Patterns of Distribution of Bivalve Populations in a Mediterranean Temporary River

Mafalda Gama [1,*]**, Filipe Banha** [1] **, Cristina Moreira** [2]**, Henrique Gama** [2]**, Manuel Graça** [3] **and Pedro Anastácio** [1]

[1] MARE—Marine and Environmental Sciences Centre, Departamento de Paisagem, Ambiente e Ordenamento, Escola de Ciências e Tecnologia, Universidade de Évora, 7000-671 Évora, Portugal; filipebanha@hotmail.com (F.B.); anast@uevora.pt (P.A.)

[2] SOMINCOR—Sociedade Mineira de Neves Corvo, S.A. Santa Bárbara de Padrões, 7780-409 Castro Verde, Portugal; cristina.moreira@lundinmining.com (C.M.); henrique.gama545@gmail.com (H.G.)

[3] MARE—Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal; mgraca@ci.uc.pt

* Correspondence: mafaldagama@uevora.pt

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Abstract: In the south of the Iberian Peninsula, many rivers are intermittent, a state most likely to be exacerbated by climate change, strongly affecting river biota. An additional challenge for native biota in this area is the arrival of new species, frequently aided by humans, and bivalves are particularly at risk. Here we assessed whether the native (*Unio delphinus*) and invasive (*Corbicula fluminea*) bivalves differed in habitat use. To address this question, we sampled populations of both species in six isolated permanent pools in the same river during summer in three consecutive years. *U. delphinus* occurred in all pools, while *C. fluminea* occurred only in the two most downstream pools. *U. delphinus*, but not *C. fluminea*, was found preferentially in patches under riparian vegetation cover. Both species were found in similar sediment types (coarse and fine gravel respectively). Although *U. delphinus* was present in all pools, recruitment was detected only in 2016, in one pool. We concluded that both species have the potential to compete for space, but a well-developed riparian vegetation cover may provide *U. delphinus* some advantage against *C. fluminea*.

Keywords: *Unio delphinus*; *Corbicula fluminea*; intermittent river; dry season; Guadiana basin; Mediterranean

1. Introduction

Bivalves have a pivotal importance in freshwater, filtering phytoplankton, bacteria and fine particulate organic matter from the water column and sediment [1]. With the exception of Invasive or exotic freshwater bivalves, bivalve biodiversity is declining rapidly at a global level [2], and most native bivalves are highly endangered [3–5]. This decline is caused mainly by habitat degradation and biological invasions [6].

Bivalve introductions were fostered by the globalization of economic trade routes, increased watershed connectivity and recreational transport [7] and most likely will continue to occur at a greater pace. In many systems the decline of native bivalves occurs concomitantly with the spread of the invasive Asian clam (*Corbicula fluminea*) or Zebra and Quagga mussels (*Dreissena* sp.) [8]. Invasive bivalves may often become dominant (by attaining high density and biomass rapidly [9,10]), and therefore alter the community structure and function of invaded systems [11–14].

The Oeiras (Guadiana basin, South Portugal; Mediterranean climate) is an intermittent river with superficial flow after winter rains but reduced to persistent isolated pools in the summer. These pools

are harsh environments for many species since they may attain high temperature and conductivity and low dissolved oxygen in the summer. According to the IPCC [15] global change predictions, an increase in the frequency and intensity of extreme events, such as droughts, is expected for this area. Under these conditions, biota in intermittent Mediterranean rivers may become extremely vulnerable since pools will be at increased risk of drying. Therefore, special conservation efforts may be needed for Mediterranean streams and rivers.

The Oeiras river has important populations of the unionids *Unio delphinus*, *Unio tumidiformis* and *Anodonta anatina* [16–18]. However, there are also large populations of the invasive *Corbicula fluminea*. *Corbicula fluminea* is a potential threat to *U. delphinus* in other Iberian rivers [9,11,19,20] since *C. fluminea* was negatively related to native mussel abundance at small spatial scales [21]. Moreover, another exotic bivalve, *Dreissena polymorpha* has been recorded in nearby rivers [22], and it is likely to reach South Portugal, further affecting native species and increasing the need to adopt urgent conservation measures for native bivalves.

To plan efficient conservation measures to protect native bivalves it is fundamental to obtain data on their current distribution, population structure and the appropriate conditions for these populations to thrive. Therefore, our objectives were: (i) to get insights on distribution of native (*Unio delphinus*) and invasive (*Corbicula fluminea*) in a 6 km stretch of the Oeiras river; (ii) to understand the microhabitat features influencing the distribution of these two species, and (iii) to estimate population parameters (densities, population structure). To fulfill these objectives, we sampled specimens and measured environmental conditions in six summer pools on three consecutive years from 2015 to 2017.

2. Materials and Methods

2.1. Environmental Conditions

We sampled six permanent pools in the Oeiras river: Ossada Montante (A), Monte Ossada (B), Monte Bentes (C), Pego do Inferno (D), Pego do Linho (E) and Pego dos Cágados (F) (Figure 1) in three consecutive summers (September) between 2015 and 2017. Each pool was mapped using satellite imagery and in loco measurements, and the extent of submerged area, the total length and width, the maximum depth, the micro habitat's composition including sediment type (see below), were determined each year.
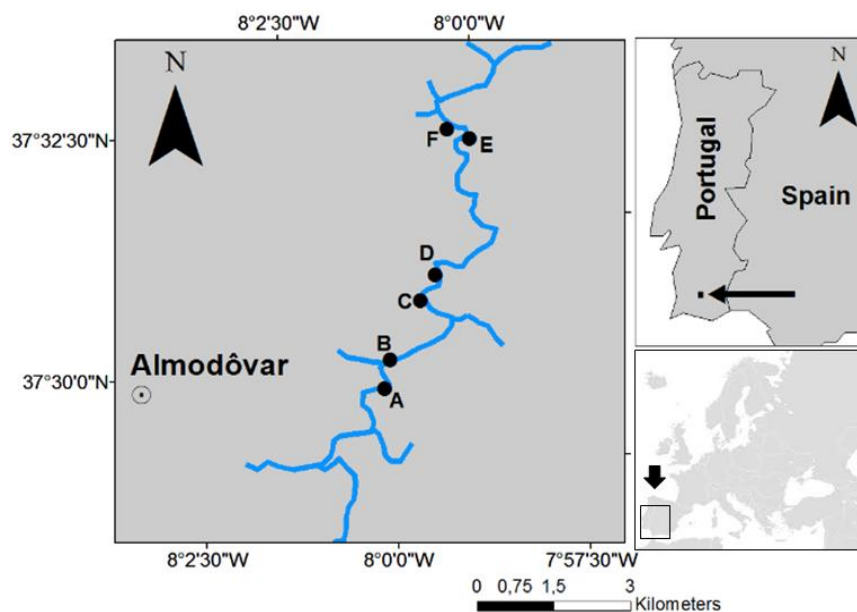

