Science of the Total Environment 624 (2018) 273–282

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

On the reintroduction of the endangered thick-shelled river mussel *Unio crassus*: The importance of the river's longitudinal profile

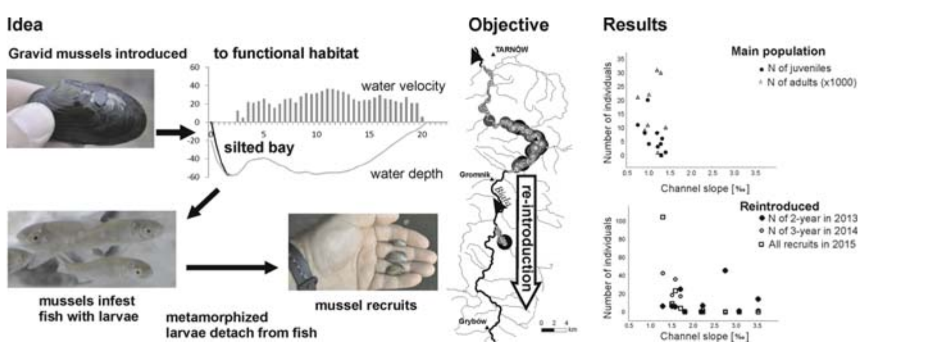
Katarzyna Zając [a,*], Jacek Florek [b], Tadeusz Zając [a], Paweł Adamski [a], Wojciech Bielański [a], Adam M. Ćmiel [a], Mariusz Klich [c], Anna M. Lipińska [a]

[a] Institute of Nature Conservation, Polish Academy of Sciences, al. Mickiewicza 33, 31-120 Kraków, Poland
[b] Department of Hydraulic Engineering and Geotechnics, University of Agriculture, al. Mickiewicza 24/28, 30-059 Kraków, Poland
[c] State Higher Vocational School in Tarnów, al. Mickiewicza 8, 33-100 Tarnów, Poland

HIGHLIGHTS

- Freshwater mussels constitute one of the most endangered groups of organisms in the world.
- The Biała River was studied with regard to existing and reintroduced populations of endangered mussel species.
- Neither physicochemical water parameters nor fish hosts distribution correlated with the mussels distribution.
- Hydromorphological variables were correlated with mussels' distribution, recruitment and success of the reintroduction.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form 27 November 2017
Accepted 29 November 2017
Available online xxxx

Keywords:
Unionidae
Channel morphology
Freshwater mussel restoration
Water chemistry
Functional habitat
Mussels recruitment

ABSTRACT

Freshwater mussels of the order Unionida provide important ecosystem functions and services. Unfortunately, some previously widespread species are now seriously endangered. To restore the historical range of the population of *Unio crassus* in the Biała River, southern Poland, the species was reintroduced into a series of 'stepping stones' joining two remnant populations. During the first phase of the study, the relationships between the abundance of *U. crassus*, physical habitat, and water quality were studied to assess reintroduction potential. In general, chemical water quality improved upstream from the existing population, favouring the decision for reintroduction, whereas morphological variables worsened. Mussel abundance was correlated negatively with the elevation and slope of channel, organic matter contents, and pH (exceeding 8.0), but positively with silt presence, water conductivity, and concentrations of HCO_3^-, Ca^{2+}, and NO_3^-. During the second phase, adult individuals were introduced into one type of functional habitat—marginal channel sectors with still water and fine sediment. Despite the initial very high rate of reproduction in some parts of the upper reach of the river, the juveniles were ultimately recruited only in the lower part of the restored range, resulting in a very rapid change in recruitment at a channel slope of 1.8‰. Recruitment was positively related to silt content, conductivity, and Ca^{2+} and HCO_3^- ions, negatively to channel elevation and slope, and water pH. The host fish species showed no correlation with abiotic habitat features within the studied reach. These results imply that most of the habitat traits related to *U. crassus* occurrence depended on the river's longitudinal profile, not on the chemical water quality, and that final success of introduction should be evaluated after several years.

* Corresponding author.
E-mail address: kzajac@iop.krakow.pl (K. Zając).

https://doi.org/10.1016/j.scitotenv.2017.11.346
0048-9697/

1. Introduction

Lotic ecosystems are, perhaps, the most heavily impacted habitats on the planet (Malmqvist and Rundle, 2002). Although the quality of freshwater systems in developed countries is steadily improving (Geist, 2014), this does not compensate for the loss of ecosystem processes and related biodiversity caused by changes in the past (Aarts et al., 2004; Riccardi et al., 2016). The direct link between ecosystem functions and services and freshwater biodiversity is frequently exemplified by freshwater mussels, which directly improve water quality (Kryger and Riisgård, 1988; Lummer et al., 2016; Pusch et al., 2001; Vaughn, 2017; Welker and Walz, 1998), while indirectly influencing other freshwater functions (Gutiérrez et al., 2003; Haag, 2012; Strayer et al., 1999; Vaughn, 2010; Vaughn and Hakenkamp, 2001; Vaughn et al., 2008). Freshwater mussels have been proposed as indicators of the ecological integrity of freshwater ecosystems (Aldridge et al., 2007; Farris and Van Hassel, 2006; but see Richter et al., 2016); thus, efficient restoration of freshwater ecosystems should be indicated by healthy mussel populations (Altmüller and Dettmer, 2006; Lundberg and Österling, 2016). However, freshwater mussels, though not long ago widespread and numerous and even used on a massive scale for commercial purposes (Haag, 2012; Williams et al., 1993), constitute one of the most endangered groups of organisms in the world (Lydeard et al., 2004). The identification of factors leading to their disappearance and, even more importantly, of factors hampering their restoration is still an unresolved scientific challenge. Mussel distribution may be influenced by many abiotic and biological factors, including deterioration of abiotic habitat (Holland-Bartels, 1990; Mueller et al., 2011), quality of water and chemical pollution (Douda, 2007, 2010; Hochwald, 2001; Hus et al., 2006; Naimo, 1995), clogging of interstitial spaces (Geist and Auerswald, 2007; Österling et al., 2010), hydraulic conditions (Gates et al., 2015; Hardison and Layzer, 2001; Moorkens and Killeen, 2014), catastrophic events (Hastie et al., 2001), and even large-scale problems within catchments (Horton et al., 2015). An additional confounding effect is the complicated life cycle of Unionida, whereby females expel large numbers of parasitic larvae into water; these larvae attach themselves to the body surfaces of fish and encyst, detaching themselves after few weeks, fall into sediments and thus start their independent life (Haag, 2012). Thus, fish host availability may affect mussel distribution (Douda, 2015; Lopes-Lima et al., 2017; Schneider, 2017; Stoeckl et al., 2015; Taeubert et al., 2012a; Watters, 1996). An in situ assessment of which intra-watercourse factors enable successful reproduction and subsequent population restoration, as advocated by Gray and Kreeger (2014), is needed.

This complicated array of factors is not easy to apply in mussel restoration projects. The fundamental questions are:

- To what degree does successful restoration of the habitat and related biota depend on general habitat features which affect large areas of the river continuum (Vannote et al., 1980)?
- Should the habitat be studied on a small scale within identified hydrological units (functional habitats; Harper et al., 1992) nested within the river channel?

Both approaches may be appropriate.

It is assumed that mussels occur in parts of a channel with more stable bed (Strayer's flow refuges; Strayer, 1999), which implies the crucial role of functional habitats. As confirmed in behavioural experiments involving endangered *Unio crassus* with respect to functional habitats, this species prefers marginal still-water areas with fine sediment deposits (Zając and Zając, 2011; see also Zieritz et al., 2014). If reintroduction of the species was conducted within one type of functional habitat in relatively uniform and discrete spatial units, it would be easier to see the influence of longitudinal processes. We used this approach to define features of the longitudinal profile of a mountain river that can influence successful reintroduction of the thick-shelled river mussel *U. crassus*, one of the most endangered European freshwater mussel species (Lopes-Lima et al., 2017). During the first phase of the study, we identified the basic longitudinal features of the river (morphology, water physicochemistry, fish distribution) which might be critical for the success of planned mussel reintroduction. During the second phase, i.e. actual reintroduction, the assumptions derived from the first phase were verified in the course of a direct field experiment involving the relocation of adults into a preferred functional habitat and monitoring of their breeding success.

2. Materials and methods

Data on reintroduction were collected in the years 2011–2015 in the course of a project devoted to convert weirs to enable migration of fish (cf. Watters, 1996) in the Biała Tarnowska, a medium-sized, low-mountain river (Carpathians, southern Poland). The upper part of the river had been heavily impacted in the past by organic pollution from the local brewery in the town of Grybów (49°37′27″ N; 20°56′52″ E) and by channel regulation started in the 1890s (Szuba, 2012). Considering the significant improvement of water quality in the river and available historical data on the occurrence of mussels in its upper course, the main aim of the project was to accelerate the process of river recolonisation up to the weirs, reintroducing the species from the main population into a series of 'stepping stones' in order to initiate its further expansion (Fig. 1).

