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Non-indigenous amphipods and mysids in coastal food webs of eastern Baltic Sea estuaries

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*The study analyses the role of non-indigenous invertebrates in the food webs of two eutrophic brackish estuarine ecosystems of the Baltic Sea: the Neva River estuary and the Curonian Lagoon, with the aim of clarifying several questions such as what trophic levels were occupied by newly established species (mainly amphipods and mysids) and whether they can affect the native benthic invertebrates as a result of their possible carnivorous nature. Stable isotope analysis ($\delta^{15}N$ values) and gut contents analysis of field-collected specimens were used to estimate trophic level and trophic links of the newly established malacostracan crustaceans, while their consumption rates when feeding as carnivores were measured experimentally. The $\delta^{15}N$ analysis allocated four trophic levels (TL) in the coastal food webs of both studied ecosystems with the lowest $\delta^{15}N$ (2–4‰) for detritus and algae and the highest for fish (12–14‰). Through their high abundance, non-indigenous crustaceans (*Pontogammarus robustoides, Gmelinoides fasciatus, Obessogammarus crassus, Gammarus tigrinus, Limnomysis benedeni *and* Paramysis lacustris*) have become important members of food chains of the studied ecosystems. Their trophic position varied significantly within species during ontogenesis. This suggests that they turned from being typically detritivores/plantivorous (TL 2–2.4) at juvenile stages to omnivores (2.5–3) or to carnivores (>3) as adults. Assessment of the predation pressure by the adult amphipods on other coexisting invertebrates (in the example of the Neva Estuary) showed a low or medium impact, depending on species of predator and productivity of its potential prey organisms.*

Keywords: macrofauna, trophic level, non-indigenous species, predation, Neva Estuary, Curonian Lagoon

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INTRODUCTION

Coastal areas of the eastern Baltic Sea are characterized by high primary production and high heterogeneity of microhabitats supporting diverse and abundant benthic communities (Lauringson & Kotta, 2006; McGlathery *et al.*, 2007; Schiewer, 2008). At the same time, species richness and abundance of these communities may be declining as a result of non-indigenous species effects. In recent decades, non-indigenous species of amphipods and mysids were recorded as key taxa in coastal communities of the Neva River estuary and the Curonian Lagoon (Berezina *et al.*, 2011). In the Neva Estuary, the non-indigenous amphipods (*Pontogammarus robustoides, Gmelinoides fasciatus* and *Gammarus tigrinus*) currently make up an important part (30–40%) of the benthic community biomass (Berezina *et al.*, 2011). Similarly, non-indigenous crustaceans (*P. robustoides, G. tigrinus, Obessogammarus crassus, Limnomysis benedeni* and *Paramysis lacustris*) were recorded as the dominant species with high occurrence frequency (>80%) among the benthic invertebrates in the coastal ecosystem of the Curonian Lagoon (Daunys & Zettler, 2006; Arbaciauskas, 2008; Lesutiene, 2009). Most of these species (the Ponto-Caspian species *P. robustoides, O. crassus, L. benedeni* and *P. lacustris*) were introduced to both studied estuarine ecosystems as part of a state-sponsored programme in the former USSR for introductions of aquatic invertebrates from southern areas (Black Sea basin) to the Baltic Sea basin (Gasiunas, 1972), implemented in the 1960s–1970s. This programme was aimed at improving the food base for local commercial fish. Other species (*G. fasciatus* and *G. tigrinus*) colonized coastal areas from other parts of the Baltic Sea and from European inland waters where they were introduced intentionally or accidentally with the ballast waters of ships (Berezina, 2007a).

Trophic connections of a newly introduced organism could provide an important clue as to whether it has already become established and in which role in the recipient ecosystem. Recent studies of the non-indigenous amphipods and mysids pointed toward possible negative effects on native species (Kelly & Dick, 2005; Bollache *et al.*, 2008; Fink *et al.*, 2012). Selective predation by some invasive amphipods is considered as the main mechanism explaining the replacement or a decrease in the abundance of invertebrate species co-existing with invasive amphipods (Dick *et al.*, 2002).

Although patterns of predation impacts of some amphipods and mysids on species and communities have been analysed in different regions (Devin *et al.*, 2005; Kelly *et al.*, 2006; Lesutiene *et al.*, 2007; Bollache *et al.*, 2008), at present there is not sufficient information on food habits and trophic links

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within communities for many non-indigenous species of malacostracan crustaceans from the Baltic region to evaluate their effectiveness as predators. Furthermore, MacNeil *et al.* (1997, 1999) demonstrated omnivory in many species of gammaridean amphipods, with a mix of different feeding strategies such as saprophagy, phytophagy and carnivory. Therefore, major variations in feeding habits, an ability to prey upon invertebrates and possible differences in position in food webs may be expected between species and within species of studied crustaceans.

We propose that their intra-species differences in food preferences may depend firstly on body size, because diet shifts with increasing proportion of animal food together with an increase of body size were found in some amphipod species from northern regions (Berezina, 2007b). Fry (1983), who measured the isotopic compositions of juvenile shrimp feeding in south Texas grass flats, also found size-dependent differences in feeding habits. The juveniles had isotopic composition values $\delta^{13}C = -11$ to -14 and 6–8‰ for nitrogen ($\delta^{15}N$) and as the shrimps grew and migrated offshore the isotopic composition converged towards offshore values of -16 and $+11.5$ for C and N, respectively. Also, Jennings *et al.* (2002) demonstrated that body mass in marine fish and benthic invertebrates was positively and significantly related to their trophic level at the community scale and also that the trophic level may change across body sizes of consumers. Until now there are no clear patterns between body size/ontogenetic stages and trophic level of indigenous and newcoming benthic invertebrates, from coastal areas of the Baltic Sea.

