water

Article

Distribution Range of the Endangered Species *Unio crassus* Philipsson, 1788 in Serbia (Western Balkans Region), Historical and Recent Data

Jelena Tomović [1,*], Vladica Simić [2], Ana Petrović [2], Ana Atanacković [1], Katarina Zorić [1], Momir Paunović [1] and Maja Raković [1]

[1] Department of Hydroecology and Water Protection, Institute for Biological Research "Siniša Stanković" National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia; adjordjevic@ibiss.bg.ac.rs (A.A.); katarinas@ibiss.bg.ac.rs (K.Z.); mpaunovi@ibiss.bg.ac.rs (M.P.); rakovic.maja@ibiss.bg.ac.rs (M.R.)

[2] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia; vladica.simic@pmf.kg.ac.rs (V.S.); ana.petrovic@pmf.kg.ac.rs (A.P.)

* Correspondence: jelena.tomovic@ibiss.bg.ac.rs

Abstract: The thick-shelled river mussel, *Unio crassus* Philipson, 1788, is considered to be one of the species with the highest conservation priority in Serbia. The study represents the first comprehensive research of the distribution of *U. crassus* in Serbian waters. The research covered a variety of waterbody types throughout Serbia, and distribution data were considered over three time periods from 1953 to 2019. The paper summarizes all the available literature data, field research and information obtained during the review of the collection of malacological material of the Natural History Museum in Belgrade. The results show a positive population trend, which is reflected in an extension of the distribution area and an increase in population density. After reviewing the museum collection, 13 synonyms for *U. crassus* were identified. The study also revealed a better insight into the habitat requirements and the limiting factors of the species. Substrate characteristics, waterbody types, altitude, and nitrate content of the water seem to be of great importance for the occurrence of the species. The results presented here can improve further measures for the conservation of *U. crassus*, not only in Serbia, but also in the Western Balkans.

Keywords: freshwater mussels; distribution history; re-identification; conservation; Serbia

Citation: Tomović, J.; Simić, V.; Petrović, A.; Atanacković, A.; Zorić, K.; Paunović, M.; Raković, M. Distribution Range of the Endangered Species *Unio crassus* Philipsson, 1788 in Serbia (Western Balkans Region), Historical and Recent Data. *Water* **2023**, *15*, 4248. https://doi.org/10.3390/w15244248

Academic Editor: Michele Mistri

Received: 7 November 2023
Revised: 5 December 2023
Accepted: 7 December 2023
Published: 11 December 2023

1. Introduction

Freshwater mussels (Unionida: Unionidae) are one of the most important and widespread groups of aquatic organisms, found in a variety of freshwater habitats, throughout the world. These bivalves are an essential component of freshwater [1] and contribute to sediment stabilization, nutrient cycling and water purification with positive effects on freshwater biodiversity [1–3]. The Unionidae (also known as bivalves, naiads and unionids) are among the most threatened faunistic groups at a global level [4–10]. Of the 16 European species of the order Unionida, nine have the status of near-threatened, endangered, or critically endangered according to the IUCN Red List [10,11]. For this reason, comprehensive environmental and population studies are very important from a conservation perspective. Mussels also have economic importance as a food source and in the ornamental industry. Their over-exploitation for industrial purposes has led to the population decline of some species in many regions [12,13] and even to their local disappearance.

The thick-shelled river mussel, *Unio crassus* Philipsson, 1788 is currently listed by the IUCN classification as endangered-EN at a global level [14]. It is listed in Annexes II and

IV of the European Commission Habitats Directive [15], and in Resolution 6 of the Bern Convention [16]) but is also covered by Serbian legislation [17]. These legislations promote the conservation of unionids, including habitat restoration and the reintroduction of mussels and host fish [18,19].

The native distribution area of *U. crassus* extends from France in the west to western Russia in the east, and from Scandinavia in the north to Asia Minor in the southeast. It was also recorded in the basins of the Baltic Sea, the Black Sea, the Azov Sea and the Caspian Sea, up to the Ural River basin in Russia and Kazakhstan [10]. The species is widespread in Europe, with the exception of Great Britain, the Apennines and the Iberian Peninsula, where its occurrence has not been recorded [10,14].

Until the first half of the 20th century, *U. crassus* was the most abundant unionid species in Europe [20]. Recently, declining population densities and the endangered status of *U. crassus* have been observed in most European countries, especially in Western and Central Europe [19]. According to the latest evidence, the decline of *U. crassus* in Europe is estimated to be more than 50% [19]. The species is listed in the national Red List as critically endangered in Switzerland, Austria and Germany with only a few intact populations remaining [21,22], endangered in the Czech Republic, Poland and Sweden, and vulnerable in Albania, Belarus, Finland and Latvia [14].

Insufficient knowledge of the unionids, their current status and the quantification of population changes over time are all problems for further research on population trends and for determining effective conservation measures not only in Serbia but also in other European countries. *U. crassus* is a strictly protected species in Serbia according to national legislation. Taking into account the new population data, we assumed that the population trend in Serbia is continuously changing. To confirm these assumptions, population changes over time need to be documented and quantified, including data on the distribution history of the species. It is also important to understand which factors are potentially responsible for the changes over time.

The aim of this study was to use a large amount of distribution data to gain a better insight into the distribution range of the species *U. crassus* in different time periods; to identify habitat preferences; to discuss anthropogenic factors affecting the distribution of the species; and to solve the problem of using many unaccepted synonyms and not confidently identifying *U. crassus* in the past in Serbia.

To achieve these goals, this manuscript compiles all known records of *U. crassus* in Serbia from the literature and unpublished sampling data up to 2019. The information presented here will significantly improve our knowledge of the current situation of *U. crassus* in Serbia and support the conservation of unionids and their habitats.

2. Materials and Methods

2.1. Data Collection

The data used for the analysis of the distribution of *U. crassus* in Serbia cover the period 1953–2019. The distribution was estimated based on all available data: (1) peer-reviewed articles, monographs, dissertations and reports [23–32]; (2) unpublished data on samples collected during field research of several national projects in Serbia (material deposited in the malacological collection of the Institute for Biological research "Siniša Stanković", University of Belgrade—further referred as the IBISS); (3) material collected during the realization of four international projects (material deposited in the malacological collection of IBISS) [33–36]; (4) BAES database—biodiversity in aquatic ecosystems in Serbia, ex situ conservation [37]; and (5) collection of unionids of the Natural History Museum in Belgrade (collector Ante Tadić)—referred to as historical data in remaining text.

2.2. Historical Data (1953–1973)

In order to determine the historical distribution of *U. crassus* in Serbia, the museum collection of unionids was reviewed (collector of Ante Tadić). The analyzed historical material consisted of 244 individuals from 36 sites in Serbia, collected in the Danube and its main tributaries in the Serbian stretch (the Sava, Tisa, Karaš, Tamiš, Nera and MlavaRivers, as well as the Velika Morava and Timok basins)—Table 1. The museum collection was inspected and the identification of each specimen was checked and verified. A re-identification was carried out and the presence of potential synonyms for the *U. crassus* was considered. Each specimen had an inventory number and a label with sampling site, the name of the collector, the date of collection and the identification. A description of the respective sampling location is given in Tadić [38]. Key features used in identification were external morphology (shell outline, color, umbo sculpture, hinge characteristics as well as the three measured linear shell distances—shell length, height and width). Review of the status and taxonomic history of the species was carried out according to databases: MolluscaBase [39], MUSSELpdb [40] and WoRMS [41].

2.3. Current Data (1990–2019)

Recently, the study of aquatic ecosystems has been intensified and covered the entire territory of Serbia. A total of 540 sites were studied, covering different types of running water—from small and medium-sized streams to large lowland rivers (Figure 1). Various techniques were used to collect mussel samples—kick and sweep sampling and the multihabitat approach (EN 27828:1994) with the FBA benthic hand net (aperture: 25×25 cm, mesh size of 500 and 250μm) according to European Standards [42], benthic dredging and in some cases visual inspection and snorkeling. To obtain comparable data, abundance was expressed as the number of individuals per sample (relative abundance). For the graphical presentation, abundance per watercourse was pooled.

The distribution data for *U. crassus* are considered over two time periods (1990–2008 and 2009–2019). The number of detections per study period and per river kilometer was carried out to examine the distribution of the species in Serbian waters.