2.1. Study site

The Biała Tarnowska River is a 101.8-km-long, right-bank tributary of the Dunajec River, which flows from a Carpathian range called the Beskid Niski northwards to the Dunajec River valley. The area of its catchment is 983 km^2. Mean water depth at low-flow conditions is 0.54 m; the average channel depth is 4.5 m, while channel width ranges from ca 15 m (in incised reaches) to 50 m (in braided reaches). The Biała Tarnowska flows through areas underlain by sedimentary rocks: thin intercalating layers of sandstones, claystones, and siltstones called Carpathian flysch, characterised by diversified resistance to erosion and supplying a specific type of bed material consisting of very flat gravels and large quantities of sand and fine sediment. Bedrock exposures also occur within the river channel. The main area of the study and mussel reintroduction (a reach ca 60 km long) was located between the village of Stróże (49°39′40.1″ N; 20°57′58.7″ E) near Grybów (303 m asl) and the city of Tarnów (50°0′43.5″ N; 20°59′9″ E) (190 m asl). The channel is characterised by a pool-riffle pattern; longer sections of plain bed occur in the upper part of the studied reach (channel classification according to Montgomery and Buffington, 1998). The area lies within a Natura 2000 site ('Biała Tarnowska', PLH120090). Under the Polish monitoring scheme (Zając, 2010), the studied population has been accorded favourable (FV) status, with an unfavourable habitat (U1), and a density of *U. crassus* locally reaching over 50 ind./m^2, with very good recruitment (Zając K., unpublished data).

2.2. Field protocol

2.2.1. Species distribution study

The Biała River and its tributaries were carefully inspected in May–June 2009 to map the distribution of existing populations of *U. crassus*. This was based on the slow penetration of the channel at low-flow periods by at least two researchers wading or floating on pontoons in deeper sections, and searching through to the bottom with aquascopes to the depth of 0.5 m (Zając and Zając, 2011). The survey was repeated in May–July 2011, at which time an exact count of the population was conducted: individuals were located using GPS and totalled for each 100-m section of the river course (Fig. 1), and the reach designated for recolonisation was inspected in search for potential receptor sites. At

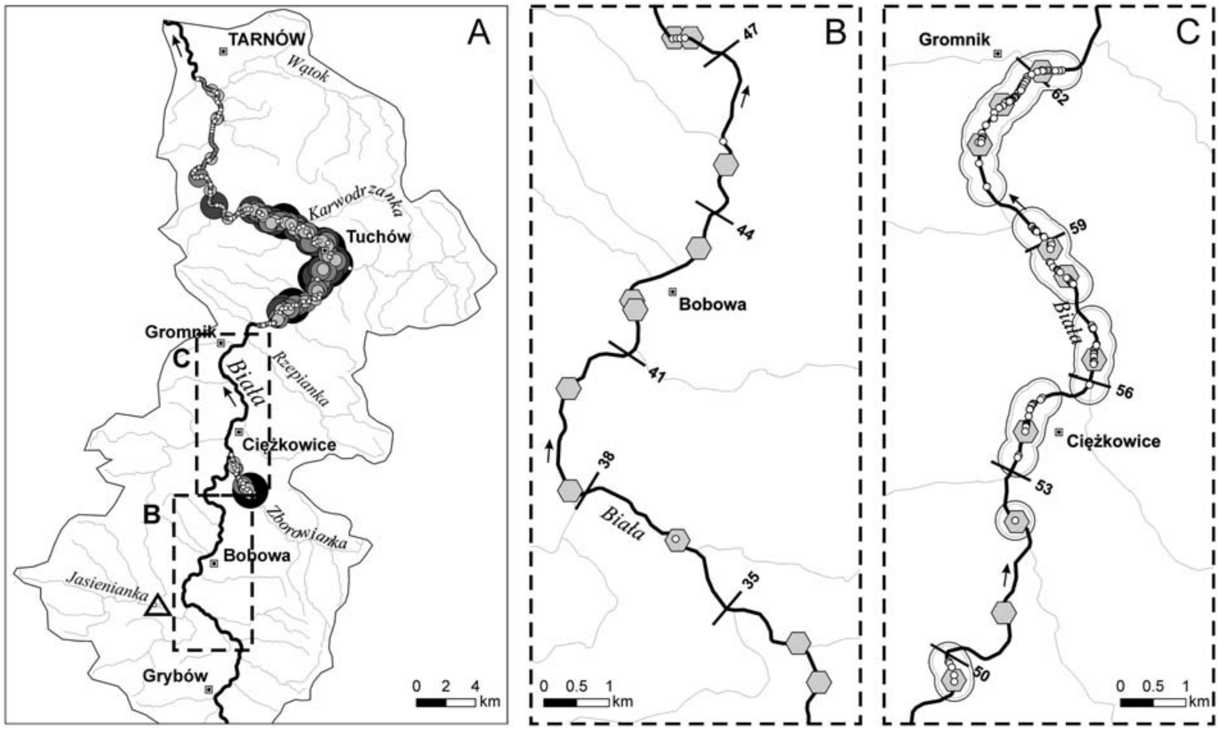


Fig. 1. General distribution of *Unio crassus* within the studied reach of the Biała River, including individuals derived from reintroduction. (A) – general view of the studied catchment with distribution of *U. crassus* remnant populations and sub-fossil sites. Circles represent the number of individuals per 100 m (the size of the circle is proportional to the number of mussels; the largest circle represents over 1000 ind./100 m); triangle represents sub-fossil records. (B, C) – details of the upper part of the reach where reintroduction was performed, with receptor sites marked with large hexagons and juveniles with small white dots; numbered dashes represent 3-km divisions of the analysed river reach; double-line buffers represent the 500–600-m potential dispersal rate of the progeny of 3-year individuals found in given locations in 2014.

the same time, the Carpathian rivers characterised by similar morphology (the San, Osława, Osławica, Jeleśnia, and Wisłok) and harbouring populations of *U. crassus* were surveyed starting from their upper parts, using the same methodology, in order to establish the upper boundary of the occurrence of the species in the Polish Carpathians.

The studied reach of the river (ca 60 km) was subdivided into 3-km sections. At downstream ends of each section, permanent sampling stations were established. At these stations, water samples were collected in order to analyse their physicochemical properties, along with samples of a layer, ca 10 cm thick, of the bed sediments in the areas of still water near the bank (in places where the occurrence of *U. crassus* was expected, from a depth of water around 20–30 cm; Zając and Zając, 2011). Samples of undisturbed sediments (ca 2 kg each) were taken to the laboratory and sieved to determine the relative proportions of gravel (>2 mm), sand (2–0.063 mm), silt (0.062–0.004 mm), and clay (<0.004 mm). At the same sampling stations, but only within the extent of the main population of the species, the nearest locations inhabited by *U. crassus* were searched, with samples of bed sediments from an area of 1 × 20 m along the bank collected and sieved (mesh size 2 mm) for the presence of juveniles. This sampling was conducted in November, to sample fully-grown (up to a shell length of 15 mm) juveniles from that year. The same methodology was used to sample juveniles at receptor sites (the sites where *U. crassus* was reintroduced; see below).

The altitude of the water level and the channel bed was measured in September–October 2013 at the sampling stations; both measurements were taken for the entire channel cross-section and for the local longitudinal profile over an average stretch, 136 m long, of the riverbed. Measurements were collected using a standard GPS RTK (Real-Time Kinematic) device with the support of the Polish ASG-EUPOS system, with an accuracy exceeding 0.03 m horizontally and 0.05 m vertically. The analysis of water level and channel bed yielded redundant results, so in subsequent analyses we show only results for the latter.

2.2.2. Water chemistry

Water samples were collected at each of the sampling stations: twice in 2011 and once in 2012. However, for the analysis we used samples collected at the Ciężkowice water-gauge station in the middle of the *U. crassus* breeding season on 5 July 2012, at the time of the lowest water level (daily water level: 164 cm; minimum water level for breeding season in 2012: 151 cm). Samples were collected in 0.33-l containers placed in a field refrigerator and transported to the laboratory, where they were analysed in a chromatograph on the following day. The water samples for suspended matter analysis were collected at each of the sampling stations in September–October 2013 and taken to the laboratory, where they were filtered, evaporated in a forced-air oven at 105 °C, and heated in a muffle furnace at 550 °C. Subsequently, suspended sediment density in water and mineral-organic composition were evaluated.

2.2.3. Electrofishing

Fish species structure was surveyed in June 2012 at 16 sampling stations over a 300-m section of the channel, using standard electrofishing procedures. We omitted 5 stations because of technical difficulties: the river was too deep for wading, therefore fish had to be sampled from a boat. The strong water current, incised channel, and difficulties with relocating fish to their previous locations biased the results and caused unnecessary fish mortality.

The electrofishing was repeated in May 2013 at 16 receptor sites where the mussels were reintroduced. The electrofishing was conducted in 50-m river sections sampled on both channel sides. Five sites were omitted because they were located too close to each other (Fig. 1); electrofishing was conducted only for one of these, chosen randomly.

2.2.4. Reintroduction project

During the field survey of the whole river in 2011, we searched for suitable receptor sites for species reintroduction (Moorkens, 2017).

We searched for still water with fine sediment near the bank (Zając and Zając, 2011), fish presence, and a stable riverbank (growing vegetation vs eroded sediment). The selected sites were local pools near banks or small bays near large woody debris, with a near-bank thick layer of fine sediment which gradually decreased with depth, with fine gravel occurring deeper in the channel or bays within an old riprap demolished by the river. The functional 'silted' habitats were usually small (<30 m along the bank). Finally, 21 receptor sites, distributed more or less evenly throughout the reach, were selected.

The reintroduction commenced in 2012. Once the presence of gravid females within the studied population had been observed, as of 20–23 May, 30 large (over 50 mm in length), randomly selected individuals were distributed at each designated receptor site. Mussels were collected from a reach expected to sustain damage due to construction work (near Lubaszowa, 49°51′46.06″N, 21° 2′13.14″E). The mussels were placed in a small car refrigerator, in a container with an oxygenating device and filled with fresh water from the river. They were transported to the receptor site and released near the bank at a depth of ca 20 cm.