Some recent studies on the food web in the Curonian lagoon and several lakes (Arbaciauskas *et al.*, 2013) have tested whether the nutrient status of environments also affects the feeding strategy and body stoichiometry of successfully spreading species and showed that invading species may have a stronger effect on the local biota in ecosystems with higher phosphorus levels (including eutrophied estuarine ecosystems of the Baltic Sea), which promote its growth, and nitrogen limitation that should favour predation. At the same time, quantitative assessment of impact of the non-indigenous species on local fauna is needed to better understand possible disruption of the community structure after establishment of new members.

This paper studies structure of food chains in littoral communities of the Neva River estuary and the Curonian Lagoon (Neman River estuary) with distinct traits of anthropogenic eutrophication and summer hypoxia events. The main questions were to clarify what trophic positions in the coastal food chains of these ecosystems are occupied by newly established malacostracan crustaceans and whether these invaders may affect benthic invertebrates as a result of their carnivorous nature. We hypothesized that trophic positions of studied species may differ between species and change during ontogenesis within the same species. Also, it was important to find some similarity or differences in trophic chains structure and position of non-indigenous species among the two largest shallow water estuarine ecosystems of the eastern Baltic Sea (Neva Estuary and Curonian Lagoon).

We evaluated the significance of non-indigenous amphipods and mysids in the food chains of these two Baltic estuaries using a combination of methods such as microscopic gut content analysis and stable isotope composition ($^{15}N/^{14}N$) measurements in the tissues of consumers. Stomach/gut contents analysis served to describe the diet of animals (Hyslop, 1980; Ahlbeck *et al.*, 2012). The nitrogen stable isotope ratio ($^{15}N/^{14}N$) in organisms' tissues, in turn, gave a more general idea of their trophic position in food chains and could be further used to understand general ecological processes in natural communities (Peterson & Fry 1987; Post, 2002; Michener & Lajtha, 2007). Finally, we estimated feeding rates of three predaceous amphipod species, and calculated production rates of their potential prey as a way to assess the potential predation impact of newly established species in the estuarine ecosystem at community level.

MATERIALS AND METHODS

Study area and sampling sites

The largest estuaries of the Baltic Sea such as the Neva River estuary (3600 km^2) and the Curonian lagoon (1584 km^2) are characterized by rather similar physical and chemical parameters. Table 1 reports the most important characteristics of both ecosystems. They are affected by irregular marine water intrusions due to stochastic water exchanges with the Baltic proper and characterized by a horizontal gradient of water salinity from 0 to 7 (Schiewer, 2008). Both ecosystems share similar biological traits such as high nutrient loads and summer micro- and macroalgal blooms. During calm summer weather intensive algal blooms develop and abundance of planktonic algae may increase exponentially over a short period (Pilkaitytė & Razinkovas, 2006). During the blooms' chlorophyll *a* reaches a maximum of 15 $\mu g\, l^{-1}$ in the Neva Estuary (Golubkov, 2009) and $>30\ \mu g\, l^{-1}$ in the Curonian Lagoon (Bresciani *et al.*, 2012; Zilius *et al.*, 2012), testifying to eutrophication. Euphotic zones of both ecosystems are dominated by fast-growing filamentous algae and higher aquatic plants proliferating on hard substrates. In the Neva Estuary production of organic matter by the macrophytes together with associated epiphytes can be as large as 2–7 g C (carbon) m^{-2} day^{-1} (Berezina *et al.*, 2005).

The study was performed at three sites in the Curonian Lagoon and at three sites in the Neva Estuary (Figure 1). Species abundance and community structure at these sites had previously been investigated (Gubelit & Berezina, 2010; Berezina *et al.*, 2011).

It was found that amphipods and mysids of studied species are usually associated with vegetated shallow littoral areas (depths of 0–3 m) over the whole ice-free season, from May

Table 1. Main characteristics of studied Baltic Sea estuaries (Golubkov, 2009; Zilius *et al.*, 2012 and own data).

Study area characters	**Neva Estuary**	**Curonian Lagoon**
River	Neva	Nemunas
Total surface area, km^2	~3600	~1600
Average (maximum) depth, m	12 (60)	3.8 (5.8)
Secchi depth, m	0.2–1.8	0.3–2.2
POC, mg l^{-1}	12.0	24.5 ± 3.5c
Salinity	0.04 -5	0.1–7
TP, mg m^{-3}	41–75	27–43
Chlorophyll *a*, μg l^{-1}	10–15	10–34
Trophic status	Eutrophic	Eutrophic/Hypereutrophic

POC, particulate organic carbon in water; TP, total phosphorus in water.

