



Histopathological screening of *Pontogammarus robustoides* (Amphipoda), an invader on route to the United Kingdom

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ABSTRACT

Biological invasions may act as conduits for pathogen introduction. To determine which invasive non-native species pose the biggest threat, we must first determine the symbionts (pathogens, parasites, commensals, mutualists) they carry, via pathological surveys that can be conducted in multiple ways (i.e., molecular, pathological, and histological). Whole animal histopathology allows for the observation of pathogenic agents (virus to Metazoa), based on their pathological effect upon host tissue. Where the technique cannot accurately predict pathogen taxonomy, it does highlight pathogen groups of importance. This study provides a histopathological survey of *Pontogammarus robustoides* (invasive amphipod in Europe) as a baseline for symbiont groups that may translocate to other areas/hosts in future invasions.

Pontogammarus robustoides (n = 1,141) collected throughout Poland (seven sites), were noted to include a total of 13 symbiotic groups: a putative gut epithelia virus (overall prevalence = 0.6%), a putative hepatopancreatic cytoplasmic virus (1.4%), a hepatopancreatic bacilliform virus (15.7%), systemic bacteria (0.7%), fouling ciliates (62.0%), gut gregarines (39.5%), hepatopancreatic gregarines (0.4%), haplosporidians (0.4%), muscle infecting microsporidians (6.4%), digeneans (3.5%), external rotifers (3.0%), an endoparasitic arthropod (putatively: Isopoda) (0.1%), and Gregarines with putative microsporidian infections (1.4%).

Parasite assemblages partially differed across collection sites. Co-infection patterns revealed strong positive and negative associations between five parasites. Microsporidians were common across sites and could easily spread to other areas following the invasion of *P. robustoides*. By providing this initial histopathological survey, we hope to provide a concise list of symbiont groups for risk-assessment in the case of a novel invasion by this highly invasive amphipod.

1. Introduction

Biological invasions are a global conservation concern, projected to increase in frequency and severity over the next three decades (Pyšek et al., 2020). Once established, invasive non-native species can cause considerable disruption to native community structure, alter ecosystem function and services (Vila and Hulme, 2017), and often generate a substantial economic cost at the national, regional, and global scale (Diagne et al. 2020). Aquatic invasive species (AIS) are particularly problematic and can cause severe native biodiversity decline (Havel et al. 2015). Crustaceans are among the most successful groups of global

aquatic invaders, consisting of ~ 40% of AIS in North America, and > 50% of AIS in Europe. Amphipoda is one of the most dominant invasive taxa (Karatayev et al., 2009), with a relatively large proportion originating from the same donor region, the Ponton-Caspian basin (Cuthbert et al., 2020).

Amphipods are a diverse group of freshwater and marine Crustacea, with many taxa exhibiting robust ecophysiological tolerances, specifically the invasive ones. *Pontogammarus robustoides* (Sars, 1894; Amphipoda: Pontogammaridae) (Mamos et al. 2021) is one species that exhibits a high range of tolerance (Grabowski et al. 2007), outcompete native taxa (Jazdzewski et al. 2004; Gumuliauskaite and Arbaciauskas

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Table 1

Sample location and collection volume of all *Pontogammarus robustoides* from across Poland. This table includes all male, female and unsexed individuals to determine geographical/population level prevalence (%).

Location and collection		Collection date	Number collected	Parasite (%)												
Collection site	Coordinates			Fouling ciliates	Fouling rotifers	Gut gregarines	HP gregarines	Gregarines with Microsporida	PrBV	Gut epithelial virus	HP epithelial virus	Haplosporidian	Microsporida	Digenea	Bacterial infection	Endoparasitic arthropod
Lucień Lake in Lucień	52.49563, 19.44469	16/06/2015	211	58.3	6.2	40.8	0.0	0.0	38.4	0.0	0.0	0.0	8.0	0.5	0.0	0.0
Włocławski Reservoir (Vistula River) in Nowy Dulinów	52.584803, 19.479901	16/06/2015	318	81.4	3.5	35.2	0.3	0.0	11.0	0.0	0.0	0.0	7.5	0.9	0.0	0.0
Włocławski Reservoir (Vistula River) in Stary Dulinów	52.571839, 19.521571	16/06/2015	66	97.0	0.0	7.6	0	0.0	4.5	0.0	0.0	0.0	6.1	1.5	0.0	1.5
Vistula River in Nieszawa	52.836048, 18.903723	16/06/2015	8	75.0	25.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Zegrzński Reservoir in Zegrze	52.460372, 21.01746	21/06/2015	139	54.0	2.9	18.7	0	3.6	23.0	0.0	0.7	0.0	3.6	0.0	0.0	0.0
Oder in Gryfino	53.25160, 14.47949	23/06/2015	122	51.6	1.6	55.8	0.8	0.0	9.0	0.0	4.9	3.3	4.1	6.6	0.0	0.0
Szczecin Lagoon in Kopice	53.69724, 14.54304	23/06/2015	287	43.6	1.4	52.3	1.0	3.8	6.0	2.4	3.1	0.3	6.6	9.4	2.8	0.3

