



IMPACT OF COPPER SUBSTRATE ON SURVIVAL, MOBILITY AND ATTACHMENT STRENGTH OF ADULT *DREISSENA POLYMORPHA* (PALL.)

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ABSTRACT: Zebra mussels were tested for their mobility and strength of attachment in six different experimental combinations. They were kept for 7 days on copper or plastic (phenoplast) plates in 4-L beakers put on the bottom, or on plastic (resocart) plates suspended 15 mm above copper ones; water used was pure or with addition of a metal ion chelator – disodium versenate (EDTA). Exposure to copper ions resulted in the highest mortality. When the ions were EDTA-removed, the animals tended to leave copper plates and stay on the glass bottom or walls of the beakers. Attachment strength was measured with a device based on pan scales, with one of the pans replaced by forceps grasping a mussel. It was significantly lower in the presence of copper ions or with metallic substrate. Both the release of ions and the surface properties of copper substrate were found to influence zebra mussels, the former factor increasing their mortality, the latter causing sublethal responses. The observations may be useful in designing fouling-resistant devices and systems.

KEY WORDS: *Dreissena polymorpha*, copper, survival, locomotion, attachment strength,

INTRODUCTION

Dreissena polymorpha (Pallas, 1771), the zebra mussel, is an invasive bivalve, living in fresh and brackish waters of Europe and North America (LEWANDOWSKI 2001). Due to its gregarious life style (density over 100 thousand individuals per metre square, WIKTOR 1963) and high filtration rate (STAŃCZYKOWSKA 1977, KARATAYEV 1994), the zebra mussel exerts a considerable impact upon both economy (fouling hydro-technical devices, O'NEILL 1997) and environment (changing sedimentation rate (DOBSON & MACKIE 1998), chemical parameters of water and sediments (EFFLER et al. 1996), substrate heterogeneity (BOTTS et al. 1996), etc.). Means of control are necessary in sites where it could cause serious damage.

Many control methods, including biological, physical and chemical ones, have been proposed (e.g. CLAUDI 1995, BOELMAN et al. 1997). Among chemical methods, besides commonly used chlorination (MALLEN et al. 1997, RAJAGOPAL et al. 2002), a very

important part is played by heavy metals, especially copper and zinc, used to prevent mussel settlement and kill already settled individuals (RACE & MILLER 1992a, b). The metals are applied as solid surfaces (e.g. pipes exposed to fouling) (RACE 1992), mesh protecting various substrates (DORMON et al. 1996) or components of anti-fouling paints and coatings (as salts, oxides or metallic powder) (MILLER 1992, RACE & MILLER 1992a, WALLER et al. 1993). There is a strong evidence that settling mussels avoid such substrates (e.g. WALZ 1973, LEWANDOWSKI 1982, RACE & MILLER 1992a, DORMON et al. 1996).

It is commonly believed that the reason for the unsuitability of these substrates for mussels is the release of toxic metal ions (e.g. DUDNIKOV & MIKHEYEV 1964, COTTRELL et al. 2000). However, it is an open question whether surface properties of copper are also important in preventing mussel settlement. Such features as surface free energy (MEYER et al. 1994, HYDE

et al. 1997), substrate wettability (DOBRETSOV & RAILKIN 1996) or roughness (ACKERMAN et al. 1996, MARSDEN & LANSKY 2000) can influence settlement of various fouling species. Differentiation between the toxic ion effects and surface properties is especially important in environments periodically experiencing fast water flow, which may remove ions from the vicinity of the substrate, but cannot change its surface properties. Such conditions are especially characteristic for hydropower plants, where turbines generate strong, temporary water currents. In our studies on the zebra mussel settlement in the hydropower facilities in Włocławek (the Włocławek Dam Reservoir on the Vistula River, Poland), we found that the density

of individuals attached to copper, though lower than on the other substrates, was still quite high (ca. 5 thousand per metre square) (KOBAK & WIŚNIEWSKI 1998). Also HANSON & MOCCO (1994) observed high densities of zebra mussels on copper plates in Lake Michigan (as much as 51 thousand per metre square). Investigations of various aspects of copper influence on the mussels could be helpful in explaining their occasional high densities observed on this metal despite its apparent toxicity.

