

Article

The Spatio-Temporal Distribution of the Freshwater Bivalves *Corbicula fluminea* and *Dreissena polymorpha* in the Lower Sector of the Danube River and the Danube Delta

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Abstract: The objective of this study is to provide an updated account of the distribution history of two invasive molluscs, *Corbicula fluminea* and *Dreissena polymorpha*, both in Europe and worldwide. In addition to this, the study also intends to review their ecological requirements to gain a better understanding of their invasive potential and distribution dynamics. Specifically, the study focuses on updating the distribution and ecological characteristics of these freshwater bivalves in the lower sector of the Danube River and the lakes of the Danube Delta. The purpose is to better understand their invasive and distribution dynamics and to develop effective measures to limit their spread in the future. To achieve this, environmental proxies such as sediment particle size and Total Organic Carbon (TOC) concentrations were used to assess their tolerances. However, the results did not show a significant correlation between the densities of these bivalves and the analyzed environmental parameters. Despite this, the species were found in high densities and formed well-developed benthic communities in some stations. The study contributes to the understanding of the invasiveness of these bivalve species and their distribution range dynamics. Nonetheless, further investigation is required to fully comprehend the role of environmental parameters in their distribution. The study covers the period between 2010 and 2020 and focuses on the lower Danube River sector and Danube Delta.



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1. Introduction

The spread of non-native invasive species in aquatic ecosystems has increased rapidly in recent decades, causing significant changes in ecology and function [1–4]. Among all the introduced species, dominating species, by density or biomass, or species bringing new traits to the ecosystem should be studied first [5,6]. Invasive bivalves play a crucial role as they can reach high densities and biomasses, affecting primary and secondary production and energy flow, and altering the community structure through ecosystem engineering. These mechanisms caused by invasive bivalves can result in significant changes to ecosystem functions and services [7,8].

This study aims to update the distribution history of two invasive molluscs, *Corbicula fluminea* and *Dreissena polymorpha*, in Europe and all over the world and to review their ecological requirements in order to understand their invasive potential and distribution dynamics. This will further serve at preparing effective measurements to limit the spread of potentially harmful species. Our new data cover the lower Danube River sector (Romania, Bulgaria, Serbia) and Danube Delta (Romania, Ukraine) between 2010 and 2020. Environmental parameters (particle size of sediments, Total Organic Carbon–TOC content) were used to examine the ecological requirements of the species. Based on the knowledge of the ecological characteristics and distribution range dynamics, we aim to contribute to the understanding of the potential invasiveness of these bivalve species.

Worldwide Historical Records of *Corbicula fluminea* (Müller, 1774) and *Dreissena polymorpha* (Pallas, 1771)

Corbicula fluminea, the Asian clam, is native to Southeast Asia and is typically found in Guangzhou, China [9]. Its native range also includes Russia, Thailand, the Philippines, China, Taiwan, Korea, Japan, Australia, and Africa [9–12]. In Southeast Asia, the clam is used for aquaculture with over 12 million tons produced per year in China [13]. The meat of the mollusc is valued for its nutritional properties and is used in pharmacology [14,15]. The Asian clam was first documented in the Columbia River in Washington (North America) in 1924 based on empty shells and in 1938 based on living individuals [16,17], and later in South America, and Europe. It was first thought to have entered the US as a food item for Chinese immigrants, but there is no evidence to support this theory [18]. Another possibility is that it entered the US with the importation of the Giant Pacific oyster *Magallana gigas* from Asia. Following rapid spread, it reached high densities in the US and has been found in Canada, Mexico, Panama, Argentina, Brazil, Uruguay, and Venezuela [19–21]. The species was first recorded in Europe near the borders of Portugal (Tagus River estuary) and in France (Dordogne River) in 1980. In 1983, it was found in the Weser River in Germany and then spread to the middle reaches of Germany in 1990 near Karlsruhe in the Upper Rhine and has since spread to most European river systems and countries, including the Norfolk Broads drainage system and the Thames and Great Ouse rivers in the UK; the Barrow, Nore, and Shannon rivers in Ireland; Austria, Bulgaria, France (Garonne, Rhone, Loire, and Seine), and Luxembourg; the delta area of the Rhine River in 1988 [22]; Poland, Portugal, and Spain by 1991; Switzerland near Basel by 1995 [23–25]; the River Po basin in Italy, the Elbe basin; and in 1997, downstream of the mouth of the Isar River in Germany, the Czech Republic, and Central Europe [26–45]. The fast spread of the species was believed to be due to transportation by cargo ships [29,46]. After appearing in the lower reaches of the Main River in 1991, its colonization of the Danube River through the Main–Danube canal, which opened for shipping in 1992, was expected [27,47]. Juvenile specimens were recorded in the Ukrainian sector of the Danube Delta in 1995. The species was introduced into the Danube River basin at different times and sectors with at least two main introductions: one in the Lower Danube and one in the Upper Danube in 1997 [48,49]. It has also been found in some of the Danube's tributaries, such as the Sava, Tisza, Prut, and Hungarian side arms [50–54]. Subfossil specimens of *C. fluminea* were reported in the Romanian sector of the Danube Delta in 1997, in Moldova Veche, Berzasca (Iron Gate area), and Vadu Oii. Nowadays, it inhabits the entire length of the Danube and Danube Delta [55–59].

Dreissena polymorpha (zebra mussel) is a Ponto-Caspian relict species native to the Black, Azov, Caspian, and Aral Sea regions [60–63]. *D. polymorpha* was once widespread throughout Europe but retreated during the last glaciations and became restricted to these areas. In the 18th century, its distribution expanded again due to human activities, first appearing in Rotterdam in 1826, Hamburg in 1830, and Copenhagen in 1840 [64,65]. In Great Britain, it was first recorded in the London Docks in 1824 and rapidly became widespread in the next 10 years [66]; however, its distribution stabilized after 1850. It was first recorded in Bulgaria's Danube River by Kreglinger in 1870. Subsequently, it was found in the Danube and its tributaries, and Black Sea coastal lakes and rivers. Fossil individuals were found in Pleistocene and Holocene sediments in North-West Bulgaria and along the Danube and the Black Sea Romanian and Bulgarian shelf zone [67–78]. The zebra mussel has also been recorded in the Ovcharitsa, Zhrebchevo, and Sopot reservoirs, Lake Chepintsi, and the Pyassachnik, Ticha, and Malko Sharkovo reservoirs [79]. *D. polymorpha* later invaded Ireland and Spain [64,80–83]. The zebra mussel was introduced from Europe to the Laurentian Great Lakes in North America in the late 1980s [84]. It soon became a problem due to their rapid growth and tendency to attach to and foul structures, causing water access difficulties for drinking water facilities, power plants, marinas, and industries [85]. Their presence also became a nuisance for boaters, fishermen, beachgoers, and water navigators as they colonized solid structures in the water [85]. The impact of the zebra

mussel invasion was revived when it was discovered in the North American Great Lakes in the 1980s and has since spread across the United States and southern Canada [86].

