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Happy together? Avoidance of conspecifics by gregarious mussels

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Abstract

Zebra mussel *Dreissena polymorpha* is a Ponto-Caspian species invasive in Europe and North America, with great environmental impact. It lives byssally attached to hard substrata in large aggregations, which is often explained by its preferences for conspecifics, though direct evidence for such preferences has been rather limited so far. We studied the reactions of zebra mussels to conspecifics, hypothesizing that they may either be attracted to one another or form aggregations only in the absence of alternative attachment sites. In Experiment 1, we tested mussel tendency to detach from existing druses depending on druse size (2–25 individuals) and substratum type (soft: sand; hard: glass). Mussels detached significantly more often on the hard substratum and from larger druses compared to soft substratum and smaller druses, respectively. This indicates that mussels tended to avoid conspecifics at high density, particularly when alternative substratum was available. In Experiment 2, we tested the responses of single mussels to distant (3 or 15 cm) conspecifics (0, 3, 15 individuals per 2.5 l tank) on the sandy substratum. The presence of conspecifics, regardless of their distance and density, resulted in single unattached mussels staying more often in their initial positions. Mussels did not move preferentially towards or away from the conspecifics. Thus, even on unsuitable substratum mussels were not attracted by conspecifics and probably exhibited an avoidance reaction by reducing their movement. This suggests that dense mussel aggregations are formed due to the lack of available alternative attachment sites rather than due to their preferences for conspecifics.

Key words: active detachment, *Dreissena polymorpha*, movement, mussel aggregations, zebra mussel.

The zebra mussel *Dreissena polymorpha* (Pallas, 1771) has been claimed to be one of the worst invasive species in the world (Gallardo 2014; McLaughlan et al. 2014), generating substantial financial and environmental impacts (Pollick 2013; Prescott et al. 2013; Karatayev et al. 1997; Ricciardi et al. 1996) due to its gregariousness (Kelly et al. 2010; MacIsaac et al. 1992). Throughout the majority of their life, zebra mussels are byssally attached to the substratum. However, metamorphosed individuals are capable of active detachment (Eckroat et al. 1993) and relocation to a better substratum (Toomey et al. 2002). This phenomenon may be a visible avoidance reaction to environmental factors such as light intensity, the presence of conspecific alarm cues, the scent of a predator or water quality (Burks et al. 2002; Toomey et al. 2002; Czarnołęski et al. 2010; Kobak and Ryńska 2014). Active movement of metamorphosed mussels undoubtedly increases their chances of survival (Kobak 2013 for a review) and must be considered in studies aiming at an explanation of their distribution and responses to environmental factors.

Studies to date mainly focused on the practical use of *D. polymorpha* as an efficient filter feeder (Stańczykowska and Lewandowski 1993; Elliott et al. 2008; McLaughlan and Aldridge 2013; Binelli et al. 2014) or bioindicator (Borcherding 2006; Kimbrough et al. 2013). Moreover, the biology, ecology, and less often behavior of this species were also tested to create effective methods of preventing and controlling its invasion (Kobak 2013; Collas et al. 2017). Most of these studies assumed that the zebra

mussel was extremely gregarious, forming dense 3-dimensional colonies (Burks et al. 2002) and aggregations of individuals byssally attached to one another, called "druses" (Stańczykowska 1964). It has often been suggested that their formation depends on active preferences of zebra mussels for conspecifics (Wainman et al. 1996; Kobak 2006), but none of the conducted studies clearly demonstrated such preferences.

A few studies have suggested that *D. polymorpha* may not exhibit such a high affinity for conspecifics as had been assumed, preferring alternative hard substrata (Kavouras and Maki 2003; Kobak and Ryńska 2014; Tošenovský and Kobak 2016). Perhaps, excessive density may make mussels actively detach from druses and, if possible, move to a more suitable substratum. Certainly, life in a colony is associated with many advantages: protection against predators, drying or hydrodynamics and suitable conditions for reproduction (Okamura 1986). On the other hand, intraspecific competition for food, space and position in the group is higher in a dense colony (Burks et al. 2002; Gascoigne et al. 2005; Wacker and Von Elert 2008; Cubillo et al. 2012). Unsuitable mussel position in the vertical structure of a colony is associated with the greater risk of becoming overgrown by other individuals (Burks et al. 2002; Wacker and Von Elert 2008). This may entail the distortion of their shells and immobilization of syphon parts (Bertness and Grosholz 1985; Cubillo et al. 2012), which finally causes organ failure and death (Griffiths and Hockey 1987; Burks et al. 2002; Czarnołęski et al. 2003). Furthermore, such a colony experiences poorer interstitial water conditions (oxygen depletion, increased waste concentration) (Burks et al. 2002; Tuchman et al. 2004). Thus, it can be assumed that life in a colony of *D. polymorpha* is a compromise between protection and successful reproduction, on the one hand, and deteriorating environmental conditions resulting from increased density on the other hand.

Apart from mussel preferences, another cause of their aggregations may be the limited availability of hard substratum in the environment, where conspecific shells may become the only choice (Stańczykowska 1964). In addition, some observations suggest that locomotor limitations, which become more conspicuous with increasing body size (Uryu et al. 1996; Toomey et al. 2002), affect the life of a sessile mussel. These limitations, stemming from the anatomy and physiology of mature individuals and the fact that they are fouled by younger conspecifics (Burks et al. 2002; Czarnołęski et al. 2003; Wacker and Von Elert 2008), may be responsible for the process of aggregation forming.