This study is an attempt to separate the effect of released ions from surface properties of copper substrate and to investigate their influence on adult zebra mussels.

MATERIAL AND METHODS

MATERIAL

The mussels used in the study were collected in the Włocławek Dam Reservoir (the Vistula River, Poland) near Dobięgniewo, from ca. 2 m depth, in October 1996. They were transported to the laboratory and kept in a 400-L aerated aquarium filled with settled tap water, at room temperature (15–18°C).

EXPERIMENTAL DESIGN

10 × 10 × 0.5 cm square plates made of copper or resocart (phenoplast plastic material used in electro-technical industry) were placed in glass beakers (diameter: 20 cm, height: 40 cm) filled with 4 litres of settled (24 h) tap water. The plates were arranged in the six following combinations: (1) copper plate; (2) copper plate with added metal ion chelator (disodium versenate, EDTA, 0.5 g/l); (3) resocart plate; (4) resocart plate with addition of EDTA; (5) resocart plate suspended 15 mm above a copper plate; (6) the same as (5), but with addition of EDTA. The plates were placed on the bottom of the beakers if not indicated otherwise. In our previous studies, resocart was found to be a good substrate for the zebra mussel settlement and subsequent growth (KOBAK & WIŚNIEWSKI 1998, and unpublished data). In the first combination both the surface properties of copper plates and the released ions influenced the mussels. The two factors were separated in combinations 2 (ions eliminated by EDTA) and 5 (resocart substrate, copper ions present in water). Combination 4 was to check possible effects of EDTA itself on the mussels, while combination 6 served as a check if EDTA was able to eliminate the effect of copper ions completely. If so, the presence of a copper plate should then have no effect on mussels (no differences compared to combination 4 should be found).

The mussels (> 15 mm shell length) were removed from the aquarium, their byssal threads were cut off

and the shells cleaned of silt and remnants of other individuals' byssus. They were then randomly divided into 6 groups of 10 individuals each, and placed on the plates in the beakers. The mean shell length of the mussels used in the experiment was 21.34 mm (SD = 2.46).

The experiment was carried out at room temperature (17 ± 1.2°C) and lasted for 7 days. Dead mussels, recognised by unnaturally wide valve gaping and failure to close when touched with a probe, were removed from the beakers. At the end of the experiment, attachment strength of mussels was measured as described below. Besides, dead individuals and those which migrated from a plate to the glass bottom or walls of the beaker (indicator of mobility), were counted. All the mussels were then measured with callipers to the nearest 0.1 mm.

The experiment was repeated three times, in December 1996 and January 1997.

MEASUREMENT OF ATTACHMENT STRENGTH

A device used to measure attachment strength of mussels was based on pan scales (Fig. 1). One pan of the scales was replaced by forceps used to grasp an attached mussel. The other pan was gradually loaded with weights (lead shot pellets, about 0.016 g each) until the mussel was detached. At this moment the attachment force, the animal's weight and the forceps weight were balanced by the weight of the shot pellets and the pan. The attachment force was calculated from the following formula:

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

where: AF – attachment force (N), M_w – mass of the weights (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 ($g \approx 9.81 \text{ m/s}^2$)

Mass of each component of the system (mussel, pan, forceps and shot pellets) was determined to the nearest 10^{-5} kg.

STATISTICAL ANALYSIS

The data were statistically analysed with STATISTICA 5.0 for Windows (STATSOFT INC. 1995).

Differences in attachment strength of mussels were tested with single-factor analysis of variance (ANOVA), followed by a posteriori Tukey's test for unequal sample sizes (SOKAL & ROHLF 1995). Square root transformation of data was used to meet the assumptions of homogeneity of variances and normality of data. The assumptions were checked with Bartlett test and Kolmogorov-Smirnov one-sample test, respectively (STATSOFT INC. 1995). Combination 1 (copper substrate without EDTA) was excluded from the analysis, because all individuals had died before the end of the experiment. The results were regarded as statistically significant at $p < 0.05$.

