

Research Article

Predatory diet and potential effects of *Orconectes limosus* on river macroinvertebrate assemblages of the southeastern Baltic Sea basin: implications for ecological assessment

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Abstract

Invasive crayfish can affect macroinvertebrate assemblages and thus alter conventional macroinvertebrate-based ecological assessment. We aimed to reveal potential impacts of the North American crayfish *Orconectes limosus* on river assessment in the Neman River basin (southeastern Baltic Sea). A laboratory experiment using identical macroinvertebrate assemblages was conducted to compare feeding selectivity and effects between *O. limosus* and the European *Astacus leptodactylus*. Field experiments were conducted to evaluate potential impacts of *O. limosus* on disturbed and undisturbed crayfish-free macroinvertebrate assemblages: one dominated by Oligochaeta vs. one co-dominated by Ephemeroptera-Plecoptera-Trichoptera and Mollusca (EPT-codominated). In the laboratory experiment, both crayfish species preferred feeding on Diptera (mostly chironomids), but *O. limosus* also selected Trichoptera and Ephemeroptera. Family richness did not change, but both species inflated the Shannon Diversity index by reducing Diptera domination. *Astacus leptodactylus* treatments had higher Shannon Diversity and percentage of EPT abundance compared to *O. limosus* treatments. Field experiments indicated (1) negative, (2) assemblage-specific, or (3) no effects of *O. limosus* on macroinvertebrate metrics. A negative effect, especially in the undisturbed assemblage, was observed on simple additive metrics based on taxa presence data, such as total or EPT family richness, or BMWP (Biological Monitoring Working Party) score. Assemblage-specific effects were indicated for some metrics based on relative abundances. In the Oligochaeta-dominated assemblage, Shannon Diversity was inflated and the percentage of Oligochaeta abundance was reduced. In the EPT-codominated assemblage Shannon Diversity was deflated while the relative abundance of Oligochaeta was not affected. No effects were observed when using the ASPT (Average BMWP Score Per Taxon) or percentage of EPT abundance. We conclude that *O. limosus* may have a more diverse predatory diet than *A. leptodactylus*, and thus can have a stronger effect on macroinvertebrate taxa sensitive to disturbances. Therefore, the invasion of *O. limosus* can alter macroinvertebrate assemblages and compromise conventional ecological assessment, even when it displaces resident *Astacus* species.

Key words: spiny-cheek crayfish, narrow-clawed crayfish, feeding preference, community-specific impact, water quality assessment

Introduction

The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817), native to North America, was intentionally introduced in Europe in 1890 and has

been gradually spreading ever since (Souty-Grosset et al. 2006; Kouba et al. 2014). Since its appearance in Lithuania (Koreiva 1994; Burba 2010) and Belarus (Alekhnovich et al. 1999), *O. limosus* has spread through the major part of Lithuania and the Western

part of Belarus (Rakauskas et al. 2010; Arbačiauskas et al. 2011b; Alekhnovich and Razlutskiy 2013). Its expansion from Poland most probably proceeded from the Vistula to Neman basin through the Augustow Canal and tributaries of the Neman (Nemunas) River (Semenchenko et al. 2009; Arbačiauskas et al. 2011b). This invader is considered superior to the native European crayfish due to faster growth with early maturation (Kozák et al. 2007), substantially higher fecundity with shorter embryogenesis (Schulz and Smietana 2001; Kozák et al. 2006; Pârulescu et al. 2015), and even the capability for facultative parthenogenesis (Buřič et al. 2011, 2013). It is also more resistant to pollution (Římalová et al. 2014) and may carry the crayfish plague pathogen *Aphanomyces astaci* Schikora, 1906 (Kozubíková et al. 2011). These traits, and high dispersal abilities (Buřič et al. 2009; Hirsch et al. 2016), frequently associated with illegal human-mediated translocations (Arbačiauskas et al. 2011b), make *O. limosus* an exceptionally successful invader.

The only native crayfish present in both Lithuania and Belarus is the noble crayfish, *Astacus astacus* (Linnaeus, 1758). Although widespread throughout most of Europe, the narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823, of Ponto-Caspian origin, is indigenous only to Southeastern Europe with the border of its presumed natural range crossing Belarus, where it is considered native (Souty-Grosset et al. 2006; Alekhnovich and Razlutskiy 2013; Kouba et al. 2014). It was introduced to Lithuania before 1900 and is currently distributed in the northeastern part of the country (Skurdal et al. 1999; Arbačiauskas et al. 2011b). *Astacus leptodactylus* is superior to and can replace *A. astacus* (Skurdal et al. 1999; Souty-Grosset et al. 2006) due to its higher fecundity, faster-growth with earlier maturation, and reduced sensitivity to pollution (Reynolds and Souty-Grosset 2012).

Invasive decapod crustaceans act as “powerful” omnivores (Strayer 2010). Large omnivores usually perform as keystone species (Reynolds and Souty-Grosset 2012), and as such, crayfish are capable of direct and indirect effects on food-webs (Momot 1995; McCarthy et al. 2006; Twardochleb et al. 2013). Probably due to less efficient digestion, non-animal materials (detritus, macrophytes, algae) are often found to make up the largest part of crayfish gut contents (Hollows et al. 2002; Chucholl 2012, 2013; Vojtkovská et al. 2014). However, some studies indicate that when possible crayfish may prefer feeding on macroinvertebrates rather than on natural plant materials (Alcorlo et al. 2004; Staszak and Szaniawska 2006), and stable isotope analyses confirm that crayfish gain most of their energy and nutrients from macroinvertebrate prey (Whitledge and Rabeni 1997; Hollows et al. 2002; Magoulick and Piercey 2016).

Thus, direct predation by crayfish should be an important factor affecting macroinvertebrate assemblages.

Large-scale ecological impacts of invasive crayfish may be more pronounced than those of native counterparts (James et al. 2015) due to their ability to utilise a wider range of physical habitats (Olsson et al. 2009; Ercoli et al. 2014, 2015b). Taxonomic distinctiveness has also been suggested to magnify the impacts of invaders in recipient aquatic ecosystems because inhabitants of these ecosystems lack evolutionary experience with taxonomically similar species (Ricciardi and Atkinson 2004; Magoulick and Piercey 2016). Thus, substantial impacts of varying scale are expected throughout Europe as resident astacid crayfish are displaced by *O. limosus* (Grabowski et al. 2005; Alekhnovich and Razlutskiy 2013) which belongs to the Cambaridae, a novel crayfish family to the continent. Unlike European crayfish, *O. limosus* is also largely active during the day (Lozán 2000; Buřič et al. 2009; Musil et al. 2010). Stable isotope analyses suggest that it is an omnivore consuming smaller macroinvertebrates (Jaschinski et al. 2011; Rakauskas 2014). Animal prey should be of key importance for invasive crayfish as it allows for better survival and faster growth (Momot 1995; Paglianti and Gherardi 2004; Bondar et al. 2005), and thus higher fitness than that of natives. However, data on the predatory diet of *O. limosus* (Vojtkovská et al. 2014) and related impacts on macroinvertebrate assemblages are rather scarce.

Shifts in macroinvertebrate assemblage structure due to pressures of invasive species may be reflected in macroinvertebrate metrics (Arndt et al. 2009; Freeland-Riggert et al. 2016; Mathers et al. 2016) and thus compromise ecological assessment of conventional disturbances, e.g. chemical water pollution (Cardoso and Free 2008). However, such impacts of crayfish may be complicated to predict, as studies indicate that impact is dependent on the physical habitat preference of the invader (Ruokonen et al. 2014), the type of metric used (Mathers et al. 2016), and the structure of the resident macroinvertebrate assemblage (Klose and Cooper 2012).

To address this problem in the Neman basin, our study aimed to reveal the potential effects of *O. limosus* on conventional macroinvertebrate metrics used for ecological assessment. Cases of displacement of *Astacus* species, and invasion into crayfish-free riverine macroinvertebrate assemblages were examined in laboratory and field experiments, respectively. First, predatory feeding preferences and impacts on the assemblage were compared between *O. limosus* and *A. leptodactylus*. Second, effects of *O. limosus* on river macroinvertebrate assemblages representing sites of different disturbance levels were collated.

Table 1. Conditions of conducted laboratory and field experiments.

Experiment	Season	Temperature (°C)	Duration	Volume (L)	Crayfish		
					Number (ind. rep. ⁻¹)	Size (cm)	Sex ratio (♀:♂)
Laboratory	mid-May	16	40 hours	10	1	7.8–9.0	0
Field (sites)							
Augustow	early Sept	12–14	3 days	40	7	4.5–9.8	0.14–0.43
Astashanka	early Sept	10–12	1 day	40	4	7.6–9.9	0.20–0.40
Neman	mid-June	20–25	4 days	40	6	5.5–8.1	0.33–0.67

Methods

We conducted a laboratory experiment using identical artificial macroinvertebrate assemblages to compare the predatory feeding habits and impacts of *O. limosus* and *A. leptodactylus*. Field experiments were performed to analyse the impact of *O. limosus* on metrics of natural macroinvertebrate assemblages representing different disturbance levels. General details of the laboratory and field experiments are summarised in Table 1.

Predatory diet and impacts of Orconectes limosus and Astacus leptodactylus (laboratory experiment)

The laboratory experiment was carried out in aquaria (23 × 21 × 23 cm) connected in a closed circulation system with a 92.4-L ammonia filter (49 × 39 × 49 cm tank filled with ceramsite granules). Nine such aquaria were used: three for control, three for the *A. leptodactylus* treatment and three for the *O. limosus* treatment. A week before the experiment, the system was filled with tap water (9.5 L per aquarium, depth – 20 cm). A bacterial starter (JBL FilterStart, JBL GmbH & Co. KG, Neuhofen, Germany) was introduced into the filter and the system was left to settle. Each aquarium was covered with transparent plastic film to prevent animal escapes. The outer walls of each aquarium and the top surface of the whole system were covered in black opaque film to prevent visual stimuli from neighbouring experimental replicates and from ambient lighting. Thus, control and experimental aquaria were maintained in darkness. The water flow of ~2.0 L min⁻¹ ensured sufficient oxygen concentration throughout the experiment (> 8.0 mg L⁻¹). The experiment was conducted at the end of spring at a constant temperature of 16 °C.

All aquaria were inoculated with identical macroinvertebrate assemblages (Table 2). This food assortment was aimed to reflect a riverine macroinvertebrate assemblage. One day before the experiment available macroinvertebrates were collected using a standard dip net and hand-picked from underwater objects in the Neris River (a tributary of the Neman river; 54.776084°N; 25.378414°E and 54.838954°N;

25.535175°E) and its tributary, the Vilnia River (54.696786°N; 25.374490°E). In the laboratory, live macroinvertebrates were sorted into families. Individuals of each family were equally distributed between 9 containers. Different sets of 9 containers were used for each macroinvertebrate group (see Table 2) to avoid predation between them before the experiment. Since it was more feasible than field collection, commercially available live chironomid larvae and own laboratory-cultivated amphipods *Gammarus pulex* (Linnaeus, 1758) and snails *Potamopyrgus antipodarum* (Gray, 1843) were additionally used for the assortment. One container of each macroinvertebrate group was released into each aquarium 1 h prior to crayfish introduction to allow the macroinvertebrates time to adjust to new conditions. No substrate was added, although large molluscs served as shelters for some taxa. The stocked biomass of macroinvertebrate prey, excluding large unionid clams and viviparid snails, varied between 9.34–9.98 g wet weight per aquarium.