Given this complex picture of mussel aggregation forming, it seems to be a paradox that few previous studies focused on the relations between *D. polymorpha* conspecifics and there is no paper which clearly hypothesizes that the zebra mussel may not prefer conspecifics. Thus, we believe the assumption that *D. polymorpha* prefers other individuals needs not only field-based correlational evidence, but also a solid experimental background. Our aim was the detailed examination of reactions of *D. polymorpha* to conspecifics in order to explain the mechanisms of aggregation forming by mussels. This is the first research where a previous conjecture concerning questionable preferences of the zebra mussel to conspecifics has been investigated.

We hypothesized that mussel aggregations were formed due to their active preferences for conspecifics or because of the absence of alternative attachment sites. In our study, we tested mussel responses to conspecifics in two situations: when a mussel is a part of druse or a singleton. Both these situations occur in the field where mussels are exposed to different environmental factors. Druses may be dragged by hydrodynamic forces to new areas and mussels may detach spontaneously to colonize new surfaces appearing in their vicinity (Lauer and Spacie 2003). Thus, mussels are likely to respond to varying conditions by adjusting their position. In the case of mussel preferences for conspecifics, the percentage of detaching and relocating mussels should be independent of the availability of the alternative hard substratum; otherwise, it should be stimulated by the presence of suitable surfaces. Moreover, for mussels physically separated from conspecifics by hydrodynamic forces or transported with pieces of debris (Lewandowski 2001), conspecific signals are likely to constitute an important cue affecting their behavior and enhancing survival. The attractancy (directional movement or increase in activity) of single mussels in the presence of conspecifics would indicate their preferences for living mussel substratum. Finally, based on the results of previous research (Stańczykowska 1964) and knowledge of conditions in dense colonies (Burks et al. 2002; Tuchman et al. 2004), we expected that with the increasing density of conspecifics in the environment, mussels would reduce or reverse their preferences for conspecifics in both experiments. Altogether, mussel responses in our experiments would indicate whether *D. polymorpha* prefers, tolerates or avoids conspecifics at particular abundances.

Materials and Methods

Mussel collection and stocking before the experiments

Dreissena polymorpha individuals were collected by a diver from the Włocławek Reservoir (a dam lake on the lower Vistula River in the central part of Poland, 52°37′04″N 19°24′28″E). They were taken from unionid clams, heavily fouled by this species. Mussels were kept in two 350-l stock tanks at a density of ~8,000 individuals per square meter, which is frequently experienced by this species in the wild. Each tank was supplied with standard aquarium filters, aerators and coolers to sustain an appropriate temperature of 19–20 °C. Mussels were acclimated in the stock tanks for at least one week before the tests and used in the experiments within 5–6 weeks after collection. They were fed with dried *Chlorella* sp. (~2 g per 1,000 mussels every second day).

Experimental conditions

We conducted our experiments in 2.5-l tanks (29/29/3 cm) filled with tap water (1.7-cm layer above the substratum surface) settled and aerated for 6 days before use, at a constant temperature of 19 °C (sustained by air-conditioning) and constant fluorescent light of 52 lx, uniform over all experimental arenas (determined with a luxometer L-20A Sonopan Ltd., Białystok, Poland). The oxygen concentration, saturation and conductivity (measured with a multimeter Multi340i; WTW GmbH, Weilheim, Germany) were similar in all experiments and treatments (oxygen concentration (mean ± *SE*) 8.8 ± 0.1 mg/l, saturation 88.1 ± 1.0%, conductivity: 588 μS/cm). The tanks were not aerated during the experiments to avoid the impact of air bubbles on mussel behavior.

We tested mussels within the length range 18–22 mm. This size falls between medium and large size classes described by Toomey et al. (2002). We chose mussels of this size due to their relatively high motility, which is not reported for larger individuals (Toomey et al. 2002; Kobak and Nowacki 2007). On the other hand, this size was sufficient for marking mussels and tracking their movements by the behavior analysis software (see below). One day before and

during the experiments, mussels were not fed. Each mussel was used only once.

Experiment 1: Dispersal of mussels from aggregations

This experiment was designed to test the stability of mussel aggregations depending on druse size and availability of alternative substratum.

In order to obtain artificial druses (aggregations consisting of groups of mussels attached to one another), one day before the experiment we placed mussels in aerated 20/30/7 cm tanks filled with a 2-cm layer of fine sand (mean grain diameter ± *SD*: 0.3 ± 0.08 mm), which is not suitable for *D. polymorpha*, due to the lack of attachment possibility (Kobak and Ryńska 2014). The sand for our experiments was collected from the Włocławek Reservoir, dried at 60 °C and kept dry for at least a month after collecting to remove any organisms and/or environmental signals that could affect mussel responses. Groups of 40 mussels were surrounded with cylinders (diameter 7 cm, height 9 cm) made of plastic 1-mm mesh, which is avoided by *D. polymorpha* (Porter and Marsden 2008). The light intensity was 52 lx, corresponding to the experimental conditions. After 24 h, the conditions led to mussels attaching to one another, irrespective of their preferences. We selected single druses consisting of 2, 4, 9, 12, or 25 mussels and carefully placed them in the central part of the experimental tanks. Zebra mussels are known to produce two types of byssal threads: temporary threads used for short-term initial anchoring and permanent threads produced after a longer time (Eckroat et al. 1993). In our study, due to the short time of druse formation, mussels produced the former type of byssus, resulting in clearly lower adhesion strength (Kobak 2006). Using mussels in their initial stage of attachment allowed their responses to the local conditions to be tested: they could stay in a druse or easily adjust their position depending on the test conditions. Handling could affect druse stability by stimulating mussel detachment. However, it equally affected all experimental treatments. A similar destabilization can be also caused by hydrodynamic forces in the field.

