

MACRO-CRUSTACEAN FAUNA OF THE VRANA LAKE BASIN IN DALMATIA (CROATIA)

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The shallow Vrana (Vransko) Lake in Dalmatia, Croatia, is a brackish water (oligohaline) lake that was artificially connected to the Adriatic Sea by the Prosika Canal, built in the 18th century. The aim of this study was to provide an updated overview of macro-crustacean diversity (class Malacostraca) in the Vrana Lake Basin. This synthesis includes all available records, combining the author's field observations with data from the scientific literature, and focuses on macro-crustacean taxa within the orders Amphipoda, Decapoda, Isopoda, and Mysida. Benthic macroinvertebrates were sampled using a hand net between 2011 and 2022, while baited crayfish traps were deployed during a one-night sampling effort in May/June 2024. Specimens of the invasive blue crab were collected manually in 2023 and 2024. In total, 32 sites were surveyed, 29 with a hand net and three exclusively with baited traps. A total of 21 macro-crustacean taxa were recorded in the Vrana Lake Basin between 2011 and 2024, comprising 16 species and five genera. The order Amphipoda was the most taxonomically diverse, with 11 taxa, followed by Isopoda (4), Decapoda (4), and Mysida (2). The highest species richness (16 taxa) was observed at the mouth of the Prosika Canal into the Adriatic Sea. The macro-crustacean fauna of the Vrana Lake Basin represents a unique assemblage of freshwater/oligohaline and brackish/marine species. The freshwater/oligohaline species, including the isopod *Proasellus coxalis*, amphipod *Niphargus dalmatinus*, and decapods *Palaemon antennarius* and *Austropotamobius fulcisanus*, were more abundant and widely distributed in the tributaries, with *P. coxalis* and *P. antennarius* also occurring in the lake. Most of the brackish/marine species were recorded only at the mouth of the Prosika Canal into the Adriatic Sea. Amphipods *Gammarus aequicauda* and *Pectenogammarus stocki*, and the isopod *Lekanesphaera hookeri*, were dominant in the lake and the Prosika Canal during, and immediately after periods of increased salinity. A recent addition to the brackish/marine group is the invasive blue crab (*Callinectes sapidus*), whose continued spread may pose a serious threat to biodiversity in the Vrana Lake Basin. The endangered white-clawed crayfish (*A. fulcisanus*) was recorded only in the freshwater Lateral Canal. Its continued monitoring is essential to assess the effectiveness of conservation measures, especially in light of planned sediment and vegetation removal to maintain flow in the canal. Salinization remains the primary threat to aquatic biodiversity in the basin. Urgent implementation of active management strategies, such as construction of a movable gate on the Prosika Canal, is necessary to mitigate this risk. The data presented in this study are critical for understanding and anticipating future changes in the macro-crustacean assemblages of this ecologically sensitive ecosystem.

Key words: freshwater, brackish water, Amphipoda, Isopoda, Decapoda, Mysida, salinization

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Vransko jezero u Dalmaciji je plitko bočato (oligohalino) jezero koje je umjetnim kanalom Prosika u 18. stoljeću spojeno s morem. Cilj ovog istraživanja bio je ažurirani pregled raznolikosti makroskopskih rakova (razred Malacostraca) u slivu Vranskog jezera. Uključeni su svi dostupni podatci, terenska opažanja autora i podatci iz znanstvene literature, s fokusom na taksone unutar redova Amphipoda,

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Decapoda, Isopoda i Mysida. Bentički makrobekralježnjaci sakupljani su ručnom bentos mrežom u razdoblju od 2011. do 2022. godine, dok su vrše za lov rakova korištene tijekom jednodnevnog uzorkovanja u svibnju/lipnju 2024. Jedinke invazivnog plavog raka sakupljene su ručno u 2023. i 2024. godini. Ukupno je istraženo 32 lokaliteta, 29 ručnom mrežicom, a tri isključivo korištenjem vrša. U razdoblju od 2011. do 2024. u slivu Vranskog jezera zabilježen je ukupno 21 takson makro-rakova, uključujući 16 vrsta i pet rodova. Red Amphipoda bio je taksonomski najraznolikiji s 11 taksona, slijede Isopoda (4), Decapoda (4) i Mysida (2). Najveća brojnost taksona (16) zabilježena je na ušću kanala Prosika u Jadransko more. Fauna makro-rakova u slivu Vranskog jezera predstavlja jedinstvenu kombinaciju slatkovodnih/oligohalinih i bočatih/morskih vrsta. Skupina slatkovodnih/oligohalinih vrsta, *Proasellus coxalis*, *Niphargus dalmatinus*, *Palaemon antennarius* i *Austropotamobius fulcisanus*, brojnija je i šire rasprostranjena u pritocima, dok vrste *P. coxalis* i *P. antennarius* također nastanjuju i jezero. Većina bočatih/morskih vrsta zabilježena je samo na ušću kanala Prosika, a samo tri vrste, *Gammarus aequicauda*, *Pectenogammarus stocki* i *Lekanesphaera hookeri*, su dominirale u jezeru i kanalu Prosika tijekom i neposredno nakon razdoblja povišenog saliniteta. Nova vrsta iz bočate/morske skupine je invazivni plavi rak (*Callinectes sapidus*), čije daljnje širenje može predstavljati ozbiljnu prijetnju bioraznolikosti sliva Vranskog jezera. Ugroženi bjelonogi rak (*A. fulcisanus*) zabilježen je isključivo u slatkovodnom Lateralnom kanalu. Kontinuirano praćenje njegove populacije ključno je za procjenu učinkovitosti mjera zaštite, osobito u svjetlu planiranog uklanjanja sedimenta i vegetacije radi održavanja protoka vode u kanalu. Zasljanjivanje ostaje glavna prijetnja biološkoj raznolikosti vodenih ekosustava u slivu. Hitna provedba aktivnih mjera upravljanja, poput izgradnje pokretne brane na kanalu Prosika, nužna je za ublažavanje ovog rizika. Podatci prikazani u ovom istraživanju ključni su za razumijevanje i predviđanje budućih promjena u zajednicama makro-rakova ovog ekološki osjetljivog ekosustava.