Figure 1. Location of the six permanent pools in the Oeiras river, flowing north in this section. Ossada Montante (**A**), Monte Ossada (**B**), Monte Bentes (**C**), Pego do Inferno (**D**), Pego do Linho (**E**) and Pego dos Cágados (**F**).

Concomitantly we characterized the pools in terms of riparian vegetation and possible human-impacted riparian features (e.g., presence of artificial structures and livestock access). We also measured turbidity and conductivity.

2.2. Qualitative Sampling

We firstly searched for bivalves with a batiscope (adapted from [12]) until the total pool area was explored or to a maximum of one hour of searching. Specimens were identified, measured, weighed and placed back at the same sampling location. For each pool, a capture- per unit of effort value (C.P.U.E.) was calculated, and results were expressed as the number of bivalves captured per person, per hour [23].

2.3. Quantitative Sampling

After the qualitative sampling, we established perpendicular transects starting at the tip of the submerged area, with a minimum distance of five meters, making sure that all micro-habitats were included. As pools length vary yearly with different submerged areas this procedure was repeated each year. In each transect, we established 0.25 m² quadrats with one quadrat randomly taken within the first meter of each margin (left and right) and two random quadrats in the middle of each transect to a maximum depth of 1.5 m [12,24,25]. The minimum distance between quadrats was three meters. Bivalves were identified, measured and weighed. We also recorded the water depth, distance to the margins, type of substrate and micro-habitat (see below) within the quadrat. Sediment grain size in each quadrat was classified according to American Society for Testing and Materials (ASTM) [26] as boulders (>300 mm), cobblers (75–300 mm), coarse gravel (19–75 mm), fine gravel (4.75–19 mm), sand (0.075–4.75 mm) and silt/clay (<0.075 mm).

Micro-habitats were defined by taking into consideration the sediment type, presence, type and extension of vegetation as well as whether quadrats were sampled under riparian vegetation shade. All native bivalves were left in their location while *C. fluminea* specimens were removed from the pools. Yearly population estimates were obtained multiplying population density by pool size.

2.4. Data Analysis

When appropriate, environmental data was tested for normality (Kolmogorov–Smirnov normality test) and homogeneity of variances (Levene's test) [27]. If normality and homogeneity of variances were not achieved, non-parametric tests were used.

Sediment data was expressed as percentages of cover at the sampling quadrats. A Multiple Correspondence Analysis (MCA) from FactoMineR package in R (Version 3.5.0, R. Core Team, 2018), was used to relate the presence/absence of bivalves and the environmental variables: sediment type, riparian gallery cover (presence or absence), distance to the nearest margin (m) and water depth (m).

We evaluated the correlation between the estimated densities and C.P.U.E. values and environmental variables using Spearman's rank correlation (SPSS, version 22.0, IBM, Armonk, New York, NY, USA). For *C. fluminea* the data used corresponded to the two most downstream pools. To assess habitat preferences in terms of sediment types we applied the Ivlev's electivity index, E_i (Ivlev, 1961) adapted for habitat preferences (e.g., [28,29]), given by:

$$E_i = \frac{r_i - P_i}{r_i + P_i} \tag{1}$$

where r_i is the proportion of individuals in a habitat with a specific sediment type (i), and P_i is the relative abundance of that habitat in the study area. Values of $E_i = -1$ indicate avoidance, $E_i = 0$ represents non-selective use of habitat type, and $E_i = 1$ indicates exclusive use of habitat type.

Population structure (sum of all pools) were compared using a G-log likelihood ratio (SPSS, version 22.0) to test for differences among years (using the post hoc *z* test). Six size classes were considered for *U. delphinus*: <30 mm, [30–40[mm, [40–50[mm, [50–60[mm, [60–70[mm and ≥70 mm. Juveniles were grouped in the first size class according to Smith [30]. *Corbicula fluminea* were also grouped in five size classes: <10 mm, [10–20[mm, [20–30[mm, [30–40[mm and ≥50 mm.

Table 1. Environmental conditions and population parameters in six pools (A to F) in the Oeiras river in 2015–2017.

Environmental Parameters-	Pools																	
	A			**B**			**C**			**D**			**E**			**F**		
Maximum depth range (cm)	72–80			75–90			90–160			200			130–160			20–20		
Maximum width range (m)	17–30			7–10			21–26			23–24			16–23			12–16		
Maximum length range (m)	84–102			28–32			226–268			100–113			159–236			37–88		
Total area (m^2)	3015			2411			16910			4065			489393			6400		
Exotic vegetation	No			No			Yes			Yes			Yes			Yes		
Riparian gallery vegetation cover	Continuous gallery composed of initial succession vegetation, reeds, presence of few trees—*Fraxinus angustifolia* or shrubs—*Nerium oleander*.			Gallery almost continuous composed mainly of shrubs—*Flueggea tinctoria* and *N. oleander*.			Gallery almost continuous in the left bank with *F. angustifolia* and Salix spp. and the exotic (Giant cane, *Arundo donax*). Discontinuous gallery of *F. angustifolia* and *N. oleander* in the right bank.			Almost continuous gallery of *F. angustifolia* and *N. oleander* in right bank, but very thin transversely. Left bank with sparse vegetation with few individuals of *F. angustifolia* and *N. oleander*. Presence of *Eucalyptus globulus*.			Sparse gallery in both banks, *N. oleander* in the right rocky bank, *F. angustifolia* Salix spp. and *E. globulus* in the left bank.			Continuous gallery composed by initial succession vegetation, reeds, presence of few trees of *F. angustifolia* Salix spp. and *E. globulus*.		
Cattle presence	Yes			Yes			Yes			Yes			Yes			Yes		
Sampling year	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Submerged area (m^2)	991	1172	1232	136	240	177	3903	5188	3659	1593	2158	1882	2859	3641	2135	488	1115	368

