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**Weak and strong invaders: which
biotic factors shape interactions among
Ponto-Caspian invasive gammaridean
species?**

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INTRODUCTION

Biological invasions in freshwater ecosystems in Europe are considered as one of major biological factors shaping the population dynamics of local biota (Wang et al. 2013). Acquiring knowledge about mechanics of this phenomenon is crucial for predicting further impacts on environment and in consequence becomes a challenge in conservation biology (Devin et al. 2003; Hesselschwerdt et al. 2008; Riel et al. 2009; Lambertini et al. 2011). Species novel to local ecosystems become an integrative part of the community, but within this process a considerable loss of biodiversity takes place. Native species are often replaced by their invasive functional equivalents and the share of native biota decreases (Kinzler and Maier, 2003). For the latest twenty years, European freshwater ecosystems have been vulnerable to constant intake of invasive species, specifically from the Ponto-Caspian region (Bij de Vaate et al. 2002). Artificial connections of inland waters made subsequent invasions of amphipods possible (Jażdżewski et al. 2002; Grabowski et al. 2003). Thus, such a violent process of enriching the biodiversity drastically decreased environmental capacity of European freshwaters. The case of Ponto-Caspian amphipod crustaceans is a particular interesting ecological case not only due to the vast biological changes in invaded ecosystems they have made up until now (Jażdżewski et al. 2004; Grabowski et al. 2007a; Bączela-Spychalska and Van der Velde 2013). Many amphipod species of Ponto-Caspian origin are characterised by similar ecological demands and represent similar behaviour and life history traits (Kley et al. 2003; Bączela and Konopacka, 2005; Grabowski et al. 2007b). It is proved that competitive reactions are in particular very intensive in the case of closely related species and sympatric Ponto-Caspian amphipods are considered as such (Chase and Leibold 2003). Based on the records of particular species, it was noted that some Ponto-Caspian species in the recent two decades have been replaced by others and this process is ongoing (Kinzler and Maier 2003; Kinzler et al. 2009). Thus, there is a supposition that in the act of negative interactions some Ponto-Caspian species are unable to coexist in a long run (Chase and Leibold 2003; Snyder and Chesson 2003). The aim of this thesis was to investigate interactions and possible biotic factors that could be agents modulating coexistence and dispersal of three most widespread Ponto-Caspian species *Dikerogammarus villosus* (Sovinsky, 1894), *Dikerogammarus haemobaphes* (Eichwald, 1841) and *Pontogammarus robustoides* (G.O. Sars, 1894). Each chapter presented in this thesis is focused on a different biotic factor that defines the boundaries of possible coexistence of the studied species. Species studied in this work differ in their ability to compete. The most successful invader is undoubtedly *D. villosus*, which often plays a role of a higher-level trophic predator

(summarised in Rewicz et al. 2014) that outcompetes weaker species. It is proved that the interspecific competition forces weaker species to migrate to less favourable habitats and this is expressed by their higher migration rate (Otsuki and Yano, 2014). Interestingly, according to historical records of appearance in Europe, *P. robustoides* and *D. haemobaphes* were spotted earlier than *D. villosus* (Bij de Vaate et al. 2002). It is likely that this might be the effect of negative interactions between those species, which resulted from excluding weaker competitors which eventually were forced to migrate to other, novel habitats. Such a statement, however, has not been confirmed by empirical studies so far. This thesis fills some gaps in knowledge about possible ecological mechanisms that stand behind the invasion of those animals. Ponto-Caspian amphipods are very similar regarding their microhabitat preferences, however there are some differences in aspect of biotic and abiotic environmental factors that determine their occurrence (e.g. nutrient richness, water flow) (Dedju 1967; Grabowski and Bączela, 2005). Thus, their temperature preferences were investigated to find whether this factor might determine coexistence of species by relocation leading to their spatial segregation among microhabitats. Indeed, temperature is an important environmental factor which may restrain the invasion success of alien species (Gherardi, 2007) but in the light of present global changes, may also facilitate the invasion (Rahel and Olden, 2008). However, thermal preferences of animals can be influenced by parasites which are capable of evoking a certain behaviour of their host (Casterlin and Reynolds 1977; Casterlin and Reynolds, 1979; Reynold et al. 1980). In general, parasites are often game changers on the ground of individual and population level, capable of shaping the structure and composition of the community (Hudson et al. 2006; Lagrue 2017). Ponto-Caspian amphipods are often highly infected with microsporidian parasites, which coevolved with them in their native region and undergo the invasion with their hosts to Europe (Berezina 2007; Grabowski et al. 2007a; Leuven et al. 2009; Grabner 2017). Infection caused by microsporidia influences behaviour of their host and contributes to changes in population structure (Wattier et al. 2007; Wilkinson et al. 2011; Bączela-Spychalska et al. 2012; Bączela-Spychalska et al. 2014; Grabner 2017). Thus, incorporation of microsporidia into study upon thermal preferences of Ponto-Caspian amphipods allowed to investigate both infection presence and temperature selection as agents capable of determining the possibility of coexistence of the studied amphipods. In aquatic environment, water itself is often an important information carrier. Thus, some animals rely predominantly on chemical signals (Ferrari et al. 2010) and infochemicals are used both in intraspecific and interspecific communication (Zimmer and Butman 2000; Krång and Baden 2004; Polo-Cavia 2009; Kobak and Ryńska 2014; Szokoli et al. 2015). The phenomenon of reciprocal recognition of Ponto-Caspian

amphipods is scarcely studied and the effect of signal interpretation is unknown. Bearing in mind the importance of this way of communication, it is likely that it may influence the alien amphipod community. Furthermore, mutual recognition may play a significant role in the invasion process, especially at the stage of establishment. Ponto-Caspian amphipods exhibit intra-guild predation and cannibalism (Dick and Platvoet, 2000; Kinzler and Maier, 2003) which suggests that proper identification of chemical stimulants may help assess whether the source of this scent should be treated as a potential threat or prey source (Chivers and Smith 1998).

Therefore, physical and chemical contact with competitors, thermal preferences and possible modulators of these factors – microsporidian parasites were chosen as important factors potentially affecting the intensity and direction of amphipod migrations. All ecological experiments were conducted with intention to define behaviour of each studied species while facing with those factors.

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The thesis consists of three articles: **Chapters 1, 2** have already been published. Chapter **3** is accepted to be published in regular issue of Aquatic Invasions and its corrected proof is available online on journal website – see appendix 1 in section supplementary materials.

Chapter 1: Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rates.

Kobak J., Rachalewski M., Bącela-Spychalska K. 2016. Biological Invasions 18:1953–1965. (MNiSW 35, IF=2.897, 40% of my participation in this article)

The chapter is based on results from microcosm ecological experiment. It was performed in an artificial system composed of a 140 cm long tank and additional equipment which allowed to investigate the migration rate and reciprocal displacement of three Ponto-Caspian amphipod species – *Dikerogammarus villosus*, *D. haemobaphes* and *Pontogammarus robustoides*. The aim of this study was to investigate whether competitive interactions among studied species affect their dispersal rate.

Chapter 2: Some like it hot: Factors impacting thermal preferences of two Ponto-Caspian amphipods *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841)

Rachalewski M., Kobak J., Szczerkowska-Majchrzak E., Bącela-Spychalska K. 2018. PeerJ 6:e4871 DOI 10.7717/peerj.4871
(MNiSW 35, IF=2.353, 50% of my participation in this article)

The chapter presents an ecological study in which thermal preferences of *D. villosus* and *D. haemobaphes* were studied using an artificial system consisting of cooling and warming equipment which created a temperature gradient across an experimental tank. The aim of this study was to investigate whether the sex and presence of intracellular microsporidian parasites influence thermal preferences of the studied species. Apart from performing ecological

experiments which allowed to achieve the main goal of this study, molecular techniques were used to determine the parasite presence in body cells of amphipods.

Chapter 3: Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods

Rachalewski M., Jermacz Ł., Bączela-Spychalska K., Podgórska M., Kobak J. *in press*.

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(https://www.reabic.net/aquaticinvasions/2019/ACCEPTED/AI_2019_Rachalewski_etal_corr ectedproof.pdf)

(MNiSW 100, IF=1.705, 45% of my participation in this article)

In this chapter the ability of *Dikerogammarus villosus*, *D. haemobaphes* and *P. robustoides* to recognize chemical scents of one another is presented and discussed. In order to verify the role of chemical communication, a Y-shaped experimental tank was constructed. Two distal arms of the tank were used to apply either scent or pure water used as a control whereas a proximal part of the tank was a place where animals were introduced and left for free migration towards one arm or another. The ultimate aim was to answer whether the studied species of amphipods express any reaction to perceived chemical odours of other species.

All studies were designed, executed and prepared mostly by me but dr hab. Karolina Bączela-Spychalska and dr hab. Jarosław Kobak contributed significantly to the final shape of each chapter, including design, production and presentation of results. Therefore, both are listed as co-authors of each chapter included here.

CHAPTER 1

Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rate

Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rates

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Abstract Ponto-Caspian gammarids have invaded European waters, affecting local communities by predation and competition. Their ranges and dispersal rates vary across Europe, which may result from their interspecific interactions, accelerating or reducing migrations. We checked this hypothesis by testing interference competition among co-occurring invaders: *Dikerogammarus villosus*, *D. haemobaphes* and *Pontogammarus robustoides*. We used 140-cm long tanks (gravel substratum), divided into seven compartments. We introduced 25 “residents” into the outermost compartment, separated with a barrier. After 1 h, we introduced 25 “intruders”. After the next 1 h, we removed the barrier and the gammarids dispersed in the tank. After 4 or 20 h, we counted the gammarids in the compartments. We tested all pairwise species combinations and single-species controls. *Dikerogammarus villosus* displaced other species (*P. robustoides* only after 4 h) and reduced its own motility after 20 h in their presence. *Pontogammarus robustoides* stimulated the short-time migrations of *D. villosus* intruders and of *D. haemobaphes*. As *P. robustoides* migrated

spontaneously much more than *Dikerogammarus* spp., its impact decreased after longer time. *Dikerogammarus haemobaphes* stimulated the short-time movement of *P. robustoides* intruders but reduced the long-time relocation of this species. In general, gammarid dispersal increased in the presence of stronger competitors (*D. villosus* and *P. robustoides*, especially residents) and decreased in response to weaker competitors (*D. haemobaphes*). Thus, competitive interactions may affect dispersal of invasive gammarids and contribute to the fastest spread of the weakest competitor, *D. haemobaphes* observed in the field, whereas the strongest species, *D. villosus* was the latest newcomer in many novel areas.

Keywords *Dikerogammarus* · *Pontogammarus* · Spatial partitioning · Species displacement · Migrations · Invasion potential

Introduction

Spatial distribution and co-occurrence of species at the microhabitat scale is a result of intra- and interspecific interactions (Waser 1985). The closer related species and the more their ecological niches overlap, the more competitive interactions can be expected, and their co-existence at the long time scale is suggested to be impossible even if a short time co-occurrence has been observed (Chase and Leibold 2003; Snyder and

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Chesson 2003). Individuals compete for the same resources which may be categorized as food or space including shelters against predators. Interference competition is observed when animals actively defend their inhabited territory including aggressive behaviour leading to injuries of the competitors (Schoener 1983). A specific type of this interaction is an intraguild predation (IGP) in which competitors representing the same guild prey on each other (Polis et al. 1989). It is known that asymmetrical IGP, when one of the species preys more efficiently on the other, strongly influences habitat selection by species in an ecosystem (Heithaus 2001). The stronger competitor and/or predator makes the weaker one to leave preferable habitats and shift to less favourable ones (e.g. Otsuki and Yano 2014). The outcompeted individuals increase their activity, even facing the risk of being preyed by a top predator or not finding an alternative suitable space to live (e.g. Shurin and Allen 2001). If the fragile species is not successful in finding a new territory, its population may decline, but in heterogeneous environments such interaction usually results in spatial partitioning of habitats by the species (reviewed in Amarasekare 2003). This avoidance of unfavourable conditions, including competition and/or predation is one of the main drivers of species dispersal (e.g. Ronce 2007). Thus, considering the above, one may expect the weaker competitor exhibits a higher dispersal rate. On the other hand, several studies showed that individuals in worse physiological condition started their density or food dependent dispersal later and moved over shorter distances than strong, healthy (thus more competitive) individuals (e.g. Bonte and de la Pena 2009; Delgado et al. 2010).

Biological invasions, which are nowadays one of the major threats to global biodiversity (e.g. Lamberini et al. 2011), are one of the greatest opportunities to study mechanisms and consequences of species dispersal (e.g. Van Riel et al. 2007, 2011). The invasive freshwater amphipods may serve as a model group for such studies. Several amphipod species with relatively similar ecological niches successfully spread into European waters (Bij de Vaate et al. 2002; Grabowski et al. 2007b). They did not colonize the same water bodies at the same time, and a sequence of invasions of particular species can be observed (e.g. Holdich and Pöckl 2007; Grabowski et al. 2007b). In most cases the newcomer significantly moderated the community

structure (e.g. Dick and Platvoet 2000; Jażdżewski et al. 2004; Krisp and Maier 2005; Kinzler et al. 2009; Chen et al. 2012; Truhlar and Aldridge 2015). In addition, several field studies showed spatial segregation of gammarid species, suggesting a habitat shift in the presence of related species (Kley and Maier 2005; MacNeil and Platvoet 2005; Hesselschwerdt et al. 2008; Żytkowicz and Kobak 2008; Platvoet et al. 2009; own unpublished data). Also experimental studies confirmed increasing swimming activity and movement of a “weaker” species (e.g. *Gammarus pulex*, *G. tigrinus* and *Pontogammarus robustoides*) to a different substratum in the presence of a stronger gammarid competitor (here *Dikerogammarus villosus*) (Van Riel et al. 2007; Platvoet et al. 2009; Jermacz et al. 2015a). These outcompeted taxa were also observed more often in a drift (Van Riel et al. 2011) or penetrating waters still not invaded by the other species, going upstream the river or entering affluents (Kley and Maier 2006), which might suggest their higher affinity for dispersal.

The influence of interference interactions on dispersal abilities of invasive amphipods has not been studied experimentally. We focused our study on three Ponto-Caspian gammarids (*Dikerogammarus haemobaphes*, *D. villosus* and *Pontogammarus robustoides*) which are widely distributed in Europe and their introduction has led to drastic changes in the macroinvertebrate community in colonized waters (Dick and Platvoet 2000; Arbaciauskas 2002; Jażdżewski et al. 2002, 2004; Berezina and Panov 2003). They are relatively large compared to native gammarids (Grabowski et al. 2007a) and exhibit several biological traits promoting their invasion, such as high reproductive abilities (reviewed in Grabowski et al. 2007a), opportunistic diet with effective predation (Platvoet et al. 2009; Bączela-Spychalska and Van der Velde 2013) as well as wide tolerance to ecological parameters (summarised in Bączela and Konopacka 2005; Rewicz et al. 2014; Bączela-Spychalska 2015).

Despite the fact that these species show slightly different preferences for hydrological conditions (Dedju 1980), they co-occur in many water bodies or were in contact in the past in native and invaded range (Carasu et al. 1955; Dedju 1980; Jażdżewski et al. 2004; Berezina 2007; Leuven et al. 2009). Although all the species could colonize new areas via similar routes (Bij de Vaate et al. 2002; Leuven et al. 2009),

they did not start to spread and establish at the same time. *Dikerogammarus haemobaphes*, which was the first *Dikerogammarus* species expanding its range via all possible migration corridors (Jażdżewski 1980), after its successful establishment faced the subsequent invasion of *D. villosus* in many European rivers: the Danube, Rhine, Oder and Vistula. The latter species outnumbered its congener very efficiently in most of these waters (Kley and Maier 2003; Müller and Hertel 2004; own unpublished data). On contrary, in the UK, *D. villosus* was recorded first (2010) and *D. haemobaphes* was notified in two year time (Environment Agency 2012). Interestingly, in this case *D. haemobaphes* is more widespread and seems to be more successful (Environment Agency 2012; 2013). *Pontogammarus robustoides*, which is very abundant in dam reservoirs, lagoons and lentic sections of rivers, e.g. the Vistula and Oder, cohabits many sites with the two *Dikerogammarus* species (Bącela and Konopacka 2005; Żytkowicz et al. 2008; own unpublished data). All three gammarids, specifically adults, in experimental studies have exhibited similar, strong preferences for stony habitats (Devin et al. 2003; Van Riel et al. 2009; Boets et al. 2010; Jermacz et al. 2015b). Thus, in the case of co-occurrence, they are likely to interact strongly with one another.

In this study we intended to check if variable dispersal rates and sequences of appearance of particular invasive Ponto-Caspian gammarid species in different parts of Europe depend on their reciprocal interspecific interactions at localities in which they co-occur. We hypothesized that weaker species would increase their dispersal rate after the introduction of a stronger competitor, particularly *D. villosus*, the largest species among them (Rewicz et al. 2014). On the other hand, the presence of related species of similar ecological demands may also indicate the suitability of a given location and result in the decrease of dispersal, particularly of stronger competitors. It is known that an individual occupying a territory (resident) is more likely to win an interference competition than an intruder, trying to seize it (Grafen 1987; Jennions and Blackwell 1996). Thus, we also hypothesized that the outcome of the interaction between gammarid species would depend on the sequence of their introductions to the environment, with the newcomer being less likely to establish at a cohabited site and moving further.

Materials and methods

Animals

We collected *Dikerogammarus villosus* and *Pontogammarus robustoides* individuals from the Włocławek Reservoir (the lower River Vistula, Central Poland), N 52°37'03", E 19°19'37" and *D. haemobaphes* from the Lucieńskie Lake, N52°29'46", E 19°26'44". In the laboratory, we placed them in stock tanks (temperature 19–22 °C) with aerated and filtered water and fed daily with frozen chironomid larvae and commercial fish food pellets. They were used in experiments 1–4 weeks after collection. We assessed gammarid sizes with ImageJ 1.40 g software (W.S. Rasband, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/i>), using photographs of 100 randomly selected individuals from each species. Mean body length \pm SD of the studied individuals was 14.2 ± 2.2 mm (*D. villosus*), 13.4 ± 2.6 mm (*P. robustoides*) and 11.8 ± 2.9 mm (*D. haemobaphes*), reflecting natural differences in body size between the species (Grabowski et al. 2007a).

