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Range extension of *Dikerogammarus villosus* (Sowinsky, 1894) in Poland (the Baltic Sea basin) and its ability to osmoregulate in different environmental salinities

by

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Abstract

The paper reports the range extension of the Ponto-Caspian gammarid *Dikerogammarus villosus* (Sowinsky, 1894) and the first occurrence of this species in new areas of the Polish part of the Baltic Sea basin: the Śmiała Vistula and the Vistula Lagoon.

The studies additionally determine the osmoregulatory ability of the species under laboratory conditions within the salinity range of 2-22 PSU. Determination of the osmoregulation of the species shows that *D. villosus* is a hyperregulator at given experimental salinities and can function in a wide range of external salinities so it is potentially able to colonize various water bodies. Osmoregulatory capacity, which is an indicator of organism's efforts to regulate the concentration of its body fluids, shows that for individuals from the Gulf of Gdańsk, 6 PSU is the best tolerated salinity. Osmoregulatory capacity is the lowest at this salinity value.

The range extension and potential osmoregulatory abilities of the species to spread to other waters are discussed in the context of pollution levels given in the literature with reference to the habitat and sensitivity of the species to e.g. fluoride and cadmium toxicity.

Key words: non-indigenous species, Ponto-Caspian gammarid, osmoregulation, osmotic capacity, Śmiała Vistula, Vistula Lagoon, Gulf of Gdańsk

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Introduction

Invasions of aquatic species occur in many aquatic ecosystems as a consequence of human activities (Leppäkoski & Olenin 2000, Chandra & Gerhardt 2008). The range extension of aquatic organisms and changes in biological diversity may be the effect of human-mediated spreading along river basins through artificial canals and/or by shipping.

D. villosus is a species of Ponto-Caspian origin, that is from the Black Sea, the Caspian Sea and the Sea of Azov's catchment area (Mordukhaj-Boltovskoj et al. 1969, Dedyu 1980). This species has been extending its geographic range, because is able to move along rivers and canals, and inhabit reservoirs and drainage systems in the vicinity of such waterways. Recently, its distribution area has been shown to cover the catchment areas of the Baltic Sea, the North Sea and the Mediterranean Sea (Bij de Vaate et al. 2002, Jażdżewski & Konopacka 2002). It has recently invaded most of Western Europe. This species was first reported in the Danube in the 1990s and since then it has been found in many countries, including e.g. Austria (Mayer et al. 2009), the Netherlands (Bij de Vaate & Klink 1995, Dick & Platvoet 2000), Belgium (Messiaen et al. 2010), France (Devin et al. 2004), Italy (Casellato et al. 2006, 2008) and England (Defra 2011). This species has also been reported in some Alpine lakes, with very low conductivity (e.g. Bącela-Spychalska et al. 2013).

In Poland, this species was first recorded in the Oder River in 1999 (Müller et al. 2001) and later in the Szczecin Lagoon and in adjacent coastal waters in 2002-2004 (Gruszka et al. 2003, Gruszka & Woźniczka 2008) (Fig. 1). These records showed that the species migrates to Poland along the southern corridor (Bij de Vaate et al. 2002).

The discovery of *D. villosus* in 2003 in the Bug River (Konopacka 2004) and later in the Vistula River – near Wyszogród in 2007 (Bącela et al. 2008) showed that the species has also migrated to Poland along the central corridor, via the Pripet-Bug connection.

Probably the floods which afflicted Poland in 2010 could have accelerated the arrival of this species into the Gulf of Gdańsk (Dobrzycka-Krahel & Rzemykowska 2010).

To date, the species has not been recorded in the

Figure 1

Records of *Dikerogammarus villosus* in Poland (based on Gruszka et al. 2003, Konopacka 2004, Grabowski et al. 2007a, Bącela et al. 2008, Gruszka & Woźniczka 2008, Dobrzycka-Krahel & Rzemykowska 2010 and recent own data)

Śmiała Vistula and the Vistula Lagoon (Jażdżewski et al. 2004, Grabowski et al. 2006, Surowiec & Dobrzycka-Krahel 2008, Dobrzycka-Krahel et al. 2013).