The experiment was conducted in tanks (1) filled with a 1-cm layer of fine sand (no alternative substratum for mussels) or (2) directly on the glass tank bottom (alternative hard substratum suitable for mussels, as shown by Kobak and Ryńska 2014). The 25-individual druse treatment was omitted on glass due to the fact that a clear effect of density was visible at lower abundances (see the "Results" section) and because of the difficulties in obtaining such large aggregations. The substrata were put in water 24 h before the tests to allow biofilm development, which makes submerged materials more suitable for mussels (Wainman et al. 1996; Kavouras and Maki 2003). We deployed simultaneously nine tanks with different experimental treatments. We replicated this procedure 35 times, randomizing the position of particular experimental treatments in consecutive trials. Tanks were cleaned and the water and substratum changed between the replicates.

After 24 h of the experiment we determined: (1) the percentage of all individuals that detached from the druse (hereafter referred to as "detached mussels"), (2) the percentage of mussels that detached and moved away, separating themselves physically from the druse, ("separated mussels") and (3) the mean crowding index of mussels after the experiment (Jarman 1974):

$$\sum_{i=1}^{k} (N_i^2) / \sum_{i=1}^{k} (N_i)$$

Where: N_i – the number of individuals in druse i, k – the number of druses. The mean crowding describes the group size experienced by an average individual in the tank or a "typical group size" according to Jarman (1974). Thus, it was an indication of the final druse size after mussel dispersal.

Due to the high heteroscedasticity of the data (resulting from different initial druse sizes), we used nonparametric Kruskal–Wallis tests (separate for each substratum) followed by a post-hoc procedure described by Sokal and Rohlf (1995) to compare the percentages of mussels detached and separated from druses of different sizes, as well as mean crowding index values. Moreover, we compared the aforementioned behavioural responses of mussels between both substrata using sequential Bonferroni-corrected Mann–Whitney U-tests, separate for each druse size.

Moreover, we compared the frequencies of modified druses (with at least one detached or separated individual) in different experimental treatments using a three-way G test of independence with the following factors: (1) druse size (2, 4, 9, or 12 individuals), (2) substratum (sand or glass), and (3) a response variable: druse status (modified or not). Obviously, the probability of druse modification is a simple function of its size, as the probability that at least one individual will detach increases with the number of individuals. Thus, we only used this analysis to check for the difference in mussel behavior between both substrata and the effect of druse size on this difference, testing substratum × druse status and druse size × substratum × druse status interactions. If their results were significant, we ran a series of appropriate sequential-Bonferroni corrected 2 × 2 G-tests to test the impact of particular conditions on druse stability.

Experiment 2: Movement of single individuals in the presence of conspecifics

This experiment was designed to test the effect of conspecific density and distance to conspecific clusters on the intensity and direction of movements of isolated mussels. In Experiment 1, mussels tended to stay in aggregations more often on unsuitable sandy substratum (see the "Results" section). Therefore, we conducted Experiment 2 using sandy substratum to check whether single, unattached individuals in such conditions would respond to conspecifics constituting potential suitable attachment sites.

One day before the experiment, mussels were marked with fast drying red nail polish to allow them to be tracked by the behavior analysis software. The painted mussels were placed for 24 h in temporary tanks with water and light conditions similar to those in the experimental tanks to recover after marking, which was associated with ~2-min. air exposure. Zebra mussels are capable of surviving several days of desiccation, therefore such a short period of air exposure is unlikely to have any long-lasting negative consequences on their health (Ricciardi et al. 1995).

The bottom of the experimental tanks (Figure 1) was covered with a 1-cm layer of fine sand to increase the probability of mussel motion and check their reactions to conspecifics on the unsuitable substratum. Zebra mussels commonly occur on sandy substrata, attached to available hard objects, such as single stones, anthropogenic solid rubbish, or hard-shelled animals (e.g. unionid clams) (Bódis et al. 2013; Garton et al. 2013), thus the conditions in our experiment reflected natural situations taking place in the wild. To prevent mussels from vertical movement, we covered the tank walls with 1-mm plastic mesh (Porter and Marsden 2008). The experimental tanks were divided into two zones: a movement zone, where we placed a single test individual and a signal zone with 3 or 15 conspecifics. These conspecifics served as a signal source, to which the

test mussel could potentially respond. These zones were separated from each other by a plastic 1-mm mesh barrier (Figure 1). The position of zones relative to the laboratory room was changed in particular trials to avoid bias resulting from some directional factors acting in the room. That said, preliminary observations of the control treatments did not reveal any tendencies for mussel movements in particular directions in the laboratory room.

