jedna od komponenti pri ocjeni stanja slatkovodnih staništa, trzalci su pronašli svoje mjesto i u paleolimnologiji. Zajedno s algama kremenjašicama, rašljoticalcima i ljuskarima njihove glavene kapsule se koriste za razumijevanje promjena u slatkovodnim ekosustavima kroz geološku prošlost (Verschuren i Eggermont, 2006).

Zbog svoje izuzetne raznolikosti i velikih brojnosti u pojedinim slatkovodnim staništima oni imaju velik indikatorski potencijal koji još nije izražen zbog nedovoljnog poznavanja njihove ekologije i taksonomije (Armitage i sur., 1995). S druge strane, zbog te iste ogromne raznolikosti i velikih brojnosti koje mogu doseći u povoljnim uvjetima, postoje nastojanja da ih se određuje do viših taksonomskih kategorija ili čak izbaci iz programa praćenja stanja vodnih tijela (Rabeni i Wang, 2001).

Jedan od glavnih nedostataka za bolje razumijevanje uloga trzalaca u ekosustavima i njihove upotrebe kao bioindikatora je slabo poznavanje lokalne faune trzalaca i njihovih interakcija s okolišem u kojem žive. Čak i u Europi, gdje su započela istraživanja trzalaca i koja se smatra najbolje istraženom regijom u smislu taksonomije trzalaca, svake godine se opisuju nove vrste trzalaca.

1.6. Ciljevi doktorskog rada

Ciljevi ovog doktorskog rada su:

1. Provesti inventarizaciju faune trzalaca na području Nacionalnog parka Plitvička jezera.

2. Odrediti promjene u sastavu zajednice, promjene u brojnosti i raznolikosti trzalaca od 2007. do 2020. godine te ustanoviti fenologiju pojedinačnih vrsta na sedrenoj barijeri Kozjak-Milanovac.

3. Odrediti stopu emergencije trzalaca na sedrenoj barijeri Kozjak-Milanovac u razdoblju od 14 godina.

4. Ukazati na indikatorsku vrijednost i važnost korištenja trzalaca u području zaštite stajaćica krškog područja.

Znanstvene hipoteze ovog doktorskog rada su:

1. Uslijed izmjene lotičkih i lentičkih elemenata na području sedrene barijere Kozjak-Milanovac očekuju se vrste specijalizirane za oba tipa staništa kao i vrste koje su generalisti.

2. Promjena udjela određenog tipa supstrata značajno utječe na zastupljenost, raznolikost i brojnost vodenih kukaca.

3. Brojnost i sastav vrsta trzalaca ovisi o abiotičkim čimbenicima staništa pa će se kao posljedica razlikovati njihova stopa emergencije tijekom godina.

4. Zbog velike raznolikosti i brojnosti koje trzalci postižu u vodenim ekosustavima, imaju veliki indikatorski potencijal.

Ova disertacija obuhvaća četiri izvorne znanstvene publikacije (**I – IV**) koje ispituju navedene hipoteze te ostvaruju navedene ciljeve istraživanja. Publikacija **I** i **III** povezane su s prvim ciljem te odgovaraju na prvu znanstvenu hipotezu istraživanja. Publikacije **II** i **III** su povezane s drugim i trećim ciljem te ispituju drugu i treću hipotezu. Publikacija **IV** povezana je s četvrtim ciljem, a ispituje četvrtu postavljenu hipotezu. U sljedećemu poglavlju, naslova **ZNANSTVENI RADOVI** predstavljene su sve četiri publikacije, a njihov objedinjeni doprinos sagledan je u poglavlju **RASPRAVA**.

2. ZNANSTVENI RADOVI

Popis publikacija

I. Ivković M, Dorić V, Baranov V, Mihaljević Z, Kolcsár L-P, Kvifte GM, Nerudova J, Pont AC (2020) Checklist of aquatic Diptera (Insecta) of Plitvice Lakes National Park, Croatia, a UNESCO world heritage site. ZooKeys 918: 99-142.

II. Dorić V, Ivković M, Baranov V, Pozojević I, Mihaljević Z (2023) Extreme freshwater discharge events exacerbated by climate change influence the structure and functional response of the chironomid community in a biodiversity hotspot. Science of the Total Environment 879: 163110.

III. Dorić V, Pozojević I, Baranov V, Mihaljević Z, Ivković M (2024) Long-Term Chironomid Emergence at a Karst Tufa Barrier in Plitvice Lakes National Park, Croatia. Insects 15: 51.

IV. Dorić V, Pozojević I, Vučković N, Ivković M, Mihaljević Z (2021) Lentic chironomid performance in species-based bioassessment proving: High-level taxonomy is not a dead end in monitoring. Ecological Indicators 121: 107041.

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RESEARCH ARTICLE

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Checklist of aquatic Diptera (Insecta) of Plitvice Lakes National Park, Croatia, a UNESCO world heritage site

Marija Ivković[1*], Valentina Dorić[2*], Viktor Baranov[3], Zlatko Mihaljević[1], Levente-Péter Kolcsár[4], Gunnar Mikalsen Kvifte[5], Jana Nerudova[6], Adrian C. Pont[7]

1 *Division of Zoology, Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia* **2** *Eko-monitoring d.o.o., Kučanska 15, 42000 Varaždin, Croatia* **3** *LMU Munich Biocenter, Department of Biology II, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany* **4** *Department of Civil and Environmental Engineering, Ehime University Bunkyo-cho 3, Matsuyama, 790-8577, Japan* **5** *Department of Arts and Education, Nord University, P.O. Box 1490, 8049 Bodø, Norway* **6** *Moravian Museum, Department of Entomology, Hviezdoslavova 29a, 627 00 Brno, Czech Republic* **7** *Oxford University Museum of Natural History, Parks Road, Oxford OX1 3PW, United Kingdom*

Corresponding author: Marija Ivković (marija.ivkovic@biol.pmf.hr)

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Abstract

Studies on aquatic Diptera in the Plitvice Lakes National Park (Croatia) conducted in the last 50 years have produced 157 species and 7 taxa of aquatic Diptera placed in 13 families. Samples were collected at 25 sampling sites representing the four main types of karst aquatic habitats: spring, stream, tufa barriers and lakes. All records of all the aquatic families of Diptera in Plitvice Lakes NP are summarized, including previously unpublished data. Twelve species new for Plitvice Lakes NP are recorded for the first time, belonging to the families: Chironomidae – *Labrundinia longipalpis* (Goetghebuer, 1921), *Nilothauma brayi* (Goetghebuer, 1921), *Potthastia longimanus* Kieffer, 1922, *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804), *Tanytarsus brundini* Lindeberg, 1963; Dixidae – *Dixella autumnalis* (Meigen, 1838); Scathophagidae – *Acanthocnema latipennis* Becker, 1894 and Stratiomyidae – *Oxycera pardalina* Meigen, 1822, *Oxycera limbata* Loew, 1862, *Oxycera turcica* Ustuner & Hasbenli, 2004, *Nemotelus pantherinus* (Linnaeus, 1758), *Oplodontha viridula* (Fabricius, 1775). The most species-rich family was the Chironomidae with 62 species (and an additional seven taxa), followed by the Empididae with 22 species and

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[*] Contributed equally as the first authors.

Limoniidae with 19 species. The highest number of species was recorded in springs. The relatively low number of species in certain families and the complete absence of some aquatic families shows that further research into the aquatic Diptera in Plitvice Lakes NP is needed.

Keywords

Barrage lake system, Chironomidae, Empididae, Limoniidae, new records, *Oxycera* spp.

Introduction

Most people probably know true flies (Diptera) mainly as a nuisance and as disease-carrying blood-sucking insects, but Diptera are also key players in the recycling of organic material in ecosystems, from the sewage of our urban communities to the leaf litter of the forest floor. In addition, Diptera provide other general ecosystem services such as pollination and pest control, but are also vectors of disease as terrestrial adults (Pape 2009; Marshall 2012; Adler and Courtney 2019).

More than any other group of macro-organisms, Diptera dominate the freshwater environment and are the most numerous group in terms of described species in freshwaters. Nearly one-third of all described fly species, roughly 46,000 species, have some connection with an aquatic environment during development process (Adler and Courtney 2019). Their abundance, omnipresence, and diversity of adaptations to the aquatic environment, position them as major drivers of ecosystem processes (Hölker et al. 2015). Fly larvae are well represented as ecosystem engineers and keystone species that alter the abiotic and biotic environments through activities such as burrowing, grazing, suspension feeding, and predation (Wotton et al. 1998; Adler and Courtney 2019). The enormous populations sometimes achieved by aquatic flies can provide the sole or major dietary component for other organisms, and as both predators and herbivores, they can serve as biological control agents (Collins and Blackwell 2000; Werner and Pont 2003; Adler and Courtney 2019). They serve as indicators of historical and future ecological and climate change while at the same time they have played a role as indicators of water quality from the earliest years of bioassessment (Walker 1987; Mihaljević et al. 1998; Larocque et al. 2001; Adler and Courtney 2019).

As holometabolous insects that undergo complete metamorphosis, all aquatic Diptera have a life cycle that includes a series of distinct stages or instars. A typical life cycle consists of a brief egg stage (usually a few days or weeks, but sometimes much longer), three or four larval instars (usually three in Brachycera, four in lower Diptera, and more in Simuliidae, Tabanidae, Thaumaleidae, some Chironomidae, and a few others), a pupal stage of varying length, and an adult stage lasting from less than two hours (Deuterophlebiidae) to several weeks or even months (Courtney et al. 2017; Lackmann and Butler 2018; Adler and Courtney 2019).

From all types of aquatic habitats, including tree holes to open oceans, and glacial meltwaters to hot springs, Diptera are the true conquerors of the aquatic environment. They have been found at elevations up to 5600 m in the Himalayas and at depths of more than 1000 m in Lake Baikal. Furthermore, the presence of Diptera species in mainland Antarctica (e.g., *Belgica antarctica* Jacobs, 1900) makes them the only group of insects inhabiting all of the world's continents (Allegrucci et al. 2006; Ferrington et al. 2008; Adler and Courtney 2019). Aquatic Diptera are free-living insects that require a wet environment in at least one life stage (Adler and Courtney 2019) or, more strictly, aquatic Diptera are considered as those associated with water bodies (Courtney et al. 2017). Out of 158 dipteran families worldwide, 41 have aquatic representatives (Adler and Courtney 2019), and in Europe there are 130 dipteran families of which about 25 are related to aquatic habitats (Oosterbroek 2006).

Plitvice Lakes form the oldest National Park in the Balkan region and is probably one of the most famous National Parks in Europe because of its exquisite beauty. Plitvice Lakes NP was established as a National Park in 1949, and from 1979 Plitvice lakes NP has been a UNESCO world natural heritage site (Stilinović and Božičević 1998). Its importance is not only scientific, as a unique karstic phenomenon, but also as a place of huge economic importance for the local community as more than a million people per year come to visit it.

Materials and methods

Study site

Plitvice Lakes National Park (NP) is a 295 km^2 forest reserve located in the karst region of the Dinaric Mountains in Croatia. The Plitvice Lakes barrage lake system consists of 16 oligotrophic, dimictic and fluvial lakes divided by tufa barriers that form an approximately 8.2 km long barrage system. The lakes are characterised by a low organic solute concentration, supersaturation with calcium salts, pH > 8.0, and the presence of algae and mosses mediating tufa barriers formation (Srdoč et al. 1985; Stilinović and Božičević 1998). After the confluence of the Bijela rijeka and Crna rijeka Rivers, they form the Matica River which is the main surface-water supplier of the lakes (Stilinović and Božičević 1998). According to the Köppen climate classification, this area is influenced by temperate and boreal climates (Šegota and Filipčić 2003).

Specimen records

This paper is based on unpublished data from our own research and on published data gleaned from the literature. Each record was georeferenced using ArcGIS software. The names of taxa presented in this checklist reflect current nomenclature and classifications (Yang et al. 2007; Ashe and O'Connor 2009, 2012; Pape and Beuk 2012; Adler and Crosskey 2018; Ivković et al. 2019; Oosterbroek 2019; Starý 2019). Locality records are listed for each species. A list of locality names including latitude, longitude, altitude, and number code for each locality is given in Table 1, and a map with all sites

Table 1. Sampling sites in National Park Plitvice Lakes, Croatia.

Site Name	Site ID	Latitude / Longitude	Elevation (m)
Spring of Bijela rijeka, Plitvice Lakes NP	1	44°49'58"N, 15°33'25"E	720
Upper reach of Bijela rijeka, Plitvice Lakes NP	2	44°50'04"N, 15°33'33"E	715
Plitvički Ljeskovac, Plitvice Lakes NP	3	44°50'27"N, 15°35'40"E	668
Spring of Crna rijeka, Plitvice Lakes NP	4	44°50'14"N, 15°36'28"E	680
Upper reach of Crna rijeka, Plitvice Lakes NP	5	44°50'10"N, 15°36'30"E	670
Crna rijeka by the bridge, Plitvice Lakes NP	6	44°50'22"N, 15°35'59"E	665
Lake Prošće, Plitvice Lakes NP	7	44°51'33"N, 15°36'09"E	635
Tufa barrier Labudovac, Plitvice Lakes NP	8	44°52'17"N, 15°35'59"E	630
Lake Okrugljak, Plitvice Lakes NP	9	44°52'23"N, 15°35'56"E	626
Lake Batinovac, Plitvice Lakes NP	10	44°52'16"N, 15°36'11"E	624
Tufa barrier Batinovac-Crno Lake-Malo Lake-Vir, Plitvice Lakes NP	11	44°52'25"N, 15°36'10"E	603
Tufa barrier Batinovac-Galovac, Plitvice Lakes NP	12	44°52'21"N, 15°36'15"E	605
Tufa barrier Galovac-Milino, Plitvice Lakes NP	13	44°52'32"N, 15°36'29"E	594
Lake Gradinsko, Plitvice Lakes NP	14	44°52'39"N, 15°36'37"E	565
Tufa barrier Burget-Kozjak, Plitvice Lakes NP	15	44°52'47"N, 15°36'53"E	547
Riječica, Plitvice Lakes NP	16	44°52'27"N, 15°36'47"E	555
Lake Kozjak, Plitvice Lakes NP	17	44°52'40"N, 15°37'07"E	535
Tufa barrier Kozjak-Milanovac,Plitvice Lakes NP	18	44°53'39"N, 15°36'32"E	545
Tufa barrier Milke Trnine, Plitvice Lakes NP	19	44°53'53"N, 15°36'39"E	540
Tufa barrier Gavanovac-Kaluđerovac, Plitvice Lakes NP	20	44°53'58"N, 15°36'39"E	537
Lake Kaluđerovac, Plitvice Lakes NP	21	44°54'02"N, 15°36'40"E	535
Tufa barrier Novakovića Brod, Plitvice Lakes NP	22	44°54'08"N, 15°36'38"E	505
Stream Sartuk, Plitvice Lakes NP	23	44º55'57"N, 15º33'10"E	765
Stream Plitvica, Plitvice Lakes NP	24	44°54'08"N, 15°36'27"E	555
Korana Village, Plitvice Lakes NP	25	44°55'33"N, 15°37'09"E	390

plotted is provided as Figure 1. Photographs of several studied sites are also given (Figs 2–7). Adult specimens were collected using emergence traps (details in Ivković et al. 2013a), sweep nets, yellow pan traps and aspirators, whereas larvae were collected by Surber sampler (25 × 25 cm) and kick-net sampler (25 × 25 cm, 500 μm mesh size). Specimens were preserved in 80% or 96% ethanol (EtOH). We identified the specimens to species level using Thomas (1997) for Athericidae; Reiss and Fittkau (1971); Bitušík (2000), Langton and Pinder (2007a, 2007b), Andersen et al. (2013), Bitušík and Hamerlík (2014), Vallenduuk (2017) for Chironomidae; Disney (1999) for Dixidae; Engel (1938–1946) for Empididae; Gorodkov (1988) for Scathophagidae; and Rozkošný and Kniepert (2000) for Stratiomyidae.

Results and discussion

List of aquatic Diptera of National Park Plitvice Lakes

The following format is used for the distribution data: literature references (name of the site and in parentheses the citation of site ID and the reference); new records (life stage in which the identifications were made, i.e., adult ♂, ♀ and larvae, name of the site and in parentheses the site ID, date of collection and the collector if possible). New

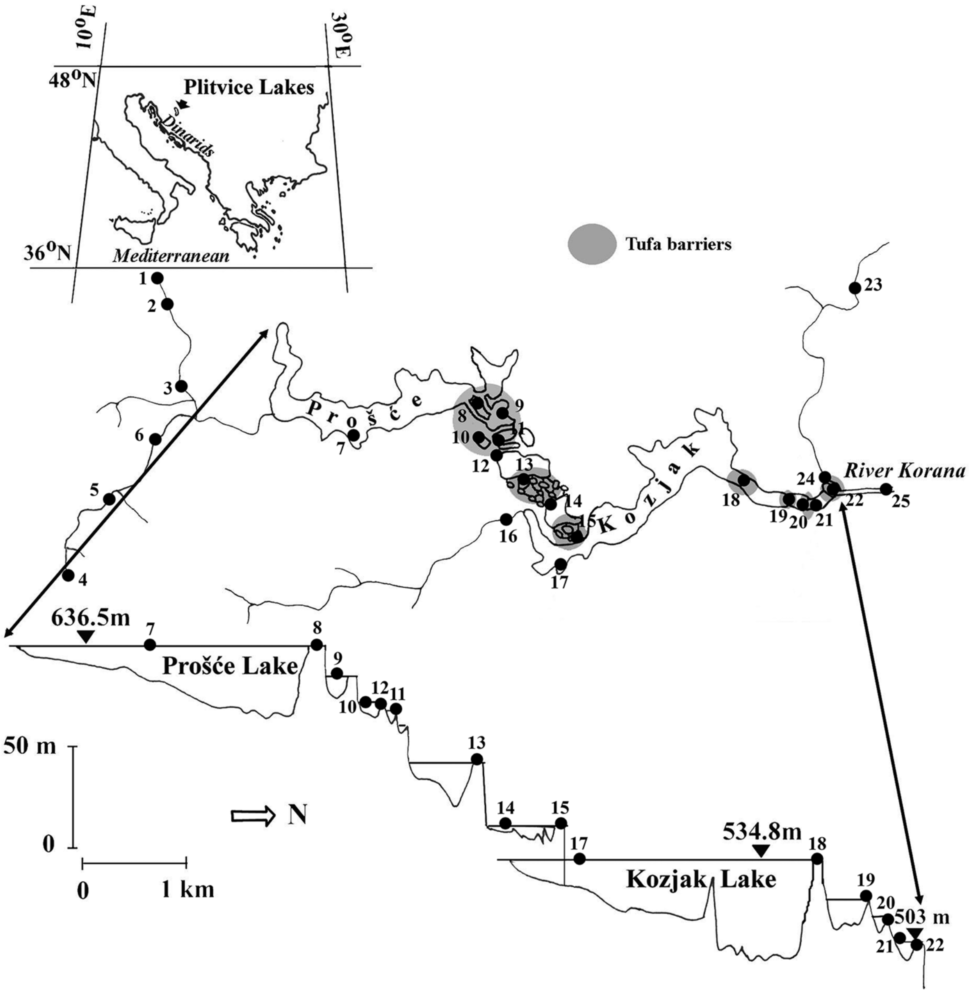

Figure 1. Sampling sites in Plitvice Lakes National Park (see Table 1 for codes).

species for Plitvice Lakes NP are listed with an asterisk before the name of the species. All the sites and site ID are listed in Table 1.

Family Athericidae

Ibisia marginata (Fabricius, 1781)

Literature reference. • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Sertić Perić et al. 2014).

New records. • 1♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2010; M. Ivković leg. • 1♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 28 Jun.

2012; M. Ivković leg. • 1♀; same site; 25 Jul. 2014; M. Ivković leg. • 3♂, 6♀; tufa barrier Novakovića Brod, Plitvice Lakes NP (22); 25 Jul. 2007; M. Ivković leg. • 10♂, 21♀; same site; 30 Aug. 2007; M. Ivković leg. • 1♂, 6♀; Korana Village, Plitvice Lakes NP (25); 29 Aug. 2008; M. Ivković leg.

Family Chironomidae
Subfamily Chironominae

Cryptochironomus (*Cryptochironomus*) *albofasciatus* (Staeger, 1839)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Dicrotendipes nervosus (Staeger, 1839)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Limnochironomus nervosus* (Staeger, 1839) in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *D. nervosus* in Spies and Saether (2013).

Endochironomus gr. *dispar sensu* Moller Pillot, 2009

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Einfeldia dissidens (Walker, 1856)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Harnischia fuscimanus Kieffer, 1921

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Cryptochironomus fuscimanus* Kieffer, 1921 in Kostić-Brnek and Brnek-Kostić (1971) a synonym of *H. fuscimanus* in Moller Pillot (2009).

Figure 2. Spring of Crna Rijeka, Plitvice Lakes National Park.

Micropsectra notescens **(Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Micropsectra uva **Giłka, Zakrzewska, Baranov & Dominiak, 2013**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Giłka et al. 2013, Ivković et al. 2015) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Microtendipes pedellus **(De Geer, 1776)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Microtendipes tarsalis (Walker, 1856)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

**Nilothauma brayi* (Goetghebuer, 1921)

New record. • 1 larva; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Paracladopelma camptolabis (Kieffer, 1913)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Paratanytarsus lauterborni (Kieffer, 1909)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Paratendipes albimanus (Meigen, 1818)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Phaenopsectra flavipes (Meigen 1818)

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

New record. • 24 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Polypedilum (*Pentapedilum*) *exsectum* (Kieffer, 1916)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentapedilum exsectum* Kieffer, 1913 in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *P. exsectum* in Spies and Saether (2013).

Figure 3. Crna Rijeka by the bridge, Plitvice Lakes National Park.

****Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804)**

New record. • 6 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

***Polypedilum* (*Tripodura*) *scalaenum* (Schrank, 1803)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Polypedilum breviantennatum* Chernovskij, 1949 in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *P. scalaenum* in Spies and Saether (2013).

***Rheotanytarsus nigricauda* Fittkau, 1960**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Rheotanytarsus pentapoda (Kieffer, 1909)

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Galovac-Milino, Plitvice Lakes NP (13) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) • tufa barrier Milke Trnine, Plitvice Lakes NP (19) (Matoničkin et al. 1971, Matoničkin 1987).

Remark. Mentioned as *Rheotanytarsus lapidicola* Kieffer, 1909 in Matoničkin et al. (1971) and Matoničkin (1987), an accepted synonym of *R. pentapoda* in Spies and Saether (2013).

Stempellina bausei (Kieffer, 1911)

Literature references. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Matoničkin 1987) • Lake Batinovac, Plitvice Lakes NP (10) (Matoničkin et al. 1971) • tufa barrier Batinovac-Crno Lake-Malo Lake-Vir, Plitvice Lakes NP (11) (Matoničkin 1987) • Lake Gradinsko, Plitvice Lakes NP (14) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Matoničkin et al. 1971).

New records. • 50 larvae; Lake Prošće, Plitvice Lakes NP (7); 17 Sep. 2018 • 92 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

**Tanytarsus brundini* Lindeberg, 1963

New record. • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 30 Jun. 2009; M. Ivković leg.

Tanytarsus heusdensis Goetghebuer, 1923

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Zavrelia pentatoma Kieffer & Bause, 1913

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Zavreliella marmorata (van der Wulp, 1859)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Subfamily Diamesinae

***Diamesa* (*Diamesa*) *thomasi* Serra-Tosio, 1970**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Baranov et al. 2012, Ivković et al. 2015).

***Diamesa* (*Diamesa*) *tonsa* (Haliday in Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

******Potthastia longimanus* Kieffer, 1922**

New record. • 1 larva; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Subfamily Prodiamesinae

***Monodiamesa bathyphila* (Kieffer, 1918)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Prodiamesa olivacea* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015) • Lake Prošće, Plitvice Lakes NP (7) (Kostić-Brnek and Brnek-Kostić 1971) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Matoničkin 1987) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971) • tufa barrier Gavanovac-Kaluđervoac, Plitvice Lakes NP (20) (Matoničkin 1987) • Lake Kaluđerovac, Plitvice Lakes NP (21) (Matoničkin et al. 1971).

Subfamily Orthocladiinae

***Acricotopus lucens* (Zetterstedt, 1850)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Acricotopus lucidus* Brundin, 1949 in Kostić-Brnek and Brnek-Kostić (1971), mentioned as a synonym of *A. lucens* in Moller Pillot (2013).

Brillia bifida (Kieffer, 1909)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Brillia longifurca Kieffer, 1921

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Chaetocladius dentiforceps (Edwards, 1929)

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Chaetocladius melaleucus (Meigen, 1818)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin, 1971).

Remark. Mentioned as *Trichocladius bicinctus* (Meigen, 1818) in Kostić-Brnek and Brnek-Kostić (1971).

Cricotopus (*Cricotopus*) *fuscus* (Kieffer, 1909)

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin, 1971).

Remark. Mentioned as *Cricotopus* (*Cricotopus*) *biformis* Edwards, 1929 in Matoničkin et al. (1971), known as a questionable synonym of *C.* (*Cricotopus*) *fuscus* in Ashe and O'Connor (2012).

Corynoneura lobata Edwards, 1924

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Eukiefferiella devonica (Edwards, 1929)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Eukiefferiella gracei (Edwards, 1929)

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971).

Remark. Mentioned as *Eukiefferiella longicalcar* Kieffer in Matoničkin et al. (1971), an accepted synonym of *E. gracei* in Spies and Saether (2013).

Eukiefferiella ilkleyensis (Edwards, 1929)

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

Remark. Mentioned as *Plectrocladius eukiefferiella quadridentata* Chernovskij, 1949 in Matoničkin (1987); see Discussion for more details.

Eukiefferiella minor (Edwards, 1929)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Epoicocladius ephemerae (Kieffer, 1924)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Heterotrissocladius marcidus (Walker, 1856)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Limnophyes gurgicola (Edwards, 1929)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Limnophyes cf. *minimus sensu* Langton & Pinder, 2007

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Metriocnemus cf. *albolineatus sensu* Langton & Pinder, 2007

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Metriocnemus eurynothus (Holmgren, 1883)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Metriocnemus intergerivus Sæther, 1995

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Orthocladius (*Mesorthocladius*) *frigidus* (Zetterstedt, 1838)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Paracladius conversus (Walker, 1856)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Paratrichocladius inserpens* Pankratova, 1970 in Kostić-Brnek and Brnek-Kostić (1971), a synonym of *P. conversus* in Moller Pillot (2013).

Parametriocnemus stylatus (Spaerck, 1923)

Literature references. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Matoničkin 1987) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Matoničkin et al. 1971, Matoničkin 1987) • tufa barrier Gavanovac-Kaluđerovac, Plitvice Lakes NP (20) • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

New records. • 11 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018 • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 30 Nov. 2009; M. Ivković leg.

Remark. Mentioned as *Limnophyes transcaucasicus* Chernovskij, 1949 in Matoničkin et al. (1971) and Matoničkin (1987), a synonym of *P. stylatus* in Moller Pillot (2013).

***Parametriocnemus* cf. *stylatus sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paraphaenocladius* cf. *exagitans sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paraphaenocladius impensus* (Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Paraphaenocladius* cf. *irritus sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paratrichocladius skirwithensis* (Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Psectrocladius* (*Psectrocladius*) *barbimanus* (Edwards, 1929)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Matoničkin 1987) • Crna Rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

***Psectrocladius* (*Psectrocladius*) *psilopterus* (Kieffer, 1906)**

Literature reference. • Crna Rijeka by the bridge, Plitvice Lakes NP (6) (Matoničkin 1987).

***Rheocricotopus effusus* (Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Synorthocladius semivirens (Kieffer, 1909)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

New record. • 1 larva; Lake Prošće, Plitvice Lakes NP (7); 26 Jul. 2019.

Thienemannia gracilis Kieffer, 1909

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Tvetenia veralli (Edwards, 1929)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Subfamily Tanypodinae

Ablabesmyia (*Ablabesmyia*) *monilis* (Linnaeus, 1758)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentaneura monilis* Linnaeus, 1758 in Kostić-Brnek and Brnek-Kostić (1971).

Apsectrotanypus trifascipennis (Zetterstedt, 1838)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Psectrotanypus trifascipennis* Zetterstedt, 1838 in Kostić-Brnek and Brnek-Kostić (1971) which is probably a misspelling.

**Labrundinia longipalpis* (Goetghebuer, 1921)

New record. • 9 larvae; Lake Prošće, Plitvice Lakes NP (7); 26 Jul. 2019.

Krenopelopia binotata (Wiedemann, 1817)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Macropelopia cf. *fehlmanni sensu* Kieffer, 1912

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Procladius (*Holotanypus*) *choreus* (Meigen, 1804)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Thienemannimyia carnea (Fabricius, 1805)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentaneura carnea* Fabricius, 1805 in Kostić-Brnek and Brnek-Kostić (1971).