D. villosus is one of the most ecologically successful and aggressive immigrants belonging to Ponto-Caspian amphipods (Bij de Vaate et al. 2002, Dick et al. 2002). It has been called the "killer shrimp" because of its predatory and extremely aggressive behavior towards other species (Dick & Platvoet 2000). Laboratory studies (Dick et al. 2002) have demonstrated its predatory habits towards invertebrates (for example: *Caenis robusta, Ischnura elegans*, *Sigara* sp., *Chironomus* sp., *Asellus aquaticus, Neomysis integer, Eurycercus lamellatus*, *Piscicola geometra)* showing that it can prey upon and replace other species. It often kills or simply bites much more prey than it can eat. *D. villosus* preys on *Gammarus duebeni* and *Gammarus tigrinus* (Dick and Platvoet 2000), and it appears that the species is able to eliminate both native and exotic species.

Discussing the biological attributes of successful Ponto-Caspian invaders, Bij de Vaate et al. (2002) report non-specific food preferences. In fact, *D.*

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villosus is omnivorous (Dedyu 1980), i.e. it feeds on detritus and also on micro-algae (Dick and Platvoet 2000, Platvoet et al. 2006). The species is known to have a high reproductive potential, expressed by rapid growth, early maturity, a short generation time and multiple reproduction (Devin et al. 2004, Kley and Maier 2006, Grabowski et al. 2007 b, Pöckl 2007). In summary, this species has been shown to have the potential to reduce the biodiversity in inhabited areas.

The species is eurythermic (Dedyu 1980). It tolerates a wide range of salinity – it can survive in salinities up to about 20 PSU, but in salinities of 15-20 PSU, the mortality is high, and 26 PSU is lethal (Bruijs et al. 2001).

Such a set of characteristics shows that this gammarid is potentially very expansive and invasive, however, there have been no previous studies based on the osmoregulatory ability of the species to predict its osmotic possibility to penetrate various water bodies. The osmoregulation pattern shows the character of osmotic possibilities of the species. Osmoregulatory capacity (osmotic capacity; OC) is the difference between the osmotic pressure of the haemolymph and of the external medium at a given salinity. Osmoregulatory capacity (OC) is a useful indicator to measure the stress of organisms exposed to different salinities (Brito et al. 2000).

Additionally, information concerning the expansion of the species and data given in the literature cited show that *D. villosus* is a relatively sensitive species to fluoride and cadmium toxicity.

The aim of the study was to determine the actual and future spread of *D. villosus* and the osmoregulatory abilities of the species.

Materials and methods

Field collection

Material for the study of the *D. villosus* distribution was collected at sampling sites in the Vistula Lagoon, the Śmiała Vistula (one of the Vistula branches) and the Gulf of Gdańsk (Fig. 2, Table 1) in 2011 and 2012. The gammarids were collected during the spring/summer period (20 May 2011, 31 May 2011, 13 July 2011, 11 July 2012, 30 July 2012, 31 July 2012). Samples for the present studies were collected in the nearshore zone, from the submerged plants and from the sandy or sandy-muddy bottom with decaying plant remains. Rush vegetation (*Phragmites* sp., *Typha* sp. and *Scirpus* sp.) occurred along the banks of the Vistula Lagoon and the Śmiała Vistula; *Pilayella* sp. and *Ulva* sp. were