Astacus leptodactylus (7.8–8.8 cm total body length) were caught in Lake Vencavas (55.711971°N; 25.972125°E) and *O. limosus* (8.3–9.0 cm length) were caught in Lake Luokesai (55.210293°N; 25.41718°E) four days before the experiment. Until transfer to the experimental setup, crayfish were maintained (without feeding) at 16 °C in species-specific 100-L aquaria. All crayfish appeared disease-free. Of each species, three intact males in their intermoult phase, with all pereopods and both well-developed claws present, were used for the experiment. These crayfish were acclimated (without feeding) to the experimental conditions for one day by placing them individually into aquaria identical to those used for the experiment (extra aquaria of the experimental system).

The experiment was started by placing a single crayfish into an experimental aquarium with prey assemblage already settled, and terminated after 40 h giving the crayfish two nights to forage. The crayfish were then removed, and the water from each aquarium was pumped out gathering the macroinvertebrates. The aquaria were then carefully inspected for attached organisms. All remaining macroinvertebrates from each aquarium were sorted and counted.

Table 2. Initial macroinvertebrate setup and their sizes (length) in each aquarium of laboratory experiment. Asterisks denote mollusc families which were excluded from feeding selectivity calculations due to improbable consumption by crayfish.

Group	Family	Individuals	Details
Hirudinea	Erpobdellidae	5	~ 20–40 mm
Crustacea	Asellidae	4	<i>Asellus aquaticus</i> (Linnaeus, 1758), 4–8 mm
	Gammaridae	3	Laboratory-cultivated <i>Gammarus pulex</i> (Linnaeus, 1758), 3–6 mm
Odonata	Calopterygidae	2	20–40 mm
	Gomphidae	1	~ 30 mm
Ephemeroptera	Baetidae	40	Various morphotypes, 5–10 mm
	Ephemerellidae	1	~ 10 mm
	Ephemeridae	1	<i>Ephemera</i> sp., ~ 15 mm
	Heptageniidae	8	Various morphotypes, 8–15 mm
	Potamanthidae	4	<i>Potamanthus luteus</i> (Linnaeus, 1767), 5–10 mm
Plecoptera	Perlodidae	2	~ 15 mm
Coleoptera	Noteridae	1	<i>Noterus</i> sp. adult, ~ 4 mm
Heteroptera	Aphelocheiridae	22	<i>Aphelocheirus aestivalis</i> (Fabricius, 1794), 4–8 mm
	Brachycentridae	4	<i>Brachycentrus subnubilus</i> Curtis, 1834 (2 ind.), ~ 7 mm, <i>Micrasema</i> sp. (2 ind.), ~ 10 mm
Trichoptera	Hydropsychidae	7	<i>Hydropsyche</i> sp., 10–20 mm
	Limnephilidae	10	Various morphotypes, ~ 10–30 mm
	Rhyacophilidae	10	10–20 mm
Diptera	Athericidae	4	10–20 mm
	Chironomidae	55	Commercially available live <i>Chironomus</i> sp., ~ 20 mm
Mollusca	Bithyniidae	12	<i>Bithynia tentaculata</i> (Linnaeus, 1758), 2–8 mm
	Hydrobiidae	16	Laboratory-cultivated <i>Potamopyrgus antipodarum</i> (Gray, 1843), 2–5 mm
	Lymnaeidae	5	<i>Radix balthica</i> (Linnaeus, 1758), 8–20 mm
	Neritidae	2	<i>Theodoxus fluviatilis</i> (Linnaeus, 1758), 4–8 mm
	Planorbidae	1	<i>Ancylus fluviatilis</i> Müller, 1774, ~ 6 mm
	Sphaeriidae	14	Various morphotypes, 5–10 mm
	Unionidae*	4	<i>Unio tumidus</i> Philipson, 1788 (2 ind.), <i>U. pictorum</i> (Linnaeus, 1758) (2 ind.), 40–80 mm
Viviparidae*	3	<i>Viviparus viviparus</i> (Linnaeus, 1758), ~ 30 mm	
Total		241	

Impacts of *Orconectes limosus* on different macroinvertebrate assemblages (field experiments)

Field experiments were conducted with macroinvertebrate assemblages from three sites located in the Neman basin: 1) the Augustow Canal at the confluence with the Neman River (Augustow: 53.886468°N; 23.758617°E), 2) the Astashanka River, a former tributary of the Neman which now connects it to the Augustow Canal (Astashanka: 53.862073°N; 23.750180°E), 3) the Neman River at Iwye town (Neman: 53.864586°N; 25.744370°E). All three sites were devoid of crayfish when experiments were conducted, but *O. limosus* occurs in the vicinities of the first two locations.

Experiments were performed in polyethylene baths (50 × 37 × 24 cm) which were placed under shade on river banks. Three control and three experimental replicates were used for the Augustow and Astashanka sites, and five control and five experimental replicates were used for the Neman site. During experiments, the baths were covered with semi-transparent plastic film to prevent crayfish

being affected by external disturbances. The dissolved oxygen level remained within the range of 7–9 mg L⁻¹ throughout the experiments.

At each study site, macroinvertebrates were caught along a 4-m stretch of river bottom using a standard hand-net. Collected macroinvertebrates together with some sampled bottom substrate (sand, detritus) were carefully mixed and distributed proportionally over experimental and control baths. Bottom substrates formed a sediment layer of about 1-cm thickness. Each bath was then gently filled with 40 L of river water filtered through a 500 µm mesh and allowed to settle for one day before crayfish introduction.

For the Augustow and Astashanka sites, *O. limosus* were caught in the Augustow Canal, at a site with sufficient crayfish abundance (53.885989°N; 23.752381°E). For the Neman experiment, *O. limosus* were caught in the Shchara River (53.429372°N; 24.769128°E), which empties into the Neman. The crayfish were starved for 24 h before the experiments.

The experiments were started by placing the same number of *O. limosus* into each site-specific experi-

Table 3. Analysed conventional macroinvertebrate metrics. All of the metrics are expected to negatively respond to increasing disturbance, except for Oligochaeta% and Diptera%, for which a positive response is expected.

Metric	Details
Total abundance	Total macroinvertebrate abundance
Shannon Diversity	$-\sum_{i=1}^{Total\#} p_i \times \ln p_i$ where <i>Total#</i> – total family richness, p_i – relative abundance of the i^{th} family (Shannon 1948)
Oligochaeta%	Percentage of Oligochaeta abundance
Diptera%	Percentage of Diptera abundance
EPT%	Percentage of Ephemeroptera, Plecoptera and Trichoptera abundance
Total#	Total family richness
EPT#	Total Ephemeroptera, Plecoptera and Trichoptera family richness
BMWP	Sum of family sensitivity scores in the Biological Monitoring Working Party system (Armitage et al. 1983)
ASPT	Average Score Per Taxon (family) of the BMWP system (Armitage et al. 1983)

mental replicate. A different number of individuals, from 4 to 7 per replicate depending on their average body size, were used at each site to maintain a similar total crayfish biomass across all sites and replicates (see Table 1). Crayfish of both sexes were present in each bath. Experiment duration varied between study sites (Table 1) and depended upon crayfish feeding activity. Each experiment was terminated when an obvious decrease of macroinvertebrate densities was observed during daily visual inspection.

As for the laboratory experiment, the crayfish were in the intermolt phase, intact and with no signs of disease. Nevertheless, all field experiments were performed while strictly avoiding potential crayfish pathogen transmission. This was achieved by preventing any contact between the experimental baths and the local natural aquatic habitat (covering them to prevent escapees and emptying them remotely from the stream) and disinfecting all used equipment with 96% ethanol after each experiment. At the end of each experiment whole bath contents (crayfish and other macroinvertebrates with sediments) were fixed in 96% ethanol and taken away for laboratory analysis.

Calculated metrics

Throughout the whole study, the highest taxonomic resolution used for the macroinvertebrate data was family-level (except the class Oligochaeta which was not identified further). Such taxonomic level is sufficient for sound water quality monitoring and helps to reduce seasonal and identification quality-driven variation in the data (Mueller et al. 2013).

The count of affected taxa was estimated for each crayfish replicate in the laboratory experiment. An

affected taxon was defined as one with abundance reduced by a crayfish by more than 5% in comparison to mean control abundance. These counts, and the number of significantly preferred macroinvertebrate groups, were assumed as proxies of crayfish diet diversity.

Feeding selectivity was evaluated for each crayfish species using Chesson index a_i (Equation 1) (Chesson 1978) in the form of ε_i (Equation 2) where values vary from -1 (avoidance) to 1 (highest preference) and 0 means random feeding on the item (Chesson 1983). To calculate this index, the macroinvertebrates were grouped into 10 main groups (m ; seven insect orders and three higher taxa – Hirudinea, Crustacea, Mollusca). Due to large body (comparable in size to that of crayfish used), hard shell, and inherent unlikeliness of consumption by crayfish, large molluscs of families Unionidae and Viviparidae were excluded from feeding selectivity calculations. The environmental abundance of each macroinvertebrate group (n_{ei}) was estimated as the average of initial and mean final abundances in the control replicates. The number of consumed items of each group (n_{ri}) was assessed as the difference between environmental abundance (n_{ei}) and final abundance in each treatment replicate.

$$a_i = \frac{n_{ri}/n_{ei}}{\sum_{i=1}^m (n_{ri}/n_{ei})} \quad (1)$$

$$\varepsilon_i = \frac{m \times a_i - 1}{(m-2) \times a_i + 1} \quad (2)$$

Conventional macroinvertebrate metrics used in river quality monitoring (Table 3) were calculated for both laboratory and field experimental data. This was done using Asterics 4.04 software (AQEM/STAR 2005, 2013).

Statistical analyses

For the analyses below, univariate analyses (*t*-tests and ANOVAs) were conducted using STATISTICA 12 software (StatSoft Inc. 2014). ANOVAs were followed by Fisher LSD tests to identify significant pairwise differences between groups (treatments or sites). Macroinvertebrate metrics denoting percentages were arcsine-transformed ($y' = \sin^{-1} \sqrt{y[\%]/100}$) to better comply with ANOVA assumptions. Appropriateness of the *t*-test comparing the counts of affected taxa and all the ANOVAs was evaluated by inspecting model residual plots for irregularities; normality of residuals was also tested by means of Shapiro-Wilk tests and the homoscedasticity assumption was tested using Bartlett tests. In case any assumption was not met, results of a parametric ANOVA were compared to those of the Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks to identify pairwise differences between groups.

Redundancy analyses (RDAs) of macroinvertebrate assemblages were run in CANOCO 5 software (ter Braak and Šmilauer 2014). Macroinvertebrate abundances were log-transformed for these ordinations to downweigh the effect of dominant taxa.