Family Dixidae

Dixa dilatata Strobl, 1900

Literature reference. • Stream Sartuk, Plitvice Lakes NP (23) (Ivković and Ivanković 2019).

Dixa maculata Meigen, 1818

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivanković et al. 2019).

Dixa nebulosa Meigen, 1830

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Ivanković et al. 2019) • Lake Kozjak, Plitvice Lakes NP (17) (Ivković and Ivanković 2019).• tufa barrier Kozjak- Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivanković et al. 2019).

Figure 4. Tufa barrier Labudovac, Plitvice Lakes National Park.

Dixa nubilipennis Curtis, 1832

Literature reference. • Korana Village, Plitvice Lakes NP (Ivanković et al. 2019) (25).

Dixa puberula Loew, 1849

Literature reference. • spring of Bijela rijeka stream, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivanković et al. 2019) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivanković et al. 2019) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak- Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivanković et al. 2019, Ivković and Ivanković 2019) • Korana Village, Plitvice Lakes NP (25) (Ivanković et al. 2019).

Dixa submaculata Edwards, 1920

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5)

• Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivanković et al. 2019).

Dixella aestivalis (Meigen, 1818)

Literature reference. • Lake Okrugljak, Plitvice Lakes NP (9) • Riječica, Plitvice Lakes NP (16) (Matoničkin 1987).

Dixella amphibia (De Geer, 1776)

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin 1987).

New record. • 1 larva; lake Kozjak, Plitvice Lakes NP (17); 11 Jul. 2019.

**Dixella autumnalis* (Meigen, 1838)

New record. • 1 larva; lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Family Empididae
Subfamily Clinocerinae

Clinocera stagnalis (Haliday, 1833)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

New record. • 1♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2013; M. Ivković leg.

Clinocera wesmaeli (Macquart, 1835)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2010, 2012a).

Dolichocephala guttata (Haliday, 1833)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Dolichocephala ocellata (Costa, 1854)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2010, 2012a).

Kowarzia barbatula (Mik, 1880)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Kowarzia bipunctata (Haliday, 1833)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2010).

Wiedemannia aquilex (Loew, 1869)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivković et al. 2010, 2012a).

Wiedemannia lamellata (Loew, 1869)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Wiedemannia zetterstedti (Fallén, 1826)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2010).

Figure 5. Tufa barrier Galovac-Milino, Plitvice Lakes National Park.

Subfamily Hemerodromiinae

Chelifera concinnicauda Collin, 1927

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a) • Korana Village, Plitvice Lakes NP (25) (Horvat 1990, Ivković et al. 2010, 2012a).

Chelifera flavella (Zetterstedt, 1838)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) (Ivković et al. 2010, 2012a).

Chelifera precabunda Collin, 1961

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) •

upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivković et al. 2010, 2012a).

Chelifera precatoria (Fallén, 1816)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2010, 2012a).

Chelifera pyrenaica Vaillant, 1981

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Chelifera siveci Wagner, 1984

Literature references. • spring of Bijela Rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna Rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Chelifera stigmatica (Schiner, 1862)

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Chelifera trapezina (Zetterstedt, 1838)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Hemerodromia laudatoria Collin, 1927

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Lake Prošće, Plitvice Lakes NP (7) (Ivković et al. 2010, 2012a).

Hemerodromia melangyna Collin, 1927

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a) • Korana Village, Plitvice Lakes NP (25) (Horvat 1990, Ivković et al. 2010, 2012a).

New record. • 1♂, 43♀; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 29 Jun. 2015.

Hemerodromia oratoria (Fallén, 1816)

Literature references. • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Hemerodromia raptoria Meigen, 1830

Literature references. • Lake Kozjak, Plitvice Lakes NP (17) (Ivković et al. 2010, 2012a) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković et al. 2013b).

Hemerodromia unilineata Zetterstedt, 1842

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Family Limoniidae

Subfamily Chioneinae

Ellipteroides (*Ellipteroides*) *lateralis* (Macquart, 1835)

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kolcsár et al. 2015).

***Gonomyia* (*Gonomyia*) *tenella* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Molophilus* (*Molophilus*) *bifidus* Goetghebuer, 1920**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kolcsár et al. 2015).

***Molophilus* (*Molophilus*) *repentinus* Starý, 1971**

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Ormosia* (*Oreophila*) *bergrothi* (Strobl, 1895)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

***Rhabdomastix* (*Rhabdomastix*) *edwardsi* Tjeder, 1967**

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Rhypholophus phryganopterus* Kolenati, 1860**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Subfamily Limnophilinae

***Eloeophila apicata* (Loew, 1871)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

***Eloeophila maculata* (Meigen, 1804)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Kolcsár et al. 2015).

***Epiphragma* (*Epiphragma*) *ocellare* (Linnaeus, 1760)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Remark. This species is not aquatic. Larvae develop in forests, woodlands, larvae associated with woody debris.

***Hexatoma* (*Eriocera*) *chirothecata* (Scopoli, 1763)**

Literature reference. • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Paradelphomyia senilis* (Haliday, 1833)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Subfamily Limoniinae

***Antocha* (*Antocha*) *vitripennis* (Meigen, 1830)**

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Dicranomyia* (*Dicranomyia*) *chorea* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

Remark. Larvae associated with rotting woody debris, but sometimes also reared from semiaquatic habitats; larvae possibly feeding in partially submerged wood.

***Dicranomyia* (*Dicranomyia*) *didyma* (Meigen, 1804)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) (Kolcsár et al. 2015).

Figure 6. Lake Kaluđerovac and Tufa barrier Novakovića Brod, Plitvice Lakes National Park.

Dicranomyia (*Dicranomyia*) *imbecilla* Lackschewitz, 1941

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kolcsár et al. 2015).

Dicranomyia (*Dicranomyia*) *mitis* (Meigen, 1830)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

Remarks. Mentioned as *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830) complex by Kolcsár et al. (2015). After re-identification of specimens by Kolcsár L.-P., using the identification key published by Starý and Stubbs (2015), it was confirmed that the specimens belong to *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830).

Limonia hercegovinae (Strobl, 1898)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Remark. Larvae unknown, but perhaps not associated with aquatic habitats as other *Limonia* species have terrestrial larvae.

Lipsothrix nobilis Loew, 1873

Literature reference. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kolcsár et al. 2015).

Family Muscidae
Subfamily Coenosiinae

Limnophora croatica Pont & Ivković, 2013

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Pont and Ivković 2013, Ivković and Pont 2016) • spring of Crna Rijeka, Plitvice Lakes NP (4) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Pont and Ivković 2013, Ivković and Pont 2016) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Limnophora olympiae Lyneborg, 1965

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016).

Limnophora pulchriceps (Loew, 1860)

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Pont and Ivković 2013).

Limnophora riparia (Fallén, 1824)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Batinovac-Galovac, Plitvice Lakes NP (12) • tufa barrier Galovac-Milino, Plitvice Lakes NP (13) • tufa barrier Burget-Kozjak, Plitvice Lakes NP

(15) (Matoničkin et al. 1971) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Milke Trnine, Plitvice Lakes NP (19) (Matoničkin et al. 1971) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Limnophora setinerva Schnabl, 1911

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • Stream Plitvica, Plitvice Lakes NP (24) (Pont and Ivković 2013).

Limnophora tigrina (Am Stein, 1860)

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Limnophora triangula (Fallén, 1825)

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković and Pont 2016) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Lispe tentaculata (De Geer, 1776)

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Ivković and Pont 2015).

Lispocephala brachialis (Rondani, 1877)

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković and Pont 2015).

Lispocephala spuria (Zetterstedt, 1838)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković and Pont 2015).

Figure 7. Stream Plitvica, Plitvice Lakes National Park.

Family Pediciidae
Subfamily Pediciinae

Dicranota (*Dicranota*) *bimaculata* (Schummel, 1829)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kolcsár et al. 2015).

Dicranota (*Paradicranota*) *pavida* (Haliday, 1833)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

Pedicia (*Amalopis*) *occulta* (Meigen, 1830)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Kolcsár et al. 2015).

Tricyphona (*Tricyphona*) *immaculata* (Meigen, 1804)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kolcsár et al. 2015).

Family Psychodidae
Subfamily Sycoracinae

Sycorax feuerborni Jung, 1954

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Sycorax tonnoiri Jung, 1953

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

Subfamily Psychodinae

Berdeniella keroveci Kvifte, Ivković & Klarić, 2013

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

Pericoma blandula Eaton, 1893

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kvifte et al. 2013).

Pericoma miljenkoi Kvifte & Ivković, 2018

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kvifte and Ivković 2018).

Pericoma pseudocalcilega Krek, 1972

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kvifte et al. 2013).

***Psychoda* (*Logima*) *albipennis* Zetterstedt, 1850 complex**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kvifte et al. 2013).

***Psychoda* (*Psychodocha*) *gemina* (Eaton, 1904)**

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

***Jungiella valachia* (Vaillant, 1963)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kvifte et al. 2013, Ivković et al. 2015).

Family Scathophagidae
Subfamily Scathophaginae

****Acanthocnema latipennis* Becker, 1894**

New records. • 4♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 26 Jul. 2016; M. Ivković leg. • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 29 Apr. 2015; M. Ivković leg.

Family Simuliidae
Subfamily Simuliinae

***Prosimulium tomosvaryi* (Enderlein, 1921)**

Literature references. • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Eusimulium*) *angustipes* Edwards, 1915**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Eusimulium*) *rubzovianum* (Sherban, 1961)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2016).

***Simulium* (*Nevermannia*) *angustitarse* (Lundström, 1911)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2016).

***Simulium* (*Nevermannia*) *costatum* Friederichs, 1920**

Literature references. • spring of Bijela Rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *monticola* Friederichs, 1920**

Literature references. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Ivković et al. 2012b, 2014).

New record. • 7♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 28 Jun. 2007; M. Ivković leg.

***Simulium* (*Simulium*) *ornatum* Meigen, 1818 (complex)**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

New records. • 4♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 29 May 2009; M. Ivković leg. • 3♂; same site; 30 Jun. 2009; M. Ivković leg.

***Simulium* (*Simulium*) *trifasciatum* Curtis, 1839**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *tuberosum* (Lundström, 1911)**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *variegatum* Meigen, 1818**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Trichodagmia*) *auricoma* Meigen, 1818**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Wilhelmia*) *pseudequinum* Séguy, 1921**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivković et al. 2012b, 2014).

Remark. Formerly this was misidentified as *Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758) in Ivković et al. (2012, 2014, 2016).

Family Stratiomyidae

Oxycera pardalina* **Meigen, 1822

New records. • 1 larva; spring of Bijela rijeka, Plitvice Lakes NP (1); 30 May 2008; M. Ivković leg. • 6 larvae; upper reach of Bijela rijeka, Plitvice Lakes NP (2); 29 May

2007; M. Ivković leg. • 1♀; same site; 26 Jul. 2010; M. Ivković leg. • 1 larva; upper reach of Crna rijeka, Plitvice Lakes NP (5); 30 Apr. 2007; M. Ivković leg. • 4 larvae; same site; 29 May 2007; M. Ivković leg. • 1 larva, 1♀; same site; 30 Jun. 2007; M. Ivković leg. • 2 larvae; same site; 30 Apr. 2008; M. Ivković leg. • 1 larva; same site; 30 May 2008; M. Ivković leg. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 30 Jun. 2008; M. Ivković leg. • 1♂; same site; 30 Jun. 2011; M. Ivković leg. • 1♀; same site; 28 Jun. 2012; M. Ivković leg.

Oxycera limbata* **Loew, 1862

New records. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 25 Jul. 2011; M. Ivković leg. • 3♂; Korana Village, Plitvice Lakes NP (25); 29 Jun. 2007; M. Ivković leg.

Oxycera turcica* **Ustuner & Hasbenli, 2004

New records. • 3♂, 2♀; Korana Village, Plitvice Lakes NP (25); 29 Jun. 2007; M. Ivković leg. • 1♂, 2♀; same site; 26 Jul. 2007; M. Ivković leg.

Nemotelus pantherinus* **(Linnaeus, 1758)

New record. • 1♀; upper reach of Bijela rijeka, Plitvice Lakes NP (1); 24 Jul. 2009; M. Ivković leg.

Oplodontha viridula* **(Fabricius, 1775)

New record. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2010; M. Ivković leg.

Family Tabanidae

Subfamily Chrysopsinae

Chrysops caecutiens **(Linnaeus, 1758)**

Literature reference. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Krčmar et al. 2008).

Chrysops viduatus **(Fabricius, 1794)**

Literature reference. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Krčmar et al. 2008).

Family Tipulidae
Subfamily Tipulinae

Tipula (*Savtshenkia*) *rufina rufina* Meigen, 1818

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

New records. • 2♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 1 Oct. 2009; M. Ivković leg. • 1♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 2 Nov. 2011; M. Ivković leg. • 1♀; spring of Bijela rijeka, Plitvice Lakes NP (1); 27 Jun. 2013; M. Ivković leg.

Species richness and assemblage composition

In total, 157 species and 7 taxa of aquatic Diptera (Table 2) belonging to 13 families, collected from 25 sites (Table 1, Figure 1) are recorded in the Plitvice Lakes NP, with twelve species new for the dipteran fauna of the National Park. New species belonging to the family Chironomidae are *Labrundinia longipalpis* (Goetghebuer, 1921), *Nilothauma brayi* (Goetghebuer, 1921), *Potthastia longimanus* Kieffer, 1922, *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804) and *Tanytarsus brundini* Lindeberg, 1963; to the family Dixidae is *Dixella autumnalis* (Meigen, 1838), and Scathophagidae *Acanthocnema latipennis* Becker, 1894. New species found in the Plitvice Lakes NP belonging to Stratiomyidae family are *Oxycera pardalina* Meigen, 1822, *Oxycera limbata* Loew, 1862, *Oxycera turcica* Ustuner & Hasbenli, 2004, *Nemotelus pantherinus* (Linnaeus, 1758), and *Oplodontha viridula* (Fabricius, 1775).

Overall, the greatest species richness was recorded within the family Chironomidae, with 62 species (and additional seven taxa) recorded so far in Plitvice Lakes NP (Table 2, Figure 8). This was expected since the Chironomidae are an extremely diverse group with more than 8000 described species (Marshall 2012), and with many more undescribed or waiting to be discovered. Some chironomid species have been mentioned in the literature, but are not included in the formal list for various reasons that are considered here. The species *Micropsectra curvicornis* (Chernovskij, 1949) listed in Kostić-Brnek and Brnek-Kostić (1971) and *Thienemanniella flaviforceps* Kieffer, 1925, mentioned from Plitvice in Matoničkin (1987), are considered to be *nomina dubia* in Ashe and Cranston (1990), as well as in Ashe and O'Connor (2012). *Ablabesmyia* cf. *tetrasticta* could possibly be a misinterpretation of the name *Pelopia tetrasticta* Kieffer, 1916 and as such is considered a *nomen dubium* in the subfamily Tanypodinae (Ashe and O'Connor 2009). Furthermore, the species *Cricotopus latidentatus* (Chernovskij, 1949), published in Matoničkin (1987) and Matoničkin et al. (1971), is considered a questionable synonym within the genus *Cricotopus* according to the Ashe and O'Connor (2012). *Cricotopus filiformis* Edwards, mentioned in Matoničkin (1987), is considered an unidentifiable error since Edwards never described a chironomid species with "fili" in the name (Ashe and Cranston 1990). Chironomidae species that are part of the formal species list, but should be addressed with caution, are also considered.

Table 2. Aquatic Diptera at different types of karstic habitats in National Park Plitvice Lakes.

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Athericidae				
Ibisia marginata (Fabricius, 1781)		•	•	
Chironomidae				
Ablabesmyia (*Ablabesmyia*) *monilis* (Linnaeus, 1758)				•
Acricotopus lucens (Zetterstedt, 1850)				•
Apsectrotanypus trifascipennis (Zetterstedt, 1838)				•
Brillia bifida (Kieffer, 1909)	•			
Brillia longifurca Kieffer, 1921				•
Chaetocladius dentiforceps (Edwards, 1929)	•			
Chaetocladius melaleucus (Meigen, 1818)	•			
Corynoneura lobata Edwards, 1924	•			
Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)				•
Cricotopus (*Cricotopus*) *fuscus* (Kieffer, 1909)		•		
Cryptochironomus (*Cryptochironomus*) *albofasciatus* (Staeger, 1839)				•
Diamesa (*Diamesa*) *thomasi* Serra-Tosio, 1970	•			
Diamesa (*Diamesa*) *tonsa* (Haliday in Walker, 1856)	•			
Dicrotendipes nervosus (Staeger, 1839)				•
Einfeldia dissidens (Walker, 1856)				•
Endochironomus cf. *dispar sensu* Moller Pillot, 2009				•
Epoicocladius ephemerae (Kieffer, 1924)	•			
Eukiefferiella devonica (Edwards, 1929)	•			
Eukiefferiella ilkleyensis (Edwards, 1929)	•			
Eukiefferiella minor (Edwards, 1929)	•			
Eukiefferiella gracei (Edwards, 1929)	•			
Harinischia fuscimanus Kieffer, 1921				•
Heterotrissocladius marcidus (Walker, 1856)				•
Krenopelopia binotata (Wiedemann, 1817)	•			
Labrundinia longipalpis (Goetghebuer, 1921)				•
Limnophyes cf. *minimus sensu* Langton & Pinder, 2007	•			
Limnophyes gurgicola (Edwards, 1929)	•			
Macropelopia cf. *fehlmanni sensu* Kieffer, 1912				•
Metriocnemus cf. *albolineatus sensu* Langton & Pinder, 2007	•			
Metriocnemus eurynothus (Holmgren, 1883)	•			
Metriocnemus intergerivus Sæther, 1995	•			
Micropsectra notescens (Walker, 1856)	•			
Micropsectra uva Giłka, Zakrzewska, Baranov & Dominiak, 2013	•			
Microtendipes pedellus (De Geer, 1776)				•
Microtendipes tarsalis (Walker, 1856)				•
Monodiamesa bathyphila (Kieffer, 1918)				•
Nilothauma brayi (Goetghebuer, 1921)				•
Orthocladius (*Mesorthocladius*) *frigidus* (Zetterstedt 1838)	•			
Paracladius conversus (Walker, 1856)				•
Paracladopelma camptolabis (Kieffer, 1913)				•
Parametriocnemus cf. *stylatus sensu* Moller Pillot, 2013	•			
Parametriocnemus stylatus (Spaerck, 1923)		•	•	
Paraphaenocladius cf. *exagitans sensu* Moller Pillot, 2013	•			
Paraphaenocladius impensus (Walker, 1856)	•			
Paraphaenocladius cf. *irritus sensu* Moller Pillot, 2013	•			
Paratanytarsus lauterborni (Kieffer, 1909)				•
Paratendipes albimanus (Meigen, 1818)				•
Paratrichocladius skirwithensis (Edwards, 1929)	•			
Phaenopsectra flavipes (Meigen 1818)	•			•
Polypedilum (*Pentapedilum*) *exsectum* (Kieffer, 1916)				•
Polypedilum (*Polypedilum*) *nubeculosum* (Meigen, 1804)				•
Polypedilum (*Tripodura*) *scalaenum* (Schrank, 1803)				•
Potthastia longimanus Kieffer, 1922				•

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Procladius (*Holotanypus*) *choreus* (Meigen, 1804)				•
Prodiamesa olivacea (Meigen, 1818)	•		•	•
Psectrocladius (*Psectrocladius*) *barbimanus* (Edwards, 1929)		•	•	
Psectrocladius (*Psectrocladius*) *psilopterus* (Kieffer, 1906)		•		
Rheocricotopus effusus (Walker, 1856)	•			
Rheotanytarsus nigricauda Fittkau, 1960	•			
Rheotanytarsus pentapoda (Kieffer, 1909)			•	
Stempellina bausei (Kieffer, 1911)		•	•	•
Synorthocladius semivirens (Kieffer, 1909)	•			
Tanytarsus brundini Lindeberg, 1963			•	
Tanytarsus heusdensis Goetghebuer, 1923				•
Thienemannia gracilis Kieffer, 1909	•			
Thienemannimyia carnea (Fabricius, 1805)				•
Tvetenia veralli (Edwards, 1929)	•			
Zavrelia pentatoma Kieffer & Bause, 1913				•
Zavreliella marmorata (van der Wulp, 1859)				•
Dixidae				
Dixa dilatata Strobl, 1900		•		
Dixa maculata Meigen, 1818		•	•	
Dixa nebulosa Meigen, 1830		•	•	•
Dixa nubilipennis Curtis, 1832		•		
Dixa puberula Loew, 1849	•	•	•	
Dixa submaculata Edwards, 1920	•	•	•	
Dixella aestivalis (Meigen, 1818)		•		•
Dixella amphibia (De Geer, 1776)			•	
Dixella autumnalis (Meigen, 1838)				•
Empididae				
Chelifera concinnicauda Collin, 1927		•	•	
Chelifera flavella (Zetterstedt, 1838)	•	•		
Chelifera precabunda Collin, 1961	•	•		
Chelifera precatoria (Fallén, 1816)	•	•		
Chelifera pyrenaica Vaillant, 1981		•	•	
Chelifera siveci Wagner, 1984	•	•		
Chelifera stigmatica (Schiner, 1862)		•	•	
Chelifera trapezina (Zetterstedt, 1838)	•	•		
Clinocera stagnalis (Haliday, 1833)	•	•		
Clinocera wesmaeli (Macquart, 1835)	•			
Dolichocephala guttata (Haliday, 1833)	•	•		
Dolichocephala ocellata (Costa, 1854)	•	•		
Hemerodromia laudatoria Collin, 1927		•		•
Hemerodromia melangyna Collin, 1927		•	•	
Hemerodromia oratoria (Fallén, 1816)		•	•	
Hemerodromia raptoria Meigen, 1830			•	•
Hemerodromia unilineata Zetterstedt, 1842		•	•	
Kowarzia barbatula (Mik, 1880)	•	•	•	
Kowarzia bipunctata (Haliday, 1833)		•		
Wiedemannia aquilex (Loew, 1869)	•	•		
Wiedemannia lamellata (Loew, 1869)	•	•	•	
Wiedemannia zetterstedti (Fallén, 1826)	•			
Limoniidae				
Antocha (*Antocha*) *vitripennis* (Meigen, 1830)		•	•	
Dicranomyia (*Dicranomyia*) *chorea* (Meigen, 1818)	•	•	•	
Dicranomyia (*Dicranomyia*) *didyma* (Meigen, 1804)	•	•	•	
Dicranomyia (*Dicranomyia*) *imbecilla* Lackschewitz, 1941	•		•	
Dicranomyia (*Dicranomyia*) *mitis* (Meigen, 1830) complex		•		
Ellipteroides (*Ellipteroides*) *lateralis* (Macquart, 1835)			•	
Eloeophila apicata (Loew, 1871)		•		
Eloeophila maculata (Meigen, 1804)		•		

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Epiphragma (*Epiphragma*) *ocellare* (Linnaeus, 1760)	•			
Gonomyia (*Gonomyia*) *tenella* (Meigen, 1818)	•	•		
Hexatoma (*Eriocera*) *chirothecata* (Scopoli, 1763)		•	•	
Limonia hercegovinae (Strobl, 1898)	•	•		
Lipsothrix nobilis Loew, 1873		•	•	
Molophilus (*Molophilus*) *bifidus* Goetghebuer, 1920	•			
Molophilus (*Molophilus*) *repentinus* Starý, 1971		•		
Ormosia (*Oreophila*) *bergrothi* (Strobl, 1895)	•			
Paradelphomyia (*Oxyrhiza*) *senilis* (Haliday, 1833)	•	•		
Rhabdomastix (*Rhabdomastix*) *edwardsi* Tjeder, 1967		•		
Rhypholophus phryganopterus Kolenati, 1860	•			
Muscidae				
Limnophora croatica Pont & Ivković, 2013	•	•	•	
Limnophora olympiae Lyneborg, 1965	•		•	
Limnophora pulchriceps (Loew, 1860)		•	•	
Limnophora riparia (Fallén, 1824)	•	•	•	
Limnophora setinerva Schnabl, 1911	•	•	•	
Limnophora tigrina (Am Stein, 1860)		•		
Limnophora triangula (Fallén, 1825)		•	•	
Lispe tentaculata (De Geer, 1776)		•		
Lispocephala brachialis (Rondani, 1877)			•	
Lispocephala spuria (Zetterstedt, 1838)	•			
Pediciidae				
Dicranota (*Dicranota*) *bimaculata* (Schummel, 1829)	•			
Dicranota (*Paradicranota*) *pavida* (Haliday, 1833)		•		
Pedicia (*Amalopis*) *occulta* (Meigen, 1830)	•	•		
Tricyphona (*Tricyphona*) *immaculata* (Meigen, 1804)	•	•	•	
Psychodidae				
Sycorax feuerborni Jung, 1954	•			
Sycorax tonnoiri Jung, 1953	•			
Berdeniella keroveci Kvifte, Ivković & Klarić, 2013	•			
Pericoma blandula Eaton, 1893			•	
Pericoma miljenkoi Kvifte & Ivković, 2018			•	
Pericoma pseudocalcilega Krek, 1972			•	
Psychoda (*Logima*) *albipennis* Zetterstedt, 1850 complex	•			
Psychoda (*Psychodocha*) *gemina* (Eaton, 1904)	•			
Jungiella valachia (Vaillant, 1963)	•			
Scathophagidae				
Acanthocnema latipennis Becker, 1894	•		•	
Simuliidae				
Prosimulium tomosvaryi (Enderlein, 1921)		•		
Simulium (*Eusimulium*) *angustipes* Edwards, 1915		•	•	
Simulium (*Eusimulium*) *rubzovianum* (Sherban, 1961)		•	•	
Simulium (*Nevermannia*) *angustitarse* (Lundström, 1911)		•		
Simulium (*Nevermannia*) *costatum* Friederichs, 1920	•	•	•	
Simulium (*Simulium*) *monticola* Friederichs, 1920		•	•	
Simulium (*Simulium*) *ornatum* Meigen, 1818 complex		•	•	
Simulium (*Simulium*) *trifasciatum* Curtis, 1839			•	
Simulium (*Simulium*) *tuberosum* (Lundström, 1911)		•	•	
Simulium (*Simulium*) *variegatum* Meigen, 1818		•	•	
Simulium (*Trichodagmia*) *auricoma* Meigen, 1818		•	•	
Simulium (*Wilhelmia*) *pseudequinum* Séguy, 1921			•	
Stratiomyidae				
Oxycera pardalina Meigen, 1822	•	•	•	
Oxycera limbata Loew, 1862		•	•	
Oxycera turcica Ustuner & Hasbenli, 2004		•		
Nemotelus pantherinus (Linnaeus, 1758)		•		
Oplodontha viridula (Fabricius, 1775)			•	

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Tabanidae				
Chrysops caecutiens (Linnaeus, 1758)		•		
Chrysops viduatus (Fabricius, 1794)		•		
Tipulidae				
Tipula (*Savtshenkia*) *rufina rufina* Meigen, 1818	•			
Number of taxa	75	71	53	38

In Kostić-Brnek and Brnek-Kostić (1971), the authors did not clearly state whether their identifications of *Endochironomus* cf. *dispar* and *Macropelopia* cf. *fehlmanni* were based on adults or larvae. If the identifications were made based on adults, then these reports can be considered valid. On the other hand, if larvae were identified then these reports are doubtful records since these species cannot be identified based on larval morphology alone. The species listed in the formal species list as *Eukiefferiella ilkleyensis* (Edwards, 1929) is mentioned as *Plectrocladius eukiefferiella quadridentata* Chernovskij, 1949 in Matoničkin (1987). This could be a typing error since a genus *Plectrocladius* does not exist within the family Chironomidae. On the other hand, *Eukiefferiella quadridentata* Chernovskij, 1949 is a synonym pro parte of *E. ilkleyensis* (Moller Pillot 2013). If this is not a typing error, then this species name is an unidentifiable error.

Families following Chironomidae by number of species are Empididae, Limoniidae, Simuliidae and Muscidae (only aquatic species identified) with 22, 19, 12 and 10 species, respectively (Table 2, Figure 8). Dixidae and Psychodidae are both present with nine species, while Stratiomyidae, Pediciidae and Tabanidae are present with 5, 4 and 2 species, respectively (Table 2, Figure 8). Families with only one recorded species are Athericidae, Scathophagidae and Tipulidae (Table 2, Figure 8).

The families Dixidae, Empididae and Simuliidae have been dealt with in detail in previous publications (Ivković et al. 2010, 2012a, b, 2016; Ivanković et al. 2019; Ivković and Ivanković 2019) so the numbers presented here might be the final species numbers for Plitvice Lakes NP. For these particular families, only a few additional species might be recorded in the future studies. Other families of aquatic Diptera have been studied sporadically and, in many cases, only a few sites have been completely processed, such as Chironomidae in Kostić-Brnek and Brnek-Kostić (1971) and Ivković et al. (2015). Aquatic Diptera families that are present in the Plitvice Lakes NP but are still unidentified to the species level are Ceratopogonidae, Culicidae, Ephydridae, Sciomyzidae and Syrphidae.