Ključne riječi: slatke, bočate vode, Amphipoda, Isopoda, Decapoda, Mysida, zasljanjivanje

INTRODUCTION

Macro-crustaceans are diverse group of crustaceans within the class Malacostraca, primarily represented by two most species-rich superorders: Peracarida and Eucarida (MARTIN & DAVIS, 2001; SCHRAM, 2013). Among these, the orders Amphipoda, Isopoda, and Mysida (Peracarida), along with Decapoda (Eucarida), constitute the most ecologically significant groups of benthic macro-crustaceans (body size > 0.5 mm) in freshwater and brackish environments. These organisms often dominate the macroinvertebrate assemblages in various freshwater habitats such as springs and streams (ŽGANEC, 2009), as well as in transitional brackish environments like estuaries and lagoons (CASAGRANDA *et al.*, 2006; DELGADO *et al.*, 2009). Ecologically, macro-crustaceans play a critical role in the decomposition of organic matter (MANCINELLI *et al.*, 2012; KRALJ *et al.*, 2025) and serve as a vital food source for fish and other aquatic organisms. Notably, most non-native and invasive macroinvertebrates in Croatian freshwater ecosystems are macro-crustaceans (ŽGANEC *et al.*, 2020). In brackish and marine waters of the Adriatic Sea, invasive species such as the blue crab (*Callinectes sapidus*) are increasingly prevalent (DULČIĆ *et al.*, 2011; GLAMUZINA *et al.*, 2023).

Scarce data on macro-crustaceans in the Vrana Lake basin exist. Few taxonomic, phylogenetic, and faunistic studies were conducted prior to a recent ecological study on the impact of salinization on benthic macro-crustacean assemblages (ŽGANEC *et al.*, 2024). The

earliest record concerns the taxonomic description of the endemic amphipod *Niphargus dalmatinus* Schäferna, 1922 (initially described as a subspecies: *N. ilidzensis* ssp. *dalmatinus*), based on specimens from the Biba and Pećina Springs, tributaries of the Vrana Lake (SCHÄFERNA, 1922). The species was later re-described from the Biba Spring (type locality) by KARAMAN (1973) and included in subsequent phylogenetic and phylogeographic studies (BORKO *et al.*, 2021, 2022). The endangered and protected white-clawed crayfish (former *Austropotamobius pallipes* complex, now *A. fulcisanus*, CRANDALL & DE GRAVE, 2017) was previously recorded in the Lateral Canal, a tributary of Vrana Lake (JELIĆ, 2014; JELIĆ *et al.*, 2016; MAGUIRE *et al.*, 2018). The white-clawed crayfish is of high conservation importance, as it is protected under national legislation and designated as a target species within the Natura 2000 ecological network. The invasive Atlantic blue crab *Callinectes sapidus*, whose population has recently expanded in the Croatian part of Adriatic Sea (DULČIĆ *et al.*, 2011; GLAMUZINA *et al.*, 2023), was first observed in Vrana Lake in 2013 (ŽUPAN *et al.*, 2016). More recently, a long-term ecological study (2011–2020) examined the effects of salinization and climate change on macro-crustacean assemblages in Vrana Lake and its associated canals, Kotarka and Prosika (ŽGANEC *et al.*, 2024). Out of a total of 18 macro-crustacean taxa identified in the previous study, only five species were more widespread and abundant in the Vrana Lake and in the Kotarka and Prosika canals.

The Vrana (Vransko) Lake is a shallow brackish (oligohaline) lake in Dalmatia (ŽGANEC *et al.*, 2024) and the largest natural lake in Croatia (30 km²). It is located in the Ravni Kotari region (North Dalmatia), near the Adriatic Sea (Fig. 1). The karst watershed of the Vrana Lake covers 515 km² (STROJ, 2012), while the lake itself occupies a geological cryptodepression with a maximum depth of 5 m and a mean depth of 2 m (ŠILJEG *et al.*, 2015). The lake is 13.6 km long and 1.4–3.4 km wide, extending parallel to the Adriatic Sea, from which it is separated by a limestone ridge 0.8–2.5 km wide. The Kotarka and Lateral Canals represent the lake's main tributaries, and the lake is hydrologically connected to the Adriatic Sea both naturally, through permeable karst (mostly through Jugovir, site 26 in Fig. 1), and artificially, via the Prosika Canal constructed in the 18th century (RUBINIĆ & KATALINIĆ, 2014). Seasonal water-level fluctuations (−0.06 to +2.35 m a.s.l.) are primarily driven by rainfall patterns and periodic droughts, whereas salinity variations result from episodic seawater intrusion and salinization of the Kotarka Canal. During periods of low water level (<45 cm at gauging station at site 25), seawater intrusions occur through the Prosika Canal and Jugovir, leading to the most pronounced lake salinization (RUBINIĆ & KATALINIĆ, 2014; ŽGANEC *et al.*, 2024).