Laboratory experiment

Feeding selectivity for each of the macroinvertebrate groups was compared to zero using *t*-tests for single samples (two-tailed) to test for non-random feeding. A *t*-test (two-tailed) was also used to compare the counts of affected taxa between *A. leptodactylus* and *O. limosus*. The effect of crayfish on the composition of artificial macroinvertebrate assemblage was explored using RDA in which macroinvertebrate abundances were used as the response and the crayfish factor (levels: control, *A. leptodactylus*, *O. limosus*) was the predictor. The main focus of this analysis was to then identify affected macroinvertebrate families using *t*-value biplots with Van Dobben circles. These circles delimit important macroinvertebrate associations with each treatment vs. the control as approximated by *t*-values larger than 2.0 of the coefficients that would be obtained in corresponding multiple regressions (Šmilauer and Lepš 2014).

Crayfish effects on macroinvertebrate metrics (total abundance, Shannon Diversity, Diptera% and EPT%) in the laboratory experiment were tested using one-way ANOVAs where each metric was the dependent variable and the crayfish factor was the independent variable.

Field experiments

Preliminary inspection after termination of field experiments showed that one control replicate from the Augustow site had an unusually high macroinvertebrate abundance (1347 vs. 293–538 in controls of all sites) due to very high number of Oligochaeta (1117 vs. 253–283), and one *O. limosus* treatment from the Astashanka experiment resulted in extreme reduction of macroinvertebrate abundance (59 vs. 140–395 in experimental replicates of all sites). Consequently, these extreme cases were treated as outliers and were excluded from analysis. To characterise macroinvertebrate assemblages of different study sites (Neman, Augustow, Astashanka) one-way ANOVAs on percentages of the dominant macroinvertebrate groups (Oligochaeta%, Mollusca%, EPT%) were applied for control data.

The effects of *O. limosus* on the composition of characterised macroinvertebrate assemblages were explored using partial RDA (pRDA). In this analysis, macroinvertebrate abundances were used as a response matrix, the main *Orconectes* (levels: control, *O. limosus*) and the interaction (assemblage × *Orconectes*) terms were included as predictors, while the main effect of assemblage (levels: Oligochaeta-dominated, EPT-codominated; see Results for assemblage characterisation) was partialled out. The intent of this analysis was to identify macroinvertebrate families substantially affected by *O. limosus* in each type of assemblage. It was achieved by producing *t*-value biplots with Van Dobben circles focused on the interaction term.

The effects of assemblage and *O. limosus* on macroinvertebrate metrics were tested using two-way ANOVAs where each analysed metric (see Table 3) was the dependent variable, and the assemblage and treatment factors were independent variables. The interaction term (assemblage × *Orconectes*) was tested in this analysis in order to identify the presence of assemblage-specific effects of *O. limosus*.

Results

Predatory diet and impacts of Orconectes limosus and Astacus leptodactylus

Feeding selectivity of both crayfish species in respect to different macroinvertebrate prey groups is depicted in Figure 1A. Significant preference for Diptera larvae was indicated for both crayfish species (*t*-tests: $t_2 > 9.7$, $P \leq 0.010$). However, *O. limosus* additionally preferred Ephemeroptera and Trichoptera larvae (*t*-tests: $t_2 > 4.9$, $P \leq 0.039$), while *A. leptodactylus* did not exhibit significant preference for these macroinvertebrates

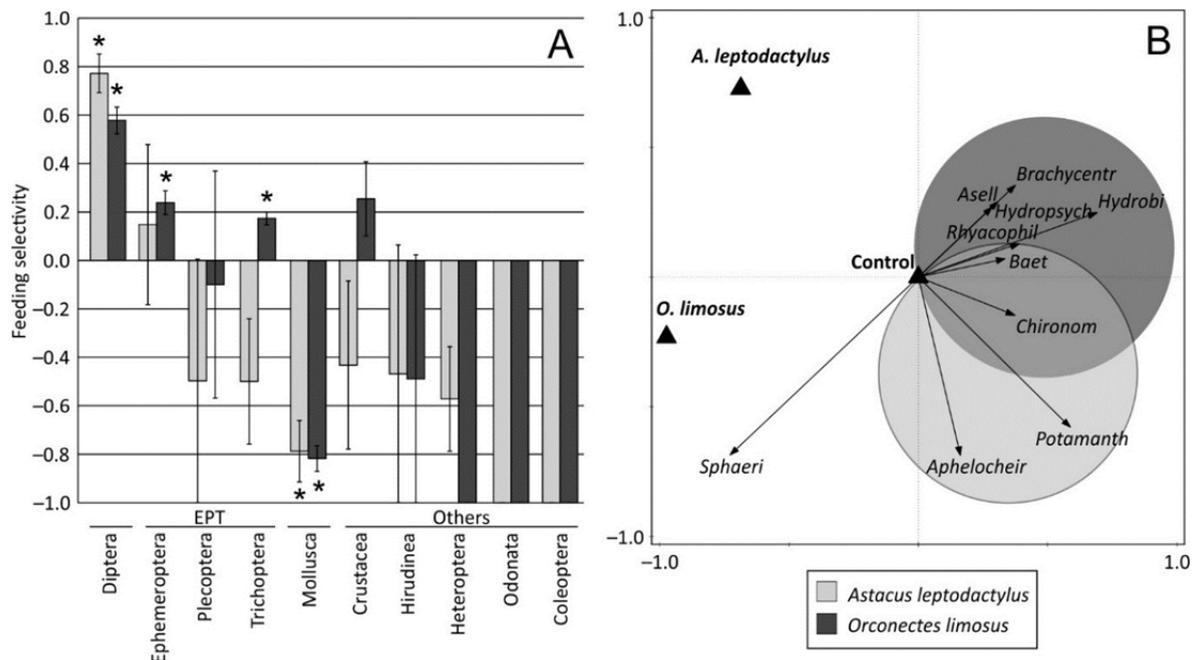


Figure 1. Results of a 40-h laboratory experiment with crayfish *Astacus leptodactylus* (light grey) and *Orconectes limosus* (dark grey): macroinvertebrate diet. (A) Chesson feeding selectivity (mean, SE) for different macroinvertebrate groups; asterisks denote significant differences from zero according to *t*-tests ($P < 0.05$). (B) *t*-value biplot from redundancy analysis (crayfish effect: pseudo- $F = 6.3$, $P = 0.002$) with Van Dobben circles delimiting macroinvertebrate families negatively associated with perpendicularly oriented crayfish treatment. Note that a significantly higher count of affected taxa was characteristic of *O. limosus* in comparison to the *A. leptodactylus* treatment (*t*-test: $t_4 = 4.4$, $P = 0.011$). Macroinvertebrate family names are abbreviated by excluding the ending “-idea”.

(*t*-tests: $t_2 = 0.4$ for Ephemeroptera and $t_2 = -1.9$ for Trichoptera, $P \geq 0.2$). In general, both crayfish species avoided feeding on molluscs (*t*-tests: $t_2 < -6.2$, $P < 0.025$), although *O. limosus* consumed the hydrobiid snail *Potamopyrgus antipodarum* (Gray, 1843) (Figure 1B). No other significant feeding patterns were identified for either species in this analysis (*t*-tests: $-2.6 \leq t_2 \leq 1.7$, $P \geq 0.12$). No changes were observed in Odonata and Coleoptera abundances after terminating the experiment in any aquarium. The invasive *O. limosus* did not eat a single individual of Heteroptera either.

Regarding the counts of affected taxa (with abundances reduced by more than 5%), *O. limosus* also affected more of them (10–11) than *A. leptodactylus* (4–7). This difference was significant (*t*-test: $t_4 = 4.4$, $P = 0.011$). Assumptions of normality and homoscedasticity were met for this test (both Shapiro-Wilk and Bartlett tests: $P = 0.20$).

The RDA described 67.6% of variation in macroinvertebrate family abundances (pseudo- $F = 6.3$, $P = 0.002$), and the families affected by crayfish during the experiment were identified using the *t*-value biplot from this analysis provided in Figure 1B. This

biplot indicated that *O. limosus* substantially fed on 7 macroinvertebrate families: three Trichoptera (Rhyacophilidae, Hydropsychidae and Brachycentridae), and one each of Ephemeroptera (Baetidae), Diptera (Chironomidae), Crustacea (Asellidae) and Mollusca (Hydrobiidae). *Astacus leptodactylus* notably fed on at most five families: two Ephemeroptera (Potamanthidae and Baetidae), one each of Diptera (Chironomidae), Heteroptera (Aphelocheiridae), and to somewhat extent on one of the Trichoptera (Rhyacophilidae).

At least one specimen of each prey family was left in all of the replicates. Therefore, there was no variation in values of macroinvertebrate metrics based on presence-absence data. However, metrics incorporating abundance were affected (Figure 2) with significant crayfish effect found in all such cases (one-way ANOVAs, $F_{2,6} \geq 6.6$, $P \leq 0.030$; Table 4). In all cases, the assumptions of normality (Shapiro Wilk tests: $P \geq 0.24$) and homoscedasticity (Bartlett tests: $P \geq 0.15$) were met. In comparison to the total macroinvertebrate abundance of 204–213 individuals in the control aquaria, both crayfish species reduced it (Fisher LSD tests: $P < 0.001$) to a similar abundance

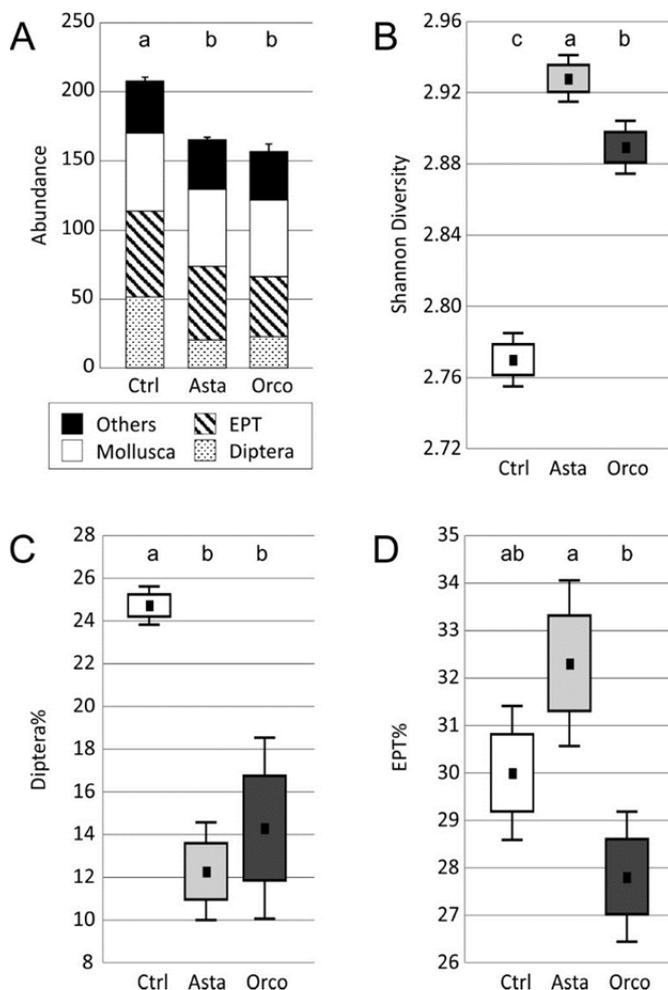


Figure 2. Results of a 40-h laboratory experiment with crayfish *Astacus leptodactylus* and *Orconectes limosus*: impacts on macroinvertebrate metrics. (A) Macroinvertebrate composition and total abundance (means, SEs), (B) Shannon Diversity, (C) percentage of Diptera abundance and (D) percentage of EPT (Ephemeroptera-Plecoptera-Trichoptera) abundance. Ctrl – crayfish-free control, Asta – *A. leptodactylus* treatment, Orco – *O. limosus* treatment. The middle points, boxes and whiskers in B–D respectively denote means, SEs and SDs. Small letters (a, b, c) denote homogenous groups with decreasing values based on Fisher LSD tests ($P < 0.05$). See Table 4 for crayfish effect sizes.