The experiment consisted of six experimental treatments: (1) control, without conspecifics in the signal zone and a test individual placed 3 cm from the mesh barrier between the zones, (2) control, without conspecifics in the signal zone and a test individual placed 15 cm from the barrier, (3) with three conspecifics in the signal zone and a test individual placed 3 cm from the barrier, (4) with three conspecifics in the signal zone and a test individual placed 15 cm from the barrier, (5) with 15 conspecifics in the signal zone and a test individual placed 3 cm from the barrier and (6) with 15 conspecifics in the signal zone and a test individual placed 15 cm from the barrier. Thus, we could test mussel responses to two conspecific densities and at two distances from the signal source.

The experiment lasted 12 h 10 min., the first 10 min. for recovery after handling followed by 12 h of behavioral observations recorded with a video camera (SNB-6004, Samsung, South Korea) suspended 60 cm above the experimental arena. We deployed simultaneously six tanks with different experimental treatments. We replicated this procedure 30 times, randomizing the position of specific experimental treatments in consecutive trials. We cleaned the tanks and changed the substratum to avoid leaving any potential signals from previous treatments in new replicates.

We determined the percentages of mussels: (1) exhibiting locomotion (relocating from one place to another), (2) exhibiting only non-locomotive movements (e.g. squirming around, swaying, etc.) without relocation, and (3) staying in their initial position without any movement in each experimental treatment. All relocating mussels (group 1) exhibited also non-locomotive movements. Moreover, we applied Noldus Ethovision® XT 10.1 software to determine the following movement characteristics: (1) total distance moved by mussels, (2) total time spent on locomotion, (3) total time spent on non-locomotive movements, and (4) the distance moved towards or away from the signal source.

We compared the frequencies of moving mussels (separately for relocating mussels and for all mussels exhibiting locomotive or non-locomotive movements pooled) in different experimental treatments using a three-way G-test of independence with the following factors: (1) signal strength (3 or 15 mussels), (2) distance from the signal source (close or far), and (3) a response variable: mussel behavior (moving or not moving). All interactions of the response variable with the other factors were tested in both analyses. If their results were significant, we ran a series of appropriate, sequential-Bonferroni corrected 2×2 G-tests to find out which groups of mussels differed from one another in their behaviors.

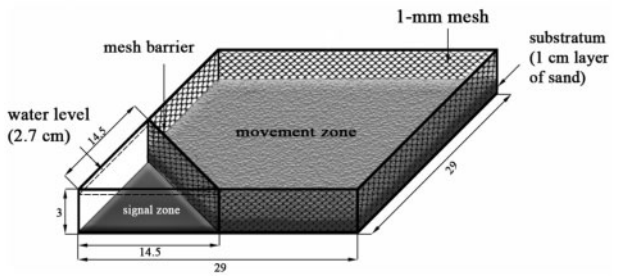

Figure 1. Experimental tank split into two zones, used in Experiment 2. Dimensions are given in centimeter .

In the analysis of movement distance and time, we only considered moving mussels. Thus, we obtained results independent of the frequency of moving individuals, to indicate potential effects of the tested factors on the behavior of those specimens which performed movement. Otherwise, we would duplicate the results of the prior analysis of movement frequency to a large extent. Due to the strong deviations of the data from homoscedasticity and normality assumptions, we compared distances travelled and times spent on moving in all experimental treatments using a nonparametric Kruskal–Wallis test. Moreover, we calculated percentages of distances moved towards and away from the signal source in particular experimental treatments and compared them with a theoretical value of 50%, using sequential Bonferroni-corrected one-sample *t*-tests. A significant departure from 50% would indicate a directional movement. These percentages (arcsine-square root transformed) did not depart significantly from the normal distribution (Shapiro–Wilk test).

Results

Experiment 1: Dispersal of mussels from aggregations

The percentage of mussels detached from druses (Figure 2A) depended on druse size on glass (Kruskal–Wallis test: $\chi^2_3 = 36.4$, $P < 0.001$) and on sand ($\chi^2_4 = 12.9$, $P < 0.012$). According to the post hoc tests, mussel detachment on sand occurred more frequently from 12 to 25-individual druses than from 2 to 4 individual druses. On the hard substratum, the threshold above which the detachment rate increased was between four and nine individuals in a druse. The percentage of mussels separated from druses (Figure 2B) depended on druse size on the hard substratum (Kruskal–Wallis test: $\chi^2_3 = 27.5$, $P < 0.001$) but not on sand ($\chi^2_4 = 4.8$, $P < 0.306$). Similarly to the detachment analysis, mussel separation from 9 to 12 individual druses on glass was greater than that from 2 to 4 individual druses (post hoc tests, Figure 2B). Detachment and separation of mussels were significantly greater on glass than on sand for 12-individual druses (Mann–Whitney U-tests: $z = 4.50$ and 4.35, respectively, $P < 0.001$) and similar on both substrata in all other cases ($z < 2.2$ and $P > 0.05$ after applying the Bonferroni correction).

The mean crowding index after the experiment (Figure 3) depended on initial druse size on glass (Kruskal–Wallis test: $\chi^2_3 = 104.2$, $P < 0.001$) and sand ($\chi^2_3 = 36.4$, $P < 0.001$). On sand, the final druse size increased monotonically with increasing initial druse size up to ~15 individuals for initial 25-individual druses (post hoc tests). On hard substratum, the final druse size reached ~approximately six individuals for initial druses of nine mussels and remained constant for larger initial druse sizes. Video recordings of the experimental trials revealed that groups of mussels were capable of active relocation, which made possible such splits of large druses into smaller aggregations. The mean crowding index was significantly greater on sand than on glass for 12-individual druses (Mann–Whitney U-test: $z = 4.4$, $P < 0.001$) and similar on both substrata in all other cases ($z > -2.1$ and $P > 0.05$ after applying the Bonferroni correction).