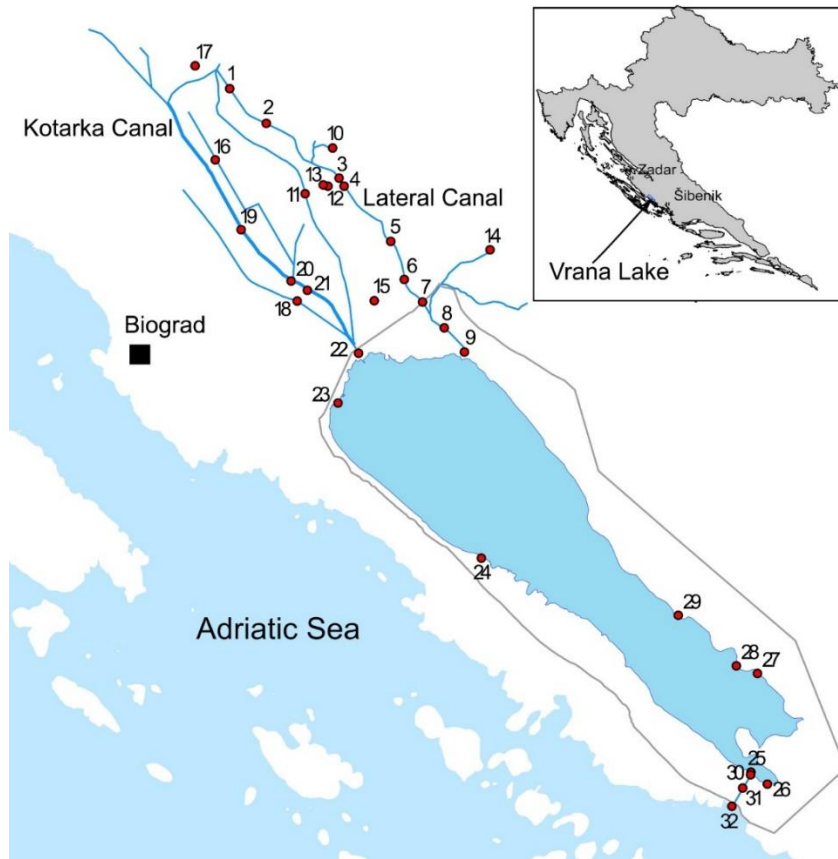


Fig. 1. All 32 study sites (their IDs) in the Vrana Lake Basin where macro-crustacean fauna was studied from March 2011 to October 2024.

Although crucial for nature conservation and ecological understanding, a comprehensive and systematic study of the macro-crustacean fauna in the Vrana Lake Basin is lacking. The present study aims to provide an updated and integrative overview of the macro-crustacean diversity in the Vrana Lake Basin. This includes compiling all available records, from both the author's field observations and from literature and biodiversity databases, pertaining to the orders Amphipoda, Decapoda, Isopoda, and Mysida up to the year 2024. The data presented in this study are crucial for the management of endangered and invasive macro-crustaceans in the basin, as well as for understanding and mitigating the impacts of salinization on future changes in the aquatic biodiversity of the Vrana Lake Basin.

MATERIALS AND METHODS

The macro-crustacean fauna of the Vrana Lake basin was investigated at 32 sites using three complementary sampling methods (Fig. 1). Benthic macroinvertebrates were collected at 29 sites using a hand net, whereas at three additional sites (1, 2, and 5) exclusively crayfish were sampled with baited traps in 2024. Invasive blue crabs were manually collected from the Prosika Canal at three sites (30, 31, and 32) during 2023 and 2024 (Tab. 1). Baited crayfish

traps were deployed at five sites (1, 2, 3, 5, and 7) in the Lateral Canal during a single overnight sampling event in May/June 2024. At each site, eight cylindrical PVC traps with funnel entrances on both ends were used. For each captured crayfish, sex, body weight (W), and total length (TL) were recorded prior to release. Benthic macro-crustaceans were sampled at 29 sites between March 2011 and August 2022 using a hand net with a 25 × 25 cm frame and 500 µm mesh size. Most sites (n = 16) were sampled during a single month, while five sites (22–26) were monitored for the longest period, spanning 14 months (Tab. 1). At the majority of sites (22), one quantitative sample (1 × 0.3125 m²) was collected from hard (predominantly sand and stones) or soft (sand and mud) substrates, along with one qualitative sample (5-minute kick sample) taken from submerged aquatic and riparian vegetation. At seven intensively sampled sites (22–26, 30 and 31), macroinvertebrate sampling occurred over 7 or 14 months (Tab. 1). During these periods, three (in 2011 and 2014) or five (in 2016–2020) replicate quantitative samples (3× or 5× 0.0652 m²) were collected, beside one qualitative sample from 2016–2020 (ŽGANEC *et al.*, 2024). All macroinvertebrate samples were fixed in the field with 96% ethanol and subsequently processed in the laboratory. Specimens were separated under a stereomicroscope and sorted by taxonomic group.

Specimens of the invasive blue crab were collected manually from the Prosika Canal (sites 30, 31, 32) on two occasions (October 2023 and October 2024) during the early night. The stretch from the canal mouth (site 32) to site 31 was carefully surveyed using a headlamp and hand net, and all crabs encountered were collected into a bucket. Additional data on blue crab removals were obtained from management activities in the Vransko jezero Nature Park. Two baited wire traps were operated by park staff at site 30 from 25 July to 18 October 2024. Carapace length (CL, distance between the centre of the anterior interorbital margin and the centre of posterior margin), carapace width (CW, maximal distance between the posterior anterolateral spines) and body mass (M), as well as sex of captured crabs were recorded that were used to describe blue crabs.

Macro-crustacean specimens were identified using standard taxonomic keys for each group: Amphipoda - PINKSTER (1993) and BELLAN SANTINI *et al.* (1982, 1993); Decapoda - FROGLIA (1978) and TZOMOS & KOUKOURAS (2015); Isopoda - ARGANO (1979), HENRY & MAGNIEZ (1983), JACOBS (1987), POORE (2001) and KEMP *et al.* (2020); Mysida - WITTMANN *et al.* (2016). In a few cases (Tab. 1), species-level identification was not possible because only juvenile or damaged specimens were present in the samples.

Tab. 1. Overview of study sites where macro-crustaceans were sampled from March 2011 to October 2024 using four different methods: I) benthic hand net (25 × 25 cm, 500 µm mesh size), II) baited crayfish traps (8 per site), III) manual collection of blue crabs, and IV) two baited wire traps for blue crabs. Abbreviations for sites 22–26, 30, and 32, used in a previous study (ŽGANEC *et al.*, 2024), are provided in parentheses next to site names. For each sampling method, the year-month of sampling and the number of months of sampling are listed, separated by a slash (/).