Table 4. Results of one-way ANOVAs testing for crayfish effect (*Astacus leptodactylus* and *Orconectes limosus* vs. crayfish-free control) on macroinvertebrate metrics (see Table 3) in a 40-h laboratory experiment. Adjusted model coefficients of determination (R^2 , %) are provided. See Figure 2 for homogenous groups based on Fisher LSD tests. Assumption tests: P_{SW} – P -values of Shapiro-Wilk tests of normality, P_B – P -values of Bartlett tests of homoscedasticity. Significant probabilities ($P < 0.05$) are in bold.

Response	ANOVA					Assumptions	
	Term	df	F	P	R^2	P_{SW}	P_B
Total abundance	Crayfish	2	57.3	< 0.001	93.4	0.394	0.350
	Error	6					
Shannon Diversity	Crayfish	2	99.4	< 0.001	96.1	0.256	0.982
	Error	6					
Diptera%	Crayfish	2	13.1	0.006	75.2	0.844	0.145
	Error	6					
EPT%	Crayfish	2	6.6	0.030	58.4	0.242	0.958
	Error	6					

of 146–168 ($P = 0.15$; Figure 2A). Both crayfish significantly inflated the values of Shannon Diversity vs. the control values of 2.76–2.79 (Fisher LSD tests: $P < 0.001$). Moreover, Shannon Diversity in the *A. leptodactylus* treatment (2.92–2.94) was significantly more inflated than in the *O. limosus* treatment

(2.87–2.90; Fisher LSD test: $P = 0.016$; Figure 2B). In both crayfish treatments Diptera% was significantly reduced in comparison to the control values of 24–26% (Fisher LSD tests: $P \leq 0.007$) and to a similar percentage (10–18%; $P = 0.4$; Figure 2C). In none of the crayfish treatments EPT% significantly differed

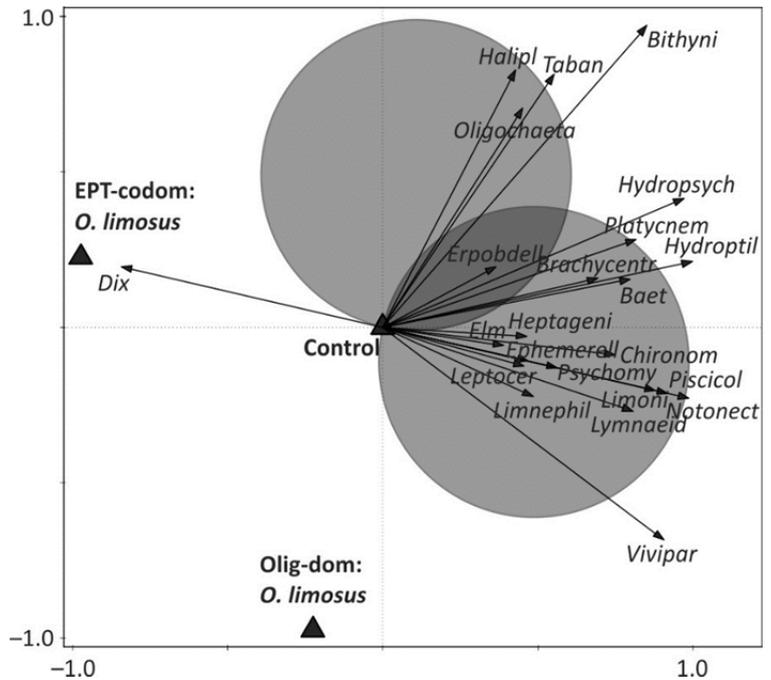


Figure 3. Results of field experiments with crayfish *Orconectes limosus*: *t*-value biplot from partial redundancy analysis with Van Dobben circles delimiting macroinvertebrate families negatively associated with *O. limosus* treatment vs. crayfish-free control in perpendicularly oriented macroinvertebrate assemblages. Assemblages: Olig-dom – Oligochaeta-dominated, EPT-codom – codominated by EPT (Ephemeroptera-Plecoptera-Trichoptera) and Mollusca. Macroinvertebrate family names are abbreviated by excluding the ending “-idea”.

from the control (Fisher LSD tests: $P > 0.11$). However, in comparison to the control (29–32%), the EPT% in *O. limosus* treatment was lower (26–29%) while in the *A. leptodactylus* treatment it was higher (31–34%), and the difference between species was significant (Fisher LSD tests: $P < 0.011$; Figure 2D).

Impacts of Orconectes limosus on different macroinvertebrate assemblages

One-way ANOVAs indicated significant differences in control percentages of all three main macroinvertebrate groups among different sites in the field experiments ($F_{2,7} \geq 20.6$, $P \leq 0.001$). In particular, there was a significantly lower Oligochaeta% (2–10% vs. 66–77%), and significantly higher Mollusca% (23–55% vs. 0–13%) and EPT% (24–41% vs. 1–6%) in the Neman controls than in Augustow or Astashanka controls (Fisher LSD tests: $P \leq 0.012$), but the latter two sites did not differ in these characteristics ($P \geq 0.091$). The residuals of all these ANOVAs were normally distributed (Shapiro-Wilk tests: $P \geq 0.23$), and homoscedasticity was maintained in cases of Oligochaeta% and Mollusca% (Bartlett tests: $P > 0.4$) but failed the formal test in case of EPT% (Bartlett test: $P = 0.010$). However, Kruskal-Wallis ANOVA also indicated significant differences in EPT% between sites ($H_{2,N=10} = 7.6$, $P = 0.022$), and multiple comparisons of mean ranks indicated similar patterns in EPT% as parametric testing: a significant difference

between Neman and Augustow controls ($P = 0.031$), a statistically insignificant difference between Neman and Astashanka controls ($P = 0.21$) and indistinguishable EPT% between Astashanka and Augustow controls ($P > 0.99$). Based on these results, the Augustow and Astashanka assemblages were characterised as Oligochaeta-dominated and the data for the two experiments were merged for further analyses. Meanwhile the Neman assemblage was characterised as EPT-Mollusca-codominated (further referred as EPT-codominated).

The pRDA described 18.9% of variation in the data remaining after the main effect of assemblage type was accounted for (pseudo- $F = 1.9$, $P = 0.012$). The *t*-value biplot from the pRDA (Figure 3) indicated that in the EPT-codominated assemblage *O. limosus* substantially reduced abundances of 14 macroinvertebrate families: four Trichoptera (Brachycentridae, Leptoceridae, Limnephilidae, Psychomyiidae), three Ephemeroptera (Baetidae, Ephemereilidae, Heptageniidae), two Diptera (Chironomidae, Limoniidae) and Hirudinea (Erpobdellidae, Piscicolidae), and one each of Coleoptera (Elmidae) and Mollusca (Lymnaeidae). The biplot also suggested that abundance of the small Dixidae larvae (Diptera) in the *O. limosus* treatment of the Neman experiment increased, which may have resulted from crayfish feeding on predatory macroinvertebrates. The biplot indicated that in the Oligochaeta-dominated assemblage, the invasive crayfish substantially reduced the abundance

Table 5. Results of two-way ANOVAs testing for effects of macroinvertebrate assemblage type (Oligochaeta-dominated vs. one codominated by EPT (Ephemeroptera-Plecoptera-Trichoptera) and Mollusca) and presence of the invasive crayfish *Orconectes limosus* (*O. limosus* treatment vs. crayfish-free control) on macroinvertebrate metrics (see Table 3) in field experiments. Adjusted model coefficients of determination (R^2 , %) are provided. See Figure 4 for homogenous groups based on Fisher LSD tests. Assumption tests: P_{SW} – P -values of Shapiro-Wilk tests of normality, P_B – P -values of Bartlett tests of homoscedasticity. Significant probabilities ($P < 0.05$) are in bold.

Response	ANOVA					Assumptions	
	Term	df	F	P	R^2	P_{SW}	P_B
Total abundance	Assemblage	1	0.6	0.433	69.7	0.344	0.488
	<i>Orconectes</i>	1	43.6	< 0.001			
	Assemblage× <i>Orconectes</i>	1	2.4	0.137			
	Error	16					
Shannon Diversity	Assemblage	1	263.7	< 0.001	93.5	0.813	0.877
	<i>Orconectes</i>	1	0.1	0.739			
	Assemblage× <i>Orconectes</i>	1	12.0	0.003			
	Error	16					
Oligochaeta%	Assemblage	1	153.1	< 0.001	89.2	0.964	0.108
	<i>Orconectes</i>	1	1.2	0.292			
	Assemblage× <i>Orconectes</i>	1	5.7	0.030			
	Error	16					
Diptera%	Assemblage	1	2.2	0.160	7.2	0.176	0.149
	<i>Orconectes</i>	1	0.1	0.805			
	Assemblage× <i>Orconectes</i>	1	2.2	0.154			
	Error	16					
EPT%	Assemblage	1	49.4	< 0.001	71.4	0.885	0.160
	<i>Orconectes</i>	1	0.6	0.459			
	Assemblage× <i>Orconectes</i>	1	0.6	0.465			
	Error	16					
Total#	Assemblage	1	35.1	< 0.001	69.3	0.750	0.295
	<i>Orconectes</i>	1	8.2	0.011			
	Assemblage× <i>Orconectes</i>	1	2.6	0.129			
	Error	16					
EPT#	Assemblage	1	26.4	< 0.001	64.2	0.884	0.811
	<i>Orconectes</i>	1	9.9	0.006			
	Assemblage× <i>Orconectes</i>	1	0.7	0.404			
	Error	16					
BMWP	Assemblage	1	88.8	< 0.001	84.2	0.972	0.850
	<i>Orconectes</i>	1	10.9	0.005			
	Assemblage× <i>Orconectes</i>	1	4.4	0.051			
	Error	16					
ASPT	Assemblage	1	19.4	< 0.001	46.7	0.942	0.301
	<i>Orconectes</i>	1	0.2	0.673			
	Assemblage× <i>Orconectes</i>	1	0.1	0.793			
	Error	16					

of dominant Oligochaeta and single families of Hirudinea (Erpobdellidae) and Coleoptera (Haliplidae) (Figure 3).

The results of two-way ANOVAs testing for the effects of assemblage and *O. limosus* on macroinvertebrate metrics are provided in Table 5. In all cases, the assumptions of normality (Shapiro-Wilk tests: $P \geq 0.18$) and homoscedasticity (Bartlett tests: $P \geq 0.11$) for the ANOVAs were met. Total macroinvertebrate abundance was similar between different types of assemblages (two-way ANOVA, assemblage effect: $F_{1,16} = 0.6$, $P = 0.4$), and significantly reduced by *O. limosus* (*Orconectes* effect: $F_{1,16} = 43.6$, $P < 0.001$).

In both types of assemblages, similar reduction was observed (~ 50%; assemblage × *Orconectes* effect: $F_{1,16} = 2.4$, $P = 0.14$; Figure 4A).

