

Preferences of invasive Ponto-Caspian and native European gammarids for zebra mussel (*Dreissena polymorpha*, *Bivalvia*) shell habitat

Jarosław Kobak · Jarosław Żytkowicz

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Abstract We investigated habitat preferences of two invasive Ponto-Caspian gammarids (*Dikergammarus haemobaphes* and *Pontogammarus robustoides*) and a native European species (*Gammarus fossarum*) in laboratory experiments. The habitats consisted of the following objects: (1) living zebra mussels; (2) empty mussel shells (clean or coated with nail varnish) with both valves glued together using aquarium silicone sealant to imitate a living mussel; (3) stones (clean or varnished); (4) empty plates. Ten objects of the same type were glued to a plastic plate (10 × 10 cm) with methyl acrylic glue. The plates were placed in experimental tanks in various combinations. A single gammarid was put into the tank and its position was determined after 24 h. The studied species responded differently to the presence of zebra mussels. *D. haemobaphes* preferred living mussels rather than

their empty shells and these two habitats over stones and empty plates. It responded positively to shell shape, selecting varnished shells rather than varnished stones, and to shell surface properties, selecting clean shells rather than varnished shells. It did not respond to waterborne mussel exudates. *P. robustoides* did not exhibit any preferences for the above-mentioned substrata. *G. fossarum* was attracted by empty mussel shells (but not by living mussels). It responded only to their shape, not to surface properties. The strong affinity for zebra mussels, exhibited by *D. haemobaphes*, might help it survive and develop stable populations in newly invaded areas.

Keywords *Dikergammarus haemobaphes* · *Pontogammarus robustoides* · *Gammarus fossarum* · Mussel bed · Interspecific interactions · Habitat selection

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Introduction

Zebra mussel, *Dreissena polymorpha* (Pallas, 1771), is a Ponto-Caspian species occurring in most of European waters since its invasion that started in the beginning of the 19th century (Stańczykowska, 1977). In 1980s, it further expanded its range to North America (Lewandowski, 2001). Due to its gregariousness and fouling life style, zebra mussel is regarded as an ecosystem engineer: a species that

considerably modifies its environment and affects various aspects of ecosystem functioning (Karatayev et al., 2002). Mussels influence their abiotic environment, e.g. water and sediment chemistry (Arnott & Vanni, 1996; Karatayev et al., 2002), sedimentation rate and water transparency (Smith et al., 1998). They also stimulate macrophyte development (Skubinna et al., 1995) and strongly affect benthic and planktonic communities (Botts et al., 1996; Stewart et al., 1998; Vanderploeg et al., 2001; Mörtl & Rothhaupt, 2003). Many taxa benefit from the presence of dreissenids, which are a source of valuable food for detritivores, feeding on mussel pseudofaeces and faeces, and provide protection against predators (Wolnomiejski, 1970; Botts et al., 1996; Stewart et al., 1998). On the other hand, zebra mussels cause declines of some taxa, such as unionid bivalves, which suffer from fouling by *Dreissena* (Ricciardi et al., 1996; Schloesser et al., 1997). Furthermore, zebra mussels are an important item in the diet of many species of fish, crayfish and waterfowl (Molloy et al., 1997; Kakareko et al., 2005).

Invasive Ponto-Caspian amphipods appeared in Europe in the 20th century (Bij de Vaate et al., 2002; Jazdzewski et al., 2002; Konopacka, 2004). The cause of their rapid expansion is unclear, but probably involves increasing salinity of large European rivers due to industrial pollution, which favours euryhaline Ponto-Caspian invaders (Jazdzewski et al., 2002; Konopacka, 2004). They often displace native gammarids due to their higher tolerance to changing environmental conditions, higher fecundity and / or more predatory nature (Arbaciauskas, 2002; Berezina & Panov, 2003; Konopacka, 2004; Kley & Maier, 2006). Usually, a combination of several ecological traits makes a given species a successful invader (Devin & Beisel, 2007). Alien gammarids play a considerable role in ecosystems to which they are not indigenous. Apart from successful competition with their local counterparts, they are predators feeding on benthic organisms, including other gammarids (MacNeil et al., 1997; Berezina & Panov, 2003; Devin et al., 2003). Moreover, they constitute a substantial item in the diet of fish (Gonzalez & Burkart, 2004; Grabowska & Grabowski, 2005; Kakareko et al., 2005).