The receptor sites were checked every month for the sole purpose of viewing the general status of the site. The final survey of the site was conducted during the period 26 September–2 October 2012, at which time all bed sediments were sieved over an area of 1 × 20 m centrally positioned within the receptor site (see above). In 2013, the same reintroduction scheme was applied. The sites were first checked at the onset of reproduction to count adults; then their number was augmented with new individuals (taken from the bridge construction zone near Burzyn 49°52′51.39″N, 21° 3′17.87″E, on 14–16 May) to reach 30 individuals per site.

Each year from 2013 to 2015, the river reach subjected to reintroduction was surveyed in September–October, in order to detect juveniles recruited as a result of the reintroduction. The juveniles in successive seasons were much larger; thus, as in adults, their siphons were visible to the naked eye and they could be easily located and counted. Because young mussels were frequently dispersed and found even at considerable distances from the original receptor site, and since 2014 continuity in their occurrence was observed between certain receptor sites, their quantity was expressed as the number found within a 3-km section, standardised according to the number of receptor sites.

2.3. Data analysis

In order to verify the suitability of the river for reintroduction, the relationships between the numbers of the *U. crassus* individuals and hydrological and physicochemical variables were checked using non-parametric tests. Each variable was analysed separately, because of the lack of independence (e.g. an increased amount of silt in a bed-material sample automatically equates to a decrease in the proportion of gravel). For variables for which tested relationships were significant, ROC curve analysis was conducted (Metz, 1978, 1986), using the pROC package (Robin et al., 2011), in order to estimate the so-called 'cut-off points', i.e. the values of an analysed parameter representing the boundary between the occurrence and non-occurrence of the studied phenomenon (Metz, 1986). The reliability of such estimation is measured by two parameters: the sensitivity and specificity of each cut-off point (Metz, 1978, 1986; Shaw, 1980). Separate analyses were conducted for the native population and for the 'stepping stone' populations originating from the reintroduction. In the case of re-introduction success, we used logistic regression to analyse absence/presence data in relation to the same set of habitat features, because bottom surveys of juvenile (1-year) recruitment (by eye, without bottom sampling) are much less reliable in relation to their number than in the second or later years of the mussel life. On the other hand, we cannot rely only on the surveys conducted in the subsequent years, because we could lose information on the 1-year recruitment as a result of later environmental changes or juvenile mortality.

3. Results

3.1. Distribution of the species

The general distribution of the main and remnant populations in the study area is shown in Fig. 1. Some sub-fossil shells were found in the Jasienianka River (312 m asl). The *U. crassus* locations with the highest elevations in Polish Carpathian rivers of similar size and morphology were, on average, at 517 m asl ($Md = 452$ m asl; $N = 5$), with the highest location (632 m asl) in the Jeleśnia River.

The distribution of the species within the main channel of the Biała River was restricted to its lower reach (Fig. 1). In total, we counted 59,889 adult individuals in the main channel, with a mean density $d = 181.5$ ind. per 100m of the channel ($SD = 308.48$, $Md = 33.5$, $d_{max} = 1786$); only 31% of the 329 sections within the range of the population were found to be unoccupied. Fourteen percent of sampled individuals were three years old or younger.

An isolated population found in the Zborowianka River, a right-bank tributary of the Biała, was composed of 4030 individuals, with a mean density $d = 57.6$ ind./100 m ($SD = 183.80$, $Md = 19.5$, $d_{max} = 1514$); only 16% of the 100-m sections ($N = 70$) within the range of the population were found to be unoccupied. Twenty-eight percent of sampled individuals were three years old or younger.

3.2. Abiotic factors

Almost all of the studied abiotic parameters of the channel were strongly correlated with the elevation of the channel above sea level (Table 1, Fig. 2). The number of *U. crassus* individuals recorded within the entire studied reach correlated negatively with elevation, channel slope, and organic content of suspended matter, but positively with clay content in the bed-material samples. The physicochemical properties of the water also correlated with the number of mussels: positively with conductance and concentrations of HCO_3^-, NO_3^-, and Ca^{2+}, and negatively with pH; however, the values of pH within the study area were all above 8.0. Based on ROC curves, cut-off points for the absence/presence data were estimated for the most significant parameters from each group (Fig. 2).

The predictive role of the above relationships was also verified within the range of the main population. Here, the number of adults counted per 3-km section was not correlated with any of the analysed predictors, except for negative correlations with the concentrations of NO_3^- ($r_S = -0.71$, $N = 11$, $p = 0.014$) and PO_4^{3-} ($r_S = -0.71$, $N = 11$, $p = 0.014$).

Within the main population, the number of 100-m-long sections per 3-km reaches of the river, which were completely deprived of *U. crassus* individuals correlated with channel morphology: empty sections were more frequent in lower elevations within this reach ($r_S = -0.84$, $N = 11$, $p = 0.001$), and in steep channel parts (although the relationship was only close to statistical significance, $r_S = 0.60$, $N = 10$, $p = 0.068$). The density of first-year juveniles per sampling station within the main population was negatively correlated only with channel slope ($r_S = -0.75$, $N = 10$, $p = 0.012$, Fig. 3) and with the content of gravel in bed-material samples ($r_S = -0.66$, $N = 11$, $p = 0.027$).

3.3. Reintroduction opportunities in relation to fish hosts

Seventy-three percent of all fish caught ($N = 6698$) belonged to three species: *Barbus barbus* (30%), *Alburnus alburnus* (29%), and *Alburnoides bipunctatus* (13%). Thus, any general analysis of mussel counts in relation to fish counts would actually refer to these three species. The number of fish species also failed to correlate with the *U. crassus* population count ($r_S = 0.26$, $N = 15$, $p = 0.35$, one outlier rejected).

Scardinius erythrophthalmus and *Cottus gobio*, widely regarded as very good hosts for *U. crassus* (Taeubert et al., 2012b), were not recorded within the studied reach at all. *Phoxinus phoxinus* occurred in large quantities

Table 1
Coefficients of Spearman rank correlation between elevation of sampling stations and the parameters of the abundance of *Unio crassus* (number of adult individuals and number of juveniles), and river environmental parameters and the occurrence of potential host fish species shown for the whole river reach before mussel re-introduction and the reach with re-introduced mussels. For the latter reach, results of logistic regression of juvenile absence/presence on river environmental parameters are also shown.

	Whole reach			*Re*-introduced reach	
	Elevation of sampling station	Number of mussels per 3 km	Number of 2012 juveniles at sampling stations in the whole reach	2012 juveniles absence/pre-sence (logistic regression)	Number of 1-year–4-year mussels at final survey in 2015 (N/3 km)
	r_S	r_S	r_S	estimate	r_S
Environmental parameter	$N = 20$			$N = 11$	
Elevation		-0.68^{***}	-0.49^{*}	13.7^{***}	-0.89^{***}
Channel slope	0.84^{***}	-0.83^{***}	-0.49^{*}	12.1^{***}	-0.88^{***}
Percent of gravel	0.48^{*}	-0.38	-0.49^{*}	7.81^{**}	-0.62^{*}
Percent of sand	-0.08	0.24	0.25	2.99	0.41
Percent of silt	-0.66^{**}	0.39	0.50^{*}	2.93	0.60
Percent of clay	-0.73^{***}	0.50^{*}	0.55^{**}	6.12^{*}	0.69^{*}
Suspended matter	0.61^{**}	-0.54	-0.14	3.61	-0.16
Percent of organic matter	0.52^{*}	-0.60^{**}	0.17	1.50	0.16
Water conductivity	-0.98^{***}	0.72^{***}	0.25	12.2^{***}	0.80^{**}
pH	0.84^{***}	-0.83^{***}	-048^{*}	11.6^{**}	-0.79^{**}
HCO_3^-	-0.71^{***}	0.62^{**}	0.18	6.77^{**}	0.30
NO_3^-	-0.76^{***}	0.48^{*}	0.09	3.27	0.22
PO_4^{3+}	-0.58^{**}	0.34	0.18	2.99	0.44
Ca^{2+}	-0.89^{***}	0.76^{***}	0.18	9.76^{**}	0.23
Fish species	$N = 16$				$N = 7$
Gobio gobio	0.44	0.14	-0.20	–	-0.28
Squalis cephalus	-0.15	0.29	0.39	–	0.14
Alburnoides bipunctatus	0.54^{*}	-0.32	-0.10	–	0.15
Phoxinus phoxinus	0.82^{***}	-0.67^{**}	-0.61^{*}	–	-0.89
Chondrostoma nasus	-0.39	0.33	0.41	–	-0.15
Alburnus alburnus	-0.89^{***}	0.59^{*}	0.60^{*}	–	0.49
Barbatula barbatula	0.68^{**}	-0.13	-0.06	–	-0.65
Barbus barbus	-0.77^{***}	0.38	0.29	–	0.92^{**}

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

(mean 34 ind./100 m; $SD = 38.8$) in the upper course of the studied reach, far from the main population (the first sampling plot where *P. phoxinus* was recorded was 12 km above the upper boundary of the main population). *Chondrostoma nasus* was distributed throughout the studied reach, but in much lower quantities (mean 3.0 ind./100 m; $SD = 5.18$).

Among fish species found in the studied reach, the number of *U. crassus* adults correlated only with *P. phoxinus* (negatively) and *A. alburnus* (positively; Table 1), and these correlations were reflected by juvenile recruitment within the main range of the population. The densities of both fish species strictly correlated with channel elevation, with sign of the correlation reversed in comparison to their correlation with the *U. crassus* population count.

