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Research Article

Invasion of the zebra mussel (*Dreissena polymorpha*) in a Dinaric karst river after formation of a new reservoir

Krešimir Žganec¹, Jasna Lajtner², Stewart Schultz³ and Damir Valić^{4,*}

¹University of Zadar, Department of Teachers Education Studies in Gospić, 53000 Gospić, Croatia

²University of Zagreb, Faculty of Science, Department of Biology, Rooseveltov trg 6, 10000 Zagreb, Croatia

³University of Zadar, Department of Ecology, Agronomy and Aquaculture, Trg kneza Višeslava 9, 23000 Zadar, Croatia

⁴Ruder Bošković Institute, Division for Marine and Environmental Research, Laboratory for Aquaculture and Pathology of Aquatic Organisms, Bijenička cesta 54, 10000 Zagreb, Croatia

Author e-mails: kzganec@unizd.hr (KŽ), jasna.lajtner@biol.pmf.hr (JL), sschultz@unizd.hr (SS), dvalic@irb.hr (DV)

*Corresponding author

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Abstract

The distribution and density of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), were studied from 2012–2017 in the Dobra River Basin, Croatia, where a new reservoir of Hydroelectric Power Plant Lešće was created in June 2010. The first zebra mussels were found in July 2012 in low density (38 ind.m⁻²) at a site 500 m downstream from the Lešće reservoir. In 2017 the density at this site had increased to 137 ind.m⁻². The Lešće reservoir was colonized by zebra mussel from two upstream and connected reservoirs constructed in 1958. Maximum recorded density in the Lešće reservoir was 12,496 ind.m⁻², with dominance of the smallest size categories. This population was the main source of propagules by which *D. polymorpha* colonized almost the whole remaining course of Gojačka Dobra through 2015. Most sites downstream from the dam showed increased densities from 2016 to 2017. The Lešće reservoir population should be monitored and managed as it represents the main “hotspot” of zebra mussels in the Dinaric karst area in Croatia from which other karst rivers could be invaded.

Key words: distribution, invasion history, density, Dobra River, Hydroelectric Power Plant, Croatia

Introduction

The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), is one of the best-known Ponto-Caspian freshwater invertebrate invaders, and has caused serious ecological and economic impacts in aquatic ecosystems across Europe and North America (reviewed in Karatayev et al. 1997, 2002). The native distribution range of *D. polymorpha* was limited to the Northern Black Sea Region and the Caspian Basin (Son 2007; Van der Velde et al. 2010). Fast westward expansion of zebra mussel in Europe started in the 18th century after construction of large canals connecting rivers of the Black and Baltic Sea basins, and by the beginning of 21st century the species had spread along northern and central inland migration corridors (Bij de Vaate 2002). Most freshwater ecosystems in Europe have been colonized

by *D. polymorpha* (Minchin et al. 2002; Pollux et al. 2010; Van der Velde et al. 2010). In 1988 large zebra mussel shells were detected in the Lake St. Clair and Lake Erie in North America (Hebert et al. 1989). By 2009 the species had spread across most eastern states in the US, as well as to some western states (Mackie and Claudi 2010). The extraordinary invasion success of *D. polymorpha* can be attributed primarily to the species' ability to disperse in both adult and larval stages. Adults can attach to hard substrate and can easily be spread for example by attaching to ship hulls or floating objects, while planktonic larvae can be transported by currents or in ballast waters (Johnson and Carlton 1996; Mackie and Claudi, 2010). Additionally, tolerance to both fresh and brackish waters (most populations 0–6 Practical Salinity Units (PSU), max 14 PSU) (Karatayev et al. 2007; Pollux et al. 2010) enables this species to survive transport in ballast waters. The ability to survive aerial exposure for a few days (Ricciardi et al. 1995a) also contributes to high dispersal potential. Furthermore, other life history traits of *D. polymorpha*, including high fecundity, 3×10^4 up to $> 10^6$ eggs/female/year (Sprung 1991; Mackie and Schloesser 1996), fast growth, and early sexual maturity (reproduction with the age of < 1 year) (McMahon and Bogan 2001; Karatayev et al. 2006, 2007), allow zebra mussel to become enormously abundant shortly after introduction into a new water body. Rapid population growth usually follows after the introduction and total biomass of a zebra mussel population can exceed 10 times that of all other native benthic invertebrates (Karatayev et al. 2002).

Zebra mussels inhabits lakes, reservoirs, rivers, and canals (Karatayev et al. 1998). Since the species reproduces by releasing both male and female gametes and producing obligate planktonic larvae, it is best adapted to stagnant waters. Unidirectional current in flowing waters makes it difficult for local populations of zebra mussels to maintain themselves, as gametes and larvae are swept downstream and cannot swim against the current (Horvath et al. 1996). However, large sink populations of zebra mussel can form in rivers and canals originating from lakes or reservoirs populated by zebra mussels (Karatayev et al. 1998). Hence, long-term maintenance of its populations in flowing waters requires propagule inputs from the upstream lakes or reservoirs (Havel et al. 2005). Allen and Ramcharan (2001) found that the presence of reservoirs significantly enhanced the likelihood of zebra mussel occurrences in downstream river reaches. Although both lakes and reservoirs can be optimal habitats for zebra mussel, studies in North America showed that interconnected water bodies such as reservoirs are more likely to be invaded by zebra mussel than more isolated lakes (Kraft et al. 2002).

The external morphology of zebra mussel is very variable (e.g. Pathy and Mackie 1993; Rosenberg and Ludyanskiy 1994), while adult shell length averages 2–3 cm (max. 4.0–4.5 cm) (Mackie and Claudi 2010). In favourable conditions first young-of-the-year can grow very fast and reach sexual

maturity only three months after their settlement with length ≥ 9 mm (Jantz and Neumann 1998). Although the age of zebra mussel cannot be assessed by size frequency distribution as age classes may not have distinct sizes (Karatayev et al. 2006), population size structure, especially abundance of smallest size categories, generally indicates occurrence of reproduction and recruitment (Chase and Bailey 1999). Morphological plasticity of zebra and quagga mussels, quantified as variation of shell size and form (measured as ratios of several morphometric variables) (e.g. Beggel et al. 2015; Teubner et al. 2016), probably represent adaptations to local environmental conditions (Claxton et al. 1998). Morphometric differences between zebra mussel populations were established between reservoir and river populations in the Drava River in Croatia (Lajtner et al. 2004).