2. Materials and Methods

From 2010 to 2020, we collected qualitative and quantitative data on *C. fluminea* and *D. polymorpha* at 153 stations located from km 1075 of the lower Danube River down to the outer delta branches (Figure 1, Supplementary Materials S2). In total, 368 samples were analyzed (Figure 1, Supplementary Materials S1). Macrozoobenthic sampling is part of GeoEcoMar’s monitoring network for the study of biodiversity and assessment of the biological quality of watercourses on the Lower Sector of the Danube River and in the lakes of the Danube Delta.

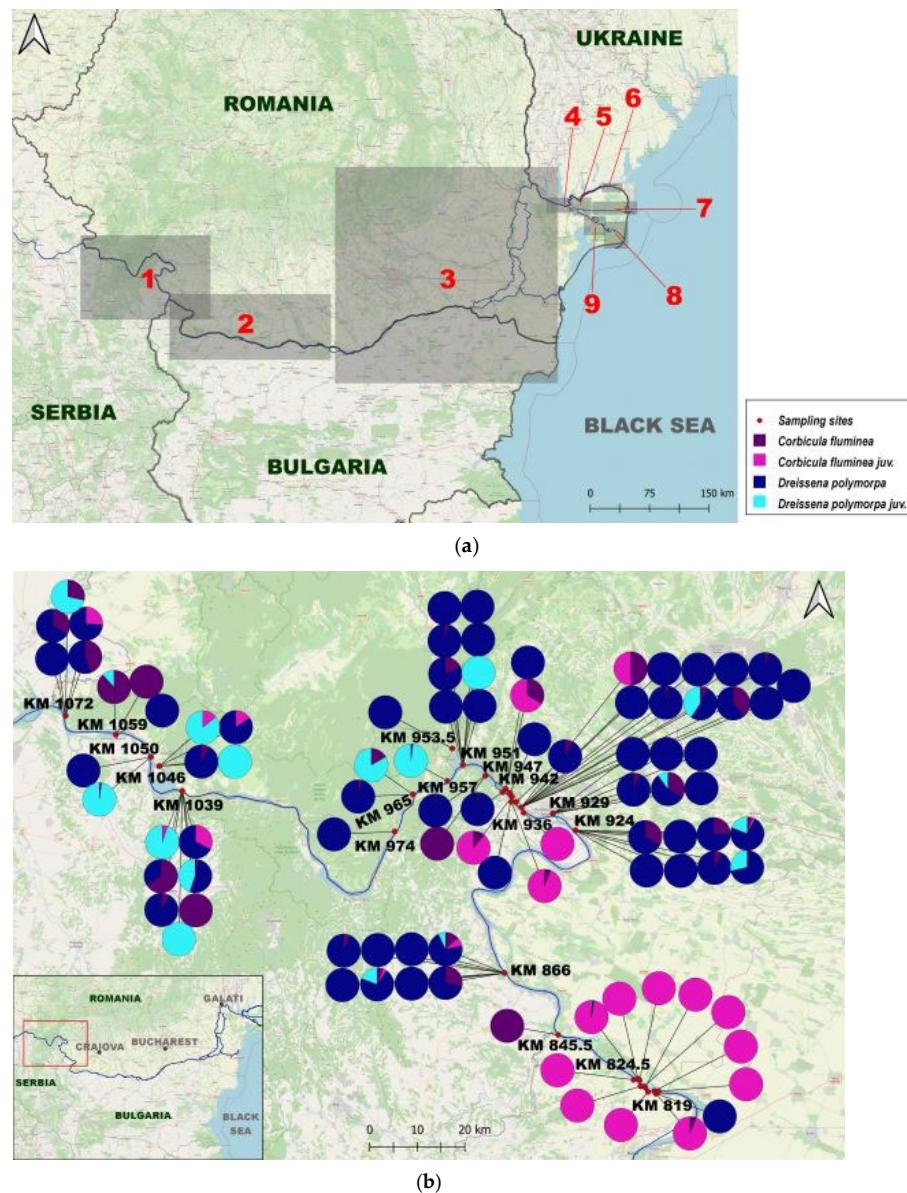


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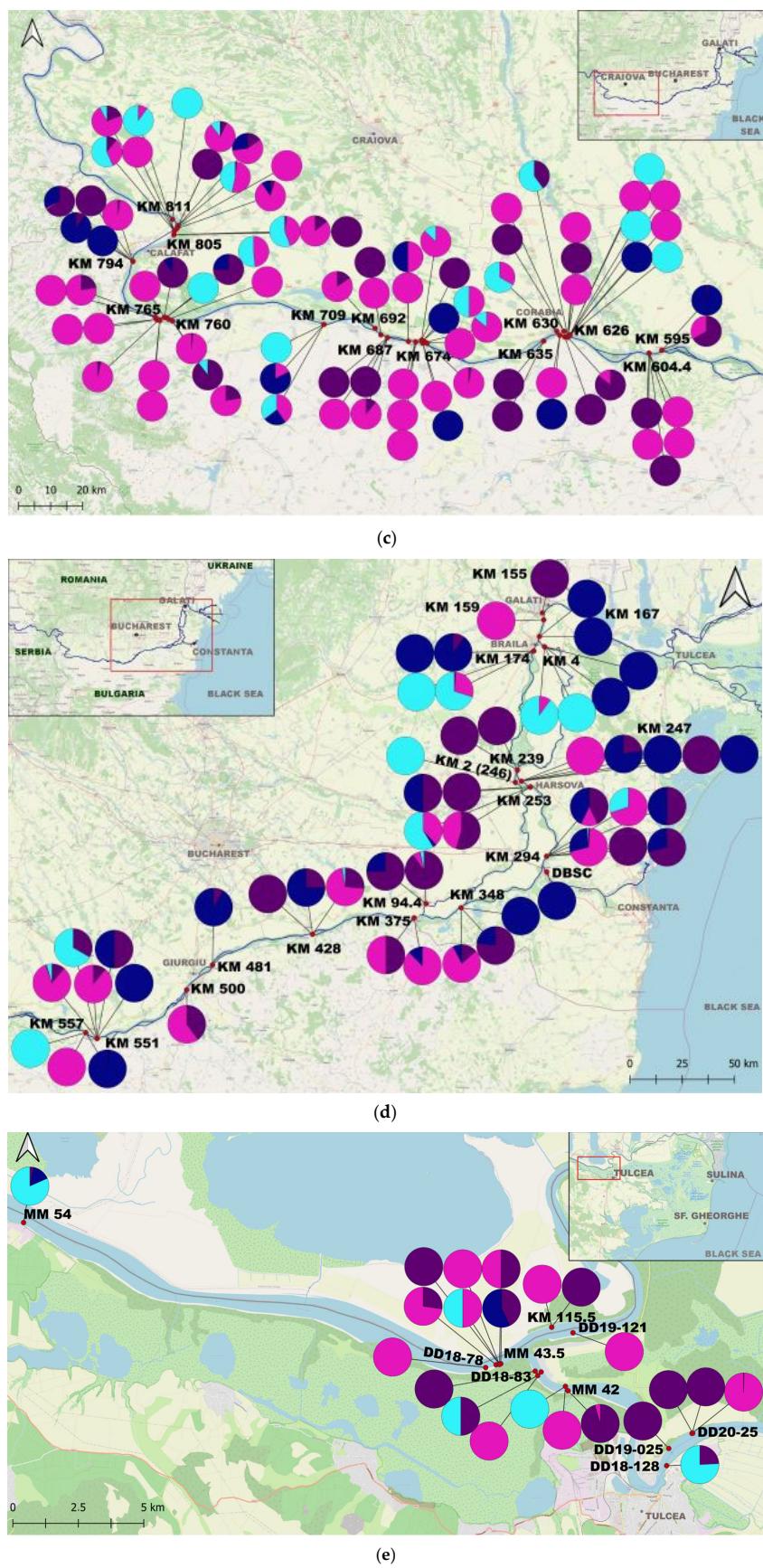


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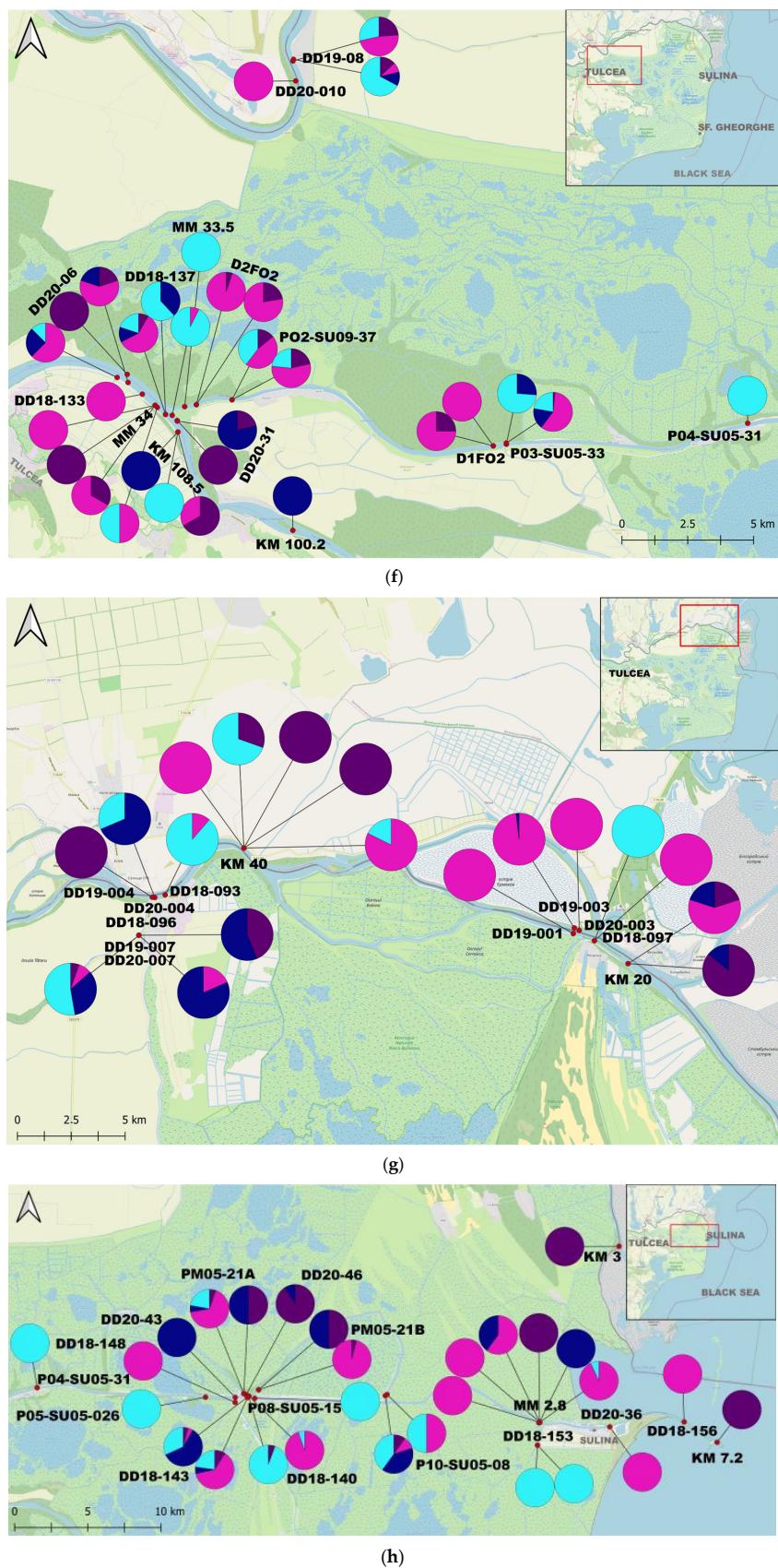


