International Aquatic Research Zebra mussels (Dreissena polymorpha) invasion affects native unionids in Lake Balaton, Hungary --Manuscript Draft--

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Zebra mussels (*Dreissena polymorpha*) invasion affects native unionids in Lake Balaton, Hungary

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Running title: Zebra mussels (Dreissena polymorpha) invasion

Abstract

Invasive species attracted great attention due to theirability to affect ecosystem integrity. Assessment of zebra mussel density, mortality and their infestation to unionids was carried out at four sites of Lake Balaton in April 2010. The relative abundance of zebra mussels was eminently exceeding those of unionids. High incidence of unionids infested with zebra mussels was reported at Balatonakali (100%), Balatonboglár (93.33 %), Balatonalmádi (66.67%) and Keszthely (43.39 %). The percentage of collected dead unionids was the highest at Balatonboglár (40 %), followed by Balatonalmádi (33.33 %) and was relatively low at Balatonakali (9.09%) and Keszthely (8.16 %). The average numbers of zebra mussels colonizing each unionids were 30±3.019, 23.0±1.411, 19.818±5.088 and 3.0±0.577 at Balatonboglár, Keszthely, Balatonakali and Balatonalmádi, respectively. Percentage of zebra mussel mass/ unionid mass is given in a decreasing order, showing high unionid infestation intensity at Balatonboglár (35.512±5.432%) and Balatonalmádi (20.191±1.638 %) whilst relatively low infestation intensity was reported for unionids at Balatonakali and Keszthely. The percentage of zebra mussel mass/ unionid mass was strictly correlated with the incidence of dead unionids at the tested sites. This might indicate that zebra mussel invasion in Lake Balaton poses actual risk on unionid mussel populations.

Keywords: Zebra mussel; Infestation; Unionid community; Lake Balaton

Introduction

Zebra mussel (*Dreissena polymorpha*, Pallas 1771) is a veneroid bivalve species of Ponto-Caspian origin that has invaded areas far beyond its native ranges to both Europe and North America (Orlova and Scherbina 2001). Its invasion induces considerable economic and ecological impacts (Miehls et al. 2009). After their initial introduction to Lake Balaton in 1930, zebra mussels spread rapidly throughout the entire Lake and presently it is the dominant invertebrate species in Lake Balaton (Muskó and Bakó 2005; Balogh et al. 2008).

Dreissena polymorpha produce a massive colony within a short period of time that exceeds those of the other native benthic invertebrates (Sinitsyna and Protasov, 1994; Burlakova et al 2000). Muskó and Bakó (2005) reported that zebra mussels in Lake Balaton (October 2000) constitute 2.48-84.85% of all animals on submerged macrophtes with a population density range 421-749.032 ind m⁻² and biomass 0.01-50.96 g m⁻²(dry weight with shell). Most recently for Lake Balaton, Balogh et al. (2008) reported massive increase for the year 2005, both in density (93,955 ind m^{-2} stone surface), and biomass (714 g m⁻² stone surface) of *D. polymorpha*. The maximal density was 220, 000 ind m⁻² of lake surface, with a biomass 1,100 g m⁻² of lake surface. Lower densities (up to 11,000 ind m⁻²) were reported for Lake Barden and River Darent (Kent, UK) (Aldridge et al. 2004). In some areas of Lake Erie (USA) massive colony densities of 20,000 (ind m⁻²) to higher one was reported (O'Neil 2008). The average zebra mussel density in European lakes falls in the range from 5,000 to about 115,000 ind m^{-2} (Aldridge et al 2004). As such, the European population density is lower than those in US lakes, most possibly due to the fact that nutrient level is somewhat higher than in European lakes (Aldridge et al. 2004). Also, in European lakes there are several predators to zebra mussels (Balogh et al. 2008), especially roach and water fowl are of significant value, whereas in US lakes the significant predatory effects of water fowl in addition to few fish species are of minimized effect (Naddafi and Rudstam 2013).

Freshwater unionid mussels (Family: Unionidae) are an important group in the aquatic habitat. Globally, they are in serious decline and in urgent need of protection and conservation (Newton et al. 2008). Therefore, their biodiversity is an important

topic for aquatic wildlife conservation. Filtering capacity of zebra mussel is much greater (about 12 times) than that of unionids, which can possibly lead to severe changes of the aquatic ecosystem, through increased water clarity, increased plant growth, and shifts in fish communities (Nalepa et al. 1996). Further ecological impact of zebra mussel invasion can be that the bioaccumulated toxins in zebra mussels (Faria *et al.*,2010) could possibly be transferred from zebra mussels to different water fowls.