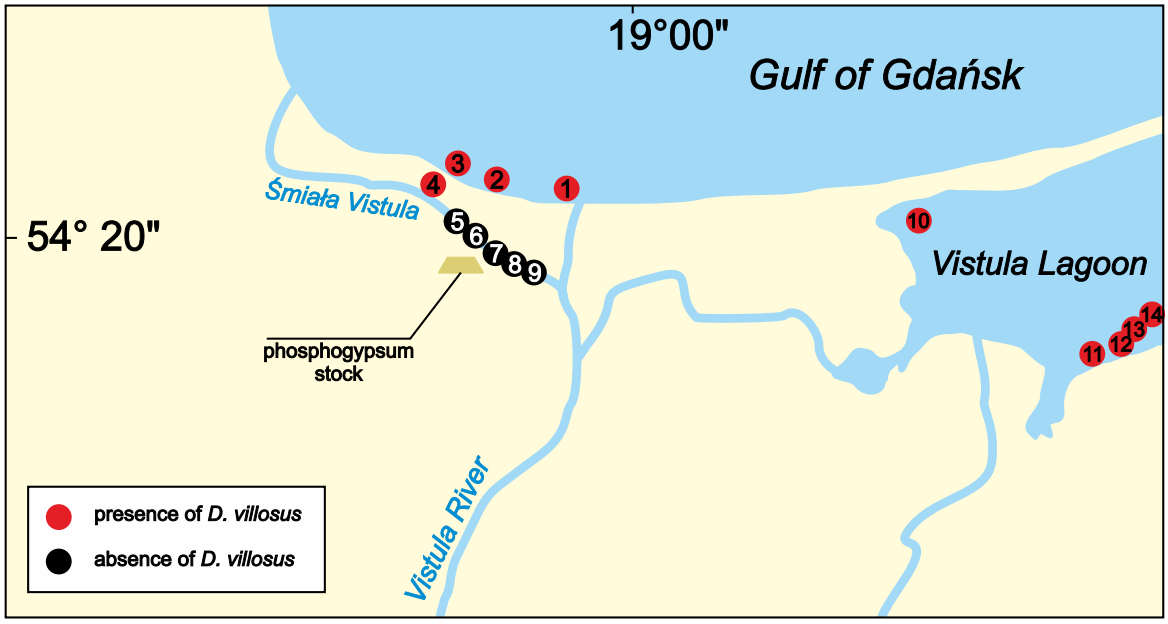

Figure 2

Sampling sites (described in Table 1) in the Gulf of Gdańsk, the Śmiała Vistula and the Vistula Lagoon

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Table 1

Location of sampling sites in the Gulf of Gdańsk, the Śmiała Vistula and the Vistula Lagoon in 2011 and 2012

Location	Site number	Site coordinates	2011	2012
Gulf of Gdańsk	1	54°21.646' N 18°56.755' E	July	
	2	54°21.108' N 18°51.190' E		
	3	54°22.645' N 18°47.256' E		
Śmiała Vistula	4	54°22'05.31"N 18°46'58.35"E	May	July
	5	54°20'27.02"N 18°49'25.28"E		
	6	54° 19' 59.88"N 18° 50' 21.15"E		
	7	54° 19'28.41"N 18° 51'13.96"E		
	8	54° 18'55.29"N 18° 53'32.74"E		
	9	54° 18'50.90"N 18° 54'37.76"E		
Vistula Lagoon	10	54° 20'22.66"N 19° 14'16.26"E	-	July
	11	54°17'09.09"N 19° 26'09.81"E	-	
	12	54° 18'01.72"N 19° 27'58.35"E	-	
	13	54° 18'24.91"N 19° 28'34.86"E	-	
	14	54° 19'34.10"N 19° 31'30.36"E	May	

observed at some sites, and *Pilayella* sp. and *Ulva* sp. occurred in the Gulf of Gdańsk.

The animals were sampled at each site by two persons for 45 minutes using a hand net of 1 mm mesh size (Jażdżewski et al. 2004). In that way, representative samples could be collected. Later, the samples were preserved in buffered 4% formaldehyde solution and transported to a laboratory for taxonomic identification. Water salinity and temperature were measured using WTW Microprocessor conductivity meter LF 196. Water salinity varied during sampling of the material from 1.4 to 3.5 PSU in the Vistula Lagoon, from 0.2 to 4.9 PSU in the Śmiała Vistula and from 5.6 to 6.8 PSU in the Gulf of Gdańsk. Water temperature varied from 12.6 to 24.0°C in the Vistula Lagoon, from 11.4 to 20.4°C in the Śmiała Vistula and from 20 to 21.8°C in the Gulf of Gdańsk.