Several aspects of zebra mussel biology and ecology have been studied in Croatia (Lajtner et al. 2004, 2008; Erben et al. 2009; Stević et al. 2013). However, information about its distribution in Croatian freshwaters is still scattered in the literature and needs to be updated. Distribution information is crucial for an understanding of the species' potential to invade the freshwaters of the Dinaric karst area in Croatia and other Balkan countries, which represent an important "hotspot" of European freshwater biodiversity, containing high numbers of endemic, rare and threatened species and unique habitats (Freyhof 2012; Tierno de Figueroa et al. 2013; Žganec et al. 2016; Carrizo et al. 2017). Zebra mussels have not yet been found in water bodies of the karst area in Croatia (Lajtner et al. *unpublished*). Reservoirs on these rivers could facilitate the spread of zebra mussel, i.e. could act as "stepping-stones" (Johnson et al. 2008) for its invasion into the freshwaters of the Western Dinaric ecoregion. Here we present a case study of zebra mussel invasion within the Dobra River Basin in a karst area of central Croatia after the creation of a new reservoir (in June 2010) of Hydroelectric Power Plant (HPP) Lešće.

The main objectives of this study were to document the distribution and infer invasion history of the invasive mussel *Dreissena polymorpha* in the Dobra River Basin. In order to do that: i) species density was estimated in three connected reservoirs and along the entire course of the Gojačka Dobra River; ii) short temporal changes in species abundance in Gojačka Dobra was assessed and iii) morphometric characteristics of all sampled populations in 2016 and 2017 were analysed. Differences in morphological traits were examined between primary source populations in two upstream reservoirs, the newly created Lešće reservoir, and populations in the downstream course of the Gojačka Dobra River. By addressing these objectives, we aim to illustrate how the formation of a new reservoir was followed by a rapid invasion of zebra mussel. Consequences of this invasion are discussed in light of the species' further spread into other karst rivers of the Western Dinaric freshwater ecoregion.

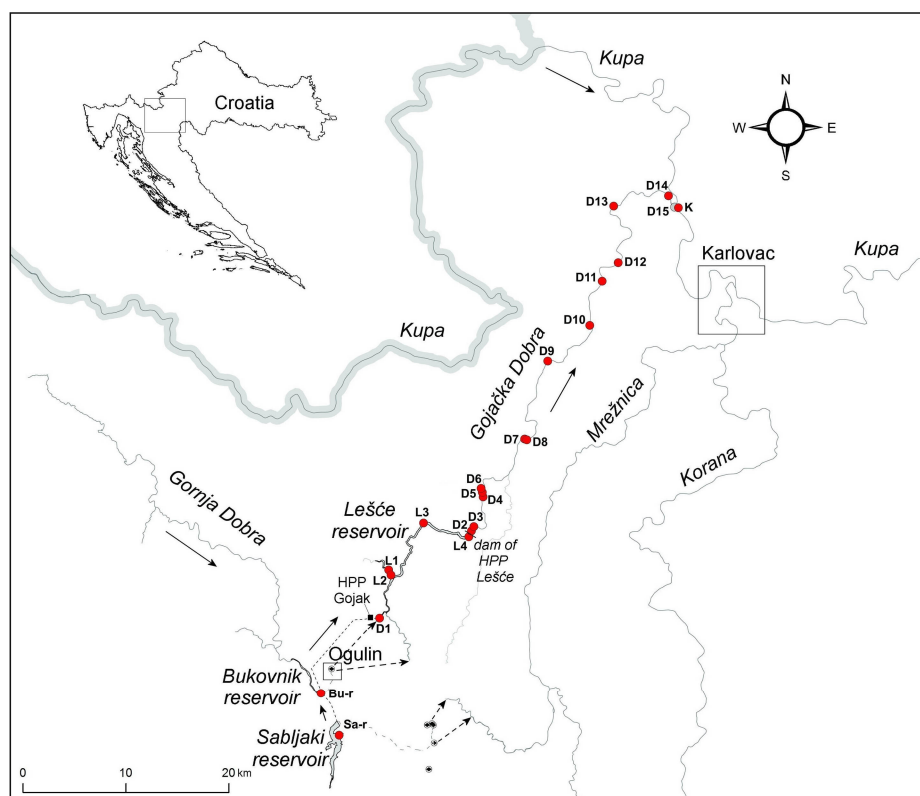


Figure 1. Sampling sites in the Dobra River Basin, with red circles showing positive and grey circles negative records (only at site D15) of *Dreissena polymorpha* in the period 2012–2017 and position of the study area in Croatia (inset). Solid arrows show the direction of river flow or water flow via pipelines (dotted lines) from reservoirs Sabljaki and Bukovnik to HPP Gojak, while dotted arrows show underground connections of sinkholes and springs (towns Ogulin and Karlovac are indicated by squares).