All this might induce a decline in the abundance of unionids and disappearing of weak species (Nalepa et al. 1996; Burlakova et al. 2000). In North American lakes and rivers, high densities (3000 m⁻²) of *D. polymorpha* have caused a massive decline of unionid population's densities and have accelerated the rate of extinction to 10 fold (Ricciardi et al. 1998). Ricciardi et al. (1995) showed that field density of zebra mussels strongly predicts the proportion of infested unionids and the mean number of zebra mussels attached to the unionids. For Lake St Clair (USA), Zanata et al. (2002) reported high incidence of zebra mussel infestation among unionids (up to 286 ind /unionid) and five species at risk. In Barden Lake (Kent, UK) zebra mussel invasion induced deleterious impacts on native unionid mussels, particularly the swan mussel (*Anodonta cygnea*) and painter's mussel (*Unio pictorum*) exhibiting high proportion of infestation (Aldridge et al. 2004). Zebra mussels' attachment to unionid bivalves can occlude their openings, preventing opening for respiration, feeding and reproduction, or preventing the closing of valves (Ricciardi et al. 1996) as well as shell deformities might develop (Hunter and Baley 1992).

In laboratory experiment, Baker and Hornbach (1997) found that infestation of zebra mussels (*D. polymorpha*) on two unionid mussels (*Actiononaias ligamentina* and *Amblema plicata*) can cause stress and symptoms of starvation in unionid mussels

which exhibited species specific responses. Ricciardi et al. (1995) and Schloesser et al. (1996) reported that high zebra mussel density in lake water (5000-6000 m⁻²) and high infestation intensity among unionids (100-200 per unionid) resulted in near total mortality of unionids. In lake Balaton, the biggest shallow lake in Central Europe, although there is early report of zebra mussel infestation and induced mortality to unionids (Sebestyen 1938), but the exact recent status of unionid mussels in regards to *D. polymorpha* infestation is still not clear. The study takes a conservation interest as there is an endangered species involved (*Pseudoanodonta complanata* Rossmässler, 1835) that is recorded in IUCN red list (IUCN 2006). Therefore, the aim of this study was to assess the magnitude of the ecological risk posed by *D. polymorpha* on the native unionid community in lake Balaton.

Materials and methods

1. Tested sites

Samples were collected during April 2010 (in two times) from four sites of lake Balaton (Fig. 1) as site 1 (Balatonalmádi, N 47°02'078" - E18°01'506"), site 2 (Balatonakali, N46°52'50.93" - E17°45'17.38") and site 3 (Keszthely, N46°45'36.9"-E17°15'16.1") from the Northern shore and site 4 (Balatonboglár N46°46'04.7"-E17°37'07.3") from the Southern shore. Sampling sites were visited twice, in each time samples from all tested sites were collected, the data presented as the summation of the two field visits. Physical characteristics, indicated in Table (1), of the lake water were measured in the field using multi probe portable equipments (Multi HQ 40 d, Hach for DO, pH, conductivity and Hach Lange for turbidity). *D. polymorpha* as well as unionids mussels were hand collected from the littoral region of the selected sites. The total number of collected unionids was 86 (6 at Balatonalmádi, 11 at Balatonakali, 54 at Keszthely, 15 at Balatonboglár).

2. Mussel density and unionid infestation

Zebra mussels were counted in six stones selected randomly at each site, their size was in the range between 0.02 and 0.09 m⁻². They were grouped according to their size into < 2 mm, 2-10 mm and > 10 mm. Density was expressed as ind m⁻² stone surface using all stones surface. At the field the total numbers of the collected as well as the dead unionids were counted. Samples were individually preserved in 5 % formalin and were transferred to the laboratory for computing the percentage of unionids infested with zebra mussels. The number and biomass of zebra mussels were assessed for each live infested unionid. The biomass of the zebra mussels per each colonized unionid was expressed as g fresh weight / g fresh unionid weight. The percentage of zebra mussel's biomass to those of each live unionid host was calculated.

3. Statistical analysis

Statistical analysis was done using Biostat 2008 professional software (version 2.5). All data were expressed as means \pm standard error, except the mortality and infestation which were expressed as ratio of incidence. ANOVA followed by Tukey HSD was computed in order to find statistical differences (P \leq 0.05) between the means of different tested items (Pipkin, 1984). Pearson correlation coefficient was computed for unionids found dead vz each of Zebra mussel biomass (g/ Unionid collected) and number of Zebra mussel (number / Unionid collected) at the tested sites of Lake Balaton.