3. Results

3.1. Sites Description

The six pools differed in their physical conditions. Some locations had a well sustained riparian vegetation cover, mainly with ash and willow trees (*Fraxinus angustifolia* and *Salix atrocinerea*) and stable margins (e.g., pool C; Table 1), while others had discontinuous riparian corridors, unstable banks, some non-native tree species, modified river channels or cattle presence. Water conductivity (>600 μs cm^{-1}), and turbidity (5.69–57.9 NTU's) were high. Conductivity was high in all pools as sampling occurred in late summer with low water availability and varied between years due to shifts in water volume and depth. Turbidity varied because intrinsic pool characteristics were also different. Some pools are shallower, some pools have more fish fauna, some pools might experience increased anthropogenic pressure, all are variables that might influence turbidity differently.

3.2. Bivalve Abundance and Distribution

Four bivalve species occurred in the Oeiras river. The native *U. delphinus* and the invasive *C. fluminea* were the most abundant, while *Anodonta anatina* and *Unio tumidiformis* were scarcer. *Unio delphinus* catch per unit effort (C.P.U.E.) ranged from zero (pools B and E in 2017) to 50.5 per researcher h^{-1} (pool A in 2017; Table 2). *Unio delphinus* densities attained up to 6.0 individuals per m^2 (pool D in 2017) while maximum densities of *C. fluminea* were estimated in 12.7 individuals per m^2 (pool E; Table 2, Figure 2). For *C. fluminea* C.P.U.E. and estimated densities were related (Spearman's rank correlation: $n = 6$; $r_s = -0.829$; $p = 0.042$) unlike for *U. delphinus* ($n = 18$; rs = −0.273; $p = 0.274$).

Table 2. Densities (individuals per m^2) and catch per unit of effort (C.P.U.E., number of bivalves collected per researcher per hour) of *U. delphinus* and *C. fluminea* in six pool sites in the Oeiras river in three years.

Site	Species	2015		2016		2017	
		Individuals per m^2	C.P.U.E.	Individuals per m^2	C.P.U.E.	Individuals per m^2	C.P.U.E.
A	*U. delphinus*	1.67	3.0	2.17	5.2	4.17	50.5
	C. fluminea	0	0	0	0	0	0
B	*U. delphinus*	3.20	2.5	0.80	7.5	0.73	0
	C. fluminea	0	0	0	0	0	0
C	*U. delphinus*	0.86	18.7	1.94	4.0	1.38	5.7
	C. fluminea	0	0	0	0	0	0
D	*U. delphinus*	2.67	6.3	2.32	15.0	6.00	0.9
	C. fluminea	0	0	0	0	0	0
E	*U. delphinus*	2.27	42.3	2.40	36.0	1.57	0
	C. fluminea	9.93	51.3	9.73	11.0	12.71	0
F	*U. delphinus*	0	20.0	0	0	0	4.3
	C. fluminea	12.00	2.0	6.29	20.0	11.38	10.7

C. fluminea and *U. delphinus* density was not correlated ($n = 170$; $r_s = 0.110$; $p = 0.154$). In general *U. delphinus* density tended to increase from 2015 to 2017 in most pools (except B and E), while *C. fluminea* density increased only in pool E within the same period.

At patch scale, the presence of bivalves was explained by the distance to the margins and depth (more individuals of both species in the shallow margins) and fine gravel (*C. fluminea*) or coarse gravel substrates (*U. delphinus*) (Multiple correspondence analysis; Table 3 and Figure 3). These results were partially consistent with Spearman's rank correlation: Densities of *U. delphinus* were correlated with the presence of fine gravel ($r_s = 0.126$, $p < 0.01$) coarse gravel ($r_s = 0.160$, $p < 0.001$) and riparian vegetation cover ($r_s = 0.153$; $p < 0.01$). Densities of *C. fluminea* were positively correlated with fine gravel ($n = 170$;