Habitat	Site ID	Lat	Long	Site name	Method	Sampling Year-Month	Nu. mo.
TRIBUTARIES	1	43.9938	15.4803	Lateral canal 1	II	2024 - 5	1
	2	43.9865	15.4908	Lateral canal 2	II	2024 - 5	1
	3	43.9751	15.5113	Lateral canal 3	I/ II	2022 - 8 / 2024 - 6	1 / 1
	4	43.9733	15.5131	Lateral canal 4	I	2016 - 9	1
	5	43.9618	15.5262	Lateral canal 5	II	2024 - 6	1
	6	43.9538	15.5303	Lateral canal 6	I	2016 - 9	1
	7	43.9491	15.5354	Lateral canal 7	I/ II	2014 - 10 / 2024 - 6	1 / 1
	8	43.9437	15.5417	Lateral canal 8	I	2011 - 3, 5, 7, 10, 12 / 2022 - 8	6
	9	43.9386	15.5474	Lateral canal 9	I	2016 - 9	1
	10	43.9813	15.5097	spring Veliki Stabanj	I	2023 - 5	1
	11	43.9720	15.5018	Jablanac canal	I	2022 - 8	1
	12	43.9735	15.5084	pond Modro jezero stream next to Modro jezero	I	2022 - 8	1
	13	43.9735	15.5071	jezero	I	2022 - 8	1
	14	43.9598	15.5550	spring Pečina	I	2011 - 3, 5, 7, 10, 12 / 2016 - 9	6
	15	43.9494	15.5216	Jasen canal	I	2011 - 3, 5, 7, 10, 12	5
	16	43.9790	15.4760	Južni canal	I	2016 - 9	1
	17	43.9986	15.4704	pond Lemašac	I	2016 - 9	1
	18	43.9494	15.4993	Vrbica canal	I	2014 - 10	1
	19	43.9644	15.4833	Kotarka canal 1	I	2016 - 9	1
	20	43.9536	15.4977	Kotarka canal 2	I	2016 - 9	1
	21	43.9517	15.5023	Kotarka canal 3	I	2014 - 10	1
	22	43.9385	15.5170	Kotarka canal 4 (K)	I	2011 - 3, 5, 7, 10, 12 / 2014 - 5, 10 / 2016 - 9 / 2017 - 10 / 2018 - 7 / 2019 - 3 / 2020 - 3, 10, 12	14
VRANA LAKE	23	43.9281	15.5110	Crkvine (V1)	I	2011 - 3, 5, 7, 10, 12 / 2014 - 5, 10 / 2016 - 9 / 2017 - 10 / 2018 - 7 / 2019 - 3 / 2020 - 3, 10, 12	14
	24	43.8956	15.5519	Drage (V2)	I	2011 - 3, 5, 7, 10, 12 / 2014 - 5, 10 / 2016 - 9 / 2017 - 10 / 2018 - 7 / 2019 - 3 / 2020 - 3, 10, 12	14
	25	43.8506	15.6291	Prosika (V3)	I	2011 - 3, 5, 7, 10, 12 / 2014 - 5, 10 / 2016 - 9 / 2017 - 10 / 2018 - 7 / 2019 - 3 / 2020 - 3, 10, 12	14
	26	43.8481	15.6338	Jugovir (V4)	I	2011 - 3, 5, 7, 10, 12 / 2014 - 5, 10 / 2016 - 9 / 2017 - 10 / 2018 - 7 / 2019 - 3 / 2020 - 3, 10, 12	14
	27	43.8712	15.6312	Živača	I	2011 - 3, 5	2
	28	43.8728	15.6251	Punta	I	2011 - 7, 10, 12	3
	29	43.8834	15.6085	Bašinka	I	2011 - 3, 5, 7, 10, 12	5
PROSIKA CANAL	30	43.8499	15.6291	Prosika canal 1 (P1)	I/ III / IV	2016 - 9, 2017 - 10, 2018 - 7, 2019 - 3, 2020 - 3, 10, 12 / 2023 - 10 / 2024 - 7, 8, 9, 10	14 / 2 / 4
	31	43.8472	15.6270	Prosika canal 2	I/ III	2016 - 9 / 2023 - 10 / 2024 - 10	1 / 2
	32	43.8439	15.6237	Prosika canal 3 (P2)	I/ III	2014 - 5, 2016 - 9, 2017 - 10, 2018 - 7, 2019 - 3, 2020 - 3, 10 / 2023 - 10 / 2024 - 10	7 / 2

RESULTS

A total of 21 macro-crustacean taxa, comprising 16 species and five genera, were recorded in the Vrana Lake Basin between 2011 and 2024 (Tab. 2). The order Amphipoda was the most diverse, with 11 taxa, followed by Isopoda (4), Decapoda (4), and Mysida (2). However, only six species were relatively widespread and abundant: the amphipods *Pectenogammarus* (*Echinogammarus*) *stocki*, *Gammarus aequicauda*, and the endemic *Niphargus dalmatinus*; the isopods *Proasellus coxalis* and *Lekanesphaera hookeri*; and the decapod *Palaemon antennarius* (Fig. 2).

The highest taxa richness (16) was recorded at site 32, located at the mouth of the Prosika Canal to the Adriatic Sea. In contrast, between 3 and 7 taxa were found at six lake sites, with the exception of site 27, where only a single species (*P. coxalis*) was observed. The amphipod *N. dalmatinus* and the isopod *P. coxalis* co-occurred at 14 of 22 tributary sites (19 of which were sampled using hand net). The highest species richness in the tributaries (5 species) was recorded at site 22, located in the Kotarka Canal approximately 200 m upstream from its mouth.

The endemic amphipod *N. dalmatinus* was recorded at 16 tributary sites (Tab. 2, Fig. 3a). Additionally, unidentified juvenile specimens of *Niphargus* sp. were observed at three lake sites (24–26), but species-level identification was not possible. The isopod *P. coxalis* was the most widespread species, present at 23 sites across both the lake and its tributaries (Fig. 3b). Brackish-water amphipods *P. stocki* and *G. aequicauda*, along with the isopod *L. hookeri*, were found in both the lake and the Prosika Canal. The latter two species also occurred at site 22 near the Kotarka Canal mouth. The decapod shrimp *P. antennarius* was relatively widespread and abundant, recorded at five sites in tributaries, five sites in the lake, and two sites in the Prosika Canal (Fig. 3c). This species, recorded at a total of 11 sites, was first detected at site 22 in September 2016. It was absent from all sites sampled in 2011 and from five sites sampled in 2014. From 2016 onward, the cumulative number of new sites where it was recorded steadily increased for this species. Most other species had more limited distributions. Juvenile specimens of semi-terrestrial amphipods *Orchestia* sp. were found at five lake sites. The amphipod *Melita palmata*, another brackish-water species, was found at site 32 and at two additional lake sites (26 and 29), though only in 2011. Five amphipod species, two isopods, two mysids and one decapod were found exclusively at the mouth of the Prosika Canal (site 32) as a component of brackish water fauna. Most of these taxa were rare and only amphipod *Microdeutopus gryllotalpa* and isopod *Sphaeroma seratum* were regularly found.