Materials and methods

Study area

The study area is located in central Croatia and described in detail in previous studies (Žganec and Gottstein 2009; Žganec et al. 2011; Žganec 2012). Before the Lešće reservoir formation, the Gojačka Dobra was a 52-km long karst river and represents the most downstream part of the Dobra River system. Its upstream part is Gornja Dobra, the sinking river with a sinkhole in the town of Ogulin (Figure 1). In 1958 the Hydroelectric Power Plant (HPP) Gojak was constructed, located at the Gojačka Dobra main spring. It receives waters from reservoirs Sabljaki and Bukovnik on sinking rivers Zagorska Mrežnica and Gornja Dobra through pipelines which connect these reservoirs, both created in 1958. The new Lešće reservoir was created after the completion of a 52.5-m high dam in June 2010. Of the three reservoirs, Bukovnik is the smallest (volume = $0.24 \times 10^6 \text{ m}^3$, area = 0.21 km^2), the Sabljaki reservoir is of intermediate size ($V = 3.3 \times 10^6 \text{ m}^3$, $A = 1.39 \text{ km}^2$) and the Lešće reservoir is the largest ($V = 25.7 \times 10^6 \text{ m}^3$, $A = 1.45 \text{ km}^2$), 12.6 km long (Bonacci and Andrić 2010). At the site D1 on Gojačka Dobra, located about 300 m downstream of the Gojak spring, normal flowing conditions exist most of the time, except during very high

water levels in the Lešće reservoir. This reservoir has a maximum depth of 44.5 m near the dam, maximum daily water level fluctuations of almost 2.5 m, and maximum seasonal water level fluctuations (in the period 2010–2012) of 6 m. However, at the beginning of October 2011, the reservoir was partly emptied, resulting in water level drop of 38 m relative to the maximum water level (184–185 m a.s.l.) characteristic for the full reservoir (Filipan Vdović et al. 2012). Banks of the Lešće reservoir are at most places very steep, with soft sediment (former forest soil) between bare rocky surfaces. The forest covering the canyon of Gojačka Dobra was completely cut down (up to the altitude of the water level of the planned reservoir) by the end of 2009. As a result, stumps covering the steep littoral zone of the reservoir were present in addition to the rocky surfaces, both of which also represent an optimal hard substrate for zebra mussel attachment (Mackie and Claudi 2010). In June 2010, when the Lešće reservoir was created, the cleared part of the canyon was already densely overgrown by fresh vegetation. After the flooding, newly grown terrestrial vegetation commenced decomposition on the bottom of the new reservoir, which negatively affected water quality in Gojačka Dobra downstream of the reservoir in the next few months (Žganec et al. 2013).

Sampling

The distribution of zebra mussel in the Dobra Basin was studied by sampling at 22 sites in total between 2012–2017 (Figure 1). Several methodologies were used as multiple studies were combined (Žganec et al. 2017; Valić *unpublished, this study*), resulting that sampling consisted of five methods, including snorkelling. A systematic study of *D. polymorpha* distribution in the Dobra basin began in July 2015 when qualitative sampling was conducted at most sites, which included 15 min to 1 hour searching of mussels on hard substrate, mostly stones, on riverbanks or in the littoral of reservoirs. Samples from other studies were used: at site D2 in 2012 and 2015 (Žganec et al. 2017) and at four sites (D1, L1, L4 and D5) in 2014, 2015 and 2017 (Valić *unpublished*) (Supplementary material Table S1). In 2016 and 2017, densities of the zebra mussel were estimated at eight sites on Gojačka Dobra, downstream from the HPP Lešće dam (sites in Figure 4). All mussels were counted (usually on the surface of larger stones) within ten replicates of 0.25 m² surface area, which were randomly placed on either side of the bank. The same sampling method was used to estimate density in the Bukovnik and Sabljaki reservoirs in July 2016, and in the Lešće reservoir at site L1 in August 2017, when low water levels in these reservoirs enabled sampling of the littoral zone where *D. polymorpha* occurred. Densities were calculated as numbers of specimens collected per sampling area. At sites Sa-r, D8–D15 and at site K in 2016 and 2017, snorkelling was performed to check the presence of the species or to collect more specimens for shell morphometrics. Samples collected in 2016 and

2017 at eight sites of Gojačka Dobra downstream of the Lešće dam were used to examine longitudinal changes of zebra mussel density as well as temporal changes between two years. Temporal changes in density during four years (2012–2017) were also examined at site D2. All collected zebra mussels were fixed with 96% ethanol in the field and finally stored in ethanol (70%). Site locations were recorded using a Garmin GPS receiver and data were mapped using Quantum GIS (version 3.2.1.).

Morphometric measurements

A total of 2067 zebra mussels were collected and measured from a total of 12 sites in the Dobra River Basin in 2016 and 2017 (Figure 5). In the laboratory all collected zebra mussels from each site in 2016 and 2017 were measured: the shell length (SL) – the maximum anteroposterior dimension of the shell, shell height (SH) – the maximum dorsal-ventral dimension of the shell, shell width (SW) – the maximum lateral dimension with valves closed, and total wet weight (shell with tissue) (SM) after drying on absorbent paper for 15 min. Measurements were made with a digital Vernier calliper with precision 0.01 mm and a Kern ABS microbalance (type WB0310111) scale with precision 0.001 g.

Statistical analyses

Generalized linear models with a Poisson family (GLMp) and log link were used to test for main effects of site (eight sites in Gojačka Dobra) and year (2016 and 2017) and the interaction on abundance within the study plots. We tested for differences in mean density at site D2 among the four years with a GLM with quasipoisson family and log link (GLMqp). In order to test for differences in morphological traits between primary source population in the Bukovnik and Sabljaki reservoirs, secondary source population in the Lešće reservoir, and tertiary population in the Gojačka Dobra River, we grouped all collected zebra mussels in these three site groups. After checking for normality of residuals using the Shapiro-Wilk test, parametric nested analysis of variance was used to test for differences among site groups in morphological traits, with site nested within the group. Equality of morphological traits across Gojačka Dobra sites and between the years 2016 and 2017 was tested by two-way ANOVA with sites and years as fixed factors. Tukey honestly significant difference tests were used to compare means between sites and between years. Data was first transformed for normality using the Box-Cox transformation for length and width, the Yeo-Johnson transformation for height, and the arcsinh transformation for weight, using the bestNormalize package in R (R Core Team 2018). The parameters of the exponential curve relating shell length to mass were estimated using linear least-squares regression on the logarithms of the two variables. All analyses were performed with R 3.5.2. (R Core Team 2018) using packages MASS (Ripley et al. 2018), bestNormalize (Peterson 2019), and TukeyC (Faria et al. 2019).

Results

D. polymorpha occurrence

The first records of zebra mussels (*D. polymorpha*) in Gojačka Dobra were made in July 2012 at site D2 (500 m downstream of the HPP Lešće dam), i.e. two years after the formation of the Lešće reservoir in June 2010 (Table S1).