The animals used in the laboratory experiments aimed at determining the osmotic concentration of hemolymph were collected at Górki Wschodnie in the Gulf of Gdańsk (14 May 2012) where salinity was 6 PSU. Later the living animals were transported to the laboratory for taxonomic identification and experiments.

Laboratory procedure

The species was taxonomically determined according to Mordukhai – Boltovskoi et al. (1969) and Konopacka (2004) at the laboratory using a NIKON SMZ 800 stereoscopic microscope. The living animals were acclimated to the laboratory conditions for at least 7 days. All animals were placed in an aquarium containing water, sediment and vegetation from the collection site. The water in the aquarium was aerated (O_2 saturation = 100%), and the experiments were carried out at the constant temperature of 18°C.

Following their acclimation to laboratory conditions, the animals were acclimated to different salinities: 2, 6, 10, 14, 18 and 22 PSU. The temperature and aeration, and also the environmental conditions (e.g. a sandy bottom) remained unchanged.

Gammarids were placed in small perforated containers and these in larger containers filled with water of a given salinity (9 animals in each container). The gammarids were not fed during the experiment.

Water of a particular salinity was prepared using mains water and a relevant amount of artificial sea salt: 'Reef Salt'. The salinity in the aquaria was changed at a rate of 2 PSU/24 h. The animals were acclimated at the experimental salinity for 48 h, after which hemolymph was sampled, because the hemolymph osmolality of gammarids has stabilized after at least 24 hours (Sutcliffe 1968).

The body length of individuals ranged between 10 and 14 mm and moulting was not observed in any of them.

Determination of the osmotic concentration of hemolymph

Before the hemolymph was sampled, the animals were carefully dried on a paper towel. The hemolymph was sampled by inserting a glass capillary between the 2nd and 3rd thoracic somites. To maintain the hemolymph in the liquid state, liquid paraffin was drawn into the capillary immediately before and after drawing in the hemolymph. This

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prevented the hemolymph from evaporating of water from the capillary, ensuring the constant concentration of the hemolymph. The paraffin at either end of the capillary tube ensured that there was no contact between the hemolymph and the external environment. The osmotic concentration of the hemolymph was determined in the presence of distilled water, which served as a standard. Samples containing the standard were prepared in exactly the same way as the hemolymph samples.

The samples were kept in a freezer (at -20°C) until the measurements were conducted. Immediately prior to measuring the hemolymph, the capillaries were cooled to a temperature of -67°C using a freezer (AG CHEMIA "Freeze").

Osmotic concentrations were determined microcryoscopically, based on the method used in many studies for freezing-point determinations upon small volumes of fluid (Ramsay 1949; Ramsay & Brown 1955; Dobrzycka & Szaniawska 1995; Dobrzycka-Krahel & Szaniawska 2005, 2007; Normant et al. 2005; Dobrzycka-Krahel & Graca 2014; Dobrzycka-Krahel et al. 2014). A thermally isolated chamber was placed on the stage with a polarizing filter. The chamber had a transparent bottom and contained 95% solution of ethanol. The chamber was also fitted with a stirrer to maintain the ethanol at a constant temperature. The temperature inside the chamber was measured using a PT 100 temperature sensor in a four-phase system with a resolution of 0.01°C and an accuracy of 0.02°C. The chamber contained a holder for 5 capillaries. The rate of temperature rise was 0.05°C per minute. In order to observe the melting of hemolymph crystals, a stereoscopic microscope (NIKON SMZ 800) was used with a polarizing (C-POL) accessory.

Both liquid hemolymph and its crystals are equally capable of rotating the plane of polarization. To distinguish between the light passing through the crystal and the light passing through the liquid, the microscope eyepiece was equipped with an analyzer.

The osmotic concentration was expressed in mOsm kg^{-1} as the mean ± SD (Fig. 3).