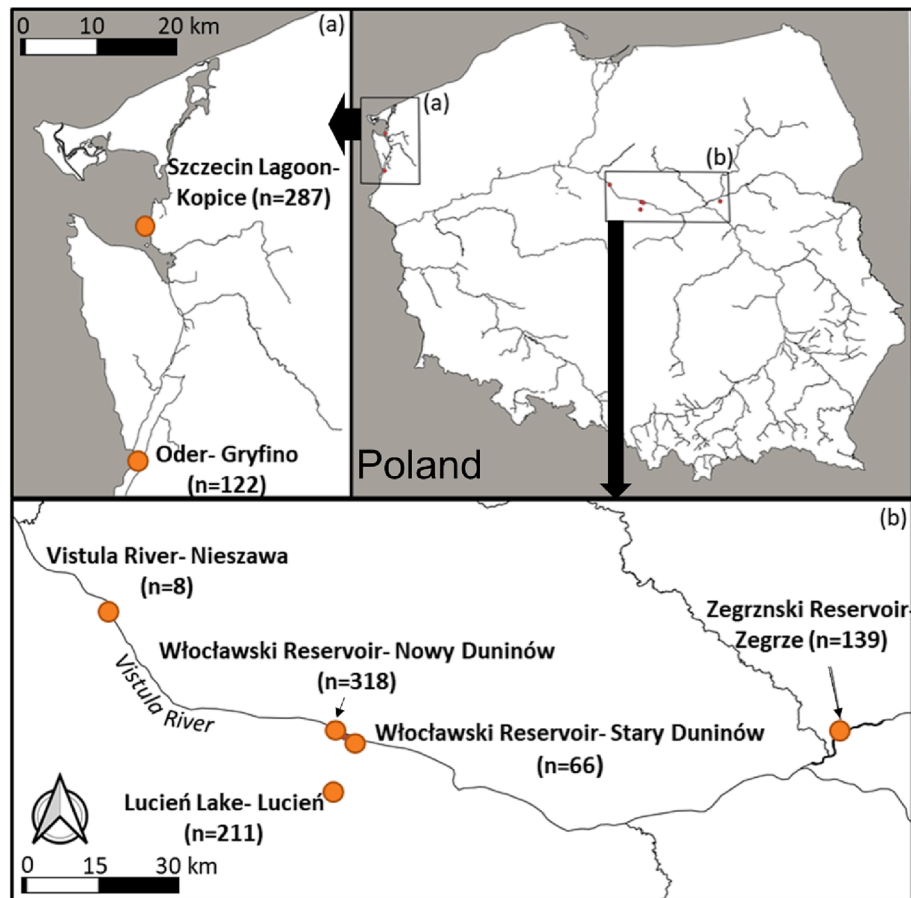


Fig. 1. Map of Poland, including the distribution of *Pontogammarus robustoides* sample sites (and the number of animals sampled) across Poland in this study (orange spots). a) Western Poland sample locations. b) Central Poland sample locations. The map was plotted in QGIS (v.3.16.11). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2008) and has now invaded most of continental Europe and adjacent areas (Copilaş-Ciocianu et al. 2023; Copilaş-Ciocianu and Šidagytė-Copilas, 2022; Perova, 2022) and its further dynamic spread into new areas is forecasted. As there have been already several Ponto-Caspian amphipods that expanded their range in the whole continental Europe and two succeeded in colonising the inland waters in UK (Copilaş-Ciocianu et al. 2023), the expanding range of *P. robustoides* raises concerns for naïve aquatic ecosystems that may soon become invaded, making this species one of several ‘hot topic’ invaders (Foster et al. 2021). Similarly worrisome are the risks posed by pathogens carried by *P. robustoides* (Roy et al. 2017; Bojko et al. 2021a; Foster et al. 2021).

Pontogammarus robustoides from its native and invasive ranges have been identified with putative nudiviruses, pathogenic bacteria (*Pseudomonas* sp.; *Vibrio* sp.), and hyperparasitic bacteria (rickettsia-like), microsporidians (*Nosema pontogammari* (unofficial); *Dictyocoela muelleri*; *Dictyocoela berillonum*; *Dictyocoela* sp.), haplosporidians (*Haplosporidium orchestiae* and *Haplosporidium echinogammari*), gregarines (*Cephaloidophora mucronata* and *Uradiophora ramosa*), trematodes (*Caudotestis skrjabini*), cestodes (Hymenolepididae and Amphilina foliacea), four unidentified nematodes, and rotifers (*Encentrum gammari*; *Proales gammari*; *Cephalodella jakubskii*) (Getsevichyute, 1970; Getsevichyute and Simonene 1974; Chernogorenko et al. 1978; Austin and Alderman, 1987; Boshko, 1994; Bauer et al. 2002; Ovcharenko et al. 2009; Wilkinson et al. 2011; Ovcharenko and Wroblewski, 2016; Bojko, 2017; Bacela-Spychalska et al. 2018; Bojko and Ovcharenko, 2019; Urrutia et al. 2019). More generally, other members of the genus *Pontogammarus* (*P. maeoticus*; *P. bosniacus*) harbour additional parasite diversity, including acanthocephalan (*Polymorphus* spp.) parasites (Bojko and Ovcharenko, 2019). The impact of these parasites on their

pontogammarid host is somewhat unknown. In this report, we unveil a range of parasitological/pathological traits alongside the biogeographical distribution of parasites from 1,141 *P. robustoides* collected from seven sites across Poland. With these information, we aim to provide a baseline survey of parasites that may be used when this amphipod invades other areas of Europe and the UK.