Within the Dobra Basin the species was found in all three reservoirs connected by pipe lines, the Sabljaki, Bukovnik, and Lešće reservoir (Figure 1). Sampling at four sites in Sabljaki reservoir in July 2016 failed to detect presence of the species, and only four specimens were found in the littoral zone at one site (Sa-r) at a depth of approximately 3 m, under larger stones. During sampling in July 2016, the Bukovnik Reservoir was emptied. As a result, shallow river flow conditions were present at the site immediately upstream of the dam of Bukovnik reservoir, where *D. polymorpha* was found on the artificial wall of the right bank, and on larger stones immediately upstream of the dam. Estimated density on the artificial wall at this site was 19 ind.m⁻². Zebra mussels were found in the Lešće reservoir for the first time in October 2014 at site L1 at a depth of 8 m, with a density of 356 ind.m⁻². In October 2015, density at the same site, using same method, was 2044 ind.m⁻². In August 2017, the species' density in the shallow littoral zone of the reservoir at site L2 using quadrat counting was 12496 ind.m⁻². Qualitative sampling on the underwater side of floating boat station in July 2015 and 2016 at site L3 revealed the presence of numerous specimens of *D. polymorpha* that were used for morphometrics. At site L4 at the depth of 40 m no zebra mussels were found, but the species was recorded there in the littoral zone where it formed dense clusters on dead branches (Figure 2a).

At site D1, flowing conditions exist most of the time. Zebra mussels were never recorded at this site during monitoring by multihabitat sampling in five months from 2014–2017 (Table S1). However, sampling on the right bank in August 2017 revealed the species' presence: estimated density at this microhabitat was 38 ind.m⁻². Downstream of the Lešće reservoir zebra mussels were found at 14 of 15 sites in total (Figure 1, including site K in Kupa river). Despite that qualitative sampling in 2015 revealed the presence of four zebra mussel specimens at site D14, in 2016 and 2017 the species could not be found at sites D14 and D15 despite very intensive search on the stony substrate in shallower areas and snorkelling in deeper sections of the river. In the Kupa River, at the mouth of the Dobra River, one zebra mussel was found attached on the surface of a large stone during a dive in July 2016, while in August 2017 the species could not be found anymore despite more than one hour of intensive search by snorkelling.

D. polymorpha density

Zebra mussel density at site D2 (Figure 3) was higher in 2017 than in 2012 (137 ind.m⁻² compared to 38 ind.m⁻²). However, differences between the



Figure 2. a) Dense overgrowth of zebra mussels (*Dreissena polymorpha*) on dead branch found in the littoral zone of the Lešće reservoir at site L4 in May 2017; b) three adult specimens of zebra mussel found attached on native and protected Natura 2000 species *Unio crassus* at site D8 in July 2016. Photo by a) D. Valić, b) K. Žganec.

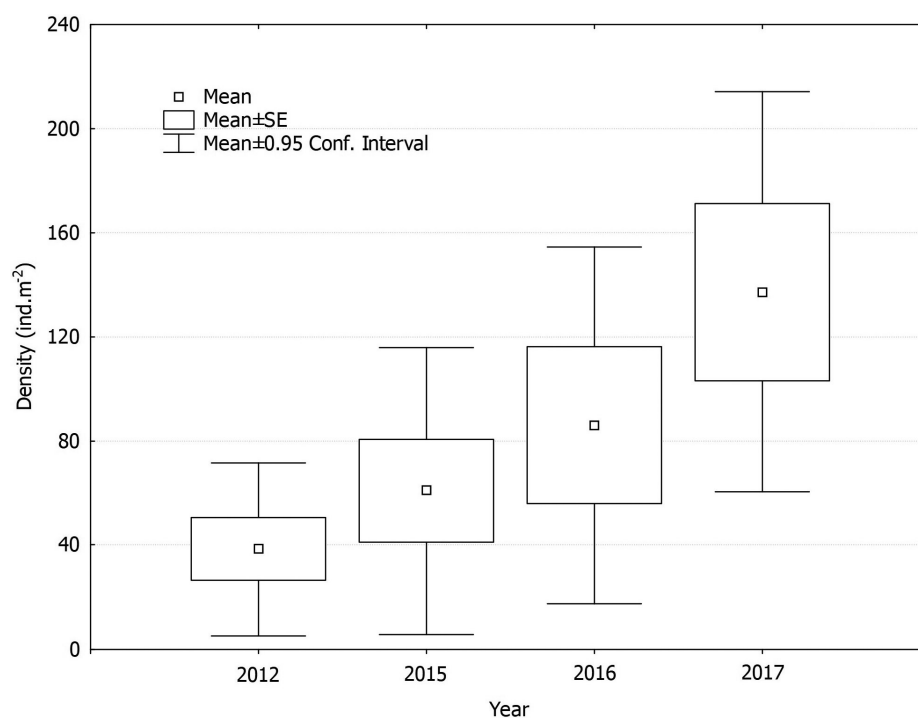


Figure 3. Density of zebra mussel at site D2 in Gojačka Dobra (500 m downstream of HPP Lešće dam) in July 2012, 2015, 2016 and 2017, i.e. 2, 5, 6 and 7 years after the formation of the Lešće reservoir.

tested years were not significant (GLMqp, $df = 3$, $\chi^2 = 456.9$, $p = 0.10$). Quadrat census of zebra mussel (surface area 0.25 m^2) on the banks of Gojačka Dobra revealed higher average densities ($25\text{--}137 \text{ ind.m}^{-2}$) at three sites (D2, D4, D8) in the first reach of 11 km in length downstream of HPP Lešće (Figure 4a) than in the more downstream river reach. Lower average densities ($2\text{--}15 \text{ ind.m}^{-2}$) were recorded at the reach from sites D9–D13 (17–39 km downstream of the dam). Of eight sites downstream of Lešće reservoir at which densities were estimated, at six sites densities in 2016 and 2017 could be compared (at sites D11 only one year was sampled and at site D13 estimated density in 2016 was zero). Zebra mussel mean abundance varied significantly among these six sites (GLMp, $df = 5$, $\chi^2 = 673.4$, $p < 0.001$) and there was also a significant difference between years (GLMp, $df = 1$, $\chi^2 = 64.9$, $p < 0.001$) and significant site \times year interaction (GLMp, $df = 5$, $\chi^2 = 40.1$, $p < 0.001$). Although abundance at five of six sites downstream of the dam was higher in 2017, only at sites D2 and D8 these differences were significant (GLMp, Tukey contrasts, $p < 0.001$) (Figure 4a).

