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# IMPACT OF SUBSTRATUM TYPE ON ATTACHMENT AND SURVIVAL OF *DREISSENA POLYMORPHA* (BIVALVIA)

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Abstract: We studied the attachment strength of *Dreissena polymorpha* on nine artificial substrata in the laboratory. The highest attachment strength (0.46 N) was found on resocart (phenoplast plastic). It was lower on aluminium, acrylic, PVC, rubber and glass (listed in the order of decreasing strength), and the lowest on zinc, Penaten cream coating and copper. Apart from reducing adhesion, copper substratum caused also heavy mortality of the mussels. Further experiments, in which mussels were exposed on resocart surfaces in the presence of the examined materials (thus being influenced only by waterborne substances released by them), revealed that zinc and copper inhibited mussel attachment primarily by means of ions released to the water column. In the case of Penaten coating, the impact of its surface properties upon mussels seemed to be more important than waterborne cues.

Key words: zebra mussel, adhesion, material, artificial substratum, mortality

## INTRODUCTION

The zebra mussel is a freshwater, invasive bivalve, attaching to hard substrata by byssal threads. Due to large densities reached under favourable conditions (Stańczykowska 1977) and high filtration efficiency (Reeders et al. 1989, Stańczykowska & Lewandowski 1993), its impact on environment is considerable (e.g. Piesik 1983, Karatayev 1994, Effler et al. 1996). It also fouls underwater devices, increasing costs of their maintenance and therefore is regarded as a nuisance (O'Neill 1997). Zebra mussels can settle on a variety of hard substrata, but preferences for certain materials (e.g. Walz 1973, Lewandowski 1982, Marsden & Lansky 2000, Kobak 2004) and shapes (Czarnołęski et al. 2004, Kobak 2005) have been found. Survival and good physiological condition of a byssate mussel is strongly influenced by its capability of creating firm bonds with a substratum. In marine bivalves, it provides protection against predators (Reimer & Harmsringdahl 1997) and dislodgement (Bell & Gosline 1997). Detached mussels are also more vulnerable to toxins (Rajagopal et al. 2005). That is why the attachment status is crucial for mussels, which try to attach also in unsuitable conditions (Clarke 1999).

Many factors influence zebra mussel attachment strength, including exposure time, shell size, light, temperature and conspecifics (Kobak in press). Another factor that could potentially affect their adhesion is substratum type. Knowledge of susceptibility of various substrata to zebra mussel attachment would help protect underwater devices from fouling (Gross 1994, Meyer et al. 1994) and, on the other hand, make use of these mussels in early warning systems (Jenner et al. 1989) or in biomanipulation (Szlauer & Szlauer 1997, Mählmann et al. 2004).

Our aim was to investigate the attachment strength of zebra mussel on several artificial materials, which could be introduced, either deliberately or by chance, to aquatic ecosystems. We hypothesised that potential differences among substrata would be brought about by their surface properties and/or substances released to the water column. We also intended to compare the mussel attachment strength with their recruitment on the same substratum types, studied previously (Kobak 2004).

## METHODS

The mussels were collected by a diver from the dam of the Włocławek Dam Reservoir (the Vistula River, central Poland) in October 2002 and kept in a 500-L aquarium filled with settled, aerated tap water. The mean shell length of the tested mussels was 13.4 mm (SD: 1.46 mm, range: 10.1–19.4 mm).

The experiments began in November 2002 and lasted until February 2003. They were conducted in 10-L tanks (bottom: 240 × 240 mm, water level: 170 mm), in settled, aerated tap water, at room temperature (ca. 18°C). The tanks were covered with dark, opaque foil to reduce access of light, which is known to modify mussel behaviour (KOBAK 2001). The mussels were tested on 100 × 100 × 5 mm plates made of the following materials: (1) resocart (phenoplast, a thermosetting plastic based on phenol-formaldehyde resin with paper as a filler), (2) methyl polyacrylate (perspex = Plexiglas, acrylic), (3) polyvinyl chloride (PVC), (4) rubber, (5) glass, (6) aluminium, (7) zinc, (8) copper, (9) resocart coated with Penaten® (Johnson & Johnson), a baby-bottom cream known for its antifouling properties (MAGEE et al. 1997). The plates were roughened with sandpaper (except for Penaten coating). To prevent the mussels from leaving their substrata, the plates were put together into boxes made of the floor and the four walls joined by rubber bands and covered with 1-mm mesh nylon netting (Fig. 1).

