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RELOCATION, ATTACHMENT AND SURVIVAL OF ZEBRA MUSSELS (*Dreissena polymorpha*) ON ALUMINUM, CHROME, IRON, GALVANIZED-IRON, ZINC, COPPER AND COPPER-ALLOY SUBSTRATES

Harun ARDALI[1], Halit KUSKU[2*], Murat YIGIT[2]

[1]Department of Aquaculture, School of Graduate Studies, Canakkale Onsekiz Mart University, Turkey
[2] Department of Marine Technology Engineering, Faculty of Marine Science and Technology, Canakkale Onsekiz Mart University, Canakkale - Turkey*

Harun Ardali: harunardali@hotmail.com; ORCID ID: 0000-0002-0652-9795
Halit Kusku: halit@comu.edu.tr, ORCID ID: 0000-0003-4109-2370
Murat Yigit: muratyigit@comu.edu.tr, ORCID ID: 0000-0001-8086-9125

**Corresponding author: Halit KUSKU, halit@comu.edu.tr, +90-542-4833797*

Abstract
The attachment strength, survival and relocation of zebra mussels (*Dreissena polymorpha*) on eight different substrates (glass, Al, Cr, Fe, galvanized-Fe, Cu, Zn, Cu-alloy) were measured. Glass substrate attracted zebra mussels, however, other materials tested were less attractive and nearly 50% of settled individuals lost attachment on Fe and Al substrates, and about 70% on Cr and galvanized Fe (gal-Fe) during the time of the experiment. The adhesion strength of mussels on different substrates decreased in the order of glass>Fe>Al>Cr>gal-Fe>Cu=Zn=Cu-alloy, with negative correlation between relocation behavior that showed an increase in the order of glass>gal-Fe>Cr>Al>Fe. All mussels were dislodged on Cu, Cu-alloy and Zn substrates by day-28. Survival of zebra mussels followed a trend with attachment strength with rates from high to low in the order of glass>Al>Fe>gal-Fe>Cr>Cu=Zn=Cu-alloy. Findings from this study provide evidence that the surface properties relevant for the adhesive conditions are influenced by the materials, and substrates made of Cu, Zn, and Cu-alloy performed best against zebra mussel adhesion, which eventually could be applied as lining material or through sheet replacement in dam lakes or hydroelectric power plants in the effort against zebra mussel infestation on underwater structures.

Keywords: Attachment, relocation, survival, zebra mussel, material selection, substrate type

Introduction

The zebra mussel (*D. polymorpha*) is an invasive species spreading in freshwater ecosystems around the world (Pollux et al., 2003; Aldridge et al., 2004), and became a significant problem through their over-seas transport by their attachment on ship hulls (Pollux et al., 2003). Zebra mussels can attach on rocks, water plants, shells of other native bivalves, and a wide range of several substances such as plastic, fiberglass, wood, concrete, iron surfaces, or even surface structures coated with traditional paints (Boelman et al., 1997), that in turn cause severe environmental consequences such as rapid colonization on new areas in the aquatic ecosystem (Nalepa et al., 2014), shifts in food web and its dynamics (Strayer et al., 1998; Vanderploeg et al., 2002; Zhu et al., 2006; Madenjian et al., 2015), decline of native bivalves (Schloesser et al., 1996; Strayer, 1999; Martel et al., 2001), changes in feeding ecology and trophic conditions of endemic fish species (Colvin et al., 2015; Smircich et al., 2017), with remarkable economic impacts (Lovell et al., 2006; Robinson et al., 2013). Due to their rapid colonization and expansion abilities to wide area, zebra mussel populations strongly influence the functioning of ecosystems and various underwater vehicles (O'Neill, 1997; Lewandowski, 2001; Karatayev et al., 2002). Along with their colonization consequences from accumulation of dead shells in underground pipes and channels are also significant concerns of zebra mussel invasion (Kilgour and Mackie, 1993). Their strong adhesion and expansion ability even in poor-quality water conditions with less food (Clarke, 1999), or even in chemically contaminated water systems (Rajagopal et al., 2002, 2005) makes is a difficult challenge of struggle against zebra mussel invasion. It is likely that the attachment power of zebra mussels responds differently to a variety of environmental factors (Rajagopal et al., 2002, 2005), with severe harmful effects on the ecosystem (Kobak et al., 2009). Several control measures for the fight against zebra mussel settlements are in use nowadays with chemical treatments using chlorine and other molluscacides, physical treatments via heating, ultrasonic vibration, electric current, variable water flow, or biological treatments such as predation, mechanical treatment with filters or scrapping, as well as sheet replacement withing pipes and channels (Mackie et al., 1989). In an earlier investigation, Walz (1973) reported a tendency of zebra mussel settlement on several materials in the order of copper < copper alloy < Plexiglas < concrete < aluminum < iron < PVC. Also, it has been reported that the attachment strength of zebra mussels can be reduced with using polystyrene instead of PVC (Van Diepen and Davids, 1986), however, the authors also underlined that polystyrene can lose its effectiveness over a certain time. Kilgour and Mackie (1993) provided useful indications regarding effective use of materials made of aluminum, copper and zinc to reduce zebra mussel settlement and mitigate colonization in pipes and tubes.

The present study investigated settlement and adhesion strength, survival, relocation on surfaces of several materials including aluminum, chrome, copper, copper-alloy, iron, galvanized iron, and zinc in order to find best-effective materials for supporting combat efforts through sheet replacement applications in dam lakes for drink water supply or hydroelectric power plants.