Tab. 2. Macro-crustacean fauna of the Vrana Lake Basin from 2011 to 2024, showing the sites' ID where each species was recorded in tributaries, the Vrana Lake, and the Prosika Canal (site ID numbers correspond to those in Tab. 1 and Fig. 1), along with the total number of sites where each species was found.

Order	Species / Taxon	Tributaries (site ID)	Vrana Lake (site ID)	Prosika Canal (site ID)	Nu. sites	
AMPHIPODA	<i>Pectenogammarus stocki</i> (G. Karaman, 1970)		23, 24, 25, 26, 29	30, 31, 32	8	
	<i>Gammarus aequicauda</i> (Martynov, 1931)	22	23, 24, 25, 26, 28, 29	30, 32	9	
	<i>Melita palmata</i> (Montagu, 1804)		26, 29	32	3	
	<i>Melita hergensis</i> Reid, 1939			32	1	
	<i>Maerella tenuimana</i> (Spence Bate & Westwood, 1862)			32	1	
	<i>Microdeutopus gryllotalpa</i> A. Costa, 1853			32	1	
	<i>Dexamine spinosa</i> (Montagu, 1813)			32	1	
	<i>Niphargus dalmatinus</i> Schäferna, 1922	3, 4, 6, 7, 8, 10, 11, 13, 14, 15, 16, 17, 19, 20, 21, 22				16
	<i>Niphargus</i> sp.		24, 25, 26			3
	<i>Orchestia</i> sp.		23, 24, 25, 26, 29			5
<i>Harpinia</i> sp.				32	1	
ISOPODA	<i>Proasellus coxalis</i> (Dollfus, 1892)	3, 4, 6, 7, 8, 10, 11, 13, 14, 15, 16, 18, 19, 21, 22	23, 24, 25, 26, 27, 28, 29	30	23	
	<i>Lekanesphaera hookeri</i> (Leach, 1814)	22	23, 24, 25, 26, 28, 29	30, 31, 32	10	
	<i>Sphaeroma serratum</i> (J. C. Fabricius, 1787)			32	1	
	<i>Cyathura</i> sp.			32	1	
MYSIDA	<i>Diamysis mesohalobia</i> Ariani & Wittmann, 2000			32	1	
	<i>Siriella</i> sp.			32	1	
DECAPODA	<i>Austropotamobius fulcisanus</i> (Ninni, 1886)	1, 2, 3, 4, 5, 7, 9			7	
	<i>Palaemon antennarius</i> H.Milne-Edwards, 1837	7, 9, 11, 12, 22	23, 24, 25, 26	30, 32	11	
	<i>Carcinus aestuarii</i> Nardo, 1847			32	1	
	<i>Callinectes sapidus</i> Rathbun, 1896			30, 31, 32	3	