The percentage of modified druses (with at least one individual changing its position) differed between substrata depending on druse size (Appendix Figure 1), as shown by significant substratum × druse size × druse status interactions (G-tests: $G_3 = 15.9$ and 22.9 for the detached and separated mussel analyses, $P < 0.001$). As shown by 2×2 G-tests, the detachment and separation of mussels

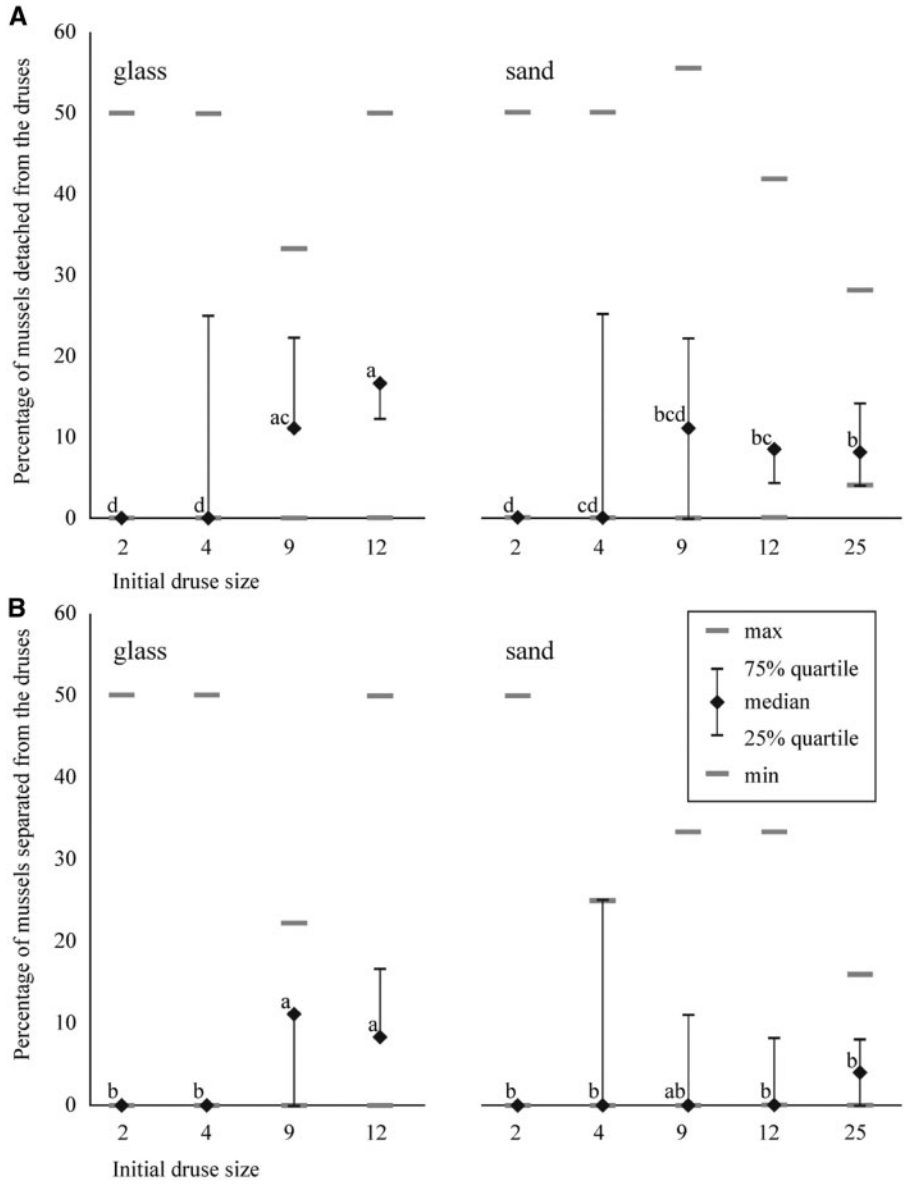

Figure 2. Percentages of mussels which detached from initially formed druses of various sizes (**A**) and mussels which separated themselves from the initial druses (i.e. lost physical contact with the druse) (**B**) in Experiment 1 (mussel dispersal from aggregations on different substrata). Different letters indicate statistically significant differences (determined by post hoc tests following the Kruskal–Wallis test).

from large druses (consisting of nine or more individuals) was greater on the hard substratum than on sand (Appendix Figure 1).

Experiment 2: Movement of single individuals in the presence of conspecifics

Percentages of moving mussels

The percentage of relocated individuals (Figure 4) depended on signal strength (i.e. number of conspecifics in the tank) (G-test: $G_4 = 17.0$, $P = 0.002$), but neither on the distance from the signal source ($G_3 = 1.5$, $P = 0.680$) nor on the interaction between these factors ($G_2 = 1.5$, $P = 0.476$). Pairwise 2×2 G-tests (Figure 4) showed that mussels in the control treatments moved more often (52%) than those exposed to conspecific signals (22%), independent of the signal strength.