Experimental setup

We conducted experiments in glass tanks 140 cm long and 15 cm wide, with the water level of 10 cm (Fig. 1). We divided the tank along its long axis into seven equal compartments and placed a Petri dish (diameter 12 cm, height 1.5 cm) filled with gravel (available commercially as aquarium substratum) in the centre of each compartment (Fig. 1). We measured the dimensions of 50 randomly selected gravel particles using ImageJ software. The gravel size, expressed as the means of the two perpendicular axes of the ellipses circumscribed on the particle shapes was 28.2 ± 3.6 mm. Gravel and stone substrata are known to be preferred by *Dikerogammarus* species (Van Riel et al. 2009; Boets et al. 2010) and *P. robustoides* (Jermacz et al. 2015b), thus we used this type substratum to induce a strong competition between them. We did not use other substrata as the three studied species show different preferences towards sand, macrophytes, etc. and this would lead to habitat partitioning and reduction of the competition, as shown by previous studies (Kley and Maier 2005;

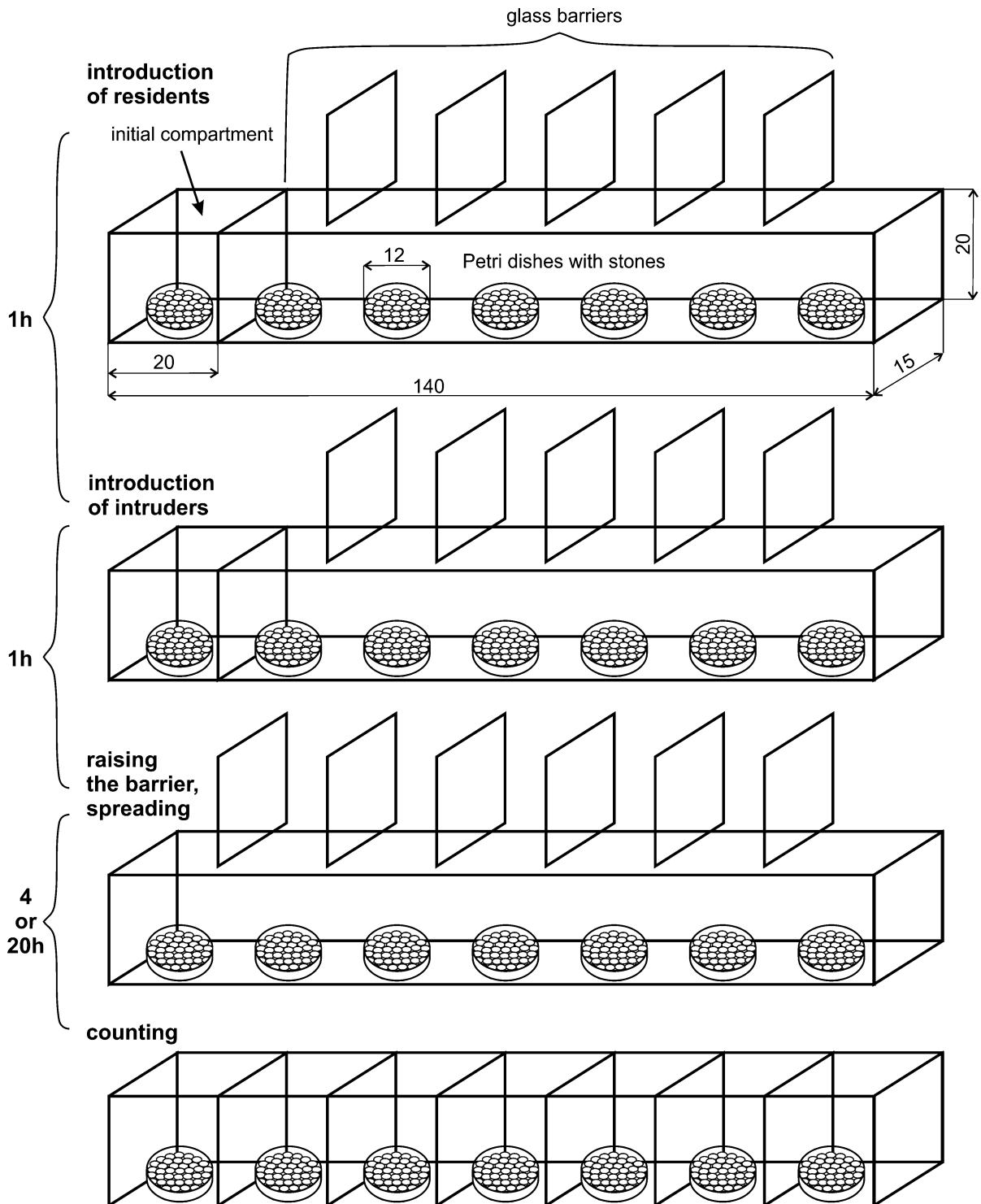


Fig. 1 Experimental tank and design. Dimensions are given in cm

Table 1 Two-way ANOVA of gammarid behaviour

Dependent variable	Species	Factor	df	MS	F	P
A % of gammarids in the first compartment	<i>P. robustoides</i> , all treatments	Accompanying species	4	0.12	9.87	<0.001
		Exposure time	1	0.09	7.40	0.008
		Interaction	4	0.10	8.00	<0.001
		Error	90	0.01		
	<i>D. villosus</i> , all treatments	Accompanying species	4	0.48	27.48	<0.001
		Exposure time	1	0.52	29.81	<0.001
		Interaction	4	0.11	6.38	<0.001
		Error	90	0.02		
	<i>D. haemobaphes</i> , all treatments	Accompanying species	4	0.32	25.77	<0.001
		Exposure time	1	0.42	33.78	<0.001
		Interaction	4	0.08	6.14	<0.001
		Error	90	0.01		
B Dispersal index	<i>P. robustoides</i> , all treatments	Accompanying species	4	2.41	8.18	<0.001
		Exposure time	1	0.83	2.81	0.097
		Interaction	4	1.76	5.98	<0.001
		Error	90	0.29		
	<i>D. villosus</i> , all treatments	Accompanying species	4	11.17	37.90	<0.001
		Exposure time	1	11.65	39.54	<0.001
		Interaction	4	2.63	8.93	<0.001
		Error	90	0.29		
	<i>D. haemobaphes</i> , all treatments	Accompanying species	4	10.07	48.34	<0.001
		Exposure time	1	8.90	42.72	<0.001
		Interaction	4	1.82	8.72	<0.001
		Error	90	0.21		
C % of gammarids in the first compartment	All species, single-species treatments	Species	2	0.37	18.58	<0.001
		Exposure time	1	1.23	61.96	<0.001
		Interaction	2	0.02	1.02	0.366
		Error	54	0.02		
D Dispersal index	All species, single-species treatments	Species	2	8.51	32.52	<0.001
		Exposure time	1	25.26	96.55	<0.001
		Interaction	2	1.09	4.18	0.021
		Error	54	0.26		

Impact of heterospecific gammarids on the behaviour of particular species (A, B) and behavioural differences among species in the single-species treatments (C, D)

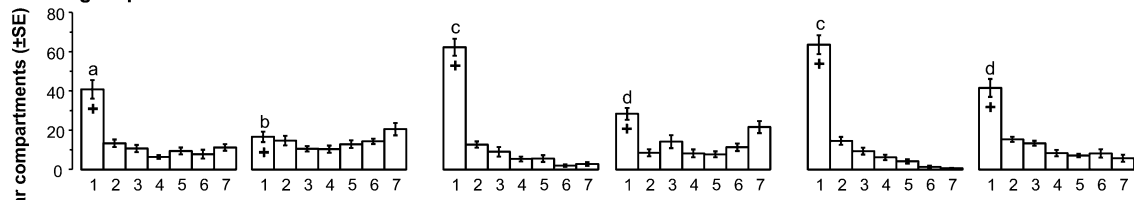
Jermacz et al. 2015a). On the other hand, in anthropogenically modified rivers, habitat heterogeneity is greatly impaired (Van Riel et al. 2007; Leuven et al. 2009) and possibilities of spatial segregation among

species are lower, which may result in different responses, such as long distance migrations. Therefore, we intended to check gammarid behaviour in a uniform habitat to simulate such conditions.

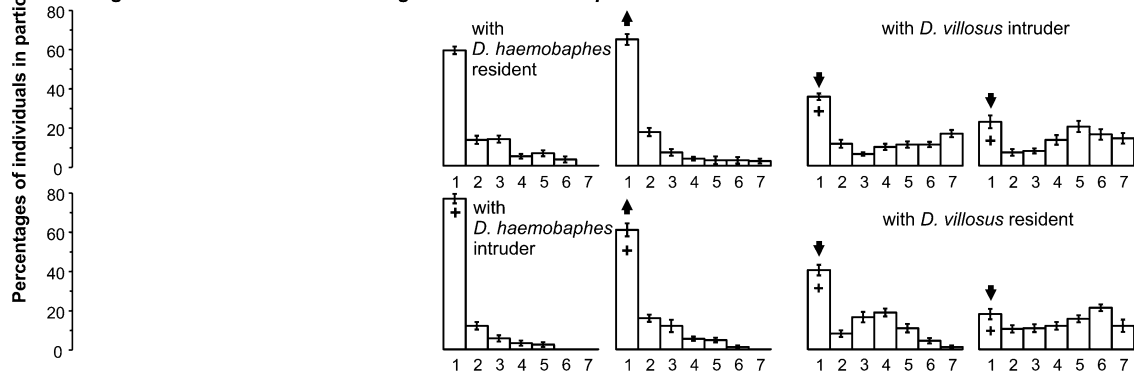
Dispersing species:

Pontogammarus robustoides short term long term *Dikerogammarus villosus* short term long term *Dikerogammarus haemobaphes* short term long term

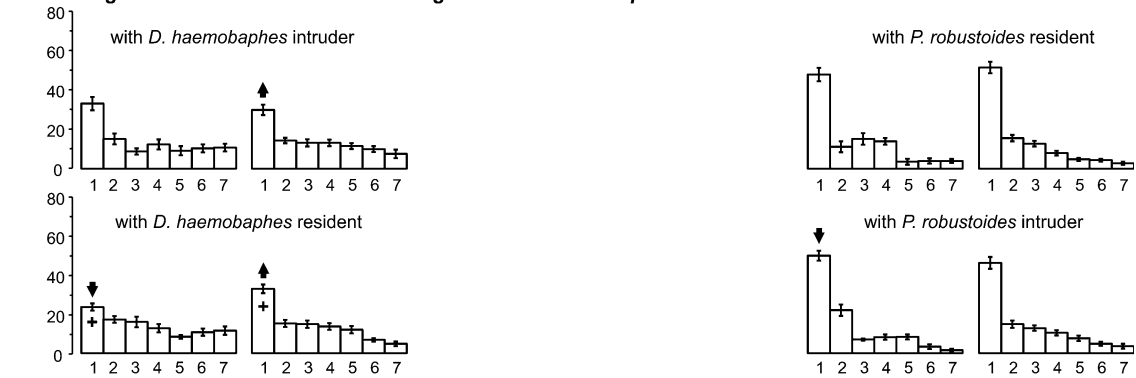
Single-species treatments



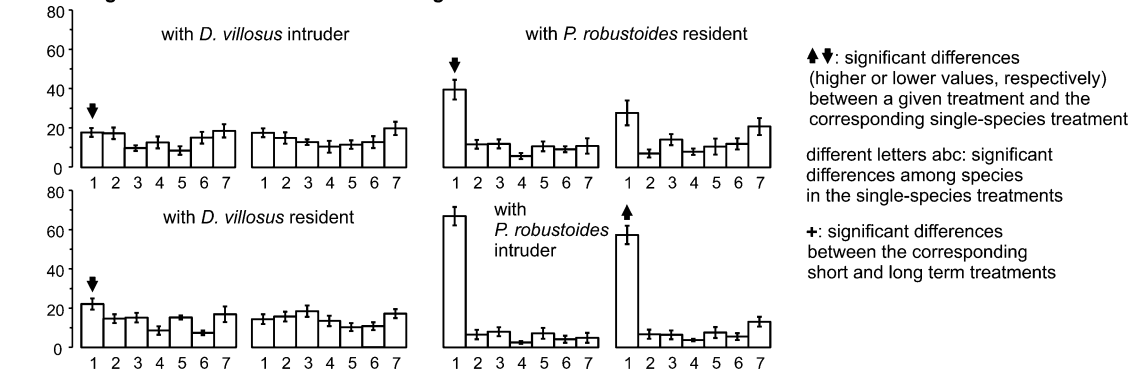
Dikerogammarus villosus vs. *Dikerogammarus haemobaphes* treatment



Pontogammarus robustoides vs. *Dikerogammarus haemobaphes* treatment



Pontogammarus robustoides vs. *Dikerogammarus villosus* treatment



▲▼: significant differences (higher or lower values, respectively) between a given treatment and the corresponding single-species treatment
 different letters abc: significant differences among species in the single-species treatments
 +: significant differences between the corresponding short and long term treatments

Consecutive compartments (1 - initial compartment to which the gammarids were introduced)

◀ **Fig. 2** Distribution of gammarids in the compartments of the experimental tank in the presence of various competitors. Behavioural responses of particular species are shown in consecutive columns, whereas particular treatments are arranged in the same rows. Accompanying species are indicated on the plots. *Arrows* pointing upwards or downwards indicate that in a particular treatment the percentage of gammarids in the initial compartment was significantly higher or lower (respectively) than in the corresponding control single-species treatment. *Plus* (+) signs indicate significant differences in gammarid percentages between the corresponding short and long term treatments. *Different letters* (a–c) above the *bars* of the single-species treatments indicate significant differences between the percentages of particular species in the initial compartment

Before the experiment, we separated the outermost compartment of the tank with a glass barrier and introduced 25 gammarid individuals, the “residents”, to this compartment. After 1 h, when the residents had established themselves in the substratum, we introduced the next 25 gammarids, the “intruders”. The

intruders were introduced to the substratum already occupied by the residents and had to outcompete them to take over the most suitable sites within the substratum. One hour is sufficient for gammarids to get used to experimental conditions and find a suitable shelter (Kinzler and Maier 2006). During subsequent manipulations, we observed that most of the individuals in our study were hidden in the substratum after 1 h, which confirms this statement. We removed the barrier after the next 1 h and allowed the gammarids to spread freely all over the tank for 4 h (short-term trials) or 20 h (long-term trials). Finally, we separated all compartments with glass barriers and counted the gammarids (Fig. 1).

We tested all pairwise combinations of gammarid species, replicated 10 times, as well as single-species controls (with the same species used as a resident and intruder). Each individual was used only once in the experiment.

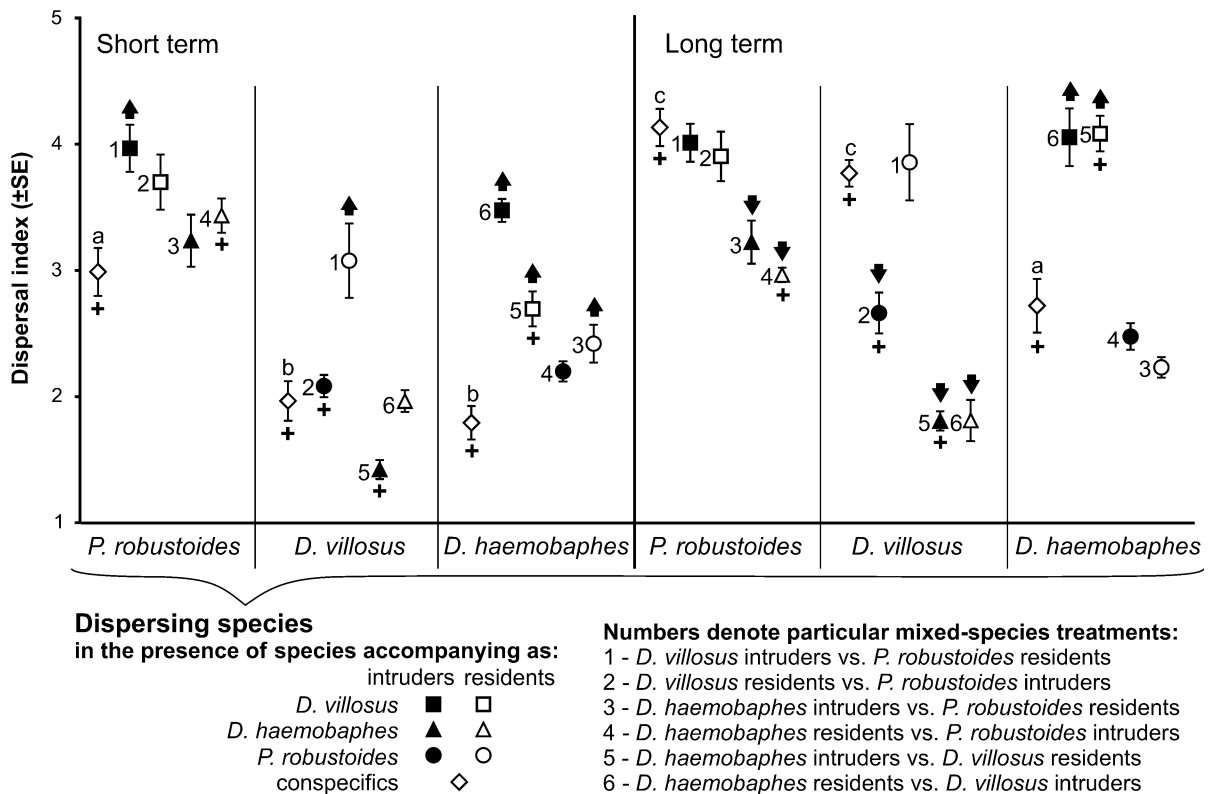


Fig. 3 Dispersal index showing the rate and distance of spread of gammarids from the initial compartment of the experimental tank in the presence of various competitors. *Numbers* 1–6

indicate particular mixed-species treatments. See Fig. 2 for the meanings of other symbols

We conducted the experiments in darkness. Water quality parameters (mean \pm SD), monitored with a multimeter Multi340i (WTW GmbH, Weilheim, Germany) were as follows: temperature 20.3 ± 0.66 °C, pH 8.0 ± 0.15 , conductivity 472 ± 41.7 μ S/cm, oxygen saturation 72.7 ± 8.46 %.

Data analysis

We analysed two dependent variables. The first variable was the percentage of individuals occupying the initial compartment of the tank, which allowed to determine the ability of gammarid species to displace one another from the occupied habitat. The second variable was a dispersal index (DI), calculated as the mean number of compartment occupied by all gammarids in the tank:

$$DI = \sum(N_i * i) / \sum(N_i)$$

where N_i is the number of gammarids in the compartment i and i is the consecutive number of the compartment (1–7, with the number of the initial compartment = 1).

Such an index allowed to analyse the dispersal of gammarids in the entire tank and to distinguish between gammarid displacement to the nearby habitat (compartment) and stimulation of spread over a longer distance.

Percentage data were arcsine square root transformed prior to the analysis. It was not possible to include all the data into a single model because the results of the mixed-species treatments were not independent of each other (both species were together in the tank), whereas those from the single-species treatments could not be paired. Therefore, we conducted separate two-way ANOVAs for each gammarid species to test the effect of (1) Accompanying species (5 variable levels: a single-species control and two other gammarid species used as residents and intruders) and (2) Exposure time (long-term and short-term trials). Moreover, we tested the differences in dispersal among gammarid species using a two-way ANOVA on the data from the single-species trials, with (1) Species (3 levels) and (2) Exposure time as factors. Significant ANOVA effects were followed by sequential-Bonferroni corrected t-tests used as a post hoc procedure.

Results

We did not observe any cases of intra-guild predation during the experiment, collecting the same numbers of individuals as had been introduced to the tanks. Behaviour of all gammarids depended on the accompanying species and exposure time, resulting in significant interactions between these factors in all analyses (Table 1A, B).