Due to strong violations of the ANOVA assumptions, which could not be removed by any data transformations, differences in the mortality and mobility of mussels in various combinations were tested with non-parametric Kruskal-Wallis ANOVA. When its results were statistically significant, pairwise Mann-Whitney U tests (SOKAL & ROHLF 1995) were used to detect differences between particular combinations. Combinations 5 and 6 were not included in the mobility analysis, because emigration of mussels tested on

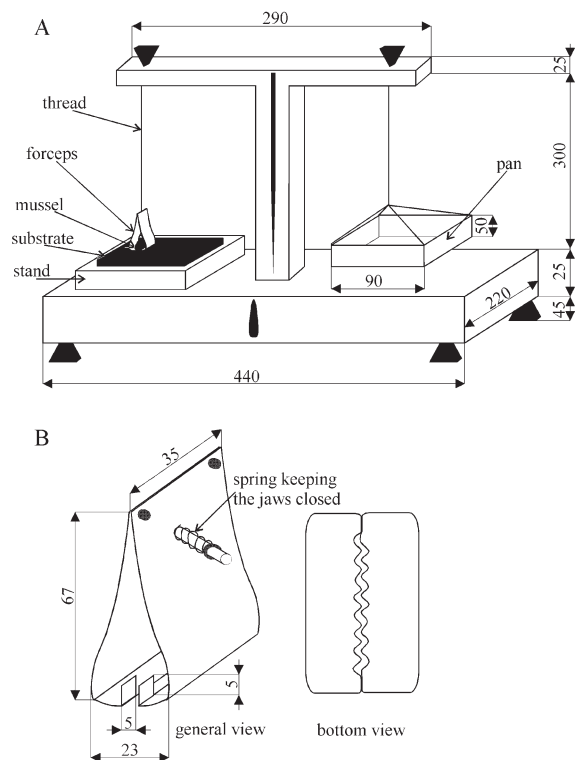


Fig. 1. Apparatus used to measure attachment strength of mussels. The dimensions are given in mm. A. Entire device. B. Forceps used to grasp a mussel

plates suspended in water and laying on the bottom could not be compared.

RESULTS

The number of surviving mussels differed significantly between the experimental combinations (Kruskal-Wallis test: $H = 14.87$, $df = 5$, $p = 0.011$). All the individuals tested on copper plates without EDTA (combination 1) died before the end of the experiment. In another combination, in which the mussels were exposed only to copper ions (combination 5), the mortality was also significantly higher than in other combinations (43.33%), though not as high as in the former one. When copper ions were eliminated from the water column by EDTA (combinations 2 and 6), the mortality was either low or none (Fig. 2).

Copper substrate, even not releasing any ions to water, seemed to be unfavourable for the mussels – in combination 2 (copper plates with EDTA) the number of individuals leaving the plates (80 %) was significantly higher than in all other combinations (Kruskal-Wallis test: $H = 9.88$, $df = 3$, $p = 0.020$). This response was observed only after removing copper ions from water – in their presence mussels did not try to find a better attachment site (Fig. 3).

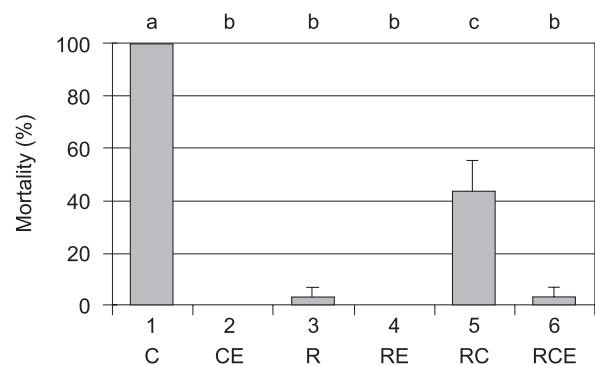


Fig. 2. Mortality of mussels in various experimental combinations. $N = 3$, error bars represent standard errors of means. Combinations with the same letters above the bars were not significantly different from each other. C – combination 1 (copper), CE – combination 2 (copper+EDTA), R – combination 3 (rescart), RE – combination 4 (rescart+EDTA), RC – combination 5 (rescart+copper), RCE – combination 6 (rescart+copper+EDTA)