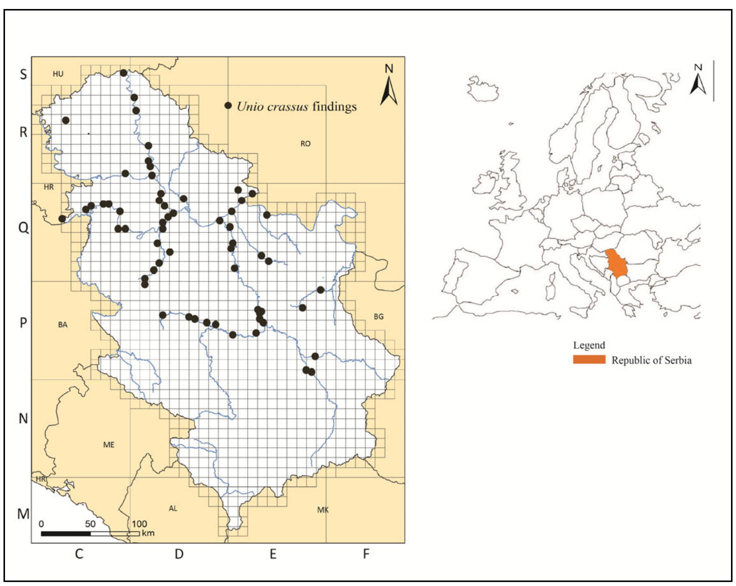


Figure 1. Map of all observed locations of *U. crassus* in watercourses in Serbia in the period (1973–2019).

2.4. Environmental Variables and Data Processing

The GPS position and elevation of each site in recent research period were recorded using a GarmineTrex 20×handheld GPS receiver (Garmin ltd). The chemical parameter(NO_3-N) considered in the study was provided by the Serbian Environmental Protection Agency (SEPA), as an official accredited institution (SRPS ISO/IEC 17025:/2017) [43] for the national water-monitoring programs. All water samples were analyzed in the accredited SEPA laboratory according to the following method: nitrates (NO_3-N): UP 1.98/PC 12. Water parameters were collected once a month. This parameter was selected based on the literature data as potentially one of the main elements affecting the *U. crassus* community.

In each watercourse the substrate type was categorized according to the AQEM protocol [44], which included: 1—megalithal (>40 cm); 2—macrolithal (20–40 cm); 3—mesolithal (6–20 cm); 4—microlithal (2–6 cm); 5—akal (2 mm–6 cm); 6—psammal/psammopelal (6µm–2 mm); 7—argyllal (<6µm) and other (organic mud, Xylal, living parts of terrestrial plants, debris) and were categorized into classes based on the percentage of cover (1–7).

According to the modified national typology [45], all surface waters in Serbia are classified into six categories: Type 1—large lowland rivers; Type 2—large rivers; Type 3—small to medium rivers with elevation below 500 m; Type 4—small to medium rivers and streams with elevation above 500 m; Type 5—watercourses of the Pannonian Plain; Type 6—small waterbodies including springs and upper stretches of streams. For this study, the material was collected in three categories of waterbodies (Types 1, 2 and 3).

2.5. Statistical and Graphical Analysis

The study of the ecological preferences of species in terms of the elevation gradient, waterbody types and the gradient of substrate types with the response curve was performed using the STATISTICA 8 software (StatSoft, Inc., Tulsa, OK) [46]. The nitrate–nitrogen content in different watercourses was analyzed using General Discriminant Analysis (GDA). The values of abundance and nitrogen content are graphically represented by mean, maximum and minimum values. The maps were created using Adobe Illustrator CC15 (Adobe Inc., 2015) [47].

3. Results

3.1. Historical Data

By analyzing historical data, 94 individuals of the 244 examined specimens were identified as *U. crassus*. A re-identification of each specimen from the museum collection was carried out and then the scientific names were validated in the database. After re-identification, 13 synonyms for *U. crassu*s were identified (Table 1).

Table 1. Re-idetification of the collection from the Natural History Museum in Belgrade (collector Ante Tadić).

Collection Number	Label	Re-Identification	Collection Data	Collection Site
63	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1963	Veliki Bački channel, Sombor
62, 65	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1955	Sava, Stara Bežanija
55	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1967	Tamiš, Pančevo
53	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1967	Dunav, Smederevo
51	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1966	Tisa, Senta
52	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1965	Karaš channel
42	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1958	Z. Morava, Ruđinci
43	*Unio crassus crassus* Philipson 1788	*Unio crassus*	1961	Dunav, Zemun
60	*Unio crassus cytherea* Kuster 1833	*Unio crassus*	/	Sava (76 rkm)
56	*Unio crassus cytherea* Kuster 1833	*Unio crassus*	1958	Z. Morava, Trstenik
46	*Unio crassus cytherea* Kuster 1833	*Unio crassus*	1961	Danube, Zemun
94	*Unio crassus batavus* (Maton and Rackett, 1807)	*Unio crassus*	1967	Danube, Golubac

97	*Unio crassus batavus* (Maton and Rackett, 1807)	*Unio crassus*	1953	Sava (35.5 rkm)
93	*Unio crassus batavus* (Maton and Rackett, 1807)	*Unio crassus*	1973	Mlava, Gornjak
99	*Unio crassus batavus* (Maton and Rackett, 1807)	*Unio crassus*	1955	Sava, Stara Bežanija
49	*Unio crassus f. Grandis*	*Unio crassus*	1967	Danube, Zemun
44	*Unio crassus crassus f. Grandis*	*Unio crassus*	1967	Tamiš, Pančevo
45	*Unio crassus crassus f. Grandis*	*Unio crassus*	1958	Z. Morava
47	*Unio crassus crassus f. Grandis*	*Unio crassus*	1958	Z. Morava, Klenjak
48	*Unio crassus crassus f. Grandis*	*Unio crassus*	1958	Z. Morava, Ruđinci
124	*Unio amnicus* Rossmässler, 1836	*Unio crassus*	6 August 1958	Z. Morava, Ruđinci
123	*Unio amnicus* Rossmässler, 1836	*Unio crassus*	/	Z. Morava, Trstenik
122	*Unio consentaneus*'Zigel' Rossmässler, 1836	*Unio crassus*	6 September 1958	Z. Morava, Ruđinci
120, 121	*Unio serbicus* Drouët, 1884	*Unio crassus*	6 August 1958; 6 September 1958	Z. Morava, Klenjak
119, 120	*Unio serbicus* Drouët, 1884	*Unio crassus*	1965; 1966	Nera, Bela Crkva
85	*Unio reniformis*'Schmidt' Rossmässler, 1836	*Unio crassus*	1961	Danube, Zemun
82	*Unio reniformis*'Schmidt' Rossmässler, 1836	*Unio crassus*	1967	Danube, Medornica confluence
87	*Unio rivalis* Drouët, 1884	*Unio crassus*	1965	Bela Crkva
68	*Unio bosnensis* Möllendorff, 1874	*Unio crassus*	1961	Danube, Zemun
69	*Unio bosnensis* Möllendorff, 1874	*Unio crassus*	1973	Mlava, Gornjak
81	*Unio savensis* Drouët, 1882	*Unio crassus*	1973	Mlava, Petrovac
73	*Unio pančići* Drouët, 1882	*Unio crassus*	1972	Crni Timok, Zaječar

The majority of species names are not considered valid based on current knowledge of the freshwater mussel diversity. Many of these synonyms were introduced into Europe by the French Nouvelle École in the late 19th century [48]. According to the historical data, considering the period from 1953 to 1973, it can be assumed that *U. crassus* was a common species in Serbia with a continuous range. The distribution range of the species based on historical data is shown in Figure 2.

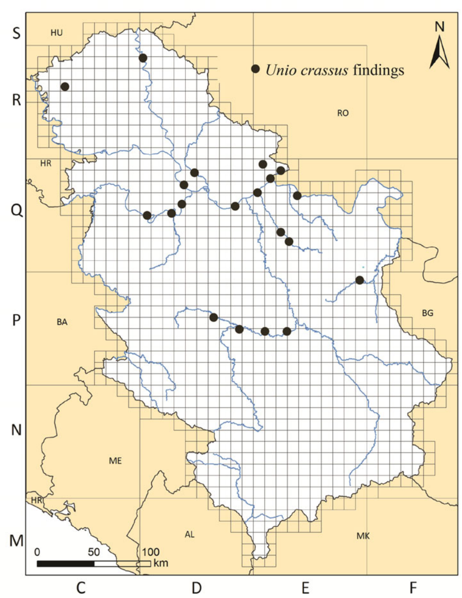


Figure 2. Map of the distribution range of *U. crassus* in studied grid squares 10 × 10 km in the period 1953–1973.