2. Materials and methods

2.1. Sample collection and preparation

Pontogammarus robustoides (n = 1,141) were collected using kick-sampling and hydrobiological nets from the riverbank at seven locations across Poland (Table 1; Fig. 1), a country with a widespread invasive population of this amphipod along the central invasion corridor *sensu* Bij de Vaate et al. (2002). Upon collection, individuals were euthanised by injection with ~ 2 ml of Davidson’s freshwater fixative (water, formaldehyde, ethanol, acetic acid) and then submerged in the same solution for up to 48 h. After 24-48hr the solution was exchanged for 70% ethanol. The samples were then placed into ethanol-soaked tissue and transported to the Centre for Environment, Fisheries and Aquaculture Science (Cefas) in Weymouth, Dorset. Each amphipod underwent whole histological preparation using a tissue processor to conduct wax infiltration, followed by embedding in a wax block and sectioning at 3–4 µm. The sections were de-waxed, stained with haematoxylin and alcoholic eosin, and then dried at ~ 40 °C on a hot plate before being cover-slipped. The resulting slides were read on a Leica light microscope, and the presence/absence of parasites in each amphipod was recorded. Amphipod sex was recorded using histology, where possible. Samples

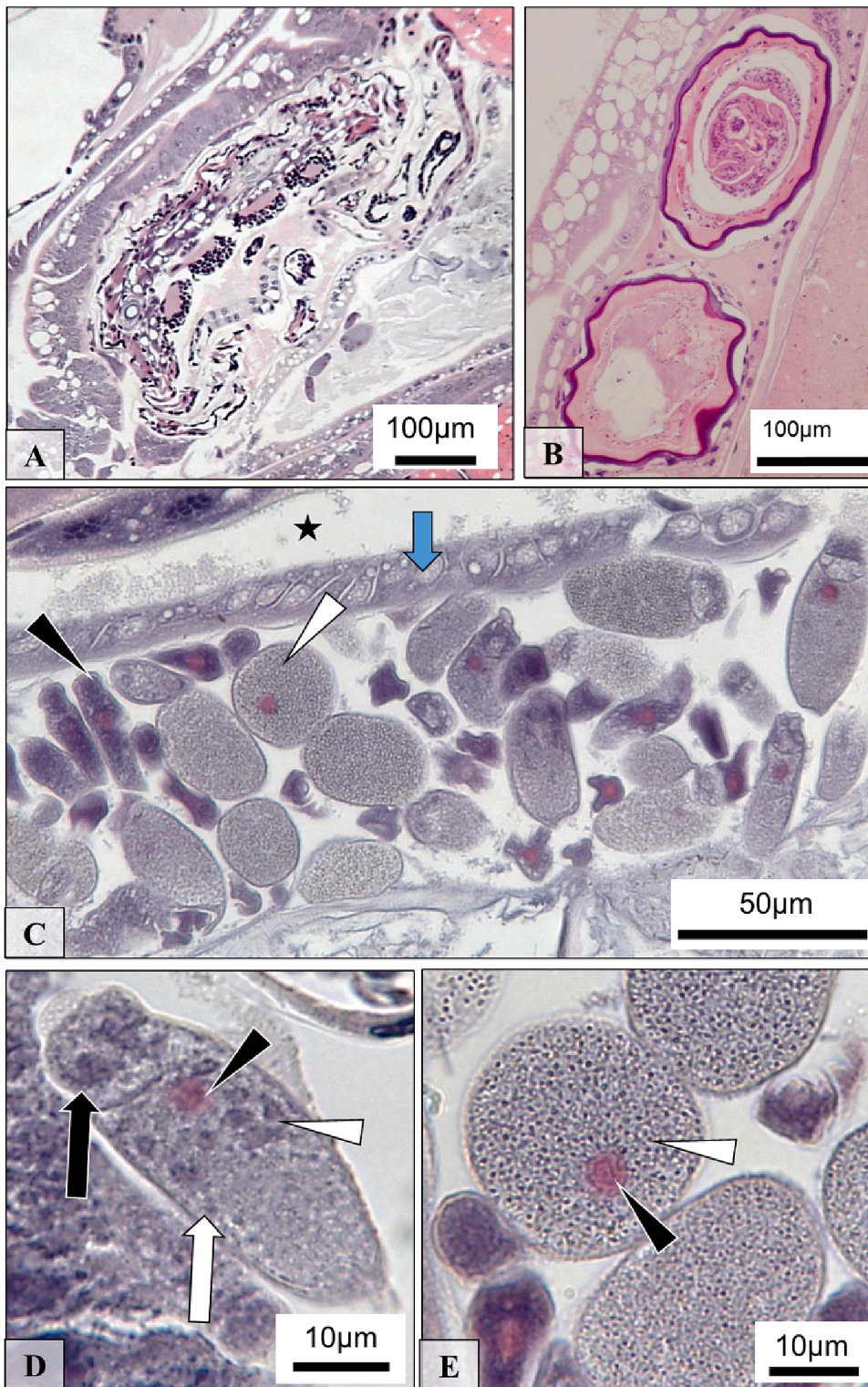


Fig. 2. Histopathological survey for parasites of *Pontogammarus robustoides*. A) An endoparasitic organism sitting between the gut and hepatopancreas of an infected host. B) Two encysted trematodes in the connective tissues and hepatopancreas. C) Gut gregarine intracellular life stages (blue arrow), extracellular life stages (black arrow), and extracellular life stages infected with an endosymbiont (white arrow) in the host gut lumen (black star). D) Early development (white arrow, black arrow) of the likely microsporidian parasite of the gregarines (white arrow). The gregarine nucleus is highlighted with a black arrow. E) Hypertrophic gregarines with visible nucleus (black arrow) are full of putative microsporidian spores. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with parasites that required electron microscopy were re-processed from wax-block into resin-block using the process described in [Bojko et al. \(2021b\)](#).