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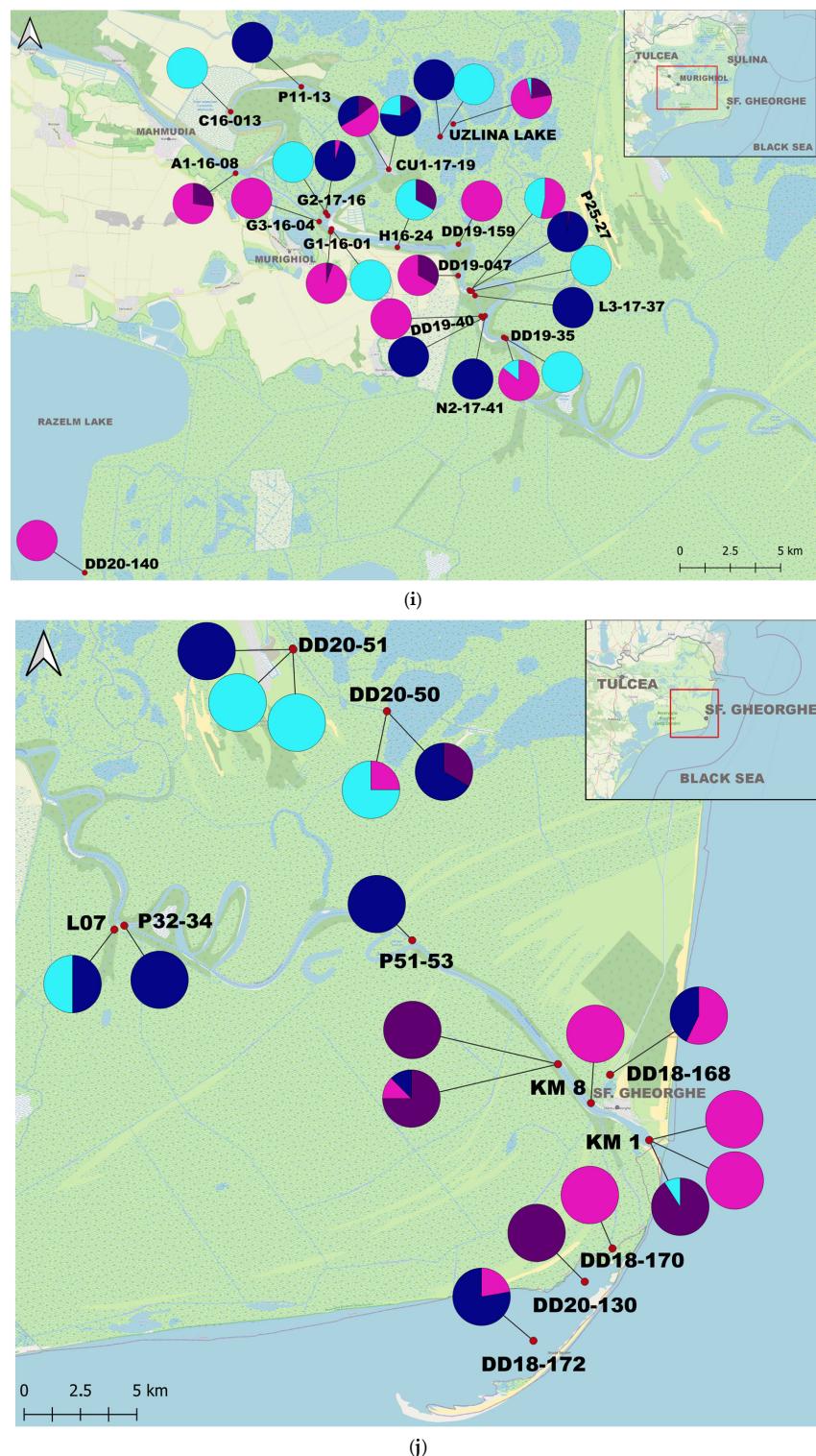


Figure 1. Map of the study area and the proportions of individuals per sample: (a) The Study Area; (b) Upstream Lower Sector of the Danube River (West); (c) Central Lower Sector of the Danube River; (d) Downstream Lower Sector of the Danube River (Est); (e) Upstream Tulcea Arm; (f) Downstream Tulcea Arm; (g) Chilia Arm; (h) Sulina Arm; (i) Upstream Sf. Gheorghe Arm; (j) Downstream Sf. Gheorghe Arm.

2.1. Sample Processing and Analysis

The sampling strategy followed the European standard [87] and employed the multi-habitat technique, which is a modified version of the AQEM method used for monitoring water bodies in Romania [88]. This multi-habitat sampling method is a European standard

for surface waters and combines techniques from the AQEM/STAR methodology and other EN and ISO standards. To perform measurements and collect samples from selected sites, we used two vessels, the research vessel Istros and a small boat. A total of 368 quantitative samples of sediments were collected using a (mouth surface 420 cm^2), for analysis of benthic fauna, particle sediment size, and TOC. Samples that had a depth of at least 10 cm and no signs of being disturbed (due to washing) were processed. Three replicates from each microhabitat were taken per station. On board the research vessel Istros, the samples were sieved through 500, 250, and 125 μm sieves to remove sediment, while on the small boat, these were filtered through a limnological net with a 125 μm mesh size. The samples were then preserved with a mixed solution of Rose Bengal and 4% buffered formaldehyde for later analysis of benthic organisms. In the laboratory, the sample processing and analysis followed the European standard [89]. Briefly, the samples were sorted under a Zeiss SteREO Discovery V8 microscope and a Carl Zeiss Axiostar microscope (Carl Zeiss Instruments SRL, Bucharest, Romania) and then species were identified at the lowest taxonomical level possible. The taxonomic identification was based on classical approaches found in the specialized literature [90]. All organisms in each sample were counted and the average of the three replicates per sample was calculated. The number of individuals per sample and unit area was then estimated using the appropriate multiplication factor related to the grab surface used [87]. The particle sediment size was analyzed using a laser granulometer (Malvern Mastersizer 2000E Ver.5.20, Malvern, UK) with a precision of 1% and reproducibility of over 99%. The particle size was separated into different classes (sand, silt, clay) and further classified into finer fractions, including fine sand, coarse sand, gravel, pebble, cobble, and boulder, based on the Udden–Wentworth logarithmic scale and detailed [91,92]. Debris such as detritus, aquatic vegetation, and mollusc shells were omitted during the particle size analysis. Total organic carbon (TOC) was measured using a titrimetric method [93,94].