Material and Method

Sampling and experimental conditions

A total of 3000 Zebra mussels (*D. polymorpha*) used in this study randomly collected from three locations of Atikhisar Dam Lake (40^{O}06'08.45" N – 26^{O}31'26.83" E; Canakkale-Turkey) within a location between surface and 60 cm depth (Figure 1). All samples were wet-transferred to the Freshwater Research Facility of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology, and stocked in 100 L volume, rectangular shaped glass aquariums of 70 x 40 x 40 cm dimensions (Figure 2).

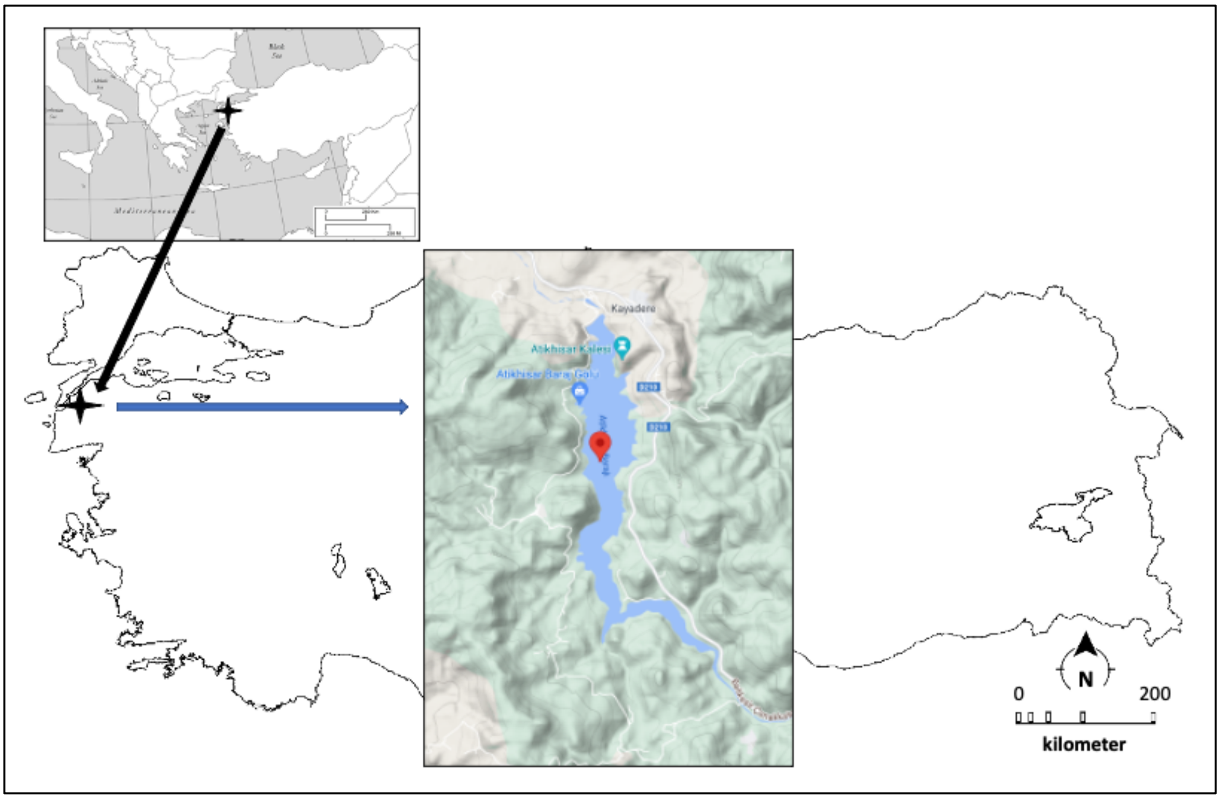

Figure 1. Sampling area of zebra mussels (Atikhisar Dam Lake, 40°06'08.45" N - 26°31'26.83" E; Canakkale-Turkey)