In the archive material, the species was detected at 20 of the 36 examined sites (55%). The species was found to be widespread in the Serbian stretch of the Danube. It was recorded at five out of nine examined sites (55%), from Apatin (1402 rkm) to Golubac (1040 rkm). A total of 16 specimens of the species were collected in the studied section of the Danube. The species was also detected in the Sava River with moderate occurrence, being recorded at three out of the eight examined sites (37%). Five individuals of *U. crassus* were collected in the Serbian stretch of the Sava (from 76 rkm to the mouth of the Danube). Thirty-nine specimens were collected at four sites. A dense population and the highest frequency of occurrence of *U. crassus* was observed in the Zapadna Morava. Even 39 specimens were collected at four sites in Z. Morava. *U. crassus* was detected in the Tamiš (in 33% of the examined samples), Mlava (in 50% of the examined samples) and Nera (in all samples) Rivers, but also detected in the Tisa and Crni Timok Rivers (at only one site). The occurrence of the species is observed in the Veliki Bački and Karaš channels.

Between the 1970s and 1990s, a period of intensive industrialization, *U. crassus* became locally and even regionally extinct. After historical data, there were no records of the species in Serbia until the early 1990s (Figure 3).

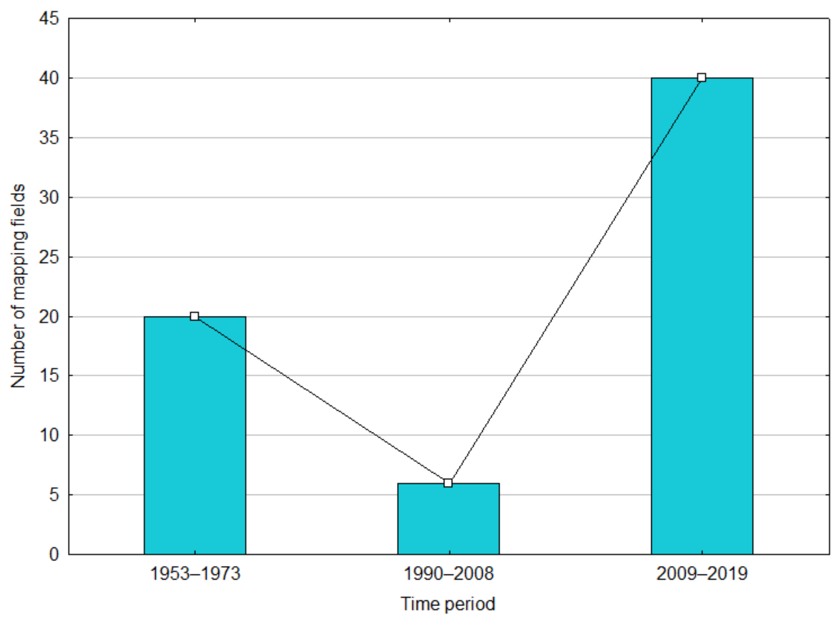


Figure 3. The number of mapping fields in different time periods.

3.2. Current Data

Of the 540 sites surveyed, mussels were detected at 46 sites. The current distribution of the species is shown in Figure 4 A, B. The number of detections in the watercourses per study period and the river kilometers are shown in Table 2 and Figure 3. *U. crassus* was detected in the Kolubara [24], Pusta reka [25], Tisa (site Novi Bečej-63rkm) Rivers in 2001 [23], Crni Timok upstream in 2004 [37] and at two sites on the Danube (Stari Banovci and Smederevo) in the period 1990–2008 [26] (Table 2, Figure 4 A).

Table 2. Findings of *U. crassus* in the period 1990–2019, according to the literature data and field investigation.

River/Site	Latitude	Longitude	Period/Year	rkm	Reference
Kolubara (downstream from the Jablanice and Obnice confluence)	44.26163	19.87572	1991–1994	No data	Marković et al. 1999 [24]
Pusta reka	43.08852	21.79819	1998–1999	No data	Živić et al. 2001 [25]
Pusta reka	43.08852	21.79819	1998–1999	No data	Živić et al. 2001 [25]
Tisa, Novi Bečej	20.13447	45.58948	2001	63	JDS-ITR Report 2002 [23]
Dunav, Stari Banovci	44.97855	20.28433	2003–2008	No data	Martinović-Vitanović et al. 2013 [26]
Dunav, Smederevo	44.65945	20.87647	2003–2008	No data	Martinović-Vitanović et al. 2013 [26]
Crni Timok-upstream	43.81826	21.74558	2004	No data	BAES database, Simić et al. 2006 [37]

Dunav, Ćerević	45.22246	19.67268	2013	1273	IBISS
Dunav, Tekija	44.68893	22.41312	2013	956	IBISS
Tisa, Titel	45.21199	20.3188	2010	11	IBISS
Tisa, Ada	45.79409	20.14725	2013	130	IBISS
Tisa, mouth	45.18785	20.31182	2019	11	IBISS
Tisa, Martonoš	46.17644	20.09552	2019	155	IBISS
Velika Morava, Brežane	44.64795	21.07092	2009	9	IBISS
Velika Morava, Varvarin	43.73424	21.37135	13 May 2010	179	IBISS
Velika Morava, Varvarin	43.73424	21.37135	20 September 2010	179	IBISS
Velika Morava, Varvarin	43.73424	21.37135	19 October 2010	179	IBISS
Velika Morava, Varvarin	43.73424	21.37135	16 November 2010	179	IBISS
Velika Morava, Ćuprija	43.94506	21.37101	20 September 2010	146	IBISS
Velika Morava, Markovački most	44.22582	21.15245	20 September 2010	93	IBISS
Velika Morava, Varvarin	43.73424	21.37135	18 January 2011	179	IBISS
Velika Morava, Markovački Most	44.22582	21.15245	31 March 2011	93	IBISS
Veliki Morava, Varvarin	43.73332	21.37018	2019	179	IBISS
Velika Morava, mouth	44.69536	21.03545	2019	2	IBISS
Zapadna Morava-upstream of the Kraljevo and upstream of the Ibar mouth	43.74022	20.73047	2009	4	IBISS
Zapadna Morava, Miločaj	43.77612	20.62904	2012	106	IBISS
Zapadna Morava, Gugaljski Most	43.86874	20.10663	2013	172	IBISS
Južna Morava	42.92038	22.03482	2011		Novaković et al. 2012 [28]
Nišava	43.30647	22.00474	2011		Savić 2012 [32]
Sava, marina	44.80639	20.4438	2010	3	IBISS
Sava, Ostružnica	44.73867	20.31975	2010	16	IBISS
Sava, Šabac	44.7924	19.69151	2011	108	IBISS
Sava, Sremska Mitrovica	44.96211	19.6088	2011	139	IBISS
Sava, Bosut confluence	44.94073	19.36989	2011	162	IBISS
Sava, Bosut confluence	44.94073	19.36989	2012	162	IBISS
Sava, Sremska Mitrovica	44.96211	19.6088	2012	139	IBISS
Sava, Jarak	44.91293	19.75402	2012	124	IBISS
Sava, Umka	44.68449	20.30589	2012	22	IBISS
Sava, Sremska Mitrovica	44.91358	19.7525	2015	139	IBISS
Sava, Šabac	44.76524	19.70304	2015	105	IBISS
Sava, Jamena	44.87813	19.08448	2019	204	IBISS
Sava, mouth	44.79289	20.39587	2019	7	IBISS
Kolubara, Draževac	44.56896	20.21381	2011	14	IBISS
Kolubara, Ćelije	44.37226	20.19992	2012	48	IBISS
Kolubara, Beli Brod	44.37083	20.19956	2013	49	IBISS
Peštan	44.42845	20.25699	2013	1	IBISS
Ljig	44.331578	20.203179	2019	No data	IBISS

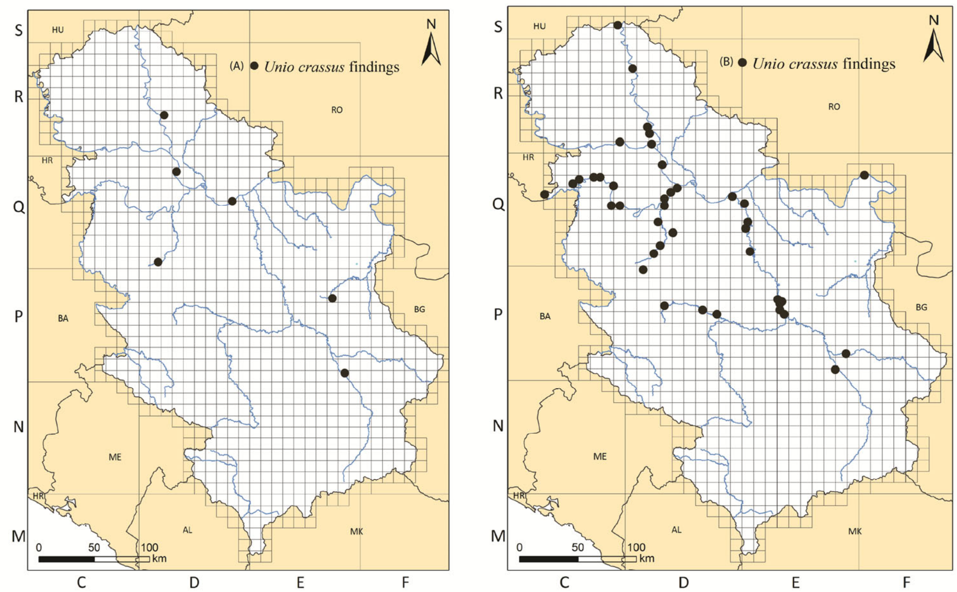


Figure 4. Maps of the distribution range of *U. crassus* in studied grid squares 10 × 10 km in the periods: (**A**) 1990–2008 (according to the literature data only); (**B**) 2009–2019.