The differences of osmoregulatory capacity of gammarids in different salinities were compared using the non-parametric Kruskal-Wallis test and Mann-Whitney U post-hoc tests in STATISTICA 10. The statistical comparisons were made at the p=0.05 level.

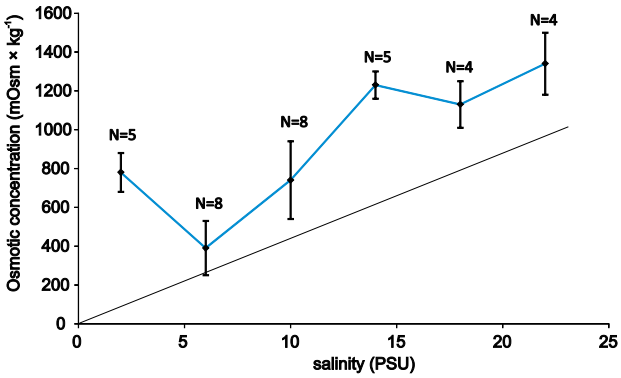

Figure 3

Osmotic concentration of *Dikerogammarus villosus* at different salinities. The dots denote the mean osmotic concentration at a given salinity. The straight line is the isoosmotic line. Error bars indicate standard deviations.

Results

Distribution of *D. villosus* in the Vistula Lagoon, the Śmiała Vistula and the Gulf of Gdańsk

On the 20th of May 2011 in the Vistula Lagoon at Tolkmicko and on the 31st of May 2011 in the Śmiała Vistula at Górki Wschodnie, individuals of *D. villosus* (Sowinsky, 1894) were recorded for the first time in these areas. Additionally, in 2011, individuals of this species were noted in the Gulf of Gdańsk at Świbno, Sobieszewo and Górki Wschodnie on the 13th of July 2011 and the 31st of July 2012 (Fig. 2, Table 2).

Osmoregulatory ability of the species

The shape of the osmoregulatory curve (Fig. 3) indicates that *D. villosus* is an osmoregulator over a wide range of salinities, which provides evidence for a hyperosmotic strategy at salinity from 2 to 22 PSU. At environmental salinity of 6 PSU, the differences in the hemolymph osmotic concentration and the external environment are the lowest. The highest differences in the hemolymph osmotic concentration and the osmotic concentration of the medium are at salinity of 14 PSU (Fig. 4). The differences in osmoregulatory capacity of gammarids at different salinities, using the non-parametric Kruskal-Wallis test, were statistically significant (χ^2=17.24201, df=5, p=0.0041).

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Table 2

Records of *Dikerogammarus villosus* in the Gulf of Gdańsk, the Śmiała Vistula and the Vistula Lagoon in 2011 and 2012 (*ns* – no specimens)

Location	Site	2011			2012		
		Water salinity (PSU)	Water temperature (°C)	Number of *D. villosus* individuals	Water salinity (PSU)	Water temperature (°C)	Number of *D. villosus* individuals
Gulf of Gdańsk	1	5.6	21	33	6.5	20	10
	2	6.1	21.8	51	6.5	21	23
	3	6.1	21.8	37	6.8	21	17
Śmiała Vistula	4	4.8	19	2	5.9	21.2	*ns*
	5	4.8	19	*ns*	5.9	21.2	*ns*
	6	4.8	19	*ns*	5.9	21.2	*ns*
	7	3.9	22.7	*ns*	4.9	25	*ns*
	8	3.9	22.7	*ns*	4.9	25	*ns*
	9	3.9	22.7	*ns*	4.9	25	*ns*
Vistula Lagoon	10	-	-	-	1.7	24.7	24
	11	-	-	-	1.8	29.3	6
	12	-	-	-	1.7	24.7	12
	13	-	-	-	1.8	29.3	11
	14	0.2	20	1	1.7	24.7	12

There were statistically significant differences between the osmoregulatory capacity ($p<0.05$), between individuals in 14 PSU and both lower (10 PSU) and higher salinities (18 and 22 PSU), using Mann-Whitney U post-hoc tests.