Attachment strength of the mussels on favourable substrates (combinations 3, 4, 6) was approximately equal to 1 N. Both the presence of copper ions (combination 5) and surface properties of copper plates (combination 2) negatively influenced attachment strength of the mussels (ANOVA: $F_{4,83} = 15.78$, $p <$

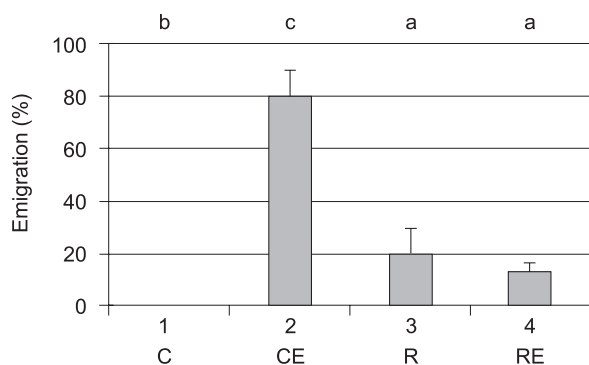


Fig. 3. Emigration of mussels from plates in various experimental combinations. N = 3. For lettering see Fig. 2

DISCUSSION

Substrate quality is one of the most important factors influencing mussel survival after settlement. Although zebra mussels are not very selective with regard to substrate type, they do prefer some materials, e.g. phenoplasts (KOBAK & WIŚNIEWSKI 1998), PVC (MARSDEN & LANSKY 2000) or conspecific shells (WAINMAN et al. 1996), and avoid others, like copper, zinc, brass or galvanized steel (WALZ 1973, LEWANDOWSKI 1982, KOBAK & WIŚNIEWSKI 1998, MARSDEN & LANSKY 2000). This selectivity may have several reasons: substances released from substrates to the water (heavy metal ions, organic particles, pheromones), surface properties of materials (hydrophobicity, roughness) and/or development of different types of biofilm on various substrates (GU et al. 1997). The above factors can influence mussels either through their toxicity (e.g. heavy metals, DUDNIKOV & MIKHEYEV 1964, COTTRELL et al. 2000) or through impeding attachment of byssal threads to the surface (e.g. low surface energy compounds, like silicone-based coatings, MEYER et al. 1994). All those factors are utilized in control of the zebra mussels and preventing their settlement. The use of heavy metals, especially copper and zinc, is one of the most popular anti-fouling methods (other metals, like mercury or cadmium, are unacceptable because of their strong negative environmental effects). According to DUDNIKOV & MIKHEYEV (1964), copper ions (electrolytically obtained) in concentration of 4 mg/l cause 100% mortality of zebra mussels within 24 hours at temperature of 20°C. On the other hand, copper sulphate turned out to be much less toxic – more than

0.001), reducing it to 0.23 and 0.15 N, respectively. These two combinations differed significantly from all the remaining ones (Fig. 4).

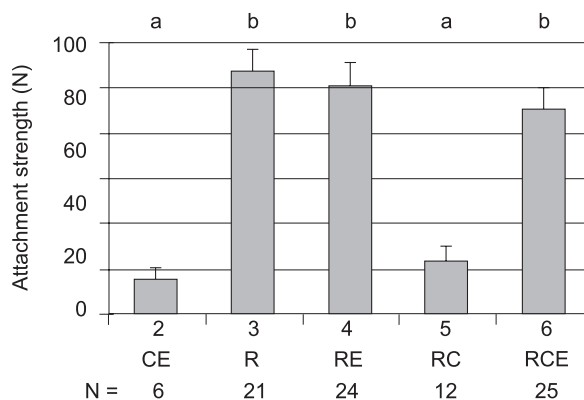


Fig. 4. Attachment strength of mussels in various experimental combinations. N (below the chart) – numbers of individuals measured in each combination. For lettering see Fig. 2

8 g/l was necessary to obtain 100 % mortality of mussels (LUKANIN 1964). This suggests that also the source of the ions influences the mussel response to contamination. In sublethal concentrations copper ions can alter mussel filtration rate (KRAAK et al. 1994, 1999), influence their metabolism (CHEN 1995) and development (AKBERALI et al. 1985). Mussels are also known to accumulate heavy metals in their tissues (GUNDACKER 1999, JOHNS 2001), which leads to further, chronic effects of these contaminants (KRAAK et al. 1993).