3.4. Success of reintroduction and juvenile dispersal

In 2012 the mean success of reintroduction to the receptor sites in terms of recruited juveniles of the year equalled 5.3 juveniles per 10-m section of the bank ($SD = 7.64$, $Md = 1$, max. = 24). In 2013, on average, 1.4 juveniles were found ($SD = 2.45$, $Md = 0$, max. = 11). The difference in recruitment between the years was significant both at receptor sites (Wilcoxon matched pairs test, $Z = 2.6$, $N = 13$, $p < 0.01$) and within the main population range (2012: $mean = 5.3$, $SD = 5.51$, $Md = 5$, $max. = 20$; 2013: 1.5, $SD = 3.27$, $Md = 0$, $max. = 11$; Wilcoxon matched pairs test, $Z = 2.0$, $N = 10$, $p < 0.047$).

Juvenile recruitment at the receptor sites ($mean = 0.8$, $SD = 1.26$, $Md = 0$, $max. = 4$) did not differ significantly from the recruitment within the main range of the population ($mean = 1.7$, $SD = 3.4$, $Md = 5$, max. = 11) for data pooled for two years (Mann-Whitney *U* test, $Z = 1.3$, $N = 22, 46$, $p = 0.18$). There were no differences for the 2012 (Mann-Whitney U test, $Z = 1.47$, $N = 11, 21$, $p = 0.137$) and 2013 data (Mann-Whitney U test, $Z = 0.44$, $N = 11, 21$, $p = 0.615$) analysed separately. The detectability of the young mussels increased in their second year due to the visibility of their siphons on the surface of the sediment; in the analysed section, we detected a total of 271 individuals recruited in 2012 in their second year of life. In the vicinity of particular sites, we found, on average, 7.6 ($SD = 33.5$, max. = 55) 2-year individuals within the morphological boundaries of the receptor site, whereas outside receptor site we found, on average, as many as 4.8 ($SD = 29.8$, $max. = 47$) 2-year individuals. In the receptor site coded UcB21, 47 2-year individuals were found outside the receptor site, compared to no young mussels within.

There were no significant correlations between the number of fish host individuals of any species found at receptor sites and the number of juveniles of introduced *U. crassus*. The maximal dispersal distance of juveniles detected in the neighbourhood of a given receptor site was >600 m. For the 2014 data, the number of juveniles per receptor site positively correlated with their maximal dispersal distance (for 2-year: $r_S = 0.83$, $N = 10$, $p = 0.003$; for 3-year: $r_S =$

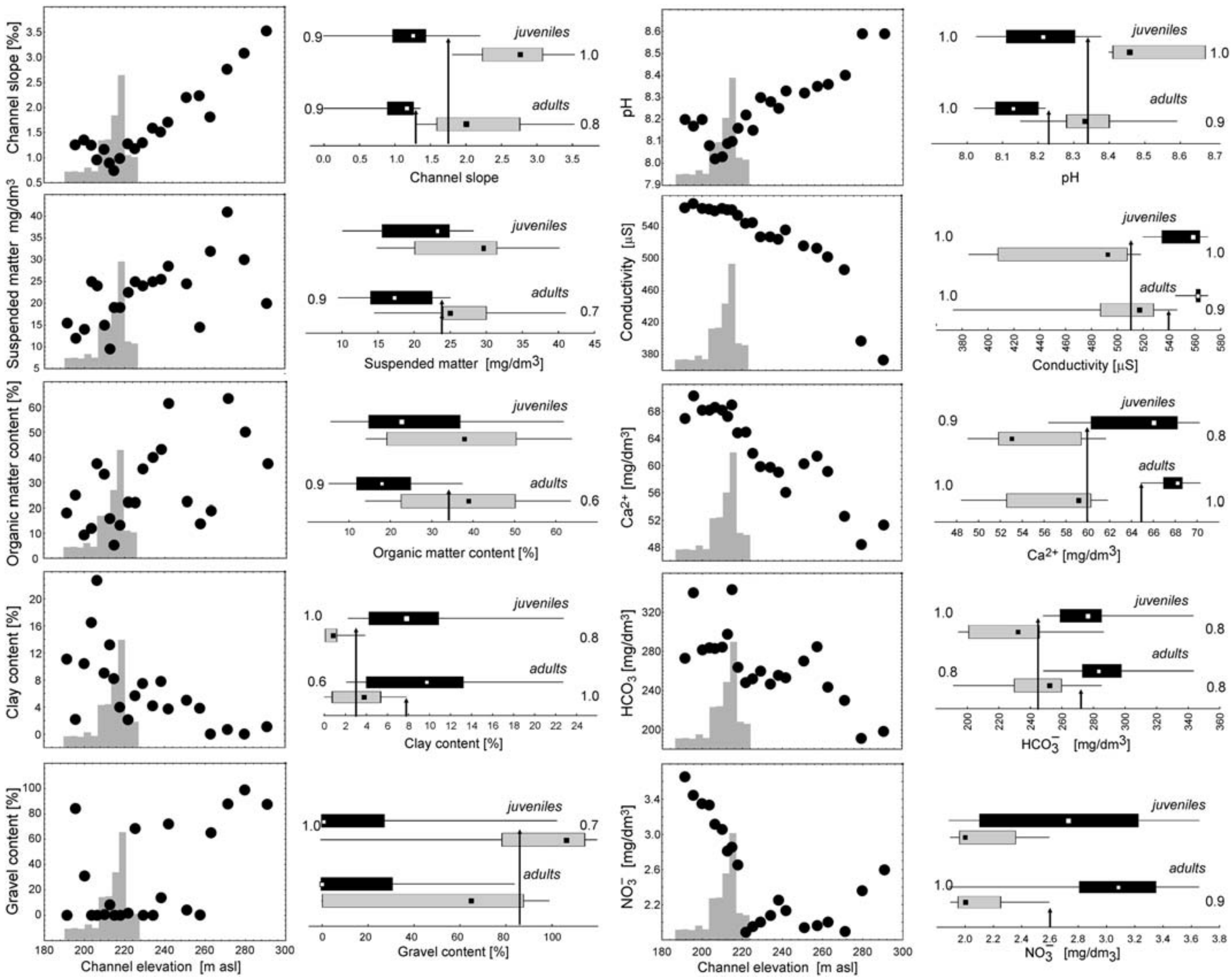


Fig. 2. Abiotic features of the Biała channel in relation to channel elevation, with indicated *U. crassus* population counts (scatter plots in left-hand columns); on the right side of the scatter plots, the box plots represent median values of the given parameter (quartiles and range) for the presence (black boxes) or absence (gray boxes) of *U. crassus*. Data is shown separately for adults from the main population and juveniles from reintroduction. The cut-off points for each abiotic feature estimated based on ROC curves are shown with black arrows. The numbers at the edges of whiskers at box plots represent sensitivity (to the left of the black bar) and specificity (to the right of the gray bar).

0.67, $N = 13$, $p = 0.012$, Fig. 4). In the same year, two groups of three and two receptor sites (Fig. 1), respectively, were practically connected by continuous distribution of young mussels (Fig. 1C), meaning that the reach of the river separating the main and Zborowianka populations was filled in 2014 with mussels colonising 86% of the channel's length.

In 2014 the morphology of 13 receptor sites was, to various extents, disrupted by a flood (lateral erosion or large transport within the channel). The flood destroyed sites with significantly larger numbers of 2-year individuals found in 2013 (the mean for the destroyed sites was 20 juveniles ($SD = 21.5$) compared to 5 juveniles at undamaged sites ($SD = 8.1$; test $Z = 2.20$, $N = 12$, $p = 0.028$)), even though in the following year juveniles recruited during the years preceding the destruction were still present in these surroundings ($mean = 11.7$, $SD = 23.0$). The number of 2-year mussels per 3-km section was much higher in 2013 in some sections located at higher elevations (with steeper channel slope) than the number of 3-year mussels recorded in the same sections in the following year (Fig. 3A), although the difference was not statistically significant (Wilcoxon matched pairs test, 2-year individuals in 2013 vs 3-year individuals in 2014: $Z = 0.06$, $p = 0.95$; 2-year individuals in 2013 vs all 1–4-year recruits in 2015: $Z = 0.420$, $p = 0.67$). Destruction of receptor sites was dependent neither on the elevation of the 3-km channel section ($Z = 0.69$, $N = 11$, $p = 0.494$) nor on channel slope ($Z = 0.23$, $N = 10$, $p = 0.820$).

3.5. Longitudinal factors related to the success of reintroduction

The density of 1-year juveniles per 3-km sampling station, counted according to the same sampling methodology and combined for already inhabited and experimentally settled reaches, significantly and negatively correlated with channel elevation, channel slope, gravel content, and pH, and positively with clay content; no other factors found significant for the main population were confirmed for reintroduced juveniles (Table 1). These relationships were confirmed by means of an absence/presence logistic regression analysis (with the exception of clay content), which additionally showed significant relationships with conductance, HCO_3^-, and Ca^{2+}. Similar results were found for juveniles aged 1–4 years (recruited in 2012–2015, counted in 2015), except for HCO_3^- and Ca^{2+} (Table 1).