2.2. Statistical Analysis

To describe the benthic population structure, the abundance and frequency were determined. Statistical analysis was conducted using XLSTAT 7.5.2 software [95]. Correlation between the abundance of *C. fluminea* and *D. polymorpha* and particle size of sediment and TOC concentrations were analyzed using the Pearson correlation and Principal Component Analysis (PCA). The significant principal components were identified as those with eigenvalues greater than one, according to Yang et al. (2014) [95].

2.3. Studied Species

2.3.1. *Corbicula fluminea*

Kingdom Animalia, Phylum Mollusca, Class Bivalvia, Subclass Autobranchia, Infra-class Heteroconchia, Subclass Euheterodonta, Superorder Imparidentia, Order Venerida, Superfamily Cyrenoidea, Family Cyrenidae, Genus *Corbicula*, Species *Corbicula fluminea* O. F. Müller, 1774 (Figure 2).



Figure 2. Specimen of *Corbicula fluminea*.

The study of *C. fluminea* started in 1774 with Müller, who described three species in the genus *Tellina* (Linne, 1758): *T. fluminalis*, *T. fluminea*, and *T. fluviatilis*. From that moment, many individuals of *C. fluminea* (Mühlfeldt, 1811) have been registered in freshwater and

estuarine habitats in Southeast Asia, the Indian subcontinent, the Pacific islands, Europe, and Africa [96]. The fossil record of *C. fluminea* is present in Europe, North America, and Japan [10,97–99].

C. fluminea is a small freshwater clam with a light-colored shell characterized by distinct concentric sulcations (growth ridges) and reaching about 5 cm in length [11,100]. The dimension of the mollusc is approximately 380 mm. Its shape is either triangular or oval, with a yellowish brown or blackish color and with concentric growth lines. The teeth on the valves are heterodont, meaning there are three cardinal teeth on each valve. The internal surface of the valves in fresh specimens is white-yellow. While this species has separated sexes, some individuals are also hermaphrodites and are capable of self-fertilization. Reproduction occurs in waters with a temperature greater than 15 °C and three-month-old individuals are capable of reproduction upon reaching 8–10 mm. Hermaphrodites produce both eggs and sperm simultaneously after reaching maturity, with first, the oocyte and then the spermatozoa being released. Multiple reproductions occur during the warm season from late May until autumn. Fertilization takes place in the paleal cavity of females and both trophophore larvae (15–20 microns) and veligers (0.2 mm) develop there. Juveniles are released when they reach 1 mm, are able to produce byssus, and can feed on phytoplankton. An individual can release up to 2000 juveniles per day and an estimated 100,000 juveniles in their lifetime. It can survive for weeks out of water and in low-oxygen environments and can be found in various habitats including stagnant or flowing water with varying water quality, from oligotrophic to eutrophic, and even in well-oxygenated silty or sandy sediments. It can withstand temperatures of between 2 and 34 °C and salinities of 5% for short periods in waters with 14 psu. However, it cannot tolerate water with high organic loads [90,101].

2.3.2. *Dreissena polymorpha*

Kingdom Animalia, Phylum Mollusca, Class Bivalvia, Subclass Autobranchia, Infra-class Heteroconchia, Subclass Euheterodonta, Superorder Imparidentia, Order Myida, Superfamily Dreissenoidae, Family Dreissenidae, Subfamily Dreisseninae, Genus *Dreissena*, Species *Dreissena polymorpha* Pallas, 1771 (Figure 3).



Figure 3. Specimen of *Dreissena polymorpha*.

The valves of the species are relatively delicate, with prominent bumps (umbones) and striped hind parts. The dorsal side is white-green with distinct brown-black stripes—although specimens without stripes can also occur. Under the umbones on the ventral side, there is a small triangular plateau. The impression of the gills lacks a sinus.

D. polymorpha is a freshwater benthic species that forms large groups on any type of natural or artificial substrate. It lives in stagnant or slow-moving water, avoids brackish and marine waters, and can grow in both plain freshwater areas and mountain lakes. The life span of the bivalve varies from 3 to 4 years, with a longer lifespan of up to 6–9 years in colder water. It reaches sexual maturity at a length of 8–10 mm and undergoes gametogenesis when water temperatures fall below 8–18 °C. Two reproductions occur in the summer, one at the end of May and the second at the end of August. When water temperatures exceed 12 °C in spring, the simultaneous release of oocytes and spermatozoa

in the water begins. Fertilization is high in *D. polymorpha*, and a female can lay between 10^4 and 1.6×10^6 eggs. The ideal temperature for fertilization is between 12 and 24 °C with a peak at 18 °C, in water with a pH of between 7.4 and 9.4, with the optimal being 8.5, and where Ca^{2+} ion concentration is over 12–15 mg/L [102]. The larval stage lasts from 8 days to 5 weeks and during this stage larvae are vulnerable. High larvae mortality is recorded (30–100%), notably caused by environmental factors such as predators and water turbidity, and internal factors such as egg quality and food availability (they cannot withstand periods of starvation longer than 2 weeks). Fixation of larvae occurs in the pediveligera stage and they can attach to any substrate; however, this can still result in high mortality (90–99%) due to strong currents, storms, low oxygenation, etc. The density of fixed larvae can vary from 0 to $10^6/\text{m}^2$ and they can be located anywhere from the surface to 30 m deep in water. The species can survive in water temperatures of between 3–6 °C and 31–32 °C, with a salinity range of 0.007–0.5‰ to 2–4‰, and a pH range of 7.9–9.4, requiring at least 12–15 mg/L of Ca^{2+} . It avoids high turbidity water but can adapt in some cases, and can last up to 10 days exposed to air under conditions of 10 °C and 95% humidity, and up to 18 days under lower temperatures and high humidity [90,101].

3. Results

The two species of mollusc were found in 153 stations out of 368 samples collected in the lower Danube sector and Danube Delta. In order to better highlight the distribution of the two bivalves, we divided them into adults and juveniles, taking into account their sizes.

C. fluminea adults were found in 180 samples and juveniles in 172 samples, while adult *D. polymorpha* were identified in 150 samples and juvenile *D. polymorpha* in 105 samples. *C. fluminea* were commonly found in areas with flowing waters from the Danube arms such as Chilia, Sulina, and Sf. Gheorghe, and the lower course of the Danube. Conversely, *D. polymorpha* was present both in the main course of the Danube and the lakes of the Danube Delta, often attached to submerged plant stems or reed roots/rhizomes in lakes (Supplementary Materials S1 and S2).

