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Biological control of invasive zebra mussel (*Dreissena polymorpha*) in a freshwater ecosystem through *Potamon ibericum*

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ABSTRACT
In the present study, possible effects of using *Potamon ibericum* for the prevention of zebra mussel (*Dreissena polymorpha*) invasion in a freshwater ecosystem were investigated. In a recirculating aquaculture system (RAS) supplied with aerated freshwater, 3 individuals of *P. ibericum* were placed in each of 3 aquariums provided by 100 zebra mussels. Throughout the 90-day monitoring study, mussel consumption was visually counted and then new mussels were introduced daily to complete the number of mussels to hundred, repeatedly each day. Throughout the monitoring study, growth rates, number of mussels consumed, and the number of broken mussel shell pieces were determined. Daily consumption rate of mussels by each crab was recorded as 11.115, 11.104, and 11.107 mussel/crab in the 3 experimental groups, respectively. The consumption rate of mussels per crab was counted as 999.78 over the 90-days experiment. As a result, the findings of this study provide strong evidence that *P. ibericum* can be used for biological control to combat zebra mussel (*D. polymorpha*) invasion in a freshwater ecosystem.

Keywords: Biological control, *Dreissena polymorpha*, Economic value, Freshwater crab, Human consumption, *Potamon ibericum*

Introduction

Zebra mussel (*Dreissena polymorpha Pallas*) is one of the two types of freshwater shellfish distributed in Eastern Europe, North America and West Asia (Drake and Bossenbroek, 2004; Kinzelbach, 1992). Several investigations have been focused on the invasion and expansion of zebra mussels spread around the freshwaters in Europe over the last 150 years and even entering North America in 1988 (Sprung and Borcherding, 1991). Zebra mussels are dominantly spread in the lakes and reservoirs of Turkey (Demirsoy, 1998; DSI, 1969; Geldiay and Bilgin, 1973). It is well known that female mussels are prolific and each individual could produce millions of eggs during spawning period, and the population of zebra mussel drastically increased in the last decade in inland waters of Turkey (Albayrak et al., 2016; Gaygusuz et al., 2007; Odabasi et al., 2019).

There is a significant damage in terms of economic impacts via the invasion and expansion of zebra mussels in freshwater reservoirs with significant impacts on the biodiversity of the aquatic ecosystem due to its sessile growth features and high reproductive characteristics (Stanczykowska, 1977; Mackie et al., 1989). Zebra mussels are settling naturally on rocks in particular and concrete materials, iron, PVC and sheet metal surfaces of facilities set in freshwater reservoirs or even on aquatic plants. Mussels can form clusters on the silty and sandy bottom, as well as rough holding places (DSİ, 2005). They form rather thick and dense layers and cause a narrowing of field they accumulate on. Even if only one zebra mussel enters an ecosystem, it is extremely difficult to control its development as the reproduction of zebra mussels is remarkably high. For instance, a zebra mussel can lay between 30 000 and 1 000 000 eggs per year, but only 2% -5% of these reach mature size, which can adhere to a suitable area within 2-3 weeks (Synder et al. 1992). Another problem of the invasive zebra mussel growth in freshwater dam lakes is the accumulation of mussels inside pipes and around valves that reduces flow velocity of water through pipes via limitation of the volume within the pipes. Moreover, massive mortalities of mussels in the long term may cause waterway pollution and filter occlusion and an increase in surface corrosion because of mussel infestation (Darrigran, 2002). It is almost impossible to eradicate the mussel once they become established in to the inland waters (Amberg et al., 2019). In Great Lakes region of the USA, the accumulation density of zebra mussel has increased from 200 adults to 700,000 adults per square meter in a year (Miller and Payne, 1992) and $500 million was spent annually for the control of zebra mussel in that region. Besides, in Mississippi Valley (USA), it was reported that the spread of zebra mussel increased 105 times in 2 years (Yager, 1994).