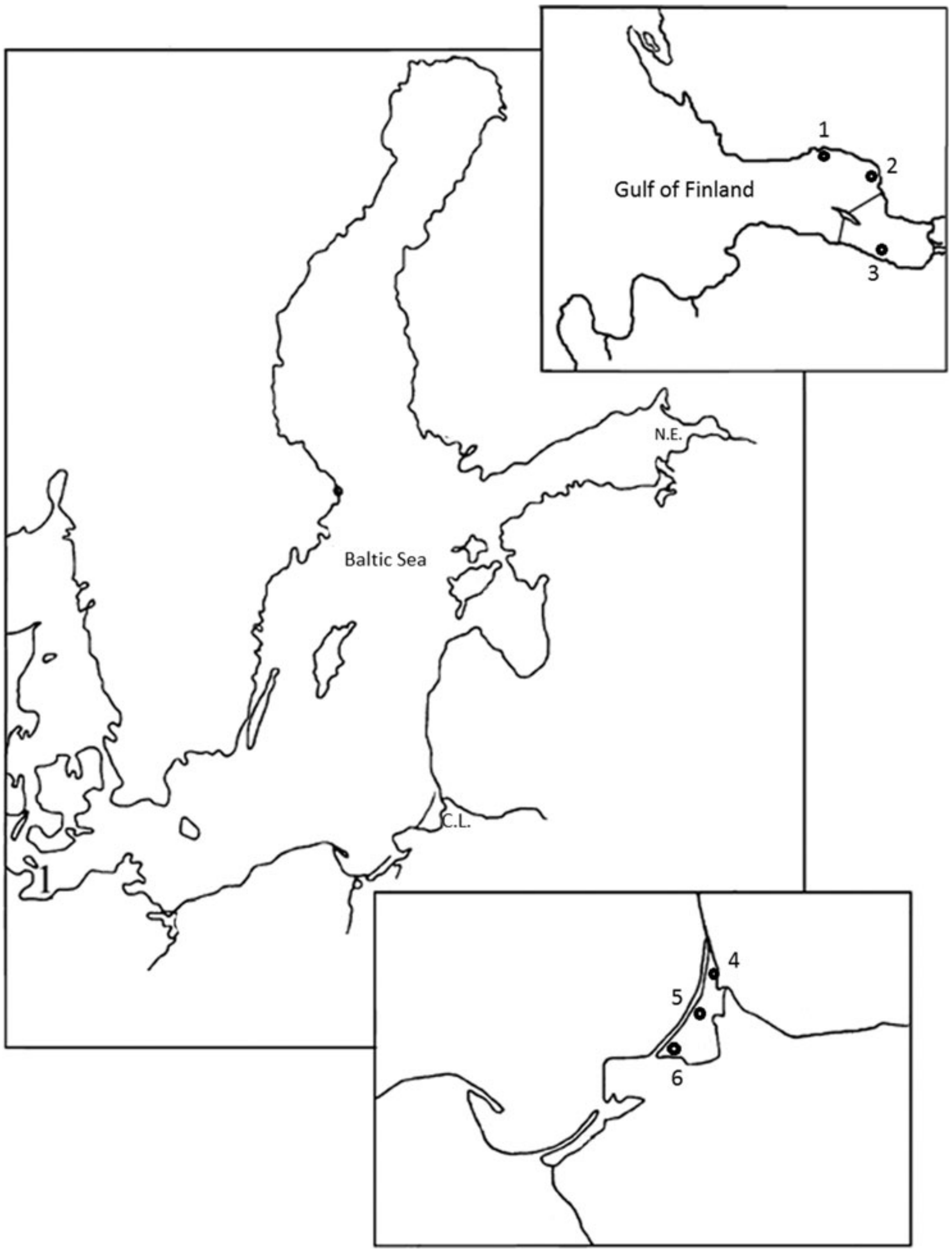

Fig. 1. Study area scheme indicating sampling sites in the Curonian Lagoon (C.L.) and in the inner Neva Estuary (N.E.).

to October. After cessation of reproduction, most of the 1-year-old adult animals die, and the remaining mature specimens born in the current year migrate to deeper areas (4–5 m) for over-wintering.

All sites selected for field sampling were located in the vegetated habitats within sub-aqual stony-sandy littoral (depths of 0–3 m, mean 1 m), and are typical for both estuarine ecosystems. The invertebrate communities at the selected sites are distinguished by the high contribution (>10% of the total abundance and biomass) of non-indigenous crustaceans.

Sample collection and processing

Samples of sediments, plants, invertebrates and fish were collected in July–August 2009 and additionally in the Neva Estuary in July 2013. Animals and plants were sampled by scuba diving or by using a hand net (mesh size 500 μm). Small fish were collected by a small hand-towed trawl net (length 3.5 m; mesh size 5 mm). To evaluate macroinvertebrate biomass we collected quantitative samples with a 0.03 m^2 cylindrical metal frame with 1 m height in three replicates per site.

The animals collected for gut content analysis were preserved in 4% formaldehyde just after collection in the field. Other samples were transported to the laboratory and stored at temperatures of 10–15°C before further analyses (see below).

Stable isotope analysis

The experimental design and the treatments used in the preparation of samples for isotopic analysis of each trophic compartment are described separately.

The 100–150 mg of live aquatic plants, filamentous algae and plant-derived detritus collected at each site were used for the sample preparations. We used a blade to remove epibionts from pond weeds, before rinsing these plants with distilled water and cutting them in pieces of ~1 cm^2 for further drying. Filamentous algae were only rinsed with distilled water to remove most of the epifauna.

Muscle tissues from legs in the case of arthropods and from other parts of bodies of other large benthic animals (molluscs, hirudineans) were collected from three to five specimens. Whole bodies of at least five individuals in the case of juvenile amphipods and mysids and other small invertebrates (II instar larvae of chironomids, *Caenis* spp.) were analysed. A piece of muscle tissue (1–2 g) was taken from the dorsal part of each of three individuals of fish belonging to each species.

Plants, detritus and animals were dried for 48–72 h in a thermostat at constant temperature 50°C. After drying, a homogeneous powder of each sample was prepared using pestle and mortar. Approximately 0.5 mg of powder of animal organisms and 1.5 mg of plants or detritus was put into small tin capsules and weighed using a Mettler Toledo MX 5 balance with a precision of ±1 μg. At least three replicates of the homogeneous powder per each type of organism or material were prepared and analysed.

Stable isotope analysis was performed using continuous flow on an isotope ratio mass spectrometer Thermo Finnigan Delta V Plus connected to an elemental analyser (Thermo Scientific Flash EA 1112) at the Scientific Center of Nature Laboratory for Stable Isotope Analysis (Vilnius Nature Center, Lithuania) in 2009 and at the Laboratory of A.N. Severtsov Institute of Ecology and Evolution (Moscow, Russia) in 2013.

The isotopic composition was expressed in δ units based on the relative difference (in parts per thousand) between the sample and conventional international standards (Vienna Pee Dee Belemnite) carbonate and atmospheric nitrogen (N_2) according to the formula:

$$\delta^{15}N(‰) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R is the ratio of heavy/light isotope content for the element corresponding $^{15}N/^{14}N$ ratio.