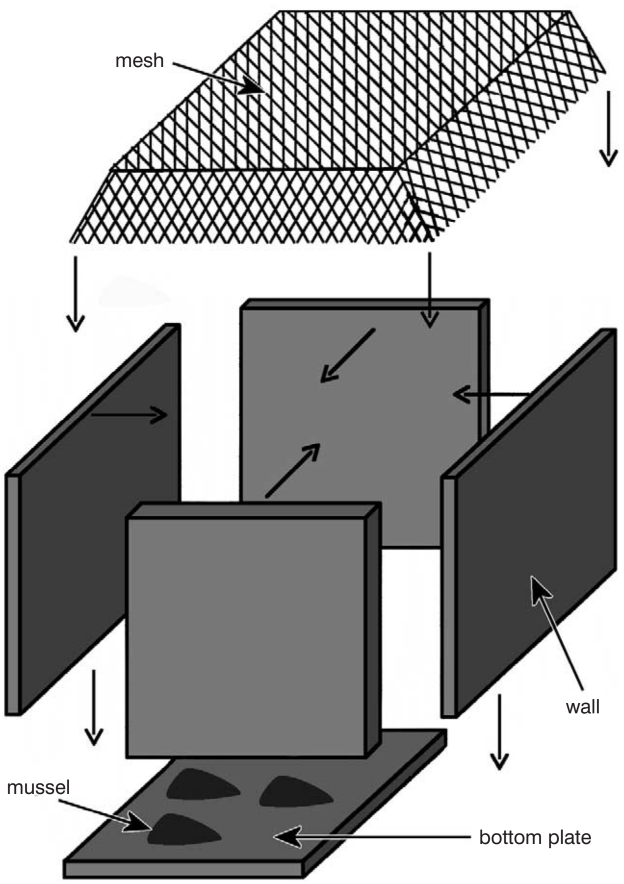


Fig. 1. Box used to study the attachment strength of mussels

Twelve mussels were put onto the bottom plate of each box, lying on the tank floor. After seven days, the boxes were removed from the water, disassembled and the mussel attachment strength was measured according to the procedure described below. Dead mussels (unable to close their valves) in each box were also counted. Since the mussel attachment strength on vertical and horizontal surfaces is similar (KOBAK in press), the individuals found on the box walls were also analysed. Those mussels that were too crowded to access them with the measuring device, or attached to conspecific shells, were excluded from the analysis. The experiment was replicated four times.

To check the mussel responses to the waterborne substances released by the studied materials, we carried out another experiment, in which mussels were tested in the boxes consisting of the resocart bottom (previously found to be a suitable substratum: KOBAK 2004 and the other results of this study) and the walls made of the examined materials. This experiment was carried out according to the procedure described above. Only the individuals attached to the resocart bottom plates of the boxes were analysed.

### MEASURING THE ATTACHMENT STRENGTH

To measure the mussel attachment strength we have modified the method of KOBAK et al. (2002). We used a device based on pan scales (Fig. 2). One pan of the scales was replaced with forceps holding the attached mussel. The other pan was gradually loaded with fine sand (at the rate of 1.26 g $s^{-1}$) until the mus-

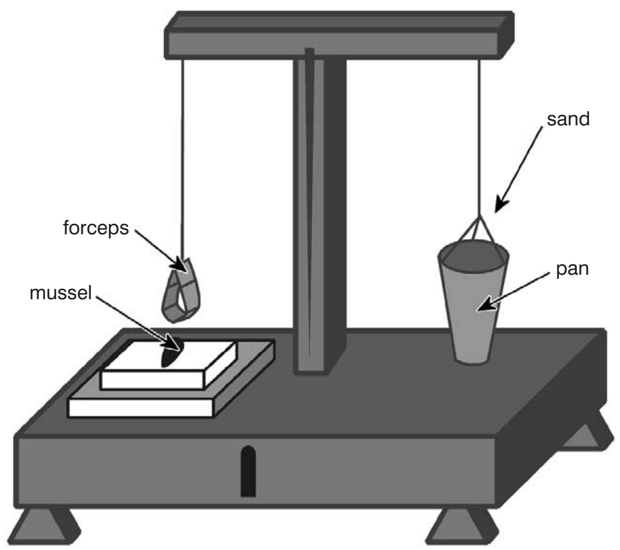


Fig. 2. Device for measuring the attachment strength of mussels

sel was detached. At this moment, the attachment force, the mussel weight and the forceps weight on one side were overbalanced by the weight of the sand and the pan on the other side. The attachment force was calculated from the following formula:

