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SHORT COMMUNICATION

The killer shrimp *Dikerogammarus villosus* (Crustacea, Amphipoda) invades Lithuanian waters, South-Eastern Baltic Sea

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Summary The killer shrimp *Dikerogammarus villosus* was recorded for the first time in Lithuanian waters in 2015. The species was detected in three sites in the Curonian Lagoon (on two buoys in the lagoon strait and the harbour, and one littoral sampling site) and in the mouth of the Šventoji River. The species presence in the buoy fouling suggests the involvement of shipping in species introduction. Most likely *D. villosus* has arrived to the Curonian Lagoon with commercial ships, while the invasion into the mouth of the Šventoji River may be associated with leisure shipping as the port situated therein is not currently functioning. Further northward expansion of the killer shrimp in the Baltic Sea basin seems very probable. As the species is highly aggressive, alterations of local macroinvertebrate assemblages can also be predicted.

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1. Introduction

The killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894) is an amphipod originating from the Ponto-Caspian region which began its range expansion across European waters in the 20th century after the re-opening of the Rhine-Main-Danube canal (Bij de Vaate et al., 2002). The crustacean is considered a highly invasive and aggressive aquatic species due to its large body, high fecundity, wide environmental tolerance and is often denoted as a voracious predator; it is capable of severe alteration of local communities and reduction or even extermination of native and other resident amphipods as well as of other macroinvertebrates (Dick and Platvoet, 2000; Grabowski et al., 2007a; Haas et al., 2002). The killer shrimp is the only amphipod included in the list of 100 most invasive non-indigenous species of Europe (Devin and Beisel, 2009).

The killer shrimp is unique for utilising all three main European invasion corridors from the Ponto-Caspian region (Bij de Vaate et al., 2002; Rewicz et al., 2014). The southern invasion corridor, connecting the basins of the Black and Northern seas along the Danube, Main and Rhine rivers, was the main pathway of expansion of *D. villosus*. The species was recorded in the lower Rhine for the first time in 1994 (Bij de Vaate and Klink, 1995). Recently it is widely distributed in all major rivers of Western Europe (reviewed in Bij de Vaate et al., 2002; Bollache et al., 2004; Jażdżewski and Konopacka, 2002; Nesemann et al., 1995). The invader originating from the southern route was also detected in the United Kingdom in 2010 (MacNeil et al., 2010; Rewicz et al., 2015). In 2003, the species was detected in the Bug River (Konopacka, 2004), indicating its expansion through the central invasion corridor along the Dnieper, Pripyat, Bug and Vistula rivers as well (see also Mastitsky and Makarevich, 2007). Molecular studies have shown that the southern and central invasion routes correspond with two distinct source populations residing in the Danube and Dnieper deltas (Rewicz et al., 2015) which may soon meet in Poland (Grabowski et al., 2007b). By the middle of the 20th century, *D. villosus* has also spread via the northern corridor (the Volga River system), within which its most northern record was made in the Kuybyshev Reservoir in 2000 (Rewicz et al., 2014; Yakovleva and Yakovlev, 2010).

A rapid further expansion of the killer shrimp across European waters is still ongoing. So far, *D. villosus* has approached the waters of the Baltic States and Lithuania in particular. In the vicinity of the Baltic Sea (Fig. 1), *D. villosus* was recorded in 2002 in the brackish-water Szczecin Lagoon, and this suggested that the lagoon was colonised by the descendants of invaders in the Oder River coming through the southern invasion corridor (Gruszka and Woźniczka, 2008). It also established populations in the Vistula Lagoon and the Gulf of Gdansk during 2010–2012 (Dobrzycka-Krahel et al., 2013, 2015).

It was already anticipated several years ago that *D. villosus* will inevitably reach Lithuania (Arbačiauskas et al., 2011), namely the middle reaches of the Nemunas River (southern Lithuania), through the Augustów canal which connects the Nemunas and the invaded Vistula basin. In this work we report the first records of *D. villosus* in Lithuanian waters, which, however, appear to be a result of a marine invasion along the Baltic Sea coast.