After the shorter exposure, individuals of *Pontogammarus robustoides* in the control treatment tended to remain near the place of their introduction. *Dikerogammarus villosus* forced them to move along the experimental tank, irrespective of the order of species introduction (Figs. 2, 3). The short-time effect of *Dikerogammarus haemobaphes* was similar to that of *D. villosus*, though less pronounced and limited only to the resident competitors and to the initial tank compartment (no significant effect for the dispersal index, Fig. 3). After the longer exposure, *P. robustoides* dispersed equally all over the tank in the control treatment and in the presence of *D. villosus*, but the presence of *D. haemobaphes* made them stay near their initial location relatively more often (Figs. 2, 3). Interestingly, the dispersal of *P. robustoides* in the presence of *D. haemobaphes* residents decreased significantly after the longer exposure compared to the short-time treatment, indicating that some individuals might return to the place of introduction after initial spreading (Fig. 2).

Dikerogammarus villosus did not respond to the short-term exposure to the other gammarids except their significant displacement by *P. robustoides* residents (Figs. 2, 3). After the longer exposure, individuals of *D. villosus* significantly decreased their dispersal in the presence of the other species and stayed in the initial compartment more often than in the control treatment.

After the short exposure, *D. haemobaphes* was forced to migrate along the tank by both heterospecific competitors, irrespective of their introduction status (Figs. 2, 3). After the longer time, only the effect of *D. villosus* remained significant.

Gammarid behaviour in the single-species treatments differed significantly among the studied species. Both *Dikerogammarus* species left their initial locations less often than *P. robustoides* at both exposure times, resulting in a significant species effect

in ANOVA (Table 1C). Also, all species were more dispersed after the longer exposure (Figs. 2, 3). However, after the longer exposure, *D. villosus* was distributed in the tank more uniformly than *D. haemobaphes* (Fig. 3), which remained mostly within the first three compartments (Fig. 2). This resulted in a significant species \times exposure time interaction in the dispersal index analysis (Table 1D). With regard to this index, the short-term dispersal of *P. robustoides* was greater from that of both *Dikerogammarus* species, whereas after the longer exposure the distribution of *D. haemobaphes* was more limited to a few first tank compartments than that of the other two species (Fig. 3).

Discussion

Gammarids are highly motile organisms, which migrate in response to a number of factors, including food and predator cues (Wisenden et al. 2009), water flow (Dennert et al. 1969) and competitor pressure (Jermacz et al. 2015a). Moreover, their dispersal is influenced by human vectors, including biofouling of artificial surfaces (Martens and Grabow 2008; Davidson et al. 2009), intentional introductions (Arbaciauskas 2002) and overland transport (Bączela-Spychalska et al. 2013; Bączela-Spychalska 2015). In our study, their dispersal rate differed between single-species and mixed-species treatments, which shows that the presence of related species is another factor influencing their movement and microhabitat occupancy. Our results support the hypothesis that, indeed, the weaker species is forced to leave its initial location and disperse more in the experimental tank. *Dikerogammarus villosus*, also called “the killer shrimp”, is a very strong competitor (Dick and Platvoet 2000; Van der Velde et al. 2000; Kinzler and Maier 2003; MacNeil et al. 2011), capable of outcompeting other species to less preferable habitats (Van Riel et al. 2007; 2009; Jermacz et al. 2015a). In our experiments, it successfully displaced *D. haemobaphes* in all treatments and *P. robustoides* in the short term treatments. It pushed out the other species even as an intruder, probably due to its aggression and active fighting for a shelter (Dick and Platvoet 2000; Platvoet et al. 2009). *Dikerogammarus villosus* can also eliminate other amphipods by intraguild predation

(Kinzler and Maier 2003; MacNeil and Platvoet 2005; Kinzler et al. 2009) but we did not observe any predation in our experiments, so this factor did not affect gammarid dispersal in our study.

The strong competition between two *Dikerogammarus* spp. could result from their similar dispersal habits (shown in single-species trials), facilitating interspecific encounters. Another reason may be a close phylogenetic relationship between them (Cristescu and Hebert 2005). Species from the same genus are more likely to compete with each other than those from different genera, which results in phylogenetic overdispersion, which is the tendency of co-occurring species to be less related with one another than expected by chance (Cooper et al. 2008; Cavender-Bares et al. 2009; Vamosi et al. 2009).

Pontogammarus robustoides also seems effective in shelter guarding, as it did not allow *D. villosus* intruders to settle successfully in the short term treatment. Moreover, it is as predatory as the killer shrimp (Bączela-Spychalska and Van der Velde 2013) and its aggression may also be similar (own observations). On the other hand, in the single-species treatments, *P. robustoides* was more active and “explorative” than *Dikerogammarus* spp. Truhlar and Aldridge (2015) observed that *D. villosus* was also relatively less active and explorative than other gammarids (e.g. *Gammarus pulex*). That is why *P. robustoides* in our study left the initial compartment faster than the other species and therefore its impact on the long-term dispersal of *D. haemobaphes* was lower than that of *D. villosus*. Such a strong tendency for spontaneous dispersal enables *P. robustoides* to test a variety of available microhabitats and finally find a suitable site in a patchy environment. It occurs on a wide range of different substrata, such as macrophytes, tree roots, mussel beds, sand or gravel (Gruszka 1999; Kobak and Żytkowicz 2007), whereas *Dikerogammarus* spp. are limited to large-grain, hard substrata or mussel beds (Boets et al. 2010). Thus, *P. robustoides* seems best adapted to locate new suitable sites in its neighbourhood and can avoid excessive competition by relocation to alternative microhabitats (Jermacz et al. 2015a). However, its long-distance spread is limited by its avoidance of flowing waters (Jażdżewski et al. 2002).

Dikerogammarus villosus and *P. robustoides*, which seem the strongest competitors, in the long

term single-species experiments spread more than in the presence of *D. haemobaphes*. It may be explained by defending local shelters by the stronger species in the presence of heterospecific interactions or by the weaker competition pressure from *D. haemobaphes* compared to that exerted by conspecifics. Interspecific competition is usually weaker than intraspecific competition (Connell 1983), and our results seem to be congruent with that hypothesis, though some exceptions from this rule do exist (Jermacz et al. 2015a).

Microhabitat heterogeneity allows competing species to co-exist when they can partition available space and avoid direct interactions (Hesselschwerdt et al. 2008; Platvoet et al. 2009; Koester and Gergs 2014). In our study, the gammarids could not switch to another microhabitat, but were able to avoid competition by dispersal. This reflects a common situation in highly regulated European rivers with decreased microhabitat diversity (Van Riel et al. 2007; Leuven et al. 2009), where weaker species would spread faster in the presence of stronger competitors. The outcompeted individuals may decline in a new area due to unsuitable local conditions, but their high invasive potential (a set of specific life history traits and wide ecological tolerance; Grabowski et al. 2007a) may allow them to thrive, leading to the faster increase of their range. A larger number of displaced individuals and/or more frequent displacement events would increase the probability of a successful invasion.

In the field, *D. haemobaphes* has often been observed to appear first, whereas *D. villosus* was the latest newcomer in most of the colonised water bodies. The spread of these species in the large part of the central migration corridor (the Dnieper, Prypiat, Bug and Vistula rivers, Bij de Vaate et al. 2002) followed that pattern. *Dikerogammarus villosus* appeared in the Vistula River system at least 4 years after *D. haemobaphes* (Konopacka 1998; Jażdżewski and Konopacka 2002; Jażdżewski et al. 2004; Grabowski et al. 2007b). In the Danube/Rhine river system, the invasion of both species was more simultaneous (Bij de Vaate et al. 2002), but *D. haemobaphes* more often spread to the affluents of the main rivers, whereas its congener was limited to the main rivers (Weinzierl et al. 1996; Kley and Maier 2003; own unpublished data). Such a pattern has usually been explained by the high dispersal rate of *D. haemobaphes*, being a “pioneer” species often colonising upstream the rivers

(Jażdżewski 1980). Our results, showing the weak dispersal of *D. haemobaphes* in the single-species treatments, shed new light on the history of invasion of the two *Dikerogammarus* spp., suggesting that competitive displacement may increase the dispersal rate of the weaker competitor and contribute to its fast spread. There, due to its highly invasive character, it can develop fast growing populations and outnumber native species (Jażdżewski et al. 2004; Grabowski et al. 2007a; Bącela-Spychalska and Van der Velde 2013).

A similar mechanism may help explain the invasion pattern of *Echinogammarus ischnus*, which was the first Ponto-Caspian gammarid colonising European inland waters (Jażdżewski 1980; Bij de Vaate et al. 2002). This species is also often outcompeted by later colonisers, such as *D. villosus* (Van Riel et al. 2006) and *P. robustoides* (Żytkowicz and Kobak 2008). Another example of that phenomenon is the case of invasive dreissenid mussels. The quagga mussel *Dreissena rostriformis bugensis* is a stronger competitor, gradually displacing the zebra mussel, *Dreissena polymorpha* when both species co-occur (Orlova et al. 2005), though the latter species is a faster colonizer with a wider range of occurrence in the early stage of invasion (Garton et al. 2013; Matthews et al. 2014).

Similar results of interactions between competitors (De Meester et al. 2014), as well as between intraguild prey and predators (Wang et al. 2013) were listed highlighting that the dispersal may be an efficient strategy leading to avoidance of adverse interactions between species. Interestingly, in most cases of biological invasions the fastest spreading species are regarded as the strongest competitors with the highest invasive potential (Lockwood et al. 2013). That is in contrast to the aforementioned phenomenon of the high dispersal rate exhibited by weaker competitors to decrease competitive pressure. Our results are congruent with the latter hypothesis, showing that negative interactions may promote spread of weaker species into novel areas, and thus contribute to their range extension.

Thus, we demonstrate two different potential mechanisms which may influence the dispersal of Ponto-Caspian gammarid species. *Pontogammarus robustoides* exhibits a natural tendency to explore new locations and spreads spontaneously even when

not affected by heterospecific competitors. *Dikerogammarus haemobaphes*, on the other hand, displays a strong affinity for the inhabited territory, but migrates in response to the presence of stronger competitors. Both these mechanisms lead to similar results: greater probability of appearance of alien species in novel areas, which in consequence increases their ranges and invasive potential. In contrast to these species, active migrations of *D. villosus* seem relatively slower, which may explain its later appearance in many invaded areas.

In accordance with the “invasional meltdown” hypothesis, positive interactions among various alien species facilitate their establishment in newly invaded areas and therefore contribute to the success of biological invasions (Simberloff and von Holle 1999). We demonstrate that negative relationships among invaders may stimulate their spread to novel areas, thus constituting another important factor promoting biological invasions and explaining the sequence of colonization of new locations.

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CHAPTER 2

Some like it hot: Factors impacting thermal preferences of two Ponto - Caspian amphipods *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841)



Some like it hot: factors impacting thermal preferences of two Ponto-Caspian amphipods *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841)

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ABSTRACT

Temperature is a crucial factor determining biology and ecology of poikilothermic animals. It often constitutes an important barrier for invasive species originating from different climate zones but, on the other hand, may facilitate the invasion process of animals with wide thermal preferences and high resistance to extreme temperatures. In our experimental study, we investigated the thermal behaviour of two Ponto-Caspian amphipod crustaceans—*Dikerogammarus villosus* and *Dikerogammarus haemobaphes*. Both species are known to live under a wide range of thermal conditions which may promote their invasion. Moreover, both these amphipods are hosts for microsporidian parasites which co-evolved with them within the Ponto-Caspian region and spread in European waters. As the presence of a parasite may influence the thermal preferences of its host, we expected to observe behavioural changes in infected individuals of the studied amphipods leading to (1) behavioural fever (selecting a warmer habitat) or (2) anapyrexia (selecting a colder habitat). The experiment ($N = 20$) was carried out for 30 min in a 100 cm. 20 cm from both sides were not available for amphipods long thermal gradient (0–40 °C), using 30 randomly selected adult amphipod individuals of one species. At the end of each trial, we checked the position of amphipods along the gradient and determined their sex and infection status (uninfected or infected by one of microsporidium species). *D. villosus* was infected with *Cucumispora dikerogammari* whereas *D. haemobaphes* was a host for *C. dikerogammari*, *Dictyocoela muelleri* or *D. berillonum*. Thermal preferences of amphipods depended on their species and sex. Females of *D. villosus* preferred warmer microhabitats (often much above 30 °C) than conspecific males and females of *D. haemobaphes*, whereas no significant differences were found among males of both species and both sexes of *D. haemobaphes*. Moreover, infected males of *D. villosus* stayed in warmer water more often than uninfected males of this species, selecting temperatures higher than 30 °C, which may be explained either as a behavioural fever constituting a defence mechanism of a host against the infection, or as a parasite manipulation of the host behaviour increasing the parasite

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fitness. On the other hand, none of the parasite species affected the thermal preferences of *D. haemobaphes*, including also *C. dikerogammari*, changing the behaviour of *D. villosus*. Our research presents the complexity of the thermal behaviour of studied amphipods and the evidence that microsporidia may trigger a change in temperature preferendum of their host species and those observations may be the result of different host-parasite coevolution time which may vary for the two host species (Poulin, 2010).

Subjects Animal Behavior, Ecology, Parasitology, Freshwater Biology

Keywords Sex, Microsporidia, Habitat selection, Parasite induced behaviour, Behavioural fever, Thermal behaviour, Behaviour modifications

INTRODUCTION

Temperature is the main variable determining multiple aspects of the functioning of poikilothermic organisms. It shapes their metabolic rate and thus their responses to other environmental factors, feeding, oxygen demands, growth rate and reproduction (Liu & Walford, 1972; Newell, 1966; Cox & Rutherford, 2000; Sardiña et al., 2017). In the aquatic environment, temperature can vary both vertically (due to stratification, usually defined as a change $>1\text{ }^{\circ}\text{C m}^{-1}$) (Wetzel, 2001), and, particularly in shallow waters, horizontally (due to variable shading and solar radiation), shaping oxygen concentration, food availability and decay rates. Moreover, daily and seasonal changes in temperature take place in water bodies (e.g., Irons III et al., 1994; Moore et al., 1997; Schindler, 1997). This gives aquatic organisms the possibility of adjustment and optimization of their microhabitat selection with regard to temperature conditions by behavioural thermoregulation (Macan, 1961; Cox & Rutherford, 2000). Temperature can also be an important factor determining the probability of success of alien species, being the main barrier for those originating from different climate zones (Gherardi, 2007), but also facilitating invasions due to global changes taking place in the present world (Van der Velde et al., 2002; Rahel & Olden, 2008). Inability to match local environmental conditions, with a leading role of thermal regime, is considered as a major reason of failures of alien species establishment in novel areas (Gherardi, 2007). Therefore, knowledge of thermal preferences of alien species is crucial for understanding their invasive potential. Among invasive aquatic organisms, crustaceans belong to the richest groups in terms of species number (Gherardi, 2007) and their recent spread can be associated with global warming (Stachowicz et al., 2002; Maazouzi et al., 2011; Hulme, 2017).

A thermal range within which animals are most frequently observed is regarded as the preferred one (Cossins & Bowler, 1987). Temperature preferences can be influenced by the presence of parasites or pyrogenic substances (Casterlin & Reynolds, 1977; Casterlin & Reynolds, 1979; Reynolds, Casterlin & Covert, 1980). A behaviour called behavioural fever, consisting in the selection of higher temperatures compared to uninfected individuals, has been reported in many invertebrate taxa (Elliot, Blanford & Thomas, 2002; Roy et al., 2006; Zbikowska & Cichy, 2012). The function of such behaviour is pathogen suppression or elimination (Roy et al., 2006). In contrast to behavioural fever, the inverted fever or anapyrexia is characterised by the change in thermal preferences to colder microhabitats

(Satinoff, 2011) which may result in the decrease in parasite growth rate or proliferation time. This form of defence against pathogens has been extensively studied in snails by Zbikowska (2004), Zbikowska (2005) and Zbikowska (2011) and Zbikowska & Cichy (2012). Up to date, invertebrate anapyrexia is better recognized as a response to low oxygen concentration (Morris, 2004; Gorr et al., 2010), but its role in parasite-induced infections cannot be excluded. In the light of the constant arm race in parasite-host interactions, both host organisms and parasites developed strategies to increase their fitness. Whereas a host organism benefits from preventing a parasite from utilization of its resources, parasites influence the behaviour of their hosts to complete their life cycle and/or stimulate their spread in the host population. Parasites are an important component of biological communities and exert a strong impact on their structure and composition (Hudson, Dobson & Lafferty, 2006; Lagrue, 2017). Thus, numerous studies show that the presence of some species in the community may depend on the action of parasites, which modify their fitness, interactions with other community members and behaviour (MacNeil et al., 2003; Dunn, 2009).

Amphipods, are one of the most important components of freshwater ecosystems (Piscart et al., 2009), often used as model organisms in ecotoxicological (e.g., Mehennaoui et al., 2016), phylogeographical (e.g., Grabowski et al., 2017) and behavioural studies, including host-parasite interactions (Rigaud, Perrot-Minnot & Brown, 2010; Petney, 2013). Like other crustaceans, they are poikilothermic organisms and temperature is a crucial abiotic agent influencing their functioning (Maranhão & Marques, 2003). They rely on behavioural thermoregulation (Lagerspetz & Vainio, 2006). Although behavioural mechanisms of thermal preference of amphipods and the impact of parasites on their thermal behaviour have been scarcely studied, there are some examples where their thermoregulation has been evidenced (Meadows & Ruagh, 1981; Timofeyev, Shatilina & Stom, 2001). Nevertheless, temperature has been reviewed by Sainte-Marie (1991) as a key environmental variable that rules reproductive bionomics of Amphipoda and therefore constitutes a major agent shaping their life history traits. Hence, for instance a negative relationship between temperature and maximum size of individuals has been reported by Panov & McQueen (1998). Additionally, it has also been shown that higher temperature results in producing more offspring, stimulates growth rate and influences amphipod activity (Maranhão & Marques, 2003; Becker et al., 2016). On the other hand, an interaction between high temperature and long day photoperiod may cause sex bias in a population by the emergence of intersexual individuals (Dunn, McCabe & Adams, 1996) and contribute to life cycle disturbances (Neuparth, Costa & Costa, 2001). Furthermore, the increase in temperature stimulates active brood care by females (Dick, Faloon & Elwood, 1998).