2.2. Statistical analysis

All statistical analyses were performed in R (version 3.5.1) and RStudio (version 2022.07.1 + 554), with a significance level of 95% ($\alpha = 0.05$). To avoid unreliable statistical estimates, caused by abnormally

high variance associated with sample size and/or parasite representation (i.e., presence/absence), data was pre-processed prior to analyses. Data obtained from amphipod specimens sampled from the Vistula River in Nieszawa (VRN) were excluded from statistical analyses due to a considerably lower sample size ($n = 8$ specimens) when compared to other locations (range = 66 – 317 specimens; [Table 1](#)). Further modifications to the data were made in relation to specific parasite types, particularly haemocyte viruses, rickettsia-like organisms (RLOs), and isopods which were excluded from analysis due to low parasitic

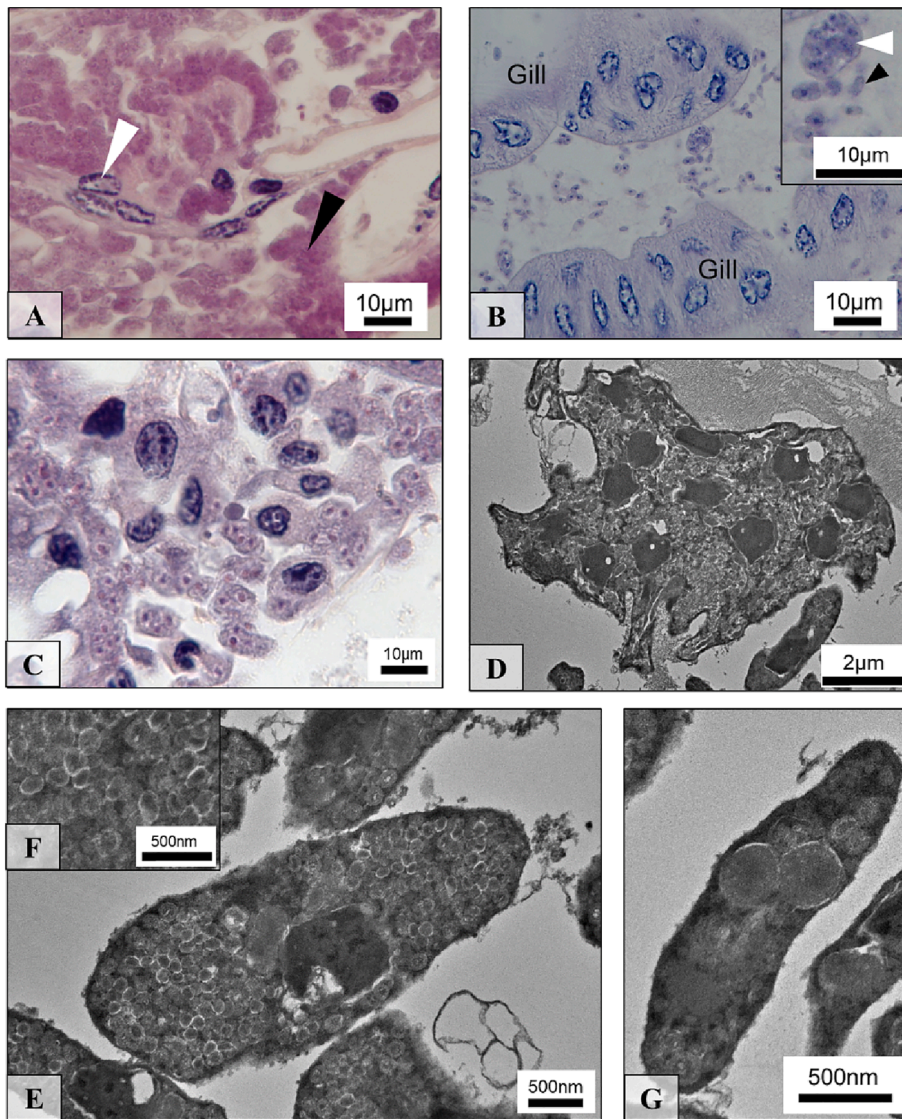


Fig. 3. *Haplosporidium echinogammari* from *Pontogammarus robustoides*. A) Masses of developing parasites are located throughout the haemocoel (black arrow) (white arrow: host nucleus). B) Individual haplosporidians (black arrow) and developing masses (white arrow) circulating the haemolymph of the gill. C) Haplosporidian stages intracellular in host connective tissue. D) An electron micrograph of a multi-nucleate developing stage of the *H. echinogammari*. E and F) A haplosporidian development stage with multiple granule-like organelles scattered throughout the cytoplasm. G) A haplosporidian development stage with enlarged granule-like organelles.

prevalence, with only a single amphipod infected by haemocyte viruses and RLOs (not the same host organism), and two amphipods infected by isopods (one internal and one external). A single unsexed amphipod specimen, sampled from Sary Duninów was also disregarded from analysis, having been identified as a host to fouling ciliates only.

