Original Research

Distribution of two invasive alien diatom species *Achnanthidium delmontii* and *Achnanthidium druartii* in Slovenia

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Abstract

The distribution of two invasive diatom species in Slovenia, *Achnanthidium delmontii* (ADMO) and *Achnanthidium druartii* (ADRU), was investigated in this study. Data from 87 rivers and 11 lakes collected between 2019 and 2024 in the frame of national monitoring of the ecological status of surface waters were included. ADMO was present in 40 rivers (46%) and was dominant (>5% relative abundance) in 27 rivers (31%), with the highest abundance reaching 77% in the Bolska River. It was rare in lakes, detected in only three lakes (27%). ADRU was detected in 12 rivers (14%) and was dominant in four rivers (5%), with the highest abundance reaching 27% in the Drava River. In contrast, ADRU was common in lakes, with a presence in eight lakes (73%), dominating Lake Slivnica and Lake Pernica. ADMO presence was associated with reduced diatom species diversity and evenness, although no direct ecological impact was observed. ADMO was present mainly in upland river sections, while ADRU was more frequent in lowland river sections and lakes. The results of this study confirmed the invasive character of ADMO, whereas ADRU did not affect species diversity and evenness of the diatom assemblages, and thus its invasive character could not be confirmed.

Keywords

Achnanthidium delmontii; *Achnanthidium druartii*; alien invasive species; diatoms; Slovenia

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Razširjenost dveh invazivnih tujerodnih vrst diatomej *Achnanthidium delmontii* in *Achnanthidium druartii* na območju Slovenije

Izvleček

V raziskavi smo proučevali razširjenost dveh invazivnih tujerodnih vrst diatomej v Sloveniji, *Achnanthidium delmontii* (ADMO) in *Achnanthidium druartii* (ADRU). V raziskavo smo vključili podatke iz 87 rek in 11 jezer zbranih med letoma 2019 in 2024 v okviru nacionalnega monitoringa ekološkega stanja površinskih voda. ADMO je bil prisoten v 40 rekah (46 %), dominantna vrsta (>5 % relativne abundance) je bil v 27 rekah (31 %), pri čemer je s 77 % v reki Bolski dosegel najvišjo zastopanost. ADMO je bil v jezerih redek, zaznan je bil le v treh jezerih (27 %). ADRU je bil prisoten v 12 rekah (14 %), dominantna vrsta je bil v štirih rekah (5 %), z največjo zastopanostjo 27 % v reki Dravi. V jezerih je bil ADRU pogost, prisoten je bil v osmih jezerih (73 %), prevladoval je v Slivniškem in Perniškem jezeru. Prisotnost ADMO je bila povezana z zmanjšano vrstno pestrostjo in enakomernostjo združb diatomej, čeprav neposrednega negativnega ekološkega vpliva nismo zaznali. ADMO se je večinoma pojavljal v povirnih delih rek, medtem ko je bil ADRU pogostejši v nižinskih delih rek in jezerih. Rezultati potrjujejo invazivni značaj ADMO, medtem ko invazivnost ADRU ni bila potrjena, saj njegova prisotnost ni pomembno vplivala na sestavo združb diatomej.

Ključne besede

Achnanthidium delmontii; *Achnanthidium druartii*; invazivne vodne vrste; diatomeje; Slovenija

Introduction

Primary producers in rivers and lakes form the basis of the food web, and as such, any change in their quantity or in the composition of their communities can result in a disturbed ecological balance in waterbodies, with a domino effect on higher trophic levels such as benthic macroinvertebrates and fish (Buczkó et al., 2022).

Among invasive benthic diatoms, the most attention in the past has been paid to one of the largest freshwater diatom species, *Didymosphenia geminata* (Lyngbye) W.M. Schmidt, which is native to Europe and North America but highly invasive and aggressive in New Zealand. It causes a lot of problems in freshwater systems, such as the formation of large and extensive mats that impact fish, aquatic plants, and insects, resulting in severe disturbances in food webs (Blanco and Ector, 2009). However, according to Taylor and Bothwell (2014), *D. geminata* blooms in New Zealand are not caused solely by the introduction of its cells in new areas, as similar, nearly synchronous blooms have occurred in areas where *D. geminata* is native, such as North America and Europe. Next to large-celled diatoms such as *D. geminata*, also small-celled diatoms (<25 µm), such as *Achnanthidium delmontii* Pérès, Le Cohu & Barthès (ADMO), and *Achnanthidium druartii* Rimet & Couté (ADRU), are considered invasive or potentially invasive species (e.g., Buczkó et al., 2022; Falasco et al., 2023; Ivanov, 2018). ADMO was discovered in 2007 and formally described in 2012, based on specimens from a French river (Pérès et al., 2012), while ADRU was first discovered in 2004 in the Rhone River in France and formally described in 2010 as a new species invading the rivers in France and Spain (Rimet et al., 2010).

Since then, ADMO has been reported from several countries in Europe (France, Germany, Hungary, Italy, Netherlands, Switzerland) and Asia (China) (Guiry and Guiry, 2022), while ADRU has been reported from several countries in Europe (Bulgaria, France, Germany, Netherlands, Serbia and Spain), North and South America, the Middle East, and Asia (Guiry and Guiry, 2022). ADMO has been reported in all Slovenian neighbouring countries, namely Hungary, Italy, Austria, and Croatia. Buczkó et al. (2022) reported the presence of ADMO in Hungary from 2015 onward and in Austria in several sections of the Danube River from 2013 onward. Falasco et al. (2023) reported on the first findings of ADMO in Italy in 2013 in the rivers of Liguria (NW-Italy). In Croatia, there are records of ADMO in the Drava River and the Danube River from 2019, which were collected in the framework of Joint Danube Survey 4 (JDS4) (ICPDR, 2019). Information on the presence of ADRU in Slovenia's neighbouring countries is scarce. There is a record of ADRU from the Danube River in Austria from 2019, collected in the framework of JDS4 (ICPDR, 2019). However, according to our information, there is no available information on the presence of ADRU in Croatia, Hungary, or Italy.

In this research, the distribution of two potentially invasive, alien benthic diatom species, namely ADMO and ADRU, in the territory of Slovenia is presented for the first time. Moreover, for the sampling sites where ADMO and ADRU were identified, nutrient and organic matter loading, as well as species diversity and evenness, are also reported.

Materials and Methods

Phytobenthos sampling

Sampling of the phytobenthos was carried out in 2019–2024 as part of the national monitoring of surface water quality according to the Water Framework Directive (WFD) (Directive 2000/60/EC). Altogether, 247 phytobenthos samples were collected at 175 sampling sites in rivers, and 35 samples were collected at 32 sampling sites in lakes and reservoirs (hereinafter referred to as lakes) across Slovenia according to the national programmes for monitoring chemical and ecological status of surface waters (ARSO, 2017, 2022). In total, 87 rivers and 11 lakes were included in this research.