The trophic level of studied animals was estimated using the ratio of heavy (^{15}N) to light (^{14}N) stable isotopes of nitrogen. The abundance of heavy isotope ^{15}N in relation to the lighter isotope (^{14}N) usually increases in food chains by about 3–5‰per trophic level. The average 'trophic enrichment' of 3.4‰ per trophic level was used in this study as suggested by Post (2002).

The trophic level (TL) of consumers was calculated with respect to $\delta^{15}N$ values using the equation:

$$TL = (\delta^{15}N_c - \delta^{15}N_b)/3.4 + 2,$$

where $\delta^{15}N_i$ is the nitrogen isotope ratio in consumers and $\delta^{15}N_b$ is the nitrogen isotopic baseline of primary consumers (i.e. trophic level 2; Post, 2002). The measured $\delta^{15}N$ values of the juvenile individuals of crustaceans (amphipods and isopods) and smallest aquatic insect larvae (<5 mm body length) were minimal (Table 2), i.e. they are definitely either herbivores or detritivores. Therefore, $\delta^{15}N$ of these animals was assumed as the baselines and used for TL calculations at each estuary and date separately.

Gut content analyses

Food spectra of the amphipods dominating study areas of both the Curonian Lagoon and the Neva Estuary were analysed using a microscopic gut content analysis.

The guts of at least 20 juvenile specimens with body length of 4–7 mm and the same number of adult specimens (>7 mm) of each studied species (*G. tigrinus, G. fasciatus, P. robustoides, O. crassus*) were analysed. The gut was carefully removed from the amphipod body cavity under a stereoscopic microscope using microneedles and microscissors and dissected on a glass slide in a glycerine drop.

Four food categories such as detritus, macrophytes, animals and inorganic matter were distinguished in the gut content. The frequency of occurrence (in % of tested animals) and relative mass proportion of each food item (in % of the total mass) in the gut content was evaluated using the methods described prior (Berezina, 2007b). Amphipod length was measured from the base of the first antenna to the base of the telson with a stereoscopic microscope equipped with an ocular micrometer.

Experimental measurement of feeding rates during predation

To assess the potential predation impact of invasive crustaceans on local communities we measured experimentally the consumption rate of three amphipod species (*G. fasciatus, G. tigrinus* and *P. robustoides*) feeding as predators and then evaluated how this rate corresponds to production (or growth rate) of their potential prey in the environment.

During 1 week, freshly collected amphipods were acclimated individually in thermostatic rooms to experimental conditions (T = 18–20°C, 12:12 light: dark) and type of prey (oligochaetes *Enchytraeus* sp. from laboratory culture). This prey was selected among other possible prey types (crustaceans, chironomids) for this rate measurement in preliminary tests because of better utilization (whole organism) by the amphipods.

The experiments were conducted in crystallizing dishes (9 cm in diameter) filled to a depth of 6 cm (400 ml) with filtered (membrane filter with pore size 1 μm) and aerated water from the natural habitats of the species. Artificial substrates such as plastic algae and stones were used as shelters for animals.

Because passage through the gut in amphipods takes ~8–10 h (own observations), the tested specimens were not fed for 12 h before experiments in order to keep their guts empty. After that, an amount of prey in excess of five times the body weight of predator was offered. Consumption rate (C) was calculated as the difference between the initial and final mass of prey after 24 h.

The relative intensity of consumption (C_w) is the ratio of the consumption rate (C) and individual wet weight of tested specimen expressed in percentages. Wet weights (W) of prey and consumers were determined using an analytical electro-balance with an accuracy of 0.01 mg. To determine the wet weight animals were carefully blotted with filter paper for 1–2 min.

Table 2. Mean $\delta^{15}N$ (‰) and trophic level of food web members in studied estuaries.

	Curonian Lagoon		**Neva estuary**			
Member year	**2008**		**2008**		**2013**	
Variables	**$\delta^{15}N$**	**TL**	**$\delta^{15}N$**	**TL**	**$\delta^{15}N$**	**TL**
Plant-derived detritus	4.4 ± 0.1	1.0	2.4 ± 0.1	1.0	5.32	1.0
Potamogeton pectinatus	4.4 ± 0.75	1.0	4.1 ± 0.26	1.0	–	1.0
P. perfoliatus	4.5 ± 0.7	1.0	4.1 ± 0.0	1.0	4.2 ± 0.1	1.0
Filamentous algae (+epifauna)	6.8 ± 0.7	–	4.9 ± 0.8	1.0	4.75 ± 0.1	–
Asellus aquaticus	6.15 ± 0.1	2.00	–	–	7.06 ± 0.1	2.08
Bithynia tentaculata	6.6 ± 0.1	2.18	6.74 ± 0.14	2.16	6.8 ± 0.1	2.06
Unio pictorum	–	–	7.28 ± 1.51	2.32	7.8 ± 0.4	2.31
Dreissena polymorpha	8.64 ± 0.1	2.76	7.61 ± 0.55	2.20	7.46 ± 0.3	2.20
Chelicorophium curvispinum	8.55 ± 0.1	2.7	–	–	–	–
Gmelinoides fasciatus						
Juveniles <5 mm	–	–	6.2 ± 0.52	2.0	6.79 ± 0.1	2.0
Females (5–9 mm)	–	–	7.07 ± 0.1	2.26	9.2 ± 0.2	2.71
Males (10–15 mm)	–	–	10.85 ± 0.8	3.37	9.3 ± 0.1	2.75
Obesogammarus crassus						
Juveniles <5 mm	6.03 ± 0.1	2.0	–	–	–	–
Females 6–10 mm	7.55 ± 0.1	2.45	–	–	–	–
Males 10–15 mm	11.9 ± 1.97	3.73	–	–	–	–
Pontogammarus robustoides						
Juveniles <5 mm	6.1 ± 0.1	2.0	6.4 ± 0.1	2.05	6.8 ± 0.1	2.01
Females 6–10 mm	8.52 ± 0.3	2.73	7.29 ± 0.26	2.32	8.5 ± 0.1	2.5
Males 10–15 mm	11.15 ± 0.1	3.51	8.7 ± 0.01	2.75	11.3 ± 0.1	3.34
Gammarus tigrinus						
Males 10–15 mm	–	–	9.1 ± 0.1	2.85	9.6 ± 0.3	2.83
Chironomidae gen sp.						
II instar larvae	–	–	–	–	7.01 ± 0.15	2.07
IV instar larvae	8.74 ± 0.1	2.9	–	–	9.1 ± 0.3	2.68
Caenis spp.	6.2 ± 0.1	2.0	6.3 ± 0.43	2.0	–	–
Ephemerella sp.	–	–	7.54 ± 0.1	2.39	8.4 ± 0.2	2.48
Heptagenia sulphurea	–	–	7.7 ± 1.26	2.43	8.5 ± 0.03	2.50
Ephoron virgo	–	–	8.03 ± 1.1	2.53	8.8 ± 0.3	2.61
Agraylea multipunctata	–	–	7.7 ± 0.14	2.44	6.8 ± 0.1	2.00
Hydropsyche sp.	–	–	9.32 ± 0.1	2.92	9.25 ± 0.1	2.73
Sialis sp.	–	–	9.53 ± 0.03	2.98	–	–
Erpobdella octoculata	10.4 ± 0.1	3.29	9.9 ± 0.1	3.09	11.5 ± 1.2	3.09
Glossiphonia complanata	–	–	10.98 ± 0.6	3.41	12.1 ± 0.4	3.50
Limnomysis benedeni	9.67 ± 0.1	3.07	–	–	–	–
Paramysis lacustris						
Adult 6–9 mm	11.3 ± 0.1	3.55	–	–	–	–
Adult >9 mm	8.5 ± 0.1	2.72	-	-	-	-
Perccottus glenii	–	–	–	–	14.25 ± 0.2	4.2
Perca fluviatilis	13.97 ± 1.5	4.34	14.34 ± 0.3	4.39	14.5 ± 0.1	4.27