We considered Ponto-Caspian amphipod species as a perfect model for studies about temperature preferences due to their tolerance to relatively wide thermal regimes (Pöckl, 2007; Bacela, Konopacka & Grabowski, 2009; Rewicz et al., 2014), which is one of the features contributing to their invasion success. Furthermore, both within the invaded territories and their native range, Ponto-Caspian gammarids are infected with several sympatric microsporidian parasites (Wattier et al., 2007; Ovcharenko et al., 2008; Ovcharenko et al., 2009; Wilkinson et al., 2011; Grabner et al., 2015) which may influence their behaviour

(*Bacela-Spychalska, Rigaud & Wattier, 2014*) or induce sex ratio distortion (Green *Green Etxabe et al., 2015*). We have chosen two Ponto-Caspian invasive gammarid species: *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841) as model organisms. They are ecologically similar and share evolutionary and invasive history as they often co-occur in their native and invaded ranges (e.g., *Dedju, 1980; Berezina, 2007; Grabowski, Jazdzewski & Konopacka, 2007; Leuven et al., 2009*). Both *Dikerogammarus* species can be infected by several microsporidian parasites: predominantly *Cucumispora* spp. and *Dictyocoela* spp. (*Wattier et al., 2007; Ovcharenko et al., 2010; Bacela-Spychalska et al., 2012; Bojko et al., 2015; Grabner et al., 2015; Green Etxabe et al., 2015*). These species often reach extremely high prevalence within the populations of their hosts (*Ovcharenko et al., 2010; Bacela-Spychalska et al., 2012; Bojko et al., 2015; Grabner et al., 2015; Green Etxabe et al., 2015*). They differ with regard to their infection pattern (vertical and/or horizontal transmission) and effect on their hosts (e.g., *Wattier et al., 2007; Krebs et al., 2010; Wilkinson et al., 2011; Bacela-Spychalska et al., 2012; Bacela-Spychalska, Rigauld & Wattier, 2014; Grabner, 2017*).

We hypothesized that (1) no differences regarding temperature preferences would be observed between amphipod species due to their common life history and similar environmental requirements. Thus, experimental protocol was designed to perform experiments separately for both species to exclude interspecific interactions among them (*Kobak, Rachalewski & Bacela-Spychalska, 2016*), which could give a false impression of thermal preferences. Furthermore, we assumed that (2) selection of the most favourable temperature by gammarids, regardless of species, could be conditioned by sex. This is due to the fact that females might be attracted to higher temperatures to hasten their maturation and oocyte development (*Panov & McQueen, 1998*) which would result in their higher fecundity (*Steele & Steele, 1973; Sheader, 1983*). Finally, we hypothesized that (3) the thermal preference of host species would be changed in the presence of a parasite, either due to the host defence mechanism (behavioural anapyrexia or fever), or due to the host manipulation by the parasite.

MATERIALS & METHODS

Animal collection

The two *Dikerogammarus* species were sampled from stable populations at two independent sites in the Vistula River (Poland) at a similar water temperature (c.a. 16 °C) in July. *Dikerogammarus villosus* individuals were captured from the Włocławek Reservoir (the lower River Vistula, Central Poland, N52.617738, E19.326453) and *Dikerogammarus haemobaphes* were collected from the middle part of Vistula River near Połaniec town (N50.423014, E21.311748). Population structure and species composition at both sites are well recognized by our research group and species determination was based on clear differences in body proportions, appendage setation (especially antennae II), as well as the arrangement and size of the conical projections of the urosome (*Eggers & Martens, 2001; Konopacka & Jazdzewski, 2002*). Animals were brought from the field in 3-L plastic buckets with aerated water which were placed in styrofoam boxes filled with ice packs.

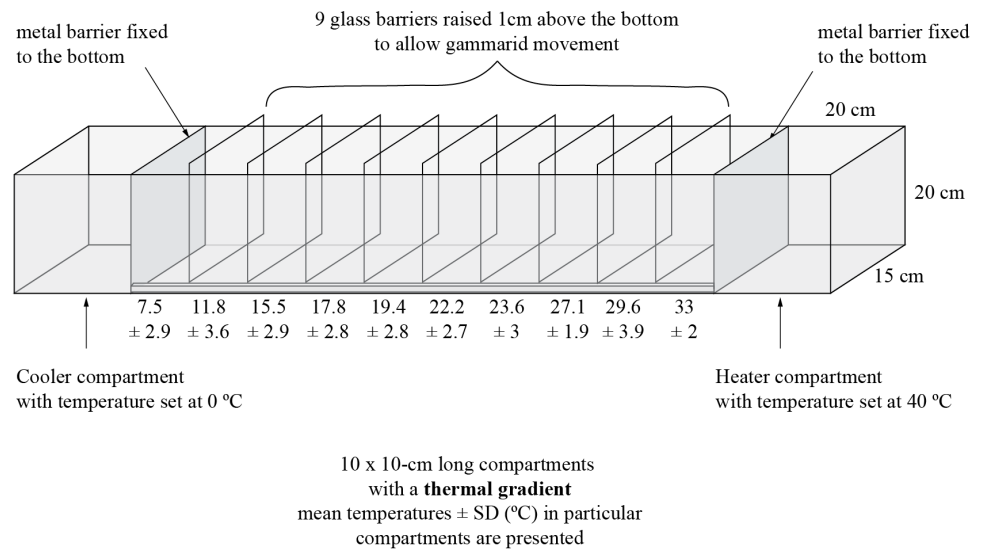


Figure 1 Experimental tank and design.

Full-size DOI: [10.7717/peerj.4871/fig-1](https://doi.org/10.7717/peerj.4871/fig-1)

Prior to the experiments, animals were acclimatized in an air conditioned laboratory for one week at 20 °C, which is considered as an optimal metabolic temperature (Brujijns *et al.*, 2001). Furthermore, we assessed the population structure, unveiling that the number of egg-carrying females was too low to include them as a separate group in statistical analyses. Knowing the life strategy of the both species (Pöckl, 2007; Pöckl, 2009; Bacela, Konopacka & Grabowski, 2009) we assume that in July all females showed high reproductive activity. Therefore, even if currently not carrying eggs, females were preparing for their release. Thus, all the females were pooled for the analysis as being likely to search for optimum conditions for oocyte/egg development.

Amphipods were fed daily with frozen chironomid larvae. After the acclimatization, the survival rate was high and animals did not exhibit any abnormal behaviour. We measured the lengths of 50 randomly selected animals under a stereoscopic microscope, from the base of the antennae to the tip of the urosome, with 0.1 mm accuracy. The mean body length was 13.2 mm (± 3.6 mm SD) and 11.2 mm (± 4.1 mm SD) for *D. villosus* and *D. haemobaphes*, respectively.

Experimental setup

The experiment was performed in a 140 cm long, 15 cm wide and 20 cm high aquarium (Fig. 1) filled with aerated dechlorinated tap water to the water level of 10 cm. At the opposite sides of the aquarium, 20 cm from both end walls, we installed aluminum barriers (enabling good heat transduction). In one end of the tank we placed a heater set to 40 °C and in the other end—a water cooler (Teco R20; Teco S.l.r. Ravenna, Italy) set to 0 °C. The remaining space within the aquarium was divided with glass plates into 10 compartments (10 cm long each), leaving a 1.5 cm gap at the bottom to allow amphipods to migrate freely along the tank. These incomplete barriers helped maintain a thermal gradient. The

bottom of each compartment was equipped with a digital thermometer (accuracy up to 0.1 °C). We ran the system for 40 min before animals were introduced which was enough for the thermal gradient to be established. Then, we introduced 30 randomly selected adult amphipod individuals of one species to the compartment where the temperature was the same as that in which the animals were kept in the laboratory. Animals were left in the experimental aquarium for 30 min to migrate towards their preferred temperature. At the end, the glass barriers were inserted down to the bottom to prevent further relocation of the amphipods, which were collected from each compartment and placed in separate vials with 96% EtOH for sex identification and molecular screening of microsporidia. Preliminary observations have indicated that gammarids did not relocate much after initial selection of a thermal zone, thus the determination of their final distribution at the end of exposure produced similar results as their continuous observation. The experimental procedure was replicated 20 times both for *D. villosus* and *D. haemobaphes*. Thus, 600 individuals of each species were tested in the experiment and, to avoid pseudoreplication, each individual was used only once in this research. Nevertheless, for the statistics we selected only individuals infected with a single microsporidium species. Thus, thermal preferences of 600 individuals of *D. villosus* and 568 individuals of *D. haemobaphes* were finally analyzed. Amphipods are highly gregarious animals ([Sornom et al., 2012](#); [Labaude, Rigaud & Cézilly, 2017](#)) and our experiment was designed taking this trait into account. That is why we decided to test them in groups. Thus, any interactions between the individuals in the experimental tank reflected their natural conditions. Nevertheless, the individuals after the experiment were not injured nor damaged, which suggested that antagonistic interactions were not common. In our study, we did not perform neither chemical nor physical analysis of water in the experimental tank. Preliminary tests and earlier experiments carried out in the same experimental setup ([Kobak et al., 2017](#)) showed that water quality (conductivity, pH, oxygen) was suitable for gammarids and did not change significantly during the trial. Oxygen concentration was not measured due to the risk of disturbance of the thermal gradient by handling the probe.

Microsporidium detection and identification

Microsporidian DNA was co-extracted with host DNA with Chelex[®] sodium procedure by Sigma-Aldrich company following the protocol of [Casquet, Thebaud & Gillespie \(2012\)](#). Afterwards, a PCR was conducted and a pair of microsporidia-specific primers V1f/530r (following [Baker et al., 1994](#) and [Vossbrinck et al., 1993](#)) was used to amplify distinctive parasite DNA fragments. PCR reactions were run in 10 µl reaction mixtures with each primer concentration of 400 nM, 200 µM dNTPs and 0.5 U/µl Promega Taq polymerase. The product was amplified under the following PCR conditions: an initial denaturing step at 95 °C for 2 min was followed by 35 cycles of 95 °C for 30 s, 62 °C for 45 s and 72 °C for 1 min. These cycles were followed by a final extension at 72 °C for 5 min. The PCR product was visualized on the 2% agarose gel in order to identify the infected individuals (positives). All the PCR products were purified with exonuclease I (Burlington, Canada) and FastAP alkaline phosphatase (Fermentas, Waltham, MA, USA) treatment and sequenced directly with the BigDye technology by Macrogen Inc., (Amsterdam,

The Netherlands) using the above mentioned primers. The obtained microsporidian sequences were edited using Geneious R10 (<http://www.geneious.com>, *Kearse et al., 2012*). Afterwards, identification of the microsporidia was determined using BLAST in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Data analysis

As both species were infected by different parasite species (see Results), we analysed their temperature selection separately, using a three-way General Linear Model with the following independent terms: (1) Infection (two levels: infected or not for *D. villosus*, three levels: two parasite species or uninfected for *D. haemobaphes*); (2) Sex; (3) Infection \times sex interaction and (4) Replicate, a random factor included to account for the assignment of various individuals to particular aquaria and thus avoid pseudoreplications. We used temperatures of aquarium compartments in which particular amphipods were found as a dependent variable.

Moreover, to check for the differences between both species, we compared uninfected individuals with a 3-way GLM with (1) Species; (2) Sex; (3) Species \times sex interaction and (4) Replicate (a random factor nested in Species) included in the model.

Post-hoc tests for significant effects were conducted using sequential-Bonferroni corrected Fisher's Least Significant Difference tests. Homoscedasticity and normality of data assumptions were checked with a Levene and Shapiro–Wilk tests, respectively. All the statistical tests were carried out with IBM® SPSS® 24.0 (Chicago, IL, USA).

RESULTS

Microsporidia species

Both *Dikerogammarus* species were infected with microsporidia; however the prevalence of microsporidiosis was higher in *D. villosus* (52%, $N = 600$) than in *D. haemobaphes* (27.4%; $N = 583$). The gammarid hosts differed in the parasite species composition: *D. villosus* was infected only with *Cucumispora dikerogammari*, whereas *D. haemobaphes* was infected by *C. dikerogammari*, *Dictyocoela berillonum* and *Dictyocoela muelleri*. Occasionally, there were mixed infections in *D. haemobaphes* by both *Dictyocoela* spp. and *C. dikerogammari* (13 individuals), and those cases were rejected from further analyses due to the risk of misinterpretation caused by ambiguous and diffuse influence of both parasites on their host. A detailed summary of microsporidiosis prevalence in the host species is presented in [Table 1](#). Sex proportion of animals used in experiments and presented as females:males ratio was 1:0.7 for *D. villosus* and 1:0.9 for *D. haemobaphes*.

Temperature preference of amphipods

The thermal behaviour of *D. villosus* ([Figs. 2A–2D](#)) depended on an interaction between sex and parasite presence ([Table 2, Figs. 2A–2D](#)). Post-hoc tests revealed that uninfected males preferred to stay at colder temperatures than other individuals, which mainly selected temperatures above 30 °C. On the other hand, uninfected males could be divided into two groups, one of them selecting low temperatures (<16 °C) and the other occupying high temperatures (>30 °C). We did not observe any significant effects of sex and parasite

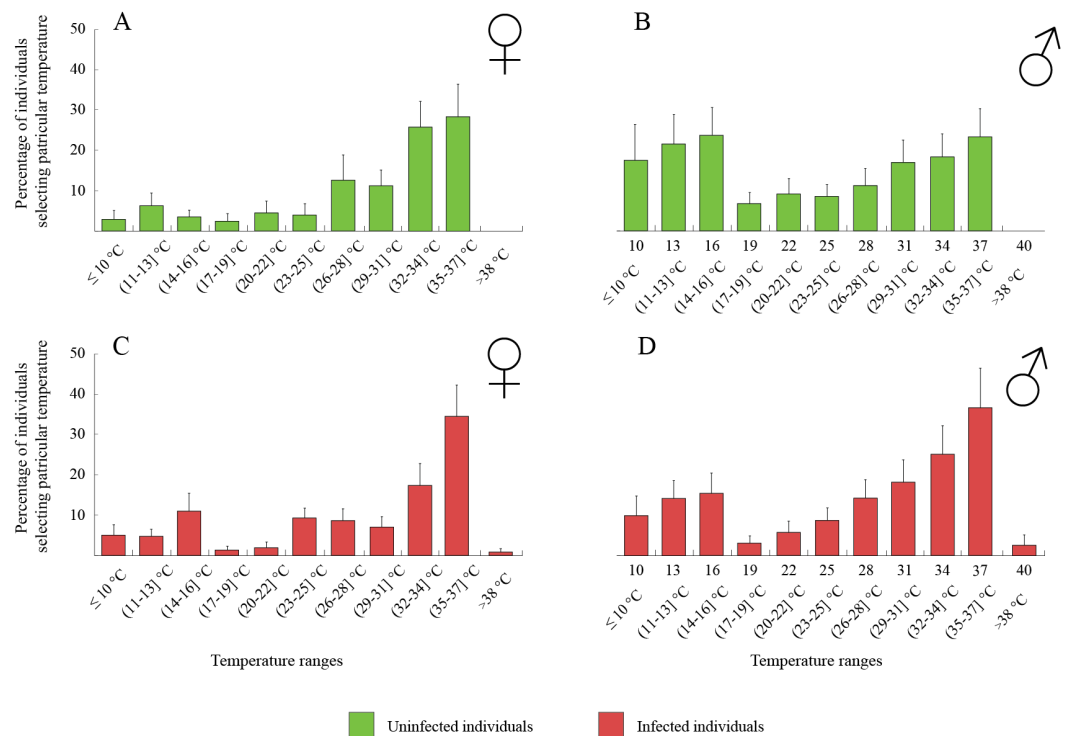


Figure 2 Temperature selection of *Dikerogammarus villosus* individuals in the thermal gradient with distinction of sex and infection presence. (A) represents total percentage of uninfected females, (C) infected females, (B) uninfected males and (D) infected males. Error bars represent standard errors of the means.

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Table 1 Percentage of uninfected host amphipods and prevalence of microsporidiosis caused by the following microsporidium species.

	<i>Dikerogammarus villosus</i>		<i>Dikerogammarus haemobaphes</i>	
	Males (N = 358)	Females (N = 243)	Males (N = 272)	Females (N = 296)
Uninfected	51%	44%	79%	72%
Infected with:				
<i>Cucumispora dikerogammari</i>	49%	56%	8%	12%
<i>Dictyocoela muelleri</i>	–	–	7%	7%
<i>Ddictyocoela berillonum</i>	–	–	6%	9%

presence or parasite species on the thermal preferences of *D. haemobaphes* (Table 2, Figs. 3A–3H), which selected a wide range of temperatures, generally similar or higher than their acclimation temperature (20–34 °C). Healthy females of *D. villosus* selected higher temperatures than female *D. haemobaphes*, whereas the preferences of uninfected males did not differ significantly between species (Figs. 2 and 3), resulting in a significant species × sex interaction in the analysis of thermal preferences of uninfected gammarids (Table 2).

Table 2 General Linear Model to test the impact of species, sex and parasite infection on thermal behaviour of the studied gammarids. Asterisks indicate statistically significant results.