Additionally, there were statistically significant differences in osmoregulatory capacity ($p<0.05$), between individuals at environmental salinity of 6 PSU and both lower (2 PSU), and higher salinities (14, 18 and 22 PSU), using Mann-Whitney U post-hoc tests.

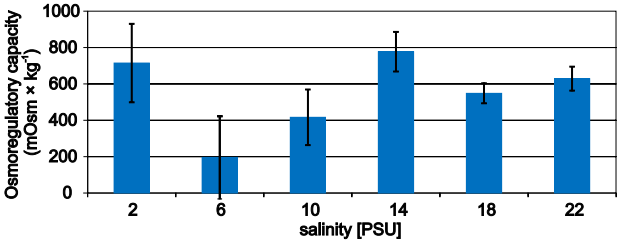

Figure 4

Osmoregulatory capacity of *Dikerogammarus villosus* at different salinities. The bars denote the mean osmoregulatory capacity at a given salinity. The plot takes account of standard deviations.

Discussion

The current study shows that *D. villosus* is a species constantly expanding its range of occurrence. In Poland, it is found in both fresh and brackish waters (Grabowski et al. 2007a). It is recorded in waters of relatively low salinity, and also in fresh waters, in the rivers: the Oder, the Vistula and the Bug (Müller et al. 2001, Konopacka 2004, Bącela et al. 2008). In brackish waters, for example, it is recorded in the Szczecin Lagoon (Gruszka et al. 2003, Gruszka & Woźniczka 2008) where the salinity (1-2 PSU) is determined by inflows of seawater and fresh waters (Majewski 1980), in the adjacent coastal waters of the Baltic Sea (about 7-8 PSU), in the Śmiała Vistula (0.2 – 4.9 PSU), in the Vistula Lagoon (1.4 - 3.5 PSU) and in the Gulf of Gdańsk (5.6 – 6.8 PSU) (Dobrzycka-Krahel and Rzemykowska 2010, this study).

On the basis of the presented results, we can conclude that *D. villosus* has established a population in the Vistula Lagoon and in the Gulf of Gdańsk. Lack of this species in the Śmiała Vistula in 2012, despite being recorded in 2011, may be the effect of the water pollution (lack of *D. villosus* individuals, although the occurrence of other alien gammarids of Ponto-Caspian origin: *Pontogammarus robustoides*, *Obesogammarus crassus* and *Dikerogammarus*

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haemobaphes and of North American origin: *Gammarus tigrinus* (own unpub. results) in the Śmiała Vistula in 2012, indicates particular adaptive constraints of *D. villosus).*

Despite the wide range of tolerance to salinity changes in *D. villosus*, most probably due to the geological history of the Ponto-Caspian region, organisms surviving the successive waves of water salinization and freshening evolved similar biological properties (Zenkievich 1959). The lack of the species in the Śmiała Vistula was observed.

The shape of the osmoregulatory curve is an excellent indicator of how the salinity of the external environment affects the homeostasis of an organism's internal environment. Various papers on this subject provide a good illustration of the strategies for adapting to ambient salinity (McLusky et al. 1970, Dobrzycka & Szaniawska 1995, Dobrzycka-Krahel & Surowiec 2011). The osmoregulatory curve of *D. villosus* indicates that individuals of this species possess osmoregulatory properties over a broad range of external salinity (2 - 22 PSU) (Fig. 3). Hyperosmotic regulation occurs throughout the whole range of salinities tested. Other investigations have indicated that *D. villosus* can regulate the water permeability and the sodium influx, both of which are important osmoregulatory mechanisms in euryhaline gammarids. The species has a serious invasion risk to areas accessible via transit through brackish water with salinity of 20 PSU or less (Brooks et al. 2008).

It is known that osmotic concentrations of body fluids may be higher in freshwater and lower in brackish conditions (Dobrzycka-Krahel and Surowiec 2011).