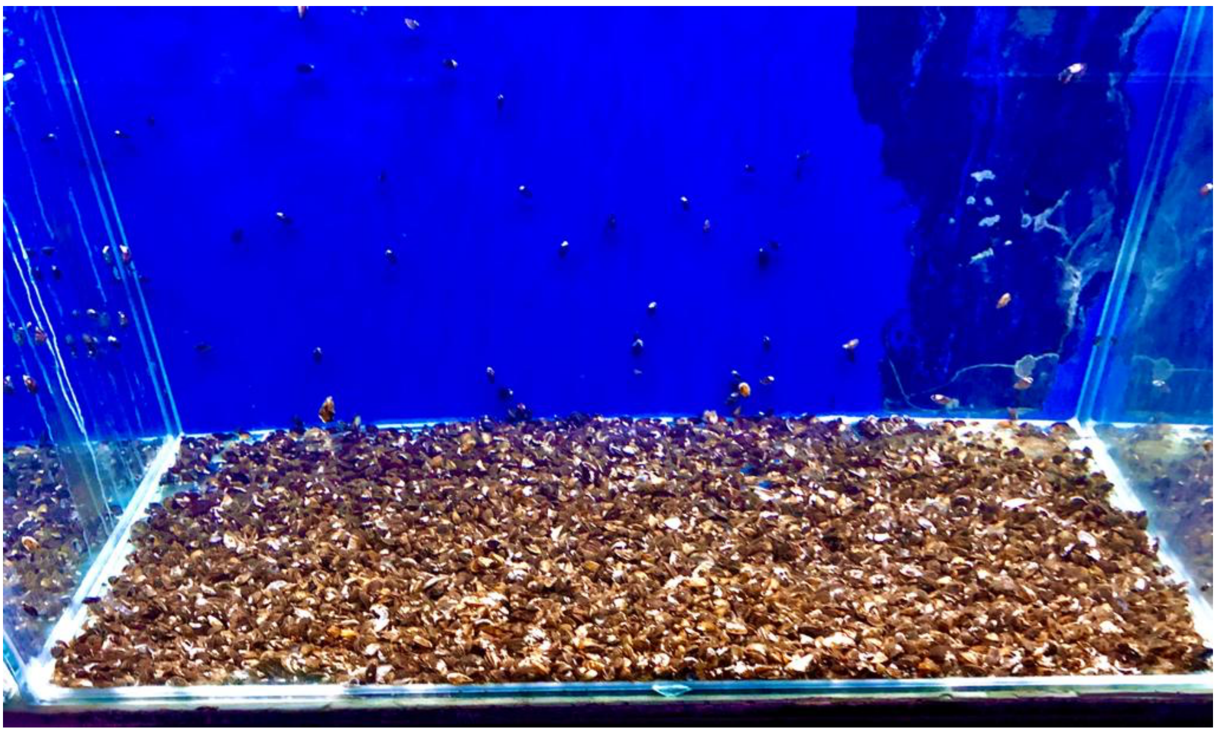

Figure 2. Stock batch of zebra mussels transferred from Atikhisar Dam Lake

Water quality parameters such as temperature, dissolved oxygen, ammonia, and pH in the experimental facility were recorded once a week using an automatic water quality measuring device (YSI brand). Oxygen concentration in water was measured as 8.3±0.2 mg/L (range: 8.0–8.5 mg/L), whereas temperature, pH, NH_3-N were recorded as 19.2±0.4 °C (18.5–19.5°C), 8.0±0.2 (7.8–8.2), and 0.02±0.002 mg/L, respectively. The conductivity was measured as 543±12 µS/cm with a range between 525 and 551 µS/cm over the course of the study period of 28 days.

In the research facility, the experimental system was set with eight-independent recirculating systems consisting of 3 aquariums in triplicate. Each of the aquariums were supplied with equal freshwater inflow of 28.5 L/min) and aeration using air stones. Lake water was used in the system, which was replaced with new lake water twice-a-week throughout the study period, in order to have similar water conditions with that of the lake environment, and also to ensure a nutrient medium for zebra mussels with life food available in the lake water, and no additional food was supplied. Additional food offer could potentially affect their attachment strength and motility as earlier described by Clarke (1999), although zebra mussels are known to survive periods of prolonged starvation without loss of tissue mass (Clarke, 1999; Chase and McMahon, 1995).

Figure 3. Experimental set-up of glass aquariums in the recirculating freshwater system.

Experimental substrates with different materials
Seven plates with dimensions of 15 x 25 cm and a thickness of 0.2 cm, made of different materials, namely aluminum (Al), chrome (Cr), copper (Cu), copper-alloy (Cu-alloy; 60:40% Cu:Zn), iron (Fe), galvanized iron (gal-Fe), and zinc (Zn) were used in the present as potential materials for in-pipe lining material to be used in shield replacement in pipes and water transmission channels.

For each of the test materials, a total of 6 plates were used and distributed into 6 aquariums as 2 plates per aquarium (3 aquariums per treatment), which then represented 1 treatment group with 3 replicates. The same set-up was made for all seven plates (Al, Cr, Cu, Cu-alloy, Fe, gal-

Fe, Zn). Further, three aquariums were set as a control without any plate. In total, twenty-one aquariums for 7 different materials and 3 for the blank test, consisting of a total of 24 aquariums were used in this study.

After an adaptation period for 5 days in the stock aquarium, 2400 individuals of alive and undamaged zebra mussels with visible byssus threads were gently withdrawn from the stock batch of 3000 mussels, and shell length of mussels were measured using a scale to the nearest 0.1 mm, which was recorded as 9.2±2.7 mm in average with a range between 4.1 and 11.8 mm.

On each of the plates set on the bottom of the aquarium, 50 individuals of zebra mussels were placed with their byssus towards bottom in 10 rows with 5 individuals per row (5 x 2 order). Fifty mussels per plate, one-hundred per aquarium and 2400 individuals of zebra mussels in total were allotted into the test aquariums in a 5 x 2 order on each plate (Figure 4).