In the 2015 survey, it appeared that, despite initial success at upper receptor sites (crosses in Fig. 3A), juveniles introduced during the relocation project were ultimately absent in reaches with channel slope ≥ 1.8‰ (Fig. 3A, B) and elevations over 245–250 m asl. This means that ultimately the southern range of the population was moved

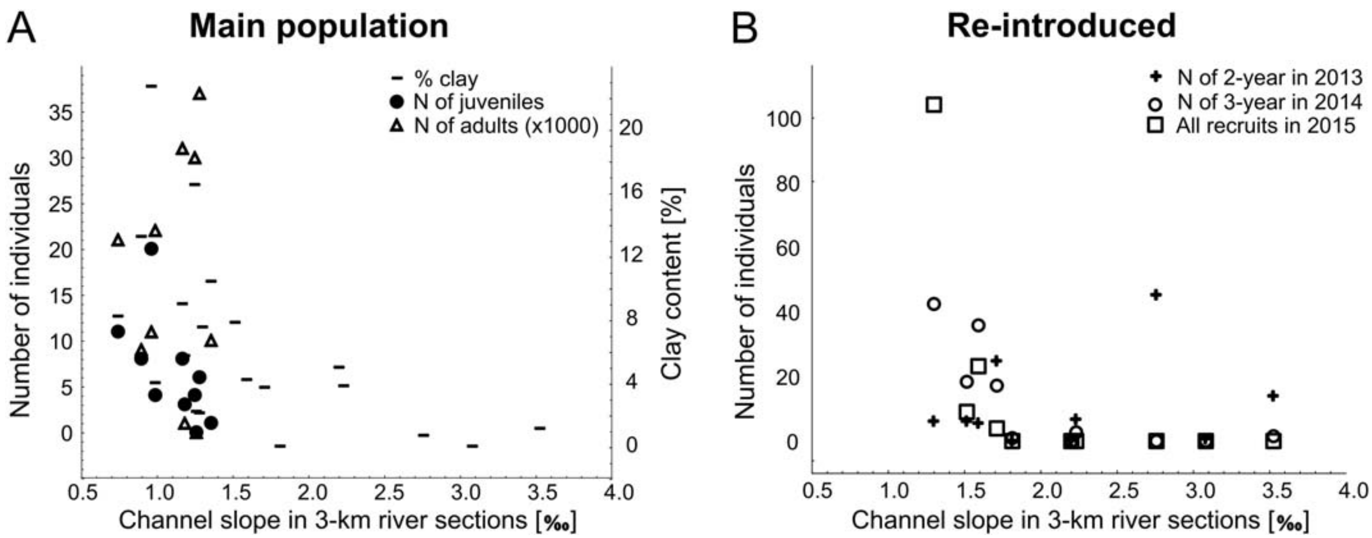


Fig. 3. (A) Relationships between the number of mussel individuals (left axis) and clay content in bed samples (right axis) and channel slope within the range of occurrence of the main population of *Unio crassus*. Solid dots indicate number of 1-year juveniles within a 10-m sample at 3-km sampling points. Empty triangles indicate number of adult individuals (×1000) per 3-km river section. (B) Relationship between the number of *U. crassus* juveniles and channel slope in the river reach with re-introduced mussels.

12 km upstream. In the main population, both juveniles of a given year and adults were restricted to reaches where the slope did not exceed 1.4‰ and where the clay content was noticeably higher (Fig. 3B).

4. Discussion

Reintroduction projects, by definition, aim at restoring a species to its former range. Usually such a decision is based on historical data on species occurrence; however, it has already been stressed by Seddon et al. (2007) that reasons for extinction are usually poorly understood. In the case of reintroduction in the Biała River, historical data, sub-fossils, and comparison to other Carpathian rivers indicated that the range of the species should extend far into the upper course of the river. This aim was strongly justified by environmental conditions. Along a mountain river, strong environmental gradients, namely chemical and hydraulic, are present. The chemical quality of the water was much higher in the upper reach of the river. Usually, water quality improvement in the upstream direction can be attributed to decreasing human density and anthropogenic impact (Allan, 2004; Donohue et al., 2006; Osborne and Wiley, 1988). In the case of the Biała, the upper parts of the catchment are mostly covered by forests with few, dispersed human settlements; thus, the chemical quality of the water in the upper river course was higher than in the lower course, which was settled by a very large and prosperous population of *U. crassus* (Table 1).

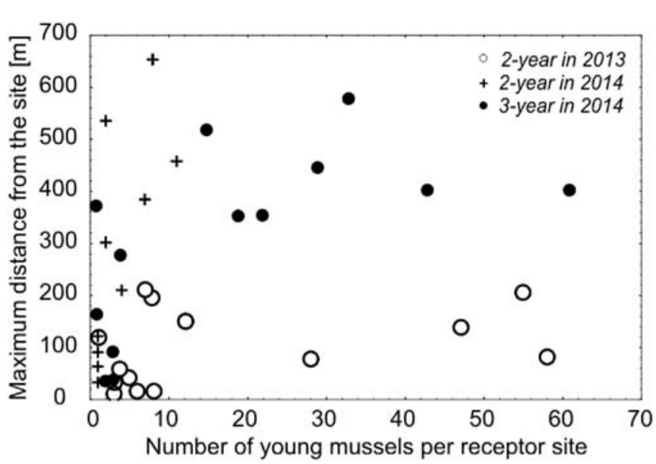


Fig. 4. Relationship between the number of young mussels attributed to a given receptor site and the maximum distance recorded between the receptor site and young mussels found in its vicinity.

Theoretically, physicochemical parameters should be good predictors of environmental conditions as they reflect many possible factors influencing riverine biota. However, as reported by Allan (2004), their indicative role depends on the character of the river and its catchment; if the environment is of good quality, or human influence is widespread and fairly uniform across the study region, physicochemical parameters reflect only natural factors. In the case of this study, physicochemical parameters were strictly correlated with the elevation of sampling points along the channel; for example, in the case of pH, they almost exactly followed the pattern of channel slope (Fig. 2). The physicochemical parameters could not be used in either case to show a relationship which might be suspected to cause mortality of *U. crassus* or block its recruitment. The only exceptions are NO_3^- and PO_4^{3-} within the range of main population, which, contrary to the relationship for the whole 60-km reach, are negatively related to the *U. crassus* count. High concentrations of NO_3^- and PO_4^{3-} are likely anthropogenic in origin; however, in this case they have little predictive value in terms of *U. crassus* occurrence, as these relationships occur only within a very prosperous population. This is in accordance with other studies reporting that *U. crassus* is not influenced by these types of pollutants within the range of natural, clear waters (Hus et al., 2006), and/or that *U. crassus* in the Biała is less sensitive to chemical pollution (Denic et al., 2014; Douda, 2010) than it was previously believed for some rivers (Denic et al., 2014; Douda, 2010; Köhler, 2006). In the most of the sampling stations, also within the main population, the concentration of NO_3^- exceeded 2 mg l^{-1} (Fig. 2), considered as detrimental for *U. crassus* populations (Köhler, 2006). It seems that high NO_3^- concentrations and the impaired vitality of *U. crassus* populations share the same causes in some rivers. Moreover, chemical parameters were not much different from those of other Carpathian rivers (Hus et al., 2006). Thus, both historical distribution and water quality favoured the decision to implement mussel reintroduction in the upper part of the river. However, the increasing elevation of the channel left mussel individuals more exposed to hydraulic forces, the strength of which increases upstream. This constituted a factor against reintroduction. In mountainous rivers, hydraulic forces directly limit the occurrence of biota (Layzer et al., 1989; Meffe, 1984). Assuming that channel slope is one of the most important factors influencing the transport of bedload (Gilbert, 1917), it is to be expected that mussels will occur mainly in flatter sections along the river profile. The collected data confirm that the main population was saved only in the area of the lowest channel slope, and that recruits from reintroduced subpopulations persisted only in reaches of the relatively low-gradient channel. A very abrupt cut-off point was found for recruit survival at a channel slope of 1.8‰, above which neither the main nor the reintroduced populations persisted (Figs. 3, 4). The cut-off point corresponds to the channel elevation at the mouth of the Zborowianka River (Fig. 1), where another part of the previously continuous population was isolated in the past; however, the cut-off point does not correspond either to the upper elevation limit of *U. crassus* in the Carpathians and to its historical distribution in the Biała River, as indicated by subfossils found in the Biała tributary, or to information from local communities on the previous range of the species. The sub-fossils originated from eroded bank material; thus, their presence reflects the distribution of this species from a point in the past at which hydrological conditions may have been quite different. This conclusion is strongly supported by recent studies which demonstrated that most Carpathian rivers, including the Biała, were subjected to major hydraulic change quite recently (Wyżga, 2008). This implies that historical data and the sub-fossil record should be treated with great caution during reintroduction projects. It also indicates that even if physicochemical changes which likely caused species extinction in the past are now absent, it is still possible for other factors limiting species range to arise.

The strong hydraulic forces of any mountainous river influence sediment transport and deposition. This takes place on both a small scale, in the cross-channel dimension, where fine sediments are deposited at channel margins even at high altitudes (Helley and LaMarche, 1973), and a large, longitudinal scale (Sullivan et al., 1987), which explains the occurrence of channels filled with fine deposits in lower, low-gradient reaches of the Biała River (Fig. 4B). These reaches are occupied by the main population of *U. crassus* (Fig. 1A); moreover, in the cross-sectional dimension, this species is concentrated along channel margins, which seems to be a widespread tendency among riverine mussels (Brim Box et al., 2002). In both situations, the river creates a functional habitat for *U. crassus*, which we defined as shallow pools in channel margins with still water and thick layers of silt. These easily identified and actually quite uniform sites (we failed to find any significant differences in reproductive success between variants of this habitat; unpublished data) were used during the project for propagating the species far outside the main population range. Nonetheless, the presence of silt can be a useful proxy for finding receptor sites for *U. crassus*, reflecting, in any case, specific hydraulic conditions such as a low Froude number (Kemp et al., 2000).