The primary reason for the problems is that the density and mass of mussels can reach very high levels. For example, densities of 30 000 - 100 000 pieces per square meter is 10 times higher than the total mass of all invertebrate bottom organisms. The economic losses caused by the zebra mussel in North America have been calculated as approximately 5 billion US dollars per year. For this reason, mussels expansion continue to be one of the most intensively researched interests (ZMIS, 2001).

To control the invasion of zebra mussels using chemicals, both physical and manual methods have been reported earlier (Singer et al., 1997). However chemical usage was not preferred due to their negative impacts on the environment and the manual collection of mussels requires huge workforce. Till now only a few studies indicated biological control methodology against zebra mussel. Singer et al. (1997) used Bacillus species against zebra mussel on their veliger stage.

In the present study, we investigated the potential use of zebra mussel as biological agent for the control of their expansion by introducing a predator crab *P. ibericum* into the freshwater ecosystem. The feeding behaviour and preferences of *P. ibericum* were also evaluated via visual and camera monitoring that might provide useful indications for possible culture efforts of *P. ibericum* in future investigations as well as farmers in the field.

Material and Methods

Ethical Statement

Regulations of Animal Behavior Society Guidelines have been followed throughout the experimental procedures of the present study, approved by the Ethical Committee of Canakkale Onsekiz Mart University via Ethical Commission Approval Number: 2021/03-04.

Sampling Area

In this study, zebra mussels were collected from Atikhisar Dam Lake, Canakkale – Turkey (Figıre 1.), while the crabs were collected from Küçükmenderes River, Canakkale - Turkey (Figure 2.) with the permission of Turkish Ministry of Agriculture and Forestry under the approval statement of E-67852565-140.03.03-232544, Ankara – Turkey.