Results

1. Physical characteristics of lakewater

Table (1) summarizes physical characteristics Lake Balaton water of the sampling sites. Significant difference was recorded only in case of turbidity, with the lowest value (9.7 FNU) at Balatonalmádi and the highest (90.8 FNU) at Balatonakali.

2. Zebra mussels' density

Highest density $(3,920.2\pm 960.4 \text{ ind m}^{-2} \text{ stone surface})$ was recorded at Balatonboglár and the lowest $(2,565.8\pm400.3\text{ ind m}^{-2} \text{ stone surface})$ at Keszthely but they nonsignificantly varied among the tested sites at Lake Balaton during April 2010 (Table 2). None of the sites had newly settled zebra mussels (< 2mm), except Balatonboglár which had low proportion (5.1 %) of newly settled larvae (Fig. 2). Populations of *D. polymorpha* at Balatonboglár and Keszthely were dominated by young zebra mussels (2-10 mm) being74.4 and 69.7%, respectively, whereas the adult (>10 mm) constituted low proportion. Adult zebra mussels were prevalent at Balatonalmádi and Balaton akali sites of lake Balaton.

3. Relative abundance of unionid species

Unionid population at Balatonalmádi and Balatonakali consisted only of the painter's mussel (*Uniopictorum*, Linnaeus 1758), even though the mortality percent was little bit higher at Balatonalmádi (Table 2). Unionid population at Keszthely appeared more diverse and exhibited the lowest mortality ratio. Of collected live specimens, swan mussels (*Anodnota cygnea*, Linnaeus 1758) amounted to the highest proportion (38.77%) followed by painters mussels (*U. pictorum*) (38.77%), duck mussels (*Anodonta anatina* Linnaeus, 1758) (14.28%),swollen river mussel (*Unio tumidus* Philipson, 1788) (12.24%) and depressed river mussels (*Pseudoanodonta complanata* Rossmässler, 1835). *Pseudoanodonta complanata*, the endangered species, constituted the lowest proportion of collected live unionids, with 2.04% (Fig.3). Ratio of dead unionids at Keszthely was the same (25%) for all collected unionids except the swan

mussels that exhibited no mortality. At Balatonboglár, the collected live unionids exhibited equal proportion for both duck and painter mussels, whereas the latter constituted the highest ratio (50%) of the collected dead unionids. *A. cygnea* was completely missing from this site. The painter mussel occurred at all the tested sites of Lake Balaton and constituted major bulk of the unionid community at Keszthely and Baltonbogár, whereas the swan mussel was only collected at Keszthely. The depressed river mussels (*P. complanata*), that is listed as near threatened on IUCN Red List (IUCN, 2006) was only collected at Keszthely amounting only 2.04% of the whole live population and showing high mortality incidence (25%). At Balatonboglár they were only found as dead shells. The numbers of collected unionid species were 5, 2, 1 and 1 at Keszthely, Balatonboglár, Balatonalmádi and Balatonakali, respectively (Fig. 3). Therefore, according to the collected data in April 2010, there is a reduction in the survival and number of unionid species at most sites of Lake Balaton.

4. Infestation of unionids

In lake Balaton, all of the tested sites were infested with zebra mussels on unionid bivalves. Infested unionids exhibited zebra mussel's colonization on the posterior third of the shell (Fig. 4) and spread all over the rest of the shell in case of heavy infestation. The percentage of infested unionids was 100% at Balatonakali, 93.33% at Balatonboglár, 66.67% at Balatonalmádi and 43.39 % at Keszthely (Table 2). Mortality of unionids was found at all the tested sites, being high at Balatonboglár (40 %) and Balatonalmádi (33.33 %), whilst low at Balatonakali (9.09%) and Keszthely (8.16%). The number of zebra mussels/unionids varied significantly (P<0.05, ANOVA) among unionids collected at all tested sites (Table 3). Its maximum number was recorded for unionids at Balatonboglár (30 \pm 3.019) followed in a descending order by those at Keszthely (23.0 \pm 1.411), Balatonakali (19.818 \pm 5.088) and Balatonalmádi (3.0 ± 0.577) . It was proportionally correlated with the biomass of zebra mussels/ living infested unionids. The biomass of zebra mussels (g fresh weight/ living infested unionid) infesting unionids at Balatonboglár (6.32±2.13) was significantly higher than those recorded for unionids at Keszthely (1.649±0.543), whereas those at Balatonakali (1.152±0.734) and Balatonalmádi (0.933±0.145) were non-significantly fluctuated (Table 3). Zebra mussel's population at Keszthely had a high proportion of youngsters (Fig.2). This may be the reason for the relatively low zebra mussel biomass, compared to their high abundance. The number of zebra mussels / living infested unionids did not show any correlation with infestation incidence of unionids (Table 3). This may be due to zebra mussels'body size fluctuations, i.e., zebra mussels infesting the unionids varied widely in their sizes. High percentage of zebra mussels' biomass (g / g of living infested unionids) was recorded at Balatonboglár (35.512±5.432) and Balatonalmádi (20.191±1.638%), they were significantly higher than those recorded at Balatonakali and Keszthely which exhibited relatively low mass proportion, being 7.224±2.159 and 4.001±1.357 %, respectively (Table 2). Also, the % of zebra mussel's biomass (g mass/ g of living infested unionid mass) was directly correlated with the individual number of infested zebra mussel /living infested unionids and the biomass of infested zebra mussels/ living unionids. It was found that % mortality of unionid bivalves directly correlated with biomass ratio of infested zebra mussels/living infested unionid. High positive Pearson correlations were reported between unionid mortality and zebra mussel biomass g/ unionid collected in case of Balatonboglár and Balatonalmádi (Pearson correlation coefficient = 0.996 and 0.993, respectively). On the other hand, no correlation was found in Keszthely (Pearson correlation coefficient = -0.67). Significant positive correlation was found between dead unionidsopposed to number of zebra mussels/ unionid collected in Balatonakali (Pearson correlation coefficient = 0.982).