$r_s = 0.260$; $p < 0.001$); sand ($n = 170$; $r_s = 0.170$; $p = 0.027$) and silt/clay ($n = 170$; $r_s = 0.210$; $p < 0.01$) and negatively correlated with depth ($n = 170$; $r_s = -0.207$; $p = 0.007$) and rock presence ($n = 170$; $r_s = -0.302$; $p < 0.001$). Although significant, all these correlations were low.
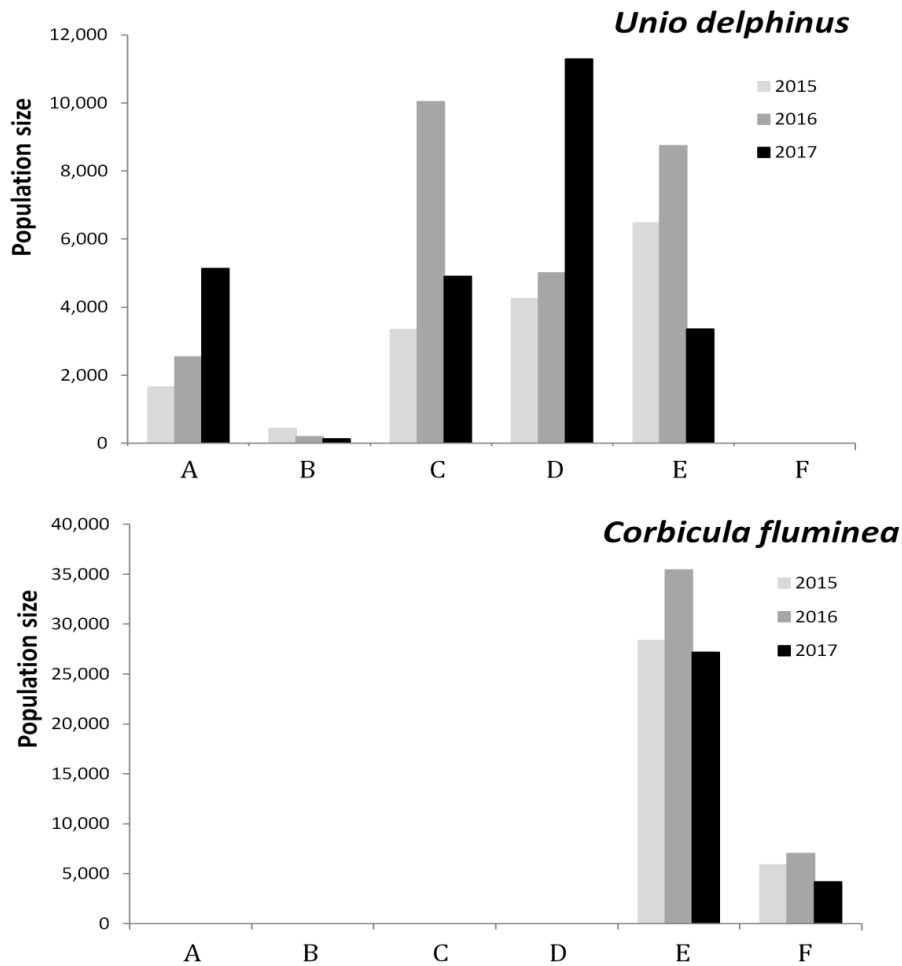

Figure 2. Estimated population size (*n*) of *U. delphinus* (top) and *C. fluminea* (below) in six pool sites (A–F) along the Oeiras river.

Table 3. Statistical parameters from multiple correspondence analysis on factors explaining the distribution of *U. delphinus* and *C. fluminea* in six pools.

| Statistical Parameters	Dimension 1		Dimension 2	
Eigenvalues	0.2		0.21	
% of variance	2.01		1.90	
Cumulative % of variance	2.01		3.91	
Variable	r^2	*p* value	r^2	*p* value
Distance to near margin (m)	0.54	3.13×10^{-47}	0.54	1.83×10^{-46}
C. fluminea	0.17	3.18×10^{-22}	0.09	2.31×10^{-12}
Silt/Clay	-	-	0.42	7.94×10^{-63}
Riparian gallery cover	-	-	0.16	5.86×10^{-22}
Water column depth (m)	0.65	7.87×10^{-79}	0.56	3.32×10^{-56}
Coarse Gravel	0.09	2.94×10^{-12}	0.17	2.12×10^{-23}
Fine Gravel	0.18	1.37×10^{-24}	0.05	1.00×10^{-06}
Boulders	0.55	8.17×10^{-93}	0.02	6.65×10^{-04}
Sand	-	-	0.08	3.73×10^{-11}
Cobblers	0.15	3.92×10^{-20}	0.15	1.76×10^{-20}
U. delphinus	0.07	1.29×10^{-09}	0.05	1.00×10^{-06}
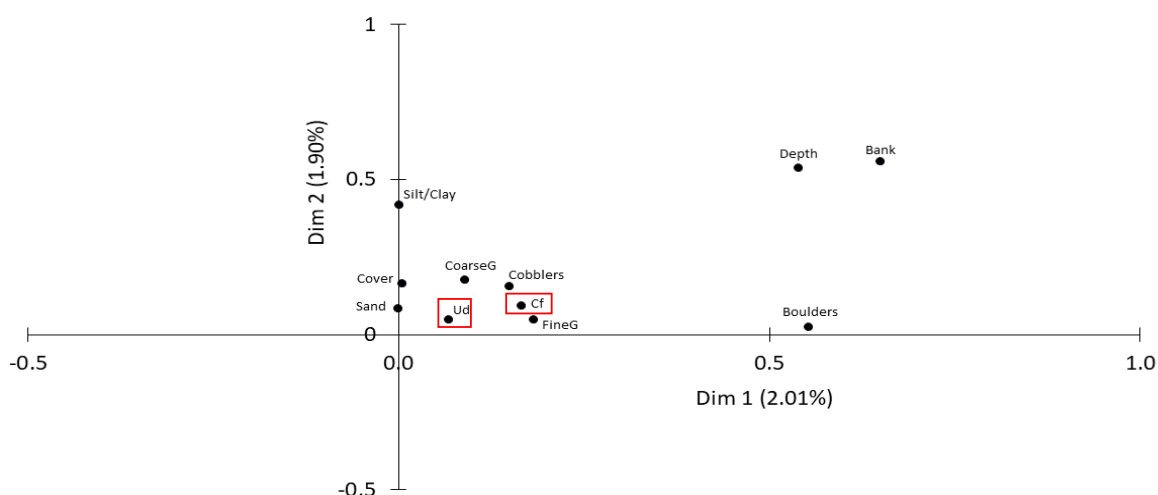
Figure 3. Multiple Correspondence Analysis (MCA) plot analysis for independent variables. Zoomed plot to 0.0 coordinates. Dim 1—Dimension 1 and Dim 2—Dimension 2. Dimensions represent categories with the highest contribution and the highest fraction of the total variance in the data. Codes for qualitative variables (presence/absence): Silt/Clay; Sand; FineG—fine gravel; CoarseG—coarse gravel; Cobblers; Boulders; Cover—Riparian gallery cover; Ud—*U. delphinus* species presence; Cf—*C. fluminea* species presence. Variable codes for quantitative variables (continuous): Bank—Distance to near margin (m); Depth—Water column depth (m).