If the release of ions were the only mechanism through which copper substrate affects the mussels, it could lose its anti-fouling properties in some conditions, for example in places with periodically strong water currents. Some results cited in the introduction seem to corroborate this hypothesis (HANSON & MOCCO 1994, KOBAK & WIŚNIEWSKI 1998). The results of the present study confirmed toxic effects of copper ions on the zebra mussel (Fig. 2). Besides, their presence reduced attachment strength of the surviving animals (Fig. 4). Furthermore, copper substrate was avoided by the mussels also when the ions were eliminated from the water column by EDTA (Fig. 3). In such a situation, tested individuals preferred glass walls of the experimental beakers, and those which eventually stayed on the copper plates, were only weakly attached to the substrate. Thus, the surface properties of copper also influenced the mussels. A similar behaviour was observed by MAGEE et al. (1997), who studied an impact of Penaten® cream coating (Johnson and Johnson Co.) on quagga mus-



sels (*Dreissena bugensis* Andrusov). They were not able to attach to the surface covered with cream, but readily attached to each other's shells, indicating that their response was caused by physical properties of the coating surface.

Theoretically, it could be possible that the chelating compound used in the study was unable to remove all copper ions from the water column, and the observed effects were caused by sublethal doses of ions remaining in the solution rather than by surface properties of the substrate. If this was true, the ions would have been also present in combination 6 (resocart plate suspended above a copper one with addition of EDTA), making it different from combination 4 (resocart plate with EDTA). No such differences were observed (Figs 2, 4), which shows that the chelator in the concentration used in the experiment eliminated completely the influence of ions on the mussels. Furthermore, assuming the highest copper ions leaching rate found in literature (about 4 µg/cm²/day, RACE & KELLY 1994, KELLY 1998), chelator concentration used in the study was sufficient to remove all ions from the solution. Another difficulty in interpreting the results could be caused by the fact that EDTA chelates, apart from copper, also other bivalent ions, e.g. calcium, which could additionally change the experimental environment. Actually, chelating compounds were found to influence positively attachment of another bivalve species – *Mytilus edulis* L. (ETOH et al. 1996). The problem was solved by comparing combinations 3 (resocart plate) and 4 (resocart plate with EDTA). The lack of differences between them (Figs 2–4) shows that mussel responses were not changed by the chelator itself (i.e. without its interference with copper).

Attachment strength of mussels not exposed to copper ions or copper surface was ca. 1 N (Fig. 4, combinations 3, 4 and 6), being comparable with that

measured by ACKERMAN et al. (1995) for individuals settled in the field on natural substrates (limestone and dolomite rocks: 1.4 N), and higher than on any artificial substrate tested by those authors (PVC, perspex, stainless steel, aluminum). It was also similar to values obtained for *Mytilus edulis* by REIMER & TEDENGREN (1997) after 100 hours of their experiment. It supports previous findings (KOBAK & WIŚNIEWSKI 1998) that resocart is a good substrate for zebra mussel development and its use as a control material in this study was appropriate.

The zebra mussel failure to attach to copper plates even after removing ions from the water column could be caused by inhibition of byssal adhesion mechanisms, like formation of hydrogen bonds and complexes with surface metals by catechol groups of amino acid components of the byssal threads (JENSEN & MORSE 1988, DEMING 1999).

The results of our study indicate that neutralization of ions strongly reduces toxic effects of copper substrate upon mussels, but its repellent properties remain. Some evidence exists that mussels successfully colonise surfaces immediately adjacent to copper coatings (MILLER 1992, KOBAK unpublished observations). It suggests that a factor influencing their site selection is different than toxicity of ions. However, surface properties of copper substrate may change with time, when its surface becomes overgrown by biofilm, facilitating settlement of various organisms (WAINMAN et al. 1996, GU et al. 1997, HAMER et al. 2001). Furthermore, algae covering the substrate may be able to complex copper and remove it from water (MILLER 1992). Then, when toxic ions are eliminated, successful colonisation of such unfavourable substrates may occur. These effects were not studied in a short-term, laboratory experiment and need further investigations.

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