Morphometrics of the zebra mussel populations in the Dobra River Basin

The largest specimen of zebra mussel was collected in the Bukovnik reservoir (max SL = 33.5 mm), and the smallest in the Lešće reservoir (min SL = 3.6 mm). Larger shells (for all four measured traits) were found in 2017 at six of seven sites of Gojačka Dobra where samples were collected in both years (Figure 4b), and only at site D8 shell decreased in 2017. In almost all cases for seven sites along Gojačka Dobra, significant main effects were found for both year and site factors, as well as significant interactions, for all four traits (ANOVA, $p < 0.0001$), except for weight, for which there was no year main effect (ANOVA, $p = 0.066$) (Table S2). Significant differences between years for all four traits were found at three sites (Tukey HSD test, $p < 0.05$; only shell length is shown in Figure 4b): significantly smaller shells in 2017 at site D8 and significantly larger shells in 2017 at sites D2 and D10.

Clear differences in size of collected shells in 2016 and 2017 among three groups of sites (upstream reservoirs Bukovnik and Sabljaki, Lešće reservoir and Gojačka Dobra) are shown on the scatterplot in Figure 5a. There were significant differences among all three groups of sites and each of the four measured morphometric variables (ANOVA, $p < 0.01$, for shell length, width, height and wet weight). Tukey post-hoc tests revealed that mussels from all three groups were significantly different ($p < 0.001$) for all four measured variables. Average shell length was smallest in the Lešće reservoir, intermediate in Gojačka Dobra and largest in Bukovnik and Sabljaki reservoirs (Figure 5b), and the same results were obtained for the other three morphometric variables. Most of the collected specimens at all sites together were in the size category 15–20 mm SL ($n = 736$), with smaller size categories $< 15 \text{ mm SL}$ being the second most frequent ($n = 744$). The largest

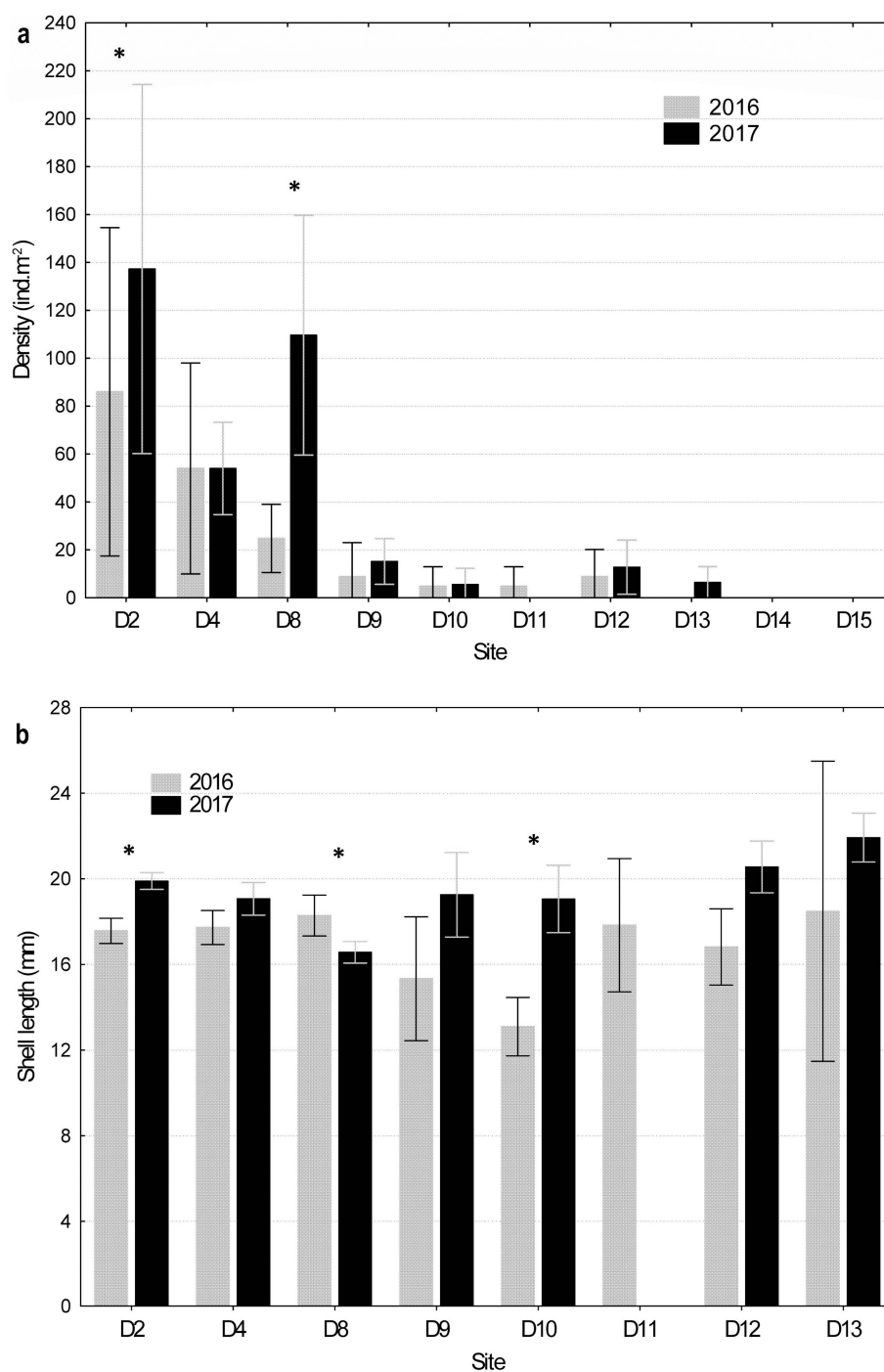


Figure 4. a) Average densities (\pm 95% CL) of *Dreissena polymorpha* at ten sites along Gojačka Dobra downstream of the Hydroelectric Power Plant Lešće dam (* significant differences between years, Tukey HSD test, $p < 0.001$); b) average shell length (\pm 95% CL) of *D. polymorpha* at eight sites along Gojačka Dobra (* significant differences between years, Tukey HSD test, $p < 0.001$).