The effect of assemblage type was significant on all other tested macroinvertebrate metrics (two-way ANOVAs, assemblage effect: $F_{1,16} \geq 19.4$, $P < 0.001$) except for Diptera% ($F_{1,16} < 2.2$, $P \geq 0.16$). In the EPT-codominated assemblage, Shannon Diversity, EPT%, Total#, EPT#, BMWP and ASPT were higher, and Oligochaeta% was lower than those in the Oligochaeta-dominated assemblage (Figure 4B–H). The main effect of *O. limosus* was significant for Total#, EPT#, and BMWP (two-way ANOVAs,

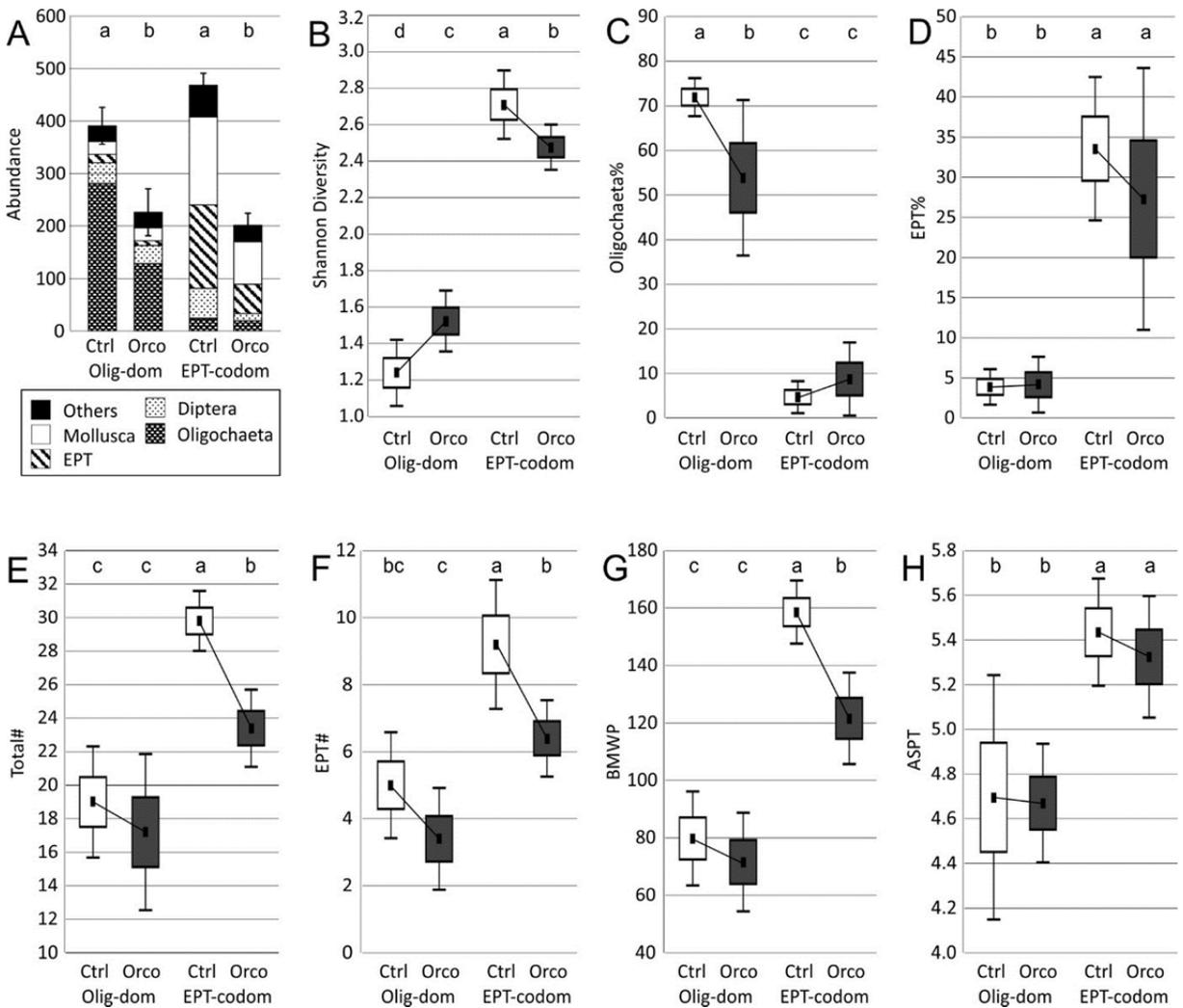


Figure 4. Results of field experiments with crayfish *Orconectes limosus*: impacts on macroinvertebrate metrics in assemblages dominated by Oligochaeta (Olig-dom) and codominated by EPT (Ephemeroptera-Plecoptera-Trichoptera) and Mollusca (EPT-codom). (A) Macroinvertebrate composition and total abundance (means, SEs), (B) Shannon Diversity, (C) percentage of Oligochaeta abundance, (D) percentage of EPT abundance, (E) total family richness, (F) EPT family richness, (G) BMWP (Biological Monitoring Working Party) score, (H) ASPT (Average BMWP Score per Taxon). Ctrl – crayfish-free control, Orco – *O. limosus* treatment. The middle points, boxes and whiskers in B–H respectively denote means, SEs and SDs. The lines connecting the boxes illustrate the direction of *O. limosus* effect. Small letters (a, b, c, d) denote homogenous groups with decreasing values based on Fisher LSD tests ($P < 0.05$). See Table 5 for *O. limosus* effect sizes.

Orconectes effect: $F_{1,16} \geq 8.2$, $P \leq 0.011$), but not for Shannon Diversity, Oligochaeta%, EPT%, ASPT, or Diptera% ($F_{1,16} < 1.8$, $P \geq 0.3$). The values of Total#, EPT# and BMWP were lower in *O. limosus* treatments (Figure 4E–G).

Significant interactions between effects of assemblage type and *O. limosus* were observed for Shannon Diversity and Oligochaeta% (two-way ANOVAs, assemblage \times *Orconectes* effect: $F_{1,16} \geq 5.7$, $P \leq 0.030$).

Orconectes limosus significantly inflated Shannon Diversity in the Oligochaeta-dominated assemblage and significantly deflated it in the EPT-codominated assemblage (Fisher LSD tests: $P < 0.042$; Figure 4B). Meanwhile Oligochaeta% was significantly reduced by *O. limosus* in the Oligochaeta-dominated assemblage (Fisher LSD tests: $P = 0.026$) but not significantly affected in the EPT-codominated assemblage ($P = 0.4$; Figure 4C). The interaction effects on

Total#, EPT# and BMWP were non-significant (two-way ANOVAs, assemblage \times *Orconectes* effect: $F_{1,16} \leq 4.4$, $P > 0.051$). However, Fisher LSD tests indicated a more negative *O. limosus* effect on these metrics in undisturbed sites: deflation was observed in the EPT-codominated assemblage ($P \leq 0.012$), but no significant effects were indicated in the Oligochaeta-dominated assemblage ($P \geq 0.13$; Figure 4E–G). Moreover, Fisher LSD tests also indicated that the EPT# values of the controls of the Oligochaeta-dominated assemblage overlapped with *O. limosus* treatment values in the EPT-codominated assemblage ($P = 0.18$; Figure 4F), indicating that this metric can become irresponsive to other disturbances due to *O. limosus* invasion. Neither interactions from two-way ANOVAs (assemblage \times *Orconectes* effect: $F_{1,16} \leq 2.2$, $P > 0.15$), nor Fisher LSD tests ($P > 0.2$) indicated any potential for assemblage-specific effects of *O. limosus* on EPT%, ASPT, or Diptera%.

Discussion

Orconectes limosus may cause shifts in macroinvertebrate assemblages due to different feeding habits not only when crayfish-free sites are invaded, but also when *A. leptodactylus* is displaced. Similarly, shifts in macroinvertebrate assemblages were observed due to predation of other invasive keystone species, such as crayfish (Klose and Cooper 2012; Ercoli et al. 2015b; Mathers et al. 2016) or benthivorous fish (Reshetnikov 2003; Lederer et al. 2006; Kloskowski 2011). Moreover, invasion of *O. limosus* may result in assemblage-specific effects on conventional macroinvertebrate metrics and thus complicate ecological assessment.

The predatory diet of *O. limosus* was more diverse than that of *A. leptodactylus*, as witnessed by a higher number of preferred macroinvertebrate groups and count of affected taxa. This is in accord with other comparative studies that found invasive species exploiting more types of prey than their native counterparts (Krisp and Maier 2005; Haddaway et al. 2012). As macroinvertebrate assemblage structure varies in relation to mesohabitat (Armitage and Cannan 2000; Rempel et al. 2000; Tolonen and Hämäläinen 2010), ecosystem productivity (Death and Zimmermann 2005; Tolonen and Hämäläinen 2010), and season (Reid et al. 1995; Šporka et al. 2006), diverse diet allows for sufficient nutrition within a broad spectrum of prey communities, and thus aids the proliferation of invasive species across a wide range of settings. In the experiment, *O. limosus* preferred all the dominant insect groups (Diptera, Ephemeroptera and Trichoptera), similar to the opportunistic predatory behaviour of the red

swamp crayfish, *Procambarus clarkii* (Girard, 1852), which switches to naïve prey faster than native European crayfish (Gherardi et al. 2001; Renai and Gherardi 2004). Diet diversification and opportunistic predatory behaviour of invasive crayfish may be especially important for competitive exclusion, which may drive the slow displacement of resident crayfish in the absence of the plague pathogen (Schrimpf et al. 2013). Interspecific differences in predatory diet also infer differential effects on macroinvertebrate metrics, and thus displacement of resident species may also affect applicability of conventional ecological assessment tools.

In the laboratory experiment, Diptera, represented almost exclusively by large chironomid larvae, were the most preferred food of both crayfish species. Consistent with our results, this food item appears to be readily selected not only by *O. limosus* (Vojtkovská et al. 2014), but various crayfish species, due to low escape abilities and soft body (Hollows et al. 2002; Chucholl 2012, 2013). Many studies also found negative crayfish effects on chironomids in the environment (McCarthy et al. 2006; Ercoli et al. 2015b; Freeland-Riggert et al. 2016). The reduction by both crayfish species of the dominant chironomids decreased Diptera%, which is expected to increase with increasing stress on communities (Freeland-Riggert et al. 2016). Thus, although the sediment-dwelling chironomid larvae were at some disadvantage due to lack of substrate in the laboratory experiment, substrate presence may not substantially change the feeding behaviour of crayfish, which are generally capable of burrowing. In comparison to crayfish-free sites, the Proportion of Sediment-sensitive Invertebrates index (PSI) was subject to inflation by invasive signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), in lakes (Mathers et al. 2016), suggesting extensive crayfish predation on flow-resistant macroinvertebrates, including sediment-dwelling taxa. Some studies also indicated that burrowing macroinvertebrates are the most affected by crayfish (Ercoli et al. 2015b). Thus, through predation on dominant Diptera larvae in disturbed sites, presence of crayfish may generally result in higher ecological quality assessments according to Diptera% in comparison to analogous sites without crayfish.