According to the Slovenian national methodologies for ecological status assessment of rivers and lakes using phytobenthos and macrophytes (MOP, 2016a, 2016b), river samples were collected once per year from June to September, up to a depth of 60 cm and at a distance of at least 1 m from the riverbanks, or in cases of smaller rivers, at least 10% of the river's width from the banks. A multi-habitat approach was used, meaning that the samples were collected from a range of habitats (e.g., gravel, mud, riffle, and pool) differing in substrate type, depth, current velocity, and shadiness. For lakes, the national methodology applies only to natural lakes (Lake Bled and Lake Bohinj). Multi-habitat sampling was also performed in these and other lakes. Phytobenthos was collected along a 50 m shoreline stretch that included various habitats characterised by varying depth, substrate, and shading conditions. In each lake, phytobenthos was sampled once per year, typically between June and September, and usually at three different sampling sites representing the dominant substrate type. An exception was Lake Vogršček, where only two sites were sampled. Sampling in Lake Pernica deviated from the recommended timeframe, as it was conducted in October, outside the June-September window defined by national methodology for natural lakes. In both rivers and lakes, phytobenthos was removed from the substrate (stones, pebbles, wood, macrophytes, etc.) using a toothbrush in a tray containing a small amount of river or lake water. The material was homogenised and poured into a wide-necked plastic bottle. Each sample was fixed with ethanol to a final concentration of 70%.

Laboratory analyses

The phytobenthos samples were transferred to the laboratory and treated with concentrated nitric acid (HNO_3) to remove cell contents and other organic matter, following the standard procedure (SIST EN 14407, 2014) and the instructions of the Slovenian national methodologies for ecological status assessment of rivers and lakes using phytobenthos and macrophytes (MOP, 2016a, 2016b). Cleaned samples were mounted in Naphrax® (Brunel Microscopes, Chippenham, Wiltshire, UK), a medium with a high refractive index, for permanent slide preparation. Permanent slides were examined using a light microscope (Leica DM RB, Germany) at 1000× magnification. Diatom identification and enumeration were performed according to the standard procedure (SIST EN 14407, 2014) and the Slovenian national methodologies (MOP, 2016a, 2016b). For each permanent slide, at least 500 valves were counted and identified to the species or lower taxonomic level. Diatom identification and nomenclature followed the identification monograph by Lange-Bertalot et al. (2017). Scanning electron microscope (SEM) images were obtained using a JEOL JSM-7500F microscope and sample preparation according to Hasle and Fryxell (1970).

Statistical analyses

The abundance of diatom species was expressed as relative counts (in %). The levels of nutrient and organic loading in the investigated rivers were evaluated using the Trophic Index (TI) (Rott et al., 1999) and Saprobic Index (SI) (Rott et al., 1997), respectively. For the investigated lakes, nutrient loading levels were evaluated using the TI (Rott et al., 1999). Both indices were calculated solely based on diatom data, followed by an assessment of the ecological trophic status (for both rivers and lakes) and the ecological saprobic status (for rivers only), in accordance with the Slovenian national methodologies for ecological status assessment of rivers and lakes using phytobenthos and macrophytes (MOP, 2016a, 2016b).

Species diversity was assessed using the Shannon-Wiener diversity index (SW), and species evenness was assessed using the Evenness index (E). Both indices were calculated using OMNIDIA 6.0.9, a software tool for the calculation of 18 diatom water quality indices. Correlations between the abundance of target species, species diversity, and species evenness were calculated using the Pearson correlation coefficient and Microsoft Excel software.

Results and Discussion

Altogether, 403 diatom taxa were identified in river samples and 238 diatom taxa in lake samples. In Slovenia, benthic diatoms are used, together with macrophytes, phytoplankton, fish, benthic macroinvertebrates, and macroalgae, as a biological quality element for the assessment of the ecological status of surface waters, namely the quality of the structure and functioning of aquatic ecosystems, as defined by the WFD (Directive 2000/60/EC). Two diatom indices, the Trophic index (Rott et al., 1999) and the Saprobic index (Rott et al., 1997), are used in Slovenia to assess nutrient and organic matter loading in rivers, respectively. For natural lakes in Slovenia (Lake Bled and Lake Bohinj), the trophic state is assessed using benthic diatoms and the Trophic index (Rott et al., 1999), in combination with phytoplankton, in accordance with national methodology (MOP, 2016b). For other lakes, where there is no national methodology, benthic diatoms and the Trophic Index (Rott et al., 1999) may still be used as an additional or indicative assessment tool, but are not formally part of the ecological status assessment under national monitoring.

Morphology of Achnanthidium delmontii and Achnanthidium druartii

The morphology of ADMO is described in detail in the work of Pérès et al. (2012) and that of ADRU in the work of Rimet et al. (2010), in which both species were first formally described. ADMO and ADRU both belong to the group of taxa related to *Achnanthidium pyrenaicum* (Hustedt) Kobayasi based on the stria density, which is around 20 µm, and the linear-lanceolate valve shape.

According to Pérès et al. (2012), the main morphological characteristics of ADMO (Figure 1) are as follows: Valves are linear with rounded apices, becoming elliptical in smaller individuals; valve length ranges from 7.3 to 21.4 µm, and valve width from 3.3 to 5.1 µm. On the raphe valve, the axial area is narrow, and the central area is irregular, typically forming a rectangular fascia, but a shortened stria may be present on one of the margins. The raphe is filiform and straight, with distinct central pores. Striae are slightly radial, numbering 20–26 in 10 µm in the central part of the valve, and up to 35 in 10 µm near the apices. On the rapheless valve, the axial area is acicular. Striae are parallel to slightly radiate near the apices; in most cases, two striae in the central part are slightly more widely spaced. Striae are slightly radial, numbering 18–22 in 10 µm in the central part and up to 25 in 10 µm at the apices.

Under light microscopy, ADMO can be distinguished from similar taxa by a rectangular fascia on the raphe valve and a typical irregular cell shape.

The main morphological characteristics of ADRU (Figure 2) according to Rimet et al. (2010) are: Valves are lanceolate with slightly subrostrate ends, never capitate; valve length ranges from 12 to 29 µm, and valve width from 3.9 to 5.8 µm. The raphe sternum is larger in the middle of the valve than in the extremities. The rapheless valve is convex, with a narrow, straight sternum that is only slightly enlarged in the centre. Striae are very weakly radiate throughout on both valves. Occasionally, short striae are inserted near the middle of the valve. For both valves, stria density is 15-22 in 10 µm in the central part of the valve and approximately 40-50 in 10 µm near the apices.

Under light microscopy, ADRU can be distinguished from similar taxa, namely *A. convergens* (Kobayasi) Kobayasi, *A. deflexum* (Reimer) Kingston, *A. japonicum* (Kobayasi) Kobayasi, *A. latecephalum* Kobayasi, *A. pyrenaicum* (Hustedt) Kobayasi and *A. rivulare* Potapova & Ponader, by its generally wider and longer valve dimensions. However, in many cases, the size may overlap (Rimet et al., 2010). According to current knowledge, only *A. pyrenaicum* and *A. rivulare* have been recorded in Slovenia among the above-listed taxa. Another interesting characteristic that differentiates ADRU is that it shows an important stria density difference between the centre and the apices.