The previous findings were also mostly consistent with the Ivlev's electivity index results regarding the native Unionidae (Figure 4; Table A1 of Appendix). *Unio delphinus* preferred coarse (E = 0.47) and fine gravel (E = 0.34) and avoided sandy substrates (E = −0.28). Results are similar for *C. fluminea*, which was found preferentially in fine (E = 0.53) and coarse gravel (E = 0.49), avoiding boulders (E = −0.22) and sand (E = −0.16; an opposite result from Spearman rank correlations).
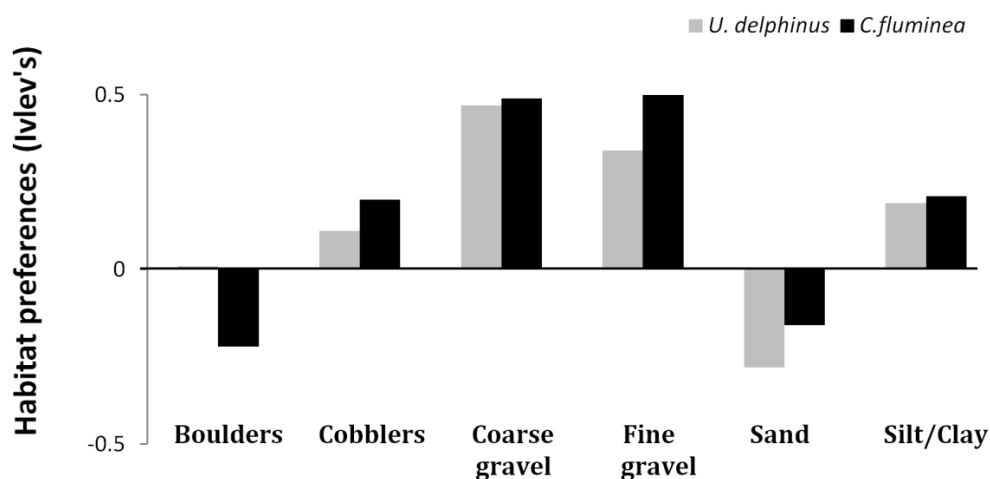

Figure 4. Habitat preferences of *U. delphinus* and *C. fluminea* according to Ivlev's electivity index. E_i is scaled so that $E_i < 0$ indicates avoidance and $E_i > 0$ indicates preference.

The dominant size classes of *U. delphinus* were [40–45[mm in 2017, [60–65[mm in 2016 and [55–60[mm in 2015, and for *C. fluminea* [20–25[mm both in 2015 and 2017 and [30–35[mm in 2016. However, for both species, population structure was different among years (G-log likelihood ratio: G = 198, *p* < 0.01, d.f. = 10, *n* = 762 for *U. delphinus* and G = 201, *p* < 0.01, d.f. = 10, *n* = 750 for *C. fluminea*; Table 4, Figure 5). In 2016 and 2017 there was an increase of *U. delphinus* smaller size classes (especially in the classes >30 mm and [30–40[mm (in 2017) (Figure 5). A similar pattern was observed for *C. fluminea* in 2017 (size classes >10 mm and [10–20[mm).

Table 4. Results from the post hoc *z* test after the G likelihood ratio was performed comparing size structure for both bivalve species. Same letters from *a, b and c* denote a subset of Year categories whose column proportions do not differ significantly from each other at the 0.05 level.

Species	Size Class	Year		
		2015	**2016**	**2017**
U. delphinus	>30 mm	*a*	*b*	*b*
	30–40 mm	*a*	*a*	*b*
	40–50 mm	*a*	*b*	*c*
	50–60 mm	*a*	*a*	*b*
	60–70 mm	*a*	*a*	*b*
	<70 mm	*a*	*b*	*b*
C. fluminea	>10 mm	*a*	*b*	*b*
	10–20 mm	*a*	*a*	*b*
	20–30 mm	*a*	*b*	*a*
	30–40 mm	*a*	*a*	*b*
	40–50 mm	*a*	*a*	*b*
	<50 mm	*a*	*a*	*b*
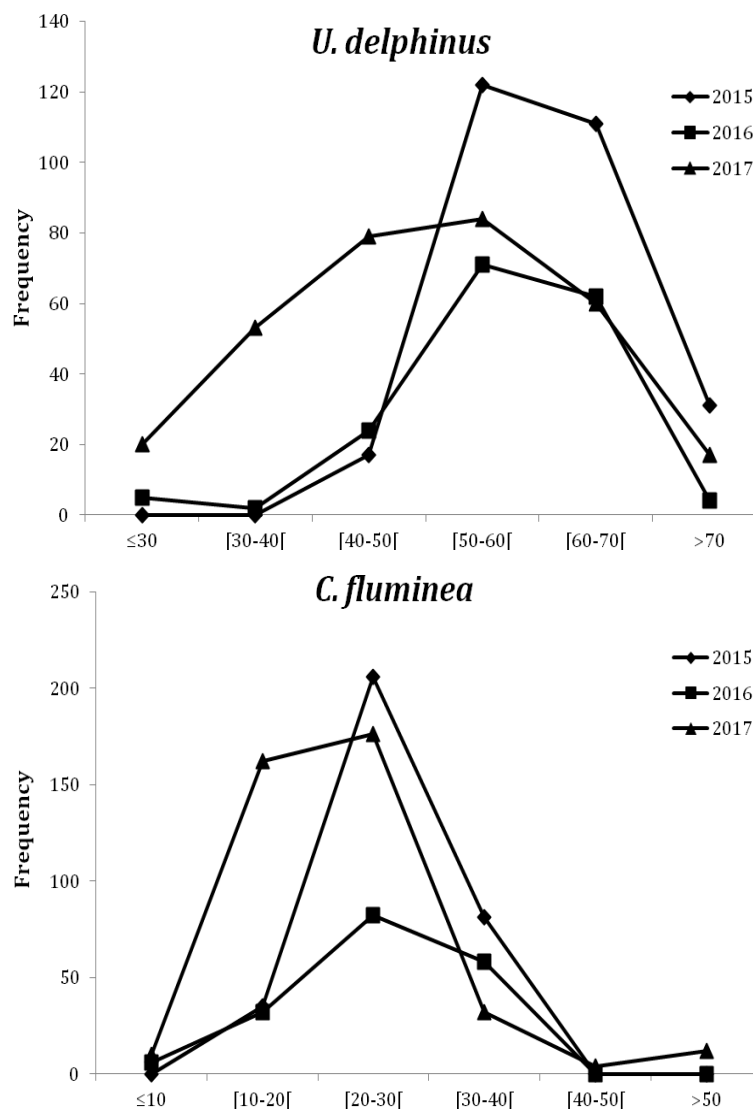
Figure 5. Size frequency distributions (according to the different size classes) of *U. delphinus* and *C. fluminea* from 2015 to 2017 (pooled data from six pools).