The latter conclusion may probably be generalised to any dominant immobile taxa typical of disturbed sites, e.g. Oligochaeta, which were absent in the laboratory experiment but were heavily reduced in their dominated Augustow-Astashanka assemblages of the field experiments. In crayfish gut contents, Oligochaeta are usually underrepresented, which is often explained by crayfish avoidance of small burrowing prey due to low profitability

(Alcorlo et al. 2004; Chucholl 2012, 2013; Vojtkovská et al. 2014). However, the importance of Oligochaeta in crayfish diet might have been simply underestimated, at least in cases when it is very abundant, due to efficient digestion of fragile soft-bodied animals (Alcorlo et al. 2004). Similarly to Diptera in the laboratory experiment, reduction of dominant Oligochaeta by crayfish in the disturbed (Oligochaeta-dominated) assemblage resulted in lower Oligochaeta%, incorrectly suggesting higher quality assessment.

The distinction between tested crayfish species in our laboratory experiment lay in the consistent preference of *O. limosus* for Trichoptera and Ephemeroptera larvae. These insect larvae were rare in the gut content of *O. limosus* in the Czech Republic (Vojtkovská et al. 2014), and studies of other crayfish reported inconsistent results in this respect (Hollows et al. 2002; McCarthy et al. 2006; Chucholl 2012, 2013). The observed effects of invasive crayfish on environmental densities of these larvae also vary (McCarthy et al. 2006; Twardochleb et al. 2013; Ercoli et al. 2015b). In the current study, substantial diet enrichment in Ephemeroptera and Trichoptera was the main reason for lower EPT% in the *O. limosus* treatment in comparison to the *A. leptodactylus* treatment. This infers that according to EPT%, sites where *O. limosus* has displaced *Astacus* species may be incorrectly assigned to a poorer ecological quality. On the other hand, *O. limosus* did not affect the EPT% in comparison to the crayfish-free control in the laboratory experiment, and the same result was obtained in both assemblages in field experiments. This suggests that EPT% may still provide correct ecological assessments. It should be noted however, that the negative effect of *Orconectes hylas* (Faxon, 1890) on EPT% in comparison to crayfish-free sites of a Missouri stream (reduction to ~ 30%) emerged only at the end of the season, when there was a generally higher EPT% in control sites (~ 50%) (Freeland-Riggert et al. 2016). In our controls EPT% reached 41% at most. Thus, we cannot entirely exclude that when *O. limosus* invades particularly pristine sites previously devoid of resident crayfish, EPT% may be significantly affected and provide falsely low assessments.

In general, simple additive (including biotic-score weighted additive) metrics based on taxa presence, such as Total#, EPT#, and BMWP score, were all negatively affected by *O. limosus* in our field experiments, indicating that crayfish eliminated some taxa in the experimental baths. Other studies found that effects of invasive crayfish on analogous metrics are expressed only in particular habitats, seasons or regions (Klose and Cooper 2012; Freeland-Riggert et al. 2016; Mathers et al. 2016). Such inconsistencies

in the results of mentioned studies could at least in part be explained by variation in macroinvertebrate assemblage structure. As undisturbed sites, such as the Neman site, harbour higher taxa richness, it was not surprising that we found a substantially higher number of negatively affected macroinvertebrate taxa in this assemblage in comparison to the disturbed Augustow-Astashanka assemblages (13 vs. 3). The negative effect on Total#, EPT#, and BMWP score was slightly apparent in the latter sites harbouring Oligochaeta-dominated assemblages but very evident in the EPT-codominated assemblage. In the current study, the EPT# could not even distinguish between *O. limosus* treatment of an undisturbed site and control of a disturbed site, indicating that this metric tends to become irresponsive to other disturbances due to invasion. Thus, according to simple additive metrics, more pronounced negative effects of invasion, or falsely low assessments, should be expected when *O. limosus* invades sites with little disturbance than when it invades disturbed sites. In general, invasive crayfish were not found to reduce macroinvertebrate diversity more than natives (James et al. 2015), and similar species richness of littoral macroinvertebrates was found between lakes harbouring native *A. astacus* or invasive *P. leniusculus* (Ercoli et al. 2015a). However, whether richness metrics can be substantially affected in a particular case when *O. limosus* displaces *Astacus* species still remains unclear and warrants further investigation. Regardless, the BMWP score should be negatively affected in such cases, as the family Cambaridae is not listed among BMWP families, while Astacidae is assigned a high sensitivity score of 8 in the grading system from 1 to 10 (Armitage et al. 1983).

In our experiments, the ASPT score was not affected by *O. limosus* in either assemblage. This contrasted with the significantly reduced non-averaged BMWP scores within the undisturbed Neman assemblage. The robustness of average score arises because *O. limosus* affects both low-scoring (Chironomidae, Erpobdellidae, Lymnaeidae, Piscicolidae, Baetidae) and high-scoring (Ephemerellidae, Heptageniidae, Leptoceridae, Brachycentridae, Psychomyiidae) families of soft-bodied macroinvertebrates. This metric was also not affected by *P. leniusculus* across different environmental settings in UK rivers (Mathers et al. 2016). It was also indicated as more robust against presence of non-indigenous macroinvertebrate species (biocontamination) than simple additive indices, including the BMWP score, in Lithuanian rivers (Arbačiauskas et al. 2011a). In contrast, displacement of a native amphipod by a more tolerant congener falsely elevated the ASPT in rivers of UK islands (MacNeil and Briffa 2009). However,

only by displacement of *Astacus* species *O. limosus* could potentially deflate ASPT assessments, as family Cambaridae is not listed among BMWP families (Armitage et al. 1983), but the robustness of this metric would most likely prevent a significant effect. Thus, out of the studied metrics, the ASPT has the most support to provide correct ecological assessments given both *O. limosus* invasion cases: either replacement of *Astacus* species or invasion into sites devoid of crayfish.

Shannon Diversity incorporates both taxonomic richness and evenness of abundance between different taxa; therefore, effects of crayfish predation on this metric are expected to be complicated and highly assemblage-specific. Most studies find negative effects of crayfish presence on Shannon Diversity of macroinvertebrate assemblages in comparison to crayfish-free sites (Moody and Sabo 2013; Ercoli et al. 2015b; Freeland-Riggert et al. 2016). According to our results, negative effects are expected in relatively undisturbed sites, such as Neman site, where Shannon Diversity is reduced by mostly negatively affecting initially high taxonomic richness and evenness. If an assemblage is characterised by a relatively low richness of dominant immobile macroinvertebrates, as in our laboratory experiment or Augustow-Astashanka field experiments, crayfish presence should inflate Shannon Diversity through reduction of dominance and thus increased evenness. After invasion of the omnivorous amphipod *Dikergammarus villosus* (Sowinsky, 1894) in the River Rhine, where the initial Shannon Diversity (~ 0.8) indicated presence of disturbance, a similar inflation effect (up to ~ 1.5) was observed (Hellmann et al. 2017). Another study indicated no effects of crayfish on Shannon Diversity (Freeland-Riggert et al. 2016). Thus, in previously crayfish-free rivers of medium ecological status, Shannon Diversity probably may be unaffected by *O. limosus* invasion in the region, while in high-status and poor-status rivers the invasion could result in falsely lower and falsely higher assessments, respectively. Shannon Diversity was demonstrated to be similarly reduced by *P. leniusculus* and *A. astacus* in lake littoral (Ercoli et al. 2015a). Our results in the laboratory experiment also indicated that the effect of *O. limosus* on Shannon Diversity in comparison to effects of *Astacus* species may differ due to differences in predatory diet. Given the highly assemblage-specific effects of crayfish presence, predictions for variation of Shannon Diversity for the specific displacement scenario in rivers of different status would be too far-fetched without further studies.

Due to good escape abilities, active swimmers are thought to be indicative of crayfish presence (Nyström

et al. 1996, 1999; Ruokonen et al. 2014, 2016). Lotic invertebrate Index for Flow Evaluation (LIFE) score was also subject to inflation following invasion of *P. leniusculus* (Mathers et al. 2016), suggesting increase of flow-sensitive taxa which include active swimmers. Accordingly, active prey, such as adult beetles, bugs and Odonata larvae, were generally avoided by both crayfish species in the laboratory, and were not substantially affected in the field experiments. However, *A. leptodactylus* consumed some *Aphelocheirus* bugs in the laboratory, which is not very surprising as it was one of the sub-dominant taxa, and occasionally some Heteroptera are found in small amounts in stomach contents of crayfish (Alcorlo et al. 2004; Vojtkovská et al. 2014).

Molluscs frequently appear in gut contents of various crayfish species (Hollows et al. 2002; Alcorlo et al. 2004; Chucholl 2013), and the negative effects on them have been rather consistent (McCarthy et al. 2006; Twardochleb et al. 2013; Ruokonen et al. 2016). Shell materials of small gastropods and the zebra mussel *Dreissena polymorpha* (Pallas, 1771) are also often found in *O. limosus* stomachs (Vojtkovská et al. 2014). However, mollusc prey was generally avoided by both crayfish species in our laboratory experiment probably because softer insect larvae were abundant and thus more profitable. However, *O. limosus* consumed some of the invasive hydrobiid snail *P. antipodarum*. As this snail was the most abundant and the smallest mollusc provided, this is in accord with the size-selective snail predation noted in crayfish (Olden et al. 2009). Using *O. limosus* as a model crayfish species in single prey experiments, crayfish were suggested as ones of few potential predators of *P. antipodarum*, a novel invasive species in Lithuanian lakes (Rakauskas et al. 2016). Our results support the fact that *O. limosus* may also prey on *P. antipodarum* in the presence of multiple prey, but indicate that *Astacus* crayfish may not feed on this snail as much. Furthermore, in the Neman field experiment, where Neritidae, Viviparidae, Sphaeridae and Lymanaeidae families of molluscs were dominant, *O. limosus* only reduced the abundance of Lymnaeidae. This could be explained by crayfish preference for soft-shelled snails *Radix balthica* (Linnaeus, 1758) which mostly represented the family (Brown 1998; Nyström et al. 1999).

Some studies have found that invasive crayfish exhibit higher feeding rates than native ones (Olsen et al. 1991; Nyström and Strand 1996; Haddaway et al. 2012), but we found no significant differences in feeding rate measured in reduction of macroinvertebrate abundance (or even translated to biomass) between *O. limosus* and resident *A. leptodactylus*. We cannot exclude, however, that the difference

would be more pronounced after a longer exposure or would emerge faster at a higher temperature (Nyström and Strand 1996; Staszak and Szaniawska 2006). When interpreting our results, we also assumed that the demonstrated effects of *O. limosus* invasion on macroinvertebrate metrics would be analogical when *O. limosus* displaces either resident *Astacus* species. However, replacing the truly native *A. astacus* instead of *A. leptodactylus* may result in even more pronounced impacts as *A. leptodactylus* is considered superior over *A. astacus* (Skurdal et al. 1999; Souty-Grosset et al. 2006). Moreover, it is important to note that crayfish effects are density-dependent (Charlebois and Lamberti 1996; Parkyn et al. 1997; Klose and Cooper 2012). Density of invasive *O. limosus* populations was noted to vary over time with its peak several years after the invasion followed by a sudden and dramatic decrease (Vaitonis et al. 2016). The varying population density may be the reason why not all effects of invasive crayfish on macroinvertebrate assemblages are consistent in the long term (Ruokonen et al. 2016). As *O. limosus* belongs to a different family than the European crayfish, more drastic ecological impacts of its invasion may be expected than in the case of *P. leniusculus* (Astacidae) (Ricciardi and Atkinson 2004; Magoulick and Piercey 2016; Larson et al. 2016).