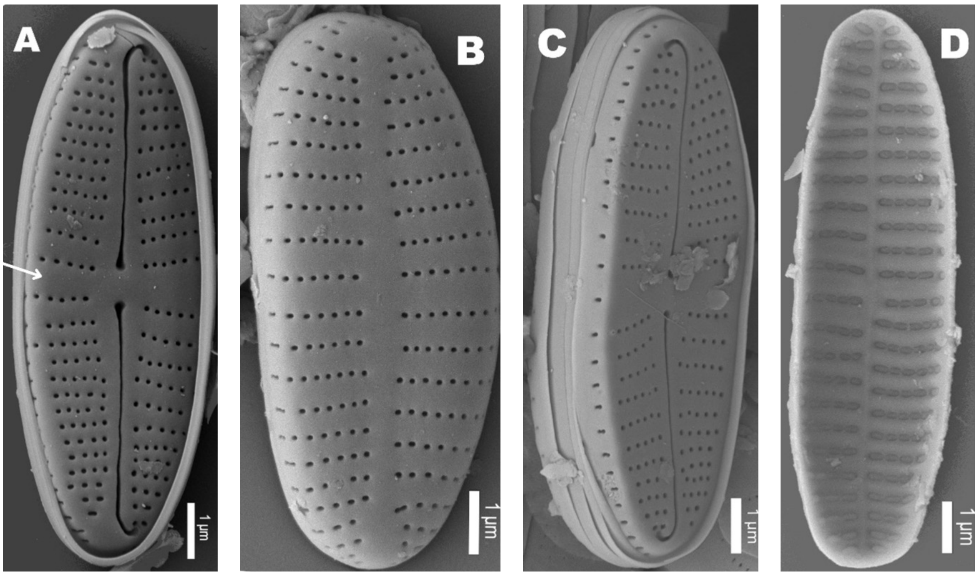

Figure 1. Scanning electron microscope (SEM) images of Achnanthidium delmontii (ADMO). SEM: external view of the raphe valve with a rectangular fascia (marked with an arrow) (A), external view of the rapheless valve (B), external girdle view of the raphe valve (C), internal view of the rapheless valve (D). One of the main characteristics of ADMO is irregular cell shape (B, C, D).

Slika 1. Fotografije z vrstičnim elektronskim mikroskopom (SEM) Achnanthidium delmontii (ADMO). SEM: pogled na zunanji del valve z rafo in dobro vidnim pravokotnim praznim prostorom (puščica) (A), pogled na zunanji del valve brez rafe (B), pogled na zunanji del valve z rafo z bočne strani (C), pogled na notranji del valve brez rafe (D). Ena glavnih značilnosti ADMO je nepravilna oblika celic (B, C, D).

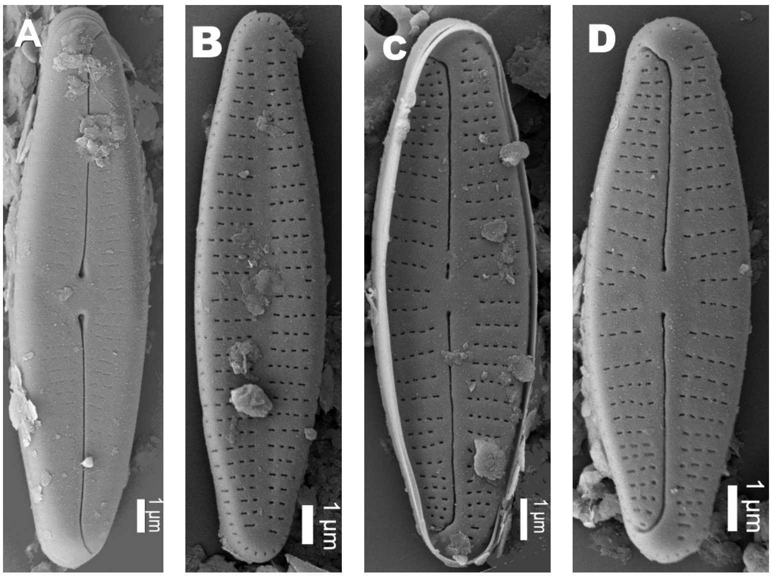

Figure 2. Scanning electron microscope (SEM) images of *Achnanthidium druartii* (ADRU). SEM: external views of the raphe valve (A, D), external view of the rapheless valve (B), internal view of the raphe valve (C).

Slika 2. Fotografije z vrstičnim elektronskim mikroskopom (SEM) *Achnanthidium druartii* (ADRU). SEM: pogled na zunanji del valve z rafo (A, D), pogled na zunanji del valve brez rafe (B), pogled na notranji del valve z rafo (C).

Occurrence of Achnanthidium delmontii and Achnanthidium druartii in Slovenia

Table 1 provides an overview of the sampling sites where ADMO and ADRU were detected, including national sampling site codes, date of sampling, and the relative abundance of both species expressed as a percentage of counted valves. The table also includes information on nutrient and organic matter loading at each site, as well as measures of species diversity and evenness, expressed as the Shannon-Wiener diversity index and the Evenness index, respectively.

Regarding rivers, a total of 247 phytobenthos samples collected at 175 sampling sites located on 87 rivers were included in the study. Among all investigated rivers, ADMO was present in 100 samples (41%) collected at 74 sampling sites (42%) from 40 rivers (46%) (Table 1). ADMO was a dominant species (more than 5% in relative abundance) in 56 phytobenthos samples (23%) collected at 45 sampling sites (26%) from 27 rivers (31%). The highest abundance of ADMO was recorded in the Bolska River (Figure 3), specifically at sampling sites Dolenja vas and Čeplje in August 2022, with relative abundances of 77% and 64%, respectively. Regarding ADMO representation, the Bolska River was followed by the Mirna River, where at the sampling site Dolenji Boštanj, the ADMO relative abundance reached 69%, also in August 2022.

ADRU was present in 32 river samples (13%) collected at 22 sampling sites (13%) from 12 rivers (14%) (Table 1). ADRU was a dominant species (more than 5% in relative abundance) in 11 phytobenthos samples (5%) collected at eight sampling sites (5%) from 4 rivers (5%). The highest abundance of ADRU was detected in the Drava River (Figure 4), specifically at sampling site Ruše in September 2021, with a relative abundance of 27%, followed by sampling site Ranca in August 2024 with 20%, and sampling site Tribej in September 2021 with a relative abundance of 19%.

In Slovenian lakes, 35 phytobenthos samples were collected at 32 sampling sites across 11 lakes. Among the two investigated species, ADMO was relatively rare, being detected in only five samples (14%) across three lakes. It was never dominant in any of the samples, with the highest recorded abundance reaching just 0.38% in the Gajševsko Lake (GaFB03, June 2023). In contrast, ADRU was more widespread and frequently encountered, being present in 15 samples (43%) in 8 of the 11 studied lakes. It showed dominance (relative abundance >5%) in 3 samples from two lakes. The highest relative abundance of ADRU was observed in Lake Slivnica (10% at SlFB04, May 2023), followed by Lake Pernica (6.6% at P2FB05, October 2024).