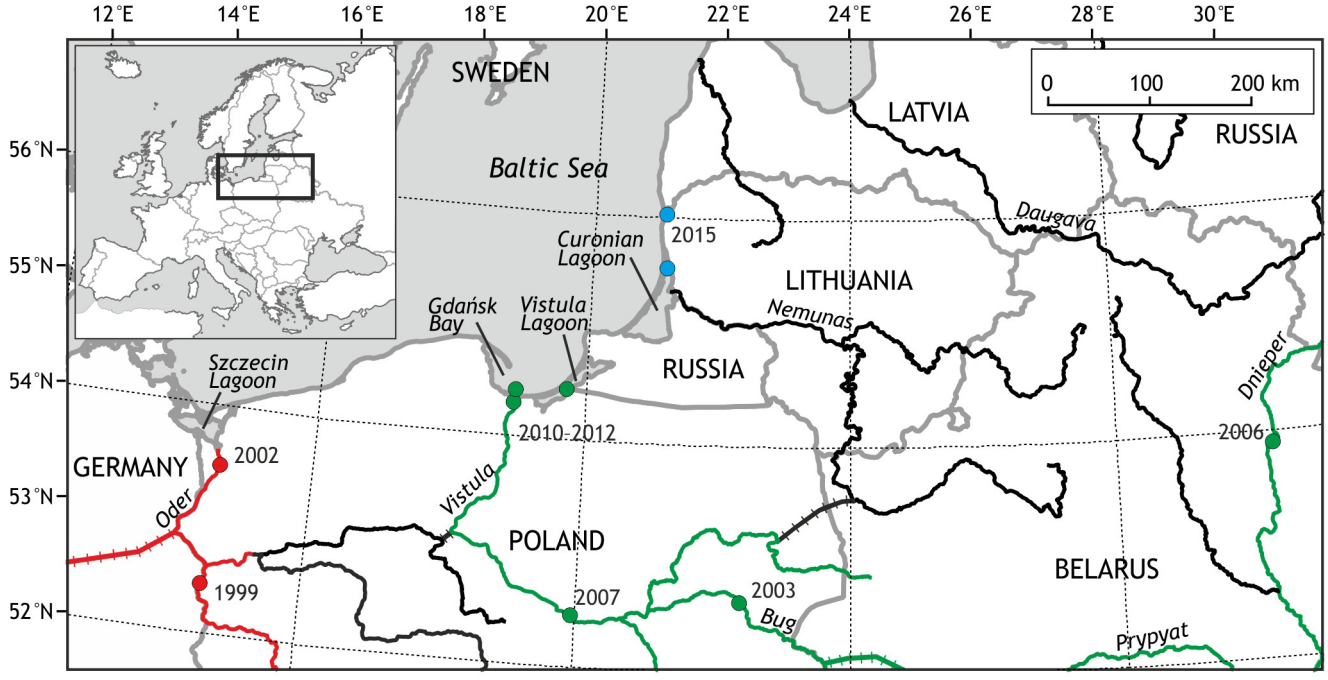

Figure 1 European invasion routes of *Dikerogammarus villosus* in the vicinity of the Baltic Sea: southern corridor denoted in red and central corridor denoted in green. Recently invaded Lithuanian water bodies are denoted by blue dots. Dates are first records of the species. Based on Rewicz et al. (2015) and Dobrzycka-Krahel et al. (2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

Investigations of amphipod assemblages were performed in the Šventoji River, which empties into the Baltic Sea, and the Curonian Lagoon during 2015. In the Šventoji River, the mouth and two other upstream sites were investigated in early September. In the Curonian Lagoon, a survey of fouling of five navigation buoys and a water level ruler located in the Klaipėda port and its vicinity was undertaken in June, and benthic sampling of three stations distributed within the lagoon was performed in September.

Samples from buoys were each taken by scraping off a 0.04-m^2 area of fouling, using a 20 cm × 20 cm sampling frame; 17 such samples in total were collected from surfaces located on buoys at different depths below the water level (Fig. 2). For composition of amphipod assemblages in the Šventoji River and the Curonian Lagoon, material was taken using a standard dip net with a 25 cm × 25 cm opening and a 0.5-mm mesh size. Semi-quantitative samples were collected in wadeable depths covering all substrate types and putting a sampling effort of 10 min per site.