and the oldest specimens (SL: 30–35 mm) were the least frequent ($n = 7$) and all were collected in the Bukovnik reservoir (Figure 5c). Maximum shell length of zebra mussels from the Lešće reservoir and downstream course of Gojačka Dobra was 28.6 mm.

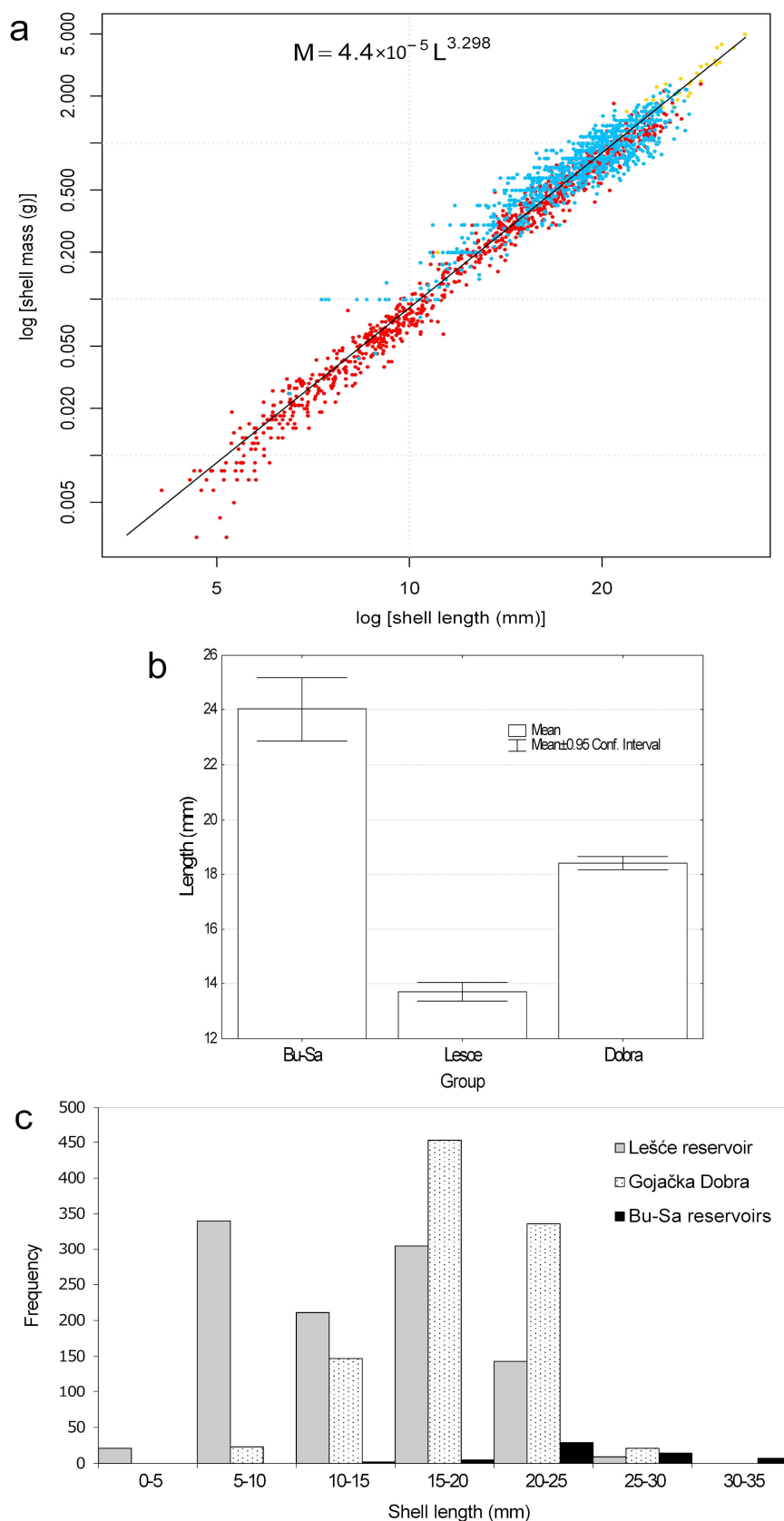


Figure 5. a) Relationship between log-transformed shell length and shell mass (shell + tissue) of all measured specimens of zebra mussels ($n = 2067$) collected at 12 sites in the Dobra River Basin in 2016 and 2017 for three different groups of sites (red-Lešće reservoir, blue-Gojačka Dobra, yellow-Bukovnik and Sabljaki reservoirs); b) average shell length of zebra mussels for three groups of sites; c) size distribution of zebra mussels shell length (SL) for the three distinguished groups of sites in 2016 and 2017.

Discussion

Zebra mussel invasion history in the Dobra River Basin

The first finding of the zebra mussel in July 2012 at site D2, 500 m downstream from Lešće reservoir dam, indicates that Lešće reservoir was invaded immediately after its formation in 2010, and soon after, the species spread along the downstream course of Gojačka Dobra. On the other hand, the first finding of empty shells of zebra mussels in July 2008 at site D1 suggests that the species was present in the Bukovnik and Sabljaki Reservoirs before the formation of the HPP Lešće reservoir. Site D1 is located at the most upstream part of Gojačka Dobra, below HPP Gojak and about 300 m downstream of the main spring Gojak. Empty shells were probably flushed through pipelines from upstream reservoirs (probably reservoir Bukovnik) to HPP Gojak and the most upstream reach of Gojačka Dobra. Interestingly, although many benthic samples ($n = 180$) were collected in the period before reservoir formation (2006–2009) in the Gojačka Dobra River, at a total of 42 sites (Žganec and Gottstein 2009; Žganec et al. 2011, 2013, 2017), zebra mussels have not been found. Therefore, the zebra mussel was either not present in the Gojačka Dobra before the Lešće reservoir was created, or due to low abundance in the first few hundred meters of the upper course below HPP Gojak, the species could not be detected until 2017, when it was found for the first time.