Springs and streams have higher numbers of species recorded than tufa barriers and lakes (Table 2). One of the reasons for this is because the springs were studied in greater detail than other sites, especially when it comes to Chironomidae (Ivković et al. 2015). On the other hand, many aquatic Diptera families have in fact a higher diversity at spring habitats and the upper reaches of streams, such as Empididae, Psychodidae, Limoniidae, etc., because most environmental parameters at those sites remain constant (Ivković et al. 2010, 2012a, 2015; Kvifte et al. 2013; Kolcsár et al. 2015).

The species list of aquatic Diptera of Plitvice Lakes NP is still not complete since many families are either dealt with partially or not at all due to the lack of available

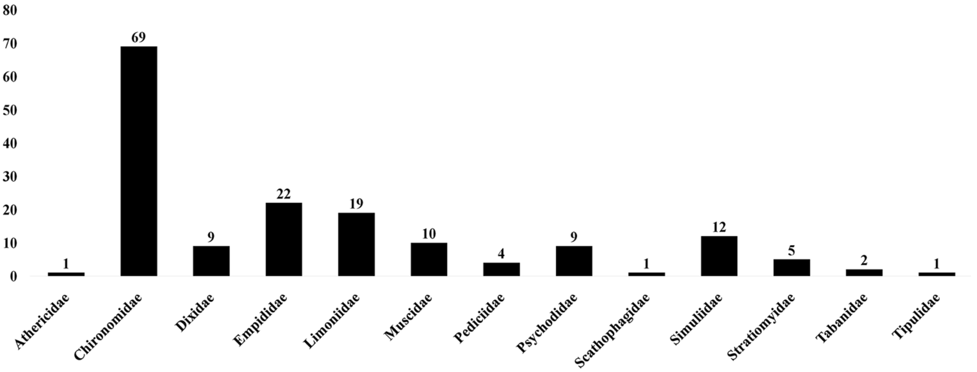

Figure 8. Species richness of Diptera families in Plitvice Lakes National Park.

experts. There is still a lot of work in front of us since we belive that about 250 species of aquatic Diptera can be expected in the unique karstic area of Plitvice Lakes NP.

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Extreme freshwater discharge events exacerbated by climate change influence the structure and functional response of the chironomid community in a biodiversity hotspot

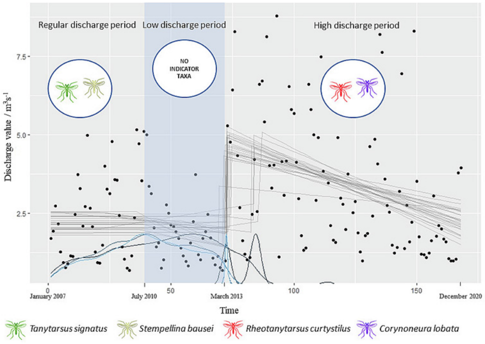
Valentina Dorić [a], Marija Ivković [a,*], Viktor Baranov [b], Ivana Pozojević [a], Zlatko Mihaljević [a]

[a] Division of Zoology, Department of Biology, Faculty of Science, Rooseveltov trg 6, 10000 Zagreb, Croatia
[b] Doñana Biological Station EBD-CSIC, C/ Americo Vespucio, 26, 41092, Isla de la Cartuja, Sevilla, Spain

HIGHLIGHTS

- Discharge was the main parameter influencing the changes in chironomid community.
- Water temperature increase of 0.1 °C was detected in the past 14 years.
- Chironomid diversity remained mostly unchanged opposed to species composition.
- With resuspension of organic material abundance of passive filtrators increased.

GRAPHICAL ABSTRACT



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ABSTRACT

Global climate change is expected to exacerbate extreme discharge events in freshwater ecosystems as a consequence of changes in precipitation volume and snow cover duration. Chironomid midges were used as a model organism in this study because of their small size and short life cycles, which enable fast colonization of new habitats and great resilience. This resilience is often expressed in easy recolonization after an extreme event. Chironomid samples together with physico-chemical water measurements were collected for 14 years, between 2007 and 2020, in a karst tufa barrier that is part of the Plitvice Lakes National Park in Croatia. More than 13,000 individuals belonging to >90 taxa were collected. Mean annual water temperature increased by 0.1 °C during this period. Multiple change-point analysis revealed three main periods by discharge patterns: the first one from January 2007 to June 2010, the second from July 2010 to March 2013, characterised by extreme low discharge, and the third from April 2013 to December 2020, characterised by an increase in extreme peak discharge values. Based on multilevel pattern analysis, indicator species of the first and the third discharge period were detected. The ecological preferences of these species indicate an environmental change related to the changes in discharge. Along with species composition, functional composition has changed with the abundance of passive filtrators, shredders and predators increasing over time. Species richness and abundance did not change over the period of observation, thus emphasizing the importance of species-level identification in detecting the earliest community response to change that would otherwise be overlooked.

1. Introduction

Extreme events are a natural and important part of many ecosystems, but because of climate change, it is expected that more areas of the world

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* Corresponding author.
E-mail address: marija.ivkovic@biol.pmf.hr (M. Ivković).

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will become exposed to these events. Many regions are already experiencing increased drought, heat waves, fires as well as extreme rainfall and floods (Douville et al., 2021). Ecosystem functionality might become disturbed and some functional groups are even partly lost with the increased frequency, duration and intensity of such events (Gulev et al., 2021). With a greater rate of change, a greater impact on species and ecosystems is expected but responses are taxonomically and geographically variable (Runting et al., 2016). Functional responses remain particularly poorly understood (Greenop et al., 2021).

Climate in general is an important determinant of insect abundance and distribution. In response to climate change, species may either adapt to new conditions or shift their distribution (Bhowmik and Schäfer, 2015; Baranov et al., 2020). Widespread, generalist species fare much better in this competition (Wilson et al., 2007).

Decline in invertebrate biodiversity may threaten key ecosystem services (Carpenter et al., 2011; Greenop et al., 2021). This process is indeed more prominent in anthropogenically influenced areas, but pristine areas are threatened as well (Baranov et al., 2020; Baker et al., 2021). Conservation planning for all habitat types and mitigating the most severe impacts of biodiversity change is only possible with knowledge provided by long-term monitoring of biodiversity change at the local level (Baker et al., 2021; Greenop et al., 2021; Jähnig et al., 2021). Centres of endemism or biodiversity hotspots represent “reservoirs” of species that are especially vulnerable to environmental changes (Wilson et al., 2007). Montane areas are particularly vulnerable, because they support a disproportionate number of endemic and relict species (Wilson et al., 2007; Previšić et al., 2009; Leps et al., 2016). Historically, taxonomic diversity was the main measure of biodiversity but today it is considered an incomplete measure that can be improved by including functional diversity (Jarzyna and Jetz, 2018; Jähnig et al., 2021). For this reason, both taxonomic and functional diversity were investigated in this paper.

The abundance, ubiquity, and disparity of adaptations of Chironomidae to various habitats make them important players in ecosystem processes in nearly all aquatic systems and they are also often used in water quality assessment (Raunio et al., 2011; Adler and Courtney, 2019; Dorić et al., 2021). Despite this, they are often neglected in ecological research due to their small size, high species richness and complex identification, which leads to them being included at family level at best (Ivković et al., 2015; Serra et al., 2016). Their feeding habits are species-specific and even within the same genus species have different food preferences (Armitage et al., 1995; Moog and Chovanec, 2000). Chironomid larvae are also an important food source for all types of aquatic predators and are key facilitators in sediment respiration (Armitage et al., 1995; Baranov et al., 2016). Adult chironomids are weak flyers and colonization of new habitats is achieved by the passive dispersion of ovigerous females (Armitage et al., 1995).

In numerous research projects taxonomic composition and abundance of lotic organisms were found to be affected by variations in discharge (Durance and Ormerod, 2009) In the long – term study of the River Thames discharge was the most important environmental variable to be correlated with chironomid taxa abundances (Ruse and Davison, 2000). Langton and Casas (1998) also reported discharge as a key driver in structuring chironomid assemblages in two Mediterranean mountain streams, whereas Grzybkowska et al. (1996) reported altered taxonomic and functional composition of chironomid community in the years with peak discharges.

In this research we sought to follow the effects of climate change on local environmental conditions and chironomid communities in an otherwise unimpacted freshwater ecosystem that has been under a strict conservation regime with a restricted number of visitors: the Plitvice Lakes National Park. In this research, this pristine background is complemented with comprehensive well-rounded biological and ecological data where chironomid emergence patterns have been monitored since 2007 with the research efforts still ongoing (Ivković et al., 2014, 2015; Ivković and Pont, 2016; Vilenica et al., 2017; Ivković et al., 2020; Vilenica and Ivković, 2021).

We hypothesized that changes in the discharge patterns would negatively affect the chironomid abundance due to increased flushing of the sediment and nutrients. We also hypothesized that discharge would affect both taxonomical and functional diversity of chironomids resulting in generalist gatherers and filterers benefiting from these new circumstances in opposition to specialist taxa such as shredders and grazers.

2. Materials and methods

2.1. Study design

Plitvice Lakes National Park (NP) (Fig. 1) is the oldest and largest national park in Croatia and is one of the UNESCO's World Heritage Sites. It is recognized as one of the biodiversity hotspots (Ivković and Plant, 2015). This research was conducted in Plitvice Lakes NP because of the minimal anthropogenic impact on the system (Vurnek et al., 2021). It consists of 16 barrage lakes connected by tufa barriers. The main tributary of the system is the River Matica which consists of Bijela rijeka and Crna rijeka streams. The site chosen for this study is the Kozjak – Milanovac tufa barrier (Fig. 1A) which connects the largest lake in the system, Lake Kozjak, to Lake Milanovac (Fig. 1B) via a 10 m high waterfall. Tufa is a product of the precipitation and incrustation of calcium carbonate from the water by aquatic plants and microorganisms. For its formation, stable water chemistry is required (Capezzuoli et al., 2013; Vurnek et al., 2021). The growth of the tufa barriers is a dynamic process that over time results in lake modification and water level fluctuation (Radišić et al., 2021).

Adult chironomids (Fig. 2) were collected monthly with emergence traps from February 2007 to December 2020, a total period of 14 years. Traps were pyramid-shaped (Fig. 1C) with a 45 × 45 cm base and 50 cm height, with 1 mm mesh nets. A collection container was placed on top of each trap, filled with a 2 % formaldehyde solution and a few drops of dishwasher soap to break the surface tension. The larvae were free to enter and exit the base of the trap. Upon collection adults were preserved in 80 % ethanol. Adult chironomids were counted and identified to species level using Reiss and Fittkau (1971), Hirvenoja (1973), Saether (1985), Wiederholm (1989), Spies (2000), Langton and Pinder (2007a,b) and Giłka (2011). Only males were used in the statistical analyses since female identification is not possible in most cases.

Chironomid species were assigned one of the nine functional feeding guilds using Moog and Hartmann (2017). Functional feeding guilds are among the most widely applied concepts for lotic ecosystems and are expected to reflect process-level aquatic ecosystem attributes with specialists such as shredders and grazers being more, and generalists such as gatherers and filterers being less sensitive to environmental changes (Moog and Chovanec, 2000; Rawer-Jost et al., 2000). They are assigned based on what type of food the organisms consume (Moog and Hartmann, 2017). The main issue when assigning functional feeding guilds is their interchangeability (e.g. younger instars of predatory subfamily Tanypodinae are obligatory detritivores) and this is adjusted for by splitting each taxon into several functional feeding guilds (Moog and Chovanec, 2000; Rawer-Jost et al., 2000).

Water temperature was recorded two times a day (14:00 h and 02:00 h) with HOBO Pendant Temperature Data loggers (Part UA-001-XX, Bourne, Massachusetts, USA). Current velocity was measured monthly during collection of the animals using P-670-M series instrument (Dostmann electronic), conductivity, oxygen saturation and pH were measured using WTW probes (WTW Oxi 330/SET, WTW pH 330 and WTW LF 330; WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Alkalinity was also estimated monthly by titration with 0.1 M HCl with methyl orange used as the titration indicator. Daily average discharge values were provided by the Croatian Meteorological and Hydrological Service and data on chemical oxygen demand (abbrev. COD), ammonia and orthophosphate concentration by Plitvice Lakes NP.

2.2. Data analysis

Average monthly water temperature followed a monotonic trend. Therefore, the Seasonal Mann Kendall test was used to determine the

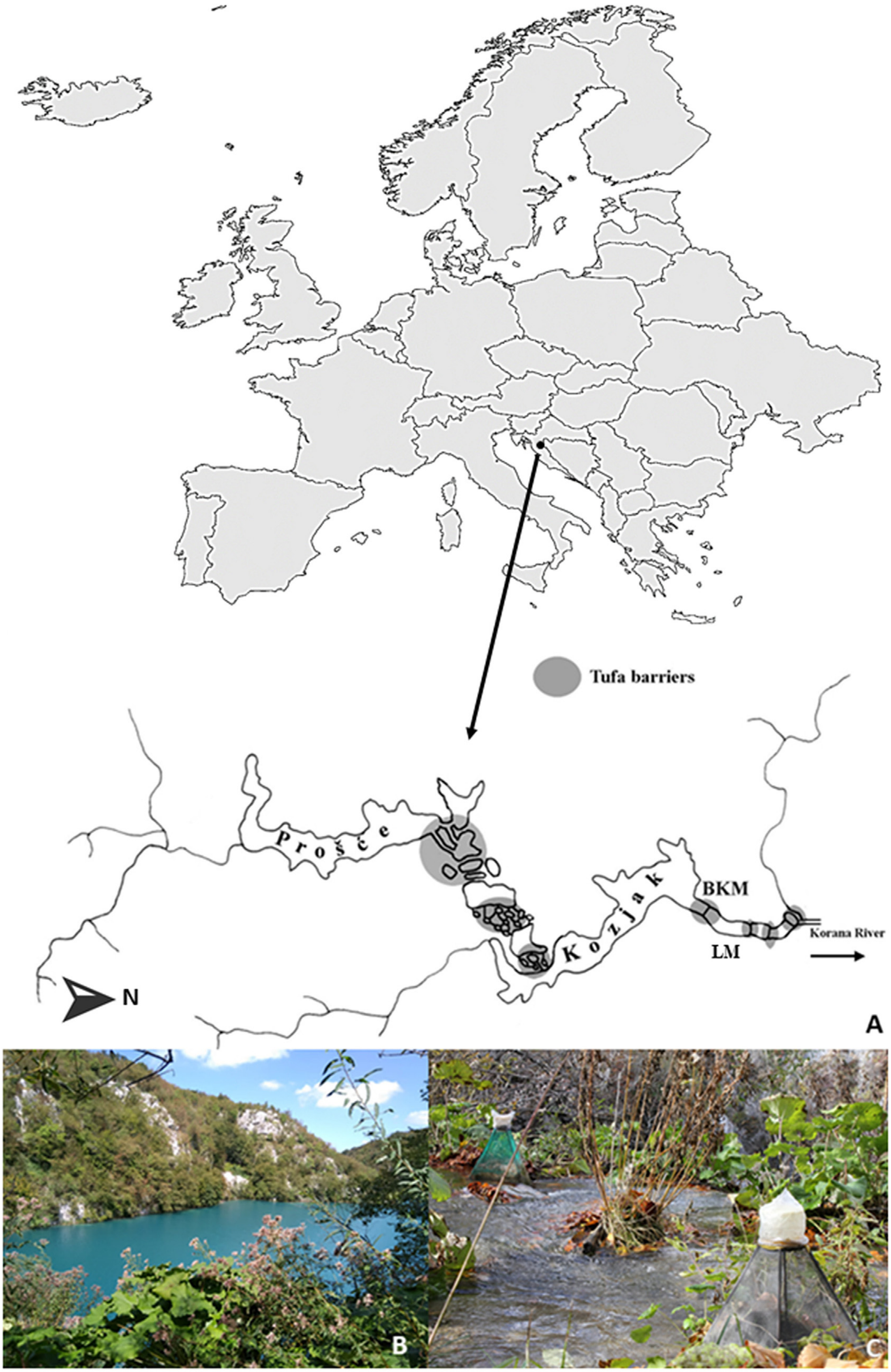

Fig. 1. Map of Europe with, A) Geographical position of the Kozjak – Milanovac tufa barrier (BKM) within the Plitvice Lakes system; arrow represents the water flow direction. B) Lake Milanovac seen from the BKM. C) Two of six emergence traps at the Kozjak – Milanovac tufa barrier.

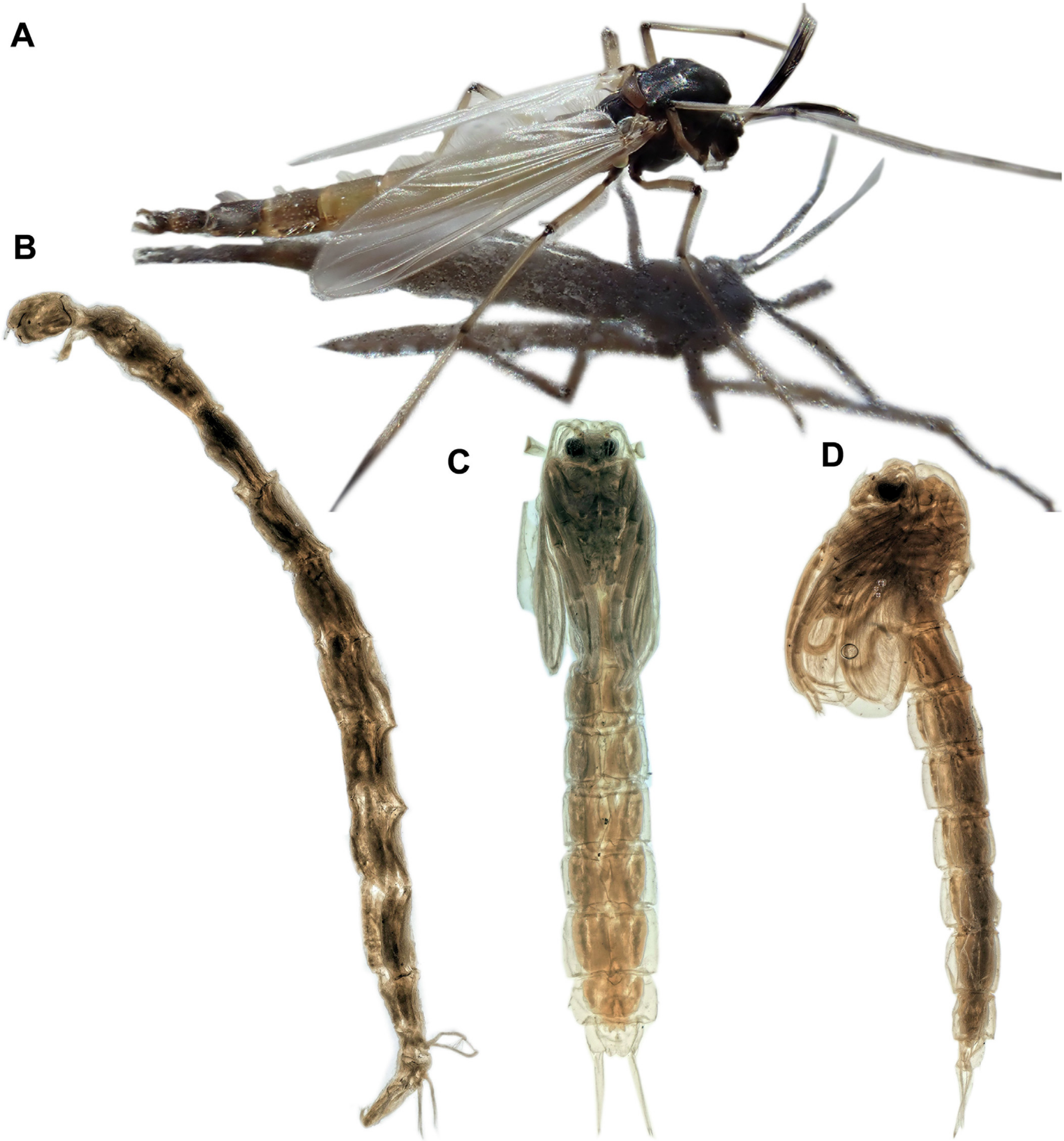

Fig. 2. Chironomid life cycle composed of different species. A - Chironomini sp., adult; Speichersee, Germany, B–D, larva and pupa of *Lasiodiamesa sphagnicola*.

direction of the trend. To quantify the magnitude of this trend, Sen's slope was calculated. Monthly chironomid abundance, species richness, Pielou's evenness index, Simpson and Shannon diversity indices as well as relative abundances of functional feeding guilds were tested for trend using the Seasonal Mann Kendall test. These analyses were performed using the R packages *Kendall* (McLeod, 2022) and *trend* (Pohlert, 2020).

Quarterly water velocity values and quarterly COD values did not follow a monotonic trend but were of an irregular nature, so the F value was used to define a breakpoint in the data based on the group means. Breakpoint analyses were calculated using the R package *strucchange* (Zeileis et al., 2022). The Lowess smoothing function was used to visualise the trend (f = 2/3).

Multiple change-point analysis (MCP) was used to detect change-points in discharge values within the 14 years. These analyses were performed using the R package *mcp* (Lindeløv, 2022). The following environmental parameters were used in an “interactive forward analysis” based on the RDA method followed by a Monte Carlo permutation test: water temperature, COD, oxygen, orthophosphate and ammonia concentration, pH; conductivity, current velocity, discharge, alkalinity and hydrological period (determined after the MCP analysis on discharge levels). This was done in order to assess the strength of the effects of different environmental variables on the chironomid species distribution and variability. This analysis was done using CANOCO package version 5.0 (Ter Braak and Šmilauer, 2012).

Diversity indices and species richness were calculated using PRIMER version 7. Multilevel pattern analysis was used to determine the relationships between chironomid species composition and different discharge patterns. Raw abundance values of all identified species were used, and discharge periods were determined according to corresponding change-points in discharge patterns, determined by MCP analysis. Multilevel pattern analysis was chosen in this case since it is not affected merely by abundance of dominant species, opposed to analysis more biased towards dominant species such as SIMPER or other similar analyses (De Cáceres et al., 2010; Ricotta and Podani, 2017). Multi-level pattern analysis was performed in the R package *indicspecies* (De Cáceres et al., 2022).

R software (R Core Team, 2022) was used to run all the above-mentioned packages with the exception of RDA.

In order to estimate the significance of the differences in chironomid diversity and functional feeding guilds between three hydrological periods, a Mann-Whitney *U* test was applied in Statistica 14.0 (TIBCO Software Inc., 2020). Mann-Whitney U test was applied only to those functional feeding guilds for which the significant Seasonal Mann Kendall trend was found. Relative abundances of functional feeding guilds for which the significant Seasonal Mann Kendall trend was found were plotted using the R package *ggplot2* (Wickham, 2016). Their relative abundances were square root transformed prior to plotting in order to downweight the differences between dominant and rare feeding guilds.

3. Results

A total of 13,522 adults belonging to 92 taxa were collected. The most abundant species was *Parametriocnemus stylatus* (Spärck, 1923) with a total of 3612 individuals, representing nearly 30 % of the community. Supplementary Fig. A.1A and B show that the overall trend in abundance and species richness of chironomids did not change during the study period. The fewest individuals were collected in 2012 (338 specimens belonging to 28 species), while the most individuals were collected in 2015 (1910 specimens belonging to 52 taxa). A total of 7858 male individuals were used for the statistical analyses.

The average monthly water temperature during the 14-year study period is shown in Fig. 3A. The Seasonal Mann Kendall test confirmed the presence of a significant positive trend (tau = 0.155, $p = 0.008$). Sen's slope was calculated and found that the water temperature in Plitvice Lakes increased by 0.008 °C/year on average, but this was not statistically significant ($p = 0.36$). A positive trend was detected for the Shannon diversity index over time, but the significance was very weak (tau = 0.133, $p = 0.03$). Positive, but non-significant trends were detected for species richness, abundance, Simpson diversity and Pielou's evenness index over time (Table A.1). Shannon diversity index was significantly different between the low and high discharge period (Fig. A.2A).

The breakpoint in water velocity was found to correspond to December 2012 (Fig. 3B), with a mean water velocity of 0.1 ms^{-1} before the breakpoint and 0.19 ms^{-1} after the breakpoint. The COD breakpoint corresponds to March 2014 (Fig. 3C), with mean COD values of 0.86 mg O_2 L^{-1} before, and 1.08 mg O_2 L^{-1} after the breakpoint.

Two change-points in discharge data were identified by the MCP analysis (Fig. 4). The first corresponds to June 2010 and the other to March 2013. With these change-points the data were divided into three discharge periods: the regular discharge period – January 2007 to June 2010 (mean discharge = 2.51 m^3s^{-1}, mean value of monthly maximums = 4.76 m^3s^{-1}), the low discharge period – July 2010 to March 2013 (mean discharge = 1.93 m^3s^{-1}, mean value of monthly maximums = 5.02 m^3s^{-1}), and the high discharge period – April 2013 to December 2020 (mean discharge = 3.36 m^3s^{-1}, mean value of monthly maximums = 7.35 m^3s^{-1}). Mean values, standard deviations, maximal and minimal values of all tested environmental variables, community and functional metrics across different discharge periods are laid-out in Table 1.

In the "interactive forward RDA analysis," eleven environmental parameters explained <15 % of the total variation in chironomid assemblages (Table 2). The only two statistically significant parameters that had an effect on the whole chironomid assemblage formation were water temperature and the hydrological period (parameter derived from the MCP analysis of discharge levels).

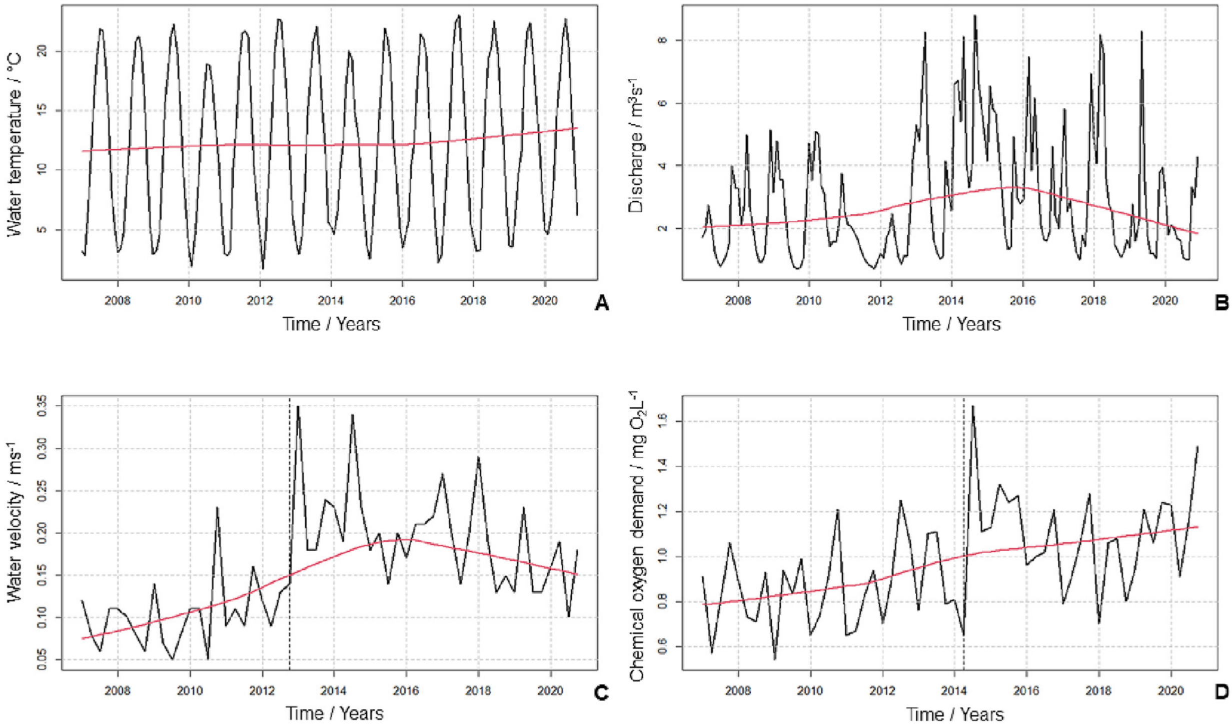

Fig. 3. Time series plots of monthly A) Temperature, B) Discharge, and quarterly values of C) Water velocity and D) COD – Chemical oxygen demand. The red line represents the Lowess smoothing algorithm, vertical black dotted line represents the breakpoint.