The same relationship was observed when locomotive and non-locomotive movements were pooled (G-test: $G_4 = 15.8$, $P = 0.003$, $G_3 = 4.9$, $P = 0.181$, $G_2 = 4.5$, $P = 0.106$ for the signal strength, distance from the signal source and signal strength × distance interaction terms, respectively). Here, mussels in the control treatments moved more often (47%–63%) than those exposed to 15 conspecifics (17%–33%) (Figure 4). The frequency of movement of individuals exposed to three conspecifics was intermediate (30%–43%) and did not differ significantly from the other experimental treatments (Figure 4).

We also checked some qualitative characteristics of mussel behavior associated with these movements by watching the video recordings manually. These observations revealed that the majority of the mussels exhibiting only non-locomotor movements (without relocation) had attempted to burrow into the sand, which was indicated by circular depressions in the sand around the individuals.

Mussel movement parameters

In all the experimental treatments, the test mussels moved on average 10.7 cm ± 16.6 *SD* (range: 0.2–95.2 cm) during 12 h (Figure 5A).

Distances moved by mussels did not differ significantly among the experimental treatments (Kruskal–Wallis test: $\chi^2_5 = 7.2$, $P = 0.207$), though the mussels exposed at a short distance to the scent of 15 individuals tended to move shorter distances.

No significant differences in the total time spent on locomotion were found among the experimental treatments (Kruskal–Wallis test: $\chi^2_5 = 4.0$, $P = 0.544$), which ranged from 4% to 7% of the total experimental time (Figure 5B). On the other hand, experimental conditions significantly affected time spent by mussels on non-locomotive movements (Kruskal–Wallis test: $\chi^2_5 = 16.0$, $P = 0.007$). The mussels located at a short distance (3 cm) from the signal emitted by three conspecifics spent the least time on non-locomotive movements (3.4% of the total time of the experiment) compared to the other groups. It differed significantly from the times observed in other experimental treatments, ranging from 7.7% to 18.3% (post hoc tests, Figure 5C).

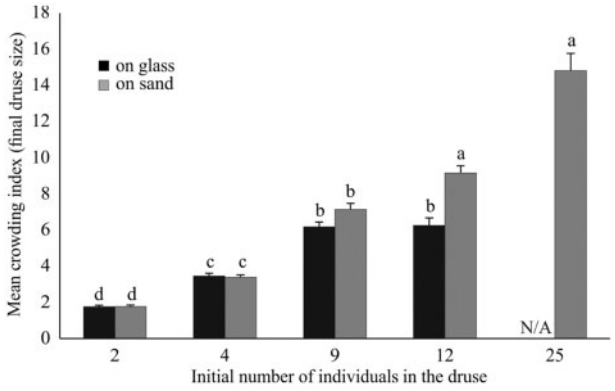

Figure 3. Mean crowding index [the final group size calculated according to Jarman (1974)] after Experiment 1 (mussel dispersal from aggregations on different substrata), depending on druse size and substratum type. Different letters indicate statistically significant differences (determined by post hoc test following the Kruskal–Wallis test). Error bars indicate standard error of the means.

The test mussels did not move preferentially towards or away from the signal source, as shown by non-significant results of the one-sample *t*-tests (Appendix Figure 2).

Discussion

Dispersal of mussels from aggregations

Mussel responses to druse density in Experiment 1 (druse dynamics) did not confirm their preferences for conspecifics, suggested by the previous studies (Wainman et al. 1996; Kobak 2006). We revealed that *D. polymorpha* avoided large druses, particularly when the alternative hard substratum was available. Moreover, mussels detaching from the druses remained in contact with conspecifics in the absence of alternative hard substrata, but otherwise preferred to stay at some distance from one another. We have confirmed earlier preliminary findings by Kobak and Ryńska (2014) and Tošenovský and Kobak (2016), suggesting that zebra mussels do not form druses until forced by the lack of alternative hard substrata, as well as those by Kavouras and Maki (2003) showing that they prefer biofilmed artificial substrata over conspecific shells. This result is against conventional wisdom, which assumes mussel preferences for conspecifics, leading to group formation. On the other hand, Kobak et al. (2009) observed that zebra mussel individuals attached in a direct contact with conspecifics were less likely to detach and move to another site than singletons. However, in the aforementioned study, mussels formed only monolayer aggregations with individuals touching one another's shell, but attached to an alternative hard substratum. Thus, our results suggest that mussels preferred other hard substrata over conspecific shells and the latter were selected only if no choice was possible. It is likely to be associated with the negative impact of a large colony on its members demonstrated in the field by Stańczykowska (1964) and Czarnołęski et al. (2003) as well as in laboratory by Burks et al. (2002) and Tuchman et al. (2004).