As well, Wilson et al. (2011) advocated 'ground-truthed' models of local habitat. Near-bed flow velocities at the scale of a microhabitat, in a space directly occupied by mussels, were measured by Hastie et al. (2000), also Moorkens and Killeen (2014) for *Margaritifera margaritifera* and Stoeckl and Geist (2016) for *U. crassus*. Unlike *M. margaritifera*, *U. crassus* was mostly found in areas with low or stagnant water flow and high concentrations of fine sediment—conditions similar to those found in this study.

Flow velocity and shear stress are usually measured at low water levels, whereas many studies indicate that high-magnitude flood flows drastically influence all biota within the channel (Hastie et al., 2001; for a general review see Piniewski et al., 2017, who also mentioned that the flood of 2014 destroyed approximately half of the receptor sites). During our study, functional habitats (silted bays) resulted in unexpectedly large juvenile production even in high-slope reaches (Fig. 3A, see points for 2013 at slopes of 2.2‰ or even 3.5‰); this likely corresponds to the range of occurrence of *Phoxinus phoxinus* within the Biała River, starting 12 km upstream of the upper range of the main population of *U. crassus*. However, after the 2014 flood and during the final survey in 2015, none of these recruits were found again (Fig. 3B). Functional habitats enabled immediate survival and reproduction, but were unsuccessful at long-term sites located at high elevations (Fig. 4B). A functional habitat is indispensable for successful species restoration; however, the final result will depend on large-scale longitudinal factors and (as must be emphasized) should be verified over the long term. This is clearly a hierarchical relationship (O'Neill et al., 1986): large-scale processes constrain the expression of processes on successively smaller scales.

The slope of the channel of the Biała River does not decrease continuously to the mouth, but increases again at the lower end of the studied reach (Fig. 2). This again reflects a common phenomenon that channel slope increases near the river mouth as a result of the lowering of base level caused by incision of the recipient river (Schumm, 1993). In this reach, the distribution of *U. crassus* again becomes discontinuous, breaking up into smaller and more dispersed sub-populations (Fig. 1); the number of sections without *U. crassus* correlates negatively with elevation but positively with channel slope. Juvenile recruitment shows the same relationship, implying that spatial distribution of the species is determined not by elevation but by channel slope.

The problem of the continuous versus discrete nature of mussel habitats has implications for considering their distribution within the channels in terms of a metapopulation (Newton et al., 2008). A metapopulation is based on a continuous process of colonisation/extinction/recolonisation of sub-populations inhabiting distinctive units (Hanski, 2002; Levins, 1969). It was demonstrated that many receptor sites were destroyed by the river; however, the high reproduction rate was positively related to the dispersal of juveniles, and young mussels colonised other sites before their own receptor site was destroyed. Their dispersal distances were surprisingly great, reaching over 0.5 km; thus within 3 years young mussels formed a near-continuous distribution (86% of the channel length, Fig. 1C) over the reach previously isolating the main population from the remnant population of the Zborowianka. Nevertheless, the young individuals were frequently single and isolated; however, this should not cause any Allee effects, considering rather long distances of sperm transport in freshwater mussels (Ferguson et al., 2013), and the strong dynamic of their dispersal indicates that new inhabitants may arrive with fish hosts very soon.

Fish host density can be a limiting factor for freshwater mussels. Convincing evidence exists to support this hypothesis (Haag and Stoeckel, 2015); however, it was not confirmed by the large-scale analyses of this study. It was to be expected that the presence of 60,000 individual mussels, with local densities exceeding 1000 ind./100 m, was related to the density of fish host species. However, nothing like this was observed. The studied reach was deprived of the optimal fish hosts for *U. crassus* (according to Douda et al., 2012), namely, *Scardinus erytrophtalamus* and *Cottus gobio*. Moreover, another very good host, *Phoxinus phoxinus* (Douda, 2015; Lamand et al., 2016), was distributed too far upstream, a long distance from the existing *U. crassus* population (a similar absence was noted by Taeubert et al., 2012b); even though its range started at 12 km upstream of the upper range of the main population, i.e. at the upper point of the successful reintroduction of juveniles, the presence of *P. phoxinus* did not allow for the effective spread of the mussel juveniles upstream. It seems that the prolonged presence of even a small number of any fish hosts in functional habitats of *U. crassus* is sufficient for the success of mussel reproduction (similar conclusion was reached by Haag and Stoeckel, 2015). In large-scale analyses conducted by Inoue et al. (2017), it was found that mussel occurrences were exclusively explained by abiotic factors, whereas fish–mussel co-occurrence was frequently mismatched. In their study, none of the 12 potential host fish species of *U. crassus* was characterised by a perfect match of abiotic responses.

An alternative explanation can be based on assumption, that different populations of *Unio crassus* can differ in their host compatibility (Douda et al., 2014), which means, that *U. crassus* from the Biała River would be more compatible with a fish species/strain not reported so far (e.g. *A. alburnus* which range in the Biała overlaps with *U. crassus*). To confirm this hypothesis, more detailed evaluation of locally specific use of hosts (see Douda et al., 2017) would be needed.

Considering that European and North American countries are combating anthropogenic sources of freshwater pollution (Skjelkvåle et al., 2005) and that the decline of freshwater mussels is ongoing in Europe (Lopes-Lima et al., 2017) and North America (Williams et al., 1993), it might be suspected that artificial disturbance of the physical structure of the channels is the main reason for this ongoing decline (Brim Box and Mossa, 1999). Some studies indicate that anthropogenic influences on channel characteristics lead to detrimental effects on freshwater mussels (as reviewed by Watters, 2000), and that results of ecological studies have to be successfully 'translated' to build a bridge between ecologists and river engineers.

This paper documents a single case of reintroduction of a threatened species, albeit one conducted on a large scale. By definition, this experiment cannot be repeated. However, this study documented and demonstrated some important habitat features which seem to influence the success of reintroduction in mountainous rivers. *U. crassus* distribution along the channel is not very strictly related to host-fish distribution. The definition of *functional habitat* seems to be the most relevant issue for practitioners: silt deposits were positively verified as an environmental signal enabling the proper selection of a specific location for species introduction and attainment of significant reproductive success and juvenile dispersal. However, the site of introduction must also be verified against longitudinal, large-scale gradients, with channel slope as the factor most likely responsible for hydraulic forces which finally regulate the long-term fate of any reintroduced population.

Acknowledgements

Our thanks to Michał Lipiński, Marta Potoczek, Zofia Książkiewicz, and Andrzej Strużyński for help in fieldwork. Funding for the study came from the Regional Water Management Authority (POIS-05.02.00-00-084/08) (RZGW) in Kraków, Poland (POIS-05.02.00-00-084/08), a grant from the Polish Ministry of Science and Higher Education (N N304 328836), and the authors. The research reported here was conducted in full compliance with the ethical codes and legislation of the Republic of Poland. Author contributions: KZ conceived the study, KZ, JF, and TZ developed the methodology, all authors collected the data, KZ, JF, TZ, WB, and PA analysed the data, and KZ and TZ wrote the manuscript.

References

Aarts, B.G.W., Van Den Brink, F.W.B., Nienhuis, P.H., 2004. Habitat loss as the main cause of the slow recovery of fish faunas of regulated large rivers in Europe: the transversal floodplain gradient. River Res. Appl. 20:3–23. https://doi.org/10.1002/rra.720.

Aldridge, D.C., Fayle, T.M., Jackson, N., 2007. Freshwater mussel abundance predicts biodiversity in UK lowland rivers. Aquat. Conserv. Mar. Freshwat. Ecosyst. 17:554–564. https://doi.org/10.1002/aqc.815.

Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35:257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122.

Altmüller, R., Dettmer, R., 2006. Erfolgreiche Artenschutzmaßnahmen für die Flussperlmuschel *Margaritifera margaritifera* L. durch Reduzierung von unnatürlichen Feinsedimentfrachten - Erfahrungen im Rahmen des Lutterprojekts (Successful species protection measures for the freshwater pearl mussel (*Margaritifera margaritifera*) through the reduction of unnaturally high loading of silt and sand in running waters – Experiences within the scope of the Lutterproject). Inform. d. Naturschutz Niedersachs. 26 (4), 192–204.

Brim Box, J., Mossa, J., 1999. Sediment, land use, and freshwater mussels: prospects and problems. J. N. Am. Benthol. Soc. 18:99–117. https://doi.org/10.1016/j.envpol.2004.12.023.

Brim Box, J., Dorazio, R.M., Liddell, D.W., 2002. Relationships between streambed substrate characteristics and freshwater mussels (Bivalvia: Unionidae) in coastal plain streams. J. N. Am. Benthol. Soc. 21:253–260. https://doi.org/10.2307/1468413.

Denic, M., Stoeckl, K., Gum, B., Geist, J., 2014. Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation. Hydrobiologia 735:111–122. https://doi.org/10.1007/s10750-013-1467-z.

Donohue, I., McGarrigle, M.L., Mills, P., 2006. Linking catchment characteristics and water chemistry with the ecological status of Irish rivers. Water Res. 40:91–98. https://doi.org/10.1016/j.watres.2005.10.027.

Douda, K., 2007. The occurrence and growth of *Unio crassus* (Mollusca: Bivalvia: Unionidae) in Lužnice River basin in respect to water quality. Acta U. Carol. Envi. 21, 57–63.

Douda, K., 2010. Effects of nitrate nitrogen pollution on central European unionid bivalves revealed by distributional data and acute toxicity testing. Aquat. Conserv. Mar. Freshwat. Ecosyst. 20:189–197. https://doi.org/10.1002/aqc.1076.