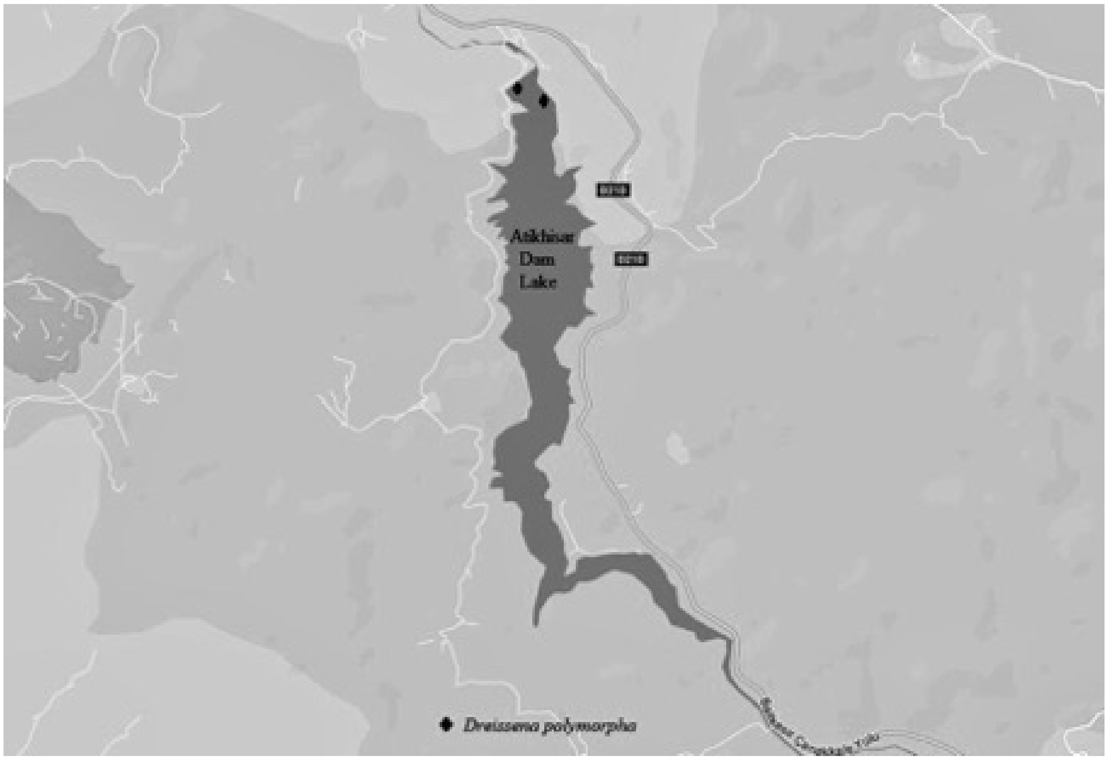

Figure 1. *Dreissena polymorpha*, natural habitat and sampling areas from Atikhisar Dam Lake, Canakkale-Turkey

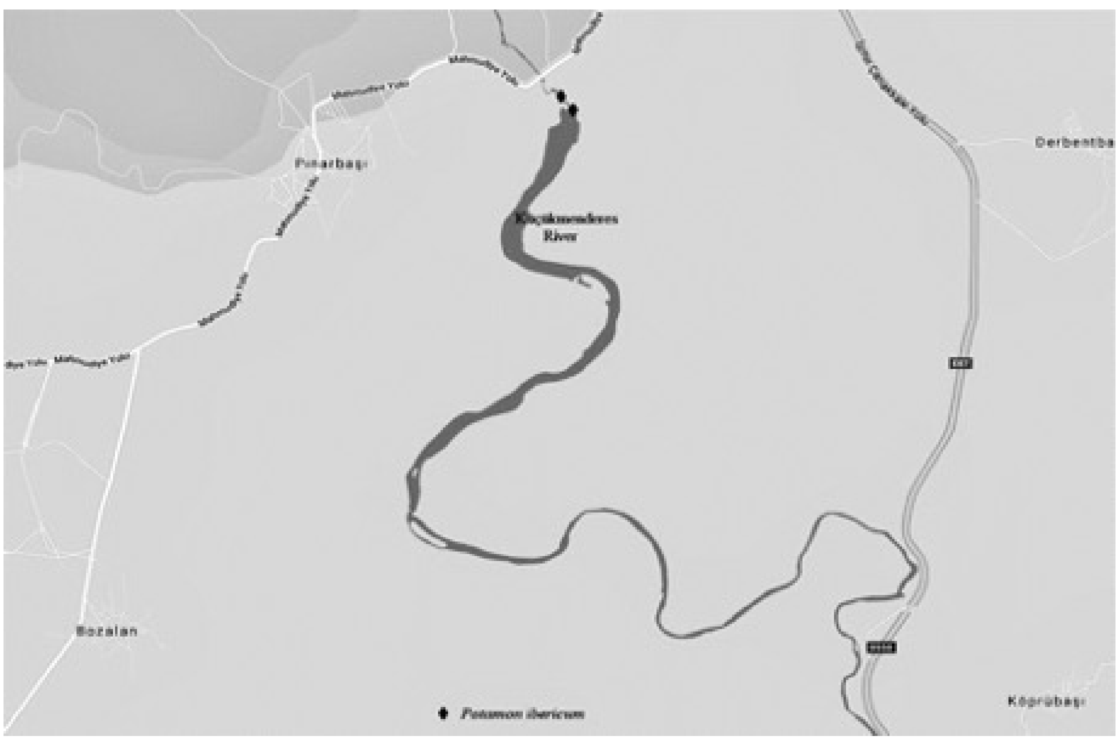

Figure 2. *Potamon ibericum*, natural habitat and sampling areas from Küçükmenderes River, Canakkale-Turkey

Experimental Conditions and Set Up

The study was carried out in the research facilities of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology (Canakkale-Turkey). A recirculating aquaculture system (RAS) consisting of glass aquariums (70 x 40 x 40 cm) supported with well-aerated freshwater with a flow rate of 30.1±0.4 L/min was used. A total of 9 predator crab (*Potamon ibericum*) (3 crab per tank) was randomly distributed into 3 glass aquariums in a triplicate design. Photoperiod followed a natural course throughout the monitoring study of 90 days.