The osmoregulatory capacity of an organism is an indicator of its efforts to regulate the concentration of its body fluids. The ability of *D. villosus* to maintain high osmoregulatory capacity at low ambient salinity (2 PSU), and also at 14 PSU, indicates that the species uses a considerable amount of energy in doing so (Fig. 4). This phenomenon probably may be connected with gill Na^+, K^+-ATPase activity in this species. In such salinity, when gill Na^+, K^+-ATPase of gammarids shows the maximum activity level, the hemolymph/medium sodium gradient has the highest value. In this case, probably the active involvement of gill Na^+, K^+-ATPase may affect the osmoregulatory process (Brooks & Mills 2006).

Many papers make reference to the relationship between the osmoregulatory capacity of an organism and its metabolic rate (J g^{-1} WW h^{-1}). The greater difference in concentration between the external and internal environments show that more energy has to be expended on metabolic processes. Hence, in salinities at which a high osmotic concentration is to be maintained, greater amounts of energy have to be expended on life processes (Normant & Lamprecht 2006, Normant & Gibowicz 2008).

An organism in a low-salinity environment is compelled to expend energy on the active transport of ions from the environment to prevent the ionic concentration of body fluids from decreasing (Styczyńska-Jurewicz 1972). With the increasing environmental salinity, tantamount to the increasing ionic concentration of the environment, the intensity of active ion transport decreases, since the organism is no longer at risk of a decrease in the ionic concentration of its body fluids. Osmoregulatory capacity (OC) is a useful test to evaluate the physiological conditions of investigated organisms and determines the environmental preferences of the species (Brito et al. 2000). Many authors agree that osmoregulatory capacity of the species can be related to its distribution according to salinity (Brito et al. 2000, Raj and Raj 1982). The osmoregulatory capacity of an organism is an indicator of its efforts to regulate the concentration of its body fluids. Osmoregulatory capacity determines the environmental preferences of the species and shows that 6 PSU is the best tolerated salinity for the individuals from the Gulf of Gdańsk. The osmoregulatory capacity has the lowest value at this salinity.

The osmoregulatory curve shows that the gammarids regulate until 22 PSU and probably above this salinity, the iso-osmotic point will be achieved.

These studies thus have shown that *D. villosus*, a species of Ponto-Caspian origin, is able to survive in a wide range of salinities and to adapt to environments with varying salinity. They have also demonstrated that the spread of this species to environments with different salinities is possible. *D. villosus* specimens used in the present investigations of osmotic regulations were sampled in the Gulf of Gdańsk where salinity is about 6 PSU. In fact, other populations of *D. villosus* in Polish waters live in

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lower salinity.

The type of hyperosmotic regulation of *D. villosus* is similar to the shape of osmoregulatory curve of *P. robustoides* (Dobrzycka-Krahel & Surowiec 2011). *D. villosus*, similarly as *P. robustoides*, is able to hyperregulate over a wide range of salinities. However there are differences in osmoregulation between individuals of the same species living in low and fully marine environments (Janas & Spicer 2010) so probably individuals of this species from other salinities can exhibit differences in osmoregulation.

It is very likely that the species will soon colonize e.g. the Danish Straits where it has not yet been recorded. Further, the continuous monitoring should be carried out in order to check the direction and extend of expansion of this species.

The highest differences between the hemolymph osmotic concentration and the osmotic concentration of the environment at salinity 14 PSU show that such salinity is less preferred by the species. There are significant differences in osmotic effort between gammarids from this salinity (14 PSU) and individuals living in 6, 10, 18 and 22 PSU. Gammarids maintain similar high osmoregulatory capacity only in the lowest salinity (2 PSU), which does not differ significantly from that of the individuals exposed to 14 PSU. There was also a statistically significant difference between the osmoregulatory capacity of individuals from the environmental salinity (6 PSU), and individuals from both lower (2 PSU) and higher salinities (14, 18 and 22 PSU). This means that gammarids after moving from environmental salinity (6 PSU) to both lower salinity (2 PSU) and higher salinities (14, 18 and 22 PSU) take a significant osmotic effort. On the basis of the above analysis, we can conclude that the survival of gammarids in different salinities is associated with osmotic stress. However, despite the osmotic effort of organisms, varied salinities are not barriers for individuals of *D. villosus*.