Due to a lack of data about reservoirs Bukovnik and Sabljaki, we can only speculate when and how these reservoirs, created in 1958, were colonized by zebra mussel. The most probable scenario for this first step of colonization of karst rivers in Croatia could be the introduction of veliger larvae with water during fish restocking (Johnson et al. 2008; Pollux et al. 2010). Another possibility is that juvenile/adult mussels were introduced on boats or were carried on fishing equipment from some invaded water bodies, probably from the Drava or Danube Basins, which are the most heavily invaded river basins in Croatia (Lajtner et al. 2004, 2008; Ćuk et al. 2019). Introduction of zebra mussel to these reservoirs by birds could be considered the least likely scenario as demonstrated by Johnson and Carlton (1996).

Rapid invasion of the Lešće reservoir was possible due to its connection with the other two reservoirs in the Dobra Basin, which were invaded sometime after they were built sixty years ago. Thus, similar to other studies (Horvath et al. 1996; Stoeckel et al. 1997; Havel et al. 2005) our results show that reservoirs represent “stepping stone” systems that greatly increase rates of zebra mussel dispersal and invasion. Reservoirs may increase the invasion likelihood for zebra mussel and other species for the following additional reasons. Size-specific growth of zebra mussels in reservoirs was shown to be consistently higher than that of mussels grown in lakes (Karatayev et al. 2006). Further, alien species are much more likely

to occur in reservoirs than in natural lakes, and reservoirs frequently support multiple invaders (Johnson et al. 2008). Several attributes of the abiotic environment and receiving biotic community make reservoirs more prone to invasions than natural lakes. Reservoirs usually have higher connectivity with river systems and other reservoirs, which increases colonization opportunities through the upstream lake or river connections (Johnson et al. 2008). Reservoirs are predicted to have higher propagule pressure since they are usually more accessible to humans than more remote and isolated lakes (Havel et al. 2005). Indeed, Johnson et al. (2008) found that the number of boat landings and/or the proximity of a water body to the nearest Great Lake positively predicted the occurrence of zebra mussels, highlighting the importance of human boating activity and distance to invader source population. Further characteristics of most reservoirs that facilitate the establishment of alien species are young age, lower niche occupancy, and often high disturbance regime (fluctuating water level, unstable thermal regime, high turbidity and sedimentation, increased nutrient loading, fish restocking). Consequently, newly assembled native communities in reservoirs may exhibit correspondingly lower biotic resistance, which makes them particularly vulnerable to species invasions (Havel et al. 2005; Johnson et al. 2008). Comparison of Croatian large rivers also showed that the Drava, with its three large reservoirs, is more heavily biocontaminated than the Sava River (without reservoirs in the Croatian river section), because Drava reservoirs, in which *D. polymorpha* and also invasive mud snail *Potamopyrgus antipodarum* (J.E. Gray, 1853) were abundant, act as sources of these two invasive species for the downstream river course (Ćuk et al. 2019). Thus, this study contributes to the large body of knowledge of how reservoirs, especially connected ones, act as “stepping stones” for zebra mussel invasion.

Distribution and abundance of zebra mussels in the Dobra River Basin

In the Lešće reservoir zebra mussels completely overgrow all objects permanently immersed in water (dead branches, boat stations, etc.). Established densities in the Lešće reservoir (site L1: 356–2044 ind.m⁻²) using an Ekman grab probably greatly underestimate zebra mussel densities in the littoral of this reservoir as this method is not appropriate for hard substrates. Much more representative estimation of species density in the littoral of the Lešće reservoir using quadrat census (Aug 2017 at site L2: 12,496 ind.m⁻²) was done only once due to high water level during other field visits to the reservoir when only the Ekman grab could be used. Nevertheless, established densities are comparable to other similar studies of reservoirs and lakes in Europe and North America (e.g. Hebert et al. 1991; Karatayev et al. 1998; Haynes et al. 1999; Burlakova et al. 2000; Leuven et al. 2014). Since the first zebra mussels in Gojačka Dobra were

detected at site D2 in July 2012, colonization of Lešće reservoir probably started immediately after its creation in summer 2010. Filling of the new reservoir started in 15th of June 2010, and it was completed (to 185 m a.s.l.) until the middle of July 2010. Until October 2010, water from the reservoir was not used much and the water level fluctuated < 1 m, i.e., it was relatively stable. In October 2011, the reservoir was partly emptied and the water level dropped 38 m (Filipan Vdovčić et al. 2012). Hence, after colonization of Lešće reservoir, zebra mussel reproduction in 2011 ended by the flushing-out of veliger larvae from the Lešće reservoir in October 2011. This event probably enabled colonization of almost the whole downstream course of the Gojačka Dobra. Further, zebra mussel invasion of the Lešće reservoir probably occurred due to similar flushing-out of veliger larvae from the Bukovnik reservoir, because water is released every summer from this reservoir through connecting pipelines into Lešće reservoir due to the usual summer repair works on HPP Gojak. Thus, probably already in 2011, the zebra mussel population in the Lešće reservoir became a source population of veliger larvae, which then colonized the downstream course of Gojačka Dobra. A similar fast increase of zebra mussel density was observed in the south part of Lake St. Clair in just one year (Hebert et al. 1991), while Strayer et al. (1996) also showed how zebra mussel invaded the Hudson River estuary in just one year. Our results also indicate that densities in the studied period of 2012 to 2017 increased in both Lešće reservoir and the downstream course of the Gojačka Dobra (Figures 3, 4a).