Water quality parameters of temperature, dissolved oxygen, ammonia, nitrite, nitrate, and pH have been recorded biweekly using an automatic water quality measuring device (YSI brand), and resulted in 19.04 ±1.21 °C, 7.2 ±1.2 mg/L, 0.01 ±0.001 mg/L NH_3, 0.023±0.011 mg/L, 2.18 ±0.17 mg/L, and 7.2, respectively.

The crab was fed with zebra mussel until satiation over the study course of 90 days, and live weight increase was determined via weighing in 30 day intervals (3 weighing in total). The number of consumed mussel was counted every 3 days. The weight of consumed mussels was determined by subtraction the number of remaining mussels the next day from the total of 100 that was introduced in each aquarium the previous day. This course of count for the remaining mussels was followed with 3 days of intervals over the course of 90-days study. At the end of the study, consumption rates of mussels and weight gain of crabs were recorded.

Statistical Analyses

Data presented in this study have been expressed as means ± SD. After meeting homogeneity and normal distribution of data, Tukey Multiple Range Test was applied in order to evaluate growth performance. SPSS 19 (IBMM SPSS Statistics 19) Statistical Software was used. Critical limits of significance were set at P<0.05.

Results and Discussion

Growth trend of *P. ibericum* given zebra mussels over the 90-days experimental period is summarized in Figure 3. It was observed and monitored that *P. ibericum* consumed *D. polymorpha* by breaking their shell. The average number of broken shells divided into pieces by *P. ibericum* is shown in Figure 4.

Result of the study showed no differences in growth performance of experimental groups at any sampling time, and each group consumed similar amounts of mussels. Weight gain and mussel consumption of the *P. ibericum* are given in Table 1.

P. ibericum consuming zebra mussels through breaking their shells is shown in Figure 5. *Small pieces of* shells of *D. polymorpha* broken by *P. ibericum* are shown in Figure 6.

The findings of the present study showed clear evidence that *P. ibericum* can be used as a biologic agent to fight for the biological control of *D. polymorpha*, and eradicate the expansion of zebra mussel in a freshwater ecosystem, that in terms may improve the biodiversity quality of the freshwater reservoirs as well as providing economic benefits through reducing expenses to combat with zebra mussel invasion, locally or worldwide.

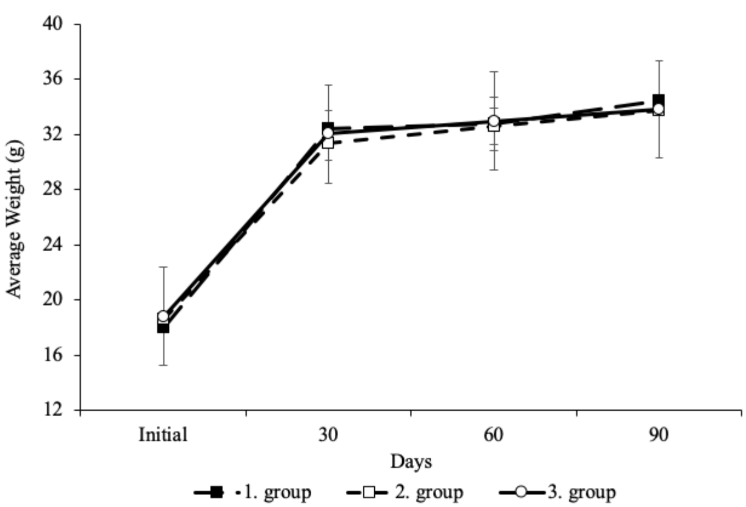

Figure 3. Growth performance of *Potamon ibericum* fed *Dreissena polymorpha*. Values are mean ± SD of 3 *Potamon ibericum*; different superscript letters in a row indicate significant differences between groups (P<0.05).