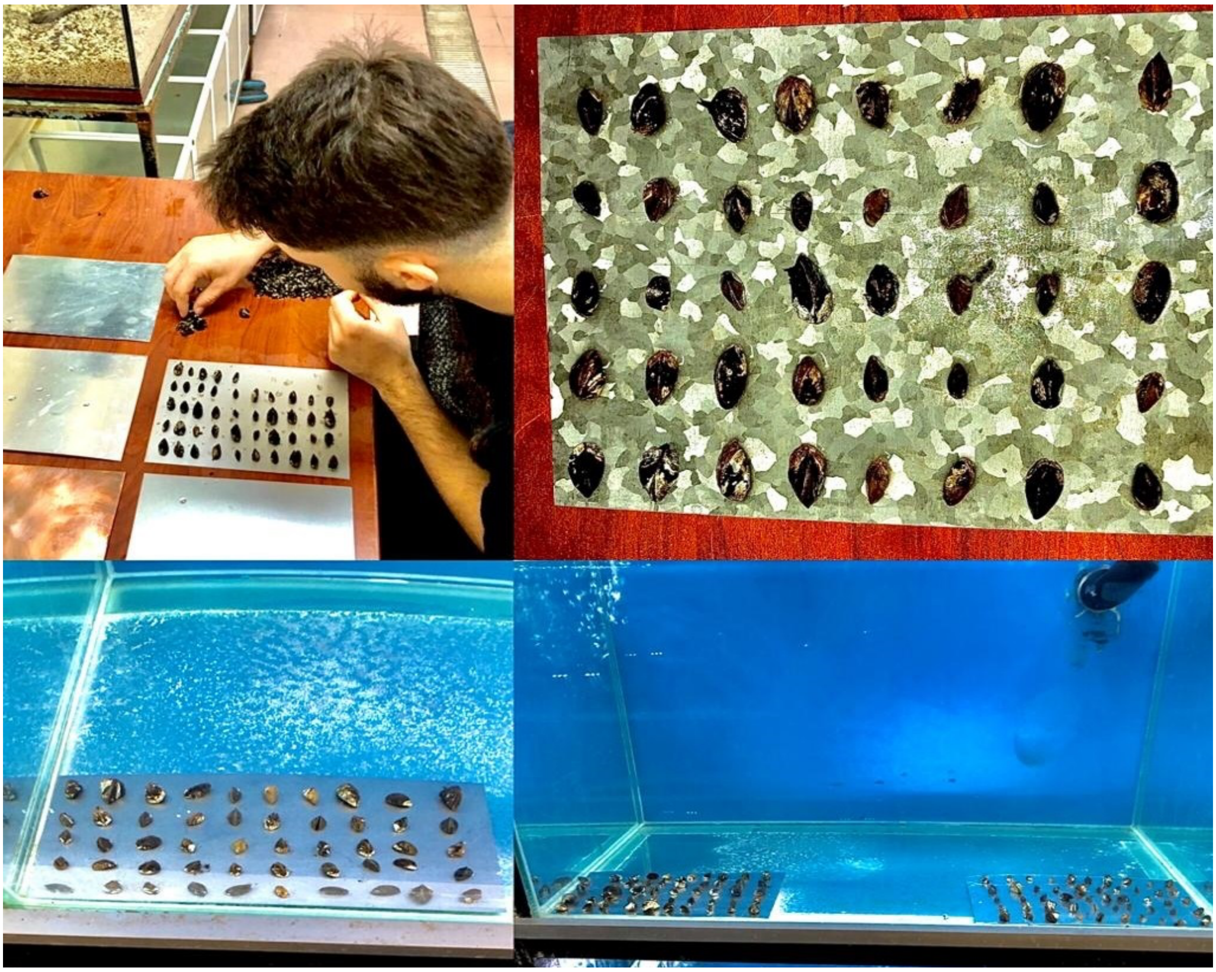

Figure 4. Placement of zebra mussels on different substrates in test aquariums.

Since mussels are known to avoid illuminated areas (Kobak, 2001; Toomey et al., 2002; Kobak & Nowacki, 2007), seeking sheltered environments (Kilgour and Mackie, 1993), it is a possibly that zebra mussels de-attach for selection of such locations of less light. However, unattached zebra mussels were found to move less frequently in light (Kobak & Nowacki, 2007), suggesting that an adverse response to lighting is also possible. Accordingly, considering earlier reports mentioned above, the indoor research facility was isolated from light for 16 hours, with a working hour period of 8 hours. Hence a photoperiod of 8:16 (light : dark) hours has been applied in this study, and all necessary control and experimental procedures were carried out during the light hours from 9:00 am to 17:00 pm throughout the course of 28-days period. An experimental period of 28 days was decided for this study following earlier reports of Chase and McMahon (1995), who provided evidence that zebra mussels can tolerate prolonged starvation with suppressed metabolic rate for over 60 days without severe tissue loss.

Attachment strength and relocation

The displacement of mussels placed on the test plates made of different materials were measured in 24-hour intervals. The point where the mussels were initially placed on the plates were marked. After 24-hours, the distance between the new point of relocation and the initial point of first placement was measured carefully without any disturbance on mussels. Video recording was performed for image tracking of mussel displacements. By the end of a 24-h period, un-attached mussels continuing movement were replaced to their initial locations with their byssus threads towards bottom, however relocated mussels attached on the plate by their byssus thread were not disturbed and remained in its position. The same protocol was performed for the following 24-h course, which was repeated throughout the 28-days of study. By the end of day-28, the total distance in cm for relocation per day for each mussel, and the number of attachments on experimental plates were recorded.

Survival rates

By daily inspections in each aquarium, any dead mussels on plates (either on plates or displaced out of the plate range) were immediately removed to prevent contamination following earlier report of Chase and McMahon (199**5**), and counted individually. The counts of dead mussels were subtracted from the total number of mussels introduced at initial for the estimation of percent survival of zebra mussels placed on different materials, which in turns also gave the number of attachment strength for the mussels in each treatment group after the end of 28-days study period.

Metal analyses

Trace metal analyses were performed at the Science and Technology Application Research Center (COBILTUM) of Canakkale Onsekiz Mart University (Canakkale-Turkey). Metal (Al, Cr, Cu, Fe, Zn) concentrations in water samples prior to start of the study and after 28 days of experimentation have been determined using ICP-OES PerkinElmer OPTİMA 8000, following the method of EPA 200.7. The initial water sample data were used to evaluate possible levels of metals in the lake water obtained from Atikhisar Dam Lake, while the data of final water samples were used for the evaluation of the status and possible leaching levels of these five metals from the plate material submerged in the aquariums for 28 days.

Final water samples were taken from the effluent-pool of the recirculating culture system. Therefore, metal concentrations in the water ambient represent a pool of accumulated metal levels for the Fe, gal-Fe, Cu, Zn, and Cu-alloy plates, as the latter one consists of 60% Cu and 40% Zn. Wavelengths for the ICP-OES Spectrophotometry were 396.153 for Al, 267.717 for Cr, 327.397 for Cu, 238.205 for Fe, and 206.202 for Zn. Prior to the metal analysis, High-Purity Quality Control Standard 27 Cat.# QCS-27 was used for the calibration of the ICP-OES.