Douda, K., 2015. Host-dependent vitality of juvenile freshwater mussels: implications for breeding programs and host evaluation. Aquaculture 445:5–10. https://doi.org/10.1016/j.aquaculture.2015.04.008.

Douda, K., Horký, P., Bílý, M., 2012. Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. Anim. Conserv. 15:536–544. https://doi.org/10.1111/j.1469-1795.2012.00546.x.

Douda, K., Sell, J., Kubíková-Peláková, L., Horký, P., Kaczmarczyk, A., Mioduchowska, M., 2014. Host compatibility as a critical factor in management unit recognition: population-level differences in mussel–fish relationships. J. Appl. Ecol. 51:1365–2664. https://doi.org/10.1111/1365-2664.12264.

Douda, K., Liu, H.-Z., Yu, D., Rouchet, R., Liu, F., Tang, Q.-Y., Methling, C., Smith, C., Reichard, M., 2017. The role of local adaptation in shaping fish–mussel coevolution. Freshw. Biol. 62:1365–2427. https://doi.org/10.1111/fwb.13026.

Farris, J.L., Van Hassel, J.H. (Eds.), 2006. Freshwater Bivalve Ecotoxicology. CRC Press, New York https://doi.org/10.1201/9781420042856.

Ferguson, C.D., Blum, M.J., Raymer, M.L., Eackles, M.S., Krane, D.E., 2013. Population structure, multiple paternity, and long-distance transport of spermatozoa in the freshwater mussel *Lampsilis cardium* (Bivalvia: Unionidae). Freshw. Sci. 32:267–282. https://doi.org/10.1899/12-028.1.

Gates, K.K., Vaughn, C.C., Julian, J.P., 2015. Developing environmental flow recommendations for freshwater mussels using the biological traits of species guilds. Freshw. Biol. 60:620–635. https://doi.org/10.1111/fwb.12528.

Geist, J., 2014. Trends and directions in water quality and habitat management in the context of the European water framework directive. Fisheries 39:219–220. https://doi.org/10.1080/03632415.2014.903838.

Geist, J., Auerswald, K., 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). Freshw. Biol. 52: 2299–2316. https://doi.org/10.1111/j.1365-2427.2007.01812.x.

Gilbert, G.K., 1917. Hydraulic-mining debris in the Sierra Nevada. US Geol. Surv. Prof. Pap. 105 pp. 1–153.

Gray, M.W., Kreeger, D., 2014. Monitoring fitness of caged mussels (*Elliptio complanata*) to assess and prioritize streams for restoration. Aquat. Conserv. Mar. Freshwat. Ecosyst. 24, 218–230.

Gutiérrez, J.L., Jones, C.G., Strayer, D.L., Iribarne, O.O., 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79–90. https://doi.org/10.1034/j.1600-0706.2003.12322.x.

Haag, W.R., 2012. North American Freshwater Mussels: Natural History, Ecology, and Conservation. Cambridge University Press, Cambridge.

Haag, W.R., Stoeckel, J.A., 2015. The role of host abundance in regulating populations of freshwater mussels with parasitic larvae. Oecologia 178:1159–1168. https://doi.org/10.1007/s00442-015-3310-x.

Hanski, I., 2002. Metapopulation Ecology. Oxford University Press, New York.

Hardison, B.S., Layzer, J.B., 2001. Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers. Regul. River 17:77–84. https://doi.org/10.1002/1099-1646(200101/02)17:1<77::AID-RRR604>3.0.CO;2-S.

Harper, D.M., Smith, C.D., Barham, P.J., 1992. Habitats as the building blocks for river conservation assessment. In: Boon, P.J., Calow, P., Petts, G.E. (Eds.), River Conservation and Management. Wiley, Chichester, pp. 311–320.

Hastie, L.C., Boon, P.J., Young, M.R., 2000. Physical habitat requirements of freshwater pearl mussels, *Margaritifera margaritifera* (L.). Hydrobiologia 429:59–71. https://doi.org/10.1023/A:1004068412666.

Hastie, L.C., Boon, P.J., Young, M.R., Way, S., 2001. The effects of a major flood on an endangered freshwater mussel population. Biol. Conserv. 98:107–115. https://doi.org/10.1016/S0006-3207(00)00152-X.

Helley, E.J., LaMarche, V.C., 1973. Historic flood information for northern California streams from geologic and botanical evidence. US Geol. Surv. Prof. Pap. 485-E, 1–15.

Hochwald, S., 2001. Plasticity of life-history traits in *Unio crassus*. In: Bauer, G., Wächtler, K. (Eds.), Ecology and Evolution of the Freshwater Mussels Unionoida. Springer Verlag, Berlin, Heidelberg:pp. 127–141 https://doi.org/10.1007/978-3-642-56869-5_7.

Holland-Bartels, L.E., 1990. Physical factors and their influence on the mussel fauna of a main channel border habitat of the upper Mississippi River. J. N. Am. Benthol. Soc. 9:327–335. https://doi.org/10.2307/1467900.

Horton, M., Keysa, I., Kirkwooda, L., Mitchella, F., Kyleb, R., Roberts, D., 2015. Sustainable catchment restoration for reintroduction of captive bredfreshwater pearl mussels *Margaritifera margaritifera*. Limnologica 50:21–28. https://doi.org/10.1016/j.limno.2014.11.003.

Hus, M., Śmiałek, M., Zając, K., Zając, T., 2006. Occurence of *Unio crassus* (Bivalvia, Unionidae) depending on water chemistry in the foreland of the Polish Carpathians. Pol. J. Environ. Stud. 15, 169–172.

Inoue, K., Stoeckl, K., Geist, J., 2017. Joint species models reveal the effects of environment on community assemblage of freshwater mussels and fishes in European rivers. Divers. Distrib. 23:284–296. https://doi.org/10.1111/ddi.12520.

Kemp, J.L., Harper, D.M., Crosa, G.A., 2000. The habitat-scale ecohydraulics of rivers. Ecol. Eng. 16:17–29. https://doi.org/10.1016/S0925-8574(00)00073-2.

Köhler, R., 2006. Observations on impaired vitality of *Unio crassus* (Bivalvia: Najadae) populations in conjunction with elevated nitrate concentration in running waters. Acta Hydrochim. Hydrobiol. 34:346–348. https://doi.org/10.1002/aheh.200500635.

Kryger, J., Riisgård, H.U., 1988. Filtration rate capacities in six species of European freshwater bivalves. Oecologia 77:34–38. https://doi.org/10.1007/BF00380921.

Lamand, F., Roche, K., Beisel, J.N., 2016. Glochidial infestation by the endangered mollusc *Unio crassus* in rivers of north-eastern France: *Phoxinus phoxinus* and *Cottus gobio* as primary fish hosts. Aquat. Conserv. Mar. Freshwat. Ecosyst. 26:445–455. https://doi.org/10.1002/aqc.2603.

Layzer, J.B., Nehus, T.J., Pennington, W., Gore, J.A., Nestler, J.M., 1989. Seasonal variation in the composition of drift below a peaking hydroelectric project. Regul. River 3: 305–317. https://doi.org/10.1002/rrr.3450030105.

Levins, R., 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull. Entomol. Soc. Am. 15:237–240. https://doi.org/10.1093/besa/15.3.237.

Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D.C., Araujo, R., Bergengren, J., Bespalaya, Y., Bódis, E., Burlakova, L., Van Damme, D., Douda, K., Froufe, E., Georgiev, D., Gumpinger, C., Karatayev, A., Kebapçi, Ü., Killeen, I., Lajtner, J., Larsen, B.M., Lauceri, R., Legakis, A., Lois, S., Lundberg, S., Moorkens, E., Motte, G., Nagel, K.-O., Ondina, P., Outeiro, A., Paunovic, M., Prié, V., von Proschwitz, T., Riccardi, N., Rudzīte, M., Rudzītis, M., Scheder, C., Seddon, M., Şereflişan, H., Simić, V., Sokolova, S., Stoeckl, K., Taskinen, J., Teixeira, A., Thielen, F., Trichkova, T., Varandas, S., Vicentini, H., Zajac, K., Zajac, T., Zogaris, S., 2017. Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biol. Rev. 92:572–607. https://doi.org/10.1111/brv.12244.

Lummer, E.M., Auerswald, K., Geist, J., 2016. Fine sediment as environmental stressor affecting freshwater mussel behavior and ecosystem services. Sci. Total Environ. 571: 1340–1348. https://doi.org/10.1016/j.scitotenv.2016.07.027.

Lundberg, S., Österling, M. (Eds.), 2016. Return of the Thick-shelled River Mussel – Restoring floodplains, habitats and connectivity by using mussels and brains. Handbook UC4LIFE project; Skåne County Administrative Board.

Lydeard, C.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., Hershler, R., Perez, K., Roth, B., Seddon, M., Strong, E.E., Thompson, F.G., 2004. The global decline of non-marine mollusks. BioScience 54:321–330. https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.

Malmqvist, B., Rundle, S., 2002. Threats to the running water ecosystems of the world. Environ. Conserv. 29:134–153. https://doi.org/10.1017/S0376892902000097.

Meffe, G.K., 1984. Effects of abiotic disturbance on coexistence of predator-prey fish species. Ecology 65:1525–1534. https://doi.org/10.2307/1939132.

Metz, C.E., 1978. Basic principles of ROC analysis. Semin. Nucl. Med. 8, 283–298.

Metz, C.E., 1986. Statistical analysis of ROC data in evaluating diagnostic performance. In: Herbert, D., Myers, R. (Eds.), Multiple Regression Analysis: Applications in the Health Sciences. American Institute of Physics, New York, pp. 365–384.