The highest density of *C. fluminea* adults, 18,125 ind./ m^2 , was recorded in 2010 at Mila 2.8 Sulina (D10-032) on clayey silt bottoms, and the highest density of juvenile *C. fluminea*, 11,289.6 ind./ m^2 , was recorded in 2019 at Km 805 (D2-19-094) on sandy gravel bottoms (Figure 4, Supplementary Materials S1).

The highest density of *D. polymorpha* adults, 30,312.5 ind./ m^2 , was recorded in 2010 at km 247 Downstream Harsova (D10-068) on sandy bottoms, and the highest density of *D. polymorpha* juveniles, 7656.25 ind./ m^2 , was recorded in 2010 at Ceatal Sf. Gheorghe Mila 35 (D10-020) on sandy bottoms (Figure 4, Supplementary Materials S1).

It is quite challenging to find a reasonable explanation for why the population has peaked or registered a much slower colonization. There are several limiting factors that can reduce the survival and longevity of zebra mussels and Asian clams (hydrodynamic conditions that may limit the ability of individuals to colonize, exposure to wind and waves, type of suitable substrate, episodes of hypoxia, higher temperatures, fish and bird predators, etc.). Continued monitoring of *D. polymorpha* and *C. fluminea* populations should provide useful data to better understand their behavior in such particular environments.

In general, the two species appear in high densities (mussel beds), thus creating a habitat for other species of organisms, which form thriving communities characterized by high biomass, abundance, and species richness [103] (Figure 4).

The composition of the macroinvertebrate community where the two species were found is formed of species such as: *Spongilla lacustris* (Linnaeus, 1758), *Eunapius fragilis* (Leidy 1851), Oligochaeta, *Hypania invalida* (Grube, 1860), *Manayunkia caspica* (Annenkova, 1928), *Anodonta cygnea* (Linnaeus, 1758), *Unio pictorum* (Linnaeus, 1758), *Viviparus viviparus* (Linnaeus, 1758), *Theodoxus fluviatilis* (Linnaeus 1758), *T. danubialis* (Pfeiffer 1828), *T. transversalis* (Pfeiffer, 1828), *Lithoglyphus naticoides* (Pfeiffer 1828), *Esperiana esperi* (Bourguignat, 1877), *E. daudebartii* (Prevost, 1821), *Chelicorophium curvispinum* (Sars 1895), *Echinogammarus tenellus behningi* (Martynov, 1919), *Dikerogammarus villosus* (Sowinsky, 1894), *Chelicorophium robustum* (G.O. Sars, 1895),

Gammaridae (Leach, 1813), *Jaera sarsi* (Valkanov, 1936), *Asellus aquaticus* (Linnaeus, 1758), *Hydropsyche bulgaromanorum* (Malicky 1977), *Palingenia longicauda* (Olivier, 1791), Polycentropodidae (Ulmer, 1903), *Gomphus flavipes* (Charpentier, 1825), and Chironomidae Larvae.



Figure 4. Examples of samples, showing the high densities of *C. fluminea* and *D. polymorpha* on different substrates.

The results of statistical analysis showed no significant Pearson correlation between the densities of *C. fluminea* and *D. polymorpha* and the analyzed environmental parameters. Based on the PCA graph, we can see that these two species do not show any correlation with the two parameters considered (substrate nature and TOC) due to the adaptability of these species to environmental conditions (Tables 1 and 2, Figure 5).

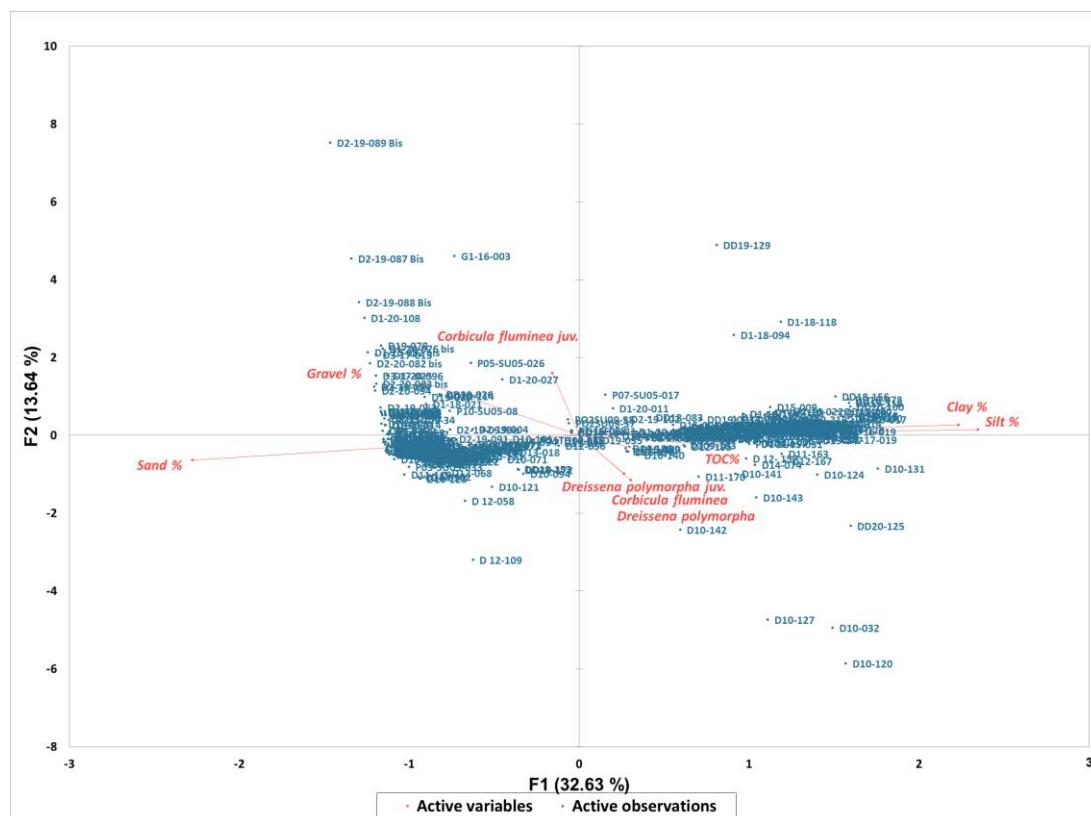
Table 1. Correlations between the abundances of the two bivalves, particle size, and TOC content in sediments.