Statistical analyses

All data evaluated in the present study are given as means ± SD. In case of homogeneity and normal distribution of data was observed, Tukey Multiple Range Test was used to evaluate relocation, survival, and attachment strength data. If data were normally distributed in case of homogeneity, Kruskal-Wallis test was used, while the Tamhane test was applied for data with no homogeneity, using SPSS 19 (IBMM SPSS Statistics 19) Statistical Software. Critical limits of significance were set at P<0.05.

Results

Survival rates

No mortalities were observed in zebra mussels placed on glass surface which showed 100% survival by the end of day-28. Among other experimental substrate groups, mussels placed on Al substrate showed highest survival rate of 94.67±2.52%, followed by the individuals placed on Fe (91.33±2.08%) and gal-Fe plates (62±1.73%). No significance was found in mussel survival on Al and Fe plates (*P>0.05*), while gal-Fe group presented significantly lower (*P<0.05*) survival compared to the Al and Fe surface groups. Lowest survival (52.67±3.06%) was recorded in mussels placed on Cr-plates, which was significantly lower (*P<0.05*) than the remaining test groups. All of the zebra mussels placed on Cu, Cu-alloy and Zn plates died (100% mortality) by the end of day-28 with 0% survival as shown in Figure 5.

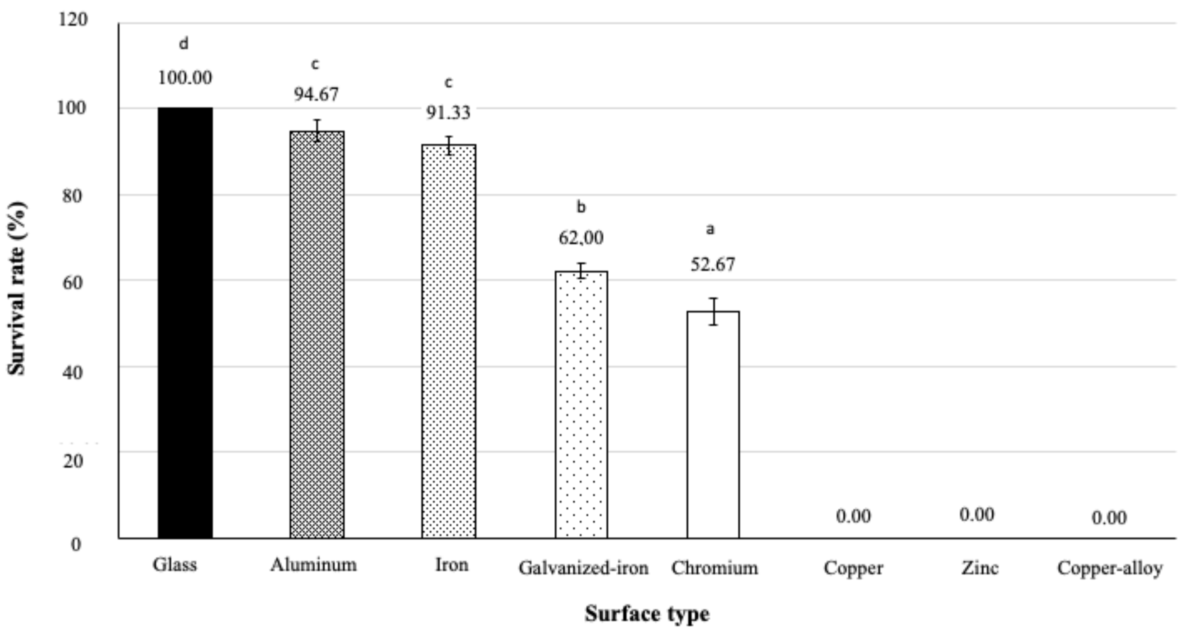

Figure 5. Average survival rates in percent of zebra mussels on different surface materials by day-28. Different superscript letters show significant differences between surface types at 0.05 level.

Attachment rates

By the end of day-28, average attachment rates were recorded as 98.67±1.15, 56.67±1.52, 51.33±3.21, 33.67±2.51, and 25.67±1.15 % on glass surface, Fe, Al, Cr, and gal-Fe surfaces, respectively, with significantly higher (*P<0.05*) on glass material compared to the other experimental substrates. The attachment rates of mussels on Fe, Al, Cr, and gal-Fe plates were also significantly different (*P<0.05*) within the groups. No attachment was observed on any of the plates made of Cu, Zn, and Cu-alloy (60:40%, Cu:Zn) materials as presented in Figure 6. Attachment rates from highest to lowest followed the order of Fe > Al > Cr > gal-Fe > Cu = Zn = Cu-alloy.

