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

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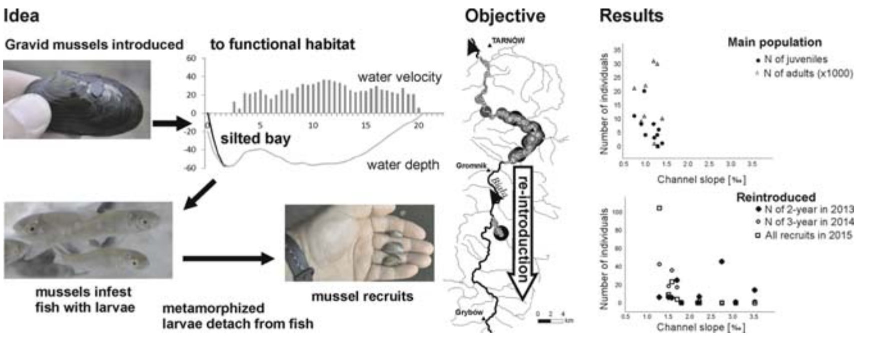
## HIGHLIGHTS

- 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.

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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