Table 1. List of sampling sites where invasive diatoms *Achnanthidium delmontii* (ADMO) and *Achnanthidium druartii* (ADRU) were detected, including national sampling site codes, date of sampling, relative abundance of both species expressed in percentage of counted valves, Trophic index (TI), Saprobic index (SI), ecological trophic status (EQR TI), ecological saprobic status (EQR SI), Shannon-Wiener diversity index (SW), and Evenness index (E). + indicates that the species was observed during the qualitative examination of the sample but not during valve counting. / indicates that the ecological status assessment methodology has not been developed. Blue colour indicates high ecological status, green indicates good ecological status, and yellow indicates moderate ecological status.

Tabela 1. Seznam vzorčnih mest z ugotovljeno prisotnostjo invazivnih diatomej *Achnanthidium delmontii* (ADMO) in *Achnanthidium druartii* (ADRU) z navedenimi nacionalnimi šiframi vzorčnih mest, datumom vzorčenja, relativno pogostostjo obeh vrst izraženo v odstotkih od preštetih polovic lupinic, trofičnim indeksom (TI), saprobnim indeksom (SI), ekološkim trofičnim (EQR TI) in ekološkim saprobnim (EQR SI) stanjem, Shannon-Wiennerjevim diverzitetnim indeksom (SW) in indeksom Evenness (E). Z znakom + je označeno, da je bila vrsta zaznana le med kvalitativnim pregledom vzorca in ne med štetjem polovic lupinic. Z znakom / je označeno, da metodologija vrednotenja ekološkega stanja ni razvita. Modra barva označuje zelo dobro ekološko stanje, zelena označuje dobro ekološko stanje in rumena zmerno ekološko stanje.

River/Lake	Sampling site	Date of sampling	ADMO (%)	ADRU (%)	TI	SI	EQR TI	EQR SI	SW	E
Mura	Bad Radkersburg	21.01.2021	33.84		2.63	2.03	0.71	0.60	3.45	0.77
Mura	Bad Radkersburg	30.08.2024	17.15		1.92	1.69	1.00	1.00	2.68	0.62
Mura	Ceršak	21.01.2021	11.04		2.90	2.09	0.61	0.58	3.96	0.82
Mura	Ceršak	30.08.2024	4.16	+	2.28	1.87	0.85	0.81	3.35	0.73
Mura	Mele	21.01.2021	6.13		2.93	2.07	0.61	0.58	3.84	0.78
Mura	Mele	30.08.2024	11.30		1.93	1.71	1.00	1.00	3.02	0.66
Mura	Mota	14.02.2023	0.78		2.71	1.87	0.69	0.80	3.85	0.78
Mura	Gibina	14.02.2023	5.86		3.15	2.16	0.47	0.55	3.75	0.77
Ledava	Domajinci	7.03.2019		2.39	2.72	1.89	0.75	0.77	4.07	0.75

River/Lake	Sampling site	Date of sampling	ADMO (%)	ADRU (%)	TI	SI	EQR TI	EQR SI	SW	E
Drava	Tribej	22.09.2021		18.70	2.52	1.94	0.75	0.71	4.4	0.82
Drava	Tribej	25.09.2024		17.81	2.34	1.89	0.83	0.77	3.85	0.74
Drava	Ruše	22.09.2021	12.08	26.53	2.28	1.79	0.85	0.93	3.86	0.75
Drava	Ruše	21.08.2024	3.28	13.68	2.19	1.76	0.89	0.96	3.96	0.77
Drava	Krčevina pri Ptuju	22.09.2021	21.97	1.98	2.04	1.66	0.96	1.00	3.12	0.67
Ptujsko jezero	Ranca	16.09.2021	0.39	13.80	1.83	1.51	1.00	1.00	3.87	0.76
Drava	Ranca	21.08.2024		19.68	2.55	1.69	0.74	1.00	4	0.75
Drava	Prepolje	6.08.2019	0.19	5.78	2.88	2.03	0.62	0.60	4.53	0.79
Drava	Prepolje	24.08.2022		1.93	2.80	1.90	0.65	0.76	4.53	0.85
Drava	Gorišnica	6.08.2019	0.77	4.44	2.67	1.90	0.70	0.76	4.37	0.82
Drava	Gorišnica	24.08.2022	5.08		2.83	1.98	0.64	0.66	4.54	0.83
Drava	Borl	16.09.2021	55.00	3.14	2.57	2.06	0.73	0.59	2.49	0.57
Drava	Borl	23.08.2024	12.28	1.56	2.52	1.71	0.75	1.00	3.84	0.75
Drava	Ormož	6.08.2019	4.79	1.80	2.63	1.98	0.71	0.66	5.01	0.83
Drava	Ormož	23.12.2020	4.23	0.38	2.54	1.99	0.75	0.64	4.41	0.79
Drava	Ormož	4.08.2022	2.47	0.38	2.95	1.94	0.59	0.70	4.61	0.83
Drava	Ormož	23.08.2024	5.81	6.97	2.54	1.77	0.75	0.95	4.39	0.79
Drava	Grabe	15.09.2021	26.21	+	1.50	1.48	1.00	1.00	2.03	0.55
Meža	Topla	17.07.2024	+		1.93	1.58	0.94	1.00	2.87	0.63
Mislinja	Mala vas	17.07.2024	0.39		1.85	1.53	1.00	1.00	2.33	0.57
Mislinja	Otiški vrh	17.07.2024	0.78		2.15	1.67	0.98	0.92	2.72	0.59
Dravinja	Videm pri Ptuju	6.08.2024	15.5		1.84	1.50	1.00	1.00	2.54	0.59
Pesnica	Zamušani	21.09.2023	1.89		2.28	1.98	0.96	0.74	3.98	0.85
Sava Dolinka	Zelenci	11.07.2023	1.75		1.43	1.41	1.00	1.00	3.07	0.61
Sava Bohinjka	nad izlivom Jezernice	29.08.2022	1.37		2.14	1.65	1.00	1.00	3.91	0.82
Sava Bohinjka	Bodešče	29.08.2022	4.89		2.54	2.02	1.00	0.75	2.66	0.58
Sava	Struževo	28.07.2021	6.9		1.39	1.51	1.00	1.00	2.72	0.72
Sava	Dragočajna	21.06.2022	1.36	0.39	1.78	1.65	1.00	1.00	3.71	0.71
Sava	Dragočajna	14.08.2024		9.23	2.45	1.79	0.70	0.77	4.68	0.87
Sava	Medno	14.08.2024	12.08		1.70	1.53	1.00	1.00	2.57	0.58
Sava	Šentjakob	26.07.2019	0.78		1.84	1.78	1.00	0.78	3.06	0.6
Sava	Kresnice	12.07.2023	4.9		1.82	1.56	1.00	1.00	2.53	0.58
Sava	Podkraj	16.08.2024	9.76		2.00	1.55	0.95	1.00	2.86	0.59
Sava	Vrhovo	20.08.2021	8.57		2.66	1.98	0.70	0.66	4.31	0.83
Sava	Vrhovo	23.06.2023	0.39		2.34	1.94	0.82	0.71	4.08	0.74
Sava	Brestanica	20.08.2021	1.86		2.81	2.03	0.65	0.59	4.04	0.74
Sava	Podgračeno	14.09.2021	0.77		2.53	1.99	0.75	0.64	3.95	0.76
Sava	Jesenice na Dolenjskem	21.08.2019	50.65		2.68	1.92	0.69	0.74	2.95	0.56
Sava	Jesenice na Dolenjskem	11.08.2020	1.36		2.23	1.59	0.88	1.00	3.1	0.6
Sava	Jesenice na Dolenjskem	14.09.2021	6.81		2.62	2.07	0.72	0.58	3.93	0.78
Sava	Jesenice na Dolenjskem	17.08.2022	4.64	0.77	2.85	2.01	0.63	0.61	3.98	0.79
Sava	Jesenice na Dolenjskem	9.10.2023	9.3		2.03	1.75	0.97	0.98	3.61	0.74
Sava	Jesenice na Dolenjskem	14.08.2024	16.01		1.61	1.48	1.00	1.00	1.93	0.49
Kokra	Kranj	26.07.2021	28.85		1.89	1.61	1.00	1.00	3.29	0.71
Sora	Medvode	22.06.2021	31.52		1.44	1.45	1.00	1.00	1.67	0.42