In the period 2009–2019, *U. crassus* was detected in 40 of the 120 examined watercourse sites (33.3%) in Serbia (Figure 4B).

The species was detected in the Danube, Tisa, Sava, Velika and Zapadna Morava Rivers, as well as in the Kolubara River basin (three sites on the main course of the Kolubara and in the Peštan and Ljig Rivers), and according to the literature data, it was also detected in the Južna Morava [39] and Nišava Rivers [40] (Table 2).

During this period, the species was sporadically detected along the Danube, with a low frequency of occurrence and abundance (up to 0.48% of the total mussel community). The species was detected in the Danube only in 2013 at two sites (Čerević-1273 rkm and Tekija-956.2 rkm). It more frequently occurred in the Tisa River. The species was detected at the sites Titel-11 km upstream of the Danube confluence with the Danube (2010), Ada-130 rkm (2013), Martonoš-155 rkm and Tisa, confluence-2 rkm (2019) with low relative abundance (up to 5.78% of the total mussel community). *U. crassus* was also detected in the Sava and Velika Morava Rivers along almost the entire stretch with a higher relative abundance (with a percentage participation of 25.42% and 11.59%, respectively) and in repeated sampling occasions. The occurrence of *U. crassus* in the Kolubara River basin was also confirmed in repeated sampling in the period 2009–2019, but with a low abundance.

The mean value of the population abundance of *U. crassus* is shown in Figure 5, with the minimum and maximum deviation of abundance in the different watercourses, with the highest population abundance recorded in the Sava River.

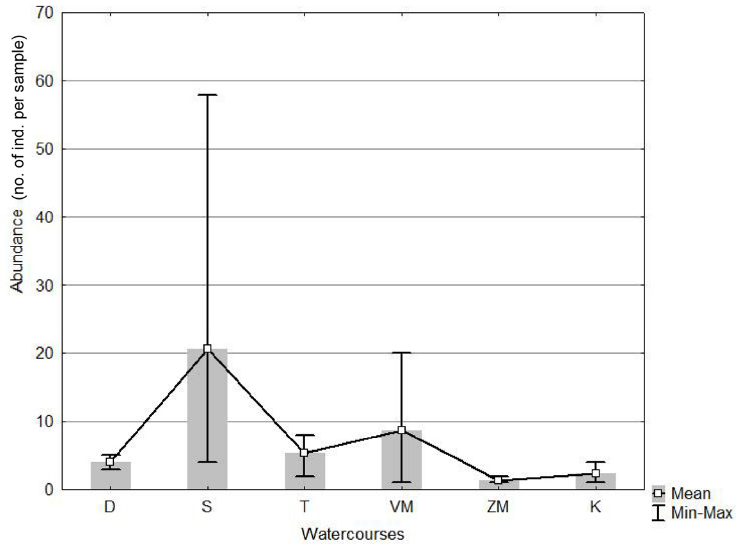
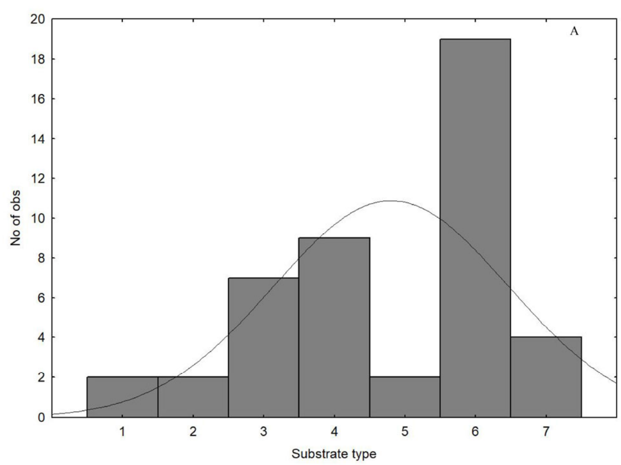


Figure 5. Population abundance of *U. crassus* in different watercourses in Serbia represented by mean, maximum and minimum abundance levels (D—Danube; S—Sava; T—Tisa; VM—Velika Morava; ZM—Zapadna Morava; K—Kolubara).

3.3. Ecological Preferences

The distribution of *U. crassus* is observed predominantly in the littoral reaches of large lowland rivers (waterbody Types 1 and 2), where fine substrate predominates (psammal/psammopelal (6μm–2 mm) and in small to medium watercourses (Type 3), where coarse substrate (mesolithal 6–20 cm and microlithal 2–6 cm) predominates, at elevations of up to 500 m (Figure 6A–C). It can be characterized as a rheo- to limnophilous species, preferring habitats with slow to moderate water flow.



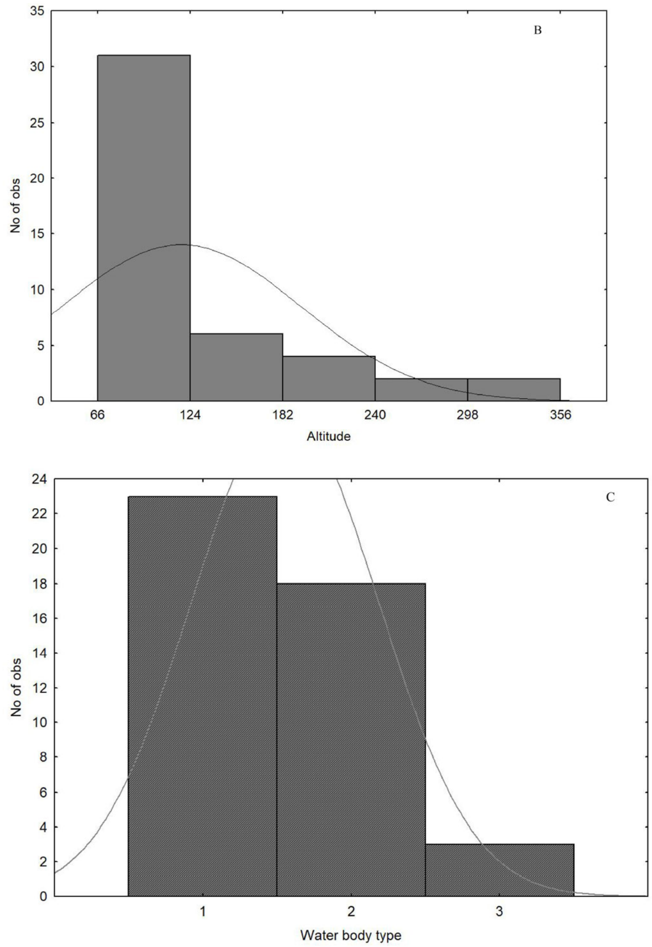


Figure 6. Preference of the mussel assemblages on the (**A**)—substrate type (1—megalithal (>40 cm); 2—macrolithal (20–40 cm); 3—mesolithal (6–20 cm); 4—microlithal (2–6 cm); 5—akal (2 mm–6 cm); 6—psammal/psammopelal (6μm–2 mm); 7—argyllal (<6μm); (**B**) altitude and (**C**) waterbody type.

Comparing nitrate–nitrogen levels for the same water bodies between the different monitoring years shows that nitrate–nitrogen levels were higher in the period 1999–2007 than in the most recent monitoring period (2011–2019) (Figures 7 and 8).

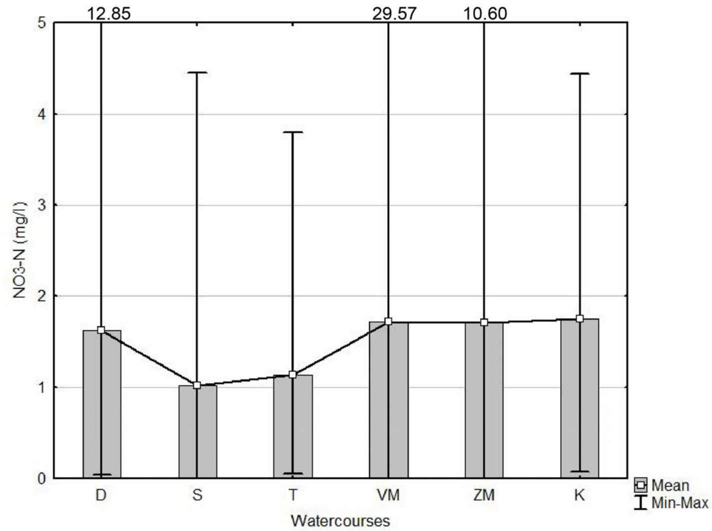


Figure 7. Nitrate–nitrogen concentration in rivers currently or formerly populated by *U. crassus* in period 1999–2007. Mean values are shown as columns with minimum and maximum deviation indicated by lines. D–Danube; S–Sava; T–Tisa; VM–Velika Morava; ZM–Zapadna Morava; K–Kolubara Rivers.