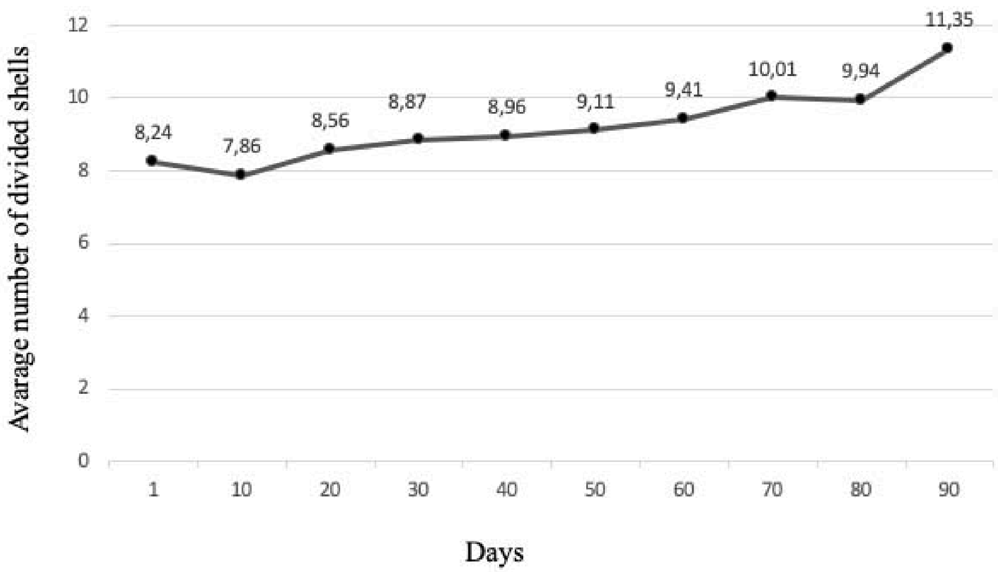

Figure 4. View of broken shells of *Dreissena polymorpha* divided into pieces by *Potamon ibericum* (Average number for 1 *D. polymorpha*). Values are in average counts

Table 1. Growth performance of *Potamon ibericum* fed on *Dreissena polymorpha* during 90 days.

Groups	WG (%)	SGR (%/days)	Mussel Consumption (counts)	
			CPC per crab	TC total
1	11.83±2.1	0.11±0.01	11,115	3001±1.8
2	10.76±1.94	0.11±0.01	11,104	2998±1.9
3	10.63±1.85	0.11±0.01	11,107	2999±1.2

CPC: mussel consumption per crab
TC: total consumption of mussels in experimental treatment group
WG (Weight gain, %) = ((final weight - initial weight) / initial weight)) x 100
SGR (Specific growth rate, %/day) = ((ln final weight – ln initial weight) / days)) x 10

P. ibericum consuming zebra mussels through breaking their shells is shown in Figure 5. *Small pieces of* shells of *D. polymorpha* broken by *P. ibericum* are shown in Figure 6.

The findings of the present study showed clear evidence that *P. ibericum* can be used as a biologic agent to fight for the biological control of *D. polymorpha*, and eradicate the expansion of zebra mussel in a freshwater ecosystem, that in terms may improve the biodiversity quality of the freshwater reservoirs as well as providing economic benefits through reducing expenses to combat with zebra mussel invasion, locally or worldwide.

Several studies have been made for the control of *D. polymorpha* expansion in freshwater reservoirs (Singer et al., 1997). Different than earlier works, the present study focused on the potential use of *P. ibericum* to combat zebra mussel expansion, and furthermore our findings provide useful information for feeding habit and growth performance of *P. ibericum* fed by *D. polymorpha*, novel information for crab culture and future investigations. Different than the present study, Singer et al. (1997) used Gramicidin S and D, and strains of Bacillus (*B. alvei, B. brevis, B. circulans, B. laterosporus*) for biological control of the *D. polymorpha*. The mussel is sensitive against the antibiotics Gramicidin S, Gramicidin D and some Bacillus strains in different life stages as well. On the mussel veliger stages, this agent was notably effective. However, using this type of the antibiotics in a natural reservoir might be hazardous in long term applications. In contrast, the use of *P. ibericum* in the biological combat against *D. polymorpha*, however is an environment-friendly approach even in long term with no hazardous influences on the aquatic ecosystem. Special care is advised to consider the biological diversity of the fresh water reservoir when introducing crab, since site specific impacts can be encountered in special locations. However, the availability and distribution of *P. ibericum* in the near-by vicinity could be a good sign and indication for a rather harmless introduction of these crabs into the selected target pitch.