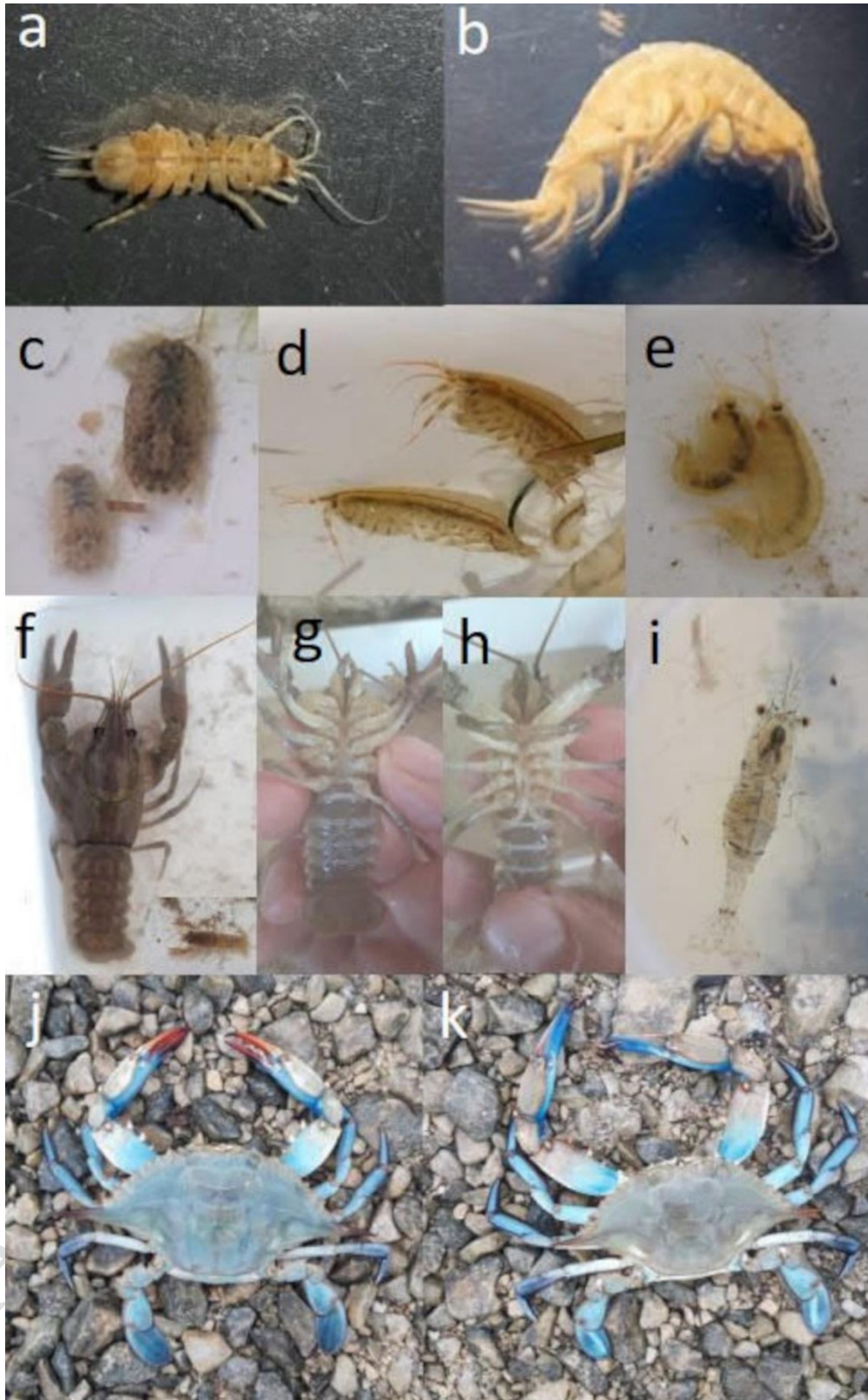


Fig. 2. Eight macro-crustacean species recorded in the Vrana Lake Basin (photo: KŽ): a) *Proasellus coxalis*, b) *Niphargus dalmatinus*, c) *Lekanesphaera hookeri*, d) *Gammarus aegicauda*, e) *Pectenogammarus stocki* (= *Echinogammarus stocki*) pair in precopula, f) *Austropotamobius fulscianus* (= *A. pallipes*), ventral views of female (g) and male (h), with juvenile visible in the lower right corner of image (f), i) *Palaemon antennarius*, j) blue crab *Calinectes sapidus* (female) and k) *C. sapidus* (male).

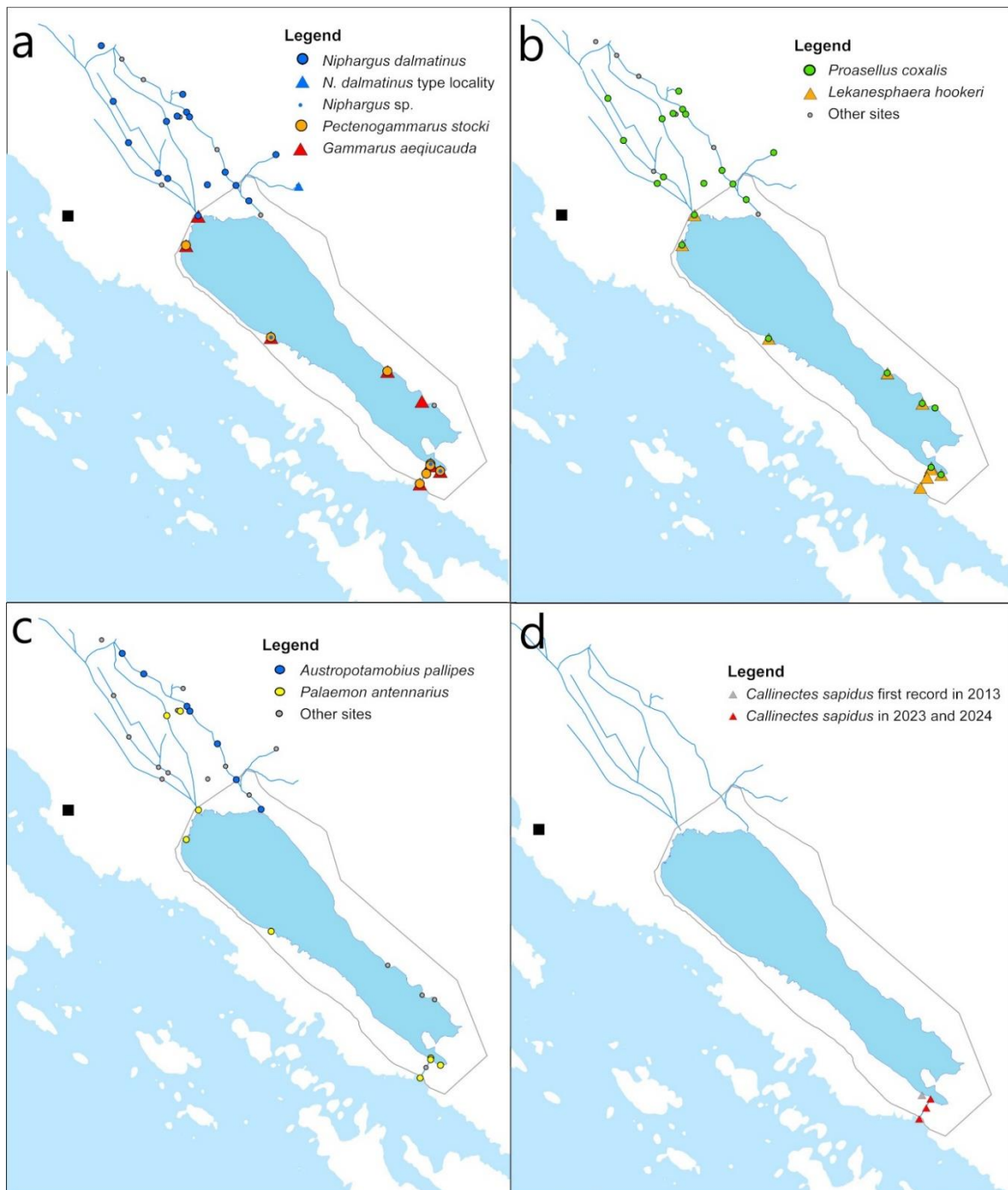


Fig. 3. Distribution of the more widespread macro-crustacean species in the Vrana Lake Basin: a) amphipods *Niphargus dalmatinus*, *Pectenogammarus* (*Echinogammarus*) *stocki* and *Gammarus aequicauda*; b) isopods *Proasellus coxalis* and *Lekanesphaera hookeri*; c) decapods *Palaemon antennarius* and *Austropotamobius fulcisanus* (*A. pallipes*); d) invasive blue crab (*Callinectes sapidus*).