Assessment of predation impact by amphipods

Mean biomass of different macroinvertebrates (calculated as wet weight) and abundance of amphipods of different sizes in field populations at each site were used for calculation of potential predation impact (PPI) of the amphipods *G. fasciatus, G. tigrinus* and *P. robustoides* on sample data from Neva Estuary in 2013. *PPI* is the ratio between consumption rate ($C_{pred.}$) of the amphipod population and total production of their prey (P_{prey}) for 24 h (Berezina, 2008): $PPI = C_{pred.}/P_{prey}$. It is ranked as high ($PPI > 1$), moderate ($0.5 < PPI < 1$) or low impact ($0 < PPI < 0.5$).

The consumption rate of the adult part of the amphipod population was estimated using the formula of mass-dependent intensity of consumption (C_w), obtained experimentally above, and biomass (B) of adult amphipods of each species in natural habitats: $C_{pred.} = \sum C_w \times B$.

Oligochaetes, isopods, juveniles of amphipods (1.5–5 mm) and larvae of chironomids, ephemeropterans, trichopterans and other aquatic insects are found as potential prey for the studied amphipods from gut content analysis (this study) and according to earlier results (Berezina, 2007b, 2008). The sum production rate (over 24 h) of these potential prey was calculated using rates of their somatic growth or specific production rate (p_s) and their biomass (B) in field: $P_{prey} = \sum p_s \times B$. The p_s rates for amphipods and insect larvae were adjusted using the following formulae (Golubkov, 2000; Berezina, 2008): Amphipoda $p_s = 0.016 \times e^{0.009T}$; Chironomidae $p_s = 0.0087 \times e^{0.142T}$; Ephemeropterans $p_s = 0.013 \times e^{0.099T}$; Trichoptera $p_{s.} = 0.013 \times e^{0.054T}$. The p_s was accepted as 0.03 for both Oligochaeta and Isopoda (cited in Berezina, 2008). The animals were collected in July when water temperature (T) averaged 20°C; this temperature was also used during *P* calculations.

Statistical analyses

All measured parameters were expressed as mean and standard error (±SE). Statistical tests included linear regression and analysis of variance (ANOVA). Possible differences in isotope signatures of species between sites as well as between proportions of food items in the gut content of amphipods were analysed by the post-hoc Fisher's least significant difference (LSD). Log-transformed values of the consumption intensity rate *vs* log-transformed body weight of amphipods were analysed by pair-wise comparisons of slope and intercept of regression lines at probability level <0.05 according to procedures proposed by Urbakh (1964).

RESULTS

Taxonomic composition of littoral communities and trophic level of invertebrates

Benthic communities in both the Curonian Lagoon and Neva Estuary consisted mainly of eurybiotic taxa. In the Curonian Lagoon, several species of amphipods *Obesogammarus crassus, Pontogammarus robustoides, Chelicorophium curvispinum*; mysids *Paramysis lacustris* and *Limnomysis benedeni*; gastropod mollusc *Bithynia tentaculata*, bivalve mollusc *Dreissena polymorpha*; different species of chironomids, and the hirudinean *Erpobdella octoculata* were recorded as the most abundant invertebrate taxa. A more diverse littoral community of invertebrates was found in the Neva Estuary, with three most abundant amphipod species (*Gmelinoides fasciatus, P. robustoides, Gammarus tigrinus*), the isopod *Asellus aquaticus*, oligochaete Lumbriculidae, hirudineans *E. octoculata* and *Glossiphonia complanata*, molluscs *D. polymorpha, Unio pictorum, Sphaeriidae* gen. sp., *B. tentaculata, Lymnaea ovata, Theodoxus fluviatilis* and several species of aquatic insects in larval stages (*Caenis* sp., *Ephoron virgo, Heptagenia* sp., *Agraylea multipunctata, Hydropsyche* sp., *Sialis* sp. and chironomids) recorded as the most abundant taxa. The common perch (*Perca fluviatilis*) and Chinese sleeper (*Perccottus glenii*) were also recorded, the latter being another non-indigenous species.