The observed reactions of mussels to conspecifics may be important for their distribution in the field. Intentional detachment from a druse and avoidance of a direct contact with a dense colony may contribute to the effective small scale spreading of this invasive species, as well as enhancing the large scale transport of mussels

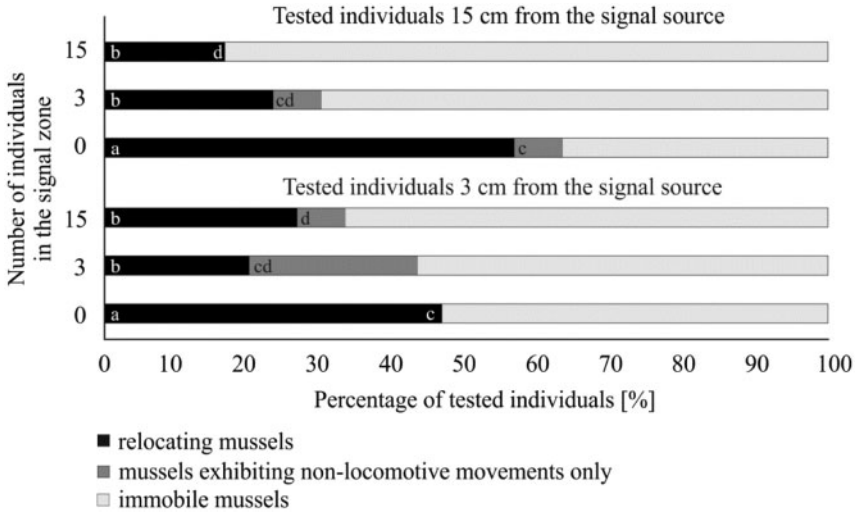

Figure 4. Percentages of mussels that relocated, exhibited only non-locomotive movements and did not move at all in Experiment 2 (mussel movement in the presence of conspecifics). Different letters on the bars indicate statistically significant differences between experimental treatments (2 × 2 G-tests with a sequential Bonferroni correction applied); a and b for relocating mussels, c and d for all moving mussels (with locomotive and non-locomotive movements pooled).

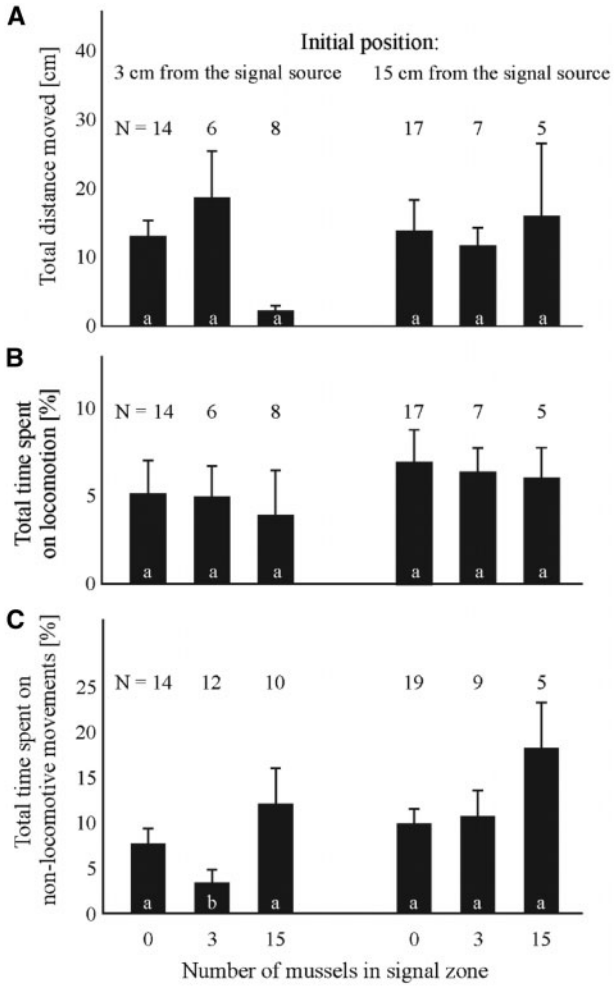

Figure 5. Total distance moved (**A**), time spent on locomotion (**B**) and time spent on non-locomotive movements (**C**) by mussels depending on their distance from the signal source and signal strength in Experiment 2 (mussel movement in the presence of conspecifics). *N* indicates the numbers of analyzed mussels (only translocating/moving individuals were used in the analysis and to calculate the means). Error bars indicate standard error of the means. Different letters on the bars indicate statistically significant differences (determined by post hoc tests following the Kruskal–Wallis test).

attached to drifting debris or boat hulls (Minchin et al. 2003) by increasing their penetration of new areas and thus the probability of their colonization (Collas et al. 2017).

Movement of single individuals in the presence of conspecifics

Character of stimulus

The nature of the conspecific cue observed in our study might be either chemical or physical, but the latter seems much less likely. As the signal donor mussels in Experiment 2 were located behind a dense mesh barrier, the test mussels had no physical contact with conspecifics and siphonal currents are clearly too weak (Ertman and Jumars 1988) to be detectable though the mesh from the distance tested in our design. Thus, given the fact that responses of zebra mussels to conspecific chemical signals were also observed in other studies (Kobak 2001; Kobak and Ryńska 2014), we assume that mussels in our experiments responded to chemical stimuli.

Percentages of moving mussels

Mussels in the presence of conspecifics clearly reduced their activity, regardless of the adverse substratum on which they were located and the lack of any shelter. A similar reduction in locomotor activity of *D. polymorpha* has been observed in response to stress factors, such as the presence of predators, alarm cues or light (Toomey et al. 2002; Kobak and Nowacki 2007; Naddafi and Rudstam 2013). On the other hand, Commito et al. (2014, 2016) observed a different behaviour of marine *Mytilus edulis*, which quickly formed aggregations even on hard substrata and irrespective of any predation cues. Perhaps, clumping is more important for marine bivalves, experiencing heavy hydrodynamic forces (Bell and Gosline 1997) and facing much more diverse predators (Reimer and Tedengren 1997). The behavior of mussels observed in our study may indicate their "passive" avoidance reaction to other individuals. An indirect reason for such activity reduction could be the fact that mussels were unable to move directionally in response to conspecific cues. As the probability of accidental encounters with other individuals during random movements increases with density, the activity reduction in response to conspecific signals seems to be the best solution if such encounters are undesirable.