$$AF = (M_s + M_p - M_m - M_F) \times g$$

where: AF – attachment force (N), $M_s$ – mass of the sand (kg), $M_p$ – mass of the pan (kg), $M_m$ – wet mass of the mussel (kg), $M_F$ – mass of the forceps (kg), g – acceleration of the free fall ($g \approx 9.81$ m $s^{-2}$). All the masses were determined to the nearest $10^{-5}$ kg.

STATISTICAL ANALYSIS

The data were logarithmically transformed ($y = \ln(x + 1)$) to reduce the violation of the homoscedasticity and normalcy assumptions. The mussel attachment strength on various substrata was analysed by two-factor ANOVA (fixed factor: substratum type, random factor: replicate), followed by Tukey test. The same method was applied to analyse the attachment in the boxes consisting of the resocart bottom and the walls made of the examined materials. The mussel mortality in both experiments was analysed using single-factor ANOVA-s followed by Tukey tests.

## RESULTS

The substratum type significantly influenced the mussel attachment strength (ANOVA: $F_{8, 24} = 13.71$, $p < 0.0001$). The highest attachment was found on resocart (Fig. 3). It differed significantly from the adhesion on all other materials except aluminium. The attachment strength on aluminium, acrylic, PVC, rubber and glass was higher than on zinc, Penaten and copper. Actually, mussels never attached to the last two substrata.

The presence of the studied materials modified the mussel attachment to resocart plates (ANOVA: $F_{8, 24} = 6.59$, $p = 0.0001$). In this case, zinc and copper still inhibited mussel adhesion, although some individuals did attach to resocart in their presence. No differences in attachment strength were found between the control, uniform resocart boxes and the boxes with PVC, acrylic and glass walls (Fig. 4). The attachment strength of the mussels tested in the presence of Penaten coating, although still lower than in the uniform resocart boxes, did not differ significantly from that in all the other treatments. The interaction between the material type and replicate in this

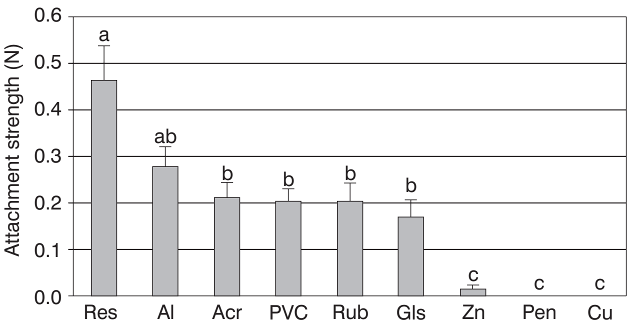


Fig. 3. Attachment strength of mussels on the examined materials. Error bars indicate standard errors of means. The items labelled with the same letter above the bar did not differ significantly from one another (Tukey test). Res – resocart, Al – aluminium, Acr – acrylic, PVC – polyvinyl chloride, Rub – rubber, Gls – glass, Zn – zinc, Pen – Penaten, Cu – copper

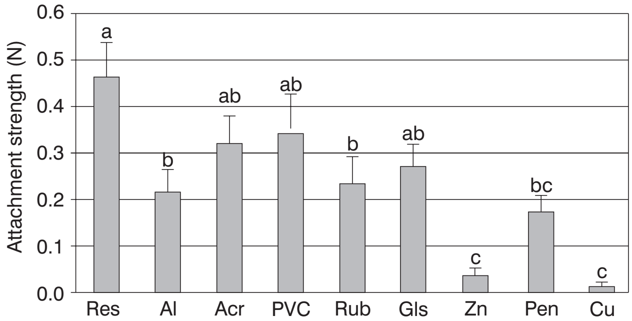


Fig. 4. Attachment strength of mussels on resocart plates surrounded by the examined materials. For lettering see Fig. 3