Aquatic plants (primary producers) in both estuaries were represented by filamentous algae (mostly *Cladophora glomerata, Ulva intestinalis*) and several species of pondweed *Potamogeton* spp.

Table 2 outlines the mean values of $\delta^{15}N$ and calculated trophic levels (TL) of the organisms in the littoral of studied sites in the Curonian Lagoon and Neva Estuary. The lowest TL was estimated for the juveniles of the amphipods and the larvae of some aquatic insects in the Neva Estuary and for juveniles of the amphipod *O. crassus* and isopod *A. aquaticus* in the Curonian Lagoon (Table 2). A significant enrichment of $\delta^{15}N$ values in studied species of amphipods, together with increasing body size between the smallest individuals (juveniles 3–5 mm) and largest males (10–15 mm and more) was found. For *O. crassus* TL increased from 2 to 3.73 between smallest and largest individuals and from 2 to 3.51 and 3.37 for *P. robustoides* and *G. fasciatus*, respectively. Similarly, notable size differences were revealed between specimens of the mysid *P. lacustris*.

The highest trophic levels were detected for fish in both estuaries (4.34 Curonian Lagoon and 4.27–4.39 Neva Estuary). The aquatic insects, numerous in the Neva Estuary were mostly first level consumers (TL 2–2.61) except for the predaceous trichopteran *Hydropsyche* sp., megalopteran larvae (2.73–2.98) and chironomids (2.68). Hirudineans were found at the second consumer level, TL 3.29 (Curonian Lagoon) and 3.09–3.5 (Neva Estuary). The mysids *L. benedeni* (TL 3.07) and *P. lacustris* (2.72–3.55) abundant in the Curonian Lagoon belong to the omnivore and predaceous consumers.

Differences in trophic level for the same species in the Neva Estuary between years were not significant (*t*-test, $P > 0.05$). At the same time, we found significantly (*t*-test, $P < 0.01$) higher values (differing by 0.26 on average) for TL of several invertebrate species such as *P. robustoides, D. polymorpha* and *E. octoculata* (only in 2013) in the Curonian Lagoon than in the Neva Estuary.

Food spectra of non-indigenous amphipods dominating in studied estuaries

Figure 2 shows mean percentage contribution of different food categories to the gut content of studied amphipods *G. fasciatus, G. tigrinus, P. robustoides* and *O. crassus*. Detritus contributed significantly more than the other types of food. The proportion of animal food varied significantly between the species and between juveniles and adult specimens within species. Ontogenetic dietary differences (in mean proportions) of all studied species were significant for detritus (1-factor ANOVA, all $P < 0.01$) and invertebrates (1-factor ANOVA, all $P < 0.001$).

Diet of *G. fasciatus* juveniles included 80% of detritus and 5% of plant food. The proportion of detritus in the gut content of adult specimens (7–11 mm) was slightly lower (67%), while the proportion of invertebrates reached 25%. Significant differences in the diet of *G. tigrinus* were confirmed by 1-factor ANOVA ($P < 0.0001$), reflecting the higher share of detritus and invertebrates. Figure 2 shows the differences in diet of *G. tigrinus* juveniles (5–7 mm) and adult (7–12 mm) specimens, with the detritus proportion in the gut decreasing from 74 to 45% whereas the proportion of animal food increased from 7 to 38%. Also, the contribution of animal food in the diet of amphipod *P. robustoides* significantly increased with size. The juveniles (5–7 mm) were mainly detritivorous (70%) while adult *P. robustoides* specimens are predominately predaceous (40%), consuming aquatic insect larvae, oligochaetes, isopods and other crustaceans; filamentous algae contributed significantly (>30%) to the diet of adult *P. robustoides*. Algae and small invertebrates (chironomids, protozoans and crustaceans) also comprised an important portion (36 and 31%, respectively) of the diet of adult specimens of another Ponto-Caspian species (*O. crassus*) while its juveniles preferred to consume mostly detritus (70%).

Experimental estimation of amphipod consumption rate

Experimentally assessed size-dependent consumption rates of *G. tigrinus* and *G. fasciatus* were similar, varying from 8–56 and 9–52% of wet weight of body, respectively (Figure 3). *F*-test did not reveal significant differences in regression lines of these two species ($F_{24,34} = 2.25$, ns), but these lines