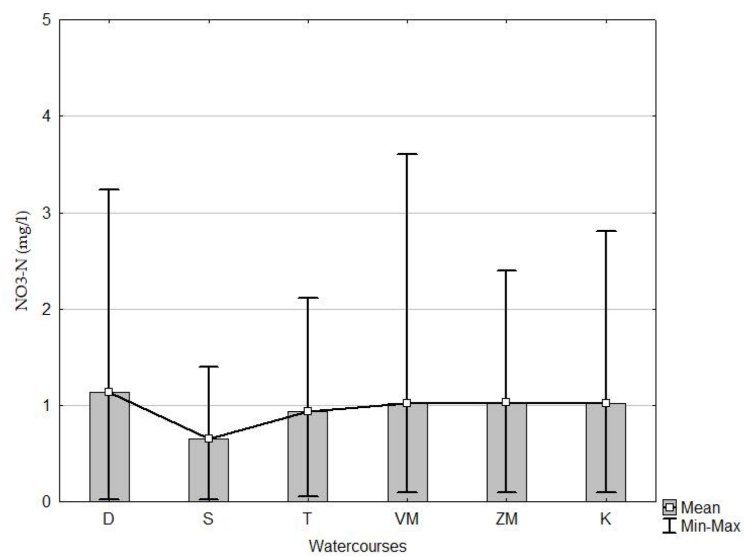


Figure 8. Nitrate–nitrogen concentration in rivers currently or formerly populated by *U. crassus* in period 2011–2019. Mean values are shown as columns with minimum and maximum deviation indicated by lines. D–Danube; S–Sava; T–Tisa; VM–Velika Morava; ZM–Zapadna Morava; K–KolubaraRivers.

4. Discussion

This study represents the first comprehensive research on the distribution of *U. crassus* in Serbian waters, based on historical, literature and field data.

Considering all the collected data, it can be observed that the distribution and abundance of *U. crassus* varies in the different study periods (Figures 2 and 4A,B) and in different watercourses (Figure 5). Re-identification of the archive samples from the Serbian Natural History Museum (period from 1953 to 1973) revealed that *U. crassus* was a common species with continuous distribution throughout Serbia until the mid-1970s. After re-identification (Table 1) of the museum collection, it was observed that there are many synonyms for *U. crassus*. Many of these taxa were first described by Henri Drouët (French'Ecole Nouvelle) in his conchological study of the unionids of Serbia, which also contains an overview of the systematics of only the unionids of Serbia [48]. The great intra-species morphological variability led to an expansion of species' descriptions in the XIX century. Based on all available data on unionids in Serbia and considering the species names according to the valid taxonomy, it can be concluded that the largest number of synonyms exists for the species *U. crassus*. European mussel diversity was also overestimated in the early 1900s due to unreliable taxonomic identification and numerous synonyms, mainly due to the influence of the French ´Ecole Nouvelle [19]. The number of described species in Europe was up to 1500 in the XIX century, but currently 16 species of Unionida are recognized after many synonymies were resolved [19].

Subsequent studies (after the 1970s) showed a decline in population density and a restriction of the distribution range, as well as sporadic findings of the species. In fact, until the early 1990s, there was no data on *U. crassus* in Serbia. Later, the occurrence of *U. crassus* was reported for the Kolubara River in the period 1991–1994 [24], for the Pustareka in the period 1998–1999 [25] and for the Crni Timok River [37]. *U. crassus* was detected in Serbian waters in the Crni Timok in 2004 [37]. Martinović-Vitanović et al. [26] reported findings at two sites in the Serbian stretch of the Danube (Stari Banovci and Smederevo) in the period 2003–2008. All of the above-mentioned findings could be characterized as rare and/or individual findings, indicating that the species was present, but with low population density.

More recent investigations (2009–2019) confirmed the presence of the species in the Danube, Tisa, Sava, Velika and Zapadna Morava Rivers, and in the Kolubara River basin (three sites on the main course of the Kolubara and in the Peštan and Ljig Rivers) (IBISS database), as well as in the Južna Morava [39] and Nišava Rivers [40] (Table 2, Figure 4B). According to the results of the survey of the Sava River in 2012 [28,29], and especially in 2019, a stable population of *U. crassus* was found in the upper and middle stretches of the river. The species was detected at all investigated sites from the site Jamena (204 rkm) to the mouth of the Danube (3 rkm). During the 2019 survey, a high abundance of the species and an almost uniform population of *U. crassus* was detected at the Jamena site. A similar distribution pattern of the species was observed in the KolubaraRiver basin and in the Velika Morava (from 179 rkm to 2 rkm) and Zapadna Morava (from 172 rkm to 2 rkm) Rivers. According to recently published data, an extension of the known range of the species in Serbia and its occurrence in the Južna Morava [28] and Nišava [32] Rivers was also detected. During the investigation of the Tisa River in 2001 [23], the presence of *U. crassus* was detected at one (Novi Bečej-63 rkm) of four investigated sites in the Serbian stretch (lower Tisa), while subsequent surveys from 2010 to 2019 showed an increasing population trend with detection of the species along almost the entire Serbian river section, from the Martonoš (155 rkm) to the Titel (11 rkm) River. Considering that the presence of the species has been confirmed in repeated sampling with significant abundance in the Velika Morava and especially in the Sava River (Figure 5), it could be assumed that the population is recovering, but stable populations are still localized. Furthermore, the permanent finding in the Kolubara River basin could indicate either a recovery of the population or that the population has reached its optimal density for that particular river type. The decreasing trend of the population and the fragmented distribution of *U. crassus* were also confirmed for Europe during studies in the second half of the XX century, with the exception of the northern part (the Baltic basin area), where the species is still considered to be relatively widespread [14]. In contrast to the current data, *U. crassus* was also formerly widespread and the most common unionid in Europe [14,].

Knowledge of the habitat requirements of endangered species is of great importance for the implementation of effective conservation strategies, which usually include habitat restoration [49]. In this study, the species was registered in different waterbody types (Types 1, 2 and 3) in areas up to 350 m a.s.l. (Figure 6), in the littoral part of rivers, mostly in fine substrate but also on larger sediment fractions. Most European unionids are lowland species, whereas *U. crassus* can inhabit higher elevations than other unionids [19,30] and can even reach very high densities in mountainous rivers [50], which supports the hypothesis of a wider niche for habitat variables than expected [51].

The population decline and local extinction may be related to the general environmental degradation due to pollution and habitat degradation in the second half of the 20thcentury. It was observed that this species is generally vulnerable to environmental degradation, especially to changes in water chemistry [14]. The high level of eutrophication caused by agricultural drainage is considered to be the main reason for the decline of *U. crassus* [10,22,30,52–54]. Our data show that the mean nitrate–nitrogen concentration varies between the study periods (Figures 7 and 8). The maximum variation in nitrogen concentration indicates highly polluted rivers and poor water quality conditions in almost all the studied rivers in the period between 1999 and 2007 (Figure 7). In the recent period (2011–2019), an improvement in water quality in terms of nitrogen concentration was observed (Figure 8). The most favorable conditions are in the Sava River where the maximum values do not exceed 1.5 NO_3-N mg/L (Figure 8). A significant improvement in water quality was observed in all the studied rivers, which is consistent with our most recent investigations. According to the latest studies, the distribution, the number of detections in selected watercourses and the localities of the first findings, clearly indicate an increasing population trend and an expansion of the distribution range in recent years in Serbia, with a focus on the Sava River basin (Figures 4B and 5). According to research by Zettler and Jueg [22], the increased nitrate–nitrogen caused by eutrophication is one of the main factors in the decline of *U. crassus*. In particular, it is a limiting factor for the growth and maturation of juveniles. A prevailing concentration below 2 mg/L throughout the year and between years indicates successful growth [22]. According to the same authors, limited recruitment of juveniles was observed in moderately polluted streams with nitrogen concentrations between 2 and 10 mg. When the nitrogen concentration exceeds 20 mg/L, the mortality of the mature *U. crassus* population strongly increases [22]. Increased mortality was observed in juveniles above concentrations of 2.3 mg NO_3-N/l [51].