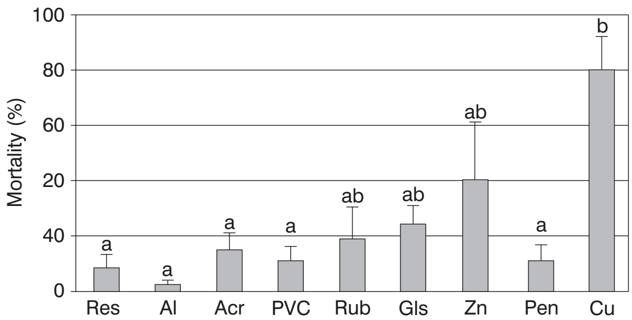


Fig. 5. Mussel mortality on the examined materials. For lettering see Fig. 3

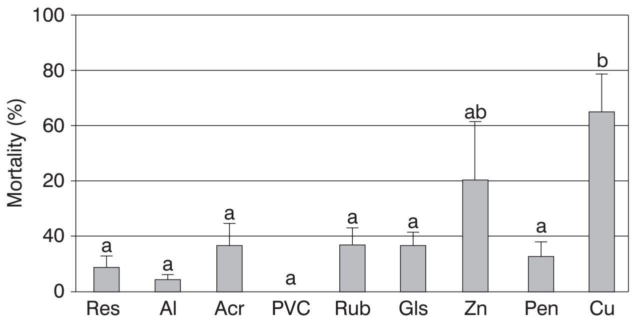


Fig. 6. Mussel mortality on resocart plates surrounded by the examined materials. For lettering see Fig. 3

analysis was also significant ($F_{24,357} = 2.00$, $p = 0.0039$), showing that the mussel responses to the applied treatments differed among replicates.

Dead mussels occurred on all materials, but survival depended significantly on the substratum type (ANOVA: $F_{8,\ 27} = 3.84$, $p = 0.0040$ for the mussels tested in thse uniform boxes and $F_{8,\ 27} = 4.23$, $p = 0.0022$ for the individuals tested in the boxes consisting of resocart plates surrounded by the studied materials). The mussel mortality was very high in the uniform copper boxes (79%) and in the presence of copper (65%). It differed significantly from the mortality noted on most of the other substrata (Fig. 5 and 6, respectively). The mortality of mussels on zinc was also considerable, but it did not differ significantly from that found on the other materials, due to a very high variability in this treatment: in one replicate the mortality on zinc was nearly 100%, while in the others it was comparable to the remaining materials.

## DISCUSSION

In general, the mussel attachment strength in our study was comparatively low. Even on a suitable resocart substratum it was less than 0.5 N (Fig. 3), while other studies often report values above 1.0 N (e.g. HUBERTZ 1994, ACKERMAN et al. 1995, KOBAK et al. 2002, KOBAK in press). It suggests a poor physiological condition of the studied mussels, which was further confirmed by their relatively high mortality, observed also on those materials that are known as suitable (e.g. resocart, PVC, acrylic). Nevertheless, the significant differences among the mussel responses to the examined substrata can be used to evaluate their suitability for mussels.

The highest attachment strength was observed on resocart. This result is consistent with high densities of zebra mussel recruits found on this substratum in the field (KOBAK 2004). On other plastic materials (PVC and acrylic), as well as on glass, the attachment strength was significantly lower (Fig. 3). On the contrary, settling mussels did not discriminate among the above materials in the field (KOBAK 2004). This discrepancy can be accounted for by different age of the involved mussels and/or differences between the field and laboratory conditions. In the field, any waterborne substances released by the substrata would be immediately diluted and removed by the water flow (rather strong due to the turbines operating at the Włocławek Hydropower Plant, where the above-mentioned field experiment took place), while in a closed laboratory tank they would remain in the water. ACKERMAN et al. (1995, 1996), studying the attachment strength of dreissenid mussels (*D. polymorpha* and *D. bugensis*) on various artificial substrata, found stronger adhesion on PVC compared to aluminium or acrylic. WALZ (1973) and MARSDEN & LANSKY (2002) observed a difference between recruit abundance on PVC and acrylic settlement plates (in favour of the former). We did not detect any significant differences among these three materials, although the attachment strength on aluminium was slightly higher (contrary to the other materials, it did not differ significantly from the adhesion on resocart). Perhaps, the impact of PVC upon mussels depends on various supplementary substances and fillers added during the production process and may vary among various kinds of this plastic.