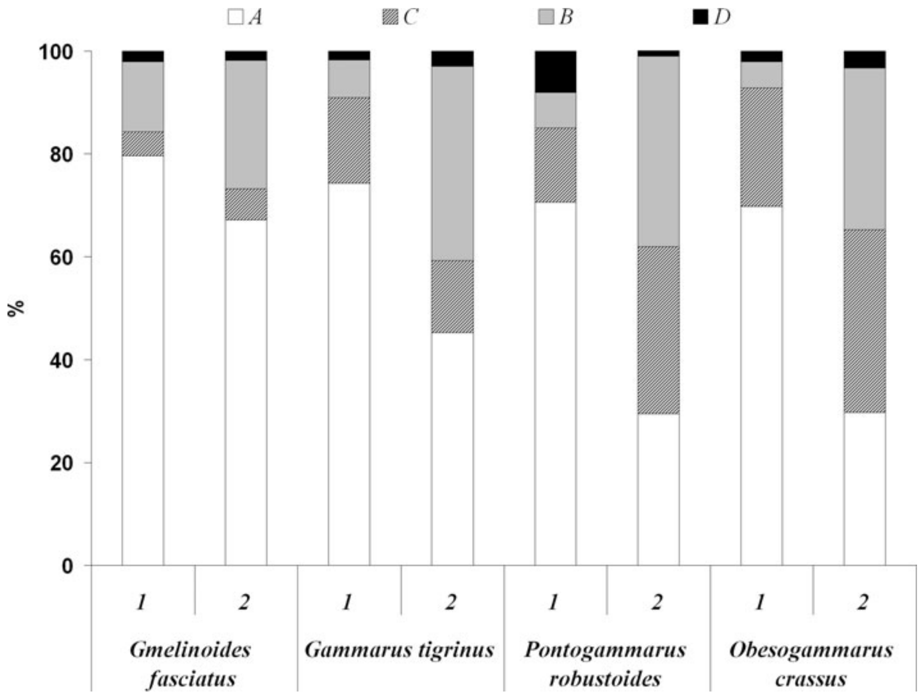

Fig. 2. Differences in proportion of food categories in gut content of juveniles with body size up to 7 mm (1) and adult with body size above 7 mm (2) for the amphipods *Gmelinoides fasciatus, Gammarus tigrinus, Pontogammarus robustoides* and *Obessogammarus crassus* collected at study sites. A – detritus, B – invertebrates, C – plants, D – others.

differed significantly at intercept level (t (intercept) $= 2.76$, $P < 0.05$).

The weight – C_w regression relationships for all studied species were approximated by a power function: $C_{w1} = 2.72 \times W_i^{-0.79}$ for *P. robustoides*, $C_{w2} = 1.24 \times W_i^{-0.78}$ for *G. tigrinus* $C_{w3} = 1.265 \times W_i^{-0.75}$ for *G. fasciatus*, where W_i is individual wet weight.

There were significant differences in the intercept of regression lines between *P. robustoides* and *G. fasciatus* ($F_{30,24} = 1.13$, ns, t (intercept) $= 11.27$, $P < 0{,}001$) although their slopes had no significant differences. Regression lines for other two species (*G. tigrinus vs P. robustoides*) differed significantly in the intercepts and slopes according to F-test (all $P < 0.001$).

Predation impact by non-indigenous amphipods on littoral macrozoobenthos

Amphipod consumption rates and production rates of their prey differed considerably between sites and species (Table 3). The calculated *PPI* indices of *G. fasciatus* and *G. tigrinus* were in the range of 0.01–0.1 testifying to a low potential impact on prey abundance due to predation. The predation pressure of *P. robustoides* was significantly higher reaching a maximum of 0.53. Combined predation impact of all studied amphipods differed significantly between sites ($P < 0.05$) reaching a maximum of 0.58 in the easternmost freshwater part of the estuary and a 4–5 fold decrease in the more marine western part.

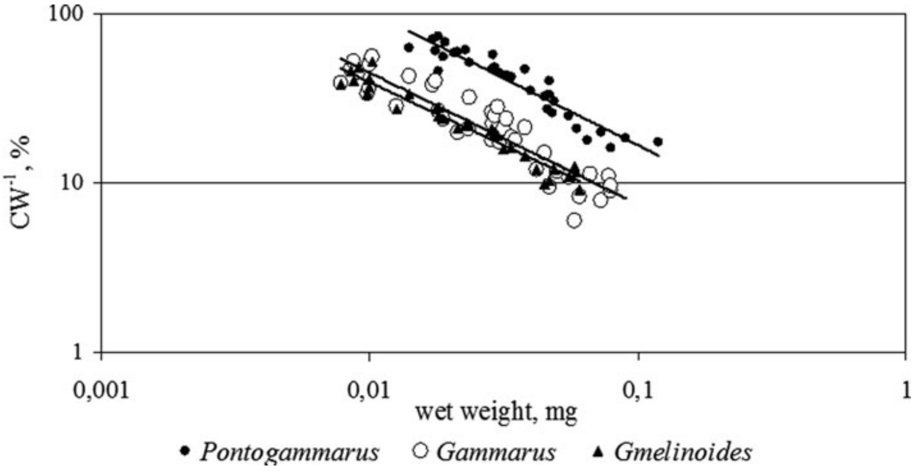

Fig. 3. Relative consumption rates (CW^{-1}, % of body mass day^{-1}) in adult specimens of *Gmelinoides fasciatus, Gammarus tigrinus, Pontogammarus robustoides* fed with oligochaetes.

Table 3. Potential predation impact (PPI) of non-indigenous amphipods in the Neva Estuary (2013).

	Site 1		Site2		Site 3	
P_{prey}	1.42		1.17		1.67	
Species/Parameter	C	*PPI*	C	*PPI*	C	*PPI*
Pontogammarus robustoides	0.051	0.036	–	–	0.896	0.54
Gammarus tigrinus	–	–	0.124	0.11	0.028	0.02
Gmelinoides fasciatus	0.158	0.11	–	–	0.051	0.03
All study amphipod species	0.21	0.15	0.12	0.11	0.98	0.58

C_1, consumption rate ($g\,m^{-2}\,day^{-1}$) for the adult amphipod *Pontogammarus robustoides* at three study sites (mean body length and weight of 12 mm and 25 mg); C_2, the same rate for *Gammarus tigrinus* (10 mm and 15 mg); C_3, the same rate for *Gmelinoides fasciatus* (9 mm and 11 mg); P_{prey}, sum production rate ($g\,m^{-2}\,day^{-1}$) of potential prey (oligochaetes, juvenile amphipods, aquatic insects, etc.).

DISCUSSION

The stable isotope ($\delta^{15}N$) analysis revealed four trophic levels in the coastal food webs of the Neva Estuary and the Curonian Lagoon. The studied non-indigenous amphipods and mysids, that are also the most abundant taxa (contributing >10% of the total macroinvertebrate biomass) in both estuarine ecosystems, are less enriched in ^{15}N than carnivorous littoral fish, and take an intermediate position between the herbivores and carnivores (third trophic level). The trophic position of the mysids *L. benedeni* and *P. lacustris* and amphipods *G. tigrinus, P. robustoides, O. crassus* and *G. fasciatus* in the littoral food webs of the Neva Estuary and the Curonian Lagoon corresponds to the level of omnivores, suggesting mixed feeding strategies. However, there were strong increases in trophic position between small juveniles and larger adults (from 2.5 to 3.5) suggesting a change of at least one trophic level during the ontogenetic development (assuming 3.4‰ as the enrichment factor).