Ponto-Caspian amphipods, expanding their range in Europe, have encountered well-estab-

lished populations of the zebra mussel, a species with which they had coevolved in the same region. It would be interesting to check for potential interactions between these taxa, which could affect their distribution and invasive potential. It was observed in situ that some of the invasive gammarids, such as *Dikerogammarus haemobaphes* (Eichwald) and *Echinogammarus ischnus* Stebbing, were especially abundant in zebra mussel beds (Palmer & Ricciardi, 2005; Wawrzyniak-Wydrowska & Gruszka, 2005). Field studies provide invaluable data on species distribution under natural conditions, but sometimes fail in determining exact causes of the observed phenomena, due to multiple factors acting simultaneously in the field. For instance, it is difficult to say whether the observed high abundance of gammarids within zebra mussel beds results only from their better growth and survival due to the obvious benefits of such habitat (providing more food and better protection) or follows also from active substratum selection. That is why we carried out a series of laboratory experiments to investigate preferences of selected Ponto-Caspian and native gammarids for zebra mussel habitat. We tested their site selection behaviour in the presence of habitats consisting of living mussels, dead mussel shells, stones or flat surfaces.

We hypothesised that alien species would actively select sites with living zebra mussels and their shells. We assumed that a similar response of native taxa, which have not coevolved with mussels, would be absent or at least weaker. We also intended to determine the mechanisms of the potential preferences for mussel shells. We hypothesised that they would involve shell shape, shell surface properties (i.e. its texture or surface-bound chemical substances) and/or waterborne substances released by mussels.

Materials and methods

Animals

Zebra mussels were collected by a diver from the dam of the Włocławek Dam Reservoir, at the depth of ca. 5 m. This reservoir is located on the lower Vistula River (central Poland), which is

part of one of the main inland corridors used by Ponto-Caspian species to migrate in Europe (Bij de Vaate et al., 2002). Mussels were kept in a 500-L aquarium filled with settled, aerated tap water, at room temperature (ca. 18–22°C).

For our study, we have chosen two invasive, Ponto-Caspian gammarid species: *Dikerogammarus haemobaphes* (Eichwald) and *Pontogammarus robustoides* (G. O. Sars), which have recently expanded their range in Europe (Jażdżewski et al., 2002; Konopacka, 2004), as well as one native European species, *Gammarus fossarum* Koch (Jażdżewski, 1975).

Individuals of *D. haemobaphes* were collected from plastic baskets suspended at a depth of 1 m in the Włocławek Reservoir for the purpose of another study. An additional source of this species was the stony bank of the lower Vistula River in the towns of Toruń and Włocławek (central Poland). Some individuals were also found in the mussel samples. Mean body length of the specimens used in this study was 10.9 mm (range: 6.5–16 mm).

Individuals of *P. robustoides* were captured from the sandy bottom near the left bank of the Włocławek Reservoir, at the depth of ca. 0.2–0.3 m, using a 1-mm mesh sieve. Mean body length of the specimens used in this study was 10.1 mm (range: 5.5–15 mm).

Individuals of *G. fossarum* were found in the Ruda River, the left tributary of the Włocławek Reservoir. They were captured with a 1-mm mesh sieve from the sandy bottom covered by emergent macrophytes, with water depth of ca. 0.2–0.3 m. Mean body length of the specimens used in this study was 8.4 mm (range: 5–12 mm).

Both invasive species were sampled from the localities in which zebra mussels or their shells were also present. Mussels did not occur in the area where *G. fossarum* was collected.

In the laboratory, the gammarids were placed (each species separately) in 50-L tanks with sandy bottoms and stony shelters, in settled, aerated tap water at room temperature (ca. 18–22°C). They were used in the experiments within 1–4 weeks after collection.