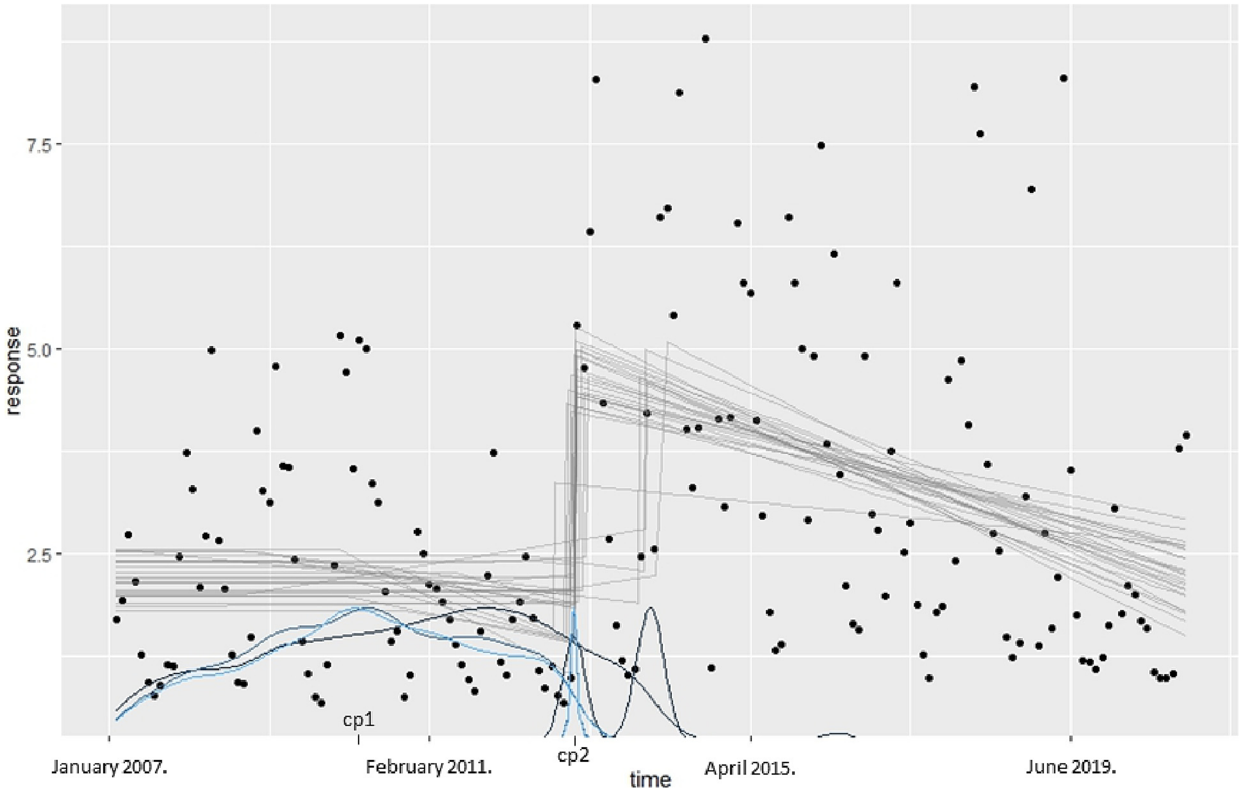

Fig. 4. Change-point model (cp = calculated change-point) based on discharge (y axis) over the 14-year period (x axis). The grey lines represent draws from the posterior distribution of the mean discharge values. The posterior distribution for the change-point is marked in dark blue on the x axis and the kernel density of the replicated datasets generated by the fitted model is shown as a light blue line. The change-points for discharge are labelled as cp1 (June 2010) and cp2 (March 2013).

The results of the Multilevel pattern analysis yielded seven indicator species for the regular discharge period and two indicator species for the high discharge period (Table 3). No indicator species were detected for the low discharge period. One indicator species was common for both the regular and low discharge periods, while four indicator species were common for both the low and extreme discharge periods (Table 3).

The results of the Seasonal Mann Kendall test detected a significant and positive trend in the relative abundance of passive filter feeders (tau = 0.478, $p \leq 0.001$), predators (tau = 0.136, $p = 0.04$) and shredders (tau = 0.208, $p = 0.002$) over time (Fig. 5). There was no significant trend detected for the rest of the feeding guilds tested (Table A.1). Mann Whitney *U* test indicates that the relative abundance of shredders (Fig. A.2B), passive filter feeders (Fig. A.2C) and predators (Fig. A.2D) is

Table 1
Mean values, standard deviation, minimal and maximal values of all tested environmental parameters, community and functional metrics across three discharge periods detected through the 14 years of research.

Discharge period	Regular			Low			High		
Metric	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Water temperature (°C)	11.67 ± 6.87	1.92	22.28	12.09 ± 7.06	1.67	22.68	12.6 ± 6.46	2.21	22.95
Discharge (m^3s^{-1})	2.51 ± 1.4	0.68	5.15	1.93 ± 1.35	0.69	6.42	3.36 ± 2.12	0.98	8.79
COD (mg O_2 L^{-1})	0.81 ± 0.16	0.54	1.06	0.9 ± 0.21	0.65	1.25	1.08 ± 0.23	0.65	1.67
Water velocity (ms^{-1})	0.09 ± 0.03	0.05	0.14	0.15 ± 0.08	0.09	0.35	0.19 ± 0.05	0.1	0.34
pH	8.27 ± 0.11	8.08	8.52	8.25 ± 0.07	8.11	8.38	8.24 ± 0.1	7.98	8.5
Conductivity (μS cm^{-1})	383.08 ± 11.28	362	403	381.21 ± 15.41	329	401	360.55 ± 44.31	323	415
Oxygen (mg O_2 L^{-1})	9.6 ± 1.03	8	12.13	9.99 ± 1.23	7.88	12.09	9.85 ± 1.23	6.43	12.54
Alkalinity (mg $CaCO_3$ L^{-1})	199.53 ± 6.6	184	212.5	203.94 ± 8.7	188.12	220.18	208.23 ± 9.72	159.5	226.5
Ammonia (mg N L^{-1})	0.03 ± 0.03	0	0.103	0.02 ± 0.01	0	0.057	0.01 ± 0.01	0	0.034
Orthophosphates (mg P L^{-1})	0.02 ± 0.04	0	0.26	0.01 ± 0	0	0.02	0.01 ± 0.01	0	0.02
Abundance	97.1 ± 152.93	0	746	46.27 ± 79.21	0	245	85.13 ± 138.02	0	729
Species richness	8.74 ± 9.1	0	33	6.06 ± 6.06	0	21	9.91 ± 9.04	0	35
Shannon diversity	1.1 ± 0.91	0	2.54	1.01 ± 0.78	0	1.96	1.35 ± 0.89	0	2.81
Simpson diversity	0.52 ± 0.38	0	1	0.53 ± 0.38	0	1	0.61 ± 0.36	0	1
Pielou's evenness	0.5 ± 0.37	0	1	0.55 ± 0.39	0	1	0.61 ± 0.35	0	1
Detritivor %	45.96 ± 77.55	0	375.8	16.77 ± 28.26	0	104.4	32.95 ± 60.87	0	390.6
Active filtrators %	4.28 ± 7.66	0	33.6	1.48 ± 2.93	0	10.7	1.85 ± 3.32	0	17.1
Passive filtrators %	0.44 ± 1.45	0	8.8	0.15 ± 0.71	0	4	5.88 ± 16.76	0	108.8
Predator %	4.1 ± 8.2	0	41.2	2.06 ± 6.02	0	33.4	8.63 ± 18.55	0	136.7
Shredder %	0.14 ± 0.47	0	2.8	0.05 ± 0.19	0	1.1	0.49 ± 1.38	0	10
Grazer %	19.9 ± 38.87	0	214.8	8.18 ± 14.18	0	51.3	16.35 ± 29.28	0	166.5

Table 2
Values of individual physico-chemical parameters in RDA "interactive forward analysis" in relation to the variability of the chironomid assemblages.

Parameter	Variation explained %	Contribution in analysis %	Pseudo-F	P
Water temperature	6	41.3	8.7	0.002
Hydrological period	2.7	18.8	4	0.002
COD (chemical oxygen demand)	1	7.2	1.6	0.158
Oxygen concentration	0.9	6.4	1.4	0.186
Ammonia concentration	0.8	5.7	1.2	0.272
pH	0.9	6.4	1.4	0.194
Conductivity	0.5	3.8	0.8	0.2
Current velocity	0.4	2.7	0.6	0.662
Discharge	0.4	2.8	0.6	0.694
Alkalinity/mg CaCO3 L^{-1}	0.4	2.7	0.6	0.618
Orthophosphate concentration	0.3	2.3	0.5	0.348

significantly higher in the high discharge period compared to other two periods.

4. Discussion

During the study period water temperature, velocity, discharge and the amount of organic material increased at our tufa barrier. Long-term analysis of nutrients by Vurnek et al. (2021) revealed negative trends indicating stable water quality in the system. Following these assumptions and results of our RDA, the changes in the chironomid community described above are associated with changes in discharge patterns that are a by-product of climate change. Vurnek et al. (2021) found positive correlations between discharge and both nutrients and COD. They explained this correlation by the re-suspension of sediments during high flow-rates. Extreme discharges also cause nutrient-rich seston inflow from the upstream lake (Špoljar et al., 2007) as well as an influx of allochthonous organic matter from adjacent emergent macrophytes and terrestrial habitats (Miliša et al., 2014). In their study of Plitvice Lakes hydrology, Radišić et al. (2021) observed an increase in the mean annual discharge, but also a decrease in the mean annual water level of the lake upstream of our tufa barrier. This observation is in complete concordance with Douville et al. (2021) who stated that a warmer climate will increase both the amount and the intensity of rainfall during wet events but will result in more drought during dry events and prolonged dry periods between rain events. For Plitvice Lakes and its tufa barriers this means an increased re-suspension of sediments and erosion rates which in extreme cases can lead to tufa barrier collapse (Radišić et al., 2021; Vurnek et al., 2021).

Table 3
Results of the Multilevel pattern analysis - indicator species for each discharge period. *p < 0.001; **p < 0.01.

Multilevel pattern analysis, α = 0,05		
Regular discharge #sps. 7	Stat	*p* Value
Tanytarsus signatus	0.65	0.002**
Stempellina bausei	0.638	0.001*
Polypedilum scalaenum	0.602	0.001*
Paratendipes albimanus	0.564	0.001*
Phaenopsectra flavipes	0.512	0.001*
Tanytarsus chinyensis	0.466	0.002**
Tanytarsus sp.	0.367	0.018*
High discharge #sps. 2	Stat	p Value
Rheotanytarsus curtistylus	0.615	0.001*
Corynoneura lobata	0.375	0.007**
Regular + Low discharge #sps. 1	Stat	p Value
Procladius choreus	0.424	0.016*
Low + High discharge #sps. 4	Stat	p Value
Rheotanytarsus reissi	0.616	0.001*
Rheopelopia maculipennis	0.599	0.002**
Stenochironomus fascipennis	0.448	0.028*
Micropsectra pallidula	0.44	0.026*

4.1. Chironomid community structure

We did not find evidence to support our first hypothesis. Chironomid abundance and the number of species did not change significantly over the period of 14 years. A redundancy analysis determined water temperature and discharge period as the only two significant environmental factors, out of eleven analysed, influencing chironomid community variability. Water temperature is directly influenced by seasonality, as is insect emergence, hence the significant relationship as shown in Vilenica and Ivković (2021) and references therein. Discharge period, on the other hand, was a factor determined after the change point analysis on discharge levels. This was determined as a significant driver of changes in the chironomid community structure. Discharge was reported as an important environmental driver for Diptera emergence by Ivković et al. (2012) and Čmrlec et al. (2013). Major changes in abundance and species richness were observed in 2012 and 2015 when the lowest and highest numbers of individuals and taxa were recorded, respectively. The year 2012 was the driest of all the studied years while 2015 had the most rainfall, most of which fell in early spring.

Community structure, on the other hand, changed significantly which supported our second hypothesis. Most indicator species of the regular discharge period (*Stempellina bausei* (Kieffer, 1911), *Paratendipes albimanus* (Meigen, 1818), *Phaenopsectra flavipes* (Meigen, 1818), *P. scalaenum*) are usually found in stagnant or slow-flowing waters and prefer fine substrate (Moller Pillot, 2009; Andersen et al., 2013). The genus *Tanytarsus* is present in most types of freshwater microhabitats and prefers soft substrates (Andersen et al., 2013). This regular discharge period was characterised by slower water velocity and weaker discharge maxima.

There were no particular species characterising the low discharge period. This was a drought period (Cindrić et al., 2016) characterised by overall lower abundance of chironomids and lower species richness. Indicator species for the high discharge period are *Rheotanytarsus curtistylus* (Goetghebuer, 1921) and *Corynoneura lobata* Edwards, 1924, both characteristic of fast-flowing streams (Andersen et al., 2013; Moller Pillot, 2013). This discharge period was characterised by more severe discharge events and increased water velocities.

There were some species that occurred in both discharge periods and can be considered drought or low discharge tolerant species. *Procladius* (*Holotanypus*) *choreus* (Meigen, 1804) is a mostly predatory chironomid that is abundant in standing or slow-flowing waters (Vallenduuk and Moller Pillot, 2007). In this study this species was characteristic of both regular and low discharge periods. In both discharge periods, water velocity was lower compared to the high discharge period. Species characteristic of both low and high discharge periods have various ecological preferences. *Rheotanytarsus reissi* Lehmann, 1970 and *Rheopelopia eximia* (Edwards, 1929) are both inhabitants of fast-flowing waters with the latter species often being found creeping around *Rheotanytarsus* cases (Vallenduuk and Moller Pillot, 2007). Extreme maximum discharge events can flush coarse woody debris on to the tufa barrier, creating favourable conditions for *Stenochironomus* (*Petalopholeus*) *fascipennis* (Zetterstedt, 1838), which has a wood mining larva (Moller Pillot, 2013). The genus *Micropsectra* inhabits various types of habitats, so all types are preferable (Wiederholm, 1989).

The above described change in species composition indicates a potential change in ecosystem functioning as one functional group is replaced by another. Although the overall abundance of chironomids has not changed, their availability as a food source has. The genus *Tanytarsus* which was abundant in the system during the regular discharge period is usually found in soft long tubes on the bottom (Andersen et al., 2013), which make them a more available prey (Syrovátka et al., 2020) than the larvae of the genus *Rheotanytarsus* which build more resistant diatom or sand incrusted cases attached to rocks or moss (Saether and Kyerematen, 2001). Given our findings it might be beneficial to investigate chironomid biomass rather than abundance as means to detect changes at the ecosystem level in the future studies.

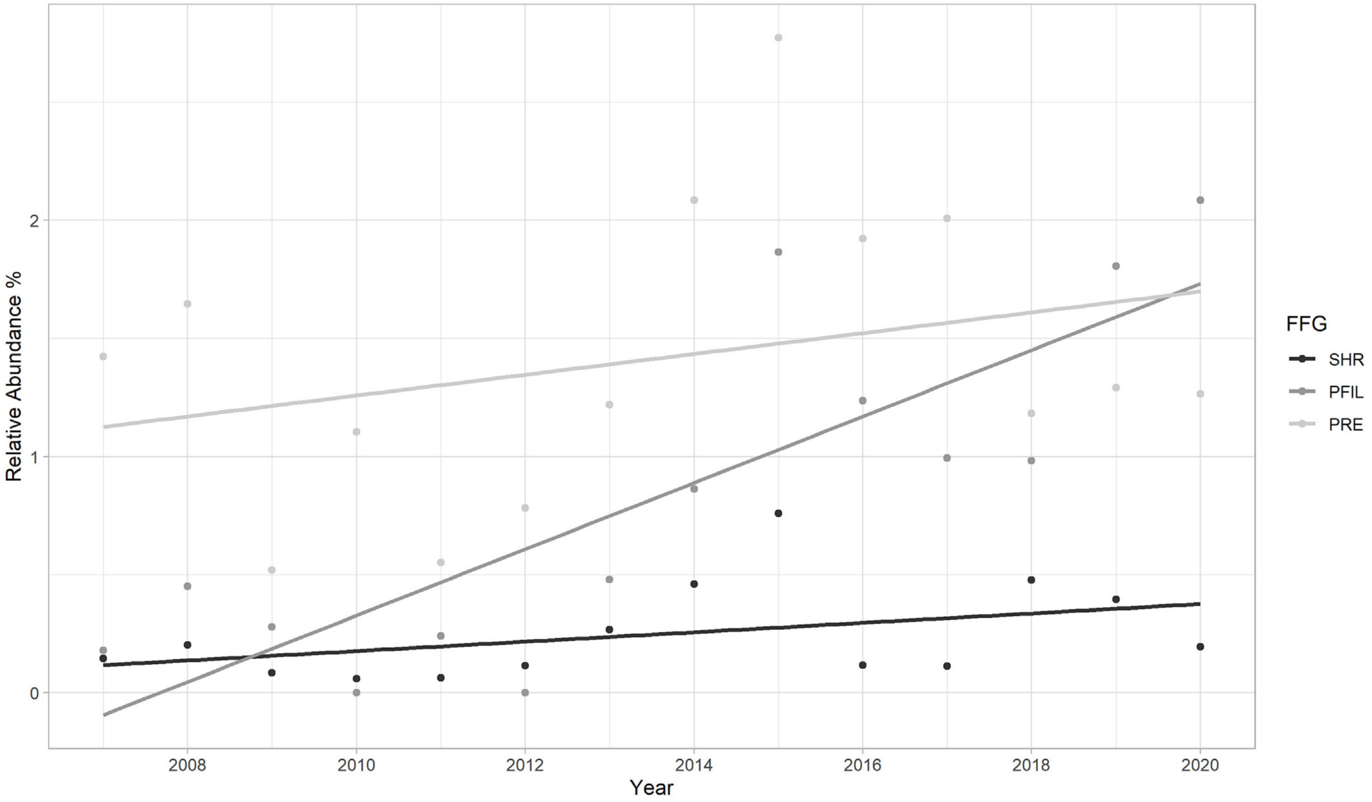

Fig. 5. Transformed average yearly relative abundances of functional feeding guilds with significant trend. SHR – shredders, PFIL – passive filtrators, PRE – predators.

4.2. Functional structure

Moderately high flows transport sediment and export organic resources that allow the restoration of communities of species with rapid life cycles. The life cycles of many aquatic species are adapted to flooding, either to avoid or to exploit variable flows (Poff et al., 1997). However, in the face of climate change, species will either have to adapt quickly or go extinct as, in our case, the barrage lake system has transitioned from predictable and gradual snow-dependent flow changes to unpredictable and severe rain-dependent flows. These processes lead to more suspended organic matter in the system and better conditions for passive filter feeders and shredders, whose relative abundance increased during our study period. This partly supports our third hypothesis that changes in discharge would affect the functional diversity benefiting generalist species. Filtrators are mostly considered as generalists since they utilize organic matter flushed from the upper parts of streams (Moog and Hartmann, 2017). However, the passive filtrating genus *Rheotanytarsus*, whose abundance increased, is also considered a specialist since it needs fast flowing waters to thrive. This exception, once more, signals the importance of species level identification and knowledge of species level ecology in chironomid studies. On the other hand, in the macroinvertebrate community shredders are considered as highly specialized trophic group. Their primary source of food is particulate organic matter (Moog and Hartmann, 2017), which from the chironomid aspect would not be considered as a specialist feeding type since they are able to utilize organic matter from different sources (Hirabayashi and Wotton, 1999). We noticed an increase in their abundance which, given the reverse role of chironomid shredders as generalists, is not surprising during turbulent environmental conditions. The increase in relative abundance of predatory chironomids was also observed and was mostly manifested in a higher abundance of *Rheopelopia eximia*. The abundance of other predatory chironomids decreased over the years, which could be a result of competition for food with new environmental and interspecific conditions better suiting *Rheopelopia*. With increased water temperature and discharge, but at the same time prolonged dry periods, Diptera may have an advantage over taxa with less favourable traits such as large body size, univoltine life cycle and lack of resistant forms (Bruno et al., 2019; Baker et al., 2021). However, not all Diptera are equally sensitive to flash floods (Rader et al., 2008) as is the case with *Rheotanytarsus* in our study. Furthermore, our study is not the first study of European freshwater ecosystems to report an increase in passive filter feeders and predators. Both Baranov et al. (2020) and Baker et al. (2021) observed similar trends in their respective studies. As reported by Baker et al. (2021), this may be an early indication that changing environmental conditions such as warming waters, as well as changes in discharge regimes are already indirectly altering fundamental ecosystem processes through their effects on biotic filtration.

The highly adaptive nature of the chironomids is visible once more in this long-term research where chironomid species richness, abundance and diversity remained virtually unchanged over the years, whereas community structure varied significantly. This long-term research proves that because of the vast taxonomical and functional diversity of the group, interactions within the chironomid assemblages can emulate interactions within the whole macroinvertebrate community. This was also proven by Dorić et al. (2021) where metrics based on chironomids solely were proven to reflect organic enrichment more precisely than metrics developed on the whole macroinvertebrate community.

5. Conclusions

Species richness, abundance and diversity indices did not indicate changes over the years, but community structure did. Functional changes would also remain hidden if species-level resolution was not applied. Species-level identification, as time-consuming as it is, remains key for achieving a fine resolution of the early community response. Morphological based taxonomy describes much more than a name of a species. It can assess the genuine “role” of the species in the ecosystem, i.e. what the animal looks like, what is its intra-specific variability, its size, its life history, its diet, etc., all those key features that allow scientists to understand what is the true function of each species in the ecosystem. A detailed morphological approach is really needed in community ecology papers to detect subtle trends and give meaning and practical context to each research. Local biodiversity changes are complex and cannot be generalised, and long-term biodiversity trends are completely unknown or poorly understood in some areas (Pilotto et al., 2020), which could lead to wrong assessments of biodiversity trends.

High seasonal discharge fluctuations pose an increasing threat to this unique habitat, exacerbated by extensive summer droughts, which lead to damage of the tufa barriers (Radišić et al., 2021). Undisturbed habitats are characterised by natural resilience and a high degree of recovery from occasional extreme events (Rader et al., 2008). However, in this case, the lake outlet (tufa barrier) is followed by another lake instead of a river, and populations are less likely to recover because these species, which are predominantly lotic, end up in a lentic system and become extinct because they are not adapted to such conditions (Čmrlec et al., 2013; Ivković et al., 2014). Global precipitation rates and extreme atmospheric events are predicted to increase as global atmospheric temperature increases (Siler et al., 2018; Douville et al., 2021), indicating major problems for this undisturbed habitat, so the earliest detection of the slightest response in the system is of great value for mitigating these impacts. This can only be achieved through a thorough taxonomic understanding of the populations that inhabit tufa barriers.

CRediT authorship contribution statement

Valentina Dorić: Conceptualization, Investigation, Software, Formal analysis, Writing – Original Draft, Project administration, Data Curation.

Marija Ivković: Project leader, Project administration, Resources, Investigation, Conceptualization, Writing – Review & Editing, Methodology, Supervision, Funding acquisition.

Viktor Baranov: Validation, Visualization, Writing – Review & Editing.

Ivana Pozojević: Investigation, Software, Writing – Review & Editing.

Zlatko Mihaljević: Investigation, Methodology, Resources, Supervision, Writing – Review & Editing, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Article

Long-Term Chironomid Emergence at a Karst Tufa Barrier in Plitvice Lakes National Park, Croatia

Valentina Dorić [1], Ivana Pozojević [1], Viktor Baranov [2], Zlatko Mihaljević [1] and Marija Ivković [1],*

[1] Division of Zoology, Department of Biology, Faculty of Science, Rooseveltov trg 6, 10000 Zagreb, Croatia; valentina.doric@biol.pmf.unizg.hr (V.D.); ivana.pozojevic@biol.pmf.unizg.hr (I.P.); zlatko.mihaljevic@biol.pmf.unizg.hr (Z.M.)
[2] Doñana Biological Station EBD-CSIC, C/Americo Vespucio, 26, Isla de la Cartuja, 41092 Sevilla, Spain; viktor.baranov@ebd.csic.es
* Correspondence: marija.ivkovic@biol.pmf.unizg.hr

Simple Summary: Chironomids or non-biting midges, which are a diverse group of insects, can be found in various freshwater habitats. Plitvice Lakes National Park in Croatia, known for its rich freshwater environment, is an ideal location for studying these insects in the long term. Our study aimed to uncover the types of chironomids present, understand their seasonal patterns, and identify factors influencing their emergence in Plitvice Lakes. For 14 years, we set up traps at a tufa barrier within the National Park to collect chironomids on a monthly basis. During this time, we discovered more than 80 chironomid species. Interestingly, we found that water temperature and organic matter were the key factors affecting chironomid emergence in this area. Toward the end of our study, we noticed that the time these insects spent in flight seemed to increase. Although we have not yet found statistical significance, this could be related to the higher water temperatures in winter. In summary, our research sheds light on the fascinating world of chironomids in Plitvice Lakes, highlighting their diversity, seasonal patterns and the environmental factors influencing their behavior.

Abstract: Chironomids are found in all types of freshwater habitats; they are a ubiquitous and highly diverse group of aquatic insects. Plitvice Lakes National Park is the oldest and largest national park in Croatia and consists of numerous and diverse freshwater habitats, making the area an ideal location for long-term research into the chironomid emergence patterns and phenology. The main objectives of this study were to identify the composition of the chironomid community, determine the phenology of the identified species, and assess the main factors influencing their emergence in Plitvice Lakes. During 14 years of research, more than 13,000 chironomids belonging to more than 80 species were recorded. The most abundant species was found to be *Parametriocnemus stylatus*. The highest abundance of chironomids was recorded in lotic habitats with faster water current over substrates of moss and algae and pebbles. Water temperature and the availability of organic matter were found to be the main factors that drive chironomid emergence at the tufa barrier studied. In the last years of this study, a prolonged flight period was observed. Although this is not statistically significant (at this stage of the study), it could be due to a higher water temperature in winter.

Keywords: non-biting midges; diversity; phenology; temperature; organic matter

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1. Introduction

The emergence of aquatic insects is the process by which immature insects complete their metamorphosis and emerge from the water [1]. This process creates aquatic–terrestrial linkages through the exchange of energy and matter between the two habitats [2]. The insects that emerge from the water are an important food source for terrestrial predators such as spiders, ground beetles, birds and bats [3–5]. Photoperiod (length of day) and water temperature are considered to be the key environmental factors influencing the duration and date of peak emergence [6].

Chironomids are among the most abundant and species-rich aquatic insects. They inhabit all types of freshwater as well as semi-terrestrial, terrestrial, marine and even subterrestrial habitats [7]. Chironomids comprise a large proportion of the benthic fauna and therefore are an important functional component in aquatic ecosystems performing crucial ecosystem functions including aquatic–terrestrial linkages, bioturbation, water remediation and carbon burial [7–9]. Due to the close link between the immature stages (larvae and pupa) and the aquatic environment, as well as the great diversity within the group, they are often used as valuable indicators of environmental health [7,10,11]. The larvae are an important food source both in aquaculture and in nature as they are rich in protein, lipids, vitamins and minerals [12]. Most species of adult chironomids have reduced mouthparts and are functionally "aphagous", meaning that some of them do not feed during the imaginal phase. They also have a very short life span of only one day, which means that their main purpose after emergence is reproduction [13,14]. Although adult chironomids are not hematophagous, they are sometimes considered as pestiferous, creating problems as nuisances and public health risks [12,15].

Plitvice Lakes National Park is a UNESCO World Heritage Site and the oldest and largest national park in Croatia. It is also recognized as one of the biodiversity hotspots [16]. Plitvice Lakes was chosen as a study site because the anthropogenic impact on the system is minimal [17]. Ongoing research on insect emergence in Plitvice Lakes has already provided some valuable information on the phenology and ecology of various aquatic insects: namely, Trichoptera [18], Ephemeroptera [19,20], Plecoptera [21], Odonata [22], Megaloptera [23], Neuroptera [24] and various Diptera families [16,25–32].

The goal of this study was to determine how the emergence phenology of chironomids, as the abundant taxon, may be controlled by environmental factors.

We hypothesized that community dynamics and structural changes in a diverse insect group (here exemplified by chironomids) will reflect the environmental conditions of the ecosystem they inhabit. To test this hypothesis, this study needed to achieve the following specific objectives: (1) identify the composition of the chironomid community and determine the phenology of individual species; (2) assess the environmental factors influencing chironomid emergence; (3) estimate species preferences for specific microhabitat types; and finally (4) determine the magnitude of barrage lake outlet-to-land linkages by emerging chironomids.

2. Materials and Methods

2.1. Study Area and Study Design

Plitvice Lakes National Park encompasses a network of 16 barrage lakes along with numerous smaller, unnamed lakes interconnected by tufa barriers. Tufa, a result of the precipitation and encrustation of calcium carbonate from the water by aquatic plants and microorganisms under stable water chemistry conditions [33], plays a pivotal role in shaping the park's landscape. The formation of tufa barriers is a dynamic process influencing lake modifications and water-level fluctuations over time [34]. Although initially perceived as natural lake outlets situated between lakes, research by Šemnički et al. [18] highlights that tufa barriers offer diverse microhabitats akin to stream environments.