River/Lake	Sampling site	Date of sampling	ADMO (%)	ADRU (%)	TI	SI	EQR TI	EQR SI	SW	E
Poljanska Sora	Na Dobravi	1.07.2019	1.32		1.90	1.72	1.00	1.00	3.21	0.67
Poljanska Sora	Na Dobravi	22.06.2021	26.21		1.48	1.48	1.00	1.00	2.27	0.58
Selška Sora	Vešter	22.06.2021	6.83		1.76	1.59	1.00	1.00	3.31	0.78
Kamniška Bistrica	Ihan	26.08.2024	28.37		2.34	1.70	1.00	1.00	3.19	0.64
Kamniška Bistrica	Beričevo	26.08.2024	3.1		2.46	1.88	1.00	0.87	2.38	0.49
Pšata	Bišče	1.07.2021	13.23		2.74	1.99	0.52	0.73	4.09	0.79
Pšata	Bišče	11.07.2024	21.79		2.74	1.97	0.52	0.74	4.18	0.77
Mirna	Dolenji Boštanj	17.06.2019	0.39		2.60	2.06	0.58	0.71	4.05	0.76
Mirna	Dolenji Boštanj	1.08.2022	68.95	0.30	2.47	1.87	0.64	0.78	1.99	0.42
Sotla	Rigonce	14.09.2021		0.39	2.64	1.92	1.00	1.00	3.44	0.69
Bistrica	Zagaj	17.06.2019	25.58		2.06	1.58	1.00	1.00	3.48	0.73
Kolpa	Osilnica	4.07.2024	6.99		1.42	1.55	1.00	1.00	3.14	0.71
Kolpa	Radenci	14.06.2021	44.53		1.51	1.53	1.00	1.00	2.75	0.62
Kolpa	Radoviči (Metlika) - Bubnjarci	4.08.2021	24.43	0.38	2.11	1.60	0.68	1.00	4.03	0.76
Kolpa	Radoviči (Metlika) - Bubnjarci	4.07.2024	14.45	0.40	2.29	1.68	0.61	1.00	4.37	0.86
Lahinja	Geršiči	12.09.2023	+	5.56	2.32	1.67	0.76	1.00	4.23	0.81
Gruberjev prekop	Ljubljana	25.07.2022	0.39		2.73	2.02	0.56	0.75	4.26	0.81
Gruberjev prekop	Ljubljana	13.09.2023	5.09		2.76	1.99	0.55	0.76	3.83	0.79
Iščica	Ižanska cesta	17.08.2022	+		2.54	1.95	0.61	0.72	3.7	0.78
Iščica	Ižanska cesta	11.07.2024	0.78		2.56	1.86	0.60	0.75	3.21	0.69
Mali Graben	Dolgi most	21.06.2022	1.15		2.40	1.73	0.67	1.00	3.16	0.64
Gradaščica	Dvor	21.06.2022	15.59		2.15	1.73	1.00	1.00	3.29	0.71
Savinja	Grušovlje	16.07.2024	1.94		2.05	1.52	1.00	1.00	2.34	0.54
Savinja	Medlog	4.09.2024	5.03		1.98	1.57	1.00	1.00	2.46	0.57
Savinja	Veliko Širje	4.09.2024	17.58		2.05	1.63	1.00	1.00	3.03	0.65
Dreta	Spodnje Kraše	10.09.2021	15.67		2.16	1.70	1.00	1.00	3.48	0.7
Paka	Ločan	10.09.2021	8.43		2.60	1.87	0.77	1.00	3.82	0.75
Paka	Šoštanj	16.07.2024	2.73		2.47	1.93	1.00	1.00	3.23	0.67
Paka	Slatina	16.07.2024	10.4		3.05	2.54	0.69	0.60	3.06	0.73
Bolska	Čeplje	2.08.2022	63.99		2.51	1.95	1.00	0.92	2.12	0.45
Bolska	Dolenja vas	2.08.2022	76.67		2.03	1.81	1.00	1.00	1.59	0.33
Gračnica	Gračnica	25.08.2023	8.54		1.87	1.64	1.00	1.00	2.59	0.61
Krka	Soteska	20.08.2020	0.19		2.38	1.94	0.68	0.78	3.31	0.64
Krka	Krška vas	20.08.2020	1.74		2.50	1.86	0.92	1.00	4.32	0.8
Radulja	Grič pri Klevevžu	25.08.2023		3.50	1.85	1.65	1.00	1.00	2.97	0.64
Soča	Solkanski jez	30.08.2019	4.14		1.73	1.37	1.00	1.00	3.43	0.7
Soča	Solkanski jez	26.08.2020	4.82		1.77	1.47	0.99	1.00	3.12	0.69
Idrijca	nad Divjim jezerom	15.06.2021	3.88		1.41	1.49	0.91	1.00	3.09	0.7
Idrijca	Hotešk	18.09.2020	59.72		2.22	1.90	0.64	0.77	2.36	0.53
Trebuščica	Most pri Sovi	5.10.2023	8.15		1.71	1.59	0.74	1.00	3.02	0.72
Bača	Grapa	5.10.2023	8.81		1.55	1.44	1.00	1.00	1.39	0.37
Vipava	Velike Žablje	27.08.2020	1.51		2.18	1.88	0.83	0.87	2.91	0.63
Vipava	Velike Žablje	18.07.2023	0.77		2.39	1.82	0.73	1.00	2.2	0.45
Vipava	Miren	27.08.2020	5.71		2.66	1.90	0.43	0.73	3.92	0.76
Vipava	Miren	18.07.2023	5.87		2.63	1.94	0.44	0.71	4.05	0.8