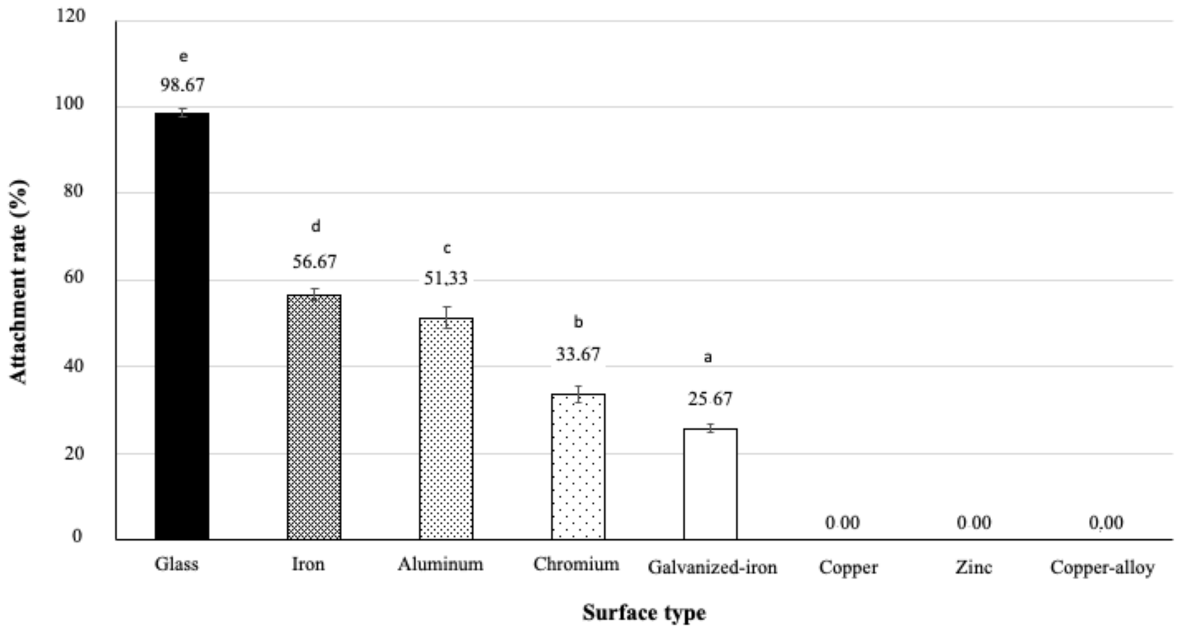

Figure 6. Average attachment rates in percent of zebra mussels on different surface materials by day-28. Different superscript letters show significant differences between surface types at 0.05 level.

Relocation
Based on monitoring through video image tracking, it was observed that zebra mussels showed relocation behavior on surfaces of Al, Cr, gal-Fe, glass, and Fe. However, zebra mussels placed on Cu, Cu-alloy (60:40%, Cu:Zn) and Zn plates did not show any relocation. Video image of the movement behavior and relocation of zebra mussel on gal-Fe plate has been recorded and presented as a video document (Video 1).

Zebra Mussel video 1.mp4

Video 1. Relocation of zebra mussels on galvanized iron substrate (shell length: 11.7 mm) (Kusku, 2022), [Video, https://www.youtube.com/watch?v=doHz6fEoBfI].

No regular relocation was observed in mussels placed on glass, gal-Fe, Al, Cr, and Fe plate surfaces, and the displacements were rather irregular towards different directions (Figure 7). By the end of day-28, the relocation of mussels in cm/day averaged 0.23±0.04, 0.41±0.06, 0.46±0.065, 0.51±0.031, and 0.63±0.042 cm/day on glass surface, Fe, Al, Cr, and gal-Fe surfaces, respectively. No movement was recorded on Cu, Cu-alloy and Zn surfaces (Figure 8). Despite the different relocations observed for the different substrates tested in this study, relocation of mussels on Al, Cr, or Fe plates were not significantly different ($P>0.05$) within the groups, however, relocation of mussels on glass substrate was significantly lower ($P<0.05$) compared to the other substrate groups.

Figure 7. Relocation directions of 50 zebra mussels placed on experimental substrates. Figure illustrates mussel relocation on Cr plate by the end of 24 hours.

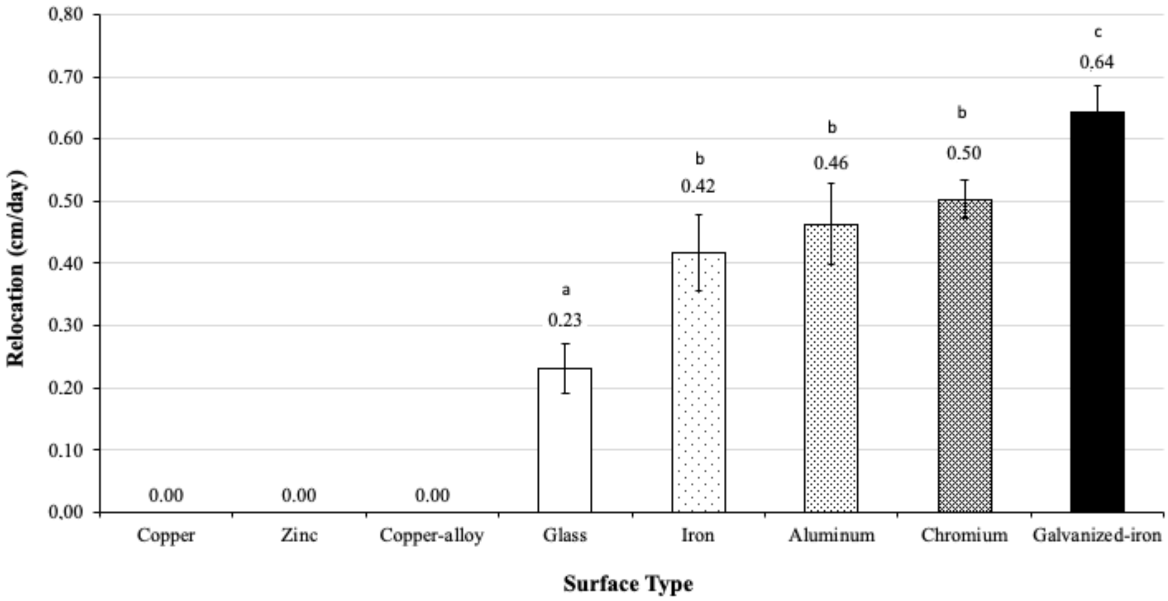

Figure 8. Average daily relocations (cm/day) of zebra mussels on different surface materials by day-28. Different superscript letters show significant differences between surface types at 0.05 level.