This study focuses on the Kozjak–Milanovac tufa barrier, linking the largest lake, Lake Kozjak, to Lake Milanovac. Refer to Dorić et al. [31] for the map of the study area.

To collect adult aquatic insects, emergence traps were deployed from February 2007 to December 2020, covering a span of 14 years. Six pyramid-shaped traps, each with a 45 × 45 cm base and a height of 50 cm, featuring 1 mm mesh nets (Figure 1), were strategically placed in different microhabitats and emptied monthly. The pyramid is slightly elevated from the river bed to allow free movement of the insect larvae. This opening was also cleaned monthly from different vegetation overgrowth in order to preserve microhabitat velocity and composition within the trap. A collecting container, housing a 2% formaldehyde solution with a few drops of detergent, was affixed to each trap. Benthic insect larvae could freely move in and out of the submerged trap base. During monthly field collection trips, adults were preserved in 80% ethanol. Statistical analyses focused on male chironomids identified to species level [35–42], as identifying females to the species level is generally not feasible. As emergence traps are not a selective method, many other groups of aquatic insects were also sampled. These were also processed in many research articles with a comprehensive overview of these findings unified in Ivković et al. [43].

Figure 1. Pyramid-shaped emergence trap at the Kozjak–Milanovac tufa barrier.

Water temperature, measured twice daily, was recorded using HOBO Pendant Temperature Data loggers (Part UA-001-XX, Onset, Bourne, MA, USA). Average daily discharge values were sourced from the Croatian Meteorological and Hydrological Service. The water levels remained stable thought the study period, meaning that all of the pyramid traps remained submerged to a level between the gathering container and the opening for free larval movement. Monthly measurements of current velocity were conducted using a P-670-M series device (Dostmann electronic, Wertheim, Germany). Simultaneously, conductivity, oxygen saturation, and pH were measured using WTW probes (WTW Oxi 330/SET, WTW pH 330, and WTW LF 330; WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Alkalinity was determined monthly through titration with 0.1 M HCl, employing methyl orange as the titration indicator. Chemical oxygen demand (COD) data were provided by the National Park.

2.2. *Assessment of Factors Influencing Chironomid Phenology in Plitvice Lakes*

Canonical Correspondence Analysis was used to quantify the total variation in the chironomid community, while Interactive Forward Selection was used to identify the key environmental parameters influencing chironomid emergence. Bonferroni correction was applied to the *p*-values. These analyses were performed in CANOCO version 5 [44]. Monthly chironomid abundance was tested for correlation with monthly water temperatures, discharge and water velocity using Spearman correlation in IBM SPSS Statistics ver. 27.0 [45].

To investigate the phenological shifts of the species over time, three phenophases for the entire chironomid community were considered:

(1) Month of peak emergence (month of occurrence of the median point of emergence, i.e., when 50% of individuals had emerged);
(2) Duration of emergence (number of months between the start and end of emergence); and

(3) The first appearance (the month in which the species emerged for the first time).

The *mblm* package [46] in R was used to fit a median-based linear model (MBLM) to examine the relationship between each of the selected phenophases and time. The MBLM approach utilizes the median instead of the mean, is robust to outliers, and therefore provides a more reliable estimation of the regression coefficients [46]. The results of the MBLM are reported as MAD (median absolute deviation) and p value. The phenophases were tested for correlation with the mean water temperature of each month and the mean annual COD using Spearman's correlation in IBM SPSS Statistics ver. 27.0 [45].

2.3. Microhabitat Preferences

The emergence traps were set at specific microhabitats present at the tufa barrier at that time. Three microhabitats were present: pebbles (1); moss and algae (2); and sand (3). Two emergence traps were set on pebbles, two were set on moss and algae, and two were set on sand. However, in June 2013, it was observed that one of the "pebble traps" became a "moss and algae trap", and in November 2013, it was observed that one of the "sand traps" became a "pebble trap". This was considered when carrying out the analyses. When linking the preference for a microhabitat type to specific chironomid species, only those that had a total abundance of more than 190 individuals throughout the study period were used, resulting in 10 species considered for this analysis (almost 50% of the total community). Differences in water velocity, total abundance, species richness and abundance of each of the 10 most abundant species between the three different microhabitat types (1/2, 1/3, 2/3) were tested using the Kruskal–Wallis test and Dunn's Multiple Comparison test for post hoc pairwise comparisons. This was performed in IBM SPSS Statistics ver. 27.0 [45].

2.4. Determination of the Magnitude of Chironomid Emergence

To determine the magnitude of chironomid emergence, chironomid abundance per square meter was calculated. The dry mass was determined only for chironomids emerging from April to August, as emergence was highest during these months and it was possible to collect at least 30 individuals in one month. The average chironomid dry mass was determined by drying at least 30 individuals in each spring/summer month and weighing them using the Mettler Toledo Gold Balance JE503G scale. To estimate the annual emergence magnitude per square meter, the chironomid abundance per square meter per year was multiplied by the average chironomid dry mass of the respective year. The results represent a very rough approximation of annual chironomid emergence.

The average annual dry mass of chironomids was tested for correlation with the following factors: average annual water temperature, average annual discharge and average annual COD using Spearman's correlation in IBM SPSS Statistics ver. 27.0 [45].

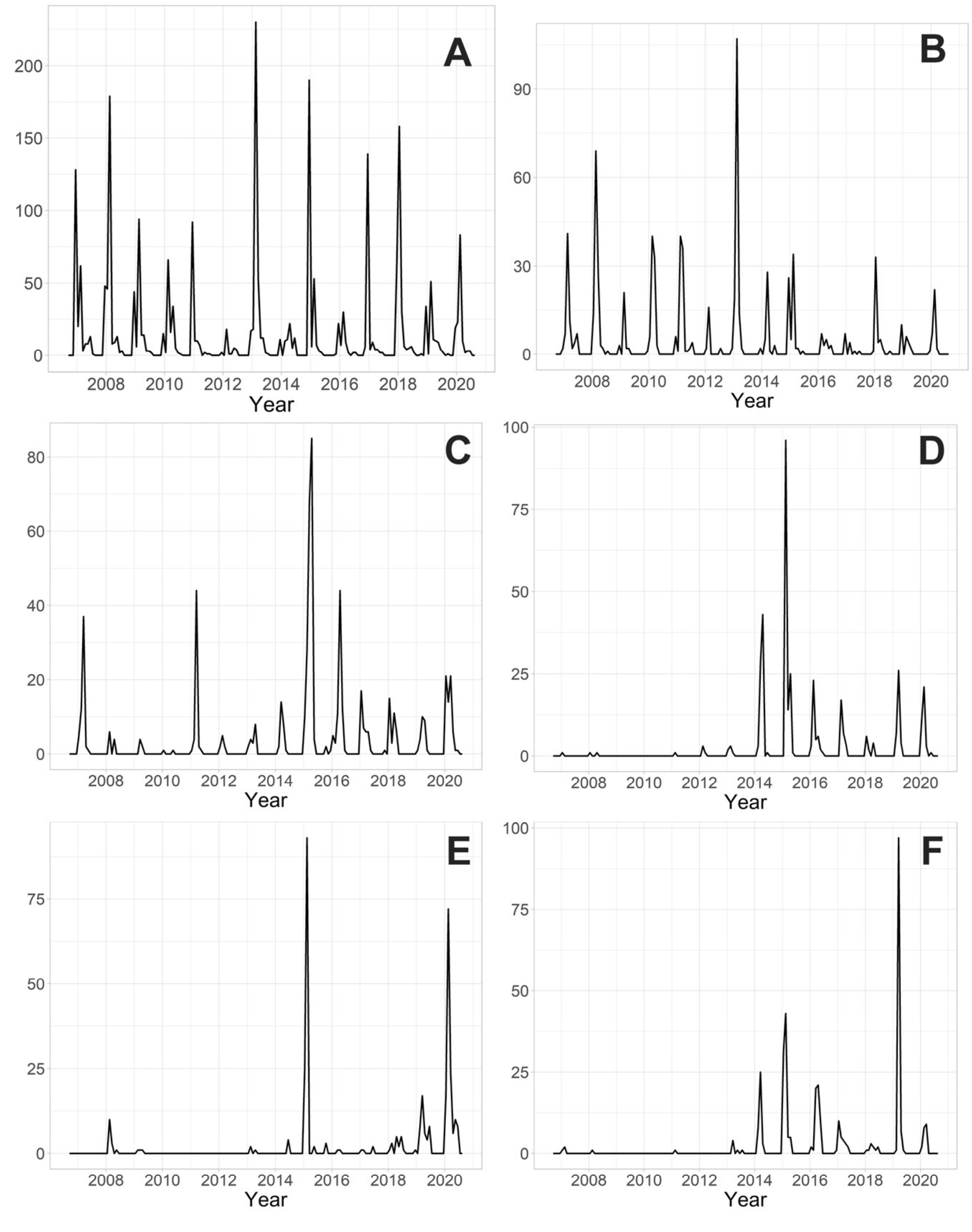
All of the plots were created using the R package *ggplot2* [47]. The R software version 4.2.3 [48] was used to run the two packages mentioned above.

3. Results

A total of 13,522 chironomid adults were collected, of which 7797 were males belonging to 81 species. In the whole study of the site, Trichoptera, Plecoptera, Ephemeroptera, Odonata and other Diptera taxa were also abundant and analyzed in detail in Ivković et al. [43]. Most chironomid species belonged to the Orthocladiinae (32 species), and the fewest species (1) belonged to Prodiamesinae. Overall, 20 species of Chironomini, 15 species of Tanytarsini, 11 species of Tanypodinae and 2 species of Diamesinae (Table S1) were recorded at the tufa barrier during the study period.

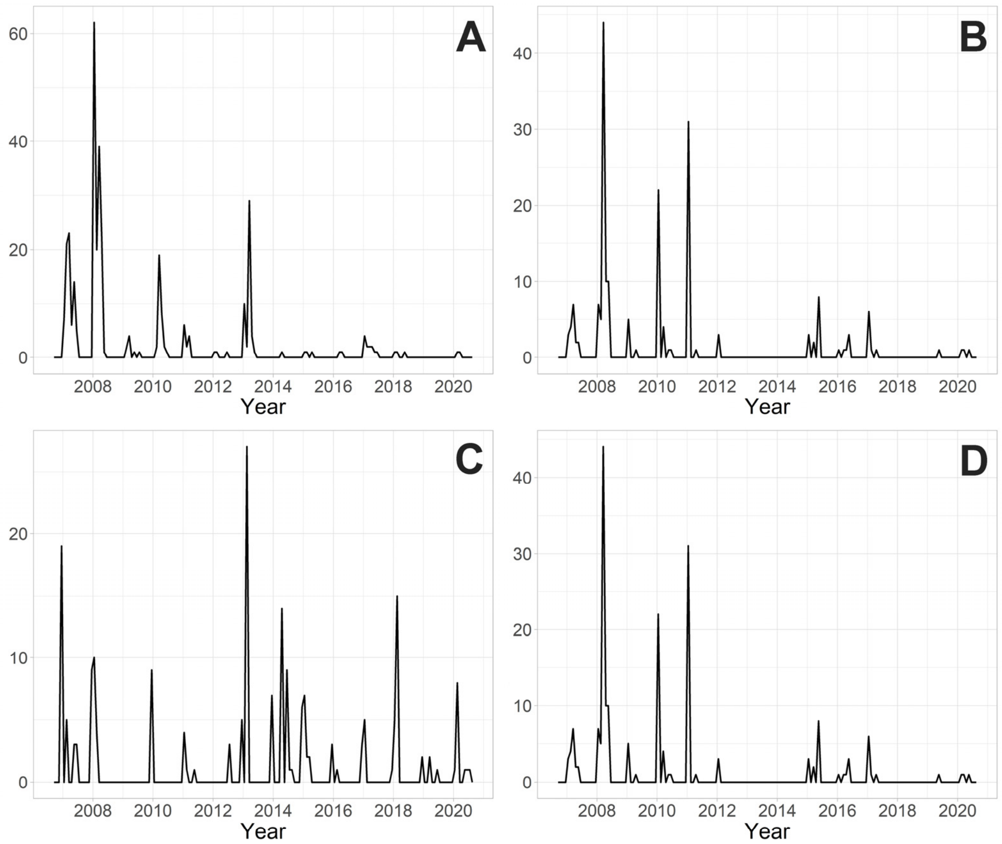
The 10 most abundant species during the 14 years of research are *Parametriocnemus stylatus* (Spaerck, 1923) (2491 individuals), *Tanytarsus brundini* Lindeberg, 1963 (799), *Tanytarsus arduennensis* Goetghebuer, 1922 (614), *Rheopelopia eximia* (Edwards, 1929) (378), *Rheotanytarsus curtistylus* (Goetghebuer, 1921) (351), *Rheotanytarsus reissi* Lehmann, 1970 (345), *Tanytarsus signatus* (van der Wulp, 1859) (344), *Paratrichocladius skirwithensis* (Edwards,

1929) (315), *Parametriocnemus* sp. 2 (202) and *Polypedilum scalaenum* (Schrank, 1803) (194). Their monthly abundances over the years are shown in Figure 2a,b.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. (**a**). Monthly abundances (the number of individuals caught in the traps from a surface area of approximately 0.4 m^2, i.e., 45 × 45 cm; in a time period of one month) of (**A**) *Parametriocnemus stylatus*; (**B**) *Tanytarsus brundini*; (**C**) *Tanytarsus arduennensis*; (**D**) *Rheopelopia eximia*; (**E**) *Rheotanytarsus curtistylus*; and (**F**) *Rheotanytarsus reissi* during 14 years of research. (**b**). Monthly abundances (the number of individuals caught in the traps from a surface area of approximately 0.4 m^2, i.e., 45 × 45 cm; in a time period of one month) of (**A**) *Tanytarsus signatus*; (**B**) *Paratrichocladius skirwithensis*; (**C**) *Parametriocnemus* sp. 2; (**D**) *Polypedilum scalaenum* during 14 years of research.

The observed seasonal succession of species over the investigated period, 2007–2020, is presented in the Supplementary Material, Table S1. The data were pooled from all traps and years. In general, chironomids were found to emerge throughout the year. The highest species richness was found in June (60), which was followed by May (52) and July (47). The lowest species richness was recorded in January (3), which was followed by December (7), March (8) and February (9).

3.1. Assessment of Factors Influencing Chironomid Phenology in Plitvice Lakes

The highest average annual water temperature (12.8 °C) was recorded in 2020 and the lowest (11.03 °C) was recorded in 2010 (Table S2). The highest average annual discharge was measured in 2014 (5.58 m^{3}s^{-1}) and the lowest was measured in 2011 (1.42 m^{3}s^{-1}).

The total chironomid abundance was positively correlated with water temperature (r(168) = 0.74; $p < 0.001$) and negatively correlated with discharge (r(168) = −0.23; $p = 0.003$) and water velocity (r(168) = −0.25; $p = 0.001$). For thorough explanations of the changes in the environmental parameters, refer to Dorić et al. [31].

The total variation in the chironomid community was 4.043 (Axis 1 = 0.26, Axis 2 = 0.14), and the explanatory variables tested accounted for 12.7% of the variation. Water temperature (Table 1) was responsible for most of the variation within the chironomid community (5.1%) compared to all other variables tested.

Table 1. The variability of the chironomid community in relation to the environmental variables selected by the Canonical Correspondence Analysis using Interactive Forward Selection.

Environmental Variable	Explains %	Contribution %	Pseudo-F	*p*	P (adj)
Temperature/°C	5.1	24.2	6.5	0.002	0.024
COD_KMnO$_4$/mg O$_2$ L^{-1}	4	19	5.3	0.002	0.024
Oxygen/mg O$_2$ L^{-1}	1.9	9.3	2.6	0.002	0.024
Nitrites/mg N L^{-1}	1.7	8.3	2.4	0.002	0.024

The earlier first appearance in the whole community was found in the later years of the study (MAD = 0.08; $p < 0.05$), as was the prolonged flight duration (Figure 3). The estimated duration of flight is 0.14 months longer each year (MAD = 0.12; $p < 0.01$). However, the peak of community emergence did not shift within the analyzed period (MAD = 0; $p > 0.05$) (Figure 3). Community flight duration was positively correlated (r (14) = 0.738; $p < 0.01$), while first appearance was negatively correlated (r (14) = −0.633; $p < 0.05$) with the COD. Community flight duration was positively correlated with higher values of mean water temperature in February (r (14) = 0.65; $p < 0.05$) and March (r (14) = 0.55; $p < 0.05$). Peak emergence was negatively correlated with higher water temperature values in May (r (14) = −0.58; $p < 0.05$).

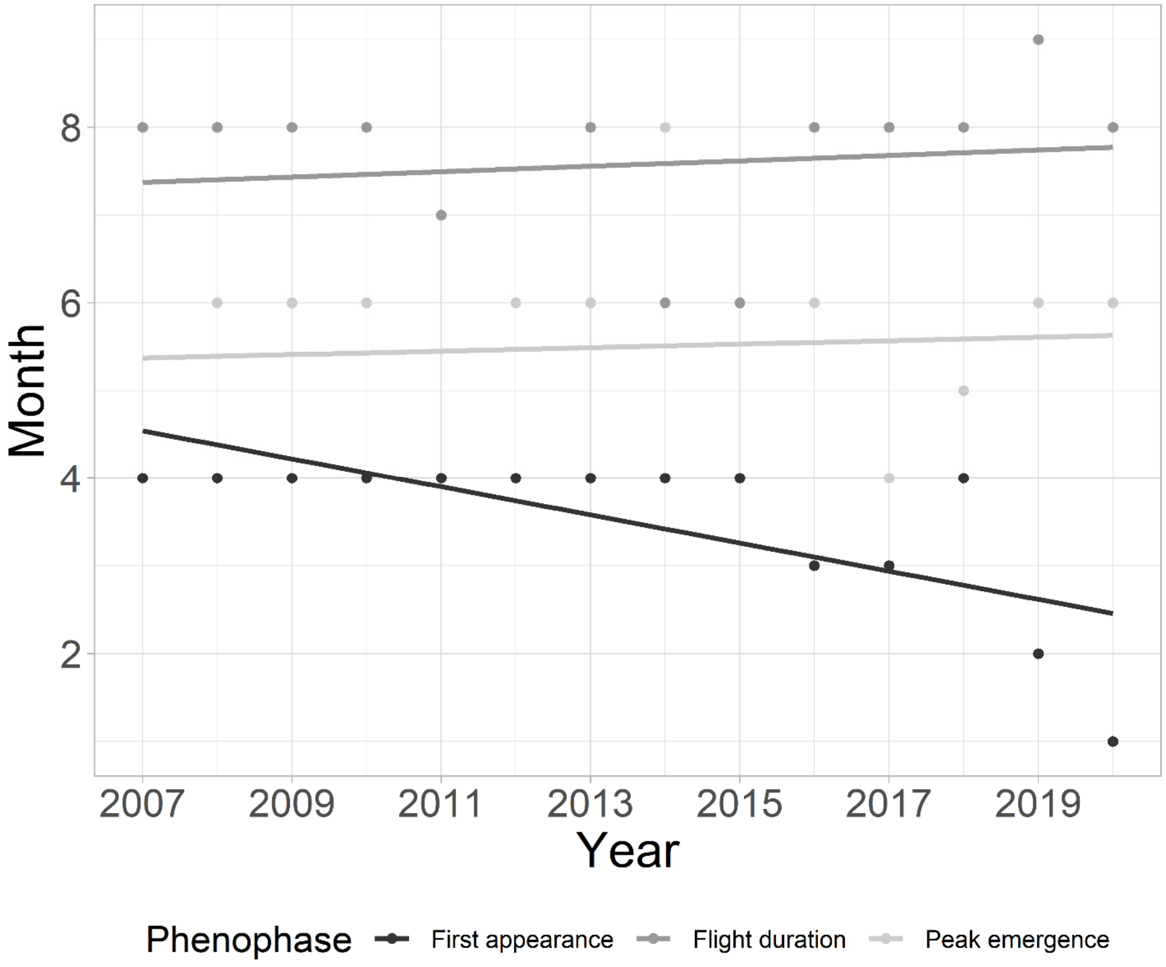


Figure 3. Mean annual shifts in phenology for the entire chironomid community studied over a 14-year period at a tufa barrier in Plitvice Lakes National Park, Croatia.

3.2. Microhabitat Preferences

A total of 10 species, accounting for 55% of the total abundance in these 14 years, were used to assess the microhabitats. Most individuals emerged on moss and algae substrate, followed by pebbles, and the fewest individuals emerged in traps attached to sandy substrate. Complete tables can be found in the Supplementary Materials (Table S3). The Kruskal–Wallis test showed significant differences between the tested microhabitats and water velocity and all tested species except *Parametriocnemus* sp. 2 and *T. brundini*. The greatest differences in species composition of the chironomid community were observed between sand and the other two microhabitats. The water velocity was lowest over the sand substrate (0.02 m s^{-1}) compared to the other two substrates (pebble: 0.17 m s^{-1}, moss and algae: 0.21 m s^{-1}) (Figure 4). Nine of the species tested preferred moss and algae and pebbles as microhabitats, while two (*Polypedilum scalaenum* and *Tanytarsus signatus*) preferred sand.

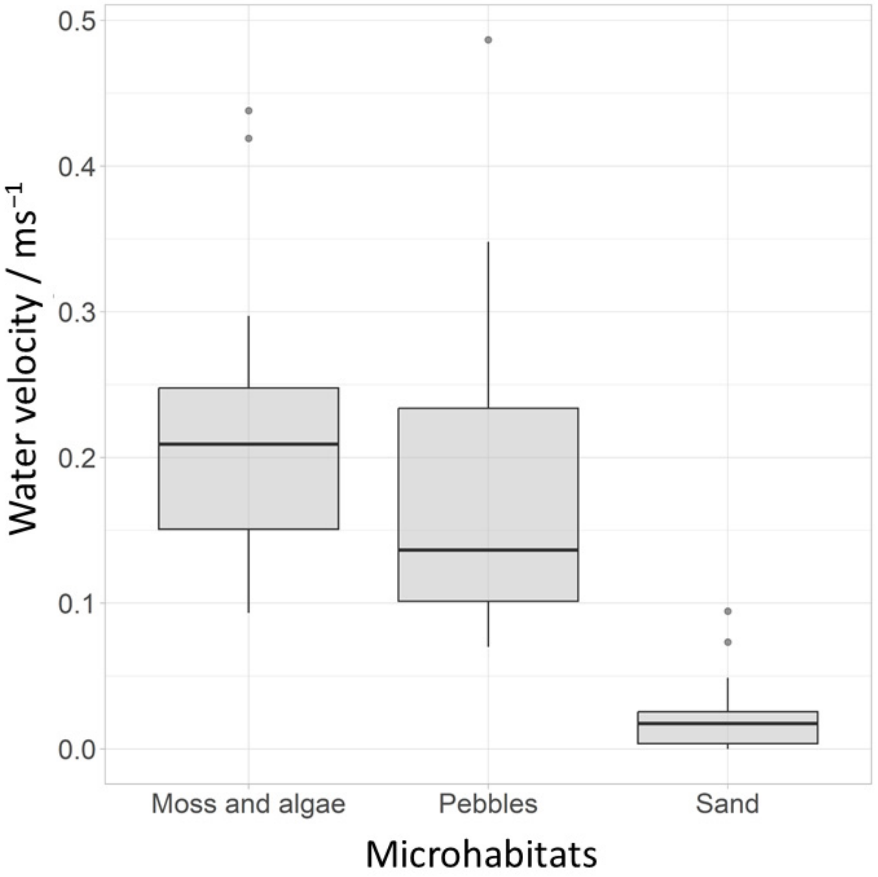


Figure 4. Average water velocity (m s^{-1}) at different microhabitats (grey dots represent outliers).

3.3. Determination of the Magnitude of Chironomid Emergence

The total chironomid emergence varied greatly during the study, as did the annual chironomid abundance. The highest production of emerged chironomids from the tufa barrier was recorded in 2008 with 4415.84 mg $m^{-2}y^{-1}$. The lowest production was recorded in 2012 with 130.19 mg $m^{-2}y^{-1}$ (Figure 5). The average annual dry mass did not correlate with any of the tested parameters (mean annual water temperature, discharge and COD).

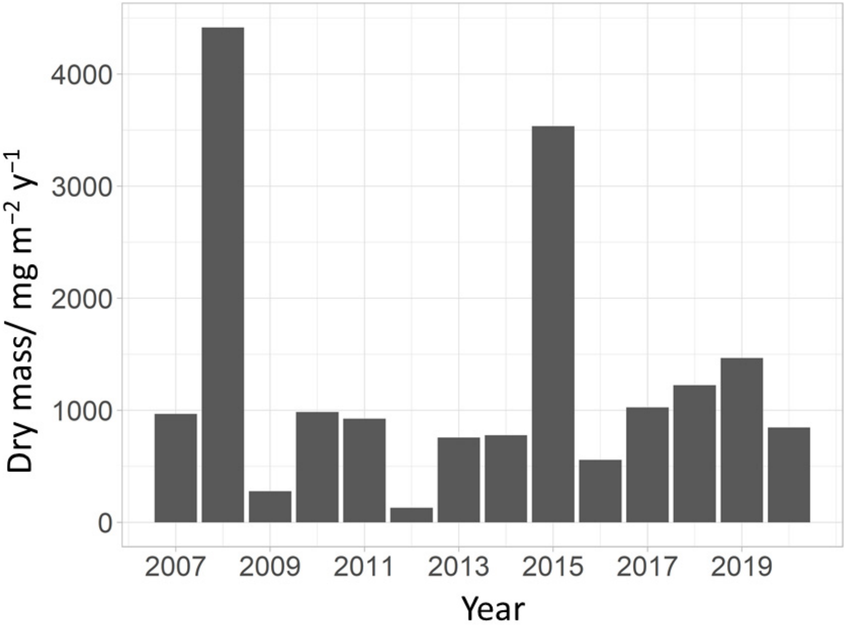


Figure 5. Annual mass of emerged chironomids (mg $m^{-2}y^{-1}$) from the six emergence traps at a tufa barrier over the analyzed period. All masses are measured as dry mass.

4. Discussion

4.1. Chironomid Community at a Tufa Barrier

In 2011 and 2012, Plitvice Lakes underwent an extreme drought [32], after which the chironomid abundances started to decline. After 2012, the period of extreme discharge generated by prolonged and more intense rainfall set in, and at the same time, the duration of winter snow cover decreased [31]. This combination of events may have led to a drying up of underground water reserves and turned Plitvice Lakes from the former snowfall and groundwater fed system into a more spurious rainfall-dependent system [31]. Discharge levels directly affected other variables such as current velocity, which in turn affected microhabitat conditions and chironomid assemblages therein [31].

These environmental changes have resulted in changes in chironomid phenology. For the most abundant species in this study, *P. stylatus*, two or three generations per year are common, as we have recorded. The species usually prefers slow flowing streams and microhabitats that are protected from fast currents. However, this species was found to be the most abundant in the samples with increased discharge and water velocity in Plitvice Lakes [31].

The main food source of *P. stylatus* is slowly decaying organic matter [49]. Although *P. stylatus* was still the dominant taxa in the community, the overall abundance of this species decreased. High discharges, that occurred frequently at the study site, caused the leaves to be washed away, which would otherwise slowly decay and provide *P. stylatus* with a preferred microhabitat as well as a food source [50]. The abundances of *Parametriocnemus* sp. 2 fluctuated over the years with the highest abundance recorded in 2013. This species is probably a new species that has yet to be described. It was also found in all types of microhabitats indicating possibly a species that is less dependent on levels of decaying organic matter then other species of the genus [49].

The genus *Tanytarsus* is very heterogeneous, but unfortunately, the ecology of the individual species of the genus is not well known. It is known that the larvae build long, soft tubes and also burrow into the sediments [51]. In our study, the abundances of *T. brundini* and *T. signatus* decreased, while the abundance of *T. arduennensis* increased over the years, possibly suggesting that the latter species is more resilient to the spurious nature of the discharge regimes in the system [31]. The abundances of *Rheopelopia eximia*, *Rheotanytarsus curtistylus* and *R. reissi* increased over the studied period. All these species prefer faster flowing streams and *R. eximia* can often be found creeping among tubes of other species of the genus *Rheotanytarsus* [14].

We observed the decline in the *P. skirwithensis* abundance, as shown in a monthly abundance graph. The species primarily inhabits spring and groundwater-dependent ecosystems [52], which means that the altered discharge regimes observed at the study site may have had an unfavorable impact on the species' phenology.

P. scalaenum is also a species for which a decline in abundance was observed. The larvae of this species inhabit sandy soils [53]. Unfortunately, the extremely high discharges recorded in Plitvice Lakes, which led to the flushing of the sand have also caused the loss of the preferred habitat for *P. scalaenum*.

4.2. Factors Influencing Chironomid Phenology in Plitvice Lakes

The water temperature is the factor that best explains chironomid community composition as previously demonstrated [6,54,55]. The rate of chironomid larval development increases with the water temperature [14]. The onset of chironomid emergence in temperate areas is associated with the rising spring temperatures [7]. However, temperature, and the other environmental variables tested, explained a small percentage of the total emergence timing variation of the chironomid community. This suggests that inter and intra-species relationships may have a stronger importance in shaping the chironomid phenology [7].