U. crassus was common in the Danube River during the period 1953 to 1973 [37,38], but according to our recent data, its presence in the river was detected only in 2013, with low abundance at only two sites (Čerević-1273 rkm and Tekija-956 rkm) (Table 2). In addition to pollution, the disappearance of this species from the Danube in recent decades could also be related to the hydromorphological changes caused by the construction of dams (Iron Gate) and their impact on the river. Dam construction is probably one of the major threats to the mussel community with direct (damage or removal) or indirect effects on mussels (loss of suitable mussel substrate and decline of host fish) [19,22]. The construction of dams creates barriers to the migration of fish that are potential obligate hosts for the unionid larvae. A lack of suitable host fish can lead to a lack of juvenile recruitment, reducing population density and can potentially lead to species disappearance from habitats or even to extinction [22,55]. The construction of the dam and the forming of a large accumulation lake on the Danube River in Serbia has led to changes in the natural river regime, i.e., the slowing down of the river flow and permanent sediment deposition [56]. Although the dam was built in the lower section, the changes in the river character are noticeable over a long distance downstream and also upstream of the dam. The change in the flow velocity of the river has led to an increased sedimentation rate in the Danube [57]. The increase in sedimentation rate and the change in substrate as a result of the dam [22,58] indirectly affects the mussel community by affecting the potential microhabitats of the species. Changes in river flow due to dam construction and their impact on mussel fauna have already been confirmed for streams and rivers in Europe [14,19,22,59].

On the territory of Serbia, the beginning of mussel exploitation dates back to the 1930s. In the 1950s, organized mussel collection for industrial purposes was performed [38]. This long-term overexploitation has certainly significantly contributed to the decline of mussel populations in our rivers, which can still be observed today. Since the 1850s, freshwater mussels have been exploited for the extraction of pearls and nacre for button making [60]. At the peak of this exploitation, up to 50,000 tons of shells were harvested from North American rivers [61]. Strict laws now prohibit these activities, but poaching continues in some countries [19]. According to Ferreira-Rodríguez et al. [62], overexploitation is only locally significant and is often of secondary importance compared to other pressures that currently exist.

Among other factors, the introduction of exotic species is a possible contributing factor to the decline of freshwater mussels [10]. Over the past 20 years, research on allochthonous species has intensified in Serbia [61]. According to Zorić et al. [63], the Danube is the main corridor for the introduction and spread of alien species in Serbia and their spread to the other major rivers, i.e., the Tisa, Sava and Velika Morava [63]. The invasive bivalve species in Serbian freshwater ecosystems include the zebra mussel *Dreissena polymorpha* (Pallas 1771), the quagga mussel *Dreissena bugensis* Andrusov, 1897, the Asian clam *Corbicula fluminea* (O. F. Müller 1774) and the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) [63]. Invasive mussels are widely recognized as an important threat to native biodiversity [64]. The ecological impact of invasive species on native communities is not well documented in Serbia but there is evidence of widespread distribution in Serbian waters, dense populations and coexistence with native fauna [63]. They can cause direct biotic interactions with the native community (e.g., predation and competition) and also indirect changes in habitat conditions (e.g., habitat structure and turbidity) [65,66].Evidence of the negative impact of invasive species on native unionids has already been observed in many European countries as well as in North America [19].

At global, regional and local levels, species important for nature conservation are selected, protected areas are designed and an ecological network is established to link protected areas important for biodiversity conservation and the remaining priority habitat types [64].

The NATURA 2000 network is the main tool for biodiversity protection in the European Union. It is now considered to be the world's largest network of protected areas, covering 30,000 sites that occupy 20% of the EU territory [67].

Nature conservation efforts in the Republic of Serbia are aimed at fulfilling obligations in the framework of preparations for accession to the European Union (EU), which mainly refers to the establishment of the NATURA 2000 ecological network. When the conditions for EU accession are met, biodiversity and habitat diversity in Serbia will become part of the European ecological network NATURA 2000, with the obligation to implement the Directive. Serbia will propose areas important for the conservation of endangered plant and animal species for the ecological network NATURA 2000 and habitat types, as well as other EU member states.

The Balkan Peninsula served as a glacial refuge for several species of freshwater macroinvertebrates [68,69]. The establishment of an ecological network of protected areas will make it possible to ensure the survival of the most valuable species and habitats, promote the protection of numerous ecosystems and ensure that the natural system of Europe, and the Balkan Peninsula in particular, remains healthy and resilient.

5. Conclusions

The results presented show considerable progress in the restoration of the former distribution range of *U. crassus* in Serbian waters. Based on a dataset that includes historical and current data, population trends of this mussel over time were identified and a better understanding of the basic ecological requirements of the species was gained. The interaction of eutrophication, hydrological changes, overexploitation as well as the introduction of invasive species may be possible factors that influenced the local disappearance of *U. crassus* in some sections or the decrease in population density in Serbia. The results of this study can be used for the further development of effective and sustainable conservation strategies for endangered *U. crassus* populations, which usually include habitat restoration. Despite the high conservation status of this species, knowledge about its biology and ecology is insufficient. To improve conservation strategies for *U. crassus*, a systematic understanding of the limiting factors in the species' life cycle is crucial. Further studies on *U. crassus* should include more comprehensive ecological, biological and genetic investigations, as well as detection of new populations on a larger geographical scale.

Author Contributions: Conceptualization, J.T., M.R. and M.P.; methodology, J.T., M.R., V.S., A.P. and M.P.; software, J.T., A.A., K.Z.; validation, M.R., M.P., V.S. and A.P.; formal analysis, J.T., K.Z., A.A. and A.P.; investigation, J.T., M.R., A.A., K.Z. and M.P.; writing—original draft preparation, J.T., M.R. and M.P.; writing—review and editing, K.Z., A.A., A.P., V.S. and J.T.; visualization, A.A., K.Z., A.P. and J.T.; supervision, M.R., V.S., M.P. and J.T.; project administration, J.T.; funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, Contract No. 451-03-47/2023-01/200007.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We would like to thank the participants of the JDS 1-4 expeditions and the International Commission for the Protection of the Danube River (ICPDR). Details of the JDS investigations, including sampling site data are available at: http://www.danubesurvey.org/jds4/, and at: https://jds4.icpdr.org/portal/ (on request from the ICPDR Secretariat, Vienna International Centre, Room D0412, WagramerStrasse 5, A-1220 Vienna, Austria, e-mail: secretariat@icpdr.org; tel.: +431 260 60 5738).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vaughn, C.C.; Hakenkamp, C.C. The Functional Role of Burrowing Bivalves in Freshwater Ecosystems. *Freshw. Biol.* **2001**, *46*, 1431–1446.https://doi.org/10.1046/j.1365-2427.2001.00771.x.
2. Howard, J.K.; Cuffey, K.M. The Functional Role of Native Freshwater Mussels in the Fluvial Benthic Environment. *Freshw. Biol.* **2006**, *51*, 460–474. https://doi.org/10.1111/j.1365-2427.2005.01507.x.
3. Vaughn, C.C.; Nichols, S.J.; Spooner, D.E. Community and Food web Ecology of Freshwater Mussels. *J. N. Am. Benthol. Soc.* **2008**, *27*, 409–423. https://doi.org/10.1899/07-058.1.
4. Bogan, A.E. Freshwater Bivalve Extinctions (Mollusca:Unionoida): A Search for Causes. *Am. Zool.* **1993**, *33*, 599–609. https://doi.org/10.1093/icb/33.6.599.
5. Araujo, R.; Ramos, M.A. Status and Conservation of the Giant European Freshwater Pearl Mussel (*Margaritifera auricularia*) (Spengler,1793) (Bivalvia:Unionoidea). *Biol.Conserv.* **2000**, *96*, 233–239. https://doi.org/10.1016/S0006-3207(00)00075-6.
6. Young, M.R.; Cosgrove, P.J.; Hastie, L.C. The Extent of, and Causes for, the Decline of a Highly Threatened Naiad: *Margaritifera margaritifera*. In *Ecology and Evolution of the Freshwater Mussels Unionoida*; Springer: Berlin/Heidelberg, Germany, 2001; pp. 337–357.
7. Lydeard, C.; Cowie, R.H.; Ponder, W.F.; Bogan, A.E.; Bouchet, P.; Clark, S.A.; Cummings, K.S.; Frest, T.J.; Gargominy, O.; Herbert, D.G. The Global Decline of Non marine Mollusks. *Bioscience.* **2004**, *54*, 321–330. https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.
8. Strayer, D.L.; Downing, J.A.; Haag, W.R.; King, T.L.; Layzer, J.B.; Newton, T.J.; Nichols, J.S. Changing Perspectives on PearlyMussels, North America's Most Imperiled Animals. *Bioscience* **2004**, *54*, 429–439. https://doi.org/10.1641/0006-3568(2004)054[0429:CPOPMN]2.0.CO;2.
9. Strayer, D.L. *Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance*; University of California Press: Oakland, CA, USA, 2008; Volume1; ISBN0520942523.
10. Soroka, M.; Wasowicz, B.; Zając, K. Conservation Status and a Novel Restoration of the Endangered Freshwater Mussel *Uniocrassus* Philipsson, 1788: PolandCase. *Knowl. Manag. Aquat. Ecosyst.* **2021**, *422*, 3. https://doi.org/10.1051/kmae/2021003.
11. IUCN. *The IUCN Red List of Threatened Species*,version 2017-1; International Union for Conservation of Nature (IUCN): Gland, Switzerland, 2017.
12. Zieritz, A.; Lopes-Lima, M.; Bogan, A.E.; Sousa, R.; Walton, S.; Rahim, K.A.A.; Wilson, J.-J.; Ng, P.-Y.; Froufe, E.; Mc Gowan, S. Factors Driving Changes in Freshwater Mussel (Bivalvia, Unionida) Diversity and Distribution in Peninsular Malaysia. *Sci. Total Environ.* **2016**, *571*, 1069–1078. https://doi.org/10.1016/j.scitotenv.2016.07.098.
13. Zieritz, A.; Bogan, A.E.; Froufe, E.; Klishko, O.; Kondo, T.; Kovitvadhi, U.; Kovitvadhi, S.; Lee, J.H.; Lopes-Lima, M.; Pfeiffer, J.M. Diversity, Biogeography and Conservation of Freshwater Mussels (Bivalvia:Unionida) in East and Southeast Asia. *Hydrobiologia.* **2018**, *810*, 29–44. https://doi.org/10.1007/s10750-017-3104-8.