Substrata

Plastic plates (100 × 100 × 5 mm) made of resocart (phenoplast-type, thermosetting plastic based

on phenol-formaldehyde resin) were used as substrata in our study. This material is suitable for periphyton organisms, especially zebra mussels (Kobak, 2004). Five types of solid objects were glued to the plates with fast-binding methyl acrylic glue:

(1) Living mussels (mean length: 20.8 mm, range: 15.4–29.2 mm), glued by the ventral surface of one of their valves (Fig. 1d), so that they could open their shells and produce their own byssal threads (as most of them did). Their shell surfaces were gently cleaned with fine sandpaper to get rid of the byssal thread remnants and other debris. Mussels were not fed during the tests, so they did not produce pseudofaeces, which was confirmed visually.

(2) Empty mussel shells (mean length: 21.3 mm, range: 15.9–28.5 mm), with both valves glued together by means of aquarium silicone sealant to imitate a living mussel shape. The shells were collected together with living mussels or obtained from individuals that died in the laboratory mussel culture during ca. six months preceding the experiments. Silicone was located only inside the shells, so the gammarids had no contact with it. Furthermore, this substance was also used to construct the experimental tanks and was present all around the tested animals, so it could not affect their movement direction. The shells were cleaned with sandpaper and attached to the plates in the same position as living mussels (Fig. 1d).

(3) Empty mussel shells (mean length: 20.4 mm, range: 15.2–28.7 mm), handled like those described above, but coated by a layer of transparent, water-resistant nail varnish (Soraya S.A., Poland), to exclude the potential effect of shell surface properties on gammarids.

(4) Stones (mean length: 21.0 mm, range: 15.1–30.1 mm), available commercially as substratum for aquarium fish keeping. We have chosen slightly elongated specimens, roughly resembling mussel shells with respect to size and proportions, but lacking their surface structure and characteristic triangular shape.

(5) Stones (mean length: 20.3 mm, range: 15.2–30.5 mm) coated with nail varnish.

Using glue to fasten the stones and empty shells was necessary to imitate the attachment of living

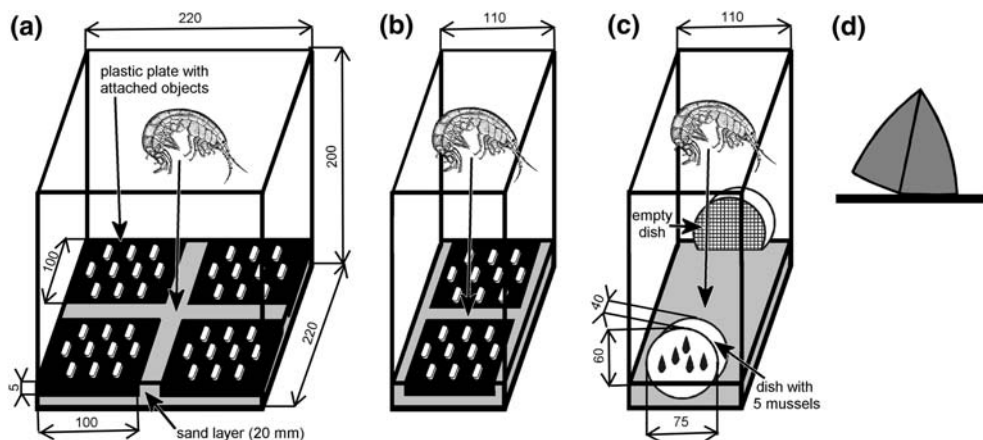


Fig. 1 The design of the experiments carried out in the study. (a) Experiment 1; (b) Experiments 2–5; (c) Experiment 6; (d) The way of fastening a mussel to a plate. Dimensions are given in mm. In Fig. b and c, only

mussels, which produced their byssal threads. The living mussels were also glued to keep the experimental conditions (*viz.* the presence of glue) in all treatments the same. Ten objects of the same type were arranged on a plate as shown in Fig. 1a–b. The sixth substratum type was an empty plate with ten drops of methyl acrylic glue in the place of the objects, to control for the potential effects of glue on gammarid behaviour. The plates were left for a week in an aquarium before use, to allow for biofilm development. Then, they were presented in various combinations to gammarids in experimental tanks.