Predation on larvae and competition for food influence the timing of emergence [7]. The highest abundance of chironomids was observed in the spring and summer months when the water temperature was higher. We can therefore confirm that water temperature is an important factor triggering chironomid emergence at the tufa barrier. A negative correlation between discharge and chironomid abundance is most likely due to the fact that higher discharge occurs during the colder, rainy period of the year (late autumn, winter and early spring) when chironomid abundance is lower. On the other hand, extremely high discharge during warmer periods of the year may result in lower chironomid emergence due to substrate disturbance and drift [27,54,56].

The chironomid flight duration Increased, and they started to emerge earlier during the study period. When the water temperature was higher in February and March and there was more organic matter in the system, chironomid emergence started earlier and lasted longer. The peak time of emergence varied over the years, but it was mostly in June. We did not detect significant shifts in peak emergence, which was most probably due to the monthly collection efforts, where daily or weekly changes can be overlooked [57]. In the period analyzed, the peak emergence occurred twice in May (2017 and 2018) and twice in July (2014 and 2019). The earlier peaks of emergence in May (in 2017 and 2018) could have been a result of the higher water temperature in the preceding months and the above average water temperature in May. The later peaks in July (in 2014 and 2019) could be the result of below average water temperatures that occurred in May of these years (12.4 °C in 2014 and 11.5 °C in 2019). In comparison, the average water temperature measured in May was almost 14 °C. Overall, peak emergence has not shifted significantly over the years, but during the last 4 years of research, a peak was detected only once in the "usual" peak abundance month, June of 2020. If this shift in peak abundance timing continues, it could lead to potential problems for terrestrial communities. Changes in the phenologies of aquatic and terrestrial insect communities could lead to asynchrony between the demand of insectivorous consumers and what is available in the environment [5].

4.3. Microhabitat Preferences

The emergence traps attached to the sand substrate were characterized by the lowest water velocity, but during 2013, the sand was washed away from the more exposed of the two traps and the pebbles were left behind. This was probably a result of the unusually high discharge and high water velocity in October 2013, which was facilitated by the unusually low water level of 2011/2012. This is yet another insight into the dynamics of the everchanging microhabitat mosaic that highlights the importance of microhabitat selection and monitoring in this type of long-term research in order to understand the dynamics of our target group of organisms. For instance, an emergence trap, which still stands on sandy substrate today, is in a very sheltered location amidst emergent macrophytes (*Cladium mariscus*) and shows distinctly different chironomid assemblages in comparison to other traps throughout the research period. Also, the moss that was observed in one of the pebble traps in the emergence trap P3 in June 2013 has most likely spread from the neighboring habitat into the emergence trap, which is most probably the cause of increased abundances of chironomid individuals in this trap in the second part of the research.

Most of the species used for the microhabitat assessment did not show a clear association to only one microhabitat type, with the exception of *P. scalaenum* and *T. signatus*, which showed a clear preference for sandy substrate and whose occurrence at the tufa barrier has decreased over the years due to the flushing of this substrate. Most of the other species seem to avoid slower flowing water and sandy substrate, which is probably due to its homogeneity. Sand does not provide a variety of microhabitats [58], and slower water velocity could lead to the deposition of organic matter and its slow decomposition [59,60].

4.4. Magnitude of Chironomid Emergence

The chironomid emergence production rate of 130 to 4400 mg m^{-2} y^{-1} corresponds to an oligotrophic to mesotrophic production of the system. However, the trophic status of the system is a rough estimate based only on a part of the fauna present in it, so it should be taken accordingly. The highest production rates correspond to years of highest chironomid abundances, and the same goes for the years with lowest rates. Lake Kozjak is known to normally fluctuate between these two trophic states [43,61]. This study shows that in years of high chironomid emergence, up to 4400 mg m^{-2} y^{-1} of chironomid biomass is available to terrestrial animals either directly as prey or indirectly as carcasses that decay on the soil surface [62]. When these chironomids die, their bodies decompose and add nutrients to the soil, mainly resulting in nitrogen and phosphate enrichment [4].

5. Conclusions

Changes in the composition of the chironomid community could affect its availability as a food source for other organisms, but they could also lead to changes in the bioturbation rate. Chironomid emergence was strongly influenced by water temperature and the amount of organic matter available in the system. Chironomids at a tufa barrier now have a longer flight duration due to higher water temperatures in winter. The ramifications of climate-driven changes include increased water temperature, which could affect peak emergence and thus decouple it from the phenology of insectivorous consumers. This is still not the case for Plitvice Lakes, but on the other hand, most studies that have found shifts in peak emergence have found shifts in days, so our monthly efforts may not be sufficient to detect these shifts. Weekly or even daily collections could lead to a more accurate detection of the shift in peak emergence. Of all species, only two showed a clear preference for sandy substrate, and their abundances are severely declining due to an increased flushing of sediments by high discharge. As chironomid larvae contribute to the recycling of organic matter and form an important trophic link between primary producers and fish, a more in-depth study of chironomid production in conjunction with their deposition on land could be a good way to continue and advance this work.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/insects15010051/s1, Table S1: The community of chironomids and their flight periods at tufa barrier Kozjak–Milanovac over the 14-year period (2007–2020); Table S2: Average monthly and annual water temperatures during the study period (2007–2020); Table S3: Microhabitat preferences of the 10 most abundant species at tufa barrier Kozjak–Milanovac.

Author Contributions: Conceptualization, V.D.,V.B. and M.I.; methodology, V.D. and M.I.; software, V.D., I.P. and V.B.; validation, Z.M. and M.I.; formal analysis, V.D. and I.P.; investigation, V.D., I.P., Z.M. and M.I.; resources, Z.M. and M.I.; data curation, V.D. and I.P.; writing—original draft preparation,

V.D.; writing—review and editing, V.D., I.P., V.B., Z.M. and M.I.; visualization, V.D., I.P. and M.I.; supervision, Z.M. and M.I.; project administration, V.D. and M.I.; funding acquisition, M.I., V.B. and Z.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data supporting reported results can be provided upon contacting the corresponding author.

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Lentic chironomid performance in species-based bioassessment proving: High-level taxonomy is not a dead end in monitoring

Valentina Dorić, Ivana Pozojević, Natalija Vučković, Marija Ivković, Zlatko Mihaljević *

University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Rooseveltov trg 6, 10000 Zagreb, Croatia

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ABSTRACT

Chironomid identification for freshwater bioassessment purposes is rarely finer than family or subfamily level. This has led to their taxonomic neglect and a lack of knowledge about their characteristics and ecology at the genus or species level, which in turn makes their implementation in bioassessment models even less appealing. The aim of this study was to object against this practice and evaluate the possibility of using chironomid assemblages solely in assessing organic and nutrient enrichment levels of lentic habitats. For this purpose, the littoral zone of 28 lentic water bodies of the Dinaric western Balkan ecoregion was sampled. Due to the scarcity of natural lakes in this region, resulting from its specific karst geology, both natural and artificial water bodies were included in this survey. Chironomids, determined mostly to species and genus level, were tested in response to variables associated with organic enrichment (dissolved organic carbon and oxygen demand measures) and nitrate concentration. A metric (Lake chironomid metric, LCM) based on 107 chironomid taxa was developed and proven to reflect organic enrichment more precisely than standard metrics that respond to organic enrichment (BMWP and different Saprobic indices). We found that the LCM strongly supports the use of chironomids with high taxonomic resolution in lentic habitat assessment, as we have shown that chironomids have the ability to improve, or even replace, already existing models for organic nutrient enrichment. We have also shown that chironomids can be used in assessing even finer levels of nitrate pollution with changes in community occurring at as low as 0.07 $mgNO_3^-/l$. This allows earlier intervention and hopefully prevention of considerable damage to the environment. We can conclude that chironomids (especially determined to species level) have great potential in monitoring of lake ecosystems.

1. Introduction

A great deal of progress has been achieved regarding the ecological assessment of inland and coastal waters since the adoption of the European Water Framework Directive (WFD), as well as the monitoring of water bodies with regard to nitrate concentrations since the adoption of Nitrates Directive (ND), but there are still many challenges in the implementation of these strategies (Hering et al., 2010; Poikane et al., 2016; Van Grinsven et al., 2016). The innovativeness of the WFD lay in the expression of results as Ecological Quality Ratios (EQR), which are relative and comparable measures of quality (Poikane et al., 2016; Reyjol et al., 2014). It also defines the deterioration and the improvement of ecological quality through the response of biota to changes in the set of physical or chemical variables (among others) in the Nitrates Directive (Hering et al., 2010). The objective of the Nitrates Directive is to reduce water pollution caused by nitrates from agricultural sources and to prevent further deterioration in order to achieve the environmental goal of protecting drinking water resources and aquatic ecosystems by reducing water pollution with nitrates from agricultural sources (Van Grinsven et al., 2016). In lake assessments, many biological quality elements (BQEs) are used. According to WFD, the main biological quality elements are fish, macroinvertebrates, phytoplankton, macrophytes and phytobenthos. Phytoplankton, phytobenthos and macrophytes are often used to assess eutrophication (Hering et al., 2010; Poikane et al., 2016), benthic macroinvertebrates are used in acidification and hydromorphological alteration assessments (Pilotto et al., 2015; Poikane et al., 2016), and fish are often used in hydromorphological alteration assessments (Birk et al., 2012). Two of the most critical and difficult tasks in implementing the WFD are the development of reliable assessment tools and the setting of ecological

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* Corresponding author.

E-mail addresses: valentina.doric@biol.pmf.hr (V. Dorić), ivana.pozojevic@biol.pmf.hr (I. Pozojević), natalija.vuckovic@biol.pmf.hr (N. Vučković), marija.ivkovic@biol.pmf.hr (M. Ivković), zlatko.mihaljevic@biol.pmf.hr (Z. Mihaljević).

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class boundaries, with work still ongoing for several taxonomic groups (Poikane et al., 2016). Even though there are many indices that assess eutrophication, there is still space to improve the process since the WFD implementation still suffers from a lack of WFD-compliant bioassessment methods (Lyche-Solheim et al., 2013; Reyjol et al., 2014).

With more than 8,000 described species, the family Chironomidae is one of the most diverse and ubiquitous Diptera families that inhabit freshwater ecosystems (Marshall, 2012). These midges are among the best indicators of organic enrichment since their abundance and community structure change rapidly in response to these environmental changes (Bazzanti, 2000). The use of profundal chironomid species in lake classification is well known and dates back to the first bioassessment attempts made by Thienemann (1922). Profundal chironomids are important for the lake classification systems, because, besides Oligochaeta, they are the only major group of benthos inhabiting profundal (Armitage et al., 1995). On the other hand, chironomids are often identified only to subfamily level in lake littoral monitoring programs (Urbanič et al., 2012; White and Irvine, 2003). There have even been attempts to omit them completely from further monitoring programs because of the difficulties and hence the cost in identifying them to lower taxonomic levels (Rabeni and Wang, 2001). Despite chironomids have proved to be useful in detecting organic pollution and eutrophication (Raunio et al., 2011; Stewart et al., 2014) they and other macroinvertebrates are seldom used in the water quality assessment of lentic habitats due to their high diversity and abundance (Solimni and Sandin, 2012) as well as their great temporal variability (Peeters et al., 2004, Solimni et al., 2003).

The natural lakes of the Dinaric western Balkan ecoregion, ER 5 (Illies, 1978) (hereinafter referred to as the Dinaric region) may lie directly on the carbonate rocks but may also be present where unconsolidated deposits overlie limestone strata (Bonacci, 2014). The permeable nature of the geological deposits of the Dinaric region is the reason why lakes are rather scarce in this region. In addition to natural lakes in the Dinaric region, there are more than 30 dams and reservoirs that have been built in order to store water, mainly for hydroelectric production. Agriculture in the Dinaric region is not as developed as in other regions but nevertheless, it has a significant impact on freshwater ecosystems due to the porous limestones and a very complex underground hydrological network (Bonacci, 2014). As part of the Mediterranean Basin Dinaric region is acknowledged as one of the biodiversity hot spots (Ivković and Plant, 2015; Myers et al., 2000) with many endemic species (e.g. Andersen et al., 2016; Giłka et al., 2013; Kvifte and Ivković, 2018; Pont and Ivković, 2012). Climate change driven eutrophication and growing use of pesticides in agriculture are becoming an ever-growing threat to the unique biodiversity in the region (Mihaljević et al., 2013).

The aim of this study was to evaluate the utility of using littoral chironomid assemblages solely in responses to organic and nutrient enrichment. In this study we have tested whether metrics based on a high taxonomic resolution of the ubiquitous chironomids could provide similar or even more precise results in lake bioassessment when compared to metrics derived from the taxonomic resolution, commonly used in the routine monitoring of the whole benthic community. Our specific objectives were: (1) to construct a littoral chironomid species-level metric that reflects organic enrichment pressures; (2) to test the ability of our metric to characterize community responses to organic enrichment and to compare it with “standard” whole community-based saprobic indices; (3) to characterize chironomid taxon-specific responses to low level nitrate enrichment, including the values at which abundances increase or decrease.

2. Materials and methods

2.1. Study area

In total, 28 lakes located in the Dinaric region were studied. Of the studied lakes, 21 are artificial and seven are of natural origin (Fig. 1). Complete list of lakes with their geographic location, surface area and other characteristics are given in Table A.1.

2.2. Macroinvertebrate and chironomid sampling and processing

This study was conducted as part of a comprehensive project, “Development of a classification system to assess lake ecological status and the ecological potential of artificial and heavily modified surface water bodies (Contract class: 325-01/16-10/22 Docket No.: 374-1-2-16-8 Contract No.: 10-034/16)”. A total of 700 samples were collected. The chironomids were collected together with other macroinvertebrates during the summer of 2016. From 1 to 6 sampling sites per lake were established according to lake surface area and lake bottom composition as well as its accessibility and heterogenic degree of the shoreline. The smaller lake surface and more uniform lake bottom composition led to less sampling sites at that lake. A modified proportional stratified sampling approach (Urbanič et al., 2012) was applied when sampling. At each sampling site, a 25 m lakeshore transect was established for 10 m towards the open water or alternatively to the point where water depth exceeded 1 m. 10 replicates were collected at each site with regard to substrate composition and distribution of water depth classes that were established *a priori* (0–0.25 m, 0.25–0.5 m, 0.5–0.75 m and 0.75–1 m). Details of the sampling protocol are explained in Urbanič et al. (2012). Replicates were conserved separately in 90% ethanol. Environmental parameters such as water temperature, oxygen saturation, pH, alkalinity and conductivity were measured on field only to ensure no extreme conditions were present at the time of sampling. The parameters used in the analyses (COD = Chemical oxygen demand (mg O_2/L); BOD = Biological oxygen demand (mg O_2/L); TOC = Total organic carbon (mg C/L); DOC = Dissolved organic carbon (mg C/L) and nitrate concentration (mg NO_3/L)) were obtained by Hrvatske vode (Croatian legal entity for water management). All macroinvertebrates were separated in the laboratory, conserved in 70% ethanol and identified (Table 1). Chironomid larvae were identified to the lowest level possible (species, groups of species, aggregates or genus) using Andersen et al., 2013; Bitušík, 2000; Bitušík and Hamerlík, 2014; Brooks et al., 2007; Cranston, 1982; Cuppen and Tempelman, 2019; Schmid, 1993; Vallenduuk and Moller Pillot, 2007; Vallenduuk, 2017.

All chironomid samples and slides are deposited at the University of Zagreb, Faculty of Sicence, Department of Biology.

2.3. Comparability of the sites

As stated earlier, because of the sparsity of lentic habitats in the Dinaric region, in this study both natural and artificial lakes were analyzed. In order to ensure that the results would be consistent between the two lentic habitat types, *t*-tests were performed for the following variables: COD, BOD, TOC, DOC and nitrate concentration between the two lentic habitat types. Prior to the analyses, the data were tested for normality using a Shapiro-Wilk test. Both tests were conducted using Statistica 13.0 (TIBCO Software Inc., 2017).

2.4. Metric selection and calculation

Three existing macroinvertebrate metrics dealing with organic pollution issues were chosen and a new metric based on chironomid species only was constructed in order to test our first prediction. The BMWP index and the Saprobic index (Zelinka and Marvan, 1961) were calculated using Asterics 4.0.3., and the Croatian saprobic index (referred subsequently in the text as saprobic index) with original indicator taxa values was calculated separately. These indices were chosen as they reflect the sensitivity of invertebrate taxa to organic pollution (Hawkes, 1997; Zelinka and Marvan, 1961).

In order to test our hypothesis on high taxonomic level chironomid bioassement possibilities, we first had to construct a chironomid-based

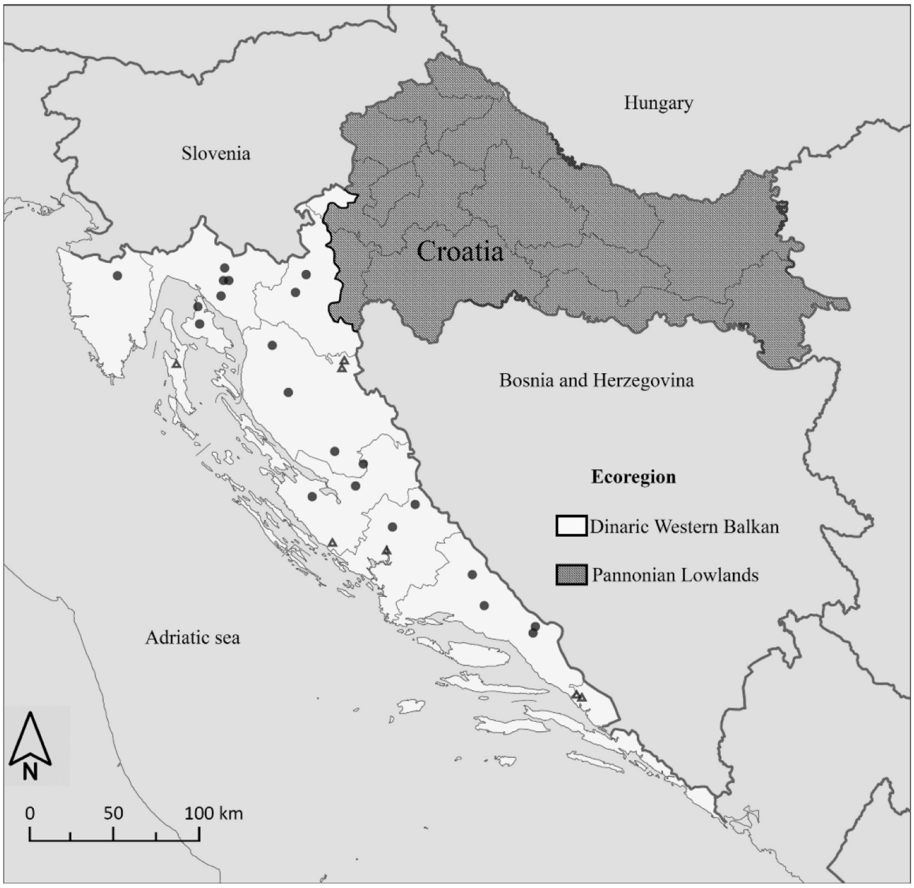

Fig. 1. Distribution of Dinaric Western Balkan ecoregion (ER 5) in Croatia and position of 21 artificial (marked with circles) and 7 natural lakes (marked with triangles) that were studied.

Table 1
Level of identification for non-chironomid macroinvertebrate groups in this study (and regular monitoring purposes used in Croatia).

Systematic group	Level of identification	Systematic group	Level of identification
Porifera	genera	Ephemeroptera	genera, species
Hydrozoa	genera	Trichoptera	genera, species
Bryozoa	presence	Odonata	genera, species
Turbellaria	genera, species	Megaloptera	genera, species
Oligochaeta	family, genera, species	Heteroptera	genera, species
Hirudinea	genera, species	Coleoptera	genera, species
Mollusca	genera, species	Diptera	family, genera, species
Crustacea	genera, species	Hydrachnidia	presence
Plecoptera	genera, species		

metric that responds to organic enrichment pressures. The metric construction follows the analytical process by Urbanič (2014) who developed an elaborate whole-community based metric for assessing hydromorphological pressures. Our version of the metric is based on mostly species-level determined chironomid assemblages and responds to organic pressures (i.e. organic enrichment disturbance).

2.5. Developing the Lake chironomid metric (LCM)

The relationship between chironomid taxa and environmental variables associated with organic enrichment disturbance (COD, BOD, TOC and DOC) was tested using Canonical Correspondence Analysis (CCA) in order to determine specific chironomid taxa sensitivity to organic pollution. Prior to analysis, chironomid abundance data were $\log(x + 1)$ transformed. A Monte Carlo test using 999 permutations ($p < 0.05$) was performed to define the significance of the correlations between taxon occurrence and environmental variables in the CANOCO package version 5.0 (ter Braak and Smilauer, 2012). The LCM was developed using organic pollution sensitivity (lake chironomid values – LCi) and resilience or niche width (organic enrichment disturbance indicative weights – OEWi) of chironomid taxa along the first CCA axis. Lake chironomid values (LCi) were determined for each taxon as the ratio of its CCA ordination axis 1 score and the maximum taxon score value in the dataset (Table 2). Organic enrichment disturbance indicative weights (OEWi) were determined using the CCA ordination axis 1 species tolerance (root mean squared deviation for species, usually referred to as niche widths). The indicative weights were transformed to whole numbers, ranging from 1 to 5: where 5 was the tolerance group < 0.2 and 1 was the tolerance group > 0.8; other values were distributed

Table 2
Example of calculating lake chironomid values (LCi) from CCA ordination axis 1 species scores (Resp. 1) and organic enrichment disturbance indicative weights (OEWi) from ordination axis 1 species tolerance (Tol.1).

Species	Resp.1	LCi (Resp.1/6.74)	Tol.1	OEWi
Ablabesmyia monilis	−0.142	−0.02106	0.6545	2
Chironomini Gen. sp.	−0.2996	−0.04445	0.3284	4
Chironomus aprilinus	6.74	1	0	5
Chironomus plumosus gr.	0.0848	0.01258	0.611	2
*	*	*	*	*
*	*	*	*	*
*	*	*	*	*
	max value = 6.74			

equidistantly. Chironomid abundances were $\log_5$ transformed into five abundance classes (ACi) where 1 represented chironomid abundances $<$ 5 and 5 represented chironomid abundances $>$ 625; other borders were 25 and 125 (Table 3). Finally, the LCM was calculated for every site as:

$$LCM = \frac{\sum_{i=1}^{n} ACi*LCi*OEWi}{\sum_{i=1}^{n} ACi*OEWi}$$

where

LCM is the Lake chironomid metric
ACi is the abundance class of a chironomid taxon
LCi is the lake chironomid value of a chironomid taxon
OEWi is the organic enrichment disturbance indicative weight of a chironomid taxon

2.6. *Testing the pressure response of the Lake chironomid metric (LCM) and other saprobic indices*

The pressure response relationship between the LCM and other saprobic indices (Zelinka & Marvan saprobic index, BMWP score, saprobic index) and environmental variables associated with organic enrichment disturbance (COD, BOD, TOC and DOC) were firstly tested by the Pearson correlation coefficient using Statistica 13.0 (TIBCO Software Inc., 2017). The values of the LCM and other saprobic indices were averaged at each site (average of 10 samples) in order to harmonize with the environmental variable dataset: here comparing LCM and saprobic indices with environmental variables of 70 sites. Linear regression was constructed in order to define the coefficient of determination (R^2) between the newly constructed metric, "standard" saprobic indices and the pressure gradients.

In order to characterize individual chironomid taxon-specific responses to low level nitrate enrichment, we used Threshold Indicator Taxa Analysis (TITAN; Baker and King, 2010). The basic premise of TITAN is that community response to environmental gradients, particularly novel environmental gradients, is best detected empirically by aggregating the responses of individual taxa rather than seeking change in community summary metrics therefore, abundances of each taxa are the input data into this analysis (all details on the methodology and analytical process in King and Baker, 2014). TITAN combines indicator species analysis and change-point analysis (Qian et al., 2003) to identify taxa whose occurrence increases or decreases along an environmental gradient. Only taxa occurring in more than three samples were included in this analysis (Baker and King, 2010). TITAN analyses were performed with 500 bootstraps on presence-absence matrices. TITAN indicated the nitrate concentration after which taxon-specific occurrences decreased or increased, thus identifying the taxa that responded most strongly to this nutrient. We used R software version 3.5.0 (R Core Team, 2018) and TITAN2 package (Baker et al., 2015) for this analysis.

3. Results

3.1. *Comparability of the sites*

No significant differences were determined for the following variables: COD, BOD, TOC, DOC and nitrate concentration between the two lentic habitat types (natural and artificial).

3.2. *Testing the Lake chironomid metric (LCM)*

The CCA of the four environmental variables associated with organic enrichment disturbance (COD, BOD, TOC and DOC) and 700 samples with 107 chironomid taxa explained 10.2% of the total chironomid taxa variability (Fig. 2). Of the 107 chironomid taxa tested in the analyses, 82 were given indicative weights and responses to variables associated with organic enrichment disturbance (see Table A.2 for taxa list and values). The eigenvalues of the first two axes were 0.268 and 0.247. A Monte Carlo permutation test showed that the ordination was statistically significant (F = 12.5, p = 0.001). The "y" axis of ordination indicated an obvious separation between chironomid taxa sensitive to and tolerant of variables associated with organic enrichment disturbance. Chironomid taxa on the left side of the ordination are considered as sensitive, whereas taxa on the right side of the ordination are considered as tolerant of organic pollution.

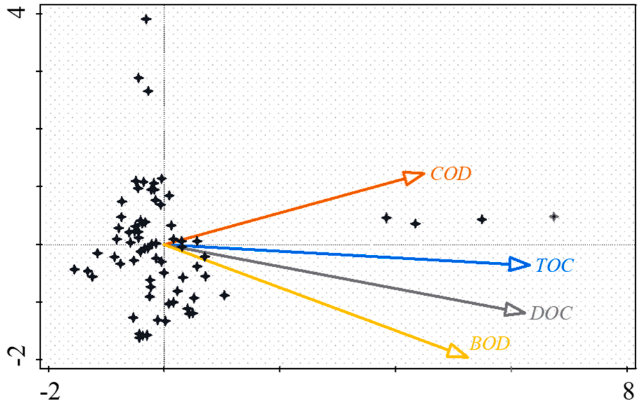

Fig. 2. CCA ordination diagram with 107 chironomid taxa (black star) and four environmental variables associated with organic enrichment disturbance (arrows): COD = Chemical oxygen demand (mg O_2/L); BOD = Biological oxygen demand (mg O_2/L); TOC = Total organic carbon (mg C/L); DOC = Dissolved organic carbon (mg C/L). Arrow length on the ordination indicates the relative importance of the explanatory variables, and their direction, relative to each other and to the taxa, indicates positive or negative correlations.

Table 3
Example of calculating abundance classes (ACi) from chironomid species abundance and the final calculation of Lake chironomid metric (LCM) for every site.

Species	LCi	Site code:	BA1-3–6	BA1-3–6	BA1-3–6	BA1-3–6	BA1-3–6
		OEWi	Abundance	Abundance classes (ACi)	ACi*LCi*OEWi	ACi*OEWi	LCM = ACi*LCi*OEWi/ ACi*OEWi
Ablabesmyia monilis	−0.021068249	2	9	2	−0.084272997	4	**−0.0416646**
Cladotanytarsus sp.	−0.036439169	3	250	4	−0.43727003	12	
Cricotopus cylindraceus festivellus gr.	−0.056543027	3	2	1	−0.16962908	3	
Cryptochironomus obreptans/ supplicans	−0.034881306	1	1	1	−0.034881306	1	
Dicrotendipes nervosus	−0.007121662	3	5	2	−0.04272997	6	
Endochironomus albipennis	0.023709199	4	3	1	0.094836795	4	
Orthocladius holsatus	−0.055712166	5	9	2	−0.557121662	10	
Paratendipes albimanus	−0.117091988	5	9	2	−1.170919881	10	
Psectrocladius limbatelus/sordidellus	−0.021409496	3	4	1	−0.064228487	3	
Tanytarsus sp.	0.044332344	1	48	3	0.132997033	3	
				Sum =	−2.333219585	56	

3.3. Testing the pressure response of the Lake chironomid metric (LCM) and other saprobic indices

The correlations between the LCM and COD ($r = 0.944$; $p < 0.001$), BOD ($r = 0.933$; $p < 0.001$), TOC ($r = 0.969$; $p < 0.001$) and DOC ($r = 0.962$; $p < 0.001$) were found to be very strong and statistically significant. Linear regressions between the LCM and pressure gradients revealed high (R^2(COD) = 0.853; R^2(BOD) = 0.721) and very high (R^2(TOC) = 0.901; R^2(DOC) = 0.947) coefficients of determination (Fig. 3).