The white-clawed crayfish (*A. fulcisanus*) was found exclusively in the Lateral Canal, at seven sites, using baited crayfish traps at five sites (1, 2, 3, 5, and 7) or only hand net at sites 4 and 9 (Tab. 2, Fig. 3c). It was first recorded in 2016 at site 4 during this study, then at sites 3 and 9 in 2022 using hand net. In 2024 it was captured using baited traps at five sites (1, 2, 3, 5,

and 7). A total of 93 individuals (46 males, 47 females) were caught in traps (8 per site), with a mean catch per unit effort (CPUE) of 2.3 (range: 1.4–3.9). Male total length (TL) ranged from 60.6 to 114.8 mm (mean \pm SD: 85.4 \pm 11.4 mm), and female TL ranged from 54.9 to 103.8 mm (85.8 \pm 10.5 mm).

The invasive blue crab (*Callinectes sapidus*) was first recorded in October 2023 during this study, when one female was manually caught at the site 30 in the Prosika Canal, and 15 specimens (13 females, 2 males) were caught between sites 31 and 32. In October 2024, 10 specimens (7 females, 3 males) were manually captured in the same area. Additionally, 38 blue crabs (23 males, 15 females) were caught by Nature Park employees at site 30 manually or using two wire traps from July to October 2024. Carapace length (CL) of males ranged from 75 to 105 mm (mean \pm SD: 91 \pm 7 mm), carapace width (CW) from 150 to 220 mm (191 \pm 17 mm), and weight from 320 to 580 g (444 \pm 82 g). CL of females ranged from 60 to 100 mm (mean \pm SD: 76 \pm 9 mm), CW from 54 to 211 mm (175 \pm 33 mm), and weight from 100 to 440 g (226 \pm 84 g).

DISCUSSION

This study extends a previous long-term investigation (2011–2020) on the effects of salinization and climate change on macro-crustacean assemblages in the Vrana Lake, and the Prosika and Kotarka Canals (ŽGANEC *et al.*, 2024). In present work we incorporated data from additional 25 sites (21 tributary sites, three lake sites and one in the Prosika Canal) sampled between 2011 and 2022, along with 2024 trapping results targeting the white-clawed crayfish in the Lateral Canal and the blue crabs in the Prosika Canal/VranaLake. Among the 21 taxa identified, five are freshwater/oligohaline species (*P. coxalis*, *N. dalmatinus*, *Niphargus* sp., *P. antennarius*, and *A. fulcisanus*), while 16 are brackish/marine species and they were predominantly present at the mouth of the Prosika Canal to the Adriatic Sea. Notably, three brackish species, amphipods *P. stocki* and *G. aequicauda*, and isopod *L. hookeri*, have colonized the lake and the Prosika Canal, with the latter two also present in the lower reach of the Kotarka Canal. The invasive Atlantic blue crab (*C. sapidus*), first recorded in 2013 (ŽUPAN *et al.*, 2016), appears to have become more abundant in recent years (2023 and 2024) and represents the fourth brackish/marine species with the potential to establish in the Vrana Lake.

The isopod *P. coxalis* and amphipod *N. dalmatinus* are the most widespread peracarid species in the lake's tributaries. Croatian endemic species, *N. dalmatinus*, inhabits freshwaters of the Adriatic Sea basin from the Cres Island in the north to the upper Cetina River and its tributaries in the south (JANDRIĆ, 2009). Its type locality is the Biba Spring (Fig. 3a), and it has

also been recorded in the Pećina Spring (site 14) (SCHÄFERNA, 1922; KARAMAN, 1974; BORKO *et al.*, 2021, 2022). Our findings indicate its widespread presence in the Vrana Lake tributaries, with high abundance in springs (Pećina-site 14, Veliki Stabanj-site 10), a stream near Modro Jezero (site 13), and sections of the Kotarka (site 19) and Lateral Canals (site 4). Adapted to cool, freshwater/oligohaline lotic habitats, *N. dalmatinus* thrives in springs and upper tributary reaches. Interestingly, *G. balcanicus*, the most prevalent freshwater epigean amphipod in the Adriatic drainage (ŽGANEC, 2009), is absent from these habitats. Species *G. balcanicus* did not colonize the Vrana Lake Basin potentially due to historical isolation or unsuitable conditions in the Vrana wetland which existed before the construction of the Prosika Canal in 1770 (RUBINIĆ, 2014). Amphipod *N. dalmatinus* occupies a similar ecological niche in the Vrana Lake tributaries, as *G. balcanicus* in other river basins of the Adriatic drainage area. The lack of competition may have facilitated the unusual dominance of *N. dalmatinus*, a subterranean depigmented, eyeless species that exemplifies subterranean adaptation to epigean environments.

Isopod *P. coxalis* is the most prevalent macro-crustacean in the Vrana Lake Basin, abundant in tributaries and consistently present along the lake's littoral zone. Its broad distribution reflects ecological tolerance, enduring brief periods of high salinity (ŽGANEC *et al.*, 2024) and summer temperatures exceeding 30 °C in the lake. Common in the Mediterranean fresh and brackish waters (KOPRNICKÁ, 2013), its salinity tolerance may explain its spread to Central and Northern Europe, where it is considered non-native/invasive (KEMP *et al.*, 2020). Given its morphological diversity and the existence of multiple subspecies (KOPRNICKÁ, 2013), further phylogenetic and taxonomic studies are warranted to clarify its classification.

The decapod glass shrimp *P. antennarius* was absent from the lake and tributaries during intensive sampling in 2011 and 2014 but appeared at site 22 in the Kotarka Canal in 2016, subsequently re-colonizing the lake and canals. This freshwater/oligohaline species inhabits freshwater and brackish environments across the Apennine and Balkan peninsulas (TZOMOS & KOUKOURAS, 2015), including the Neretva River Delta in Croatia at salinities between 0–5‰ (GOTTSTEIN MATOČEC & KEROVEC, 2002; GOTTSTEIN MATOČEC *et al.*, 2006). In the Vrana Lake Basin, *P. antennarius* was found at 11 sites across tributaries, the lake littoral, and the Prosika Canal. Although absent in earlier records, it may have previously inhabited the area, with its disappearance potentially linked to elevated salinity (up to 18.3 PSU at site 25 in the lake in 2012), high summer temperatures, and low oxygen levels in 2012 (ŽGANEC *et al.*, 2024). Freshwater/oligohaline refugia, such as the Lateral Canal, may have facilitated its survival and

subsequent recolonization, likely through passive dispersal of juveniles by birds and active movement from the Kotarka and Lateral Canals into the lake's northwest regions.