Experimental setup

The experiments were carried out in tanks with square (220 × 220 mm) or rectangular (110 × 220 mm) bottoms (Fig. 1a–c). The tanks were filled with settled (at least 24 h) tap water, which was aerated before the experiments, but not during them, to avoid influence of air bubbles on gammarid behaviour. The tank bottom was covered with 2-cm layer of fine sand. The tested substrata were placed horizontally in the corners of the square tanks (four plates, Fig. 1a) or adjacent to the both shorter walls of the rectangular ones (two plates, Fig. 1b). The plates were immersed in sand so that their surfaces were at the level of the sand surface (Fig. 1a–b). The experiments were carried out at room tempera-

ture (18–22°C) under ambient light conditions. Arrangement of the plates and their position relative to the laboratory room were changed in various replicates.

A single gammarid was put in the centre of each tank. Animals were tested individually to avoid interactions between them (*e.g.* cannibalism, aggregation or competition for space). After 24 h, the tanks were divided into quarters (four-plate treatments) or halves (two-plate treatments) with glass plates, tightly matching the tank width, and the gammarid location was determined. We assumed that if a gammarid did prefer a given substratum, it would spend more time on it, so the probability of finding the animal in the corresponding part of the tank would be higher.

After the test, the animals were measured to the nearest 0.5 mm under dissecting microscope using plotting paper and identified to species according to Jażdżewski (1975), Konopacka (1998) and Konopacka (2004). The numbers of individuals tested in each experiment ranged from 28 to 115. Exact values are shown in the Results section.

Experiment 1: Preferences for various substratum types

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This was an initial experiment, designed to check whether gammarids preferred any of the tested objects. Four types of substrata: (1) with living

mussels, (2) empty shells, (3) stones and (4) an empty plate were put in the corners of a square tank (Fig. 1a). All three species were tested in this experiment.

The results of this experiment were used to plan Experiments 2–6, designed to examine cues used by gammarids in their habitat selection behaviour. Only those species, which showed appropriate preferences in Experiment 1, were used in these experiments. Thus, *D. haemobaphes* and *G. fossarum*, selecting mussel shells (see Results), were tested in Experiments 2–5, while only *D. haemobaphes*, preferring living mussels, was included in Experiment 6.

Experiment 2: The quality of stones

It was possible that the avoidance of stones, observed in Experiment 1 (see Results), was caused by unsuitability of the stones used in this study, rather than by actual preferences of gammarids for shells. We examined the behaviour of animals that were given a choice between plates with attached stones and empty plates, placed in a rectangular tank (Fig. 1b). Preferences for the stones would indicate that this habitat was not avoided by gammarids.

Experiment 3: The quality of the varnish

To check whether the nail varnish used in Experiments 4 and 5 (see below) did not affect gammarid behaviour, plates with varnished and clean stones were presented to gammarids in a rectangular tank (Fig. 1b). We assumed that, if the varnish repelled or attracted gammarids, they would either avoid or prefer the varnished stones, respectively. The lack of any preferences in this experiment would be an indication of the neutrality of the varnished surface for gammarids.

Experiment 4: The effect of the shall shape

In this experiment, we checked whether gammarids preferring mussel shells were guided by shell shape. Animals could choose between varnished shells and varnished stones in a rectangular tank (Fig. 1b). The varnish made surfaces of both

object types similar, while their shapes remained different. Preferences of gammarids for shells would indicate that they responded to shell shape.

Experiment 5: The effect of the shell surface quality

To check whether gammarids responded to surface properties of mussel shells (i.e. texture and/or chemical substances bound to their surface), we tested them in the presence of varnished and clean shells in a rectangular tank (Fig. 1b). We assumed that the varnish excluded stimuli associated with the quality of shell surface, while its shape remained unchanged. Thus, preferences of gammarids for clean shells would indicate the effect of shell surface properties on their behaviour.