Correlation Matrix Pearson (n):									
Variables	<i>Corbicula fluminea</i>	<i>Corbicula fluminea juv.</i>	<i>Dreissena polymorpha</i>	<i>Dreissena polymorpha juv.</i>	Gravel %	Sand %	Silt %	Clay %	TOC%
<i>Corbicula fluminea</i>	1	-0.009	0.182	0.066	-0.028	-0.061	0.074	0.038	0.017
<i>Corbicula fluminea juv.</i>	-0.009	1	-0.042	-0.027	0.198	-0.035	-0.031	-0.018	-0.078
<i>Dreissena polymorpha</i>	0.182	-0.042	1	-0.009	-0.067	-0.065	0.086	0.059	-0.005
<i>Dreissena polymorpha juv.</i>	0.066	-0.027	-0.009	1	-0.032	-0.075	0.081	0.068	0.013
Gravel %	-0.028	0.198	-0.067	-0.032	1	0.041	-0.345	-0.301	-0.119
Sand %	-0.061	-0.035	-0.065	-0.075	0.041	1	-0.934	-0.859	-0.234
Silt %	0.074	-0.031	0.086	0.081	-0.345	-0.934	1	0.804	0.272
Clay %	0.038	-0.018	0.059	0.068	-0.301	-0.859	0.804	1	0.181
TOC%	0.017	-0.078	-0.005	0.013	-0.119	-0.234	0.272	0.181	1

Values in bold are at an alpha level <0.05.

Table 2. Eigenvalues, variability, and cumulative score in the PCA matrix.

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	2.937	1.228	1.150	1.002	0.908	0.795	0.779	0.201
Variability (%)	32.628	13.640	12.780	11.138	10.085	8.835	8.659	2.235
Cumulative %	32.628	46.268	59.048	70.186	80.271	89.106	97.765	100.000

**Figure 5.** Principal Component Analysis on the species abundances and particle size of sediments and TOC concentrations.

4. Discussion

4.1. Historical and Modern Distribution Range Shifts

The Danube River is an important shipping route, and, hence, a significant aquatic invasion pathway in Europe that connects the North Sea and the Black Sea through the Rhine–Main–Danube Canal, [54]. The process of *C. fluminea*'s colonization of Europe has undergone multiple stages since its first appearance on the continent in 1980. This process involved overcoming the isolation between large and small river basins, and spreading within occupied freshwater systems [22,24,25,28–31,33–36,38–40,42–45,48–55,104–111]. There have been three main stages in the distribution of *C. fluminea* across Europe: the first is the continental European stage, with spreading to most European countries, starting from the first discovery of the mollusc in 1980–1983 [26,27]; the second is the Danube stage, when the mollusc invaded the large basin of the Danube River in the mid-1990s [29,48]; and the third is the British stage, when colonization began later, in 1998 [31,112,113]. It is widely believed that *C. fluminea* entered European river systems through ship ballast water, and the initial sightings of the species in the lower reaches of navigable rivers support this theory. The species initially spread inward from the Atlantic coast and dispersed within river systems. The completion of the Main–Danube Canal in 1992 facilitated the eastward spread of the species along the Danube and into the Black Sea region. However, the first discovery of *C. fluminea* in the Danube Delta in Ukraine in 1995, followed by its appearance in the upper reaches and

tributaries in Germany in 1997, raises the possibility of independent entry into the Danube through the canal or lower Danube ports [34,48,49]. The rate at which molluscs settle upstream is influenced by human activities such as sport fishing, aquaculture, and transportation of sand and gravel, and depends on the intensity of navigation [44]. The colonization of the Rhine, one of Europe's largest navigable rivers, by *C. fluminea* took seven years, with the species first discovered in the estuary in 1988 and in the upper reaches near Basel, Switzerland in 1995 [22,24]. The average rate of migration for these molluscs is 63 km per year, with some areas experiencing a maximum rate of 276 km per year [114].

The zebra mussel was first discovered in the Caspian Sea by Pallas in 1769 and is believed to be native to the Caspian and Aral Seas, as well as the low-salinity lagoons of the Black Sea and adjacent rivers [49]. During the early 19th century, *D. polymorpha* spread rapidly through Europe by invading canals connected to the Danube and Dnieper basins and reached England and Prussia by 1825. The mussel continues to invade new lakes in Europe and is abundant in low-salinity (0–6 ppt) estuaries from Finland and Baltic Russia to Ireland, Britain, France, and Spain [115]. In North America, zebra mussels were transported to various lakes and river systems during the 1990s and 2000s [20,116,117]. By 2008, they were found in the upper Colorado and Arkansas drainages in Colorado, as well as a lake in Utah and a reservoir in the Monterey Bay watershed, California. The spread of zebra mussels between river basins was facilitated by canals and barges in the eastern and midwestern regions, while isolated rivers and lakes were infested through the transport of mussels on trailered boats or fishing gear [118–121]. According to our study results, *C. fluminea* dominate the lower Danube sector and the arms of the Danube with flowing waters while *D. polymorpha* were identified along the Danube course and in the lakes of the Danube Delta.

Ecological and Biological Prerequisites for Successful Invasion of *D. polymorpha* and *C. fluminea* and Implications of Their Invasiveness Potential

The rapid spread and success of zebra mussels and Asiatic clams across Europe, North America, and South America can be attributed to their biological characteristics (fast growth, early sexual maturity, high fertility, multiple reproductive methods, planktonic larvae, and ability to reach high population densities) and physiological tolerance [102,122]. The species can survive in fresh and brackish water [48,123–126] and are found in various water bodies such as rivers, lakes, reservoirs, and canals. They thrive in large water bodies and reach higher densities in flowing water [25,44]. When present in high numbers, they can displace other benthic species, compete for food resources, limit food for other species, consume many individuals of unionid mussel sperm, glochidia, and juveniles due to their high filtration rate, and transfer parasites and pathogens [102]. Their biofouling impacts on water used for power plants, drinking water systems, industrial water systems, irrigation systems, and canals have been reported in the US [127–129] and Europe [130]. Zebra mussels are highly reproductive filter feeders that can produce up to half of a million eggs per year. They have two life stages, a planktonic veliger larva and an adult form that attaches to hard surfaces or forms colonies by attaching to each other. Their high abundance in aquatic ecosystems can result in a significant impact, reducing plankton populations and affecting benthic invertebrates and fish [131–145]. The zebra mussel, *D. polymorpha*, has been shown to have a significant impact on aquatic ecosystems as a result of its ability to alter habitats in both an autogenic and allogenic manner [82,146–151]. This species modifies the physical and morphological properties of sediments and changes the suspended material, affecting the availability of resources in the ecosystem [138,152–154]. In addition, zebra mussel clumps can increase the colonizable benthic surface area, provide stress-free spaces, control the transport of particles and solutes in the near-bottom environment, alter boundary layer characteristics, increase the amount of organic material in the sediment through the deposit of feces and pseudofeces, and affect dissolved nutrient concentrations by excreting or removing seston from the water column [149,150,152,155–159]. The spatial and temporal distribution of *C. fluminea* populations may depend on a variety of factors,