Analysis	Effect	df	MS	F	P
<i>A. D. villosus</i>	Sex (S)	1	2038.1	26.81	<0.0001*
	Infection (I)	1	402.5	5.30	0.0220*
	Replicate	19	299.5	3.94	<0.0001*
	S × I	1	995.3	13.09	<0.0001*
	Error	578	76.0		
<i>B. D. haemobaphes</i>	Sex (S)	1	218.1	3.83	0.0508
	Infection (I)	3	52.8	0.93	0.4268
	Replicate	19	154.5	2.72	0.0001*
	S × I	3	81.8	1.44	0.2310
	Error	543	56.9		
C. Healthy individuals of both species	Species (Sp)	1	169.4	2.62	0.1062
	Sex (S)	1	2632.8	40.70	0.0000*
	Replicate	19	203.6	3.15	0.0000*
	Sp × S	1	575.9	8.90	0.0030*
	Error	620	64.7		

DISCUSSION

We demonstrated that *Dikerogammarus villosus* and *D. haemobaphes* differed with regard to their thermal preferences. Females of *D. villosus* preferred warmer habitats than females of *D. haemobaphes*. In fact, females of *D. villosus* often occupied the warmest part of the gradient, at temperature of more than 32 °C, likely to cause their death after a longer exposure (Wijnhoven, Van Riel & Van der Velde, 2003). This might have resulted from a thermal shock experienced by animals entering a lethal temperature zone, making them unable to move. Nevertheless, as they were always introduced to the gradient at the location thermally matching their acclimation temperature, they entered this zone by active movement. Thus, they were not repelled by extremely high temperatures. We suggest that both species may exhibit different strategies of temperature selection. Is it likely that *D. villosus* selects the highest available temperature, as also confirmed by Kobak et al. (2017), whereas *D. haemobaphes* avoids extreme temperatures. Under natural conditions, both strategies would possibly lead to a similar effect, i.e., occupation of warm microhabitats of suitable thermal conditions. This is because extreme temperatures, as those used in the warm part of our gradient, are missing in the environment where both species live. Nevertheless, under artificial laboratory conditions it was possible to detect interesting differences in the mechanisms underlying habitat selection by both species. Males of *D. villosus* preferred to stay in similar thermal conditions as *D. haemobaphes* (regardless of sex). Elevated temperature is especially beneficial for females because it accelerates growth and development rate and there is a positive correlation between female body size and fecundity (Bacela, Konopacka & Grabowski, 2009; Pöckl, 2007). Furthermore, during the reproductive period, large, receptive females are more attractive to males (Dick & Elwood, 1990; Borowsky, 1991; Krång & Baden, 2004). On the other hand, the higher

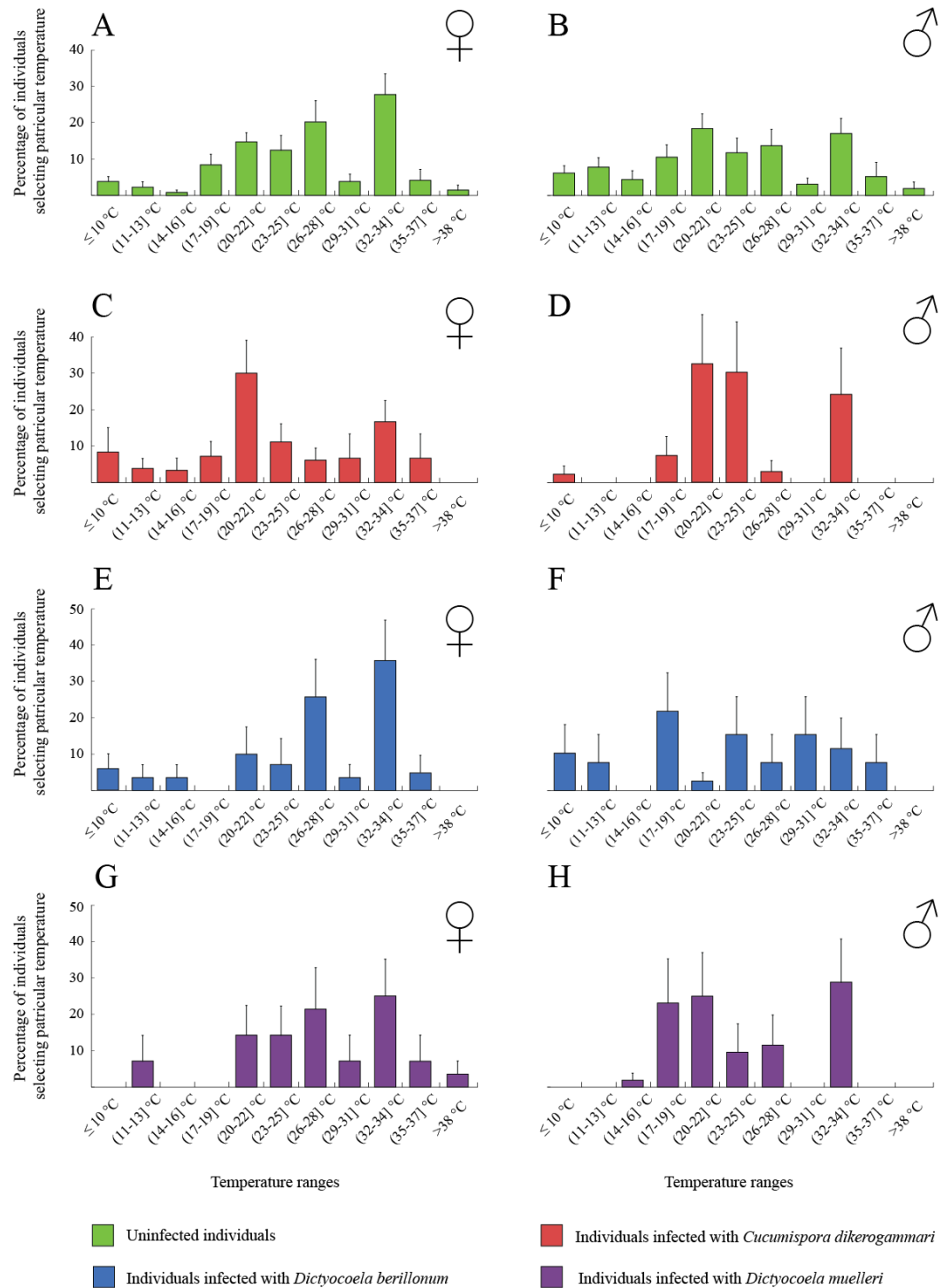


Figure 3 Temperature selection of *Dikergammarus haemobaphes* individuals in the thermal gradient with distinction of sex and infection presence. (A) represents total percentage of uninfected females, (C) females infected with *Cucumispora dikerogammari*, (E) females infected with *Dictyocoela berillonum*, (G) females infected with *Dictyocoela muelleri*, (B) uninfected males, (D) males infected with *Cucumispora dikerogammari*, (F) males infected with *Dictyocoela berillonum*, (H) males infected with *Dictyocoela muelleri*. Error bars represent standard errors of the means.

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metabolic rate at higher temperatures results in the higher oxygen consumption and increase in the frequency of respiratory pleopod movements (Dick, Faloon & Elwood, 1998; Wijnhoven, Van Riel & Van der Velde, 2003; Maazouzi et al., 2011). At the same time, due to the lower solubility of oxygen in water, the probability of oxygen deficiencies increases with temperature. Thus, temperature-based habitat selection must be a trade-off between the acceleration of maturation and energy expenditure associated with higher metabolic demands. Oogenesis is a more energetically expensive process than spermatogenesis (Bedulina et al., 2017), thus the faster metabolism at the high temperature is likely to stimulate the former to a greater extent. Perhaps that is why most of the females selected high temperatures, whereas males exhibited two opposite thermal strategies, selecting either cold or warm water.

Due to the high impact of ecological disturbances caused by *D. villosus* in invaded territories, its ecology and biological limitations are well studied. Hence, Wijnhoven, Van Riel & Van der Velde (2003) reported that critical temperature threshold for *D. villosus* is estimated at 31 °C whereas Maazouzi et al. (2011) showed in a short time experiment that the temperature tolerance is lower: 26 °C. However, it has been demonstrated that *D. villosus* has reduced tolerance to higher temperature in waters of low conductivity (Wijnhoven, Van Riel & Van der Velde, 2003). Interestingly, Hupalo et al. (2018) showed that the temperature of 27 °C induces a thermal stress for *D. villosus*, resulting in the production of heat shock proteins (HSP70). Nevertheless, Hupalo et al. (2018) proved that the resistance of *D. villosus* to thermal stress was different in two European genetically distinctive populations. This difference can be explained by different experiences of those populations, faced along two independent migration routes representing two different climatic-geographical regions. The western route is composed of the Danube, Rhine and the major French rivers, ending in the German river systems connected by an artificial channel with the Oder river in Poland. The eastern route is shorter and composed of the Dnieper, Prypiat, Bug and Vistula rivers. The eastern route is characterized with the harsh continental climate, whereas the western route is influenced by the calm Mediterranean and Atlantic climate. Hereby, Hupalo et al. (2018) found that animals migrating through the eastern route were less tolerant to high temperatures than those from the western route. However, although our experiments were performed on the population from the eastern route, individuals of *D. villosus* appeared to group at warm temperatures, often exceeding the stressful temperature of 27 °C (Hupalo et al., 2018). This supports our above-mentioned hypothesis on the strategy of choosing the highest available temperature, used by this species to select an optimum microhabitat. In contrast to *D. villosus*, *D. haemobaphes* tended to stay at intermediate temperatures in our gradient and seemed to be generally less selective with regard to temperature. Accurate data on temperature tolerance for this species are missing except those reported by Kititsyna (1980), who stated that it was capable of surviving in a wide range of temperatures (6–30 °C). However, until now there have been no experimental data on the thermal preferendum of this species. ”Both *Dikerogammarus* species preferred to stay at temperatures higher than that to which they were previously acclimatized. This is congruent with observations made on various crustacean species (Kivivuori, 1994; Chen & Chen, 1998; Lagerspetz, 2000; Díaz et al., 2002; Lamkemeyer, Zeis & Paul, 2003; Kobak et

al., 2017) whose thermal preferendum is also elevated in relation to an acclimatization temperature.

An important factor, which was expected to influence the thermal behaviour of amphipods was the presence of microsporidian parasites. Only in the case of *D. villosus* males were we able to detect the impact of microsporidian parasites on thermal behaviour: infected males tended to stay at higher temperature than uninfected ones. The observed temperature preferences could be an effect of either an action of the parasite or defensive response of the host to the infection. Hence, the explanation should be presented within such a dualistic scenario. This is because the sort of behavioural changes expected for a behavioural fever (a host act) may be similar to that expected if the parasite mediates the host behaviour for its own needs (Poulin, 1995). Behavioural changes triggered by parasitism in amphipods have been widely studied (e.g., Bethel & Holmes, 1973; Cezilly, Gregoire & Bertin, 2000; Lagrue, Kaldonski & Perrot-Minnot, 2007; Lefèvre *et al.*, 2009; Bakker, Frommen & Thünken, 2017; Labaude, Rigaud & Cézilly, 2017). Parasites which are transmitted to another host trophically (e.g., acanthocephalans) are particularly considered as inducing behavioural modifications of their hosts to increase their susceptibility of being preyed by another, final host (Holmes & Bethel, 1972; Lafferty, 1999). These modifications include changes in the preference to light, staying outside shelter and entering the water column (Cezilly, Gregoire & Bertin, 2000; Perrot-Minnot *et al.*, 2012). In our study, only the presence of microsporidium *Cucumispora dikerogammari* in *D. villosus* significantly modified amphipod behaviour. This parasite species relies mainly on trophic transmission and its development takes place mainly in its host muscles (Ovcharenko *et al.*, 2009; Bacela-Spychalska *et al.*, 2012). Therefore, its presence may lead to locomotion inability of amphipods (Fielding *et al.*, 2005). However, contrary to this observation, Bacela-Spychalska, Rigaud & Wattier (2014) found that amphipods were more active when infected by this parasite. Muscle necrosis is a result of the fast spore development in muscle tissue and consequently can be beneficial for the parasite transmission as the host becomes more vulnerable to predation by cannibalistic conspecifics. We suggest that analogous situation could be observed in our research, where infected individuals of *D. villosus* aggregated in warmer water where metabolic expenditure increases, which in the wild would likely lead to the decrease in their survival time. Hence, the higher mortality of host individuals may in turn increase the possibility of microsporidia to transfer to another host, as *D. villosus* often expresses scavenging behaviour (Dick, Platvoet & Kelly, 2002).

Another explanation for the preference of infected *D. villosus* males towards warmer water may be the host defence response, which seems to better explain the observed behaviour. It is known that microsporidium virulence and survival is limited in both high and low temperatures, but the value of this temperature may vary in different microsporidian species (Maddox, 1973). Although extensive studies of the temperature thresholds affecting those parasites in amphipod hosts are missing, there are however examples of such studies in other invertebrates (Raun, 1961; Olsen & Hoy, 2002; Martín-Hernández *et al.*, 2009). Undeen, Johnson & Becnel (1993) estimated the thermal tolerance of a microsporidium *Edhazardia aedis* infecting yellow fever mosquito (*Aedes aegypti*) as ranging from 0–40 °C. On the other hand, (Olsen & Hoy, 2002) showed that a temperature

of 33 °C in the aquatic environment is sufficient to cure the midge *Metaseiulus occidentalis* infected by microsporidia. [Weiss & Becnel \(2014\)](#) summarised on the basis of numerous studies that 35 °C is enough to severely reduce viability of microsporidian spores. As these are the values selected by infected *D. villosus* males in our study, it is possible that their observed affinity to higher temperature is a defence response to infection. Although animals present in the warmest compartments were less vital, it is possible that we observed a trade-off between a thermal stress for the host and possibility to decrease the viability of the parasite. Up to now, behavioural fever induced by microsporidia has been found in few invertebrates ([Boorstein & Ewald, 1987](#); [Campbell et al., 2010](#)). The thermal behaviour of *D. villosus* presented in this study could likely be considered as new evidence for behavioural fever in invertebrates. Interestingly, we did not observe any changes in the thermal behaviour of females of *D. villosus*. As we have demonstrated above, uninfected females of this species also selected warm water, so further increase in selected temperature by infected females was not possible.

None of the microsporidium species detected in *D. haemobaphes* affected thermal preferences of their host, including also *Cucumispora dikerogammari*, previously shown to change the behaviour of male *D. villosus*. The difference in responses between the gammarid species can be accounted for by their different habitat selection strategies, as suggested earlier, with *D. villosus* choosing the warmest available temperature and being generally more thermophilic than *D. haemobaphes*, which tends to avoid extremes. It may also be explained by the different host-parasite coevolution time which may vary for the two host species ([Poulin, 2010](#)).

Two other microsporidia infecting *D. haemobaphes*: *Dictyocoela berillonum* and *Dictyocoela muelleri* disseminate vertically (parental transmission to offspring), transovarially across maternal line ([Haine et al., 2004](#); [Terry et al., 2004](#)). Compared to *C. dikerogammari*, *Dictyocoela* spp. are supposed to be less virulent, as their transmission depends on the reproductive success of their host ([Fine, 1975](#); [Dunn & Smith, 2001](#)). They may lead to sex ratio distortion of *D. haemobaphes* population by feminizing the individuals ([Terry et al., 2004](#); [Green Etxabe et al., 2015](#)), which however has not been found in our study. The lack of any action of the infected host to eliminate the microsporidium could be explained by its costs.

CONCLUSIONS

In conclusion, we observed that *D. villosus* expressed a more complex thermal behaviour compared to *D. haemobaphes*. Both sex and microsporidian infection played an important role in the distribution of this species within a thermal gradient. Furthermore, our study showed that *D. villosus* was a more thermophilic species than *D. haemobaphes*. Using a temperature gradient we were able to unveil probable differences in temperature selection strategies between the tested amphipod species. We suggest different temperature preferences may contribute to the spatial segregation of these species and in consequence lead to the reduction in interspecific competition between them. The tendency to select high temperatures is likely to be especially beneficial when facing progressive climatic changes

(Gallardo & Aldridge, 2013; Kernan, 2015). Furthermore, *D. haemobaphes*, in contrast to *D. villosus*, is vulnerable to infection by more than one microsporidium species but, despite this, its thermal preferences are not affected by those parasites.

Knowledge about abiotic limitations of microsporidium species occurring in communities of invasive amphipods is particularly important for the conservation biology and for understanding the links between invasion patterns and disease transmission. This in consequence might help predict the impact of invaders on local biota (Prenter *et al.*, 2004). It has been shown that the transmission of spores infecting Ponto-Caspian amphipods to native species is possible (Bacela-Spychalska *et al.*, 2012, A Quiles, pers. comm., 2018).

Our study helps broaden the knowledge upon thermal preferences of two highly invasive species in Europe, taking into account an extremely important, inseparable factor affecting those animals—the presence of microsporidium parasites. Our results emphasize that the analysis of animal behaviour should be performed in such a wide perspective, especially because microsporidia are permanently associated with the invasive amphipod community in invaded territories.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Michał Rachalewski conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft, sequence editing and analysing.
- Jarosław Kobak conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

- Eliza Szczerkowska-Majchrzak authored or reviewed drafts of the paper, approved the final draft, laboratory work.
- Karolina Bącela-Spychalska contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft, sequence editing and analysing.

Data Availability

The following information was supplied regarding data availability:

The raw data are provided in a [Supplemental Information 1](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.4871#supplemental-information>.

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CHAPTER 3

Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipod

CORRECTED PROOF

Research Article

Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods

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Abstract

Chemical communication plays a significant role in the aquatic environment, enabling the mutual recognition of mates, predators and food items. Invasive species facing new communities are subjected to a huge variety of new scents. Their ability to recognize and interpret them adequately could be the reason of their successful establishment. We studied reciprocal chemical recognition among invasive freshwater Ponto-Caspian gammarids (*Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*) that often co-occur in native and invaded areas, to test their ability to avoid via chemical signals possible competitor and/or top intraguild-predator. We used a Y-maze with inflow of water to the Y-arms from two source tanks containing the signal (scent of living animals or the alarm cues released by crushed individuals) or a control (dechlorinated tap water), respectively. Time spent in the Y-maze zones, number of visits to the Y-arms and animal activity were analysed. Only *D. villosus* was attracted to conspecific and heterospecific alarm cues as well as to living heterospecifics. The other two species avoided the scent of *D. villosus*. We conclude that *D. villosus*, as the strongest competitor and intra-guild predator (including cannibalism) among the tested species, perceived various scents as potential food cues and posed a threat to other gammarids.

Key words: biological invasions, Ponto-Caspian gammarids, preference, avoidance, chemical communication, kairomones

Introduction

Interactions among species are often preceded by mutual recognition, based on the information sent by donors and received by recipients (Ferrari et al. 2007). Depending on the environment and the organism itself, stimuli can be chemical, visual or/and mechanical (Smith and Harper 2003). In the aquatic environment, animals rely predominantly on chemical stimuli (Ferrari et al. 2010) due to the low visibility, high density and high viscosity of the medium (Sehr and Gall 2016). Thus, the role of chemical communication cannot be underestimated and there are numerous studies confirming that different aquatic taxa respond to minute concentrations of

chemical substances released by other organisms (Brönmark and Hansson 2000). The composition of chemical signals is a highly diverse blend of compounds (Ingvarsdóttir et al. 2002; Rittschof and Cohen 2004), enabling a fine-tuned recognition of the identity of odour sources by recipient individuals. Infochemicals are used for intraspecific communication in reproductive behaviour (Krång and Baden 2004), aggregation forming (Zimmer and Butman 2000), territorialism (Jaeger and Gergits 1979), signalling the presence of a predator to conspecifics (Kobak and Ryńska 2014), and in interspecific interactions, such as identification of predators (Szokoli et al. 2015), food detection (De Lange et al. 2005) and assessment of the strength of interspecific competition (Polo-Cavia et al. 2009).

A specific situation takes place when alien species colonize a novel habitat. They form new interactions with local biota and can re-create reciprocal links among organisms originating from the same region (Strauss et al. 2006). Progressive biotic homogenization followed by subsequent invasions of alien species constitute a perfect model for studying mutual recognition among them since, as a consequence, it might facilitate the establishment of subsequent invaders. For example, this may be due to the increased possibility to detect “known” food sources, enemies or interspecific alarm signals.

Although the chemical nature of these signals perceived by peracarid crustaceans is scarcely known, their effects have been extensively studied from the aspect of sexual behaviour and food path detection (Thiel and Breithaupt 2011), but less so from the aspect of competition avoidance and antipredator responses (Baumgärtner et al. 2003; Hesselschwerdt et al. 2009). Despite the chemical communication within an invasive community in a newly invaded territory being studied by several authors (Acquistapace et al. 2004; Corkum and Belanger 2007; Jermacz et al. 2017b), knowledge about this phenomenon is still insufficient. We studied the reciprocal recognition of chemical stimuli released by three species of freshwater gammarid crustaceans (Amphipoda, Gammaroidea) originating from the Ponto-Caspian region: *Dikerogammarus villosus* (Sowinsky, 1894), *Dikerogammarus haemobaphes* (Eichwald, 1841) and *Pontogammarus robustoides* (G.O. Sars, 1894). These species have colonised all the main rivers of Europe outside their native range (Grabowski et al. 2007b) and often co-occur in their native and invaded waterbodies (Jażdżewski et al. 2004; Berezina 2007; Leuven et al. 2009). They share over one million years of co-evolution and geological history, consisting of widely varying environmental conditions that have shaped their plasticity, which has consequently contributed to their invasion success (Reid and Orlova 2002). Thus, bearing in mind their common history, the communication among these species may increase their fitness in their new range (Jermacz et al. 2015; Kobak et al. 2016; Jermacz and Kobak 2017). These Ponto-Caspian gammarids are omnivorous, occupying variable trophic positions in

various communities (Hellmann et al. 2015), but many studies have pointed out their strong inclination for being carnivorous (van Riel et al. 2006; Platvoet et al. 2009; Bacela-Spychalska and van der Velde 2013). They exhibit intra-guild predation (IGP), feeding on related species (Kinzler and Maier 2003), as well as cannibalism (Dick and Platvoet 2000). Chemical interactions among aquatic crustaceans seem to be complex and difficult to predict. They can simultaneously perceive both hetero- and con-specifics as potential predators, prey, competitors and/or sources of alarm cues in addition to detecting conspecifics as sexual receptive mates (Wudkevich et al. 1997; Chivers and Smith 1998; Dunn et al. 2008; Thiel and Breithaupt 2011). Proper identification of available information allows an individual to assess the cost/benefit trade-off between multiple chemical cues, which announce the presence of other species as either prey or a potential threat (Chivers and Smith 1998).