A significant difference in food preference between juvenile and adult non-indigenous species revealed by stable isotope analysis was also confirmed by the gut content analysis. All studied amphipod species (*G. tigrinus, P. robustoides, O. crassus* and *G. fasciatus*) were mainly detritivorous and algivorous at juvenile stages and showed increased contributions of animal food in the diet with maturity and increase in size, so that above 7 mm of body length specimens of all four species were omnivorous, with the ability to prey upon small invertebrates.

Detailed studies by Jankauskiene (2003), Lesutiene *et al.* (2007) and Fink *et al.* (2012) of the feeding habits of mysids *P. lacustris* and *L. benedeni* at different locations, based on both stomach content analysis of field-collected animals and of $\delta^{15}N$ analysis, confirmed that these mysids at the adult stage are carnivorous consuming zooplankton and epibenthic animals. Our results also confirm carnivory of both mysids at adult stages. According to Lesutiene *et al.* (2007), the juvenile mysid diet is composed largely of phytoplankton, immature or sub-adult individuals had mixed diets and only specimens at or above the threshold size of 8–9 mm become largely carnivorous and started to feed actively on zooplankton.

We found some notable differences in the trophic positions of the same non-indigenous species (*P. robustoides*) and other species between studied estuarine ecosystems. The invertebrate species in the Curonian Lagoon were at slightly higher trophic levels than the same species in the Neva Estuary. Although *PPI* of non-indigenous species in the Curonian lagoon was not evaluated, we suggest a stronger effect on the local biota due to higher phosphorus levels in water, which can promote growth of consumers and nitrogen limitation that can favour predation (Arbaciauskas *et al.*, 2013). Other studies showed that the feeding behaviour of the amphipod *P. robustoides* differed between the Curonian Lagoon and Lake Plateliai, with more predatory habits in the lagoon than in the freshwater lake (Arbaciauskas *et al.*, 2013). Another example of diet variation between different ecosystems was detected for the mysid *L. benedeni.* Arbaciauskas *et al.* (2013) showed that it feeds mostly on detritus in lakes. Contradictory to this, according to our results this species is typically omnivorous/carnivorous in Curonian Lagoon. The trophic state differences between the two ecosystems are the most probable explanation of these differences in food habits of the same species.

As the share of animal food significantly increases during the ontogenetic development of different amphipod and mysid species, their impact on planktonic and benthic invertebrates may change significantly as well (Dick & Platvoet, 1996; Bollache *et al.*, 2008). Our estimation of consumption rates for adult predaceous amphipods from the Neva Estuary revealed that *P. robustoides* was a more effective predator than the other studied species. Similarly, Bacela-Spychalska & Van der Velde (2013) estimated comparative consumption rates in different amphipod species and found that *P. robustoides* is a more effective predator than the other amphipods *Dikerogammarus haemobaphes* and *G. fossarum.*

In the studied areas of the Neva Estuary the predation impact by the non-indigenous amphipods on invertebrates was assessed as low (<0.5) being limited by high production and abundance of *r*-strategic species (chironomids, oligochaetes) in the macroinvertebrate community. Predation pressure may change during season (Berezina, 2008) and depends on the heterogeneity of the habitat. For example, the rocky/cobble shore amphipod *Echinogammarus marinus*, naturally consuming both algae and macroinvertebrates according to gut content analysis, was an active predator in experiments even with alternative food presence (Dick *et al.*, 2005). At the same time, predation impacts by predaceous *E. marinus* reduced significantly when substrate heterogeneity increased. The relationship between abundance-dependent feeding is still unclear and needs further investigation.

Also, dietary differences in marine animals may depend on type, availability and concentration of food, varying among habitats and seasons (Jennings *et al.*, 2002), but these patterns can only be assumed for non-indigenous amphipods in Baltic estuaries and these questions warrant further studies. In addition, the opportunistic strategy of feeding allows the non-indigenous crustaceans to turn from predation to non-predatory feeding on less nutritious food items (detritus or plants) in situations of low prey availability. Different species of amphipod (including non-indigenous species) are known as omnivorous (predatory) feeders with an ability to use alternative food sources (Cruz-Rivera & Hay 2001; Arbaciauskas *et al.*, 2013; Bacela-Spychalska & Van der Velde, 2013). We suggest that diversity of diets allows these species to be more adaptable and to successfully compete with native species that tend to be more stenophagous. In addition, the capability of these non-indigenous crustaceans to change their food habits from typically plantivorous to carnivorous, preying upon other invertebrates (e.g. chironomids, isopods, oligochaetes) and low food selectivity might be considered as an adaptation of taxa to live in highly eutrophic estuaries featuring algal 'blooms' and hypoxia (Gubelit & Berezina, 2010). Arbaciauskas *et al.* (2013) also proposed that omnivorous habits combined with the possibility to switch towards predatory feeding when adjusting to ontogenetic nutrient demands and resource availability could be optimal.

CONCLUSION

The coastal community of the eastern Baltic Sea is dominated by non-indigenous species that are well adapted to eutrophication (mid-summer algal blooms and temporary near-bottom hypoxia) and other unfavourable threats. The non-indigenous amphipod and mysid species are well integrated in the coastal food webs and are characterized as opportunistic, omnivorous feeders in both the Neva Estuary and Curonian Lagoon. In spite of specific dietary differences, all studied amphipod and mysid species are predominantly grazers and collector-gatherers consuming detritus and plants at juvenile stages, then turning to omnivore feeding at adult stages. As they become more predatory during ontogenetic development, this in turn could affect abundance of other coexisting invertebrates. This study revealed low or moderate predatory impact of non-indigenous species, depending on species. It is likely that further increase of the impact of non-indigenous species may be expected in the case of acceleration of their abundance in the estuaries.

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