Experiment 6: The effect of substances released by living mussels

This experiment was designed to check whether gammarids preferring the vicinity of living mussels were attracted by substances released by mussels to the water column. Five living mussels (mean shell length: 18.7 mm, range: 16.4–21.1 mm) were put into a circular glass dish (diameter: 75 mm, height: 40 mm). The dish was then closed with 1-mm nylon mesh fastened by a rubber band, and placed at one of the shorter walls of a rectangular tank, with the mesh directed to the water column (Fig. 1c). A similar empty dish was put in the opposite part of the tank. Preferences for the tank zone with living mussels would indicate that gammarids were attracted by waterborne mussel exudates.

Statistical analysis

Distributions of various gammarid species within tanks in each experiment were compared with one another using *G*-tests of independence. To test hypotheses of uniform gammarid distributions within tanks, we used *G*-tests of goodness of fit (Sokal & Rohlf, 1995). In Experiment 1, when the results of a global test were significant, pairwise *G*-tests were also carried out to check

which substrata differed significantly from one another with respect to gammarid number. The sequential Bonferroni correction was applied to these tests to control for multiple comparisons (Rice, 1989). Body lengths of animals occupying various substrata were compared using Kruskal–Wallis ANOVA (Experiment 1) or Mann–Whitney U test (Experiments 2–6).

Results

Experiment 1: Preferences for various substratum types

Distributions of the studied species on various substrata differed significantly from one another (G -test: $G = 22.77$, $df = 6$, $P = 0.0001$).

D. haemobaphes preferred living mussels rather than their empty shells and these two habitats over stones and empty plates. No significant differences were found between the two latter substrata (Table 1). Abundances of *P. robustoides* on the studied substrata, including empty plates, did not differ significantly from one another (Table 1). *G. fossarum* showed preferences for dead shells. Its abundance in this habitat differed significantly from those found on all other substrata, including living mussels (Table 1).

No significant differences in gammarid body length were found among the studied substrata (Kruskal–Wallis test: $H = 2.75$, $df = 3$, $P = 0.432$; $H = 3.09$, $df = 3$, $P = 0.378$ and $H = 1.35$, $df = 3$, $P = 0.717$ for *D. haemobaphes*, *P. robustoides* and *G. fossarum*, respectively).

Experiment 2: The quality of the stones

Distributions of *D. haemobaphes* and *G. fossarum* did not differ from each other (G -test: $G = 0.12$, $df = 1$, $P = 0.729$). Both species strongly preferred stony substratum rather than empty plates (Table 2). Thus, the stones did not exhibit any repelling activity against gammarids and could be used for comparisons with shells in other experiments.

Body lengths of gammarids occupying both substrata were similar (U test: $Z = 0.19$, $P = 0.844$ and $Z = 1.16$, $P = 0.247$ for *D. haemobaphes* and *G. fossarum*, respectively).

Experiment 3: The quality of the varnish

Distributions of *D. haemobaphes* and *G. fossarum* were similar to each other (G -test: $G = 0.29$, $df = 1$, $P = 0.588$). None of them showed any preferences for clean or varnished stones (Table 2), confirming that the varnish used in our study did not affect their behaviour. Thus, its application in Experiments 4 and 5 was justified.

Body lengths of gammarids occupying both substrata were similar (U test: $Z = 0.44$, $P = 0.658$ and $Z = 0.88$, $P = 0.378$ for *D. haemobaphes* and *G. fossarum*, respectively).

Experiment 4: The effect of the shall shape

Distributions of *D. haemobaphes* and *G. fossarum* did not differ from each other (G -test: $G = 0.54$, $df = 1$, $P = 0.463$). Both species preferred varnished shells rather than varnished stones (Table 2).

Gammarid sizes on both substrata were similar (U test: $Z = 0.84$, $P = 0.397$ and $Z = 0.04$, $P = 0.963$ for *D. haemobaphes* and *G. fossarum*, respectively).

Experiment 5: The effect of the shell surface quality

The difference between the distributions of *D. haemobaphes* and *G. fossarum* was not significant, though the P -value was suspiciously low (G -test: $G = 3.53$, $df = 1$, $P = 0.060$). On the other hand, a significant departure of the observed gammarid distribution from the theoretical, uniform one was observed only in the case of *D. haemobaphes*. Its abundance among clean shells was higher than the number of individuals found among varnished shells. Such a difference was insignificant in the case of *G. fossarum* (Table 2).