Figure 5. *Potamon ibericum* feeding on zebra mussel *Dreissena polymorpha*

Figure 6. Consumed *Dreissena polymorpha* at the end of the day-3 with shells shredded in the containment.

Considering the solution methods of zebra mussels' damages, several methods have been reported, namely, cleaning by high pressure water spraying, application of low frequency electromagnetism, heat applications, high flow rate, the use of repellent building materials, biological control agents, mechanical cleaning, the use of chemicals and various dyes are some methods that could be applied (Sprecher and Getsinger 2000; Bobat et al., 2004; Aksu and Yıldız, 2017). Earlier reports underlined that all mussel larvae are retained with a 40 µm filter application (DSİ, 2012).

It was detected that application of 8-methyl-Nvanillyl-trans-6-nonenamide, N-vanillylnonanamide, and N-benzoylmonoethanolamine benzoate had an antifouling effect of up to 90% for Zebra mussels (Angarano et al., 2007). Furthermore, it was reported that encapsulated biocide-loaded particles led to death of more than 90% of Zebra mussels (Costa et al., 2011). Similarly, application of potassium chloride, poly-DADMAC, niclosamide ethanolamine salt and 2- (thiocyanomethylthio) benzothiazole (TCMTB) showed 60% -90% molluscicid effect in Zebra mussels (Costa et al., 2011; Costa et al., 2012). Waller and Bartsch (2018) applied carbon dioxide as a pressure-free infusion of 1000-3000 µatm PCO2 to get zebra mussels under control, and reported that 100% mortality was achieved after 96 hours. In a recent study, steam and flame applications carried out for the control of zebra mussels achieved 100% mussel death (Coughlan et al., 2020). However, there is still remarkable concern and question mark for the technical, economic or environmental applicability of these above-mentioned methods. These earlier investigated methods are still not in application so far, besides in small locations for testing.

Conclusion

Inland water crabs are ecologically significant in global scale (Dobson et al., 2007). Not only in terms of their ecological importance but also in terms of medicinal usage (Rinaudo, 2006), in addition, as a potential food source also, crabs are very critical (Padghane et al., 2016). The study results indicated that crabs could consume the mussel which is very hazardous for the environment, and at the same time increasing growth performance of crabs, which resulted positively in two ways; namely (a) crabs could consume huge numbers of mussels and prevent mussel expansion in the freshwater environment, (b) crab could be harvested from the freshwater habitat where they have been introduced to consume the mussels and thereafter be used as medicinal compounds and human consumption as a food source.

Based on our knowledge so far, there is no any study on the biological control of *D. polymorpha* by using *P. ibericum* as a biological agent. This is the first attempt for an environment-friendly biological control method for the combat against *D. polymorpha*, an invasive species threatening freshwater reservoirs all around the world. In conclusion the findings of this study provide significant evidence for the successful use of *P. ibericum* to fight against invasion and eradicate the expansion of Zebra mussel *D. polymorpha* in freshwaters around the world. Further, the harvest of crabs fed and enriched with zebra mussels in freshwater reservoirs, may provide a good source high quality food for the increasing world population. Thus, the nutritional value of *P. ibericum* as a potential for human consumption is encouraged to be evaluated in future investigations.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Canakkale Onsekiz Mart University Ethical Commission Approval Number: 2021/03-04.

Funding disclosure: -

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Disclosure: -

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