including the season and method of sampling, as well as abiotic factors such as substrate type [44]. *C. fluminea* prefers sandy silt and sandy clay sediments [44,102,160,161]. At Danube sites in Bulgaria, the highest species density was found in areas with a substrate made of coarse sand and sand with gravel. At Zagrazhden, where the highest density of juvenile specimens was observed, the substrate was coarse sand mixed with clam shells. Tributaries of the Danube with a sandy substrate, such as the Tsibritsa River, had higher population densities compared to other tributaries. In turn, in lakes and reservoirs with a substrate of sand, gravel, clay, and mud, the population density was lower than in the rivers [114]. The shells of the zebra mussel, *D. polymorpha*, provide a favorable environment and protection for aquatic life. This leads to shifts in the macroinvertebrate community [158,162–165]. The invasive bivalves introduce new shell substrates to the ecosystem, altering the availability and suitability of substrates for colonization by some mud/sand-dwelling benthic organisms [8]. Empty bivalve shells were found to generate a diverse range of microhabitats, attracting a macroinvertebrate community that was distinct from the natural macroinvertebrate community [166,167].

Our study found that the dominant macroinvertebrates were amphipods, corophidae, gammaridae, gastropods, oligochaeta, polychaeta, and insect larvae (trichoptera, chironomidae, etc.). *C. fluminea* registered high densities where the substrate was clayey silt or sandy gravel bottoms, and *D. polymorpha* preferred sandy bottoms. Different taxonomic groups may be attracted to empty shells for various reasons. Empty shells can provide a hard surface for the growth of amphipods, found in high abundance individuals, such as *Corophium curvispinum* that live in sediment tubes that are attached to the empty bivalve shells of *Dreissena* or *Corbicula*. The shells also offer a hard surface and shelter for the tubes of polychaetes and trichopteres, which are fixed to the valves of *Dreissena* or *Corbicula*. Gastropods, leeches, several crustacean species, and other organisms also find attachment sites on the hard shells' surface.

4.2. The Species Populations' Dynamics and Trends

The density of the zebra mussel, *D. polymorpha*, and the Asian clam, *C. fluminea*, has been reported in various North American and European rivers. In North America, the maximum density of *C. fluminea* can range from 10,000 to 20,000 ind./m² and can dominate the benthic community [168]. The highest recorded density was 131,000 ind./m² in California [33,99]. In Europe, *C. fluminea* has also been observed to attain high densities, such as 736 ind./m² in the Hungarian stretch of the Danube River, 5000 ind./m² in the Vén-Duna side arm of the Danube River in Hungary (km 1480–1483), and up to 9636 ind./m² in the Barrow River in Ireland [53,54,150]. Also, high densities were registered in an artificial channel near the Elbe River, 1045–4224 ind./m² [169]. In 2004, a maximum density of 1680 ind./m² was estimated in the Ukrainian section of the river (km 39) according to Lyashenko and Makovskii (2011) [111]. The densities in other European rivers have been estimated as the following: up to a maximum of 1500 ind./m² in the Upper Rhine River (2003–2005) [111], 2120 ind./m² in the Mero River basin (Calicia–Northwest on the Iberian) [42], up to 2500 ind./m² in the Chet River, Norfolk (UK), dominated by small individuals (2 to 9 mm in length) [168], and up to 9636 ind./m² in the Barrow River in Ireland [113]. In 2003, the river Altrhein (7 km downstream of Basel) had 200–600 clams ind./m². In Basel, the density of *C. fluminea* ranged from 5 to 200 ind./m² and at upstream locations from 1 to 20 ind./m². In the Canal de Huningue, densities of 10–50 ind./m² were recorded. *C. fluminea* was most abundant (up to 600 clams ind./m²) on sandy substrates near the banks of the river. *D. polymorpha* has been present and dominant in Lake Balaton since the 1930s, with densities reaching 220,000 ind./m² [170,171]. In the Szczecin Lagoon in Germany, the species forms large aggregations with a density of up to 10,000 ind./m² [170]. A large and stable population of *D. polymorpha* was found in the 1950s in the Szczecin Lagoon with an average density of 15,400 ind./m² [171]. In the eastern Gulf of Finland, in the Neva estuary, *D. polymorpha* registered up to 3,000 ind./m² [172]. *D. polymorpha* also registered high densities in the Danube River at 1821–1825 km of 188–1322 ind./m² [173].

In our study, the highest density of *C. fluminea* adults, 18,125 ind./m², was recorded in 2010 at Mila 2.8 Sulina (D10-032) on clayey silt bottoms, and the highest density of juveniles, 11,289.6 ind./m², was recorded in 2019 at Km 805 (D2-19-094) on sandy gravel bottoms. *D. polymorpha* adults recorded their highest density, 30,312.5 ind./m², in 2010 at km 247 Downstream Harsova (D10-068) on sandy bottoms, and *D. polymorpha* juveniles recorded their highest density, 7656.25 ind./m², in 2010 at Ceatal Sf. Gheorghe Mila 35 (D10-020) on sandy bottoms.

The density of both species can vary depending on environmental factors such as bottom structure, slope, and water conditions. In favorable conditions, these two species can quickly become the dominant species, with harmful consequences for the native species and the functioning of the ecosystem [174].

Even if these two species are found in great abundance on certain types of substrates, they can be found in all types of sediment, thus making them the two alien invasive species one of the biggest threats to biodiversity worldwide.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su15118526/s1>. Supplementary Materials S1. The distribution of the freshwater bivalves *Corbicula fluminea* and *Dreissena polymorpha* on the Lower Danube River and Danube Delta (abundance, granulometry of sediments, carbon organic total); Supplementary Materials S2. The distribution of the freshwater bivalves *Corbicula fluminea* and *Dreissena polymorpha* on the Lower Danube River and Danube Delta (Coordinates).

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