In the field, all collected samples were fixed in 4% formaldehyde solution. In the laboratory, they were examined for the presence of amphipods that were sorted and preserved in 70% ethanol. The amphipod *D. villosus* was identified based on the shape of conical protuberances on urosomes I–II and dense tufts of long setae on the flagellum of antennae II and on the propodus of gnathopods in males (Dobson, 2013).

Figure 2 A buoy in the Malkų Bay, the Klaipėda port. Photograph by M. Orlov.

3. Results

Locations of study sites where *D. villosus* was recorded are given in Table 1 and Fig. 3. In the Šventoji River, *D. villosus* (Fig. 4A) was present in the mouth and absent from the other two upstream study sites. There were only two specimens per sample that comprised around 1% of amphipods in the sample. Alien amphipods *Gammarus tigrinus* Sexton, 1939 of North American origin, and *Pontogammarus robustoides* (Sars, 1894) and *Chaetogammarus warpachowskyi* (Sars, 1894) originating from the Ponto-Caspian region dominated in the mouth of the river.

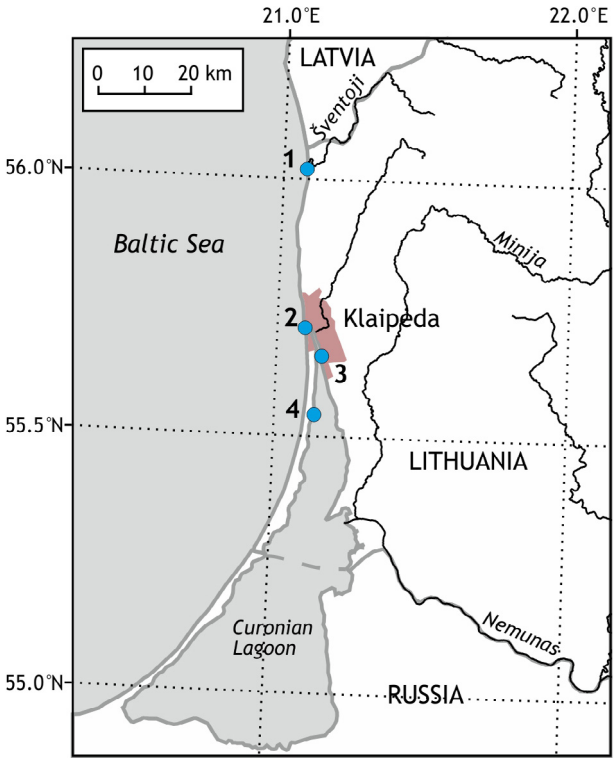

Figure 3 Geographic location of sampling sites in Lithuanian waters where *Dikerogammarus villosus* was recorded. Site numbers correspond to those in Table 1.

Table 1 Description of sampling sites in the Šventoji River and the Curonian Lagoon where *Dikerogammarus villosus* was recorded. Sampled substrates: buoy fouling (BF), sand (SA), silt (SI), stones (ST), macrophytes (MA); number of specimens per sample (*N*), proportion among amphipods (*P*).

No	Site	Coordinates	Date	Substrate	*N*	*P*
	Šventoji River					
1	River mouth	56°01′45″N, 21°04′22″E	2015-09-04	SA, SI, MA	2	0.01
	Curonian Lagoon					
2	Lagoon strait	55°43′12″N, 21°06′05″E	2015-06-22	BF	16	0.18
3	Malkų Bay, Klaipėda port	55°39′15″N, 21°09′10″E	2015-06-18	BF	48	0.10
4	Near Juodkrantė town	55°33′14″N, 21°07′44″E	2015-09-25	ST, SI, MA	44	0.19