The substrata did not differ from each other with respect to gammarid size (U test: $Z = 0.16$,

Table 1 Abundances of gammarids on various substrata in Experiment 1

Species	Number of individuals				G-test (df = 3)	
	Mussels	Shells	Stones	Empty	G	P
Dik	53 ^a	32 ^b	15 ^c	13 ^c	35.50	<0.0001*
Pont	30 ^a	38 ^a	26 ^a	21 ^a	5.33	0.1490
Gam	15 ^a	42 ^b	20 ^a	8 ^a	28.73	<0.0001*

Asterisks indicate statistically significant differences in gammarid distribution among substrata ($P < 0.05$). Values labelled with the same superscript letters did not differ from one another (Bonferroni-corrected pairwise G-tests). Dik—*Dikerogammarus haemobaphes*, Pont—*Pontogammarus robustoides*, Gam—*Gammarus fossarum*

Table 2 Abundances of gammarids on various substrata in Experiments 2–6

Experiment	Species	Number of individuals		G-test (df = 1)	
				G	P
2		Clean stones	Empty plate		
	Dik	28	9	10.24	0.0014*
3	Gam	34	13	9.72	0.0018*
		Varnished stones	Clean stones		
4	Dik	14	14	0.00	1.0000
	Gam	16	21	0.68	0.4104
5		Varnished shells	Varnished stones		
	Dik	29	11	8.40	0.0038*
6	Gam	38	20	5.68	0.0172*
		Varnished shells	Clean shells		
5	Dik	14	36	10.02	0.0015*
	Gam	28	34	0.58	0.4457
6		Dish with mussels	Empty dish		
	Dik	18	17	0.03	0.8658

Asterisks indicate statistically significant differences in gammarid distribution among substrata ($P < 0.05$). See Table 1 for the species names

$P = 0.869$ and $Z = 0.85$, $P = 0.397$ for *D. haemobaphes* and *G. fossarum*, respectively).

Experiment 6: The effect of substances released by living mussels

D. haemobaphes was not attracted by the dish containing living mussels (Table 2).

Sizes of animals found in different tank zones were similar (U test: $Z = 1.34$, $P = 0.179$).

Discussion

Dikerogammarus haemobaphes

This species exhibited strong preferences for living mussels and their shells (Experiment 1). Its abundances among stones and on empty

plates were similar to each other. It was possible that gammarids never sought refuge among stones and treated them similarly to flat plates devoid of any solid objects. Otherwise, shells could be so attractive for gammarids that they did not select stones in their presence. Experiment 2, in which gammarids preferred stony habitats over empty plates, supported the latter hypothesis.

When the effect of surface properties was excluded by varnish, gammarids preferred mussel-shaped objects (varnished shells rather than varnished stones) in Experiment 4. Thus, the object shape was an important cue in their habitat selection behaviour. Moreover, gammarids discriminated between clean and varnished shells (Experiment 5). Experiment 3 demonstrated that they did not avoid the varnish in itself, indicating

that their behaviour in Experiment 5 was a positive response to cues associated with shell surface properties. Altogether, these results show that *D. haemobaphes* really prefers mussel shell surfaces, and not only just any objects of appropriate shape.

The behaviour of *D. haemobaphes* observed in our experiments corroborates with other studies, demonstrating associations of this species with zebra mussel beds or stony habitats (Muskó, 1993; Bij de Vaate et al., 2002; Wawrzyniak-Wydrowska & Gruszka, 2005). However, it is often difficult to say whether observed differences in distribution of organisms follow from their variable survival inside and outside a mussel bed, or from their active habitat selection. Our study showed that *D. haemobaphes* was able to select zebra mussels actively and clearly preferred this habitat over other types of solid objects.