Densities of zebra mussels in Gojačka Dobra downstream of the Lešće reservoir were estimated only at microhabitats with slower currents, mostly on stony substrate in shallow bank areas. It was shown that densities of zebra mussels in Gojačka Dobra (at microhabitats where the species was more abundant) on average declined downstream. Since zebra mussel populations in rivers strongly depend on upstream lakes or reservoirs which act as a source of the species larvae, the density usually declines in downstream river reaches (Horvath et al. 1996; Stoeckel et al. 1997). Thus, the observed decline supports the fact that Lešće reservoir represents the source of zebra mussel larvae for downstream river reaches. Larvae concentration in Gojačka Dobra probably decreases in the downstream direction due to dilution and mortality of larvae during transport, but also probably due to filtering effects of mussels and other filtering macroinvertebrates (e.g. Trichoptera larvae, Simuliidae, *Hydra* sp.) (Horvath et al. 1996 and references within). Similar downstream decrease of zooplankton released from a reservoir was demonstrated by Ward (1975). Furthermore, since higher densities of zebra mussels were observed in the first reach of Gojačka Dobra downstream of the Lešće reservoir (D2–D8, 11 km in length) than in the second more downstream river reach (D9–D13), better survival of juveniles and adults in the former is probably connected to

greater abundance of food (plankton from the reservoir). In the last reach of Gojačka Dobra (D14–D15, 1 km in length) as well as in the Kupa river at the mouth of the Dobra river, *D. polymorpha* had a very low abundance and could not be detected most of the time.

Comparison of zebra mussel densities in Gojačka Dobra downstream of the Lešće reservoir with other studies of zebra mussel density in rivers (Strayer et al. 1996; Ricciardi et al. 1995b) revealed that observed densities were very low, especially when compared with studies of river courses downstream of lakes and reservoirs (Horvath et al. 1996; Clevlen and Frenzel 1993). Its densities in Gojačka Dobra are comparable to those of the Croatian section of the Danube River, and somewhat lower than its densities in the Croatian section of the Drava River (Ćuk et al. 2019), perhaps due to the brief time interval since its appearance in Gojačka Dobra. Increases in density were observed from 2012 to 2015 at site D2 (Figure 3) and at six sites from 2016 to 2017, indicating that due to a relatively short time after Lešće reservoir formation it has not yet managed to reach higher densities. Furthermore, a low concentration of larvae in released water could also be the reason for its low densities in the downstream river course. Zebra mussel depth distribution varies among habitats, but dense populations are usually restricted to well-oxygenated waters above the epilimnion (McMahon and Bogan 2001; Karatayev et al. 2018). Although its distribution by depth in Lešće reservoir was not established, we can assume that adults were most abundant in the epilimnion and metalimnion layers (i.e. littoral and sublittoral zone) of the reservoir and that the highest concentrations of its planktonic larvae are found there. Therefore, the release of hypolimnion water (at a depth of 20 m when Lešće is fully filled), probably results in a low concentration of zebra mussel larvae and consequently causes low densities in the downstream course of Gojačka Dobra.

Future monitoring of zebra mussel abundance in Gojačka Dobra is needed in order to examine the time trajectory in population density, and its effect on native unionid mussels and the associated assemblage of benthic macroinvertebrates. Interestingly, even now with very low abundance and despite the dominance of hard substrate along the whole course of Gojačka Dobra, zebra mussel probably negatively affects native unionids on which it is attaching (Figure 2b). Since many negative effects of such attachment on native unionid mussels have been demonstrated (Strayer and Smith 1996; Ricciardi et al. 1995b, 1996; Burlakova et al. 2000), future monitoring of zebra mussel in Gojačka Dobra should focus on its effects on Natura 2000 species *Unio crassus*, which was present at many sites in Gojačka Dobra in the reach more downstream from the dam (D8–D15) during this study.

Morphometrics of the zebra mussels in the Dobra River Basin

There was a clear difference in morphometric traits between three groups of sites in the Dobra River Basin: the largest and the oldest specimens were

found in the Bukovnik and Sabljaki reservoirs. This supports the idea that the connecting reservoirs were primary source populations whose larvae formed a population in the Lešće reservoir. In the latter reservoir, zebra mussel finds optimal conditions for intensive reproduction, inferred from the dominance of the smallest size categories (Figure 5). However, in the downstream course of Gojačka Dobra, where recruitment is much less successful, larger size categories of the zebra mussel, 15–25 mm in length (*ca.* 1–2 years old), were the most abundant. This could indicate that the recruitment of the river population is variable interannually. Interestingly, an increase in mean shell length from 2016 to 2017 was observed for the most sites downstream of HPP Lešće dam (D2–D13, Figure 4b). These results indicate that growth of zebra mussel is an important factor that increases competitive pressure with native unionids in reaches of Gojačka Dobra downstream of the dam. Thus, a future increase of zebra mussel abundance in reaches more downstream of the Lešće dam, where native unionids were abundant (especially *U. crassus*), could further increase the negative impact on native species.

Measures to reduce further invasions of the zebra mussel in Dinaric karst waters

The Lešće reservoir now represents the main “hotspot” of zebra mussel density in Dinaric karst areas in Croatia. Population control can be achieved with a reduction or extermination of the small but significant source population in the Bukovnik reservoir and manipulation of the water level in the Lešće reservoir. By reduction of the water level in the Lešće reservoir, lower than regular during usual summer repair works in July and August every few years, for at least two weeks to one month (longer than zebra mussel survival time in the air, Ricciardi et al. 1995a; Pollux et al. 2010), the population of zebra mussel in the reservoir could be controlled. This would decrease the chance of its further natural dispersal into other stagnant and flowing waters in the Dinaric karst. Of course, human-mediated dispersal should be controlled by “check, clean and dry” practice for boats and fishing equipment, while anglers should be educated not to use fish baits or transport water from the Lešće reservoir.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Records, time, methods of sampling and presence-absence/density of *Dreissena polymorpha* at 22 sites in the Dobra Basin (Croatia).

Table S2. Test statistic (df, F-values and P-values) for nested ANOVA and two-way ANOVA.