Mussel movement parameters

The individuals placed near 15 conspecifics (thus potentially experiencing the strongest signal) displayed a clear, though non-significant tendency for a substantial decrease in travelled distance and a significant increase in non-locomotor movement, revealing their ability to estimate the strength of conspecific signals. The non-locomotor movement seems to be associated with preparation for attachment in the current position, as shown by visual examination of the video recordings. The increase in bivalve byssogenesis in the presence of conspecifics is a known phenomenon (Uryu et al. 1996; Kobak 2006) and up to now has been considered as a reaction showing preferences of mussels to conspecifics. Our study suggests that non-locomotive movement of mussels can also be the effect of "passive avoidance": mussels experiencing a high density of conspecifics and being unable to move directionally reduced their locomotion and searched for the nearest suitable substratum to attach.

Apparently, the intraspecific relations of *D. polymorpha* are the result of interactions among a number of factors varying in different environments, including the presence of predators (Kobak and Kakareko 2009) and strong water currents (Tošenovský and Kobak 2016), which may stimulate aggregation forming. Nevertheless, our study has shown that conspecific avoidance reactions, as well as the availability of hard substratum, are among the major forces forming these relationships.

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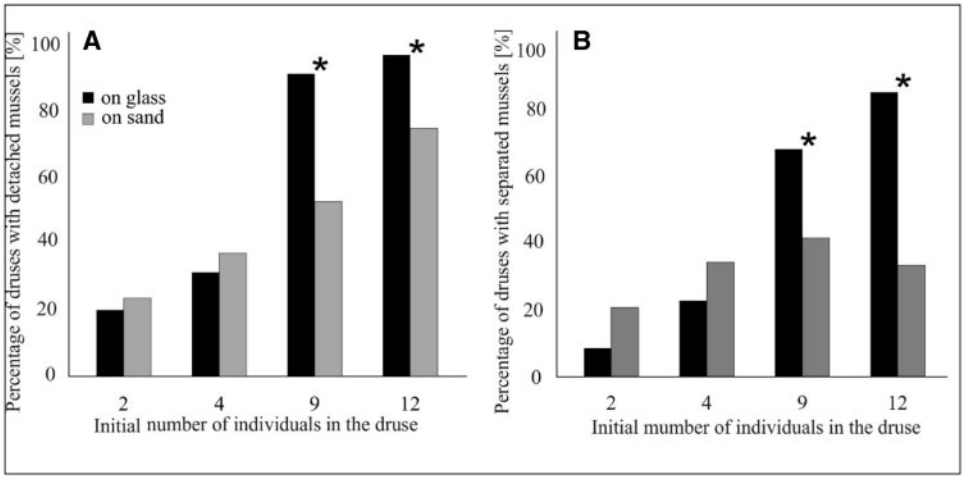
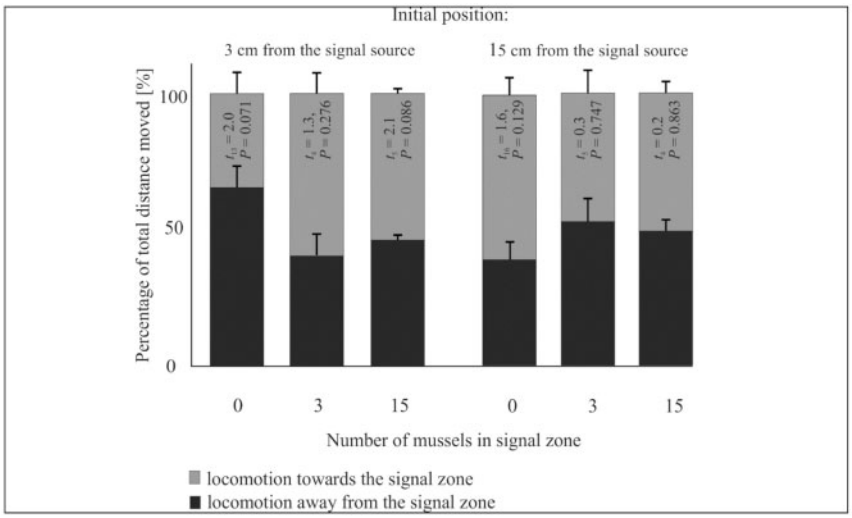


Appendix Figure 1. Percentages of druses changed by (**A**) detachment and (**B**) separation (i.e., detachment and losing physical contact with the druse) of mussels in Experiment 1 (mussel dispersal from aggregations on different substrata). Asterisks indicate statistically significant differences between substratum types for a given druse size (2 × 2 G-tests with a sequential Bonferroni correction applied).



Appendix Figure 2. Percentages of distances moved towards and away from the signal source in Experiment 2 (mussel movement in the presence of conspecifics). The results of one-sample *t*-tests comparing the percentages with the theoretical value of 50% (denoting the lack of directional movement) are shown on the bars. Error bars indicate standard error of the means.