We conducted Y-maze experiments to determine gammarid responses to various conspecific and heterospecific scents. We applied scents of live or crushed animals to simulate the presence of an active predator that may be detected as alarm cues released from injured individuals (Sih 1986; Wudkevich et al. 1997; Chivers and Smith 1998; Abjörnsson et al. 2000). We hypothesized that *D. villosus*, regarded as the strongest competitor and very efficient intraguild predator among the studied species (Rewicz et al. 2014), would respond positively to heterospecific signals; identifying them as weaker, potential prey. Furthermore, the presence of another species may provide information about a suitable shelter since all the tested species share the same preferences towards substrata – gravel and stones (Boets et al. 2010; van Riel et al. 2009; Jermacz et al. 2015). This hypothesis is congruent with the outcome of an experimental study upon induced dispersal of Ponto-Caspian amphipods, where displacement of weaker competitors was observed (Kobak et al. 2016). Moreover, we expected a *D. villosus* scent would be avoided by the other species, which could contribute to the reduction of direct negative interactions; such as competition and predation (Jermacz et al. 2015). Furthermore, we assumed that the alarm cues released by injured conspecifics, and possibly also by related species, should elicit an avoidance reaction. However, attraction of predatory and cannibalistic species to alarm signals may be regarded as a response to potential food stimuli (Jermacz et al. 2017b).

Materials and methods

Animals

We collected *Dikerogammarus villosus* and *Pontogammarus robustoides* from the Włocławek Reservoir (lower Vistula River, central Poland) (52°37'03"N; 19°19'37"E). *Dikerogammarus haemobaphes* was obtained from the Lucieńskie Lake situated in the Vistula valley (52°29'46"N; 19°26'44"E). The sampling was conducted in May–June 2014. Animals

were transported to the laboratory in 10 L plastic containers with aerated water. In the laboratory, we placed them into stock tanks with aerated and filtered water at 18 °C (sustained by air-conditioning) and fed them daily with frozen chironomid larvae and commercial fish food pellets. We kept the animals in separate tanks before experimentation to avoid their habituation to the scents of heterospecifics. This allowed us to observe their real preference for or avoidance of the applied cues. They were used in experiments 1–4 weeks after collection. Preliminary observations confirmed that gammarids survived well during this period and did not exhibit any behavioural symptoms of exhaustion due to prolonged captivity. We assessed the size of 100 randomly selected gammarids of each species with ImageJ 1.40 g software (W.S. Rasband, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/i>), using freeze-frames from video recordings. Measurements were made from the base of the first pair of antennae to the end of the urosome in still images. We chose adult individuals within the mean body lengths of $16.2 \pm \text{S.D. } 2.4$ mm (*D. villosus*), 14.8 ± 2.8 mm (*P. robustoides*), and 13.6 ± 2.4 mm (*D. haemobaphes*), which reflected natural differences in body size between the species (Jazdzewski 1975; Grabowski et al. 2007b). We used individuals of both sexes (receptive ones and those used to prepare species odours, see below) as we aimed at studying reactions at the species level, excluding the impact of sex on the behaviour. Our field work was performed in accordance with the Polish Water Law, which considers all lotic waters in Poland as public and provides Polish citizens unhindered access to them. Our study was performed only on invasive invertebrates and did not involve any endangered or protected species. Therefore, no institutional permits were necessary.

Experimental setup

We conducted our experiments in a glass Y-maze constructed according to an established design (Baumgärtner et al. 2002) (Figure 1) used in various other behavioural studies (Lee 1992; Pearl et al. 2000; Jutfelt et al. 2017; Johannesen et al. 2017). Gravitational inflow of water (0.5 L min^{-1}) to the Y-arms was provided by plastic tubes (12 mm in diameter) from two separate 40 L source tanks containing appropriate signals or control water. The suitability of the Y-maze was preliminarily tested with dyes, which were applied to the source tanks in order to investigate the distribution of currents in the arms. This provided us with a time needed for the signal to pass through the arms. These tests determined that the water currents in both Y-arms were independent of each other and it only took several seconds for the dye to pass through the Y-maze arms. The water currents from the arms mixed only in the mixing zone. This observation has been further confirmed by the results of an earlier test, where water of various salinity was added to the source tanks of a Y-maze of the same design (Kobak et al. 2017). Kobak et al. (2017) showed that both Y-arms clearly

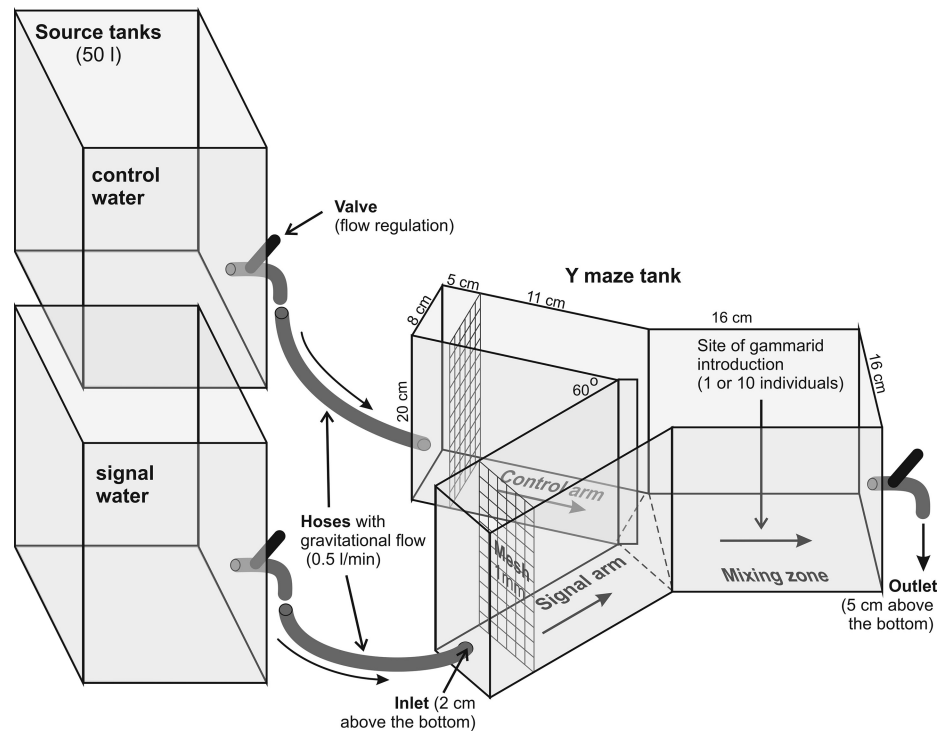


Figure 1. A diagrammatic representation of the Y-maze tank, including any accessories, used in the experiments. The arrows with full tips indicate the flow direction of the liquid containing the scent or control.

differed in salinity level, with the mixing zone having an intermediate concentration of ions. In order to keep the same flow rate and concentration of infochemicals, water in the source tanks was replaced and replenished to the initial level. Fresh signal was added to the signal tank before each replicate. After each trial, the source tanks were dried and washed with 96% ethanol before proceeding. The signal and control arms were switched between each replicate. The outlet from the mixing zone was covered by 1 mm mesh preventing tested animals from leaving the tank. Both inlets were separated from the part available for gammarids by mesh barriers which prevented the gammarids from moving upstream and also provided them with shelters. Homogenous indirect light conditions (520 lx) were established and controlled with a light meter (L-20A, Sonopan Ltd., Białystok, Poland). Water quality parameters (mean \pm SD), monitored with a multimeter Multi340i (WTW GmbH, Weilheim, Germany) were as follows: temperature: 17.7 ± 0.7 °C, pH: 7.69 ± 0.3 , conductivity: 473 ± 8.64 μ S/cm, oxygen saturation: $80.7 \pm 2.5\%$. Similar values were measured in the stock tanks.

Water containing living gammarid signals was obtained by incubation of ca. 400 individuals of a given species in a filtered and aerated 200 L tank filled with dechlorinated tap water (added to the tank 24 h before the start of the incubation) for 24 h at 18 °C. Gammarids in the incubation tank were not fed to avoid food cues in the signal water. The entire source tank was filled with water from the incubation tank. The control water was dechlorinated tap water prepared according to the same procedure as described above, but without any gammarids. The alarm cues of injured

gammarids were obtained by manual crushing of 20 individuals with 10 ml of control water. Such a mixture was filtered (40 μm gauze) and immediately added to the source tank filled earlier with control water. To adjust the experimental setup, preliminary tests were performed using established numbers of incubated and crushed individuals (Jermacz et al. 2017b), as well as an incubation time sufficient to obtain active infochemicals.

The signal water or alarm substance was added to the source tank immediately before each trial, ensuring the activity of the chemical signal. We tested each species in the presence of one of the above-mentioned cues (2 types of cues: 3 alarm signals and 3 scents of living animals, obtained from individuals of *D. villosus*, *D. haemobaphes*, *P. robustoides*) in one of the Y-arms, with control water in the other. We also conducted a control treatment with control water in both arms of the Y-maze to check for equality. Such a design allowed us to test whether gammarids responded to the detection of a signal positively, negatively or not at all. We intended to detect the presence of gammarid responses to particular signal sources, rather than their relative strength and/or interactions, thus we did not conduct treatments with two different gammarid signals in the Y-arms.

After stabilizing the flow, we introduced 10 gammarids into the mixing zone of the Y-maze. Each experimental trial lasted for 35 min, including 5 min for adaptation and 30 min of behavioural observations. Our experimental protocol was adjusted to the results of a study that estimated the activity time of gammarid chemical cue as approximately 3 h (Wisenden et al. 2009). Nevertheless, we applied the signal water immediately after it was prepared to reduce the risk of degradation. The experiment was recorded using an IP video camera (SNB-6004, Samsung, South Korea) placed above the tank. The experiment was replicated 10 times for each signal type.

In each treatment, we tested 10 groups of 10 gammarids according to the protocols of similar experiments already performed by other authors (Jermacz et al. 2017b; Wisenden et al. 2009). We were not able to follow individuals separately due to technical difficulties, but our experimental protocol allowed us to obtain the mean activity of the 10 gammarids. Group testing allowed us to avoid the influence of abnormal behaviour of particular individuals and the effect of their “personality” on the results (Kaldonski et al. 2007). Furthermore, amphipods always occur in high densities reaching up to a few thousand ind/m² (Dedju 1967). Thus, the experiment was set with respect to their natural conditions, since being alone would have been a deviation for those animals. It should be noted that in our experiment, when a group of gammarids were exposed to the conspecific cue, it was possible that the scent from the source tank (and that of the other individuals being tested) may have interfered with each other, weakening gammarid responses to the cue from the Y-arm. Nevertheless, tests with healthy conspecific signals were necessary to check whether gammarids reacted differently to conspecific and heterospecific

scents of particular taxa, rather than responding similarly to a general “gammarid cue” released by any species. Every individual in the experiment was used only once to avoid pseudoreplication.

Data analysis

We analysed the video recordings using Noldus Ethovision® XT 10 software. The individuals present in a single replicate were not independent of one another and the software sometimes switched the identities of the individuals that had touched during the experiment. To avoid pseudoreplications, all 10 individuals used in a single replicate were treated as a unit. Cumulative values (summed up for all group members) for each replicate were divided by 10 to obtain the average behaviour of gammarids in the group and were used as data points in any subsequent analyses (Jermacz et al. 2017b).

We assumed that gammarids sensing a cue from the signal zone would change: (1) the amount of time spent in this zone (relative to the control zone) and (2) the number of visits to the zone. We calculated an electivity index based on the Ivlev’s index (Ivlev 1961):

$$E = (R_s - R_c) / (R_s + R_c)$$

where R_s and R_c are the values of a response variable measured in the signal and control Y-arms from a given replicate, respectively. This approach considers the difference relative to the overall magnitude of gammarid behaviour (occupation time or number of visits to both Y-arms). Positive and negative difference values would indicate a preference for, or avoidance of, the signal. For the control treatment, we randomly assigned the Y-arms as signal or control. We assumed that the reduction in the time spent in the signal arm (compared to the control arm) and/or the reduction in the number of entrances to the signal arm (negative values of electivity indices) would indicate avoidance of a particular signal. The increase in one or both of these parameters (positive values of electivity indices) would indicate the preference. Moreover, an increase in the time spent in the signal zone accompanied by the reduction in the number of entrances to this zone could indicate a freezing response: the reduction in activity after signal detection.

We also expected that the signals might affect gammarid behaviour throughout the entire experimental tank, regardless of their zone selection. Such responses could not be detected by comparisons between the maze arms. Therefore, we also analysed (3) gammarid activity in the signal zone (where we expected the strongest response), expressed as the percentage of time spent while moving, relative to the total time spent in the zone. Moreover, it was possible that gammarids decreased their time spent in both Y-arms, staying in the mixing zone throughout particular treatments. To check this, we compared (4) the time spent in the mixing zone during each treatment.

Table 1. Statistical analysis of the behavioural response data from the groups of gammarids to conspecific and heterospecific signals (one-way ANOVA). Values in bold indicate statistically significant differences.

	Variable	Species	df ¹	MS ¹	F	P
A	Electivity based on the occupancy time of the signal and control y-arm	<i>D. villosus</i>	6, 63	0.53, 0.07	7.27	< 0.001
		<i>D. haemobaphes</i>	6, 63	0.59, 0.17	3.55	0.004
		<i>P. robustoides</i>	6, 63	0.28, 0.08	3.71	0.003
B	Occupancy time of the mixing zone	<i>D. villosus</i>	6, 63	0.42, 0.05	8.44	< 0.001
		<i>D. haemobaphes</i>	6, 63	0.61, 0.13	4.67	0.001
		<i>P. robustoides</i>	6, 63	0.10, 0.06	1.81	0.111
C	Electivity based on the number of visits to the signal and control y-arm	<i>D. villosus</i>	6, 63	0.15, 0.04	3.34	0.006
		<i>D. haemobaphes</i>	6, 63	5.49, 2.59	2.12	0.064
		<i>P. robustoides</i>	6, 63	1.42, 0.68	2.09	0.067
D	Activity in the signal zone	All species, only control treatments	2, 27	21.72, 0.49	44.29	< 0.001
E	Activity in the signal zone	<i>D. villosus</i>	6, 63	1.49, 0.26	5.81	< 0.001
		<i>D. haemobaphes</i>	6, 63	0.40, 0.25	1.63	0.155
		<i>P. robustoides</i>	6, 63	4.99, 0.20	25.46	< 0.001

¹ values for effect and error terms, respectively.

We used one-way ANOVAs to compare the following response variables among treatments (including the control treatment without signal in both arms): (1) electivity index based on the time spent in each of the Y-arms, (2) electivity index based on the numbers of visits to both Y-arms, (3) activity (log-transformed to stabilize variance and normality) and (4) the time spent in the mixing zone (log-transformed). We checked the normality and homoscedasticity of the data with Shapiro-Wilk and Levene tests, respectively. We analysed the behaviour of each species separately, as particular cues were not equivalent for them in terms of perceiving conspecific and heterospecific signals (i.e. for each species, different signals are either conspecific or heterospecific signals). For significant ANOVA effects, we compared gammarid behaviour in the presence of particular signals with those observed in the control treatment (in which we assumed no zone selectivity and basic activity unaffected by signals) using sequential Bonferroni corrected Fisher LSD tests.

To check interspecies differences in activity, independent of the applied signals, we applied a one-way ANOVA on the data using the control treatments only.

Results

Time spent in the zones

The analysis of the Y-arm electivity revealed that all the species responded to gammarid cues (Table 1A). *Dikerogammarus villosus* stayed longer in the signal zone in the presence of the cues of living *P. robustoides* and alarm substances released by crushed conspecifics and crushed *D. haemobaphes* (Figure 2A). *Dikerogammarus haemobaphes* avoided the signals of living *D. villosus* and *P. robustoides* (Figure 2B). *Pontogammarus robustoides* was repelled from the signal zone by the cues of living *D. villosus* and crushed conspecifics (Figure 2C). The time spent by gammarids in the mixing zone

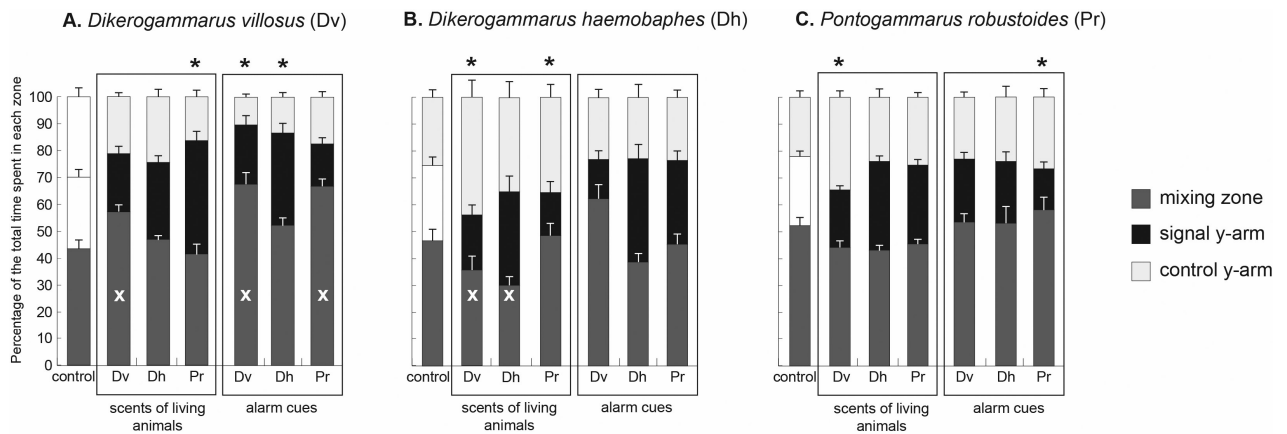


Figure 2. Percentage of time spent by gammarids in the mixing zone, as well as in the signal and control Y-arms. Asterisks indicate which treatments significantly different from the control via the electivity index and based on the time spent by gammarids in each Y-arm. “X” symbols indicate which treatments significantly different from the control, and are expressed as the time spent by gammarids in the mixing zone. Dv, Dh and Pr are abbreviations of *Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*, respectively. The error bars reflect the standard deviation of the mean.

differed among treatments for *D. villosus* and *D. haemobaphes*, but not for *P. robustoides* (Table 1B). Compared to the control treatment, *D. villosus* stayed in the mixing zone more often in the presence of crushed conspecifics and crushed *P. robustoides*, as well as in response to living conspecifics. *Dikerogammarus haemobaphes* exposed to the cue of living conspecifics and live *D. villosus* stayed in the mixing zone for a shorter time period than the control individuals (Figure 2B).