Leaching of metals

According to the results obtained from water samples prior to start of the experimentation and by the end of day-28 of the study, Al, Cr, and Cu could not be detected in both initial and final water samples. Zn in the sample taken at the beginning of the experiment was recorded as 3,135 µg/L. Based on metal analyses performed in this study, Fe, Cr, Al, Cu values could not be detected. Prior to the start of the study, no Fe was detected in the water, whereas Fe concentration in water by the end of day-28 increased to 77.6 µg/L. Initial level of Zn in the lake water was found as 3.135 µg/L, whereas increased Zn concentration was recorded as 56.46 µg/L, by the end of the experimentation conducted for 28 days (Table 1).

Table 1. Metal concentrations in water samples at initial and final (day-28) of the study.

Element	Initial Water Sample day-0	Final Water Sample day-28
Al	ND	ND
Cr	ND	ND
Cu	ND	ND
Fe	ND	77.6 µg/L
Zn	3.135 µg/L	56.46 µg/L

ND: not detectable

Discussion

In the present study, all of the zebra mussels placed on Cu, Cu-alloy and Zn plates died showing 100% mortality by the end of the study period of 28 days, whereas zebra mussels placed on other materials such as glass, Al, Fe, gal-Fe, and Cr presented survival rates between 52 and 100%, with highest rates for glass, and lowest for Cr surface. This was in line with the attachment rates of zebra mussels on different materials, namely, the average attachment rates for these materials were found between 25 and 98 %, again with highest rates for the glass media and lowest for Cr surface. Further, no attachment was observed on Cu, Zn, and Cu-alloy (60:40%, Cu:Zn) plates. The results of the present provide evidence that substrate structure is important in the habitat selection of zebra mussels, which is in agreement of findings of Lewandowski (2001), who reported that fates of settled postveligers mainly depend on the type of the substrate. The reason for mussels showing no survival and no attachment in the present study, could be attributed to the antimicrobial properties of Cu and Cu-Zn brass alloy materials as reported earlier for antifouling properties of Cu-alloy mesh nets in cage aquaculture (Yigit et al., 2018a, b, c; 2020). Further, the antimicrobial properties of Cu-alloys were also reported in health care applications by Grass et al. (2011) and Arendsen et al. (2019). This can also explain the inactivity of zebra mussels in the present study when the mussels were placed on Cu, Zn, and Cu-alloy surfaces, which showed no relocation as was observed for the individuals set on other materials such as glass, Al, Fe, gal-Fe, and Cr.

Once the mussels reach the adult phase following the postveliger stage that persists for around ten days, the mussels are attached to the substrate with their byssus threads, however under extreme conditions they can detach the byssus threads and actively relocate for convenient habitat search (Lewandowski, 2001). For instance, Kobak et al. (2009) stated that zebra mussels showed relocation behavior through crawling towards darker locations and displacement frequency changed according to mussel size, where large mussels crawled less often than smaller ones. The authors also underlined that either small or large mussels attached for 1-6 days relocated from their initial positions less frequently than unattached individuals. However, Kobak et al. (2009) reported that the attachment status and relocation behavior in zebra mussels can change according to various factors with different responses to environmental stimuli. Therefore, relocation is an important indication for the evaluation of behavioral features and habitat selection of zebra mussels.

Petersen et al. (2020) indicated that roughness even at the micro scale can impact the fouling release ability of a surface, and barnacles showed longer attachment durations on materials with higher roughness size. Earlier investigations underlined a positive relation between fouling accumulation and the surface characteristics for different barnacle species (*Chthamalus fragilis*, Wethey, 1986; *Chthamalus anisopoma*, Raimondi, 1988; *Semibalanus balanoides*, Hills and Thomason, 1998). Surface roughness has also been reported to influence biofouling dynamics of *Balanus improvises* (Berntsson et al., 2000, 2004) and *Balanus amphritite* (Schumacher et al., 2007; Aldred et al., 2010). Ackerman et al. (1996) reported that the strongest attachment was observed on macroscopically rough natural (rock) and metallic (steel) substrates, whereas lowest attachments were noted on smooth polymeric surfaces. In contrast, Marsden (1992) indicated that zebra mussels did not show remarkable preferences among various materials such as wood, fiberglass, concrete, limestone, aluminum, steel, Plexiglass, glass, or PVC, however, the author underlined that zebra mussels strongly avoided galvanized steel substrates. The finding of Marsden (1992) regarding avoidance of mussels on galvanized steel is in close agreement with the present study, where zebra mussel attachment was lowest (25.67%) and relocation was highest (0.64 cm/day) on galvanized iron substrate. Kobak (2010) reported that the attachment strength on aluminum material increased with time between day-2 and 6 after of exposure, with the higher gain for aluminum compared to phenoplast and PVC materials. In the present study, highest survival of mussels was found on glass surface, followed by aluminum substrate with a rate of 94.67%, which is in line with Kobak (2010) for aluminum media. James et al. (2021) reported consistent attachment of zebra mussels on glass (borosilicate), polyvinyl chloride (PVC), and Polydimethylsiloxane (PDMS) substrates in aquarium conditions, without significant differences among substrate types. The consistent attachment of zebra mussels on glass media reported by James et al. (2021) was in line with the findings in this study for the attachment of mussels on glass surface, which resulted in >98% adhesion. This was also supported by Kobak et al. (2009), who found that zebra mussels strongly attach to glass surfaces. In the present study, significantly higher attachment strength ($P<0.05$) obtained for the zebra mussels place on glass surface (>98%), than the other materials (Fe, >56%; Al, >51%; Cr, >33%; gal-Fe, >25%; Cu, 0%; Cu-alloy, 0%; Zn, 0%), could be attributed to the smooth topography of the glass surface compared to the other materials tested in this study. Although surface roughness, and microstructure of the applied substrate materials were not measured in this study, all substrates of Al, Fe, gal-Fe, Cr, Cu, Cu-alloy, and Zn plates used, were macroscopically smooth, and thus mechanical interlock is not expected to play a significant role in the adhesion of zebra mussels.