4. Discussion

The native *U. delphinus* and the introduced *C. fluminea*, when co-occurring, they roughly coexist in the same substrate types, preferring coarse and fine gravel, respectively. Nevertheless, *U. delphinus* was more abundant in locations under riparian tree cover, unlike *C fluminea*. The importance of vegetation for *U. delphinus* is unclear, but it may be related to protection against high temperatures caused by direct sunlight. Other authors reported *U. delphinus* burrowing in banks between tree roots in hydraulically more stable locations [16]. Related species, such as *U. tumidiformis*, *U. mancus*, and *U. ravoisieri* seem to have the same preference for river locations shaded by riparian vegetation [31,32].

Densities of *U. delphinus* were higher than the reported for the northern Portuguese rivers Tua and Sabor (0.015–0.121 ind/m^2; [33]), although sampling techniques differed, and those rivers were permanent. In the Oeiras river, while *U. delphinus* occurred in all the sampled pools, *C. fluminea* was only present in the two most downstream pools. It is possible that *C. fluminea* had fewer capabilities to expand upstream due to the river intermittency despite studies suggesting *C. fluminea* can move upstream up to 2.4 km per year [34]. In our study site the closest pool is approximately 4 km upstream (with the section in between pools being dry most of the year). Native bivalves are very adapted to summer conditions, unlike *C. fluminea,* which is known to be sensitive to summer environmental stress, suffering mass mortality events [35] making upstream dispersal more difficult. Some studies also suggest that the success of *C. fluminea* invasion decreases with increasing abundance of adult native mussels, probably due to lack of space for the invaders, physical displacement by actively burrowing mussels and locally reduced food and oxygen resources [21]. *C. fluminea* is a hermaphroditic species. Larvae are incubated until being released as juveniles into the water column, settle and bury [9]. Before settling they can be dragged through currents or move attached to other organisms [9]. Unlike native mussels this invasive species does not need a fish host to successfully reproduce, which is an enormous competitive advantage. On the contrary, glochidium larvae of freshwater Unionidae need to find suitable fish hosts to attach themselves to and metamorphose into free-living juveniles [17].

Our results were consistent with previous studies reporting *C. fluminea* preference for sediments with large organic matter content (lower grain sized sediments) such as sand mixed with silt and clay [10]. Organic material in sediments may be important for *C. fluminea* as this species is known for high filtration and growth/turnover rates, exacerbated during summer conditions [10,36]. Nonetheless in our pools sand was very uncommon, only detected in 2016. Therefore, the Ivlev's index avoidance and the association observed in Spearman's rank correlation have to be interpreted very carefully. Karatayev and co-workers [37], found a correlation between *C. fluminea* occurrence and of other three unionids. The same study reported that both were mostly found in coarse detritus (as *U. delphinus* in our study) and clay substrates (*C. fluminea*), similar to this study, due to the higher organic matter content and at the same depth.

Corbicula fluminea presence can exacerbate the pressure on *U. delphinus* by competing for food [21,38] and reducing available habitats for juvenile unionids. Suspension and deposit feeding negatively impact unionid recruitment, and the ingestion of unionids sperm, glochidia and juveniles may contribute to population declines [9,20]. Also, the introduction of new parasites and diseases [39], and increased ammonia toxicity as a result of massive *C. fluminea* die-offs, especially in the summer, may also increase native bivalves' mortality [40].

Changes in environmental conditions due to global warming may enable the colonization by new fish, which may not be suitable hosts for some native bivalve's glochidia [41]. Additionally, invasive invertebrate species may predate or compete with *U. delphinus* [1,9,42–45]. Several invasive predatory species are already abundant in this river, such as the Pumpkinseed sunfish (*Lepomis gibbosus*), the Chameleon cichlid (*Australoheros facetus*) and the red swamp crayfish (*Procambarus clarkii*), which may partially explain the low number of juveniles detected during this three-year survey.

Finally, as a consequence of global warming, and water deviation for irrigation and livestock, a reduction in water availability is expected in pools in the coming years [14] further increasing the pressure on *U. delphinus*.

5. Conclusions

In conclusion, the knowledge about *U. delphinus* preference for locations under riparian vegetation, with coarse and fine gravel, may aid conservation efforts. In this context, improvement of riparian vegetation should lead to better-quality habitat, potentially decreasing suitability for invasive species such as *C. fluminea*. Mitigation or protection measures should start by protecting or increasing riparian areas, enabling protection from high summer temperatures, and maintaining areas with coarse and fine gravel. Since our results suggest that native and invasive species prefer similar sediment types and may compete for space, extreme caution should be taken not to allow *C. fluminea* expansion upstream.

Appendix A

Table A1. Percentage of occurrence of *U. delphinus* and *C. fluminea* in sediment types and percentage of occurrence of each sediment type (yearly).