To sum up, our results indicate that invasion of *O. limosus* should result in impacts on the structure of macroinvertebrate assemblages when crayfish-free sites are invaded, as well as when resident *Astacus* species are displaced. In the latter case, the shifts in assemblages would be caused by differences in predatory diet between the resident and novel crayfish species. The latter was indicated to have a more diverse predatory diet enriched in macroinvertebrate taxa sensitive to disturbances. The results also indicated that when *O. limosus* invades crayfish-free river sites, Shannon Diversity, Total#, EPT# or BMWP score may provide falsely lower assessments for undisturbed sites, and using Shannon Diversity and Oligochaeta% may misclassify disturbed sites as of better ecological status. ASPT and EPT% seem to have the highest potential to remain informative of disturbance levels and unaffected by *O. limosus* in assemblages containing no resident crayfish. However, EPT%, and Shannon Diversity may not provide correct ecological assessment when *Astacus* species are displaced. Thus, in agreement with other authors (Mathers et al. 2016), we would suggest incorporating different types of metrics into a multimetric approach for robust ecological assessment using macroinvertebrate assemblages.

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References

- Alcorlo P, Geiger W, Otero M (2004) Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. *Crustaceana* 77: 435–453, <https://doi.org/10.1163/1568540041643283>
- Alekhovich AV, Ablov SE, Kulesh VF, Pareiko OA (1999) The American spiny-cheek crayfish, *Orconectes limosus* in the fauna of Belarus. In: Gherardi F, Holdich DM (eds), *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation?* (Crustacean Issues, 11). A. A. Balkema, Rotterdam, The Netherlands, pp 237–242
- Alekhovich A, Razlutskij V (2013) Distribution and spread of spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) in Belarus. *BiolInvasions Records* 2: 221–225, <https://doi.org/10.3391/bir.2013.2.3.08>
- AQEM/STAR (2005) ASTERICS (AQEM/STAR Ecological River Classification System).
- AQEM/STAR (2013) ASTERICS einschließlich Perloides (deutsches Bewertungssystem auf Grundlage des Makrozoobenthos), Version 4 Software-Handbuch für die deutsche Version. 2013, 120 pp
- Arbačiauskas K, Višinskienė G, Smilgevičienė S (2011a) Non-indigenous macroinvertebrate species in Lithuanian fresh waters, Part 2: macroinvertebrate assemblage deviation from naturalness in lotic systems and the consequent potential impacts on ecological quality assessment. *Knowledge and Management of Aquatic Ecosystems* 402: 13, <https://doi.org/10.1051/kmae/2011076>
- Arbačiauskas K, Višinskienė G, Smilgevičienė S, Rakauskas V (2011b) Non-indigenous macroinvertebrate species in Lithuanian fresh waters, part 1: distributions, dispersal and future. *Knowledge and Management of Aquatic Ecosystems* 402: 12, <https://doi.org/10.1051/kmae/2011075>
- Armitage PD, Moss D, Wright JF, Furse MT (1983) The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Research* 17: 333–347, [https://doi.org/10.1016/0043-1354\(83\)90188-4](https://doi.org/10.1016/0043-1354(83)90188-4)
- Armitage PD, Cannan CE (2000) Annual changes in summer patterns of mesohabitat distribution and associated macroinvertebrate assemblages. *Hydrological Processes* 14: 3161–3179, [https://doi.org/10.1002/1099-1085\(200011/12\)14:16/17<3161::AID-HYP140>3.0.CO;2-8](https://doi.org/10.1002/1099-1085(200011/12)14:16/17<3161::AID-HYP140>3.0.CO;2-8)
- Arndt E, Fiedler S, Böhme D (2009) Effects of invasive benthic macroinvertebrates on assessment methods of the EU Water Frame Work Directive. *Hydrobiologia* 635: 309–320, <https://doi.org/10.1007/s10750-009-9923-5>
- Bondar CA, Bottrill K, Zeron K, Richardson JS (2005) Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2632–2639, <https://doi.org/10.1139/R05-167>
- Braak C.J.F. ter, Šmilauer P (2014) CANOCO 5 (software for multivariate data explanation, testing, and summarization).
- Brown KM (1998) The role of shell strength in selective foraging by crayfish for gastropod prey. *Freshwater Biology* 40: 255–260, <https://doi.org/10.1046/j.1365-2427.1998.00350.x>

- Burba A (2010) The dispersal of the invasive spinycheek crayfish, *Orconectes limosus*, throughout Lithuanian waters. *Freshwater Crayfish* 17: 67–72
- Buřič M, Kozák P, Kouba A (2009) Movement patterns and ranging behavior of the invasive spiny-cheek crayfish in a small reservoir tributary. *Fundamental and Applied Limnology/Archiv für Hydrobiologie* 174: 329–337, <https://doi.org/10.1127/1863-9135/2009/0174-0329>
- Buřič M, Hulák M, Kouba A, Petrusek A, Kozák P (2011) A successful crayfish invader is capable of facultative parthenogenesis: a novel reproductive mode in decapod crustaceans. *PLoS ONE* 6: e20281, <https://doi.org/10.1371/journal.pone.0020281>
- Buřič M, Kouba A, Kozák P (2013) Reproductive plasticity in freshwater invader: from long-term sperm storage to parthenogenesis. *PLoS ONE* 8: e77597, <https://doi.org/10.1371/journal.pone.0077597>
- Cardoso AC, Free G (2008) Incorporating invasive alien species into ecological assessment in the context of the Water Framework Directive. *Aquatic Invasions* 3: 361–366, <https://doi.org/10.3391/ai.2008.3.4.1>
- Charlebois PM, Lamberti GA (1996) Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. *Journal of the North American Benthological Society* 15: 551–563, <https://doi.org/10.2307/1467806>
- Chesson J (1978) Measuring preference in selective predation. *Ecology* 59: 211–215, <https://doi.org/10.2307/1936364>
- Chesson J (1983) The estimation and analysis of preference and its relationship to foraging models. *Ecology* 64: 1297–1304, <https://doi.org/10.2307/1937838>
- Chucholl C (2012) Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae). *Knowledge and Management of Aquatic Ecosystems* 404: 4, <https://doi.org/10.1051/kmae/2011082>
- Chucholl C (2013) Feeding ecology and ecological impact of an alien “warm-water” omnivore in cold lakes. *Limnologia* 43: 219–229, <https://doi.org/10.1016/j.limno.2012.10.001>
- Death RG, Zimmermann EM (2005) Interaction between disturbance and primary productivity in determining stream invertebrate diversity. *Oikos* 111: 392–402, <https://doi.org/10.1111/j.0030-1299.2005.13799.x>
- Ercoli F, Ruokonen TJ, Hämäläinen H, Jones RI (2014) Does the introduced signal crayfish occupy an equivalent trophic niche to the lost native noble crayfish in boreal lakes? *Biological Invasions* 16: 2025–2036, <https://doi.org/10.1007/s10530-014-0645-x>
- Ercoli F, Ruokonen TJ, Erkamo E, Jones RI, Hämäläinen H (2015a) Comparing the effects of introduced signal crayfish and native noble crayfish on the littoral invertebrate assemblages of boreal lakes. *Freshwater Science* 34: 555–563, <https://doi.org/10.1086/680517>
- Ercoli F, Ruokonen TJ, Koistinen S, Jones RI, Hämäläinen H (2015b) The introduced signal crayfish and native noble crayfish have different effects on sublittoral macroinvertebrate assemblages in boreal lakes. *Freshwater Biology* 60: 1688–1698, <https://doi.org/10.1111/fwb.12601>
- Freeland-Riggert BT, Cairns SH, Poulton BC, Riggert CM (2016) Differences found in the macroinvertebrate community composition in the presence or absence of the invasive alien crayfish, *Orconectes hylas*. *PLoS ONE* 11: e0150199, <https://doi.org/10.1371/journal.pone.0150199>
- Gherardi F, Renai B, Corti C (2001) Crayfish predation on tadpoles: a comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bulletin Français de la Pêche et de la Pisciculture* 361: 659–668, <https://doi.org/10.1051/kmae:2001011>
- Grabowski M, Jazdzewski K, Konopacka A (2005) Alien Crustacea in Polish waters – introduction and Decapoda. *Oceanological and Hydrobiological Studies* 14: 43–61
- Haddaway NR, Wilcox RH, Heptonstall REA, Griffiths HM, Mortimer RJG, Christmas M, Dunn AM (2012) Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. *PLoS ONE* 7: e32229, <https://doi.org/10.1371/journal.pone.0032229>
- Hellmann C, Schöll F, Worischka S, Becker J, Winkelmann C (2017) River-specific effects of the invasive amphipod *Dikerogammarus villosus* (Crustacea: Amphipoda) on benthic communities. *Biological Invasions* 19: 381–398, <https://doi.org/10.1007/s10530-016-1286-z>
- Hirsch PE, Burkhardt-Holm P, Töpfer I, Fischer P (2016) Movement patterns and shelter choice of spiny-cheek crayfish (*Orconectes limosus*) in a large lake’s littoral zone. *Aquatic Invasions* 11: 55–65, <https://doi.org/10.3391/ai.2016.11.1.06>
- Hollows JW, Townsend CR, Collier KJ (2002) Diet of the crayfish *Paranephrops zealandicus* in bush and pasture streams: insights from stable isotopes and stomach analysis. *New Zealand Journal of Marine and Freshwater Research* 36: 129–142, <https://doi.org/10.1080/00288330.2002.9517076>
- James J, Slater FM, Vaughan IP, Young KA, Cable J (2015) Comparing the ecological impacts of native and invasive crayfish: could native species’ translocation do more harm than good? *Oecologia* 178: 309–316, <https://doi.org/10.1007/s00442-014-3195-0>
- Jaschinski S, Brepohl DC, Sommer U (2011) The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus* L.): stable isotope and fatty acid analyses. *Aquatic Sciences* 73: 91–101, <https://doi.org/10.1007/s00027-010-0163-6>
- Klose K, Cooper SD (2012) Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities. *Freshwater Biology* 57: 526–540, <https://doi.org/10.1111/j.1365-2427.2011.02721.x>
- Kloskowski J (2011) Impact of common carp *Cyprinus carpio* on aquatic communities: direct trophic effects versus habitat deterioration. *Fundamental and Applied Limnology* 178: 245–255, <https://doi.org/10.1127/1863-9135/2011/0178-0245>
- Koreiva Č (1994) *Orconectes limosus* in Lithuania. *Crayfish News: IAA Newsletter* 16: 7
- Kouba A, Petrusek A, Kozák P (2014) Continental-wide distribution of crayfish species in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems* 413: 5, <https://doi.org/10.1051/kmae/2014007>
- Kozák P, Buřič M, Polícar T (2006) The fecundity, time of egg development and juvenile production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. *Bulletin Français de la Pêche et de la Pisciculture* 380–381: 1171–1182, <https://doi.org/10.1051/kmae:2006019>
- Kozák P, Buřič M, Polícar T, Hamáčková J, Lepičová A (2007) The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. *Hydrobiologia* 590: 85–94, <https://doi.org/10.1007/s10750-007-0760-0>
- Kozubíková E, Viljamaa-Dirks S, Heinikainen S, Petrusek A (2011) Spiny-cheek crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague pathogen *Aphanomyces astaci*. *Journal of Invertebrate Pathology* 108: 214–216, <https://doi.org/10.1016/j.jip.2011.08.002>
- Krisp H, Maier G (2005) Consumption of macroinvertebrates by invasive and native gammarids: a comparison. *Journal of Limnology* 64: 55–59, <https://doi.org/10.4081/jlimnol.2005.55>
- Larson ER, Twardochleb LA, Olden JD (2016) Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. *Limnology* 18: 275–286, <https://doi.org/10.1007/s10201-016-0505-8>
- Lederer A, Massart J, Janssen J (2006) Impact of round gobies (*Neogobius melanostomus*) on dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) and the associated macroinvertebrate community across an invasion front. *Journal of Great Lakes Research* 32: 1–10, [https://doi.org/10.3394/0380-1330\(2006\)32\[1:1 ORGNM\]2.0.CO;2](https://doi.org/10.3394/0380-1330(2006)32[1:1 ORGNM]2.0.CO;2)