2.2.1. Parasite diversity

Parasite diversity (i.e., the total number of parasite types identified in each amphipod specimen, sampled from each geographic location) was analysed using a generalised linear model (GLM) fitted with a Poisson distribution error. Parasite diversity was analysed in relation to host sex (male/female) and sampling site, both as individual predictors and as a two-way interaction term (host sex \times sampling site). If non-significant, the interaction term was removed, and a simplified model was run. To determine whether model simplification significantly affected the model predictions, changes to residual deviance were analysed using χ^2 . If a significant effect was identified (main effect or interaction term), *post-hoc* analyses were carried out using the *emmeans*

package (version 1.8.0; Lenth, 2022) to assess between-group variance based on estimated marginal means (EMMs).

2.2.2. Parasite prevalence

Parasite prevalence (i.e., the proportion of amphipods infected by a parasitic organism) was calculated based on binary presence/absence scores for each parasite type and analysed using a generalised linear mixed effect model (GLMM) fitted with a binomial error structure (*glmmTMB*, v.1.1.4; Brooks et al., 2017). Parasite prevalence was analysed in relation to parasite type, host sex, and sampling site, with each categorical variable provided as a main effect only. Interaction terms were not included in the model, given that the number of levels associated with each independent variable (parasite type = 12 levels, host sex = three levels, sampling site = six levels) caused the model to fail due to a lack of convergence. To account for the presence of multiple parasite types per host organism, per sampling site, 'Host-ID' was included as a random effect, nested within the fixed factor 'sampling site':

$$\text{InfectionStatus}[0, 1] \sim \text{ParasiteType} + \text{HostSex} + \text{SamplingSite} + (1|\text{SamplingSite} : \text{HostID})$$

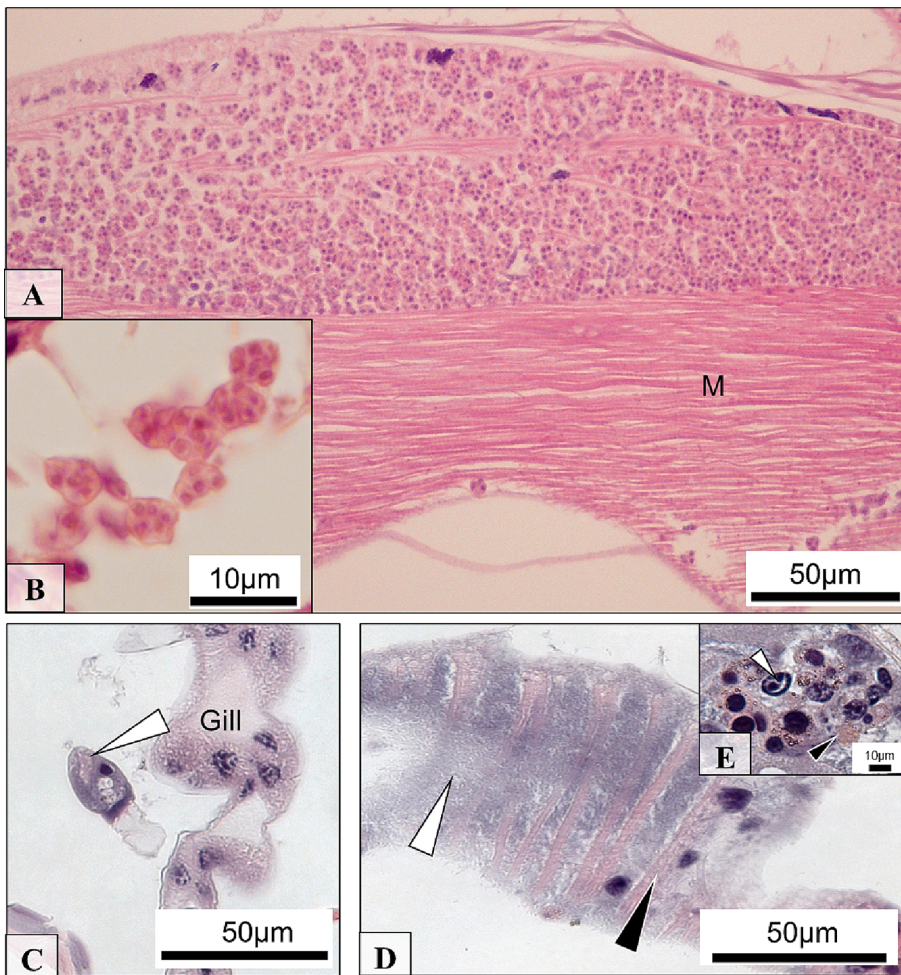


Fig. 4. Parasites of *Pontogammarus robustoides*. A) A microsporidian parasite of the musculature (M). B) Development of octosporous stages in the host muscle sarcolemma. C) A ciliated, stalked, protozoan (white arrow) on the gill. D) Systemic bacterial infection (white arrow) most prevalent in the host haemolymph. The heart muscle (black arrow) is imaged. E) Pockets of melanisation (black arrow) identify a host response to the presence of the bacterial pathogen. Host nuclei (white arrow).