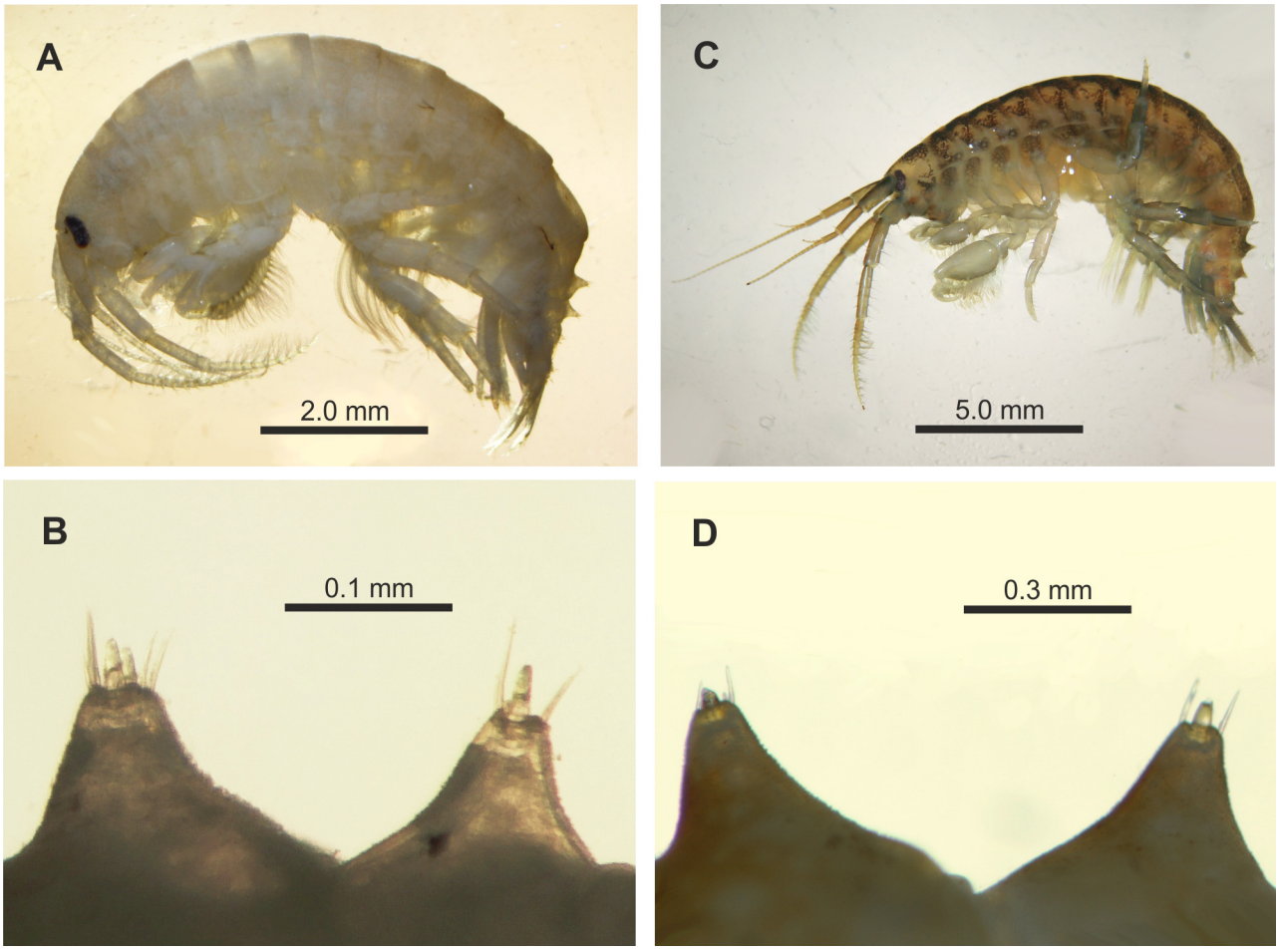

Figure 4 *Dikerogammarus villosus* from Lithuanian waters: ethanol preserved 11-mm male from the mouth of the Šventoji River (A) and its urosomal protuberances (B); defrosted 21-mm male from the Curonian Lagoon (C) and its urosomal protuberances (D). Note that both specimens have only two spines on each of the protuberances, the characteristic typical of most male *D. villosus* currently observed in Lithuanian waters. Photographs by E. Šidagytė.

In the Curonian Lagoon, the species (Fig. 4C) was detected on two (out of five studied) navigation buoys located in the strait and the port in the Malkų Bay. The abundance of the killer shrimp per fouling sample was larger on the buoy from the port in the Malkų Bay, however, due to large abundance of other amphipods the proportion of the new invader here was about twofold lower than that observed on the buoy from the strait (Table 1). The dominant amphipods on the buoys were other alien amphipods, *Obesogammarus crassus* (Sars, 1894) and *Chelicorophium curvispinum* (Sars, 1894) of Ponto-Caspian origin, and *G. tigrinus*.