River/Lake	Sampling site	Date of sampling	ADMO (%)	ADRU (%)	TI	SI	EQR TI	EQR SI	SW	E
Hubelj	Ajdovščina	5.03.2021	+		1.69	1.67	1.00	1.00	3.17	0.67
Nadiža	Robič	3.07.2024	3.14	0.39	1.65	1.65	0.98	1.00	3.18	0.76
Reka	Podgraje	10.03.2021	2.09		1.75	1.78	1.00	0.78	3.56	0.78
Reka	Topolc	21.06.2021	27.72		1.94	1.63	1.00	1.00	2.86	0.6
Reka	Topolc	7.09.2023	25.48		2.48	1.77	0.75	1.00	3.33	0.72
Reka	Cerkvenikov mlin	8.03.2021	6.73		2.39	1.98	1.00	0.82	4.21	0.83
Klivnik	Brid	25.02.2020		1.17	1.54	1.48	1.00	1.00	2.28	0.48
Molja	Zarečica	13.09.2023	6.65		2.36	1.81	0.81	0.77	4.33	0.8
Rižana	Dekani nad pregrado	17.06.2022	12.4		2.34	1.81	0.75	1.00	3.43	0.72
Rižana	Dekani nad pregrado	7.09.2023	14.85		1.60	1.53	1.00	1.00	3.25	0.72
Dragonja	Planjave	8.03.2021		4.31	1.37	1.46	1.00	0.80	3.29	0.71
Dragonja	Podkaštel	8.03.2021		5.34	1.65	1.71	0.98	0.72	3.74	0.74
Blejsko jezero	BlFB08	12.08.2019		0.20	2.41		0.43		3.52	0.68
Bohinjsko jezero	BOFB05	21.06.2022	+		1.32		0.75		3.87	0.74
Bohinjsko jezero	BOFB08	21.06.2022	+		1.25		0.77		3.77	0.74
Šmartinsko jezero	SmFB04	26.05.2023		1.23	2.02				4.64	0.87
Šmartinsko jezero	SmFB05	26.05.2023		0.97	1.59				3.04	0.61
Šmartinsko jezero	SmFB06	26.05.2023		1.95	1.57				3.77	0.77
Slivniško jezero	SlFB02	29.05.2023		5.34	2.12				3.8	0.76
Slivniško jezero	SlFB04	29.05.2023		10.02	2.30				3.77	0.75
Slivniško jezero	SlFB05	29.05.2023		4.30	2.43				3.91	0.76
Gajševsko jezero	GaFB02	14.06.2023	+		2.67				3.85	0.75
Gajševsko jezero	GaFB03	14.06.2023	0.38	+	2.64				4.05	0.77
Mola	MoFB02	1.08.2024		0.38	1.80				2.23	0.54
Vogrščček	V2FB04	7.08.2024		0.60	1.62				2.65	0.65
Vogrščček	V2FB06	7.08.2024		0.38	1.61				3.45	0.68
Klivnik	KLFB03	8.08.2024	+	+	1.68				2.62	0.63
Perniško jezero 2	P2FB03	23.10.2024		2.94	3.17				4.36	0.8
Perniško jezero 2	P2FB05	23.10.2024		6.61	2.98				2.3	0.54
Perniško jezero 2	P2FB06	23.10.2024		+	3.06				2.85	0.64

For the purpose of this study, SW and E of the samples with ADMO and/or ADRU were recorded (Table 1). Moreover, the correlations between ADMO and ADRU abundance and SW and E were calculated using the Pearson correlation coefficient. The results showed moderate negative correlations of -0.46 and -0.50 between ADMO abundance and SW, and ADMO abundance and E, respectively. These results indicate that the presence of ADMO in the samples is associated with lower diatom species diversity and lower diatom species evenness, which is most evident in the samples with the highest abundance of ADMO (Table 1). High abundance of ADMO in the samples, reaching up to 77%, is most probably due to its invasive character. According to Buczkó et al. (2022), AD, MO has a wide ecological range, which serves to confirm its potential invasive behaviour. High biodiversity and high species evenness are often associated with balanced environmental conditions and low anthropogenic pressure, which reflect good or high ecological status of water bodies. However, in the dataset used in this research, the river sections in which ADMO abundance was the highest (more than 60%) and diatom species diversity and evenness were the lowest are associated with good or high ecological saprobic and ecological trophic status (Table 1). Moreover, ADMO was present mainly in the upland river sections (Figure 5), which are, in most cases, hydromorphologically undisturbed, which corresponds with the findings of Falasco et al. (2023). We can conclude that even if ADMO is very abundant, the consequent disturbance of the aquatic environment is not noticed, at least as far as the physical appearance of river sections (Figure 3) and ecological saprobic and trophic status assessment are concerned. These findings are in accordance with the findings from the literature (Buczkó et. al., 2022). In the lakes, ADMO was detected mainly during the qualitative inspection of the samples (Lake Bohinj, Gajševsko Lake and Lake Klivnik). With a slightly higher abundance of 0.4% ADMO was detected only in one sample in Gajševsko Lake in June 2023 (Table 1).

The results of the correlations between ADRU abundance and SW and E were the opposite compared with ADMO, showing a weak positive correlation of 0.25 and 0.23 between ADRU abundance and SW, and between ADRU abundance and E, respectively. Rimet et al. (2010) considered ADRU as an invasive species; however, in the dataset used in this research, ADRU was present in much lower proportions (up to 27%) compared to ADMO (up to 77%) and did not affect the species diversity and evenness of the diatom assemblages in the samples. These findings are in accordance with other studies in which the invasiveness of ADRU could not be confirmed (Ivanov, 2018). In the dataset used in this research, ADRU was present mainly in the lowland river sections, lakes and reservoirs (Figure 6), which corresponds with the findings of Ivanov (2018). ADRU reached the highest abundance in the Drava River, a river with a high number of reservoirs above the dams of Hydro-Power Plants, namely at the sampling sites Ranca, Ruše, and Tribej. In the dataset used in this research, the river sections in which ADRU abundance was the highest (>5%) are associated with good or high ecological saprobic and ecological trophic status (Table 1). Among the 11 lakes included in this study, ADRU was confirmed in eight, with the highest abundance of 10% recorded in Lake Slivnica in May 2023. In Slovenia, there are two natural lakes, namely the subalpine Lake Bled and the alpine Lake Bohinj. ADRU was found in Lake Bled in August 2019 with a low abundance of 0.2%; however, in Lake Bohinj, which is considered one of the most ecologically pristine lakes in Slovenia (good ecological status; Table 1), it was not found. Moreover, Lake Bled was included in the dataset used in this research in the years 2019 and 2022; however, ADRU was found only in one sample in 2019 and was not detected again in 2022.

The distribution of ADMO and ADRU in Slovenian rivers and lakes in the years 2019–2024, accompanied by the relative abundance of both species expressed in percentage, is presented in Figures 5 and 6. Buczkó et al. (2022) found that ADMO started to spread in the Danube River from the source downstream. In 2013, it was found in Germany and Austria, and three years later, it was found in the Hungarian section of the Danube River. However, this pattern was not confirmed in Slovenian rivers.