The attachment strength on rubber was lower than on the most suitable materials, although it differed significantly only from the adhesion on resocart. In the field recruitment study (KOBAK 2004), differences between rubber and other substrata (including resocart, PVC, aluminium, acrylic and glass) were much stronger: recruit density on rubber was considerably lower than on the above-mentioned materials.

The attachment on zinc, copper and Penaten coating (containing zinc oxide) was the weakest among the studied materials. This is compatible with a number of studies demonstrating toxicity of zinc and, especially, copper ions and compounds (e.g. DUDNIKOV & MIKHEEV 1964, KRAAK et al. 1994, COTTRELL et al. 2000). These metals are often used in various anti-fouling measures, such as coatings applied to susceptible underwater devices and constructions (RACE & MILLER 1992, GROSS 1994, DORMON et al. 1996). Our study confirmed high toxicity of copper, as most of the mussels tested on this substratum or in its presence did not survive (Figs 5 and 6). The mortality on zinc, although it also seemed to be higher than on the other substrata, was not significantly different. Thus, we were able to demonstrate only sublethal effects of this metal. These results agree with the difference in the toxicity between copper and zinc. Copper (in the form of electrolytically obtained ions in concentration of 4 mg $l^{-1}$) causes 100% mortality of zebra mussels within 24 hours at the temperature of 20°C, while the exposure of mussels to zinc ions in the same conditions results in only 5% mortality (DUDNIKOV & MIKHEEV 1964). The mortality of mussels on Penaten coating, which was another substratum inhibiting the attachment in our study, was similar to that on the most suitable materials. MAGEE et al. (1997) have also shown that quagga mussels (*D. bugensis*) survived exposure to this coating, but did not attach to it. On the other hand, they noted a high mortality of veligers exposed to the Penaten leachate. Probably, earlier developmental stages are more vulnerable to toxic compounds, due to their smaller size and the lack of a hard shell.

The observed differences among the studied substrata could be caused by waterborne substances released to the water (e.g. toxic ions) and/or by their surface properties (GROSS 1994, MEYER et al. 1994). The results of our second experiment, with the mussels tested on the resocart plates surrounded by various materials, allowed for discrimination between these two potential factors. Some of the differences between resocart and the other materials, observed in the experiment involving the uniform boxes (Fig. 3), disappeared (Fig. 4: resocart vs. PVC, acrylic and glass), suggesting that the lower attachment strength on these materials resulted from their surface properties. For instance, sanding, which was applied to all substrata, did not affect glass to the same extent as the other plates. It is known that mussels prefer rough rather than smooth surfaces (ACKERMAN et al. 1996, MARSDEN & LANSKY 2000) and this may be the reason for their lower attachment strength on glass, compared to resocart. The mussels tested in the presence of Penaten were attached much more firmly than those exposed directly on this coating, although their attachment strength was still lower than on clean resocart (Figs 3 and 4). Probably, mussels were unable to attach to a Penaten-coated surface due to its slippery nature and/or chemical composition, preventing formation of firm chemical bonds with it. Thus, this material influenced mussels mainly through its surface properties (inhibiting their attachment but not survival), but the impact of leachate cannot be unambiguously excluded.

Zinc and copper strongly influenced also those mussels that had no direct contact with them, acting by means of the toxic ions released to the water. In the previous study, copper was found to affect zebra mussels through both surface properties (when ions were eliminated with EDTA) and toxic ions (KOBAK et al. 2002). The former factor caused only attachment inhibition, and the latter reduced both attachment and survival.

The attachment of mussels to resocart in the presence of rubber was also lower than to resocart alone, suggesting that some waterborne substances released by rubber negatively influenced the mussels. However, this effect was much weaker and less clear than that of the toxic metals.

It is difficult to account for the weaker mussel attachment to resocart plates surrounded by aluminium walls (Fig. 4), compared to their adhesion to uniform aluminium boxes (Fig. 3). Thus, further research is needed to fully explain the impact of this metal upon mussels. Furthermore, one should note that in our second experiment the interaction between the substratum type and replicate was significant, indicating that the mussel responses were somewhat different in the consecutive replicates. It suggests an impact of some uncontrolled factors on the results of this part of the study, which should be treated with particular care.

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