Where analysis indicated a statistically significant effect by a given variable(s) *post-hoc* analyses were carried out using the *emmeans* package, with p-values adjusted using the default Tukey HSD.

2.2.3. Parasite Co-occurrence

As well as addressing how parasite prevalence might differ in relation to parasite type, host sex, and sampling site, we also assessed whether parasite types in *P. robustoides* exhibited similar co-occurrence patterns across all sampling sites. For this, we used the R package *cooccur* (v.1.3; Griffith et al. 2014) and applied a probabilistic model, first developed by Veech (2013), to a species by site co-occurrence matrix. This model utilises distribution-free probabilistic combinatorics to determine whether species pairs co-occur in the same site more or less often than expected at random. Here we defined species based on each parasite type identified in *P. robustoides* and site as each host individual (Galen et al., 2019).

3. Results

3.1. Histopathological parasite survey

The histopathological survey of *P. robustoides* revealed the following parasites: an endoparasitic metazoan (putatively Crustacea: Isopoda), Digenea (Trematoda), apicomplexan gregarines (and their hyperparasitic microsporidians), Haplosporidia, muscle-infecting microsporidians, ciliated protozoans, bacteria, and viruses (Figs. 2-5), at a range of prevalences across seven sites in Poland (Table 1).

One individual amphipod host was observed with a large (~0.55 mm

in length) endoparasitic metazoan parasite occupying the internal cavity of the animal, displacing the hepatopancreas, gonad, and gut of the host (Fig. 2A; Table 1). No visible host response was observed, including a lack of melanisation or haemocyte aggregation. Other metazoan parasites include Digenea, which were encysted in the connective tissues of the host (Fig. 2B; Table 1). Haemocyte aggregation was common among specimens observed histologically; however, a lack of host immune response was often observed. The digenean trematodes were commonly present with a basophilic layer surrounding their cyst, which included the parasite centrally (Fig. 2B).

Protozoa, or protozoan-like, groups from *P. robustoides* included gregarines (Fig. 2C-E), Haplosporidia (Fig. 3), Microsporidia (Fig. 4A-B), and ciliated protozoa (Fig. 4C). Gregarines were common in the gut, and less common in the hepatopancreas, of amphipod hosts (Table 1), often present in large numbers aligned against the gut epithelia as well as developing within the epithelial cells (Fig. 2C). In addition to the gregarines, some appeared to have an intracellular hyperparasite likely to be a microsporidian, which was refractile and present in multiple predicted developmental stages (Fig. 2D-E). Haplosporidia (*Haplosporidium orchestiae* and *Haplosporidium echinogammari*) were present in the haemolymph (Fig. 3A) of the amphipod host as well as visible in the gill (Fig. 3B) and connective tissues (Fig. 3C) (Table 1). Transmission electron microscopy confirmed infection to be haplosporidian in nature, including multi-nucleate development stages (Fig. 3D-F), and follow-up molecular diagnostics were presented in Urrutia et al. (2019). Microsporidian parasites were observed in the muscle tissue of *P. robustoides* from multiple sites (Fig. 4A-B; Table 1), some with octosporous developmental stages observable using histology (Fig. 4B). Finally, stalked/