Number of visits to the zones

The gammarids moved from the mixing zone to one of the Y-arms 13.8 ± 10.6 times (mean per one individual \pm SD) during the analysed period. The notable exception was *D. haemobaphes*, which was much less active and switched between the mixing zone and Y-arms 1.1 ± 0.4 times on average. We observed significant differences in the electivity index based on the numbers of visits between the zones only for *D. villosus* (Table 1C), which entered the zone containing the living conspecific cues less frequently than the control Y-arm (Figure 3A). It was noted that the results for the other species were only marginally non-significant (Table 1C).

Activity

Activity of the species in the control treatments differed from one another (Table 1D). *Dikerogammarus villosus* was the most active and *D. haemobaphes* was the most immobile (Figure 4). *Dikerogammarus villosus* and *P. robustoides* modified their activity in response to gammarid cues, whereas the activity of *D. haemobaphes* did not vary significantly among treatments (Table 1E). *Dikerogammarus villosus* and *P. robustoides* reduced their activity in the presence of *D. haemobaphes* (both living and crushed). Additionally, *D. villosus* spent less time moving in response to living *P. robustoides* (Figure 4).

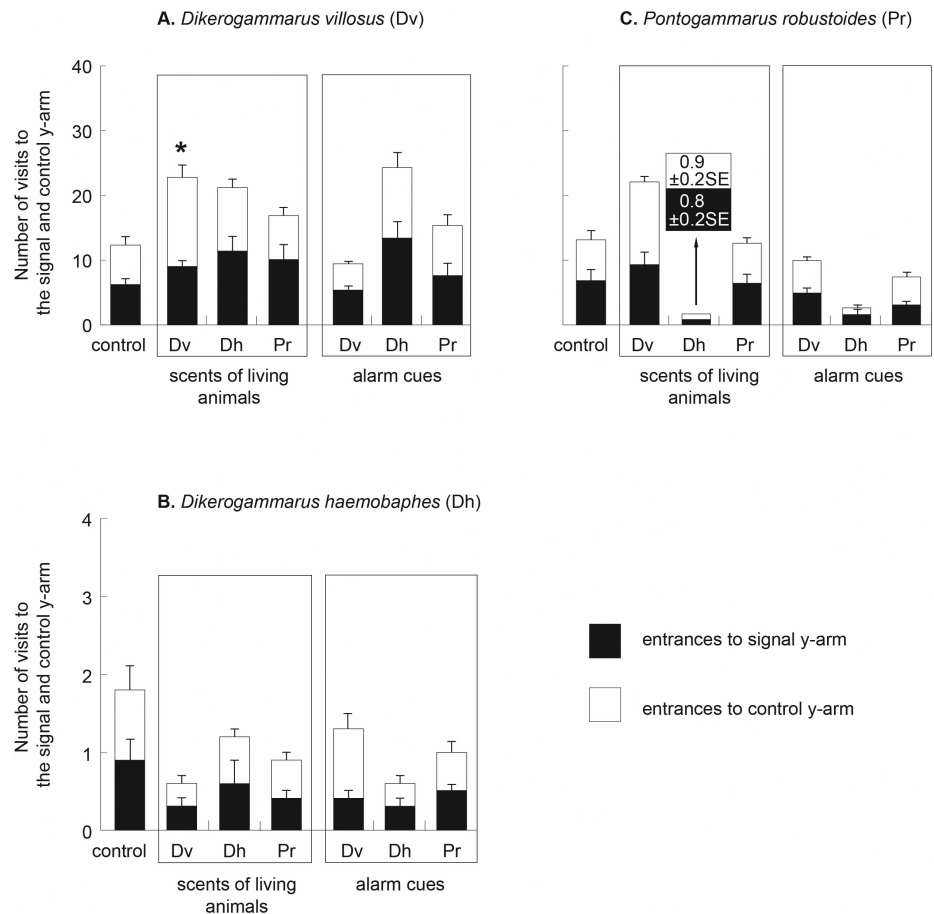


Figure 3. The number of entrances by amphipods to the signal and control Y-arms. Asterisks indicate which treatments significantly different from the control using the electivity index and based on gammarid visits to the Y-arms. Dv, Dh and Pr are abbreviations of *Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*, respectively. The error bars reflect the standard deviation of the mean.

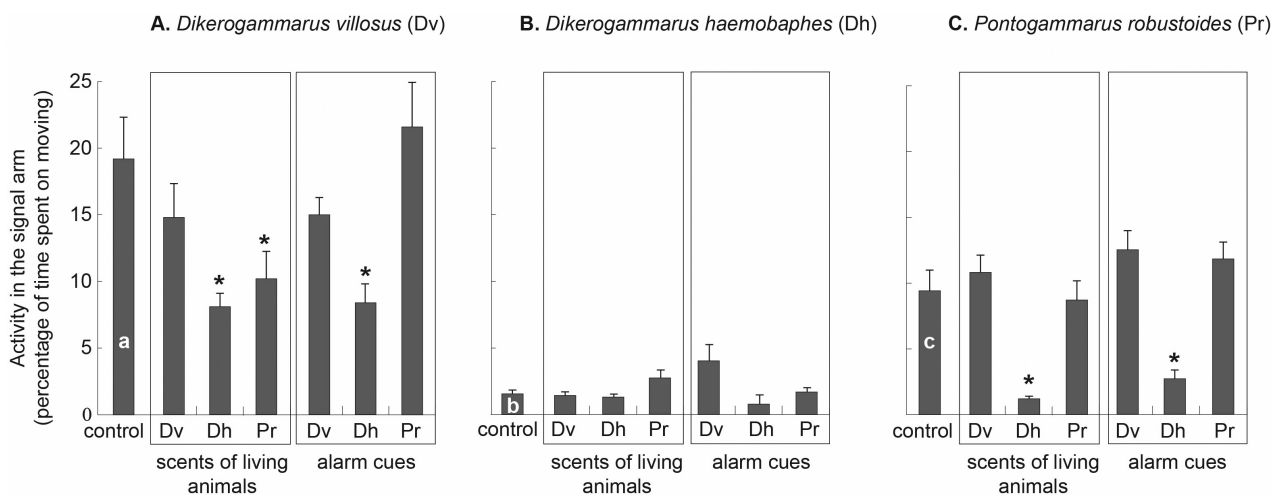


Figure 4. The activity of the gammarids (percentage of time spent on moving) in the signal Y-arm. Asterisks' indicate which treatments differ significantly from the control. Different letters on the control bars indicate which species significantly differing from another regarding their activity, under control conditions. Dv, Dh and Pr are abbreviations of *Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*, respectively. The error bars reflect the standard deviation of the mean.

Table 2. A summary of gammarid behavioural responses to conspecific and heterospecific signals (compared to the control treatment).

Species tested	Variable	Signal source					
		Living individuals			Alarm substances		
		Dv	Dh	Pr	Dv	Dh	Pr
<i>Dikerogammarus villosus</i> (Dv)	Time spent in the mixing zone	+	0	0	+	0	+
	Electivity (time in the y-arms)	0	0	+	+	+	0
	Electivity (visits to the y-arms)	–	0	0	0	0	0
	Activity in the signal y-arm	0	–	–	0	–	0
<i>Dikerogammarus haemobaphes</i> (Dh)	Time spent in the mixing zone	–	–	0	0	0	0
	Electivity (time in the y-arms)	–	0	–	0	0	0
	Electivity (visits to the y-arms)	0	0	0	0	0	0
	Activity in the signal y-arm	0	0	0	0	0	0
<i>Pontogammarus robustoides</i> (Pr)	Time spent in the mixing zone	0	0	0	0	0	0
	Electivity (time in the y-arms)	–	0	0	0	0	–
	Electivity (visits to the y-arms)	0	0	0	0	0	0
	Activity in the signal y-arm	0	–	0	0	–	0

+ stands for a preference or value increase, – stands for an avoidance or value decrease, 0 stands for no significant response.

Discussion

The study species differed from one another with respect to their responses to gammarid scents. They responded differently to the cues of various species (Table 2). *Dikerogammarus villosus* was the only species attracted to alarm cues and the scents of living heterospecifics. The other species either avoided or did not respond directionally to the infochemicals.

Responses of Dikerogammarus villosus

Numerous examples of avoidance behaviour induced by alarm signals have been reported so far (Ferrari et al. 2010; Verheggen et al. 2010; Richter et al. 2018; Ferzoco et al. 2019). Nevertheless, *D. villosus* were not only unrepelled, but were attracted by such stimuli released by conspecifics and the congener species, *D. haemobaphes*. Theoretically, a similar outcome might have been obtained as an effect of a freezing response to the alarm cue. For example, an animal entering the zone with the predation signal would have stopped its movement, which may eventually have resulted in an aggregation in this zone, giving a false impression of a positive response. However, we can exclude this explanation because the number of visits of *D. villosus* to the signal Y-arm was never lower than the number of visits to the control Y-arm in all the preference cases. This was not expected for a freezing response, which would have been associated with a lower number of visits to the zone.

Conversely, we found that *D. villosus* stayed in the mixing zone in the presence of a crushed gammarid signal. This could happen because *D. villosus* exhibits “sit and wait behaviour” with low locomotor activity (Becker et al. 2016), a high affinity for inhabited territory, and its tendency for spontaneous migrations is reduced in the presence of other species (Kobak et al. 2016). Nevertheless, when moved, *D. villosus* still did not avoid the signal Y-arm containing the alarm cues of various gammarid species.

Actually, it even selected the alarm cues released by conspecifics and *D. haemobaphes* (though, interestingly, it did not respond to those released by *P. robustoides*). Regardless of the time spent by this species in the mixing zone, we concluded that positive responses of *D. villosus* to conspecific and congener alarm cues were viable. It is likely that *D. villosus*, which is an omnivore, a scavenger and a cannibal, followed all these scents, perhaps by interpreting them as suitable food items (van Riel et al. 2006; Jermacz et al. 2017b), such as fresh carrion and/or wounded individuals that are easy to prey on.

Conspecific alarm odour emerges when a predator captures its prey, so should be recognized by conspecifics as potential danger. Błońska et al. (2015) found that *D. villosus* was not frequently consumed by goby fish when alternative gammarid prey was available. This is because this species has been found to have harder exoskeleton (Błońska et al. 2015), avoid fish predation more efficiently (Kobak et al. 2014; Beggel et al. 2016), be less mobile (Becker et al. 2016; Beggel et al. 2016) and to form protective aggregations more effectively (Jermacz et al. 2017 a) than other gammarids. Accounting for this, we suggest that *D. villosus* individuals in our experimental system might have not been under any eminent threat, probably due to the relatively low predatory pressure at the sampling site. The sampling site had an abundant bottom community including chironomid larvae, oligochaetes and other potential alternative prey for fish (Poznańska et al. 2009). A similar positive response detected in the isopod *Caecidotea intermedius* (Forbes, 1876) was observed to wounded conspecific cues. This has been attributed to a low predatory pressure in the wild (Spivey et al. 2015). Therefore, the scent of a wounded conspecific might indicate a potential food source for this gammarid, which has been frequently observed to feed on members of its own species (summarised in Rewicz et al. 2014). This might be particularly important at high densities, where the probability of encountering a wounded conspecific is relatively high and the availability of other food sources may be reduced due to high intraspecific competition, promoting cannibalism. Moreover, it has been observed that *D. villosus* is also attracted to the scent of fish predators that feed on gammarids, including *D. villosus* itself (Jermacz et al. 2017b). In our case, the crushed conspecific scent may be recognized by *D. villosus* as a food-related signal rather than a typical alarm cue, triggering avoidance responses.

Cannibalism seems to be the likely reason for the positive response of *D. villosus* to the crushed conspecific cue. This is further evidenced by *D. villosus* lacking a positive response to living conspecifics in our study. The latter signal was the only one to trigger the avoidance behaviour of *D. villosus*, indicated by the lower number of visits to the Y-arm with the scent of living conspecifics. This could be explained by its tendency to be cannibalistic (Kinzler et al. 2009), or possibly reveals an attempt to

decrease intraspecific competition, which is likely to be stronger than interspecific interactions. *Dikerogammarus villosus* tended to follow the scent of living *P. robustoides*. It is likely that *D. villosus* could select areas or shelters with existing gammarid communities as suitable habitat for its development, possibly due to their similar habitat demands but also because inhabited shelters are preferable by gammarids due to the benefits from aggregation forming (Abjörnsson et al. 2000; Musko 1994). Alternatively, this could have resulted from the antagonistic relationship between those species, particularly those with similar predatory habits. *Dikerogammarus villosus* has been commonly reported to exhibit intra-guild predation (Kinzler and Maier 2003).

All things considered, *D. villosus* is presently the most widespread invasive Ponto-Caspian crustacean and has recently dominated European freshwaters. Its apparent boldness, expressed by the ability to recognize and follow various amphipod scents, including those of crushed conspecifics, clearly differentiates it from other related species. Such behaviour is likely to help it find suitable habitats and food sources and suggests that it seems relatively safe from predation pressures. These traits may contribute to its invasive potential and increased success in newly invaded areas.

Responses of the other Ponto-Caspian species

Both *D. haemobaphes* and *P. robustoides* expressed avoidance behaviour in response to the scent of living *D. villosus*, suggesting that such infochemicals were interpreted as a potential threat. This also corroborates the positive response of *D. villosus* to the scents of heterospecific gammarids observed in our study. It has been shown that both *P. robustoides* and *D. villosus* share similar microhabitat preferences, but their interactions result in the displacement of *P. robustoides* (Jermacz et al. 2015). Moreover, these results, combined with the aforementioned preference of *D. villosus*, suggest that the tendency of these species to form single-species aggregations can be explained by the efficient avoidance of mixed groups by the weaker *P. robustoides*, rather than by the behaviour of *D. villosus* (Jermacz et al. 2017a). Both *Dikerogammarus* spp. exhibit similar habitat demands (Musko 1994) and the weaker species (*D. haemobaphes*) has been shown experimentally to be displaced by stronger *D. villosus* (Kobak et al. 2016). *Dikerogammarus haemobaphes* in our study also avoided the scent of living *P. robustoides*, which is another highly aggressive species (Bacela-Spychalska and van der Velde 2013) that is capable of displacing *D. haemobaphes* from its shelters (Kobak et al. 2016). We have shown that weaker gammarid species are able to recognize and avoid the scents of their stronger competitors, reducing the probability of direct encounters.

Pontogammarus robustoides was the only species in our experiments that avoided crushed conspecific alarm cues. Other species in our experiments

did not express such behaviour, despite the fact that this may indicate the presence of a foraging predator in the direct vicinity (Sehr and Gall 2016; Wudkevich et al. 1997).

Dikerogammarus haemobaphes appeared to be less active than the other species and did not switch the Y-maze arms often. The reason for this could be due to its tendency to remain within an established habitat. This was found to be higher in *D. haemobaphes* than in the other species (Kobak et al. 2016). A physical contact with a stronger competitor (*D. villosus*) is the primary reason for it to leave its site and migrate (Kobak et al. 2016).

Summary

Our study has demonstrated the importance of intra- and inter-specific chemical recognition with respect to the behaviour of freshwater invasive amphipods. It has shed new light on the nature of their reciprocal interactions from multiple perspectives. This is the first study researching the ability of gammarids to recognize chemical stimuli from other gammarid individuals and was tested on the most ubiquitous invasive species of the Ponto-Caspian gammarid community. We have shown that *D. villosus* is perceived as a threat by other amphipod species and that, even without a direct physical contact, other Ponto-Caspian species (*D. haemobaphes* and *P. robustoides*) avoid *D. villosus*. This may be one of the mechanisms of their displacement from occupied habitats (Kobak et al. 2016). Subsequently, *D. villosus* might follow displaced species, which could contribute to the large-scale spread of the Ponto-Caspian gammarids. Understanding the outcomes of the chemical perception in these species may contribute to the knowledge of factors that influence amphipod community composition in European fresh waters.

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Authors' Contribution

ŁJ, KBS and JK designed the study. ŁJ and JK designed the experiments. MR, ŁJ, MP and JK collected animals and carried out the experiment. MR and JK analysed and interpreted data. KBS and JK secured the funding. MR and JK wrote the manuscript with contributions from all the authors.

Declarations of interest

None

Ethics and Permits

All the authors acknowledge that they have complied with the institutional and/or national policies governing the humane and ethical treatment of the experimental subjects, and that they are willing to share the original data and materials if so requested. All research pertaining to this article did not require any research permit.

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CONCLUSIONS

This thesis enriches the knowledge about interactions among invasive species of Ponto-Caspian amphipods. Each of the chapters presented here has a significant input on possible explanation of the amphipod behaviour and predicts the consequences that may arise in natural environment.

- *Pontogammarus robustoides* is characterized by higher affinity for spontaneous dispersion compared to *Dikerogammarus* spp. It migrates intensively even without stimulation from other competitors (chapter 1).
- *Dikerogammarus haemobaphes* is a weak competitor which can be easily displaced by stronger *Dikerogammarus villosus*. Its spontaneous dispersion rate is the lowest among the studied species, but it is easily displaced and forced to migrate by competitor species (chapter 1)
- *Dikerogammarus villosus* is considered as the strongest competitor. Its spontaneous dispersion rate is the lowest and this can be explained by the fact that none of the studied species was able to outcompete it (chapter 1).
- Low dispersal rate of *D. villosus* could be the reason why it appeared in central and western Europe later, whereas other, weaker species (here *D. haemobaphes*) appeared earlier – possibly due to the fact that they were outcompeted by the stronger competitor *D. villosus* (chapter 1) and competition for limited resources (preferable microhabitat) is a potential stimulus causing migration of a weaker competitor. This may increase invasion process as the dispersion is accelerated.
- Different thermal behaviour of various gammarid species can enable their coexistence due to possible spatial segregation in the environment (chapter 2).
- Infection by microsporidia modifies thermal behaviour of *D. villosus*, but not that of *D. haemobaphes* (chapter 2).
- Chemical stimulants bear information used by the studied amphipod species (chapter 3)
- Amphipods are capable of distinguishing scents of one another and link the scent of a stronger competitor (*D. villosus*) with a threat (chapter 1). Thus, weaker species are capable of avoiding spots inhabited by *D. villosus* based on chemical recognition (chapter 3).

My PhD thesis unveils traits of Ponto-Caspian amphipod species which have an impact on their behaviour and their ability for competition. Ecological experiments presented here shed the new light on interactions between invasive amphipods and allowed to broaden knowledge about possible mechanisms modulating the rate of the invasion process. Thus, studied amphipod species are capable to distinguish chemical stimuli produced by related species and in a consequence, they reduce the risk competition. Furthermore, my thesis provides that negative interactions may be a factor that gives an impulse for acceleration of the invasion process. It happens when weaker competitors are forced to move to other, less preferable or new microhabitats.