- Lozán JL (2000) On the threat to the European crayfish: a contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). *Limnologia* 30: 156–161, [https://doi.org/10.1016/S0075-9511\(00\)80010-9](https://doi.org/10.1016/S0075-9511(00)80010-9)
- MacNeil C, Briffa M (2009) Replacement of a native freshwater macroinvertebrate species by an invader: implications for biological water quality monitoring. *Hydrobiologia* 635: 321–327, <https://doi.org/10.1007/s10750-009-9924-4>
- Magoulick DD, Piercey GL (2016) Trophic overlap between native and invasive stream crayfish. *Hydrobiologia* 766: 237–246, <https://doi.org/10.1007/s10750-015-2457-0>
- Mathers KL, Chadd RP, Extence CA, Rice SP, Wood PJ (2016) The implications of an invasive species on the reliability of macroinvertebrate biomonitoring tools used in freshwater ecological assessments. *Ecological Indicators* 63: 23–28, <https://doi.org/10.1016/j.ecolind.2015.11.051>
- McCarthy JM, Hein CL, Olden JD, Zanden MJ Vander (2006) Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology* 51: 224–235, <https://doi.org/10.1111/j.1365-2427.2005.01485.x>
- Momot WT (1995) Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science* 3: 33–63, <https://doi.org/10.1080/10641269509388566>
- Moody EK, Sabo JL (2013) Crayfish impact desert river ecosystem function and litter-dwelling invertebrate communities through association with novel detrital resources. *PLoS ONE* 8: e63274, <https://doi.org/10.1371/journal.pone.0063274>
- Mueller M, Pander J, Geist J (2013) Taxonomic sufficiency in freshwater ecosystems: effects of taxonomic resolution, functional traits, and data transformation. *Freshwater Science* 32: 762–778, <https://doi.org/10.1899/12-212.1>
- Musil M, Buřič M, Polícar T, Kouba A, Kozák P (2010) Comparison of diurnal and nocturnal activity between noble crayfish (*Astacus astacus*) and spinycheek crayfish (*Orconectes limosus*). *Freshwater Crayfish* 17: 189–193
- Nyström P, Brönmark C, Granéli W (1996) Patterns in benthic food webs: a role for omnivorous crayfish? *Freshwater Biology* 36: 631–646, <https://doi.org/10.1046/j.1365-2427.1996.d01-528.x>
- Nyström P, Strand JA (1996) Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biology* 36: 673–682, <https://doi.org/10.1046/j.1365-2427.1996.d01-508.x>
- Nyström P, Brönmark C, Granéli W (1999) Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* 85: 545–553, <https://doi.org/10.2307/3546704>
- Olden JD, Larson ER, Mims MC (2009) Home-field advantage: Native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). *Aquatic Ecology* 43: 1073–1084, <https://doi.org/10.1007/s10452-009-9244-9>
- Olsen TM, Lodge DM, Capelli GM, Houlihan RJ (1991) Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1853–1861, <https://doi.org/10.1139/f91-219>
- Olsson K, Stenroth P, Nyström P, Granéli W (2009) Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology* 54: 1731–1740, <https://doi.org/10.1111/j.1365-2427.2009.02221.x>
- Paglianti A, Gherardi F (2004) Combined effects of temperature and diet on growth and survival of young-of-year crayfish: a comparison between indigenous and invasive species. *Journal of Crustacean Biology* 24: 140–148, <https://doi.org/10.1651/C-2374>
- Parkyn SM, Rabeni CF, Collier KJ (1997) Effects of crayfish on in-stream processes and benthic faunas: a density manipulation experiment. *New Zealand Journal of Marine and Freshwater Research* 31: 685–692, <https://doi.org/10.1080/00288330.1997.9516798>
- Părvulescu L, Pirvu M, Moroşan LG, Zaharia C (2015) Plasticity in fecundity highlights the females' importance in the spiny-cheek crayfish invasion mechanism. *Zoology* 118: 424–432, <https://doi.org/10.1016/j.zool.2015.08.003>
- Rakauskas V, Ruginis T, Arbačiauskas K (2010) Expansion of the spinycheek crayfish, *Orconectes limosus* (Rafinesque 1817), in the Nemunas River Basin, Lithuania. *Freshwater Crayfish* 17: 73–76
- Rakauskas V (2014) Trophic position of non-indigenous crustaceans and their impact on food webs in lakes. PhD Thesis, Vilnius University and Nature Research Centre, Vilnius, Lithuania, 32 pp
- Rakauskas V, Butkus R, Merkytė E (2016) Consumption of the invasive New Zealand mud snail (*Potamopyrgus antipodarum*) by benthivorous predators in temperate lakes: a case study from Lithuania. *Hydrobiologia* 775: 213–230, <https://doi.org/10.1007/s10750-016-2733-7>
- Reid RA, Somers KM, David SM (1995) Spatial and temporal variation in littoral-zone benthic invertebrates from three south-central Ontario lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1406–1420, <https://doi.org/10.1139/f95-136>
- Rempel LL, Richardson JS, Healey MC (2000) Macroinvertebrate community structure along gradients of hydraulic and sedimentary conditions in a large gravel-bed river. *Freshwater Biology* 45: 57–73, <https://doi.org/10.1046/j.1365-2427.2000.00617.x>
- Renai B, Gherardi F (2004) Predatory efficiency of crayfish: comparison between indigenous and non-indigenous species. *Biological Invasions* 6: 89–99, <https://doi.org/10.1023/B:BINV.000010126.94675.50>
- Reshetnikov AN (2003) The introduced fish, rotan (*Perccottus glenii*), depresses populations of aquatic animals (macroinvertebrates, amphibians, and a fish). *Hydrobiologia* 510: 83–90, <https://doi.org/10.1023/B:HYDR.0000008634.92659.b4>
- Reynolds J, Souty-Grosset C (2012) Management of Freshwater Biodiversity: Crayfish as Bioindicators. Cambridge University Press, Cambridge, UK, 388 pp, <https://doi.org/10.1017/CBO9781139031790>
- Ricciardi A, Atkinson SK (2004) Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. *Ecology Letters* 7: 781–784, <https://doi.org/10.1111/j.1461-0248.2004.00642.x>
- Římalová K, Douša K, Štambergová M (2014) Species-specific pattern of crayfish distribution within a river network relates to habitat degradation: implications for conservation. *Biodiversity and Conservation* 23: 3301–3317, <https://doi.org/10.1007/s10531-014-0784-5>
- Ruokonen TJ, Karjalainen J, Hämäläinen H (2014) Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific. *Freshwater Biology* 59: 12–25, <https://doi.org/10.1111/fwb.12242>
- Ruokonen TJ, Ercoli F, Hämäläinen H (2016) Are the effects of an invasive crayfish on lake littoral macroinvertebrate communities consistent over time? *Knowledge and Management of Aquatic Ecosystems* 417: 31, <https://doi.org/10.1051/kmae/2016018>
- Schrimpf A, Maiwald T, Vrålstad T, Schulz HK, Śmietana P, Schulz R (2013) Absence of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of European and American crayfish in central Europe. *Freshwater Biology* 58: 1116–1125, <https://doi.org/10.1111/fwb.12112>
- Schulz R, Śmietana P (2001) Occurrence of native and introduced crayfish in northeastern Germany and northwestern Poland. *Bulletin Français de la Pêche et de la Pisciculture* 361: 629–641, <https://doi.org/10.1051/kmae:2001009>
- Semenchenko VP, Rizevsky VK, Mastitsky SE, Vezhnovets VV, Pluta MV, Razlutsky VI, Laenko T (2009) Checklist of aquatic alien species established in large river basins of Belarus. *Aquatic Invasions* 4: 337–347, <https://doi.org/10.3391/ai.2009.4.2.5>
- Shannon CE (1948) A mathematical theory of communication. *Bell System Technical Journal* 27: 379–423, <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
- Skurdal J, Taugbøl T, Burba A, Edsman L, Söderbäck B, Styrrishave B, Tuusti J, Westman K (1999) Crayfish introductions in the Nordic and Baltic countries. In: Gherardi F, Holdich DM (eds),

- Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation? (Crustacean Issues, 11). A.A. Balkema, Rotterdam, The Netherlands, pp 193–220
- Šmilauer P, Lepš J (2014) *Multivariate Analysis of Ecological Data using Canoco 5*. Cambridge University Press, New York, 376 pp
- Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P (2006) Atlas of Crayfish in Europe (Patrimoines naturels, 64). Muséum national d'Histoire naturelle, Paris, 187 pp
- Šporka F, Vlek HE, Bulánková E, Krno I (2006) Influence of seasonal variation on bioassessment of streams using macroinvertebrates. *Hydrobiologia* 566: 543–555, <https://doi.org/10.1007/s10750-006-0073-8>
- Staszak K, Szaniawska A (2006) Feeding rates and food preferences of the spiny-cheek crayfish *Orconectes limosus* at two different temperatures. *Freshwater Crayfish* 15: 148–154
- StatSoft Inc. (2014) STATISTICA (data analysis software system).
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152–174, <https://doi.org/10.1111/j.1365-2427.2009.02380.x>
- Tolonen KT, Hämäläinen H (2010) Comparison of sampling methods and habitat types for detecting impacts on lake littoral macroinvertebrate assemblages along a gradient of human disturbance. *Fundamental and Applied Limnology / Archiv für Hydrobiologie* 176: 43–59, <https://doi.org/10.1127/1863-9135/2010/0176-0043>
- Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32: 1367–1382, <https://doi.org/10.1899/12-203.1>
- Vaitonis G, Alekhnovich A, Razlutskiy V, Rybakovas A, Šniaukštaitė V, Šidagytė E (2016) Spread of spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) in Lithuania and Belarus. In: Dapkus D (ed), Lietuvos biologinė įvairovė: būklė, struktūra, apsauga. Lietuvos edukologijos universiteto leidykla, Vilnius, Lithuania, pp 114–119
- Vojtkovská R, Horká I, Ďuriš Z (2014) The diet of the spiny-cheek crayfish *Orconectes limosus* in the Czech Republic. *Central European Journal of Biology* 9: 58–69, <https://doi.org/10.2478/s11535-013-0189-y>
- Whitledge GW, Rabeni CF (1997) Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2555–2563, <https://doi.org/10.1139/cjfas-54-11-2555>