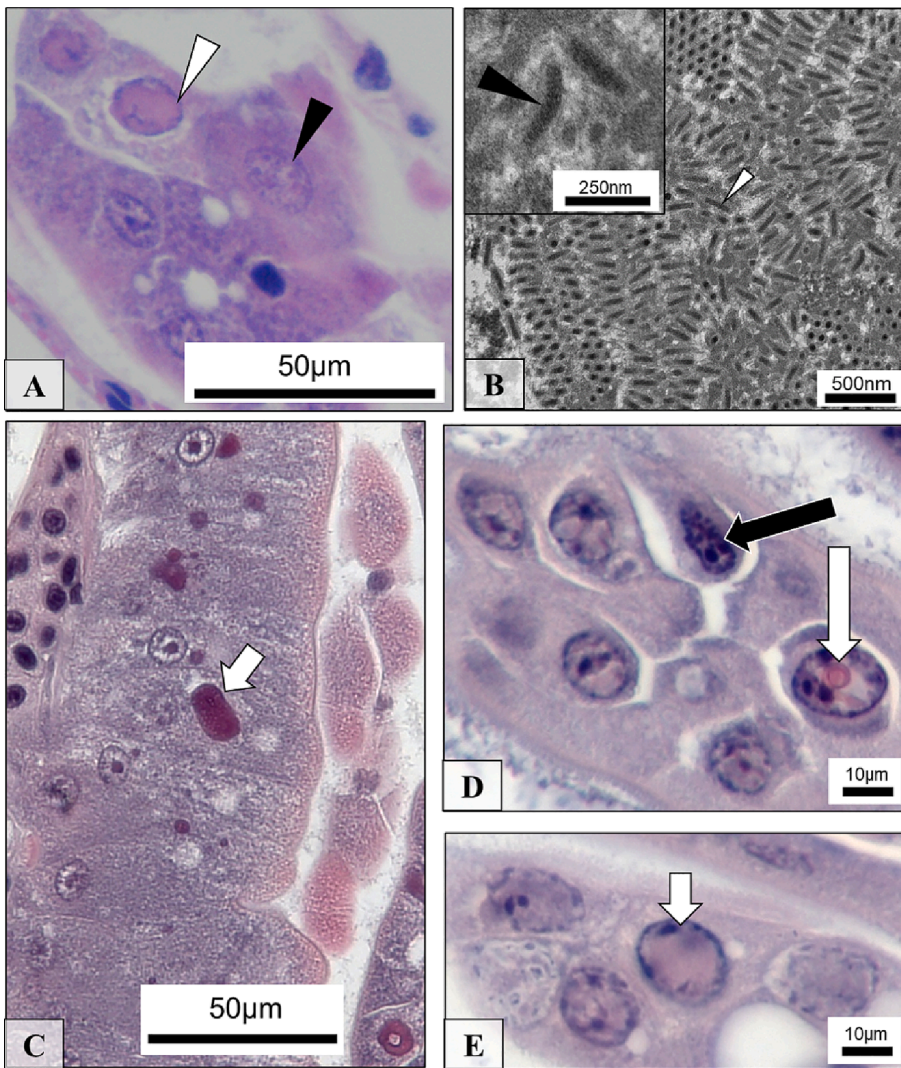


Fig. 5. Viral associations in *Pontogammarus robustoides*. A) Nuclear hypertrophy due to a growing viroplasm in an infected hepatopancreatic cell (white arrow). An uninfected nucleus is identified (black arrow). B) The bacilliform virus (putative *Nudiviridae*) (white/black arrow) that was causing the hypertrophy observed in image A, can be visualised in an electron micrograph. C) Large basophilic cytoplasmic inclusions in hepatopancreatic cells (white arrow), considered putatively viral. D and E) Hypertrophic nuclei of the gut epithelial layer appear with translucent or eosinophilic inclusions (white arrow). Unaffected cell (black arrow).

ciliated protozoa were common externally on the gills and appendages of the host (Fig. 4C; Table 1). No observable immune response was noted for gregarines, haplosporidians, or ciliated protozoa, but small pockets of haemocyte aggregation or melanisation were common in microsporidian infection.

Systemic bacterial infections, most notable in the haemolymph of the host, were observed (Fig. 4D; Table 1). The individual bacteria stained basophilic and were common in large numbers in the haemolymph around the heart, muscle, and hepatopancreas. The infection triggered haemocyte aggregation and melanisation responses throughout the haemolymph (Fig. 4E).

Finally, observations of viral or viral-like pathologies included a bacilliform virus from *P. robustoides*, termed 'Pontogammarus robustoides Bacilliform Virus' (PrBV). Electron micrographs of the infected nuclei revealed rod-shaped virions, which measured 37.5 ± 5.7 nm in core width and 166.4 ± 20.6 nm core length, and 72.7 ± 8.0 nm virion width and 217.8 ± 25.3 nm virion length (Fig. 5 A-B). A putative cytoplasmic viroplasm was observable in the hepatopancreas of infected individuals, displaying as a purple-staining mass (Fig. 5C). Gut epithelial cells were noted with small eosinophilic inclusions, which may become larger and translucent in later stages (Fig. 5D-E) and are considered putative viroplasmic inclusions. No observable inflammatory responses were present in relation to the viruses or putative viral-like pathologies.

3.2. Parasite diversity

There was no significant association between parasite diversity and host sex (Poisson GLM; $\chi^2 = 1.63$, $df = 1$, $p = 0.20$), with male and female amphipods containing a similar number of parasite types (Mean \pm SEM = 1.41 ± 0.04 and 1.32 ± 0.03 parasite types respectively). Comparisons across sampling sites highlighted a significant difference in the number of parasite types identified in amphipod hosts (Poisson GLM; $\chi^2 = 16.04$, $df = 5$, $p < 0.01$; Fig. 6). Parasite diversity was higher in amphipods sampled from Lucień Lake, compared to those sampled from Włocławski Reservoir in Stary Duninów ($\beta = 0.261$, $z = -2.017$, $p = 0.044$) and Zegrzynski Reservoir in Zegrze ($\beta = 0.363$, $z = 3.562$, $p < 0.001$), and comparable to those sampled from Szczecin Lagoon in Kopice, Włocławski Reservoir in Nowy Duninów and Oder in Gryfino ($p > 0.05$ for all). Conversely, amphipods sampled from Zegrzynski Reservoir were predicted to host, on average, significantly fewer parasite types than Włocławski Reservoir (Nowy Duninów; $\beta = -0.290$, $z = -2.986$, $p = 0.003$), Oder ($\beta = -0.291$, $z = -2.499$, $p = 0.013$) and Szczecin Lagoon ($\beta = -0.242$, $z = -2.479$, $p = 0.013$). There was no significant interaction between host sex and sampling site when predicting parasite diversity in *P. robustoides* (Poisson GLM; $\chi^2 = 1.64$, $df = 5$, $p = 0.90$), although when removed, the loss of this interaction term did not significantly improve model predictability ($p > 0.05$).