14. Lopes-Lima, M.; Kebapçı, U.; Van Damme, D. *Unio crassus*. In *The IUCN Red List of Threatened Species*; International Union for Conservation of Nature (IUCN): Gland, Switzerland, 2014. https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T22736A42465628.en.
15. Directive, Habitats. Council of Europe Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora. Brussels. **1992**, *206*, 7, 50.
16. European Council. *Revised Annex I of Resolution 6 of the Bern Convention Listing the Species Requiring Specific Habitat Conservation Measures*; European Council: Brussels, Belgium, 1998.
17. Official Gazette of RS 98/2016. *The Regulation on Proclamation of Strictly Protected and Protected Wild Species of Plants, Animals and Fungi*; Ministry Agriculture, Forestry and Water Management: Belgrade, Serbia, 2016.
18. Collier, K.J.; Probert, P.K.; Jeffries, M. Conservation of Aquatic Invertebrates: Concerns, Challenges and Conundrums. *Aquat. Conserv. Mar. Freshw. Ecosyst*. **2016**, *26*, 817–837. https://doi.org/10.1002/aqc.2710.
19. Lopes-Lima, M.; Sousa, R.; Geist, J.; Aldridge, D.C.; Araujo, R.; Bergengren, J.; Bespalaya, Y.; Bódis, E.; Burlakova, L.; VanDamme, D.; et al. Conservation Status of Freshwater Mussels in Europe: State of the Art and Future Challenges. *Biol. Rev*. **2017**, *92*, 572–607. https://doi.org/10.1111/brv.12244.
20. Taeubert, J.; Gum, B.; Geist, J. Host-Specificity of the Endangered Thick-Shelled River Mussel (*Unio crassus*,Philipsson1788) and Implications for Conservation. *Aquat. Conserv. Mar. Freshw. Ecosyst*. **2012**, *22*, 36–46. https://doi.org/10.1002/aqc.1245.
21. Engel, H.; Wächtler, K. SomePeculiaritiesin Development Biology of Two Forms of the Freshwater Bivalve *Unio crassus* in-Northern Germany. *Arch. Hydrobiol*. **1989**, *115*, 441–450. https://doi.org/10.1127/archiv-hydrobiol/115/1989/441.
22. Zettler, M.L.; Jueg, U. The Situation of the Freshwater Mussel *Unio crassus* (Philipsson,1788) inNorth-East Germany and ItsMonitoring in Terms of the EC Habitats Directive. *Mollusca.* **2007**, *25*, 165–174.
23. *Report on theJoint Investigation of the Tisza River on the Territory of the FR Yugoslavia within the International JDS-ITR Program*; JDS-ITR National Tisza Report; Republic of Serbia, Ministry for Protection of Natural Resources and Environment, Federal Hydro-Meteorological Institute: Belgrade, Serbia, 2002; pp.1–52.
24. Marković, Z.; Miljanović, B.; Mitrović-Tutundžić, V. Macrozoobenthos as an Indicator of the River Kolubara Water Quality. [Serbia, Yugoslavia] water quality. In Proceedings of the 28 Konferencija o Aktuelnim Problemima Zastite voda. Zastita voda'99, Soko Banja, Yugoslavia, 12–15 October 1999; Jugoslovensko Drustvo za Zastitu Voda: Belgrade, Serbia, 1999; Volume 28, pp. 261–266.
25. Živić, I.; Marković, Z.; Brajković, M. Macrozoobenthos in the Pusta Reka River, Left Tributary of the South Morava River. *Arch. Biol. Sci*. **2001**, *53*, 109–122.
26. Martinovic-Vitanovic, V.M.; Rakovic, M.J.; Popovic, N.Z.; Kalafatic,V.I. Qualitative Study of Mollusca Communities in the Serbian Danube Stretch (RiverKm1260–863.4). *Biologia.* **2013**, *68*, 112–130. https://doi.org/10.2478/s11756-012-0137-3.
27. Tomović, J.; Paunović, M.; Atanacković, A.; Marković, V.; Gačić, Z.; Csányi, B.; Simić,V. Biotic Typology of the Danube River-Based on Distribution of Mollusc Fauna as Revealed by the Second Joint Danube Survey (2007). *Acta Zool. Bulg*. **2014**, *66*, 527–537.
28. Novaković, B. Indicative Ecological Status Assessment of the Južna Morava River Based on Aquatic Macroinvertebrates. *Water Res. Manag*. **2012**, *2*, 45–50.
29. Paunović, M.; Tomović, J.; Kovačević, S.; Zorić, K.; Žganec, K.; Simić, V.; Atanacković, A.; Marković, V.; Kračun-Kolarević, M.; Hudina, S. Macroinvertebrates of the Natural Substrate of the Sava River–Preliminary Results. *Water Res. Manag*. **2012**, *2*, 33–39.
30. Marković, V.; Gojšina, V.; Novaković, B.; Božanić, M.; Stojanović, K.; Karan-Žnidaršič, T.; Živić, I. The freshwater mollusks of Serbia: Annotated checklist with remarks on distribution and protection status. *Zootaxa.* **2021**, *5003*, 1–64. https://doi.org/10.11646/zootaxa.5003.1.1.
31. Lucić, A.; Paunović, M.; Tomović, J.; Kovačević, S.; Zorić, K.; Simić, V.; Atanacković, A.; Marković, V.; Kračun-Kolarević, M.; Hudina, S.; et al. Aquatic Macroinvertebrates of the Sava River. In *The Sava River*; Milačić, R., Ščančar, J., Paunović, M., Eds.; The Handbook of Environmental Chemistry; Springer:Berlin/Heidelberg,Germany;NewYork,NY, USA;Dordrecht,The Netherlands; London, UK, 2015; Volume 31, pp.335–359.
32. Savić, A.V. Ekološka Analiza Zajednice Makrozoobentosa Reke Nišave [Ecological Analysis of the Macrozoobenthos Community of the Nišava River]. Ph.D. Thesis, Biološki Fakultet, Univerzitet u Beogradu [Faculty of Biology, University of Belgrade]: Belgrade, Serbia, 2012; p. 174.
33. Literáthy, P.; Koller-Kreiml, V.; Liška, I. *Final Report of the Joined Danube Survey*; International Commission for the Protection of the Danube River—ICPDR: Vienna, Austria, 2002; p. 262.
34. Liška, I.; Wagner, F.; Slobodnik, J. *Joint Danube Survey—Final Report ICPDR*; International Commission for the Protection of the Danube River—ICPDR: Vienna, Austria, 2008; p.235.
35. Liška, I.; Wagner, F.; Sengl, M.; Deutsch, K.; Slobodník, J. *Joint Danube Survey 3—A Comprehensive Analyses of Danube Water Quality*; International Commission for the Protection of the Danube River—ICPDR: Vienna, Austria, 2015; p.369.
36. Liska, I.; Wagner, F.; Sengl, M.; Deutsch, K.; Slobodnik, J.; Paunovic, M. Joint Danube Survey 4 Scientific Report. *A Shared Analysis of the Danube River*; International Commission for the Protection of the Danube River: Vienna, Austria, 2021; ISBN978-3-200-07450-7.
37. Simić, V.; Simić, S.; Petrović, A.; Paunović, M.; Šorić, V.; Dimitrijević, V. *Biodiversity in Aquatic Ecosystems in Serbia, Ex Situ Conservation (BAES Ex Situ)*; Faculty of Science: Kragujevac, Serbia, 2006.