From the three littoral sites sampled in the Curonian Lagoon, *D. villosus* was detected only in one site located close to Juodkrantė Town. Here, 44 specimens of the killer shrimp were found. They comprised 19% of all sampled amphipods which were represented almost exclusively by alien species *G. tigrinus*, *O. crassus*, *C. curvispinum* and *C. warpachowskyi*.

4. Discussion

Our results clearly indicate that Lithuanian waters in the close vicinity of the Baltic Sea have been invaded by the killer shrimp *D. villosus*. A survey of macroinvertebrate assemblages in 17 sites along the Nemunas River in Lithuania, the northern branch of the central invasion corridor, failed to detect *D. villosus* in 2015 (unpublished results), thus we conclude that the newcomer had arrived through the sea. The most probable source of this amphipod invader into Lithuanian waters is the Polish coastal waters. Because of possible intermixture of invaders from the two invasion routes in the region, fine-scale molecular studies are warranted to determine the origin of Lithuanian populations. However, their origination from the central invasion corridor seems more presumable. So far, the Šventoji River mouth is the most northern recorded site of *D. villosus* presence in the Baltic Sea basin.

The presence of the species in the fouling of navigation buoys located in the Klaipėda port and its vicinity suggests that *D. villosus* probably was transferred by commercial ships, whether within the hull fouling or in ballast waters. Introduction of the species into the mouth of the Šventoji River may be associated with leisure shipping as the port at this site is not currently functioning. On the other hand, a possibility of natural dispersal over the coastal waters of the Baltic Sea should not be excluded. This is possible as the species proved

to be an osmotic hyper-regulator capable of resisting a wide range of external salinities (Bruijs et al., 2001; Dobrzycka-Krahel et al., 2015).

While quite abundant (when present) in the samples from the Curonian Lagoon, this amphipod only occasionally occurred in the mouth of the Šventoji River. In June 2016, abundant individuals of various size, precopula pairs and ovigerous females of *D. villosus* were observed in the stony littoral habitats of the Curonian Lagoon near Juodkrantė, while in the sandy mouth of the Šventoji River its abundance did not seem to be high. Only 16 specimens of the killer shrimp (just 3% of all sampled amphipods) were identified after rigorous sampling carried out by three people. This pattern suggests that the invasion of *D. villosus* into the lagoon may have occurred at least a year before its first detection, while colonisation of the Šventoji River has started only recently. However, the differences in abundance may also be related to the scarcity of hard substrates preferred by *D. villosus* (Devin et al., 2003; Kley and Maier, 2005) in the predominantly sandy habitat of the mouth of the Šventoji River.

The characteristic temperature and salinity ranges of the Curonian Lagoon (respectively 0—24°C and 0—8 PSU; Olenin and Daunys, 2004), as well as these conditions in most Lithuanian inland waters, should be easily tolerated by such a plastic species as *D. villosus* (Bruijs et al., 2001; Devin and Beisel, 2009; Dobrzycka-Krahel et al., 2015). It has been reported that the species largely prefers hard substrates (from gravel to boulders, artificial embankments, roots, dreissenid colonies) and avoids sand and silt (Boets et al., 2010; Devin et al., 2003; Kley and Maier, 2005; MacNeil et al., 2010). Most of invasive pontogammarids are oxyphilic species, however, the lethal oxygen concentration for the killer shrimp (0.38 mg L^{-1}) is the highest among them (Dedyu, 1980). As a result, the species has also been noticed to be more restrained by pollution than other pontogammarid invaders (Boets et al., 2010; Dobrzycka-Krahel et al., 2015). It has been suggested that *D. villosus* prefers slow-flowing waters and therefore should not invade small rivers, even if the water quality is good (Boets et al., 2010). Thus it can be preliminarily concluded that ecosystems from large rivers, reservoirs and lakes to lagoons and bays of the eastern Baltic Sea, especially habitats with any kind of hard substrate and no substantial pollution, are vulnerable to the new invader.