Discussion

The density of zebra mussels in the tested sites of Lake Balaton (April 2010) was 2365.773 to 3920.225 ind m⁻² rock surface which fell in the lower range previously recorded by Muskó and Bakó (20005) and Balogh et al. (2008) for lake Balaton during 2005. The zebra mussel's density was non-significantly varied among the tested sites of the lake. This is although the lowest density was reported for Keszthely and the highest one for Balatonbogár. This is may be related to variations of the physical parameters of the water.

Unionids mortality was reported throughout the all tested sites of lake Balaton with maximum (40%) at Balatonbogár and minimum (8.16%) at Keszthely, yet it was also high at Balatonalmádi (33.33%) and relatively low at Balatonakali (9.09%). They were in parallel with high zebra mussels' infestation incidence and field density range (2114.22 - 3919.19 ind m⁻² rock surface). These data were in agreement with those reported by Aldridge et al. (2004) that inlakeBarden (UK) the reduction in the proportion of painter's mussel (*U. pictorum*) from 85% in 2002 to 71% in 2003 with high incidence of mortality of swan mussel (*A. cygnea*) is related to zebra mussels' density (2921±944 ind m⁻²). In severe condition, Ricciardi et al. (1995) recorded severe unionid mortality (> 90%) in St. Lawrence River (USA) when zebra mussels density reach 6000 m⁻² and infestation intensity (100 ind / infested unionid). The present data evoked a reduction in the unionids survival rate and number of collected species at the tested sites of lake Balaton, except at Keszthely. This unionids reduction due to zebra mussel's invasion is well reported for both European and North American water bodies (Nalepa et al. 1996; Schloesser et al., 1996; Ricciardi et al. 1998;

Burlakova et al. 2000; Aldridge et al. 2004). In the present study, incidence of unionids infested with zebra mussels was prominent at all the tested lake Balaton sites with severe (100%) and lowest (33.33%) records at Balatonakali and Balatonalmádi, respectively. Recently, it is reported that the potential impacts of zebra mussel's invasion on unionids are positively correlated with their population density (Wu *et al.*, 2010).

The incidence of unionids mortality reported herein for lake Balaton was correlated with infestation intensity (% biomass of infested zebra mussels to those of unionid host). Unionids mortality versus infestation intensity correlation was reported previously (Burlakovaet al., 2000; Schloesser et al., 2000). In the present study, the maximum unionids infestation intensity for lake Balaton was 30±3.019 (no of zebra mussels/infested unionids) and 35.512 ± 5.432 as а % of zebra mussels' biomass/infested unionid biomass. Similar infestation intensity 26.5±5.6 as no of zebra mussels/ infested unionid and 30 as % of zebra mussels biomass / unionid biomass was reported earlier for unionids in lake Naroch in 1990 (Belarus) (Burlakova et al., 2000). In contrast, higher zebra mussels intensities 219.6±24.5 (no/unionid) and 40 % (% of zebra biomass/unionid biomass) were reported earlier for lake Clark (USA) in 1996 (Burlakova et al. 2000) and 6805±623 (no/unionid) and 257 % (% of zebra mussel biomass /unionid biomass) for Western lake Erie (USA) in 1989 (Schloesser and Nalepa, 1994). According to Burlakova et al. (2006) the impacts of zebra mussels are more liable to be coupled to biomass than numbers of the mussels. Schloesser and Kovalak (1991) indicate that the biomass of infested zebra mussels may exceed host weight by a factor of four (dry weight), this is due to the largest proportion of the young mussels that continue to grow and impedes the locomotion of the unionids which may sink down into the muddy sediments. Thus,

zebra mussels infestation cause starvation of the unionids when sank into the sediment, impair movement and induce metabolic costs of carrying zebra mussels that adversely affect the health of the host (Schloesser and Kovalak 1991; Schloesser et al. 1996).