Montgomery, D.R., Buffington, J.M., 1998. Channel processes, classification and response. In: Naiman, R.J., Bilby, R.E. (Eds.), River Ecology and Management. Lessons from the Pacific Coastal Ecoregion. Part I. The Physical Environment. Springer-Verlag, New York, pp. 13–36.

Moorkens, E.A., 2017. Short-term breeding: releasing post-parasitic juvenile *Margaritifera* into ideal small-scale receptor sites: a new technique for the augmentation of declining populations. Hydrobiologia https://doi.org/10.1007/s10750-017-3138-y.

Moorkens, E.A., Killeen, I.J., 2014. Assessing near-bed velocity in a recruiting population of the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in Ireland. Aquat. Conserv. Mar. Freshwat. Ecosyst. 24:853–862. https://doi.org/10.1002/aqc.2530.

Mueller, M., Pander, J., Geist, J., 2011. The effects of weirs on structural stream habitat and biological communities. J. Appl. Ecol. 48:1450–1461. https://doi.org/10.1111/j.1365-2664.2011.02035.x.

Naimo, T.J., 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4:341–362. https://doi.org/10.1007/BF00118870.

Newton, T.J., Woolnough, D.A., Strayer, D.L., 2008. Using landscape ecology to understand and manage freshwater mussel populations. J. N. Am. Benthol. Soc. 27:424–439. https://doi.org/10.1899/07-076.1.

O'Neill, R.V., Deangelis, D.L., Waide, J.B., Allen, T.F.H., 1986. A Hierarchical Concept of the Ecosystem. Princeton University Press, Princeton, New Jersey.

Osborne, L.L., Wiley, M.J., 1988. Empirical relationships between land use cover and stream water quality in an agricultural watershed. J. Environ. Manag. 26:9–27. https://doi.org/10.1016/j.watres.2005.10.027.

Österling, M.E., Arvidsson, B.L., Greenberg, L.A., 2010. 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. 47:759–768. https://doi.org/10.1111/j.1365-2664.2010.01827.x.

Piniewski, M., Prudhomme, C., Acreman, M.C., Tylec, L., Oglęcki, P., Okruszko, T., 2017. Responses of fish and invertebrates to floods and droughts in Europe. Ecohydrology 10, e1793. https://doi.org/10.1002/eco.1793.

Pusch, M., Siefert, J., Walz, N., 2001. Filtration and respiration rates of two unionid species and their impact on the water quality of a lowland river. In: Bauer, G., Wachtler, K. (Eds.), Ecology and Evolution of the Freshwater Mussels Unionoida. Ecological Studies Vol. 145. Springer-Verlag, Berlin, Heidelberg:pp. 317–326. https://doi.org/10.1007/978-3-642-56869-5_17.

Riccardi, N., Lopes-Lima, M., Froufe, E., Mazzoli, C., 2016. When and how? Freshwater mussel recolonization in Lake Orta. J. Limnol. 75:120–130. https://doi.org/10.4081/jlimnol.2016.1286.

Richter, A., Stoeckl, K., Denic, M., Geist, J., 2016. Association between the occurrence of the thick-shelled river mussel (*Unio crassus*) and macroinvertebrate, microbial, and diatom communities. Freshwat. Sci. 35:922–933. https://doi.org/10.1086/687811.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.C., Müller, M., 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 12 (1):77. https://doi.org/10.1186/1471-2105-12-77.

Schneider, L.D., 2017. Conservation Ecology of the Thick-Shelled River Mussel *Unio crassus* - the Importance of Parasite-Host Interactions. Karlstad University Studies, Doctoral thesis.

Schumm, S.A., 1993. River response to baselevel change: implications for sequence stratigraphy. J. Geol. 101:279–294. https://doi.org/10.1086/648221.

Seddon, P.J., Armstrong, D.P., Maloney, R.F., 2007. Developing the science of reintroduction biology. Conserv. Biol. 21:303–312. https://doi.org/10.1111/j.1523-1739.2006.00627.x.

Shaw, M.L., 1980. Identifying attentional and decision-making components in information processing. In: Nickerson, R.S. (Ed.), Attention and Performance VIII. Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp. 277–295.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannio, J., Monteith, D.T., Mosello, R., Rogora, M., Rzychon, D., Vesely, J., Wieting, J., Wilander, A., Worsztynowicz, A., 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. Environ. Pollut. 137:165–176. https://doi.org/10.1016/j.envpol.2004.12.023.

Stoeckl, K., Geist, J., 2016. Hydrological and substrate requirements of the thick-shelled river mussel *Unio crassus* (Philipsson 1788). Aquat. Conserv. Mar. Freshwat. Ecosyst. 26:456–469. https://doi.org/10.1002/aqc.2598.

Stoeckl, K., Taeubert, J.E., Geist, J., 2015. Fish species composition and host fish density in streams of the thick-shelled river mussel (*Unio crassus*) - implications for conservation. Aquat. Conserv. Mar. Freshwat. Ecosyst. 25:276–287. https://doi.org/10.1002/aqc.2470.

Strayer, D.L., 1999. Use of flow refuges by unionid mussels in rivers. J. N. Am. Benthol. Soc. 18:468–476. https://doi.org/10.2307/1468379.

Strayer, D.L., Caraco, N.F., Cole, J.F., Findlay, S., Pace, M.L., 1999. Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. BioScience 49:19–27. https://doi.org/10.1525/bisi.1999.49.1.19.

Sullivan, K.L., Lisle, T.E., Dolloff, C.A., Grant, G.E., Reid, L.M., 1987. Stream channels: the link between forests and fishes. In: Salo, E.O., Cundy, T.W. (Eds.), Streamside Management: Forestry and Fishery Interactions. Inst. Forest Res. Contr. No 57. Univ. of Washington, Seattle, Washington, USA, pp. 39–97.

Szuba, K., 2012. Przemiany stosunków wodnych w zlewni i dolinie Dunajca w kontekście poszukiwania środków ograniczenia zagrożenia powodziowego (Changes of water relations in the valley and the Dunajec River catchment as means of seeking for methods of flood threat reduction). Czasopismo Techniczne Środowisko 109, 237–251 (in Polish).

Taeubert, J.E., Gum, B., Geist, J., 2012a. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation. Aquat. Conserv. Mar. Freshwat. Ecosyst. 22:36–46. https://doi.org/10.1002/aqc.1245.

Taeubert, J.-E., Martinez, A.M.P., Gum, B., Geist, J., 2012b. The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. Biol. Conserv. 155: 94–103. https://doi.org/10.1016/j.biocon.2012.06.005.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Gushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130–137.

Vaughn, C.C., 2010. Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. BioScience 60:25–35. https://doi.org/10.1525/bio.2010.60.1.7.

Vaughn, C.C., 2017. Ecosystem services provided by freshwater mussels. Hydrobiologia (In press). https://doi.org/10.1007/s10750-017-3139-x.

Vaughn, C.C., Hakenkamp, C.C., 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshw. Biol. 46:1431–1446. https://doi.org/10.1046/j.1365-2427.2001.00771.x.

Vaughn, C.C., Nichols, S.J., Spooner, D.E., 2008. Community and foodweb ecology of freshwater mussels. J. N. Am. Benthol. Soc. 27:409–423. https://doi.org/10.1899/07-058.1.

Watters, G.T., 1996. Small dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. Biol. Conserv. 75:79–85. https://doi.org/10.1016/0006-3207(95)00034-8.

Watters, G.T., 2000. Freshwater mussels and water quality: a review of the effects of hydrologic and instream habitat alterations. In: Tankersley, R.A., Warmolts, D.I., Watters, G.T., Armitage, B.J., Johnson, P.D., Butler, R.S. (Eds.), Freshwater Mollusk Symposia Proceedings. Part II. Proceedings of the First Freshwater Mollusk Conservation Society Symposium. Ohio Biological Survey Special Publication, Columbus, pp. 261–274.

Welker, M., Walz, N., 1998. Can mussels control the plankton in rivers? – a planktological approach applying a Lagrangian sampling strategy. Limnol. Oceanogr. 43:753–762. https://doi.org/10.4319/lo.1998.43.5.0753.

Williams, J.D., Warren Jr., M.L., Cummings, K.S., Harris, J.L., Neves, R.J., 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6–22. https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2.

Wilson, C.D., Roberts, D., Reid, N., 2011. Applying species distribution modelling to identify areas of high conservation value for endangered species: a case study using *Margaritifera margaritifera* (L.). Biol. Conserv. 144:821–829. https://doi.org/10.1016/j.biocon.2010.11.014.

Wyżga, B., 2008. A review on channel incision in the Polish Carpathian rivers during the 20th century. In: Habersack, H., Piegay, H., Rinaldi, M. (Eds.), Gravel-Bed Rivers VI: From Process Understanding to River Restoration. Elsevier, Amsterdam, pp. 525–556.

Zając, K., 2010. Thick-shelled river mussel *Unio crassus*. In: Makomaska-Juchiewicz, M. (Ed.), The Monitoring of Animal Species. Guidelines I. GIOŚ, Warszawa, pp. 157–179 (in Polish).

Zając, K., Zając, T., 2011. The role of active individual movement in habitat selection in the endangered freshwater mussel *Unio crassus* Philipson 1788. J. Conchol. 40, 446–461.

Zieritz, A., Geist, J., Gum, B., 2014. Spatio-temporal distribution patterns of three stream-dwelling freshwater mussel species: towards a strategy for representative surveys. Hydrobiologia 735:123–136. https://doi.org/10.1007/s10750-014-1897-2.