It is of interest to note that Ponto-Caspian amphipod *O. crassus* usually dominated amphipod assemblages in the northern part of the Curonian Lagoon, at least in the areas affected by the brackish sea water. However, in over 50 years the species did not manage to colonise the mouth of the Šventoji River located just 35 km north (Arbačiauskas et al., 2011; current study), although it was once observed in the Baltic coastal waters at Palanga town, around 20 km north from the Curonian Lagoon (Solovjova, unpublished results). *Dikerogammarus villosus*, on the other hand, was recorded in both ecosystems simultaneously, indicating a huge potential of the species to spread across marine waters. Therefore, it is not surprising that the killer shrimp is considered the most probable future amphipod invader in the North American waters (Ricciardi and Rasmussen, 1998). Future northward expansion of *D. villosus* in the basin of the Baltic Sea and invasion of the Latvian waters seems inevitable. It is also likely that the species is already there (as well as in the Russian Kaliningrad region) but is not yet recorded.

There are three species of the genus *Dikerogammarus* invading the European waters, the other two being *Dikerogammarus haemobaphes* (Eichwald, 1841) and *Dikerogammarus bispinosus* Martynov 1925; the latter only recently being promoted to a species from a subspecies of *D. villosus* (Müller et al., 2002). It is often noted that adult males of *D. villosus* are easily distinguishable from the congeneric invaders using a combination of three identification features: (1) short setation of the peduncle but dense and long setation on the flagellum of antennae II, (2) long setation of the propodus of gnapthopods, and (3) 3—5 spines on each of the two pointed dorsal protuberances of the urosomes I—II (Dobson, 2013; Müller et al., 2002). In Lithuanian populations, however, male individuals confined to the first two identification features very clearly, but most of them had two main spines on both protuberances (Fig. 4B and D). MacNeil et al. (2010) have mentioned personal observations of D. Platvoet that only adult males of *D. villosus* over 16-mm body length (may reach up to 30 mm; Dobson, 2013) usually have 3—5 spines. The largest individuals in our study reached slightly over 20 mm. They all, however, had two spines on at least one of the protuberances, and very often two spines on both protuberances. Smaller males of 10—11-mm length consistently had only two spines on both protuberances, while variation from 2 + 2 to 3 + 5 spines was observed in medium 14—15 mm long males. Thus, it seems that the number of spines on dorsal urosomal protuberances should be used with caution for species identification. So far, only A. Konopacka (2004) has clearly pointed out that urosomal protuberances of *D. villosus* may contain from 2 to 5 spines.

The killer shrimp has been repeatedly reported as a voracious predator (Dick and Platvoet, 2000; Grabowski et al., 2007a; Haas et al., 2002), thus it is ecologically aggressive towards resident macroinvertebrate species and is capable of impacts on their communities. The other Ponto-Caspian amphipod, *P. robustoides*, is the dominant alien amphipod species in the inland freshwaters of Lithuania (Arbačiauskas et al., 2011) and the central part of the Curonian Lagoon (Solovjova, unpublished results). This amphipod species is also characterised as an ecologically aggressive species which is capable of extermination of native amphipods and alteration of composition of macroinvertebrate assemblages (Arbačiauskas and Gumuliauskaitė, 2007; Gumuliauskaitė and Arbačiauskas, 2008). In Polish dams, *P. robustoides* has been reported to out-compete the preceding invader, the daemon shrimp *D. haemobaphes* (Jażdżewska and Jażdżewski, 2008). Some experimental studies confirm that *P. robustoides* stands close to *D. villosus* according to interference competitiveness (Kobak et al., 2016).

Although *P. robustoides* prefers more lentic habitats than *D. villosus* (Jazdzewski et al., 2002), localities recently invaded by *D. villosus* and *P. robustoides* coincide in some reservoirs of Poland and Russia. In these reservoirs, they are currently able to coexist by occupying different habitats: *P. robustoides* dwells in the sandy shallows while *D. villosus* prevails in higher depths (Kobak et al., 2014; Yakovleva and Yakovlev, 2010). It may be that *D. villosus* is a stronger competitor but is more restricted to hard substrates (Boets et al., 2010) and less resistant to desiccation

(Poznańska et al., 2013) and pollution (Boets et al., 2010; Dobrzycka-Krahel et al., 2015) than *P. robustoides*.

These two aggressive invaders will definitely clash in Lithuanian waters as well, and it would be of interest to reveal the outcomes of their interaction. Alterations, even severe ones, of local macroinvertebrate assemblages in waters suitable for the killer shrimp can definitely be expected in the near future.

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