The lack of *D. villosus* in the Śmiała Vistula may be explained by location of sampling sites. The phosphogypsum waste dump is located on the bank of the Śmiała Vistula (Fig. 2), near the sampling sites. The concentrations of different toxic substances are very high in the phosphogypsum stack area and lower in the Śmiała Vistula river near the phosphogypsum waste dump (Räike et al. 2015) (Table 3). During the high-flow periods (e.g. snow melt season in spring), however, water accumulates in the ditches, whereas the excess water, not taken up in vegetative processes, evaporates in the summer season (Räike et al. 2015).

The results (Gonzalo et al. 2010) indicate that *D. villosus* is a very sensitive species to fluoride toxicity. 96h-LC_{50} for this species = 5 800 µg F^- l^{-1}.

Table 3

Concentrations of F and Cd in the Śmiała Vistula (ŚV) and near phosphogypsum waste dump (Lethal concentrations for *Dikerogammarus villosus*: 96h-LC_{50} = 5 800 µg F^- l^{-1} (Gonzalo et al. 2010) 96h-LC_{50} = 4.7 µg Cd l^{-1} (Boets et al. 2012))

Pollutant	Location	Concentration (µg l^{-1})	Reference
F	in the ŚV river near the phosphogypsum waste dump	< 500	Räike et al. 2015
		260-430	Regional Inspectorate for Environmental Protection, Gdańsk 2013
	in the heap's vicinity	5.6	Greenpeace 2007
	in melioration ditch	990	Räike et al. 2015
	in the reserve moat	35 000	
	in filtration pond	120 000	
	in evaporation pond	230 000	
Cd	in the ŚV river near the phosphogypsum waste dump	0.013-0.043	
		0.0375-0.25	Regional Inspectorate for Environmental Protection, Gdańsk 2013
	in the heap's vicinity	< 10	Greenpeace 2007
	in melioration ditch	0.18	Räike et al. 2015
	in the reserve moat	1 200	
	in filtration pond	1 400	
	in evaporation pond	2 000	

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The concentrations of fluoride are relatively high near the phosphogypsum waste dump (Table 3). In general, the fluoride (F^-) concentration in unpolluted fresh waters ranges from 0.01 to 0.3 mg F^- l^{-1} [10 to 300 µg F^- l^{-1}] whilst in unpolluted seawater: from 1.2 to 1.5 mg F^- l^{-1} [1 200 to 1 500 µg F^- l^{-1}] (World Health Organization 2002).

The results (Boets et al. 2012) indicate that *D. villosus* is the most sensitive to Cd toxicity (96h-LC_{50} = 4.7 µg Cd l^{-1}) followed by *Gammarus pulex* (96h-LC_{50} = 20.3 µg Cd l^{-1}), *G. fossarum* (96h-LC_{50} = 64.2 µg Cd l^{-1}), *G. roeseli* (96h-LC_{50} = 8.6 µg Cd l^{-1}), *G. tigrinus* (72h-LC_{50} = 146.5 µg Cd l^{-1}) and *Echinogammarus berilloni* (96h-LC_{50} = 88.9 µg Cd l^{-1}). It might be that this ion disturbs the iono-regulatory homeostasis.

In the Śmiała Vistula, fluoride and cadmium levels generally ranged within regular values (Table 3): fluoride concentrations are in the second class, cadmium concentrations are in the first class of water quality (Regional Inspectorate for Environmental Protection, Gdańsk 2013), but higher concentrations of pollutants were not recorded due to reduced frequency of sampling and determination (samples are currently collected and determined only sporadically).

Tests on fluoride and cadmium toxicity on *D. villosus* combined with monitoring data should be continued in the Śmiała Vistula.

Conclusions

- *D. villosus* is a species constantly expanding its range of occurrence. The species spreads in the Polish Baltic coastal waters.
- Marine waters do not present an osmotic barrier to the species, which may facilitate its spread to the seas.

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