All correlations between the Zelinka & Marvan saprobic index and variables associated with organic enrichment disturbance (COD, BOD, TOC and DOC) were found to be statistically non-significant. The BMWP score was found to significantly correlate only with TOC ($r = 0.337$, $p = 0.024$) and DOC ($r = 0.333$, $p = 0.026$). The saprobic index was found to correlate significantly with all the variables associated with organic enrichment disturbance: COD ($r = 0.350$; $p < 0.018$), BOD ($r = 0.371$; $p < 0.012$), TOC ($r = 0.363$; $p < 0.014$) and DOC ($r = 0.374$; $p < 0.011$). Linear regressions between only the saprobic index and pressure gradients were constructed as it was the only variable with significant correlations with all the stressors. The regression revealed relatively low (R^2(COD) = 0.099; R^2(BOD) = 0. 077; R^2(TOC) = 0.105; R^2(DOC) = 0.140) coefficients of determination (Fig. 4).

Based on TITAN, 15 chironomid taxa were found to decrease with growing levels of nitrate concentration (Fig. 5). For taxa which decreased in occurrence, change-points were detected at nitrate levels of 0.07 mg/L, with the highest magnitude changes occurring for *Nubensia nubens*, *Cladopelma viridulum* gr. and *Kiefferulus tendipediformis*. Most of the 31 taxa that increased in occurrence with higher nitrate concentrations increased between 0.2 and 1 mg/L.

4. Discussion

LCM was developed as a response to the lack of littoral-specific assessment metrics and was proven to be more correlated to organic enrichment than the saprobic index. The main factor that makes lake assessment in the Dinaric region challenging is the scarcity of lakes due to the unique karst geology. For this reason, both natural and artificial lakes were included in the LCM development. Many benthic invertebrate indices are based on tolerances or preferences for specific, measurable stressors, but rarely are they focused on a single macroinvertebrate group. This is because very few invertebrate groups are as ubiquitous, have such great taxon diversity and show such a vast array of responses to different types of stressors as do chironomids. Nevertheless, in this study we found that chironomid communities to a great extent reflect the water chemistry conditions of lentic habitats. Our results suggest that the use of taxon-specific optima along a stressor gradient provides a solid foundation with which to support the development of metrics for ecological classification (Dodkins et al., 2005; Donohue et al., 2009).

The satisfaction of researchers with chironomid identification only to family or subfamily level in bioassessment projects has led to their taxonomic neglect and the loss of knowledge of their traits at the genus or species level, which then makes them even less attractive as indicators in bioassessment (Serra et al., 2016). In this paper we have shown that chironomids can and should be used in bioassessment and we therefore strongly believe that they should be included in every bioassessment approach. Our results show that for proper lake trophic classification, based on littoral macroinvertebrates, chironomids may be prime indicators but only if both their abundance and their taxonomic diversity are considered. This is in accordance with Bazzanti et al. (2012) who recommended using the numerical abundance and the lower taxonomic levels (e.g. genus, species) of chironomids to increase the discriminatory power of existing bioassessment models.

Even though our results show that chironomids can play an integral role in lake classification, we are aware of their high tolerance and adaptability to hydromorphological change and this is where some other groups may prove to be useful (Miler et al., 2013). We should therefore focus on the community as a whole and not just on one or a few groups in order to obtain a complete set of information about systems and the processes that occur in them. Our results corroborate what was already

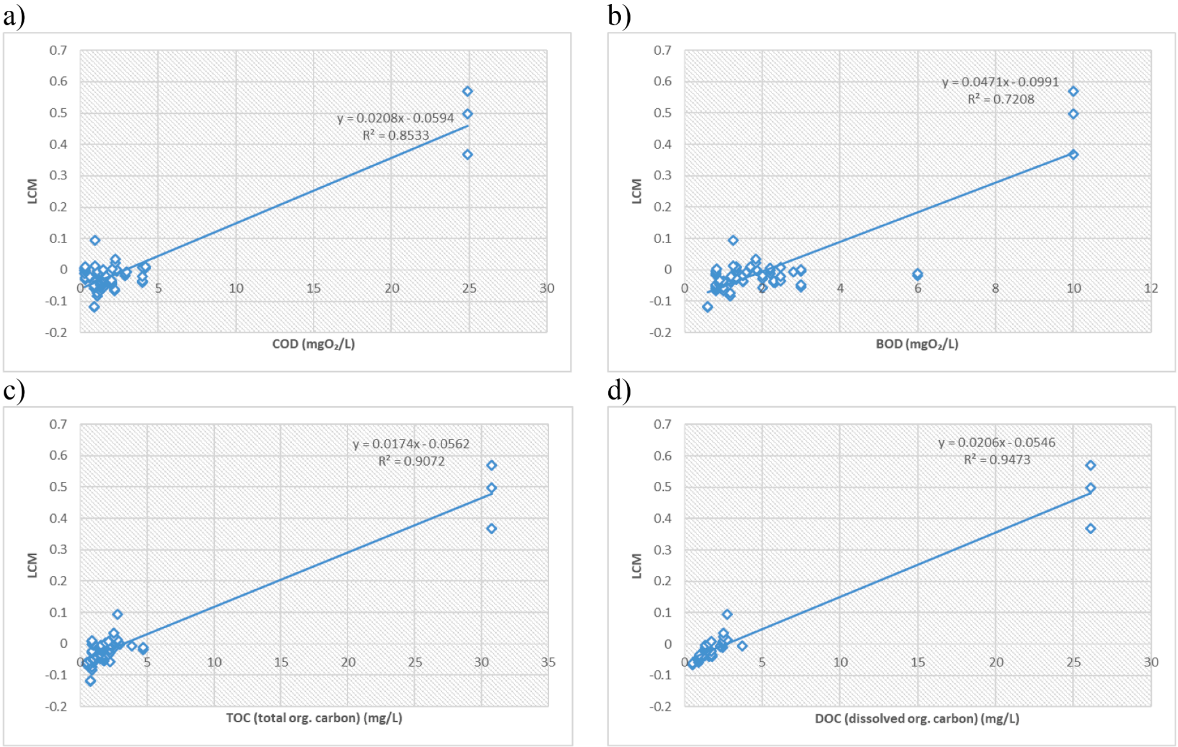

Fig. 3. Linear regression between the LCM (independent variable) and pressure gradients: a) COD = Chemical oxygen demand (mg O_2/L); b) BOD = Biological oxygen demand (mg O_2/L) and c) TOC = Total organic carbon (mg C/L); d) DOC = Dissolved organic carbon (mg C/L).

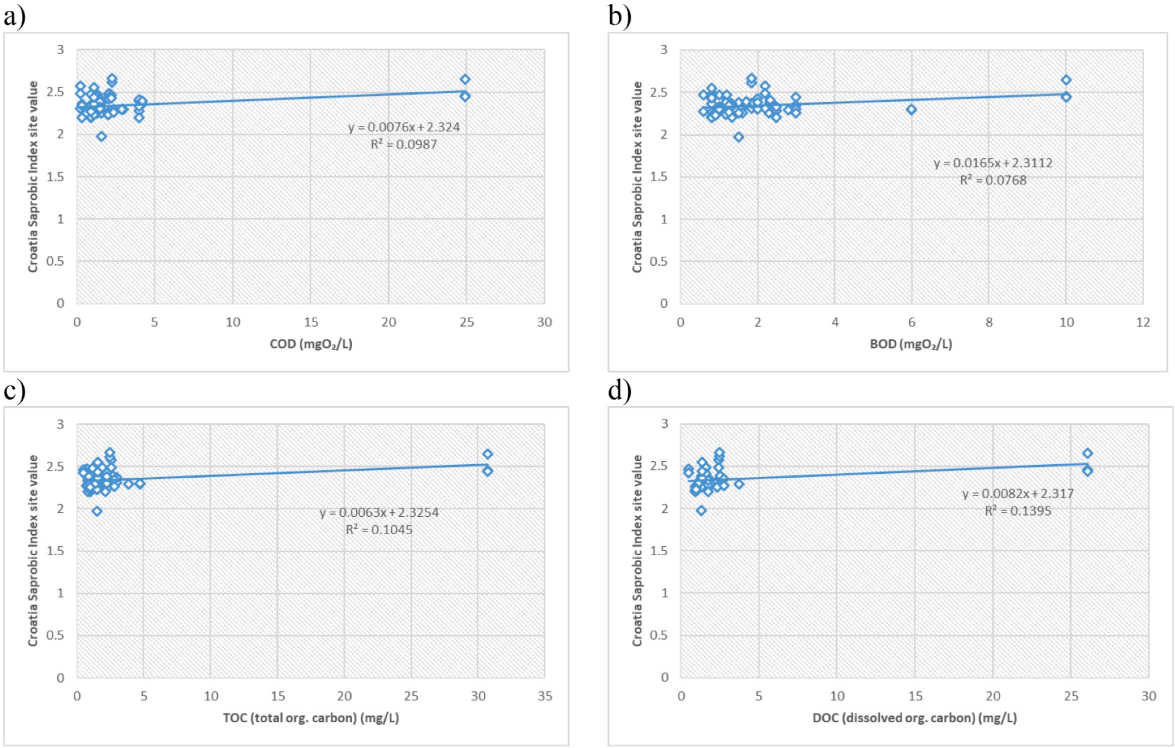

Fig. 4. Linear regression between the saprobic index (dependent variable) and pressure gradients: a) COD = Chemical oxygen demand (mg O_2/L); b) BOD = Biological oxygen demand (mg O_2/L) and c) TOC = Total organic carbon (mg C/L); d) DOC = Dissolved organic carbon (mg C/L).

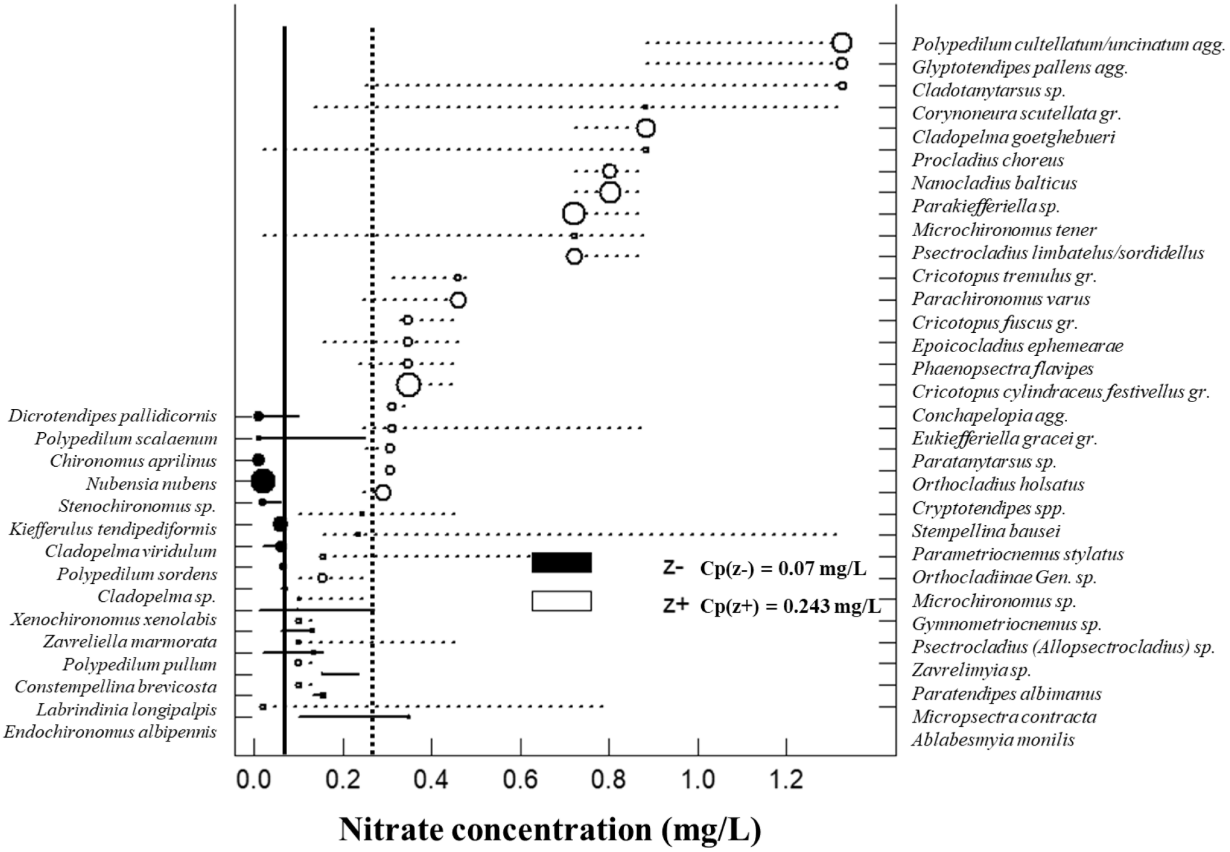

Fig. 5. TITAN results indicating taxa for which occurrence decreased ("sensitive" taxa, left-hand axes, black circles and solid lines) and increased ("tolerant" taxa, right-hand axes, white-filled circles and dotted lines) with nitrate concentration (mg/L). Circles indicate mean change-points (50th quantile) in the occurrence of each taxon (bigger circles indicate more significant changes), whereas horizontal lines delimit the 5th and 95th quantiles calculated based on 500 bootstraps. The changing point (cp) for sensitive (z−) taxa was calculated as 0.07 mg/L, whereas the cp for tolerant (z+) taxa was calculated at 0.243 mg/L.

strikingly pointed out by Raunio et al. (2011), namely: the exclusion of chironomids may lead to biased findings about the functioning relationships within the biodiversity-ecosystem in freshwater ecosystems (i.e. the importance of species diversity in ecosystem functioning), flawed estimates of the local diversity of macroinvertebrates, and insufficient information for conservation biology and environmental assessment. Furthermore, determining chironomids only to subfamily, or even just to family level, may underestimate waterbody's species richness by up to 50% which was the case in two of our study sites: Lepenica and Prološko Blato. This gives a false projection of low local diversity and consequently biased ecological quality scores.

Wherever possible, species or species-group level identification is desirable since different species of the same genus may exhibit different preferences. For example, in our dataset, the *Cladopelma goetghebueri* gr. is listed as tolerant of nitrate pollution whereas the *Cladopelma viridulum* gr. is listed as sensitive to the same pollutant. It is stated in Moller Pillot (2009) that both groups of *Cladopelma* can be found in a wide range of trophic and oxygen conditions. Despite our data being slightly at odds with the current literature (Moller Pillot, 2009), karstic geology and the low nitrate level must be considered. Most of the chironomid ecological preferences in the study of Moller Pillot (2009) are derived from the Netherlands and lowland habitats, whereas in our case the data are from the Dinaric region where the extreme heterogeneity of habitats and an unknown interplay between the karst surface and underground systems makes it very hard to obtain local information and to draw reliable conclusions about water circulation (Bonacci, 2014). On the other hand, agriculture in the Netherlands is much more developed and intensive (Van Grinsven et al., 2016) than in the Dinaric region of Croatia, which results in a much higher influx of nitrates. Consequently, the waters of the Dinaric region have a lower nitrate load. We found that in water bodies with a low nutrient level, such as those covered in our research, change-points for sensitive chironomid taxa were detected at very low nitrate levels of 0.07 mg/L. It is the position of the authors of this paper that chironomids have an important role to play in the implementation of the EU Nitrates Directive as a tool for discriminating among lakes with lower nitrate levels and in preventing nitrate influx in the early stages as it is always cheaper to prevent the potential damage to the environment than it is fixing it.

The other two species for which a much greater level of change in abundance occurs with an increase in nitrate concentration are *Kiefferulus tendipediformis* and *Nubensia nubens*. *K. tendipediformis* is a species usually associated with decaying plant material but with good oxygen conditions (Moller Pillot, 2009). Due to nitrate influx, denser macrophyte coverage and more abundant algal communities are able to develop, thereby causing occasional oxygen depletion. As for the species *N. nubens*, there are little data on its distribution and ecology, but in our case its distribution was limited to the oligotrophic Lake Vrana on the Island of Cres which by nature has very low nitrate concentrations and is used as a drinking water supply (Tomec et al., 2002).

We strongly encourage future work to focus more on educating new taxonomists as well as implementing DNA barcoding (Carew et al., 2007; Montagna et al., 2016) and eDNA-aided identification to accomplish a synergy of classical taxonomic expertise and modern taxonomic tools. Nevertheless, the use of DNA barcoding in identification often proves to be too expensive for some parts of the world, and so the use of Convolutional Neural Networks seems to be a good way to obtain both cheap and accurate chironomid identification (Milošević et al., 2020).

5. Conclusions

Our results undoubtedly suggest that a taxonomic group as abundant and diverse as chironomids, not only have their place in the freshwater monitoring, but can vastly improve current biomonitoring efforts. In the development of proper freshwater assessment tools in regions with specific environmental conditions and with problems such as small number of comparable sites, reaching for fundamental, species-based ecology is the key for successful implementation of the WFD. Since our results show great promise for the implementation of classical taxonomy in bioassessment and, even more importantly, in detecting very precise responses to even slight anthropological pressure, the research efforts described here will continue.

CRediT authorship contribution statement

Valentina Dorić: Conceptualization, Investigation, Writing - original draft. **Ivana Pozojević:** Conceptualization, Software, Formal analysis, Investigation, Data curation. **Natalija Vučković:** Investigation, Data curation, Visualization, Project administration. **Marija Ivković:** Validation. **Zlatko Mihaljević:** Investigation, Methodology, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107041.

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3. RASPRAVA

3.1. Inventarizacija faune trzalaca na području Nacionalnog parka Plitvička jezera

Prema publikaciji **I** na području NP Plitvička jezera na ukupno 25 lokacija zabilježene su 62 vrste trzalaca i dodatnih sedam svojti. Pet novih vrsta trzalaca je zabilježeno po prvi puta na području NP Plitvička jezera: *Labrundinia longipalpis* (Goetghebuer, 1921), *Nilothauma brayi* (Goetghebuer, 1921), *Potthastia longimanus* Kieffer, 1922, *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804) i *Tanytarsus brundini* Lindeberg, 1963. Publikacija **I** je objavljena prije dovršetka determinacije svih uzoraka s barijere Kozjak-Milanovac. U publikaciji **III** nalazi se popis vrsta i svojti sa sedrene barijere Kozjak-Milanovac. U tom popisu nalazi se ukupno 79 vrsta i dvije svojte, od toga 54 nove vrste (i dvije nove svojte) za faunu NP Plitvičkih jezera. Na kraju ove doktorske disertacije u NP Plitvička jezera zabilježeno je ukupno 116 vrsta i devet svojti trzalaca. Od toga je 60 vrsta zabilježeno na sedrenim barijerama, 33 vrste su zabilježene u jezerima, 32 vrste u izvorskim tipovima staništa, a svega pet vrsta je zabilježeno u potocima. Vrlo visoka raznolikost vrsta zabilježena na sedrenoj barijeri Kozjak-Milanovac posljedica je izmjene lotičkih i lentičkih staništa, različitih tipova supstrata (Giller i Malmqvist, 1998), ali i višegodišnjeg lovnog napora kojim je omogućeno skupljanje rijetkih vrsta. Unatoč dugoj tradiciji limnoloških istraživanja na području Nacionalnog parka, potoci su istraživani sporadično i u budućim istraživanjima bi se veća pažnja trebala posvetiti upravo njima. Bez obzira na točkastu istraženost, porodica trzalaca je najraznolikija porodica vodenih kukaca na području Nacionalnog parka Plitvička jezera. Za usporedbu, na području Plitvičkih jezera do sada zabilježeno 17 vrsta vodencvjetova, 14 vrsta vretenaca, 31 vrsta obalčara, 91 vrsta tulara, 23 vrste vodenih kornjaša i 95 vrsta dvokrilaca koji spadaju u 12 porodica ne uključujući trzalce (Ivković i sur., 2023). Kroz rezultate opisane u publikacijama **I** i **III** ostvaren je prvi cilj ove disertacije, a to je inventarizacija faune trzalaca na području Nacionalnog parka Plitvička jezera.

3.2. Zajednica trzalaca od 2007. do 2020. godine na sedrenoj barijeri Kozjak-Milanovac

3.2.1. Promjene u sastavu zajednice trzalaca od 2007. do 2020.

U publikacijama **II** i **III** glavni ciljevi su bili odrediti promjene u sastavu zajednice, brojnosti i raznolikosti trzalaca tijekom 13 godina istraživanja te ustanoviti fenologiju pojedinačnih vrsta na sedrenoj barijeri Kozjak-Milanovac. Kroz navedene publikacije ispitivane su druga i dio treće hipoteze.

Tijekom 13-ogodišnjeg istraživanja došlo je do porasta temperature vode, brzine strujanja vode i količine organske tvari u sedimentu. Osim navedenih, došlo je i do promjena u protoku vode s izraženijim ekstremnim protocima. Prema RDA analizi temperatura vode i period protoka su najznačajniji faktori koji utječu na varijabilnost zajednice trzalaca. U analizi nutrijenata koju su nakon 12 godina praćenja stanja proveli Vurnek i sur. (2021) uočeni su negativni trendovi koji upućuju na stabilno stanje sustava Nacionalnog parka, ali uočena je i pozitivna korelacija između protoka i količine organske tvari. Mogući razlozi za to su resuspenzija organske tvari iz sedimenta tijekom bujičnih protoka (Vurnek i sur., 2021), pojačan utok sestona bogatog hranjivim tvarima iz uzvodnog jezera Kozjak (Špoljar i sur., 2007), kao i ispiranje alohtone organske tvari iz susjednog kopnenog staništa i makrofitske vegetacije (Miliša i sur., 2014).

U hidrološkom istraživanju Plitvičkih jezera Radišić i sur. (2021) su opazili povećanje srednje godišnje vrijednosti protoka, ali i istovremeno smanjenje srednje godišnje razine vode u jezeru Kozjak. Ovo opažanje je u potpunosti podržano u izvješću IPCC-a za 2021. godinu u kojem stoji kako se očekuje da se povećanjem temperature atmosfere očekuje pojačana količina i intenzitet oborina u kišnom razdoblju godine, a učestaliji i duži sušni periodi u ljetnom razdoblju (Douville i sur., 2021). U kontekstu Plitvičkih jezera ovo znači povećanu resuspenziju sedimenta i pojačanu eroziju koje u ekstremnim slučajevima mogu dovesti do urušavanja sedrenih barijera kao što se dogodilo 2018. godine na Milinom jezeru (Radišić i sur., 2021; Vurnek i sur., 2021).

Brojnost i bogatstvo vrsta trzalaca nisu se promijenili tijekom 13 godina istraživanja. Tijekom 2012. godine uočene su rekordno niska abundancija i bogatstvo vrsta trzalaca. Te godine uhvaćeno je svega 338 jedinki koje su pripadale u 28 svojti. U razdoblju od 2011. do 2012. godine na području cijele Hrvatske pa tako i Plitvičkih jezera zabilježena je rekordna suša (Cindrić i sur., 2016; Pozojević i sur., 2023) koja je rezultirala vrlo niskim protokom i razinom vode pa je zajednica trzalaca te godine bila desetkovana. S druge strane, rekordno visoka abundancija i bogatstvo vrsta uočene su 2015. godine. Tada je uhvaćeno 1910 jedinki trzalaca, a pripadale su u 52 svojte. Ta godina je bila jedna od godina s najviše padalina te je glavnina padalina zabilježena u rano proljeće.

Unatoč tome što tijekom 13 godina istraživanja sedrene barijere Kozjak-Milanovac nije došlo do promjene u abundanciji i bogatstvu vrsta, došlo je do promjene u sastavu zajednice trzalaca. Vrste karakteristične za prvi period protoka vode (od početka 2007. do srpnja 2010.) preferiraju finiji supstrat, stajaću ili sporije tekuću vodu (Moller Pillot, 2009; Andersen i sur., 2013), a radi se o vrstama *Stempellina bausei, Paratendipes albimanus* (Meigen, 1818), *Phaenopsectra flavipes* (Meigen, 1818) i *Polypedilum scalaenum* (Schrank, 1803). Osim navedenih vrsta, za ovaj period protoka karakteristične su i vrste roda *Tanytarsus* kojeg je moguće pronaći u većini vodenih staništa, a preferiraju finiji supstrat (Andersen i sur., 2013). U kasnijem periodu visokog protoka (od ožujka 2013. do kraja 2020.) dominiraju vrste *Rheotanytarsus curtistylus* (Goetghebuer, 1921) i *Corynoneura lobata* Edwards, 1924. Obje vrste su karakteristične za brzu struju vode (Andersen i sur., 2013; Moller Pillot, 2013). Za period vrlo niskog protoka (od srpnja 2010. do ožujka 2013.) nije bilo karakterističnih vrsta, ali je utvrđena smanjena abundancija i bogatstvo vrsta.

Iako nije došlo do promjene u brojnosti vrsta trzalaca moguće je da je zbog promjene u sastavu zajednice došlo do promjene u njihovoj dostupnosti kao izvoru hrane. Naime, vrste roda *Tanytasrus* obitavaju na finom supstratu od kojeg grade mekane cijevolike kućice što ih čini dostupnijim izvorom hrane od vrsta roda *Rheotanytarsus* koje grade čvrste kućice od pijeska koje su zalijepljene za kamenje ili mahovinu (Saether i Kyerematen, 2001; Syrovátka i sur., 2020).

3.2.2. Promjene u funkcionalnoj strukturi zajednice trzalaca

Umjereno jaki protoci uzrokuju resuspenziju sedimenta i organske tvari u njemu što pogoduje zajednicama s brzim životnim ciklusima. Mnoge vrste slatkovodnih makrobeskralješnjaka imaju životne cikluse prilagođene izbjegavanju poplava ili njihovom iskorištavanju (Poff i sur., 1997) međutim, u svjetlu klimatskih promjena vrste će se morati brzo prilagoditi novonastalim uvjetima ili nestati iz staništa. U publikaciji **II** jasno je vidljivo da sustav Plitvičkih jezera prelazi iz sustava koji se vodom primarno opskrbljivao iz snježnog pokrivača koji je polako punio podzemne spremnike u sustav koji se vodom opskrbljuje kišom. To nikako nije dobro, jer su protoci u sustavima koji ovise o snijegu predvidivi, a taj snijeg se postupno tali, puni podzemne spremnike i otpušta vodu u sustav. S druge strane, sustavi ovisni o kiši su nepredvidivi i često rezultiraju ekstremnim protocima (Poff i sur., 1997). Ovakvi uvjeti staništa rezultiraju većom količinom otopljene organske tvari koja pogoduje pasivnim filtratorima i usitnjivačima, čija se brojnost povećala tijekom istraživanja prezentiranog u publikaciji **II**. Filtratore se općenito smatra generalistima, jer koriste organsku tvar koja se ispire iz gornjeg dijela toka (Moog i Hartmann, 2017), ali u slučaju ovog istraživanja rod *Rheotanytarsus*, koji je pasivni filtrator, se isto tako smatra i specijalistom, jer preferira vrlo brzu struju vode (Andersen i sur., 2013). Ovaj izuzetak ukazuje na važnost određivanja trzalaca do najnižih mogućih taksonomskih kategorija, jer samo vrste (u rijetkim slučajevima rodovi) imaju ekološke značajke. Usitnjivači se u zajednici makrozoobentosa smatraju vrlo specijaliziranom grupom, a njihov glavni izvor hrane je usitnjena organska tvar (Moog i Hartmann, 2017). Gledano iz perspektive trzalaca, usitnjivači se ne smatraju specijalistima, jer mogu koristiti organsku tvar različitog podrijetla (Hirabayashi i Wotton, 1999). Tijekom istraživanja uočeno je povećanje njihove brojnosti što nije toliko iznenađujuće tijekom nepredvidivih uvjeta u okolišu ako se na usitnjivače trzalce gleda kao generaliste. Ovime je potvrđena prva hipoteza ovog rada.

Osim navedenih funkcionalnih skupina tijekom 13 godina istraživanja došlo je do porasta brojnosti predatora što je rezultat povećane brojnosti vrste *Rheopelopia eximia* (Edwards, 1929). Brojnost ostalih predatornih vrsta trzalaca smanjila se tijekom istraživanja što može biti posljedica povećane kompeticije za hranu uz nove okolišne uvjete i interspecijske odnose koji pogoduju vrsti *R. eximia*.

U novonastalim uvjetima povišene temperature vode i pojačanog protoka, uz istovremene duže sušne periode, dvokrilci, a time i trzalci, imaju prednost nad skupinama koje imaju veće tijelo, univoltni životni ciklus ili nemaju oblike otporne na isušivanje (Bruno i sur., 2019; Baker i sur., 2021). Naravno, nisu niti svi dvokrilci jednako osjetljivi (ili neosjetljivi) na bujične poplave (Rader i sur., 2008) što je vidljivo iz promjene sastava zajednice trzalaca tijekom ovog istraživanja. Vrste roda *Rheotanytarsus* te vrste *Rheopelopia eximia* i *Tanytarsus arduenennsis* su bolje prilagođeni novonastalim životnim uvjetima. Ovakve promjene u funkcionalnoj strukturi zajednica uočene su u istraživanjima koja su trajala više desetljeća na području Njemačke (Baranov i sur., 2020; Baker i sur., 2021). Baker i sur. (2021) čak sugeriraju da bi promjena u funkcionalnoj strukturi zajednice mogla biti vrlo rana naznaka da promjene okolišnih čimbenika već utječu na temeljne procese unutar ekosustava kroz njihov utjecaj na biotičku filtraciju.