Year	Species	Sediment Type					
		Boulders	Cobblers	Coarse Gravel	Fine Gravel	Sand	Silt/Clay
2015	*U. delphinus*	8	15	42	25	1	8
	C. fluminea	4	16	41	8	0	31
2016	*U. delphinus*	29	16	24	10	4	18
	C. fluminea	13	22	30	22	8	5
2017	*U. delphinus*	23	11	11	43	0	12
	C. fluminea	19	13	10	44	0	13
% of occurrence							
	2015	29	14	7	0	0	10
	2016	13	10	8	7	1	10
	2017	14	10	8	12	0	6

References

1.　Vaughn, C.C.; Hakenkamp, C.C. The functional role of burrowing bivalves in freshwater ecosystems. *Freshw. Biol.* **2001**, *46*, 1431–1446. [CrossRef]

2.　Sala, O.E.; Chapin, F.S.; Armesto, J.J.; Berlow, E.; Bloomfield, J.; Dirzo, R.; Huber-Sanwald, E.; Huenneke, L.F.; Jackson, R.B.; Kinzig, A.; et al. Global biodiversity scenarios for the year 2100. *Science* **2000**, *287*, 1770–1774. [CrossRef] [PubMed]

3.　Bogan, A.E. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. *Hydrobiol* **2008**, *595*, 139–147. [CrossRef]

4.　Osterling, M.E.; Arvidsson, B.L.; Greenberg, L.A. Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: Influence of turbidity and sedimentation on the mussel and its host. *J. Appl. Ecol.* **2010**, *47*, 759–768. [CrossRef]

5.　Lopes-Lima, M.; Burlakova, L.E.; Karatayev, A.; Mehler, K.; Seddon, M.; Sousa, R. Conservation of freshwater bivalves at the global scale: Diversity, threats and research needs. *Hydrobiologia* **2018**, *810*, 1–14. [CrossRef]

6.　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.; et al. The global decline of nonmarine mollusks. *BioScience* **2004**, *54*, 321–330. [CrossRef]

7.　Aldridge, D.C.; Elliot, P.; Moggridge, G.D. The recent and rapid spread of the zebra mussel (*Dreissena polymorpha*) in Great Britain. *Biol. Conserv.* **2004**, *119*, 253–261. [CrossRef]

8.　Strayer, D.L.; Malcom, H.M. Effects of zebra mussels (*Dreissena polymorpha*) on native bivalves: The beginning of the end or the end of the beginning? *J. N. Am. Benthol. Soc.* **2007**, *26*, 111–122. [CrossRef]

9.　Sousa, R.; Antunes, C.; Guilhermino, L. Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: An overview. *Int. J. Lim.* **2008**, *44*, 85–94. [CrossRef]

10.　Sousa, R.; Nogueira, A.J.A.; Gaspar, M.B.; Antunes, C.; Guilhermino, L. Growth and extremely high production of the nonindigenous invasive species *Corbicula fluminea* (Müller, 1774): Possible implications for ecosystem functioning. *Estuar. Coast. Shelf Sci.* **2008**, *80*, 289–295. [CrossRef]

11.　Sousa, R.; Gutiérrez, J.L.; Aldridge, D.C. Non-indigenous invasive bivalves as ecosystem engineers. *Biol. Invasions* **2009**, *11*, 2367–2385. [CrossRef]

12.　Sousa, R.; Varandas, S.; Cortes, R.; Teixeira, A.; Lopes-Lima, M.; Machado, J.; Guilhermino, L. Massive die-offs of freshwater bivalves as resource pulses. *Ann. Limnol.-Int. J. Lim.* **2012**, *48*, 105–112. [CrossRef]

13.　Ilarri, M.; Sousa, R. Corbicula fluminea Müller (Asian clam). In *A Handbook of Global Freshwater Invasive Species*; Francis, R.A., Ed.; Routledge: London, UK, 2012; Chapter 15; p. 484.

14.　Anastácio, P.M.; Ribeiro, F.; Capinha, C.; Banha, F.; Gama, M.; Filipe, A.F.; Rebelo, R.; Sousa, R. Non-native freshwater fauna in Portugal: A review. *Sci. Total Environ.* **2019**, *650*, 1923–1934. [CrossRef] [PubMed]

15.　IPCC. Summary for policymakers. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2014; pp. 1–32.

16.　Araujo, R.; Toledo, C.; Machordom, A. Redescription of *Unio gibbus* Spengler, 1793, a west palaearctic freshwater mussel with hookless glochidia. *Malacologia* **2009**, *51*, 131–141. [CrossRef]

17.　Reis, J.; Machordom, A.; Araujo, R. Morphological and molecular diversity of Unionidae (Mollusca, Bivalvia) from Portugal. *Graellsia* **2013**, *69*, 17–36.

18.　Lopes-Lima, M.; Sousa, R.; Geist, J.; Aldridge, D.C.; Araujo, R.; Bergengren, J.; Bespalaya, Y.; Bódis, E.; Burlakova, L.; Van Damme, D.; et al. Conservation status of freshwater mussels in Europe: State of the art and future challenges. *Biol. Rev.* **2017**, *92*, 572–607. [CrossRef] [PubMed]

19.　Novais, A.; Dias, E.; Sousa, R. Inter- and intraspecific variation of carbon and nitrogen stable isotope ratios in freshwater bivalves. *Hydrobiologia* **2016**, *765*, 149–158. [CrossRef]

20.　Modesto, V.; Castro, P.; Lopes-Lima, M.; Antunes, C.; Ilarri, M.; Sousa, R. Potential impacts of the invasive species *Corbicula fluminea* on the survival of glochidia. *Sci. Total Environ.* **2019**, *673*, 157–164. [CrossRef] [PubMed]

21.　Vaughn, C.C.; Spooner, D.E. Scale-dependent associations between native freshwater mussels and invasive Corbicula. *Hydrobiologia* **2006**, *568*, 331–339. [CrossRef]

22.　Rajagopal, S.; Pollux, B.J.A.; Peters, J.L.; Cremers, G.; Moon-van der Staay, S.Y.; van Alen, T.; Eygensteyn, J.; van Hoek, A.; Palau, A.; bij de Vaate, A.; et al. Origin of Spanish invasion by the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) revealed by amplified fragment length polymorphism (AFLP) fingerprinting. *Biol. Invasions* **2009**, *11*, 2147–2159. [CrossRef]