The differences of the mortality of unionid species reported herein may provoke species sensitivity to the invasive effects of zebra mussels. This species specific sensitivity is proven experimentally by Baker and Hornbach (1997).They found *Actinonaias ligamentina* (subfamily Lampsilinae) to be more sensitive to *D. polymorpha* infestation than *Amblemaplicata* (subfamily Ambleminae) as indicated by changes in oxygen uptake rate and grazing rate.In contrary, interspecific competition for a limited food resource may negatively impact not only the unionids, but the attached dreissenids as well (Baker and Hornbach 2008).

Thus, it could be concluded that in lakeBalaton, there is high incidence of zebra mussels infestation to the native unionids that is coupled with high unionid mortality, infestation intensity and a reduction of theunionid species diversity. However, the magnitude of impacts on unionids in Lake Balaton was not high as those recorded for some European water bodies or extreme as recorded for some North American one.

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Table (1). Physical characteristics of water at different sites of Lake Balaton (April 2010).

Sites	Temperature (°C)	pН	DO, mg $L^{-1}(\%)$	Conductivit $y (us Cm^{-1})$	Turbidity (FNU)
Balatonalmádi	13.45 ± 1.85^{a}	8.44 ± 0.52^{a}	7.83 ± 1.28^{a} (75.75 ± 9.85)	832.0 ±7.0 ^a	7.40 ± 1.30^{a}
Balatonakali	14.8 ±2.0 ^a	7.645 ±0.295 ^a	9.575±0.125 ^a (97.80±1.80)	792.0 ± 4.0^{ab}	73.30 ±17.50 ^b
Keszthely	16.65 ±2.45 ^a	8.46 ±0.20 ^a	9.68±0.75 ^a (101.925±4.22 5)	$756.50 \pm 3.50^{\rm b}$	38.65 ± 12.36^{ab}
Balatonboglár	17.1 ±1.3 ^a	8.445 ± 0.135^{a}	11.22 ± 0.41^{a} (118.65±1.55)	790.50 ± 15.50^{ab}	14.90 ± 0.80^{a}

All data are mean \pm SE. In same column means not sharing superscript articles are significantly different at P \leq 0.05. DO; dissolved oxygen.

Items	Balatonalmádi	Balatonakali	Keszthely	Balatonboglár
Density of	3110.213	2972.379	2565.773	3920.225
zebra mussels (Ind. m ⁻²)	±717.071 ^a (6)	$\pm 673.357^{a}$ (6)	$\pm 300.263^{a}$ (6)	±960.347 ^a (6)
% of unionids colonized by zebra mussels	66.66	100	43.39	93.33
% unionids found dead	33.33	9.09	8.16	40
Number of	3.0 ± 0.577^{a}	$19.818{\pm}5.088^{b}$	23.0 ± 1.411^{b}	30 ± 3.019^{d}
zebra mussels/ living infested unionid	(4)	(11)	(23)	(14)
Biomass of zebra mussel/ living infested unionids (g)	0.933±0.145 ^a (4)	1.152±0.734 ^a (11)	1.649±0.543 ^a (23)	6.32±2.13 ^b (14)
% biomassof zebra mussel / living unionid host	20.191±1.638 ^a (4)	7.224±2.159 ^b (11)	4.001±1.357 ^b (23)	35.512±5.432 ^c (14)

Table (2). The infestation of unionids by zebra mussels (*D. polymorpha*) in Lake Balaton, April, 2010.

Data expressed as mean \pm SE. Means in the same row carrying different superscript are significantly different at P \leq 0.05. Number of replicates indicated between brackets.



Fig. (1). Location of sampling sites at Lake Balaton.



Fig. (2). Relative abundance (%) of different sizes of zebra mussels (Dreissena polymorpha) collected at different sites of Lake Balaton, April, 2010.



Fig. (3). Relative abundance (%) of live and dead unionids collected at different sites of Lake Balaton, April 2010.



Fig. (4). Unionid bivalves (*Unio pictorum*) infested with zebra mussels (*Dreissena polymorpha*) showing infestation at the posterior edge.