Three dominant brackish/marine peracarid species (*P. stocki*, *G. aequicauda*, and *L. hookeri*) had colonized the lake prior to 2011, likely during the initial salinization event in 2008 (ŽGANEC *et al.*, 2024). Other brackish species, such as *Melita palmata*, appeared in 2011 at two sites in the lake, but vanished during the low salinity period from 2016 to 2020. This species is, thus, next potential colonizer of the lake if salinity of the lake increases permanently. Semi-terrestrial amphipods like *Orchestia* sp. were infrequently observed in the lake, as they primarily inhabit the upper supralittoral zone and only occasionally enter the water. While abundant under shoreline plant debris, the study's methodology was not suited for detailed analysis of these semi-terrestrial amphipods.

Eastern Adriatic populations of the white-clawed crayfish were previously considered part of the *Austropotamobius pallipes* complex (JELIĆ, 2014; JELIĆ *et al.*, 2016), until CRANDALL & DE GRAVE (2017) classified these populations as *Austropotamobius fulcisanus*, and this species is now listed and mapped in the World of Crayfish database (ION *et al.*, 2024). However, nomenclatural issues in *A. pallipes* complex still remain unresolved (MANGANELLI *et al.*, 2006; JELIĆ, pers. comm.). Former species *A. pallipes*, distributed from Spain, France and United Kingdom to the Balkan Peninsula (JELIĆ *et al.*, 2016), is listed as endangered by the IUCN Red List (FÜREDER *et al.*, 2010) and is protected under the Bern Convention and EU Habitat Directive. In Croatia, it is safeguarded by the Nature Protection Act (NN 80/13). Our findings indicate its presence throughout the Lateral Canal, which, along with its tributaries, represents the basin's most significant remaining freshwater habitat. Long-term monitoring (Hrvatske vode, monthly from 2015–2020) at site 7 in Lateral Canal revealed salinity levels ranging from 0.2 to 0.7 PSU. We recorded the highest salinity of 1.7 PSU at site 9 in August 2022, when one juvenile white-clawed crayfish was found. In contrast, higher salinity levels were observed in the Kotarka and Jablanac Canals (4.2 and 5.7 PSU, respectively) in August 2022. Salinity is potentially the major limiting factor of the species' distribution in the Vrana Lake Basin. Temperature may also be a limiting factor, as cooler summer temperatures, like those in the Lateral Canal (9.0–23.0 °C), are optimal for this species, whereas higher temperatures (up to 29.4 °C at site 22 in the Kotarka Canal) in combination with higher salinity and lower oxygen levels may hinder establishment. Monitoring in 2024 was prompted by planned riverbed cleaning along the Lateral Canal by Hrvatske Vode, which could threaten the crayfish population. Adherence to prescribed measures, such as leaving one side of the canal

undisturbed, is crucial to minimize mortality and population decline. Post-cleaning monitoring will assess habitat recovery and population resilience.

Although the presence of the invasive Atlantic blue crab (*C. sapidus*) is not a recent discovery, its population have significantly increased compared to its first recorded in the Vrana Lake in 2013 (ŽUPAN *et al.*, 2016). The earliest documented records of this species in the Croatian Adriatic date back to 2004, from the Neretva River delta and the Pelješac Peninsula (ONOFRI *et al.*, 2008). Notably, the first record in the Adriatic Sea was reported much earlier, in 1949, from Grado, Italy, in the northern Adriatic (DULČIĆ & DRAGIČEVIĆ, 2010). Since 2004, records of *C. sapidus* along the Croatian coast have steadily increased, with the species gradually spreading to the north. The 2013 record in the Vrana Lake (ŽUPAN *et al.*, 2016) coincides with initial observations on the Istrian Peninsula, which likely occurred in 2013 or 2014 (DUJMOVIĆ *et al.*, 2023). As of 2025, the species has been documented along the entire Croatian Adriatic coastline, from the Mirna estuary in Istria to Dubrovnik (DULČIĆ *et al.*, 2011; GLAMUZINA *et al.*, 2023; see also iNaturalist database, URL 1). Due to its opportunistic and omnivorous feeding habits (MANCINELLI *et al.*, 2016), *C. sapidus* may exert a strong ecological impact on benthic macroinvertebrate communities and benthic algae within the Vrana Lake. By 2024, the species had likely established a reproducing population in the south-eastern part of the lake near the Prosika Canal. However, further spread of the blue crab northward into the Vrana Lake could likely be prevented through continued removal efforts, such as those initiated by the Vransko Jezero Nature Park in 2024.

In conclusion, the macro-crustacean fauna of the Vrana Lake Basin represents a unique combination of freshwater/oligohaline and brackish/marine species. The first group, including *P. coxalis*, *N. dalmatinus*, *P. antennarius*, and *A. fulcisianus*, is more abundant and widespread in the tributaries, with *P. coxalis* and *P. antennarius* also occurring within the lake. The second group, composed of brackish and marine species such as *P. stocki*, *G. aequicauda*, and *L. hookeri*, dominated the lake during periods of increased salinity and immediately afterward (ŽGANEC *et al.*, 2024). A recent and potentially disruptive addition to the second group is the invasive blue crab (*C. sapidus*), which may have a significant negative impact on biodiversity within the Vrana Lake Basin if its spread continues unchecked. Continued monitoring of the endangered white-clawed crayfish (*A. fulcisianus*) is essential to evaluate the effectiveness of conservation measures aimed at mitigating disturbances in the Lateral Canal. Furthermore, the implementation of active management strategies, such as the installation of a movable gate on the Prosika Canal, is urgently needed to reduce the anticipated increasing salinization and

thermal stress in the Vrana Lake. The data presented in this study are crucial for understanding and predicting future changes in the macro-crustacean assemblages in the Vrana Lake Basin.

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