Gammarids living in mussel beds may feed on mussel faeces and pseudofaeces as well as on other detritivorous organisms. Perhaps that is why we observed their preference for living mussels rather than for empty shells. For certain organisms (e.g. chironomids or a snail *Ferrissia rivularis* (Say)), living mussels are a better habitat than empty shells, while other taxa (e.g. a snail *Gyraulus* sp., a caddisfly *Helicopsyche* sp. and hydrozoans) do not discriminate between these two substratum types, preferring them over other habitats (Stewart et al., 1998; Botts et al., 1996; Ricciardi et al., 1997; Mörtl & Rothhaupt, 2003). The former are regarded to benefit from food provided by mussels and the latter use their shells only as a shelter. Gonzalez & Downing (1999) have shown that amphipods (mainly *Gammarus fasciatus* Say, but the authors suspect an admixture of *Echinogammarus ischnus*) were more abundant on substrata with living zebra mussels or their shells, compared to empty rocks, at least in certain parts of the year. This distribution was caused by better protection against fish predation provided by shells, which was demonstrated in an additional laboratory experiment. Another amphipod, *Gammarus roeseli* Gervais, native to the Balkan region (Jażdżewski, 1980; Konopacka, 2004), was more abundant in habitats consisting of living mussels rather than their dead shells (Mörtl & Rothhaupt, 2003), similarly to the

behaviour of *D. haemobaphes* in our study. A Ponto-Caspian species, *E. ischnus*, occurred in higher numbers on substrata covered by zebra mussels than on those fouled by a filamentous alga *Cladophora* sp., and actively selected the former habitat in laboratory (Van Overdijk et al., 2003). Furthermore, *E. ischnus* kept in the presence of predators survived better in shelters provided by zebra mussel shells than in other types of hideouts (Gonzalez & Burkart, 2004). Another example of an active selection of zebra mussel shell habitat is a snail *Physella heterostropha* (Say), which prefers such substratum, especially in the presence of predator kairomone (Stewart et al., 1999).

It was unclear from Experiment 1, whether gammarids discriminated between living mussels and empty shells using waterborne substances released by mussels or stimuli acting during a direct contact with them. The latter seemed more probable, as the appearance of a chemical gradient in a relatively short experimental tank was rather unlikely. Experiment 6 confirmed these expectations, as gammarids did not respond to mussels separated from them by mesh. We cannot conclude that gammarids are totally unable to detect waterborne mussel exudates, but in our study they were clearly guided by other cues. Mussels did not produce pseudofaeces during the tests, so this factor also could not stimulate gammarids to move towards them. Probably, they were able to discriminate between surfaces of living mussels and dead shells. Alternatively, they might detect byssal threads (not present in the dead shell treatment) or respond to siphonal currents generated by mussels. Undoubtedly, further studies on mechanisms of this discrimination are necessary.

D. haemobaphes is a Ponto-Caspian species, indigenous to the region inhabited also by native populations of zebra mussels (Bij de Vaate et al., 2002). Thus, there was enough time for evolution of interactions between these taxa. Another Ponto-Caspian *Dikerogammarus* species, *D. villosus* (Sowinsky) is also known for its associations with zebra mussel beds, which are especially pronounced in the case of larger individuals, while smaller specimens are found mainly on macrophytes (Devin et al., 2003). In our study,

body sizes of gammarids occupying various substrata did not differ from one another. Probably, differences observed by Devin et al. (2003) resulted from variable survival of small gammarids in various habitats, e.g. due to cannibalism. Such interactions were excluded by our experimental design.

Perhaps, preferences of *D. haemobaphes* for *Dreissena* shell habitat could stimulate its successful invasion in Europe, in addition to environmental changes in European water bodies (Jazdzewski et al., 2002). It would support the invasional meltdown hypothesis, assuming that ecosystems become more susceptible to invasions, as the number of newcomers increases (Simberloff & Von Holle, 1999; Ricciardi, 2001). New immigrants benefit from the presence of other aliens, especially those originating from the same regions of the world, by finding suitable habitat, valuable food source or both. Such interactions seem to be more common than antagonistic ones, like competition or negative habitat alterations (Ricciardi, 2001). Zebra mussel plays an important role in such interactions, due to its gregariousness and strong impact upon environment (Ricciardi, 2001; Karatayev et al., 2002). Invasive gammarids, supported by zebra mussels, can themselves facilitate introduction of other aliens, e.g. neogobiid fish, being a considerable item in their diet (Grabowska & Grabowski, 2005; Kakareko et al., 2005).