23.　McRae, S.E.; Allan, J.D.; Burch, J.B. Reach and catchment scale determinants of the distribution of freshwater mussels (Bivalvia: Unionidae) in south-eastern Michigan, USA. *Freshwat. Biol.* **2004**, *49*, 127–142. [CrossRef]

24.　Obermeyer, B.K. Comparison of Quadrats Versus Timed Snorkel Searches for Assessing Freshwater Mussels. *Am. Midl. Nat.* **1998**, *155*, 307–320. [CrossRef]

25. Schloesser, D.W.; Metcalfe-Smith, J.L.; Kovalak, W.P.; Longton, G.D.; Smithee, R.D. Extirpation of Freshwater Mussels (Bivalvia: Unionidae) Following the Invasion of Dreissenid Mussels in an Interconnecting River of the Laurentian Great Lakes. *Am. Midl. Nat.* **2006**, *155*, 307–320.42. [CrossRef]
26. *ASTM D2487—1966 Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System)*; Reapproved; ASTM International: West Conshohocken, PA, USA, 2006.
27. Zar, J.H. *Biostatistical Analysis*, 4th ed.; Prentice-Hall, Inc.: Upper Saddle River, NJ, USA, 1999; p. 663.
28. Anastácio, P.M.; Banha, F.; Capinha, C.; Bernardo, J.M.; Costa, A.M.; Teixeira, A.; Bruxelas, S. Indicators of movement and space use for two co-occurring invasive crayfish species. *Ecol. Indic* **2015**, *53*, 171–181. [CrossRef]
29. Loughman, Z.J.; Skalican, K.T.; Taylor, N.D. Habitat selection and movement of *Cambarus chasmodactylus* (Decapoda: Cambaridae) assessed via radio telemetry. *Freshwat. Sci.* **2013**, *32*, 1288–1297. [CrossRef]
30. Smith, D.G. Sistematics and distribution of the recent Margaritiferidae. In *Ecology and Evolution of the Freshwater Mussels Unionoida*; Springer: Berlin/Heidelberg, Germany, 2001; pp. 33–49.
31. Araujo, R.; Unio tumidiformis. The IUCN Red List of Threatened Species. 2011. Available online: http://dx.doi.org/10.2305/IUCN.UK.20112.RLTS.T171935A6810869.en (accessed on 10 November 2011).
32. Rovira, Q.; Campos, M.; Feo-Quer, C.; Camós, I.; Martí, J.; Angelats, I.; Cros, C.; Casadevall, R.; Dalmau, G.; Cruset, E.; et al. Habitat preferences of *Unio mancus* and *U. ravoisieri* in northeast of Catalonia. In Proceedings of the 2nd International Seminar on the Rearing of Unionoid Mussels, Clervaux, Luxembourg, 24 November–27 November 2015.
33. Patrício, C.I.M. Contribuição para o estudo da bioecologia dos mexilhões de água doce (Unionoida) do Nordeste de Portugal. Master Thesis, University of Oporto, Oporto, Portugal, 2013.
34. Beran, L. Spreading expansion of *Corbicula fluminea* (Mollusca: Bivalvia) in the Czech Republic. *Heldia* **2006**, *6*, 187–192.
35. Vohmann, A.; Borcherding, J.; Kureck, A.; bij de Vaate, A.; Arndt, A.; Weitere, M. Strong body mass decrease of the invasive clam *Corbicula fluminea* during summer. *Biol. Invasions* **2010**, *12*, 53–64. [CrossRef]
36. Way, C.; Hornbach, D.; Miller-Way, C.; Payne, B.; Miller, A.C. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). *Can. J. Zool.* **2011**, *68*, 115–120. [CrossRef]
37. Karatayev, A.Y.; Burlakova, L.E.; Kesterson, T.; Padilla, D.K. Dominance of the Asiatic clam, *Corbicula fluminea* (Müller) in the benthic community of a reservoir. *J. Shellfish Res.* **2003**, *22*, 487–493.
38. Ferreira-Rodriguez, N.; Pardo, I.; Sousa, R. Negative effects of *Corbicula fluminea* over native freshwater mussels. *Hydrobiology* **2018**, *810*, 85–95. [CrossRef]
39. Graczyk, T.K.; Conn, D.B.; Marcogliese, D.J.; Graczyk, H.; de Lafontaine, Y. Accumulation of human waterborne parasites by zebra mussels (*Dreissena polymorpha*) and Asian freshwater clams (*Corbicula fluminea*). *Parasitol. Res.* **2003**, *89*, 107–112. [PubMed]
40. Cherry, D.S.; Scheller, J.L.; Cooper, N.L.; Bidwell, J.R. Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) I: Water-column ammonia levels and ammonia toxicity. *J. N. Am. Benthol. Soc.* **2005**, *24*, 369–380. [CrossRef]
41. Taeubert, A.; Martinez, J.E.; Gum, M.P.B.; Geist, J. The relationship between endangered thick-shelled river mussel (Unio crassus) and its host fishes. *Biol. Conserv.* **2012**, *155*, 94–103. [CrossRef]
42. Correia, A.M.; Bandeira, N.; Anastácio, P.M. Predator-prey interactions of *Procambarus clarkii* with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica* **2005**, *28*, 337–343. [CrossRef]
43. Reis, J.; Araújo, R. Life history of the freshwater mussel *Unio tumidiformis* (Bivalvia: Unionidae) in a temporary Mediterranean-type stream. *Invert. Biol.* **2016**, *135*, 31–45. [CrossRef]
44. Weiss, S.; Marić, S.; Snoj, A. Regional structure despite limited mtDNA sequence diversity found in the endangered Huchen, *Hucho hucho* (Linneaus, 1758). *Hydrobiologia* **2010**, *658*, 103–110. [CrossRef]
45. Meira, A.; Lopes-Lima, M.; Varandas, S.; Teixeira, A.; Arenas, F.; Sousa, R. Invasive crayfishes as a threat to freshwater bivalves: Interspecific differences and conservation implications. *Sci. Total Environ.* **2018**, *649*, 938–948. [CrossRef]