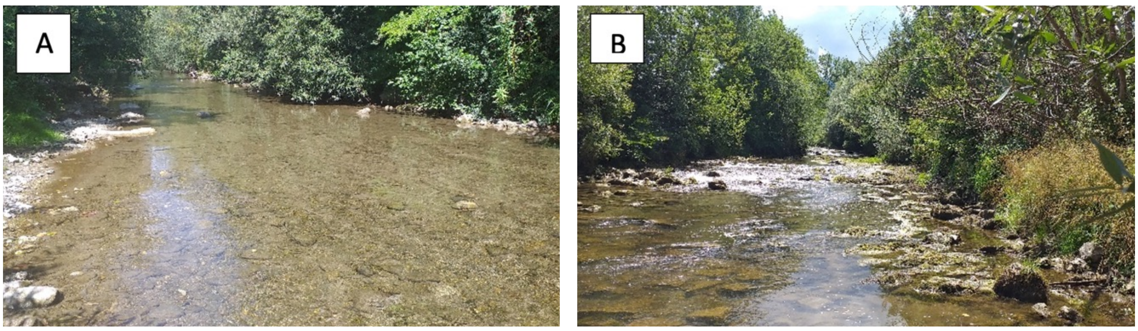

Figure 3. River Bolska has the highest occurrence of invasive benthic diatom *Achnanthidium delmontii* (ADMO) in Slovenia. A-Bolska-Čeplje and B-Bolska-Dolenja vas in August 2022. Photo: archive ARSO.

Slika 3. Reka Bolska z najvišjo številčnostjo invazivne bentoške diatomeje *Achnanthidium delmontii* (ADMO) v Sloveniji. A-Bolska-Čeplje in B-Bolska-Dolenja vas avgusta 2022. Foto: arhiv ARSO.

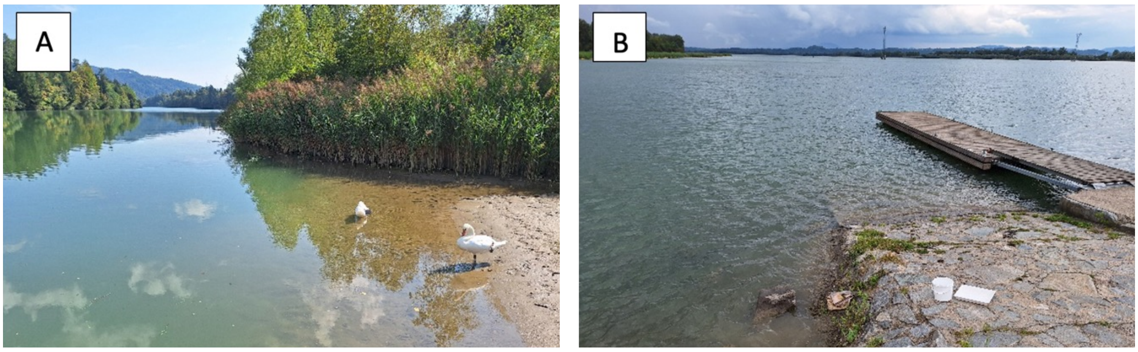

Figure 4. River Drava has the highest abundance of invasive alien diatom species, *Achnanthidium druartii* (ADRU), in Slovenia. A-Drava-Ruše and B-Drava-Ranca in August 2024. Photo: archive ARSO.

Slika 4. Reka Drava z najvišjo številčnostjo invazivne tujerodne vrste diatomeje *Achnanthidium druartii* (ADRU) v Sloveniji. A-Drava-Ruše in B-Drava-Ranca avgusta 2022. Foto: arhiv ARSO.

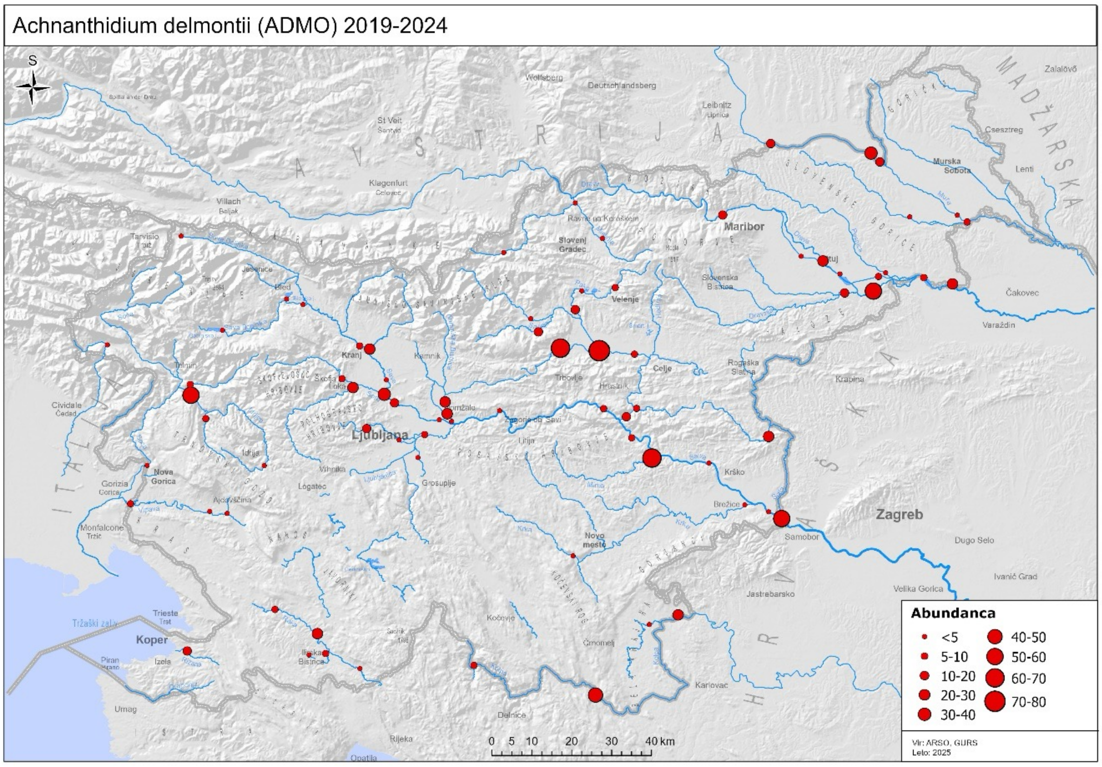

Figure 5. Distribution and relative abundance (in percentage) of *Achnanthidium delmontii* (ADMO) in Slovenia between 2019 and 2024.

Slika 5. Porazdelitev in relativna abundanca (v odstotkih) vrste *Achnanthidium delmontii* (ADMO) v Sloveniji med leti 2019 in 2024.