3.2.3. Promjene u zastupljenosti mikrostaništa

Unutar emergencijskih klopki pričvršćenih na pjeskoviti supstrat zabilježena je najsporija struja vode. Tijekom 2013. godine uslijed naleta bujica nakon dugotrajnog sušnog perioda iz manje zaštićene klopke pijesak je ispran, a ostao je samo šljunak. Unutar zaštićenije emergencijske klopke koja se nalazi okružena emerznim makrofitom vrste *Cladium mariscus* (L.) Pohl i danas se nalazi pjeskoviti supstrat, a zajednica trzalaca koja se uhvati u toj klopki različita je od ostatka i manje brojna.

Osim ove promjene mikrostaništa tijekom godina došlo je i do širenja mahovine u jednu od piramida koja se prije toga nalazila na šljunkovitoj podlozi. Ova promjena je uočena u lipnju 2013. godine, a vjerojatno se mahovina proširila u piramidu iz susjednog staništa. Nakon toga u toj piramidi je zabilježena veća brojnost jedinki trzalaca (Baranov i sur., 2017).

Većina vrsta korištenih u procjeni mikrostaništa nije pokazivala preference za jedan tip staništa osim vrsta *Polypedilum scalaenum* i *Tanytarsus signatus* koje pokazuju jasne preference za pjeskoviti supstrat. Brojnost ovih vrsta se tijekom godina smanjila zbog ispiranja supstrata čime je potvrđena druga hipoteza. Većina drugih vrsta izbjegava sporiju struju vode i pjeskoviti supstrat vjerojatno zbog homogenosti tog tipa supstrata. Pijesak ne pruža zaštitu koju pružaju ostali tipovi mikrostaništa (Giller i Malmqvist, 1998), a sporije strujanje vode može dovesti do nakupljanja i sporije razgradnje organske tvari što u nekim slučajevima može dovesti i do anoksije (Hamerlík i sur., 2006; Miliša i sur., 2006).

3.2.4. Fenologije pojedinačnih vrsta na sedrenoj barijeri Kozjak-Milanovac

Promjene u okolišu opisane u prethodnim poglavljima dovele su do promjena u fenologiji trzalaca čime je potvrđen prvi dio treće hipoteze. Za najbrojniju vrstu u ovom istraživanju, vrstu *Parametriocnemus stylatus*, zabilježene su dvije do tri generacije godišnje, a glavni izvor hrane ove vrste je raspadajuća organska tvar (Moller Pillot, 2013). Vrsta *P. stylatus* bila je dominantna svojta, ali njena ukupna brojnost se tijekom godina smanjivala. Vrlo visoki protoci koji su učestali na ovoj lokaciji uzrokovali su ispiranje listinca koji bi se inače sporo raspadao i pružao vrsti *P. stylatus* preferirano mikrostanište i izvor hrane.

Brojnost jedinki vrste *Parametriocnemus* sp. 2 je varirala tijekom godina s najvišim brojnostima zabilježenima tijekom 2013. godine. Ovo je vrlo vjerojatno nova vrsta koja tek treba biti opisana, a bilježene su dvije do tri generacije godišnje. Jedinke ove vrste pronađene su u različitim tipovima mikrostaništa što upućuje da je vrsta možda manje ovisna o količinama raspadajuće organske tvari u odnosu na vrstu *P. stylatus* (Moller Pillot, 2013). Do kraja 2021. obje vrste, *P. stylatus* i *Parametriocnemus* sp. 2 bile su prisutne na Plitvičkim jezerima tijekom cijele godine.

Rod *Tanytarsus* je vrlo heterogen, ali je ekologija pojedinih vrsta vrlo slabo poznata. Ono što je zajedničko većini vrsta ovog roda jest da ličinke grade dugačke mekane cijevi od čestica supstrata i ukopavaju se u sediment (Andersen i sur., 2013). Tijekom ovog istraživanja brojnosti jedinki vrsta *T. brundini* i *T. signatus* se tijekom godina smanjila dok se brojnost jedinki vrste *T. arduennensis* povećala. Ovo upućuje da su jedinke vrste *T. arduennensis* otpornije na nagle promjene u protoku i bolje prilagođene životu u bržoj struji vode nego prve dvije vrste. Vrste *T. brundini* i *T. signatus* pojavljuju se od travnja do studenog, dok se vrsta *T. arduennensis* pojavljuje od ožujka do listopada.

Brojnost jedinki vrsta *Rheopelopia eximia*, *Rheotanytarsus curtistylus* i *R. reissi* se povećala tijekom godina. Sve navedene vrste preferiraju bržu struju vode, a uz to, vrsta *R. eximia* preferira tražiti svoj plijen između kućica vrsta roda *Rheotanytarsus* (Vallenduuk i Moller Pillot, 2007).

Pad brojnosti jedinki vrste *Paratrichocladius skirwithensis* je zabilježen tijekom istraživanja. Ova vrsta nastanjuje izvore i sustave koji su ovisni o podzemnim izvorima vode (Jyväsjärvi i sur., 2018) pa je moguće da su promjene u režimu protoka na ovoj lokaciji nepovoljno utjecale na fenologiju ove vrste.

Za vrstu *P. scalaenum* je također uočen pad brojnosti jedinki. Ličinke ove vrste nastanjuju pjeskoviti supstrat (Moller Pillot, 2009) koji je uslijed ekstremno visokih protoka ispran s ove lokacije što je negativno utjecalo na brojnost jedinki.

Na razini zajednice također su opažene promjene. Trajanje leta trzalaca tijekom istraživanja se produžilo i to zato što su oni počeli ranije emergirati. Uočeno je da je s višim temperaturama vode u veljači i ožujku povezana veća količina organske tvari u vodi pa emergencija trzalaca tih godina započinje ranije i traje duže. Prema literaturnim podacima temperatura vode jedan je od ključnih čimbenika koji utječu na sastav i strukturu zajednice trzalaca (Čmrlec i sur., 2013; Ivković i sur., 2013a; Finn i sur., 2021). Povećanjem temperature vode ubrzava se rast i razvoj ličinki trzalaca (Vallenduuk i Moller Pillot, 2007), a povećanje temperature vode u proljeće ključan je okidač za početak emergencije trzalaca na prostorima s umjerenom klimom (Armitage i sur., 1995). Međutim, u ovom istraživanju temperatura vode i drugi testirani okolišni čimbenici (količina organske tvari, nitrita i kisika u vodi) objasnili su samo maleni postotak (12,7 %) varijabilnosti u početku emergencije zajednice trzalaca što upućuje na to da inter i intraspecijski odnosi imaju važnu ulogu u kontroliranju emergencije trzalaca. Predacija nad ličinkama i kompeticija za hranu također utječu na početak emergencije trzalaca (Armitage i sur., 1995).

Vrhunac emergencije trzalaca je bio varijabilan tijekom godina, ali uglavnom se to događalo tijekom lipnja. Značajna promjena u vrhuncu emergencije nije opažena, ali moguće je to posljedica mjesečnog skupljanja uzoraka. U tom slučaju dnevne ili tjedne promjene vrhunca emergencije se ne mogu zabilježiti (Baranov i sur., 2020). Tijekom istraživanja vrhunac emergencije je dva puta zabilježen u svibnju i dva puta u srpnju. Raniji vrhunac emergencije u svibnju (2017. i 2018. godine) je moguće povezati sa iznadprosječno visokim temperaturama vode u svibnju i prethodnim mjesecima dok je kasniji vrhunac emergencije u srpnju (2014. i 2019. godine) moguće povezati sa ispodprosječnim temperaturama vode u svibnju tih godina (12,4 ˚C 2014. godine i 11,5 ˚C 2019. godine). Prosječna temeperatura vode u svibnju iznosi skoro 14 ˚C. Vrhunac emergencije se tijekom ovog istraživanja nije značajno pomaknuo, ali zanimljivo je istaknuti da je tijekom zadnje četiri godine istraživanja vrhunac emergencije samo jedne godine zabilježene u “svoje uobičajeno vrijeme” i to u lipnju 2020. godine. Ako se ovakve promjene u vrhuncu emergencije nastave to može dovesti do problema za kopnene kao i vodene zajednice organizama. Shipley i sur. (2022) zabilježili su da promjene u fenologijama slatkovodnih i kopnenih zajednica kukaca mogu dovesti do izostanka sinkronizacije između potreba insektivora i zajednice kukaca koja je dostupna u okolišu.

Najviša brojnost trzalaca uočena je tijekom proljeća i ljeta kada je i temperatura vode najviša tako da je moguće zaključiti da je temperatura vode važan faktor koji kontrolira početak i trajanje emergencije trzalaca na sedrenoj barijeri Kozjak-Milanovac što je već zabilježeno kod drugih vodenih dvokrilaca (Ivković i sur., 2012; 2014).

Negativna korelacija prikazana u publikaciji **III** između protoka i brojnosti trzalaca vjerojatno je posljedica manjih brojnosti trzalaca tijekom hladnijeg i kišnog dijela godine kada su i visoki protoci učestaliji. Međutim, ne treba isključiti i važnost ekstremno visokih protoka tijekom toplijeg dijela godine koji može dovesti do poremećaja u strukturi supstrata i otplavljivanju jedinki trzalaca što može rezultirati njihovom smanjenom brojnošću (Čmrlec i sur., 2013; Ivković i sur., 2013b; Ivanković i sur., 2019).

3.3. Stopa emergencije trzalaca na sedrenoj barijeri Kozjak-Milanovac

Stopa emergencije na sedrenoj barijeri Kozjak-Milanovac varirala je od 130 do 4400 mg m^{-2} g^{-1} što odgovara statusu oligotrofnog do mezotrofnog sustava (Armitage i sur., 1995). Prema literuturnim podacima fluktuacija između ova dva stupnja trofije je uobičajena (Hanžek i sur., 2021; Ternjej i sur., 2023). Najniža stopa emergencije je zabilježena 2012. godine kada je zabilježena i najniža brojnost jedinki trzalaca, a najviša 2015. godine kada je zabilježena njihova najviša brojnost. Te godine 4400 mg m^{-2} trzalaca bilo je dostupno za transfer u kopneno stanište i dostupno kopnenim organiznima za korištenje. Kopneni organizmi trzalce mogu koristiti direktno, kao izvor hrane, ili indirektno, kao nitrate i fosfate koji nastaju razlaganjem njihovih trupala (Gratton i sur., 2008; Dreyer i sur., 2015). Ono što je važno napomenuti jest da je stopa emergencije trzalaca izložena u ovom doktorskom radu vrlo gruba procjena stope emergencije trzalaca na samo jednoj lokaciji na području Nacionalnog parka Plitvička jezera. Ono što je vidljivo jest činjenica da je stopa emergencije korelirana s brojnosti jedinki trzalaca pa je samim time podložna ekstremnim promjenama u okolišu uvjetovanim klimatskim promjenama. U ovom dijelu istraživanja do kraja je potvrđena treća hipoteza.

3.4. Indikatorska vrijednost trzalaca na području stajaćica krškog područja

U publikaciji **IV** prikazan je indikatorski potencijal trzalaca u stajaćicama Dinarida, a potvrđena je četvrta hipoteza. Razvijen je novi LCI (Lake Chironomid Indeks) indeks kao metrika za procjenu stanja litorala, a pokazalo se da je bolje koreliran s organskim opterećenjem nego saprobni indeks. Glavna prepreka koja otežava procjenu stanja prirodnih jezera u Dinaridima jest specifičnost krškog reljefa koja za posljedicu ima malen broj prirodnih jezera. Iz tog razloga su za razvoj LCI indeksa korištene prirodne i umjetne stajaćice. Rijetko kada su indeksi koji se koriste za procjenu stanja bentosa usredotočeni na samo jednu skupinu makrozoobentosa. To je zato što je rijetko koja skupina organizama toliko raznolika, sveprisutna i pokazuje cijeli niz odgovora na različite pritiske u okolišu kao trzalci. Zbog njihove male veličine biološka procjena utjecaja hidromorfoloških promjena je slaba i ne pokazuju zadovoljavajući odgovor, ali pokazuju odlične rezultate u procjeni organskog opterećenja hranjivim tvarima u stajaćicama (Dodkins i sur., 2005; Donohue i sur., 2009).

Determinacija trzalaca samo do razine porodice ili potporodice kako bi se dobilo na brzini za potrebe procjene stanja ekosustava dovelo je do taksonomskog zanemarivanja trzalaca i gubitka znanja o njihovim značajkama na razini vrste ili roda što ih čini još manje privlačnima kao indikatorima u procjeni stanja ekosustava (Serra i sur., 2016). U publikaciji **IV** jasno je pokazano da se trzalci mogu koristiti za klasifikaciju stupnja trofije stajaćica, ali samo ako se koristi njhova brojnost i raznolikost. Ovo je u skladu sa zaključcima Bazzanti i sur. (2012) koji su preporučili korištenje brojnosti nižih taksonomskih razina trzalaca za pojačanje razlučivosti već postojećih modela za biološku procjenu stanja.

Iako je u publikaciji **IV** istaknuta uloga trzalaca u klasifikaciji stajaćica istaknuta su i njihova ograničenja kao što je mogućnost prilagodbe i tolerancija hidromorfoloških promjena te je tada idealno koristiti neke druge skupine organizama (Miler i sur., 2013). Najbolje je koristiti podatke koje nam pruža cijela zajednica kako bismo dobili najbolji skup informacija o sustavima i procesima koji se u njima odvijaju. Ovi rezultati potkrijepljuju rezultate iznesene u Raunio i sur. (2011) kako isključivanje trzalaca iz sustava ocjene može rezultirati nepotpunim procjenama lokalne raznolikosti makrozoobentosa, krivim zaključcima o tome kako funkcioniraju slatkovodni ekosustavi i naposljetku, nedostatkom informacija za donošenje funkcionalnih planova upravljanja. Determinacijom trzalaca samo do razine porodice može se podcijeniti bogatstvo vrsta i za 50 % (primjer Lepenice i Prološkog Blata) što može rezultirati lažnim zaključkom o maloj lokalnoj raznolikosti vrsta i potpuno krivom ocjenom ekološke kakvoće.

Kada god je moguće, determinacija do razine vrste ili grupe vrsta je poželjna, jer ponekad čak i vrste unutar istog roda pokazuju drugačije značajke. Primjerice, u ovom istraživanju grupa vrsta *Cladopelma goetghebueri* gr. je navedena kao svojta tolerantna na onečišćenje nitratima dok je grupa vrste *Cladopelma viridulum* gr. navedena kao osjetljiva na onečišćenje nitratima. Prema podacima iz literature obje grupe vrsta iz roda *Cladopelma* je moguće pronaći u širokom rasponu koncentracija kisika i hranjivih tvari (Moller Pillot, 2009). Naši podaci jesu u suprotnosti s literaturom, ali mora se uzeti u obzir geologija krša i zaista niske koncentracije nitrata. Ekološke značajke navedene u najopsežnijem izvoru takvih podataka za trzalce (Moller Pillot, 2009) uglavnom potječu iz nizinskih staništa s područja Nizozemske, gdje je i poljoprivreda intenzivnija (Van Grinsven i sur., 2016), što može rezultirati višim vrijednostima nitrata nego što je slučaj u Dinaridima. Staništa Dinaridske ekoregije karakteristična su po svojoj iznimnoj heterogenosti, vrlo kompleksnom sustavu podzemnih voda (Bonacci, 2014), slaboj naseljenosti i u većini slučajeva i dalje tradicionalnom obliku poljoprivrede što za posljedicu ima vrlo niske koncentracije nitrata u stajaćicama te ekoregije.

Za još dvije vrste je zabilježena značajna promjena u brojnosti s povećanom koncentracijom nitrata, a to su vrste *Kiefferulus tendipediformis* (Goetghebuer, 1921) i *Nubensia nubens* (Edwards, 1929). Jedinke vrste *K. tendipediformis* moguće je pronaći unutar djelomično razgrađenog biljnog materijala, ali s umjerenim koncentracijama kisika (Moller Pillot, 2009). Uslijed priljeva nitrata moguć je razvoj gušće makrofitske vegetacije i brojnije zajednice algi koje mogu dovesti do povremenih hipoksija. Što se tiče druge navedene vrste o njenoj rasprostranjenosti i ekologiji ima vrlo malo podataka. U Hrvatskoj je ova vrsta do sada pronađena samo u oligotrofnom Vranskom jezeru na otoku Cresu koje je prirodno vrlo siromašno nitratima i koristi se kao izvor pitke vode (Tomec i sur., 2002).

4. ZAKLJUČAK

1. Na području Nacionalnog parka Plitvička jezera zabilježeno je 116 vrsta i devet svojti trzalaca. Visoka raznolikost se može pripisati mozaiku staništa na području parka koja se očituju kroz različite sastave supstrata i brzine strujanja vode.
2. Na barijeri Kozjak-Milanovac izmjenjuju se lotička i lentička staništa, a s njima i sastav vrsta pa tako na barijeri nalazimo generaliste poput vrste *T. arduennensis*, ali i vrste specijalizirane za život u pjeskovitom supstratu (*P. scalaenum*) ili brzoj struji vode (*R. reissi*, *R. curtistylus* i *R. maculipennis*).
3. Tijekom 13 godina istraživanja trzalaca na barijeri Kozjak-Milanovac došlo je do porasta temperature vode, ali i režima padalina i jačine protoka vode.
4. Uslijed promjena u režimu padalina i jačini protoka vode došlo je do ispiranja vrsta trzalaca koje su prilagođene sporijoj struji vode, a posebno onih specijalizirane na život u pjeskovitom supstratu. Te vrste se i dalje povremeno nalaze u uzorcima s barijere Kozjak-Milanovac upravo zahvaljujući velikoj raznolikosti staništa koja služe kao refugij rijetkim vrstama. Na mjesta potisnutih vrsta naselili su novi specijalisti i to oni prilagođeni brzoj struji vode.
5. Brojnost trzalaca se tijekom 13 godina istraživanja mijenjala isključivo u ekstremnim slučajevima poput izražene suše ili tijekom vlažnih godina s pogodnim režimom padalina, a stopa emergencije se mijenjala zajedno s brojnošću.
6. Uslijed povišene temperature vode vrijeme trajanja leta trzalaca na sedrenoj barijeri Kozjak-Milanovac je produženo i tijekom istraživanja oni nisu izlijetali samo tijekom prosinca i siječnja.
7. Trzalci se mogu koristiti za procjenu stanja vodenih ekosustava. Unatoč tome što se trzalci mogu koristiti za procjenu stanja vodenih ekosustava njihova upotreba je nedovoljna pa je razvijen LCI indeks kojim je moguće uočiti razlike među jezerima s vrlo niskim koncentracijama nitrata zahvaljujući odgovoru u sastavu zajednice trzalaca koje nastaju uslijed dospijeća vrlo niskih koncentracija nitrata u sustav. Tako se omogućuje brže postupanje nadležnih institucija u slučaju zagađenja po principu "bolje spriječiti nego liječiti".

8. Trzalci imaju iznimnu indikatorsku vrijednost u istraživanju utjecaja klimatskih promjena, ali samo ako se oni određuju do najniže taksonomske razine. U suprotonom se njihov indikatorski potencijal gubi, jer samo na razini vrste u potpunosti su jasni njihovi ekološki zahtjevi i prilagodbe, što je neophodno za adekvatnu valorizaciju promjena u okolišu.

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ŽIVOTOPIS

DORIĆ, VALENTINA

Valentina Dorić rođena je 9. studenoga 1991. godine u Zagrebu. Osnovnu školu završila je u Vrbovcu, a opću gimnaziju u Srednjoj školi Vrbovec. Godine 2010. upisuje prijediplomski sveučilišni studij Biologije, a 2013. diplomski sveučilišni studij Eksperimentalne biologije, smjer Zoologija na Prirodoslovno-matematičkom fakultetu Sveučilišta u Zagrebu gdje 2016. stječe zvanje magistre eksperimentalne biologije. Od 2018. godine upisana je na poslijediplomski doktorski studij Biologije na istom Sveučilištu. Valentina Dorić je od 2019. do 2020. sudjelovala u izvođenju nastave na Zoologijskom zavodu kao naslovni asistent. Od 2020. do 2022. zaposlena je na Zoologijskom zavodu kao viši tehničar na projektu „Klimatska ranjivost Hrvatske i mogućnost prilagodbe urbanih i prirodnih okoliša“, a od travnja 2023. zaposlena je kao asistentica na istom zavodu. Sudjeluje u izvođenju nastave na kolegijima Opća ekologija, Ekologija i Terenska nastava iz biološke, geografske i geološke zaštite prirode. Područje znanstvenog interesa Valentine Dorić je makrozoobentos s naglaskom na ekologiju i raznolikost trzalaca (Diptera, Chironomidae) te njihovu primjenu u redovnom sustavu monitoringa i potencijal kao indikatorske skupine. Valentina Dorić je 2019. dobila DAAD stipendiju u kategoriji Short-Term Research Grant za usavršavanje u izradi preparata i determinaciji odraslih trzalaca na Sveučilištu Ludwig Maximilian u Münchenu, Njemačka. Uz to, usavršavala se u determinaciji ličinki trzalaca na radionici održanoj 2019. u Banskoj Bystrici, Slovačka. Tijekom 2022. godine usavršavala se na online radionicama iz područja statistike. Objavila je 13 znanstvenih radova, jedno poglavlje u knjizi i sudjelovala na 12 međunarodnih skupova sa 21 kongresnim priopćenjem. Do sad je sudjelovala na osam stručnih projekata pod vodstvom prof. dr. sc. Zlatka Mihaljevića i dva znanstvena projekta.

CROSBI PROFIL: Valentina Dorić (CROSBI Profil: 39025)

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PROŠIRENI SAŽETAK

Trzalci (Diptera, Chironomidae) su najrasprostranjenija i često najbrojnijih skupina vodenih kukaca slatkovodnih ekosustava, a koriste se u analizama procjene kvalitete vode. Detaljnije istraživanje njihove ekologije i taksonomije je prijeko potrebno s obzirom na njihovu indikatorsku važnost. Nacionalni park Plitvička jezera je odabran kao primarno područje istraživanja i uzorkovanja odraslih jedinki, jer je moguće provesti sveobuhvatno i detaljno istraživanje biologije i ekologije trzalaca. Osim Plitvičkih jezera uzorkovanja ličinačkih stadija su provedena u prirodnim i umjetnim stajaćicama Dinaridske ekoregije.

Na području Plitvičkih jezera ukupno je zabilježeno 116 vrsta trzalaca čime je ukupan broj vrsta u Hrvatskoj povećan na 258 vrsta. Dvije nove svojte i 54 nove vrste zabilježene su za faunu Nacionalnog parka. Najviše vrsta je zabilježeno na sedrenim barijerama, a najmanje u potocima što ukazuje na potrebu za daljnjim istraživanjima faune Parka.

Globalne klimatske promjene su se pokazale kao jedan od glavnih faktora koji utječu na sastav zajednice trzalaca na sedrenoj barijeri Nacionalnog parka Plitvička jezera. Utvrđeno je kako je tijekom 13 godina istraživanja na području Parka došlo do povišenja temperature vode, povećanja brzine strujanja vode, protoka i količine organske tvari što je utjecalo na zajednicu trzalaca. Dugotrajni podaci ukazuju na povećanje srednjeg godišnjeg protoka vode uz istovremeno smanjenje razine vode što je u potpunosti u skladu s predviđanjima o učestalijim i snažnijim padalinama te izraženijim i dugotrajnijim sušnim razdobljima. U ovakvim uvijetima povećava se erozija i mogućnost oštećenja sedrenih barijera.

Tijekom istraživanja, brojnost jedinki i raznolikost vrsta nisu se značajno promijenile osim 2012. godine kada su zabilježene najniža brojnost, raznolikost vrsta i stopa emergencije te 2015. godine kada su zabilježene najviša brojnost, raznolikost trzalaca i stopa emergencije. Značajne promjene u sastavu i strukturi zajednice su uočene, a objašnjene su kroz različite odgovore vrsta na promjene u protoku vode. Sastav zajednice mijenjao se kroz razdoblja sporijeg, vrlo niskog i brzog protoka vode što ukazuje na sposobnost zajednice trzalaca da vrlo brzo odgovori na hidrološke promjene.

Funkcionalna struktura trzalaca također je promijenjena. Visoki protoci u kasnijem razdoblju istraživanja uzrokuju ispiranje supstrata i organske tvari čime se stvara povoljno stanište za vrste s brzim životnim ciklusom. Tijekom istraživanja uočen je porast brojnosti vrsta koje pripadaju funkcionalnim skupinama pasivnih filtratora i usitnjivača što ukazuje na to da generalisti bolje iskorištavaju promjenjive protoke. Međutim, nekim specijalistima poput roda *Rheotanytarsus* su pogodovali novonastali uvijeti što ukazuje za potrebom determinacije do razine vrste u ekološkim istraživanjima. Brojnost grabežljivih vrsta trzalaca također se promijenila. Tako je u kasnijim godinama značajno porasla brojnost vrste *Rheopelopia eximia*. Visoka sposobnost prilagodbe trzalaca na različite životne uvijete očitovala se kroz skoro nepromijenjenu brojnost jedinki i raznolikost unatoč značajnim promjenama unutar strukture same zajednice.

U ovom istraživanju praćena je također fenologija trzalaca i odgovor na promjene u okolišu. Snažne kiše nakon dugotrajnih sušnih razdoblja dovode do erozije. Upravo zbog toga na barijeri Kozjak-Milanovac značajno se smanjila brojnost vrsta *Polypedilum scalaenum* i *Tanytarsus signatus*. Visoka temperatura vode može dovesti do ubrzanog rasta i razvoja ličinki trzalaca i time ubrzati emergenciju. Međutim, okolišni čimbenici su objasnili vrlo malen postotak varijabilnosti u početku emergencije što ukazuje na to da inter i intraspecijski odnosi imaju važnu ulogu u kontroliranju početka i trajanja emergencije trzalaca. Tijekom istraživanja utvrđeno je produljenje faze leta trzalaca. Uslijed povišene temperature vode trzalci s emergencijom započinju ranije. Promjene u vrhuncu emergencije nisu uočene, ali prema trenutnim literaturnim podacima te promjene se mjere u danima stoga ih mjesečnim uzorkovanjem nije moguće zabilježiti.

Izradom novog LCI indeksa (Lake Chironomid Indeks) kao metrike za procjenu stanja litorala ponuđeno je rješenje za nedostatak metrika koje su specifične za litoral. Razvojem LCI indeksa dokazana je važnost trzalaca kao indikatorske skupine. Indeks ima mogućnost otkrivanja vrlo niskih koncentracija nitrata i organskog opterećenja što je od velike koristi u Dinaridskoj ekoregiji u kojoj je stanovništvo malobrojno pa je samim time i poljoprivredna djelatnost rijetkost. Glavni izazov za razvoj indeksa predstavljala je jedinstvena geologija krša čiji rezultat je regija siromašna jezerima. Iz tog razloga za razvoj indeksa korištena su umjetna, izmijenjena i prirodna jezera. Na taj način je dobiven gradijent različitih utjecaja i povećana je otpornost indeksa čime je dobiven koristan alat za procjenu ekološkog stanja stajaćica Dinaridskog krša. Mala veličina tijela trzalaca te visoka tolerancija na hidromorfološke promjene slivnog područja i samog litorala ograničavaju mogućnost njihovog korištenja za procjenu organskog opterećenja ili obogaćenja hranjivim tvarima. Razvojem ovog indeksa ukazalo se na važnost taksonomske rezolucije u zaštiti i upravljanju stajaćicama, čijom primjenom se mogu poboljšati postojeći modeli procjene stanja ekosustava.

Ovim istraživanjima ukazano je na potrebu za dugotrajnim praćenjem staništa kako bi se dobilo što bolje razumijevanje utjecaja klimatskih promjena na staltkovodne ekosustave. Trzalci su se pokazali kao odlični indikatori upravo zbog svoje raznolikosti, mogućnosti brze prilagodbe i različitih odgovora na promjene u okolišu, ali samo kada se determiniraju do najniže moguće taksonomske kategorije. U budućnosti bi valjalo posvetiti pažnju školovanju novih taksonoma, ali i pokušati postići ravnotežu između korištenja modernih tehnologija, poput DNA barkodiranja ili korištenja okolišne DNA, i tradicionalne taksonomije. Istraživanje je doprinijelo budućoj zaštiti vodenih ekosustava i naglašava važnost visoke taksonomske rezolucije u provedbi istog.