SUMMARY

Animal dispersion is a natural phenomenon occurring worldwide and constantly. This process finally leads to gene flow and, apart from fitness consequences for the individual, changes in population characteristics take place. Population dynamics are considered as overriding determinants of species distribution. In recent decades, however, there is growing attention to species movement beyond their natural range, enhanced often by side effects of human activities. Uncontrolled species movements outside their natural range of occurrence often lead to biological invasions, which constitute a great threat to biodiversity e.g. due to biotic homogenization. In my research, I have focused on the case of three successful invasive Ponto-Caspian amphipod species – *Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*. The ultimate goal of this thesis was to investigate possible agents which on one hand can facilitate further invasion, but on the other hand can maintain a stable coexistence within invaded areas. Thus, the following questions were answered: (1) do the physical contact, which leads to the interference competition, influences the dispersal of weaker competitors? (chapter 1); (2) Can the temperature be an agent determining spatial segregation of the studied species and provide conditions for their coexistence? (chapter 2); (3) do the microsporidian parasites influence the thermal preferences of their host, which in result may stabilize the host coexistence? (chapter 2); (4) does the chemical signalling allow to avoid stronger competitors, and in a result prevent interference competition? (chapter 3).

Results of the study presented in chapter 1 (Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rates. Kobak J., Rachalewski M., Bączela-Spychalska K. 2016. Biological Invasions 18:1953–1965) showed that

spatial distribution on the microhabitat scale is a result of inter- and intraspecific interactions. Experiments on three Ponto- Caspian species – *D. villosus*, *D. haemobaphes* and *P. robustoides* clearly showed their tendencies for spontaneous and induced migration. *P. robustoides* showed the highest affinity for spontaneous migration. Such highly explorative tendencies of this species can be explained by its tendency for searching microhabitats which are free of other, stronger competitors. Indeed, *P. robustoides* is characterized by having the widest spectrum of preferred microhabitats, whereas occurrence of other *Dikerogammarus* species is limited rather to large grain substrata or mussel beds. Both *D. villosus* and *D. haemobaphes* were less explorative than *P. robustoides*, which can be explained by their high affinity to defend a presently occupied shelter. Out of all species tested in this experiment, *D. villosus* appeared to be the strongest competitor capable of displacing the other species. Its highly aggressive behaviour along with effective defence of an occupied habitat resulted in pushing weaker competitors towards less preferable habitats. Chapter 1 enriches the knowledge on the role of interference competition and points it as one of the important agents determining induced migration and, in a result, further expansion of amphipods to novel habitats.

In chapter 2 (Some like it hot: Factors impacting thermal preferences of two Ponto- Caspian amphipods *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841). Rachalewski M., Kobak J., Szczerkowska-Majchrzak E., Baćela-Spychalska K. 2018. PeerJ 6:e4871 DOI 10.7717/peerj.4871), an experiment on thermal preferences of *D. villosus* and *D. haemobaphes* were tested. Amphipods are poikilothermic animals and their activity predominantly relies on the surrounding temperature. In consequence, this agent determines the population dynamics by influencing maturation time and breeding period. Thus, it influences the condition of the population and may restrict its potential for further invasion. Elevated temperature is beneficial for growth and development, but as the metabolic rate rises, the consumption of oxygen increases. On the other hand, as the temperature rises, the availability of oxygen decreases. Thus, temperature-based habitat selection must be a trade-off between acceleration of maturation and energy expenditure related to higher metabolic demands. The results of the experiment performed in chapter 2 showed that both studied *Dikerogammarus* species differed in their thermal preferences. *D. villosus* expressed a strategy of selecting the highest available temperature in an experimental tank, whereas *D. haemobaphes* tended to avoid extreme temperatures and preferred to stay in intermediate ones. Apart from temperature as habitat-selection factor, the presence of microsporidia parasites was incorporated in this study. There were two premises which suggested linking of those factors

into one experiment. First of all, microsporidiosis may cause far-reaching changes in the host behaviour including increase in activity or in contrast, movement impairment. Secondly, the development of the disease is linked to temperature and an infected host individual may express either anapyrexia or behavioural fever to cope with the disease. Eventually, it may influence ability for migration. The only case where microsporidia influenced thermal preference of their host was spotted in males of *D. villosus*. Uninfected individuals tended to stay at lower temperatures than infected ones. There are two possible explanations for this result. It is either an action of a parasite, or a defence mechanism of a host. The answer is however speculative and clear statement cannot be posted until more extensive studies will be performed on this topic. Nevertheless, the results presented in chapter 2 explain that coexistence of both *Dikerogammarus* species is possible as long as microhabitats with different thermal regimes are available nearby.

The ability to detect chemical signals secreted by other amphipods was tested in chapter 3 (Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods. Rachalewski M., Jermacz Ł., Bączela-Spychalska K., Podgórska M., Kobak J. in press. Aquatic Invasions 14). This is a factor that can possibly shape the coexistence of *D. villosus*, *D. haemobaphes* and *P. robustoides*. Chemical communication is common in aquatic ecosystems and constitutes a significant source of acquiring information about surroundings, potential threat, availability of food patches or in case of intraspecific signalling – finding a sexually receptive mate. Furthermore, detection of such stimulants may determine species composition, population dynamics and finally – migratory tendencies which were studied in chapter 1. This study is the first one, up to date which tested the ability of scent recognition in Ponto-Caspian amphipods. The results showed that all species were able to distinguish scents of one another. This suggests that interspecific chemical communication exists between tested species. The scent of *D. villosus* was interpreted by all studied animals as a threat. This outcome confirms the results of the first study (chapter 1) presented in this thesis – *Dikerogammarus villosus* is not only an effective competitor but also is perceived as such by other, weaker species. Interestingly, *D. villosus* tended to avoid scent of its conspecifics which could be explained by avoidance of equally strong competitors (intraspecific competition is stronger than interspecific), which may result in cannibalistic behaviour which is relatively common in this species. Ability of odour recognition can finally lead for interaction avoidance which as a result can facilitate coexistence in a long run.

The final conclusion of this thesis is the fact that negative interactions, thermal preferences and chemical signalling are significant agents influencing the co-existence of the studied species. It was shown that different temperature preferences can stabilize co-existence whereas competition and odour recognition can be considered as agents capable of enhancing the invasion process by forcing weaker competitor to migrate.

STRESZCZENIE

Dyspersja jest naturalnym, powszechnym i ciągłym zjawiskiem w świecie zwierzęcym, które w efekcie prowadzi do przepływu genów i wpływa na kondycję i dynamikę populacji. Dynamika populacji jest jednym z kluczowych determinantów wpływających na rozmieszczenie gatunków w przestrzeni. W ciągu ostatnich dekad dyspersja gatunków inwazyjnych stała się jednym z ważniejszych tematów badań ekologów środowiskowych. Inwazje biologiczne mają szczególną dynamikę, bowiem działalność człowieka umożliwiła ten proces. Jedną z bardziej dotkliwych konsekwencji inwazji biologicznych jest spadek bioróżnorodności, między innymi poprzez homogenizację składu gatunkowego. W mojej rozprawie doktorskiej skupiłem się na przypadku trzech gatunków kielży ponto-kaspijskich, które stanowią modelowy przykład na sukces inwazji – *Dikerogammarus villosus*, *Dikerogammarus haemobaphes*, *Pontogammarus robustoides*. Nadrzędnym celem niniejszej rozprawy doktorskiej było zbadanie czynników, które z jednej strony mogą sprzyjać dalszemu procesowi inwazji, z drugiej zaś mogą wpływać na utrzymanie równowagi i możliwość współwystępowania badanych gatunków w nowo zajętych ekosystemach. W pracy postawiono pytania:

- (1) Czy w wyniku konkurencji międzygatunkowej słabsi konkurenci ulegają dyspersji? (rozdział 1)
- (2) Czy temperatura może być czynnikiem wpływającym na rozmieszczenie gatunków w przestrzeni i zapewniać warunki do współwystępowania? (rozdział 2)
- (3) Czy mikrosporydia zmieniają preferencje termiczne swoich żywicieli (badanych gatunków kielży) i w ten sposób wpływają na ich zdolność do współwystępowania? (rozdział 2)
- (4) Czy na drodze sygnałów chemicznych badane gatunki mają zdolność do wzajemnego rozpoznawania się i w efekcie unikania silniejszego konkurenta? (rozdział 3)

Wyniki badań przedstawionych w rozdziale 1 (Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rates. Kobak J., Rachalewski M., Bącela-Spychalska K. 2016. Biological Invasions 18:1953–1965) pokazały, że rozmieszczenie gatunków w skali mikrohabitatatu jest wynikiem konkurencji wewnątrz- i międzygatunkowej. Przeprowadzone eksperymenty na trzech gatunkach kielży

pontokaspjskich – *D. villosus*, *D. haemobaphes* i *P. robustoides* wykazały ich zdolność do dyspersji spontanicznej oraz tej, indukowanej obecnością konkurenta. *P. robustoides*, spośród badanych gatunków wykazał się największą zdolnością do dyspersji spontanicznej. Ten wynik może wskazywać na tendencję przedstawicieli tego gatunku do poszukiwania mikrohabitatów wolnych od innych, silniejszych konkurentów. *Pontogammarus robustoides* wykazuje szerokie spektrum preferencji względem siedliska, podczas gdy występowanie gatunków z rodzaju *Dikerogammarus* ogranicza się zwykle do kamienistego podłoża lub przestrzeni między muszlami racicznicy zmiennej. Zarówno *D. haemobaphes* jak i *D. villosus* posiadały znacznie mniejszą tendencję do eksploracji przestrzeni eksperymentalnej, co można wyjaśnić silnym przywiązaniem i obroną zajmowanych przez siebie siedlisk. Spośród wszystkich badanych gatunków *D. villosus* okazał się najsilniejszym z konkurentów, zdolnym do wypierania pozostałych gatunków. Jego agresja połączona z silnym przywiązaniem do zajmowanego schronienia sprawiała, że zmuszał słabszych konkurentów do ustąpienia i zmiany mikrohabitatu na mniej preferowany. Rozdział 1 wzbogaca wiedzę o znaczeniu konkurencji międzygatunkowej i wskazuje, że ten typ interakcji odgrywa znaczącą rolę w dyspersji innych gatunków. Indukowana dyspersja słabszych gatunków może prowadzić do ich migracji do nowych siedlisk, a więc do dalszej inwazji.

W rozdziale 2 (Some like it hot: Factors impacting thermal preferences of two Ponto-Caspian amphipods *Dikerogammarus villosus* (Sovinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841). Rachalewski M., Kobak J., Szczerkowska-Majchrzak E., Bącela-Spychalska K. 2018. PeerJ 6:e4871 DOI 10.7717/peerj.4871) zbadano preferencje termiczne *D. villosus* i *D. haemobaphes*. Obunogi są organizmami zmiennocieplnymi, a więc ich aktywność i rozwój zależą w znacznej mierze od temperatury środowiska, w którym się znajdują. Czynniki te mają istotny wpływ na dynamikę populacji, wyznaczając czas rozrodu czy czas osiągnięcia dojrzałości płciowej. Temperatura środowiska determinuje to, w jakiej kondycji jest populacja, a więc w znaczący sposób może wpływać na jej potencjał do dalszej inwazji. Podwyższona temperatura sprzyja osobniczemu wzrostowi i rozwojowi, ale wraz ze zwiększonym tempem metabolizmu, wzrasta również zapotrzebowanie na tlen, jednakże w wyższej temperaturze jego dostępność spada. Wybór siedliska w oparciu o jego warunki termiczne musi być więc wynikiem kompromisu między zwiększonym tempem dojrzewania i wzrostu, a zapotrzebowaniem energetycznym i tlenowym związanym ze zwiększonym tempem metabolizmu. Wyniki przedstawione w rozdziale 2 wykazały różnice w preferencjach termicznych obydwu badanych gatunków kielży. *Dikerogammarus villosus* wykazał się

strategią polegającą na wyborze siedliska z możliwie najwyższą dostępną temperaturą, podczas gdy *D. haemobaphes* unikał miejsc o skrajnych wartościach temperaturowych i przebywał w okolicach średniej z dostępnego w eksperymentach zakresu. Poza temperaturą jako czynnikiem wpływającym na wybór siedliska, zbadano również rolę pasożytów – mikrosporydiów jako czynnika determinującego wybór preferowanej temperatury przez ich gospodarzy – kielży. Dwie przesłanki stały za tym, aby połączyć czynnik temperatury i pasożyta w prowadzonym eksperymencie. Pierwszą z nich był fakt, że mikrosporydia mogą powodować daleko idące zmiany w zachowaniu swoich gospodarzy powodując zwiększenie ich aktywności lub przeciwnie – wywołując upośledzenie ruchowe. Drugą przesłanką był fakt, iż rozwój choroby wywołanej przez mikrosporydia wiąże się z temperaturą, toteż u zainfekowanych osobników kielży może występować gorączka behawioralna lub anapireksja, których celem jest zwalczenie lub zahamowanie choroby. W rezultacie, może mieć to wpływ na zdolność kielży do dyspersji. Tylko w jednym wypadku stwierdzono wpływ mikrosporydiów na preferencje temperaturowe kielży. Niezainfekowane samce *D. villosus* przebywały w niższych temperaturach niż zainfekowane. Istnieją dwa, równie prawdopodobne wyjaśnienia dla tej obserwacji. Rezultat ten mógł być wynikiem działalności pasożyta lub efektem mechanizmu obronnego gospodarza. Odpowiedź, który był to mechanizm byłaby jednak spekulacją i konieczne jest przeprowadzenie większej liczby badań. Niemniej jednak, wyniki przedstawione w rozdziale 2 wyjaśniają, że współwystępowanie obydwu gatunków z rodzaju *Dikerogammarus* jest możliwe, pod warunkiem występowania wyraźnych różnic temperaturowych w obrębie siedliska.

W rozdziale 3 (Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods. Rachalewski M., Jermacz Ł., Bącela-Spychalska K., Podgórska M., Kobak J. in press. Aquatic Invasions 14) zbadano zdolność do wzajemnego odbierania sygnałów chemicznych wydzielanych przez trzy gatunki kielży – *D. villosus*, *D. haemobaphes* i *P. robustoides*. Zdolność do odbierania i interpretacji sygnałów chemicznych może mieć wpływ na współwystępowanie tych gatunków. Komunikacja oparta na tego rodzaju sygnałach powszechnie występuje w wodnych ekosystemach i pozwala na odbiór informacji o środowisku, w którym znajduje się organizm: o obecności zagrożenia, dostępności pożywienia, a w przypadku wewnątrzgatunkowej komunikacji – pozwala na odnalezienie partnerów zdolnych do reprodukcji. Detekcja sygnałów chemicznych może mieć wpływ na skład gatunkowy w siedlisku, wpływać na dynamikę populacji, a także wywoływać dyspersję, którą zbadano w rozdziale 1 niniejszej rozprawy. Badania przedstawione w rozdziale 3 są pierwszymi

tego typu, w których badano zdolność do rozróżniania sygnałów chemicznych u kielży pontokaspijskich. Wyniki wykazały, że wszystkie badane gatunki kielży mają zdolność do rozróżniania wzajemnych sygnałów chemicznych. Sygnały *D. villosus* były interpretowane przez pozostałe badane gatunki jako zagrożenie. Wynik ten potwierdza wyniki z rozdziału 1, ukazując, że *D. villosus* jest nie tylko silniejszym konkurentem, ale jest jako taki odbierany przez pozostałe, słabsze gatunki. Co ciekawe, osobniki *D. villosus* również unikały sygnałów przedstawicieli tego samego gatunku, co pozwala twierdzić, że unikały równie silnych konkurentów – konkurencja wewnątrzgatunkowa jest silniejsza od międzygatunkowej. Unikanie sygnałów tego samego gatunku to prawdopodobnie efekt tego, że u *D. villosus* występuje dość powszechnie zjawisko kanibalizmu.

Konkluzją z przeprowadzonych przeze mnie badań jest to, że spośród wszystkich badanych czynników mogących potencjalnie wpływać na zachowanie badanych gatunków kielży, wszystkie okazały się w tym istotne. Czynniki te modyfikują interakcje między badanymi gatunkami oraz wpływają na zdolność do współwystępowania gatunków. Badania wskazały, że temperatura jest czynnikiem mogącym skutecznie wpływać na zdolność gatunków do współwystępowania na tym samym obszarze, ale w obrębie niepokrywających się nisz określonych poprzez temperaturę. Zdolność do interpretacji sygnałów chemicznych oraz konkurencja międzygatunkowa są czynnikami, które mogą sprzyjać inwazji, poprzez zintensyfikowanie migracji u słabszych konkurentów.

AUTHORSHIP STATEMENTS

Hereby, I declare that all co-authors of included in this PhD thesis scientific articles made a signature under commonly established agreement about percentage contribution in each of presented here studies. They agreed to use presented here scientific articles as a part of my PhD thesis. Signed documents of agreement are included in this thesis.

APPENDIX 1

Temat: AI19-039: pre-proof, figures in high resolution

Nadawca: "Managing Editor" <ai_editor@reabic.net>

Data: 28.08.2019, 13:07

Adresat: "Karolina Bącela-Spychalska" <karolina.bacela@biol.uni.lodz.pl>

Dear Karolina,

My congratulations with acceptance of your paper for publication in "Aquatic Invasions" (Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods).

I am sending you pre-proof version of your paper in Word format for final editing.

Specifically, please:

1. provide figures for your paper in high resolution in separate files (min 300 dpi, preferably in the initial tiff format, high quality jpg or pdf also acceptable) - please upload these files to your editorial Dropbox (in new subfolder);
2. check references highlighted in red - they can be missing in text or References section;

Please confirm.

Thanking you in advance
and with best wishes,

Vadim

Vadim E. Panov, Ph.D.

Managing Editor of INVASIVESNET/REABIC open-access journals

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From: Karolina Bącela-Spychalska <karolina.bacela@biol.uni.lodz.pl>

Sent: 8/13/19 1:19 PM

To: Elena Tricarico <elena.tricarico@unifi.it>, Michał Rachalewski <rachalewski@me.com>, Jarosław Kobak <jkob73@umk.pl>

Cc: "ai_editor@reabic.net" <ai_editor@reabic.net>

Subject: Re: AI19-039: update

Dera Elena,

thank you for this great news:)

all the best

karolina

W dniu 04.08.2019 o 22:48, Elena Tricarico pisze:

Dear Karolina and coauthors,

I am pleased to inform you that your manuscript "Friends or enemies? Chemical recognition and reciprocal responses among invasive Ponto-Caspian amphipods" has been formally accepted for publication in Aquatic Invasions.

Your manuscript will now be sent for technical process and you will be contacted for the publishing issues.

My very best congrats for this achievement!

Cheers

Elena

Elena Tricarico

Thematic-Deputy Editor in Chief: Aquatic Invasions: <http://www.aquaticinvasions.net>

Associate Editor: *BioInvasion Records*: <http://www.reabic.net/journals/bir/>

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