The highest attachment strengths on surface materials were recorded in the order of glass > Fe > Al > Cr > gal-Fe, whereas no attachments were observed on Cu, Zn, and Cu-alloy surfaces by the end of the 28-days experimentation in this study. The relocation in cm per day was negatively correlated with the attachment strength, and followed the order of gal-Fe > Cr > Al > Fe > glass substrates. Mussels on substrates with higher attachment rates demonstrated less relocation, an indication that less displacement was needed. The lowest relocation of mussels on glass substrate showed that mussels were most conformed on glass surface compared to the other substrate materials tested in this study.

The deviations of attachment rates on different metallic plates could be related to the chemistry of the substrate surfaces, where leaching or corrosion may have influenced attachment, as reported earlier by Ackerman et al. (1996). It was reported that the leaching of aluminum ions from the surface may have limited the attachment of mussels (Pillai and Ravindran, 1988; Ackerman et al., 1992). Therefore, despite the fact that the second highest survival rate of mussels after glass surface was found for aluminum substrate (>94%) in the present study, and high attachment strength of mussels were found on aluminum substrate by Kobak (2010), this should be considered with care, since aluminum may corrode over long exposure, and the corrosion of aluminum could change its properties, thus possibly resulting in changes of attachment strength of mussels on this material, as also reported earlier by Kobak (2004). Lewandowski (1982) and Walz (1973) reported high rates of attachments on aluminum and PVC materials, with higher preference of mussels on PVC (Walz, 1973). Copper liberates cupric ions (Cu) that act by reducing algal fixation initiating the ecological succession of biological encrustation (Noyce et al., 2007). In the seawater, the Cu metal oxidizes to copper oxide (CuO), which dissociates to release the Cu^+ ion. The toxic mechanism of Cu^+ and Cu^{2+} ions in marine animals (Fisher et al., 1984) is reported to be related with the inhibition of Na^+ and K^+, causing an intracellular ion imbalance, and the enzymatic activity inhibition of carbonic anhydrase, impairing gas exchange and acid-base regulation (Lopes et al., 2011). The results with no mussel attachments observed for the Cu, Zn, and Cu-alloy surfaces in the present study could be explained by the toxic mechanism of Cu^+ and Cu^{2+} ions as addressed earlier by Fisher et al. (1984), and Lopes et al. (2011). Also, Zn and its compounds are known to be toxic to zebra mussels and widely used for the prevention of biofouling (Kraak et al., 1994; Race and Miller, 1992). It was stated that Cu, which is more toxic than Zn (Race and Miller, 1992) prevented attachment of mussels by both surface properties and releasing ions, that caused mortality in mussels (Kobak et al., 2002). These earlier reports support the findings in the present study, where 100% mortality was observed when mussels were placed on Cu, Zn and Cu-alloy substrates, where the latter material consisted of 60% Cu and 40% Zn.

In the present study, water samples by the end of the experimentation were taken from the effluent-pool of the recirculating culture system. Hence, metal concentrations in the water ambient represented a pool of accumulated metals of Fe, gal-Fe, Cu, Zn, and Cu-alloy plates, as the latter one consisted of 60% Cu and 40% Zn. Therefore, the leaching level of Zn in the final water samples could not be distinguished among the Zn and Cu-alloy plates. However, Cu could not be detected in the water samples, and only Zn was found at a level of 56.46 µg/L, that might the amount of Zn leaching from the Zn plate instead of Cu-alloy material that consisted of both Cu and Zn, (60% Cu and 40% Zn), which however needs further clarification in future studies.

Conclusion

Zebra mussels show distinct adhesion characteristics on different materials, and attachment strength strongly depends on substrate type. The adhesion strength of zebra mussel decreased on substrates in the following order of glass > Fe > Al > Cr > gal-Fe > Cu = Zn = Cu-alloy. The relocation of mussels was also dependent on substrate material and showed an increase in the order of glass > gal-Fe > Cr > Al > Fe, whereas zebra mussels on Cu, Cu-alloy and Zn substrates did not show any relocation at all. Survival of zebra mussels were also influenced by the substrate material, with highest rate on glass surface, and lowest on Cr substrate. Mussels placed on Cu, Cu-alloy and Zn materials did not survive by the end of the study for 28 days. This study presents important information for understanding the impacts of different substrates on biofouling dynamics and attachment of zebra mussels, with indications for selecting proper material in underwater structures exposed to zebra mussel adhesion. For instance, sheets made of the best performing materials against mussel adhesion (Cu, Zn, or Cu-alloy), could be used as lining material or sheet replacement in pipes and water weirs of underwater structures of hydroelectric power plants in the effort against zebra mussel infestation. Information on the complex relation between mussel attachment and surface properties may help decision makers in preventive policies against aquatic invasive species such as zebra mussels. Measuring substrate roughness in future studies may provide further explanation and deeper understanding of the results presented in this study.

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Ethical approval
All procedures applied in this study have been approved by the Commission of Ethics at Canakkale Onsekiz Mart University (Turkey) with the Approval Number of 2021/09-01, dated 10 November 2021.

Informed consent
Not available.

Data availability statement
The authors declare that data are available from authors upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing of this study.

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Contribution of authors
Harun Ardali: Conceptualization, Data curation, Formal analysis, Writing original draft
Halit Kusku: Investigation, Methodology, Writing original draft, Software
Murat Yigit: Resources, Supervision, Validation, Visualization, Review, Editing.

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