Pontogammarus robustoides

Another non-indigenous gammarid of Ponto-Caspian origin, *P. robustoides*, did not show any significant preferences, neither for mussel shells nor even for any other solid objects on a flat surface (Experiment 1). That is why it was not tested in Experiments 2–6. This species is associated with very shallow sandy areas (Gruszka, 1999; Żytkowicz, unpublished data) or macrophytes (Bij de Vaate et al., 2002). In our experiments, these gammarids were usually found buried in sand covering the tank floor. Macrophytes were not tested in our study, while sand was present in the tanks around all kinds of the applied substrata. This may be a reason for the lack of choice made by *P. robustoides* in our

study. It was also possible that the plates in themselves were sufficiently good shelters for this species, independently of the quality of objects attached to them. Nevertheless, it does not seem to prefer shell habitat over other solid substrata tested in this study.

Gammarus fossarum

Similarly to *D. haemobaphes*, this native European species sought refuges among solid objects present on the flat substratum (Experiments 1 and 2). Surprisingly, it exhibited preferences for zebra mussels, although they were limited only to their empty shells. Contrary to *D. haemobaphes*, it selected all objects resembling mussel shape (Experiment 4), independent of their surface properties (Experiment 5). Avoidance of living mussels by this species, compared to dead shells, is rather difficult to understand. As the shell structure did not affect gammarid behaviour (Experiment 5), one can speculate that such factors as siphonal currents or byssal threads were involved in discrimination between living and dead mussels. However, explaining this phenomenon will need further studies.

Gammarus fossarum came into contact with zebra mussels at the beginning of the 19th century, when the latter species suddenly expanded its range in Europe. Such a comparatively short time may be sufficient for appearance of evolutionary changes in a population (Reznick et al., 1997; Kinnison & Hendry, 2001). On the other hand, it is possible that gammarids simply search for the most suitable shelter and a mussel shell shape, just by chance, predestines it for that purpose. This speculation is supported by the fact that varnished shells were as good substratum for this species as clean ones (Experiment 5).

The North American species, *G. fasciatus*, did not show any preferences for zebra mussel habitats, but selected equally all types of complex substrata, contrary to the Ponto-Caspian species, *E. ischnus*, that preferred mussel shells (Van Overdijk et al., 2003). In our study, we observed a positive response of a native gammarid to mussel shells, but, contrary to that of *D. haemobaphes*, it was based only on shell shape and not on its structure (Experiment 4 and 5). Furthermore, *G. fossarum*

did not respond positively to living mussels, but only to dead shells. Thus, its affinity for mussels was weaker than that of invasive *D. haemobaphes*.

Real advantages gained by the studied gammarids from living in a zebra mussel bed are still to be checked. Nevertheless, it is possible that a native species also can benefit from the presence of zebra mussels. Palmer & Ricciardi (2005) observed even the lower predation pressure on American *G. fasciatus* than on Ponto-Caspian *E. ischnus* in the presence of zebra mussels. On the other hand, Gonzalez & Burkart (2004) obtained opposite results: lower predation of fish on *E. ischnus* than on *G. fasciatus* in *Dreissena* shell habitat. It may be explained by the better ability of the Ponto-Caspian species, coevolving with the zebra mussel for a long time, to find a refuge among shells. Probably, the contrasting outcomes of these studies resulted from different predators involved in each of them, or from differences in experimental design (field versus laboratory study), affecting the quality of mussel bed refuges and their influence on examined gammarids.

Conclusions

We have demonstrated that a Ponto-Caspian gammarid, *D. haemobaphes*, actively selected zebra mussel shell habitat, guided by shell surface properties and shape. Another Ponto-Caspian species, *P. robustoides* does not show such preferences. The native European species, *G. fossarum*, also exhibits preferences for *Dreissena*, but limited only to dead shells. Shell shape is the main stimulus used by this species to find the suitable habitat.

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