38. Tadić, A. *Najvažniji Predstavnici Školjaka Roda Unio iz Save, Dunava I Kopačkog Jezera*; Posebna izdanja; Prirodnjački Muzej: Belgrad, Serbia, 1960; Volume 28, pp. 1–46.
39. MolluscaBase. 2023. Available online:https://www.molluscabase.org (accessed on 5 December 2023).
40. Graf, K.S.; Cummings, D.L. The Freshwater Mussels (Unionoida) of the World (and Other Less Consequential Bivalves); Updated9August2018. Available online: http://www.mussel-project.net/(accessed on 30 November 2023).
41. WoRMSEditorialBoard. World Register of Marine Species, 2023. Available online: https://www.marinespecies.orgatVLIZ. (accessed on 3 December 2023).
42. *ISO 7828:1985*; Water Quality—Methods of Biological Sampling—Guidance on Hand net Sampling of Aquatic Benthic Macro-Invertebrates; European Committee for Standardization: Brussels, Belgium, 1994.
43. *SRPS ISO/IEC 17025: 2017*; General Requirements for the Competence of Testing and Calibration Laboratories. Institute of Standardization of Serbia: Belgrade, Serbia, 2017.
44. AQEM. *Manual for the Application of the AQEM System. A Comprehensive Method to Assess European Streams Using Benthic Macroinvertebrates, Developed for the Purpose of the Water Framework Directive, Version 1*; The AQEM Consortium: Duisburg-Essen, Germany, 2002; pp. 619–630.
45. Regulative on Parameters of Ecological and Chemical Status of Surface Waters and Parameters of Chemical and Quantitative Status of Ground waters). *Off. Gaz*. Republic of Serbia. **2011**, No. 67/2011. Available at: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2011/74/4/reg
46. "StatSoft, Inc., Tulsa, OK.: STATISTICA, Version 8," AStA Advances in Statistical Analysis, Springer;German Statistical Society, vol. 91, 3, pp. 339-341.
47. Adobe Inc., 2015. Adobe Illustrator, Available at: https://adobe.com/products/illustrator.
48. Drouët, H. Suppl. Unionidae de Serbie. s.n., Paris, pp. 159–174.
49. Stoeckl, K.; Geist, J. Hydrological and Substrate Requirements of the Thick-Shelled River Mussel *Unio crassus* (Philipsson1788). *Aquat. Conserv. Mar. Freshw. Ecosyst*. **2016**, *26*, 456–469. https://doi.org/10.1002/aqc.2598.
50. Zając, K. *Unio crassus* Philipsson,1788. In *Skójka Gruboskorupowa. Pol. czerwona księga zwierząt, Bezkręgowce*; Instytut Ochrony Przyrody PAN: Kraków, Poland, 2004; pp. 353–355.
51. Tatoj, K.; Ćmiel, A.M.; Kwaśna, D.; Lipińska, A.M.; Zając, K.; Zając, T. The Endangered Thick-Shelled River Mussel (*Unio crassus*): A New Host Species for the European Bitterling (*Rhodeus amarus*). *Biodivers.Conserv*. **2017**, *26*, 1217–1224.
52. Patzner, R.A.; Müller, D. Effects of Eutrophication on Unionids. In *Ecology and Evolution of the Freshwater Mussels Unionoida*; Springer: Berlin/Heidelberg, Germany, 2001; pp. 327–335.
53. Köhler,R.Observations on Impaired Vitality of *Unio crassus* (Bivalvia:Najadae) Populations in Conjunction with Elevated Nitrate Concentrationin Running Waters. *ActaHydrochim.Hydrobiol*. **2006**, *34*, 3, 46–348. https://doi.org/10.1002/aheh.200500635.
54. Lyubas, A.A.; Kondakov, A.V.; Tomilova, A.A.; Gofarov, M.Y.; Eliseeva, T.A.; Konopleva, E.S.; Vikhrev, I.V.; Yunitsyna, O.A.; Pešić, V.; Bolotov, I.N. Taxonomic Reassessment of Freshwater Mussels from the Western Balkans Reveals an Overlooked butCritical Refugium and Defines Conservation Priorities. *Diversity*, **2022**, *14*, 935. https://doi.org/10.3390/d14110935.
55. Zale, A.V.; Neves, R.J. Reproductive Biology of Four Freshwater Mussel Species (Mollusca:Unionidae) in Virginia. *Freshw.Invertebr. Biol.* **1982**, *1*, 17–28.
56. Mladenović, M.B.; Kolarov, V.; Damjanovic, V. Sediment Regime of the Danube River in Serbia. *Int. J. Sediment Res*. **2013**, *28*, 470–485.
57. Nedeljkovic, R. Zoobentos Dunava u Godinama Posle Izgradnje Brane u Djerdapu [Zoobenthos of the Danube in Period after the Construction the Dam in Iron Gate Sector]. In Proceedings of the II Kongr. Ekol. Jugoslavije, Zb. Rad. Zagreb [2nd Congress of the ecologists of Yugoslavia, Conference proceedings] Zagreb, 1979; pp. 1881–1888.
58. Bril, J. *Measuring Mussel Behavior and Analyzing High Frequency Nitrate Data to Explore New Phenomena in Dynamic Nutrient Cycling*; University of Iowa: Iowa, IA, USA, 2010.
59. Beran, L. Distribution and Recent Status of Freshwater Mussels of Family Unionidae (Bivalvia) in the Czech Republic. *Knowl. Manag. Aquat. Ecosyst.* **2019**, *420*, 45. https://doi.org/10.1051/kmae/2019038.
60. Humphries, P.; Winemiller, K.O. Historical Impacts on River Fauna, Shifting Baselines, and Challenges for Restoration. *Bioscience.* **2009**, *59*, 673–684. https://doi.org/10.1525/bio.2009.59.8.9.
61. Haag, W.R. *North American Freshwater Mussels: Natural History, Ecology, and Conservation*; Cambridge University Press: New York, NY, USA, 2012; ISBN1139560190.
62. Ferreira-Rodriguez, N.; Akiyama, Y.B.; Aksenova, O.V.; Araujo, R.; Barnhart, M.C.; Bespalaya, Y.V.; Bogan, A.E.; Bolotov, I.N.; Budha, P.B.; Clavijo, C.; et al. Research priorities for freshwater mussel conservation assessment. *Biol. Conserv.* **2019**, *231*, 77–87. https://doi.org/10.1016/j.biocon.2019.01.002.
63. Zorić, K.; Atanacković, A.; Tomović, J.; Vasiljević, B.; Tubić, B.; Paunović, M. Diversity of Alien Macroinvertebrate Species inSerbian Waters. *Water.* **2020**, *12*, 3521. https://doi.org/10.3390/w12123521.
64. Ricciardi, A.; Neves, R.J.; Rasmussen, J.B. Impending Extinctions of North American Freshwater Mussels (Unionoida) Following the Zebra Mussel (*Dreissena polymorpha*) Invasion. *J. Anim. Ecol*. **2002**, *67*, 613–619. https://doi.org/10.1046/j.1365-2656.1998.00220.x.
65. Sousa, R.; Novais, A.; Costa, R.; Strayer, D.L. Invasive Bivalves in Fresh Waters: Impacts from Individuals to Ecosystems andPossible Control Strategies. *Hydrobiologia.* **2014**, *735*, 233–251.

66. Crooks, J.A. Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos.* **2002**, *97*, 153–166.
67. Popović, M.; Rajkov,S.; Ružić, M.; Savić, D.; Šćiban, M.; Simović, A.; Mesaroš, G.; Radišić, D. *Vrste Biljaka I Životinja Značajne za Zaštitu Prirode u Evropskoj Uniji*; Mesaroš,G., Ed.; Protego: Subotica, Serbia, 2014.
68. Pešić, V.; Glöer, P. The Handbook of Environmental Chemistry. In *The Skadar/Shkodra Lake Environment*; Pešić, V., Karaman, G., Kostianoy, A., Eds.; Springer:Cham, Switzerland, 2018; pp. 295–310.
69. Raković, M.; Tomović, J.; Popović, N.; Pešić, V.; Dmitrović, D.; Stamenković, V.S.; Hinić, J.; Stefanovska, N.; Lajtner, J.; Paunović, M. Gastropods in Small Water Bodies of the Western Balkans—Endangerments and Threats. *Small Water Bodies West. Balk.* **2022**, 227–249.