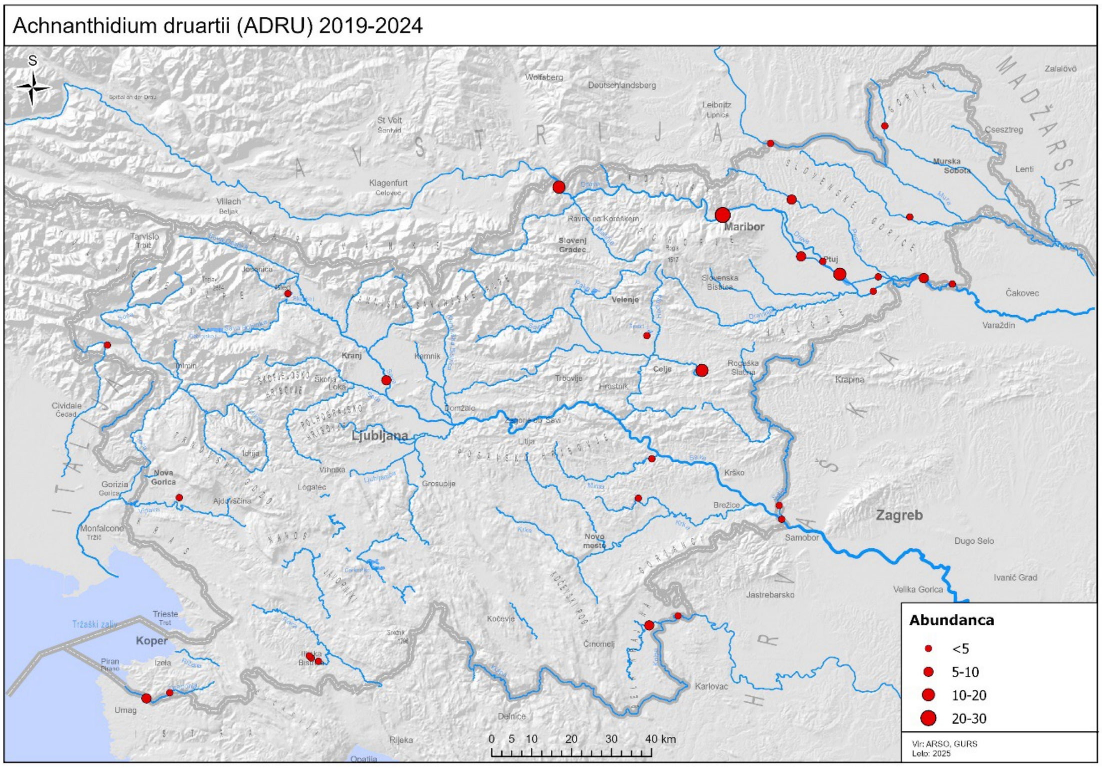

Figure 6 Distribution and relative abundance (in percentage) of *Achnanthidium druartii* (ADRU) in Slovenia between 2019 and 2024.

Slika 6. Porazdelitev in relativna abundanca (v odstotkih) vrste *Achnanthidium druartii* (ADRU) v Sloveniji med leti 2019 in 2024.

The first record of ADMO and ADRU in Slovenia is from the national monitoring of surface waters in 2019. The most probable reason is that in 2019, the Slovenian Environment Agency switched from the diatom taxonomy of Süßwasserflora von Mitteleuropa (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b) to the taxonomy of Freshwater Benthic Diatoms of Central Europe (Lange-Bertalot et al., 2017). In the monograph of Lange-Bertalot et al. (2017), both ADMO and ADRU were already included, while in older monographs, they are missing since both species were only formally described as new diatom species in 2012 (ADMO) and 2010 (ADRU). Although ADMO was formally first observed in Slovenia in 2019, it was most likely present in Slovenian freshwaters several years earlier and had been counted under *Achnanthes biasolettiana* Grunow, which is currently regarded as a synonym of *Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi. One reason to assume the earlier occurrence of ADMO in Slovenia is that it had already been reported in the freshwaters of neighbouring countries: as early as 2013 in Austria (Buczkó et al., 2022) and Italy (Falasco et al., 2023), and in 2015 in Hungary (Buczkó et al., 2022). Buczkó et al. (2022) reported that in 2013, when ADMO was first recorded in German and Austrian sections of the Danube River, it was present in low abundances (<3%); however, by 2019, ADMO had become one of the most abundant and frequent diatom species found in the Danube River. Given that ADMO, when first recorded in Slovenian samples in 2019, was already present with very high abundances, reaching up to 51% in Sava - Jesenice na Dolenjskem (Table 1, Figure 5), we can conclude that it had already been present in Slovenia well before 2019. In contrast, according to our information, there are no records of ADRU occurring in neighbouring countries before 2019, and thus it may not have been present in Slovenian waters earlier. Furthermore, ADRU, when first recorded in Slovenian samples in 2019, showed a relative abundance of up to 6% in Drava - Prepolje, which is 8.5 times lower than that of ADMO. There is a possibility that ADRU will be more widely distributed in Slovenian waters with a higher relative abundance after a certain amount of time, as was the case with ADMO. The data presented in this study show only the present state, which might be temporally biased. Although there are no records of ADRU in Croatia, Hungary, and Italy, we can conclude from its presence in Slovenian rivers near the border of the above-mentioned countries, such as the Kolpa, Sotla, Dragonja, Ledava, and Nadiža (Table 1, Figure 6), that ADRU is most likely present in the territory of those countries.

Although diatoms are useful indicators of various pressures such as nutrient and organic loading, acidification, or salinity in running and standing surface waters, ADMO and ADRU are not yet considered as indicator organisms. However, ongoing research is exploring their potential as indicator organisms, which would be reasonable given their increasing prevalence across Europe.

Conclusions

Altogether 247 phytobenthos samples collected at 87 rivers and 35 phytobenthos samples collected at 11 lakes in the frame of the national monitoring of surface water quality from 2019 to 2024 were included in this study. *Achnanthidium delmontii* (ADMO) was present in 100 river samples (41%) from 40 rivers (46%) and was a dominant species in 56 phytobenthos samples (23%) from 27 rivers (31%). The highest abundance of ADMO was recorded in the Bolska River at sampling sites Dolenja vas and Čeplje with relative abundances of 77% and 64%, respectively. *Achnanthidium druartii* (ADRU) was present in 32 river samples (13%) from 12 rivers (14%) and was a dominant species in 11 phytobenthos samples (5%) from 4 rivers (5%). The highest abundance of ADRU was detected in the Drava River at sampling site Ruše with a relative abundance of 27%. In lakes, ADMO was relatively rare, being detected in only five samples (14%) across three lakes and was never dominant in any of the lake samples. ADRU was present in 15 samples (43%) from 8 lakes and was a dominant species in 3 samples from two lakes. The highest relative abundance of ADRU was observed in Lake Slivnica with a relative abundance of 10%.

The results of this study show that the presence of ADMO in the samples is associated with lower diatom species diversity and evenness, indicating, together with very high relative abundances, its invasive character. However, the river sections in which ADMO abundance was the highest and diatom species diversity and evenness were the lowest are associated with good or high ecological saprobic and ecological trophic status. Moreover, ADMO was present mainly in the upland river sections, which are, in most cases, hydromorphologically undisturbed. Although ADMO was present in the investigated rivers in very high numbers, the consequent disturbance of the aquatic environment was not noticed, at least as far as the physical appearance of river sections and ecological saprobic and trophic status assessment are concerned. ADRU, which was present mainly in the lowland river sections and lakes, did not affect the species diversity and evenness of the diatom assemblages in the samples, and thus its invasive character could not be confirmed.

Author Contributions

Conceptualization, A.K.K.; methodology, A.K.K. and T.Š.; investigation, A.K.K. and T.Š.; resources, A.K.K.; data curation, A.K.K. and T.Š.; writing—original draft preparation, A.K.K.; writing—review and editing, A.K.K. and T.Š.; funding acquisition, A.K.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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