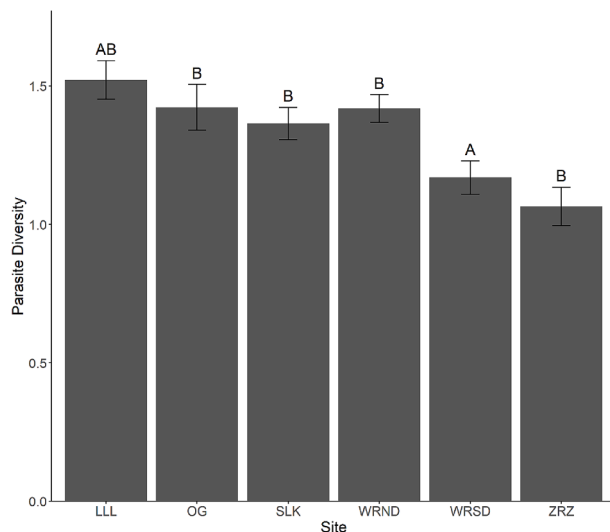


Fig. 6. Average parasite diversity estimated for *P. robustoides* specimens (excluding unsexed individuals) sampled from six locations in Poland. Error bars represent the standard error of the mean, and letter notations indicate statistically significant differences between sampling sites at a significance level of 0.05. Note: Codes represent sampling sites, with LLL = Lucień Lake in Lucień, OG = Oder in Gryfino, SLK = Szczecin Lagoon in Kopice, WRND = Włocławski Reservoir in Nowy Duninów, WRSD = Włocławski Reservoir in Stary Duninów, and ZRZ = Zegrzynski Reservoir in Zegrze.

3.3. Parasite prevalence

Parasite prevalence varied considerably depending on parasite type and sampling site (GLMM $p < 0.05$, Fig. 7). Parasite prevalence did not vary with respect to host sex, with 85.2% of males (375/440) and 84.6% of females (494/584) infected by at least one parasite type ($\beta = 0.121$, t -ratio = 1.687, $p = 0.092$) (Fig. 7). Of the different parasite types screened, when unsexed hosts are excluded, we see the following prevalence data; fouling ciliates (61.9%; 634/1024), gut gregarines (39.6%; 406/1024), and bacilliform virus (16.3%; 167/1024) were the most prevalent and detected in a significantly greater proportion of *P. robustoides* specimens, relative to: systemic bacteria (0.8%; 8/1024), Digenea (3.7%; 38/1024), external rotifers (2.7%; 28/1024), putative viruses found in both the gut epithelia (0.6%; 6/1024) and cytoplasm of hepatopancreatic cells (1.5%; 15/1024), hyperparasitic microsporidians (parasitizing gregarines) (1.6%; 16/1024), Haplosporidia (0.3%; 3/1024), hepatopancreatic gregarines (0.4%; 4/1024), and muscle microsporidians (6.5%; 67/1024) (Table S1). Comparisons amongst fouling ciliates, gut gregarines, and bacilliform viruses revealed significant differences in prevalence (all $p < 0.001$), whereas parasites with lower prevalence (i.e., Haplosporidia, hepatopancreatic gregarines, putative gut epithelial virus, and systemic bacteria) were detected in a similar number of amphipod hosts (all $p > 0.05$).

Across the six different sampling sites included in the analysis, parasite prevalence was generally lowest in Zegrzynski Reservoir, with 75.5% of individuals (105/139) found to be infected by at least one parasite type. Parasite prevalence was significantly lower in Zegrzynski Reservoir, when compared to Szczecin Lagoon (80.7%; 217/269), Oder (87.5%; 91/104), Lucień Lake (86.3%; 157/182) and Włocławski Reservoir (Nowy Duninów) (88.7%; 235/265; $p < 0.05$ for all; Table S2), but not Włocławski Reservoir (Stary Duninów) which, despite having the highest prevalence (98.5%; 64/65), was identified as non-significant ($p = 0.943$); a factor likely attributable to similarities in the types of parasites found across both sites, and the number of amphipods infected by them (Fig. 7).

3.4. Parasite Co-occurrence

The probabilistic co-occurrence model identified statistically significant co-infection patterns between six parasite types (Fig. 8). Positive co-infection patterns (i.e., co-infection occurring at a rate greater than expected by chance) were found between fouling ciliates and external rotifers, gut epithelia viruses and gut gregarines, and gut gregarines and gregarines infected by microsporidia ($p < 0.05$ for all pairs). Negative co-infection patterns (i.e., co-infection occurring at a rate lower than expected by chance) were observed between fouling ciliates and both gut epithelial viruses and gut gregarines, and 'Pontogammarus robustoides bacilliform virus' and digenea ($p < 0.05$ for all pairs). All other parasite pairings were expected to occur at random (Table S3).

4. Discussion

Biological invasions consisting of crustaceans and their parasites remain largely unexplored, where only a fraction (~1/3) of the parasite diversity is thought to have been catalogued from classified invasive or non-native crustaceans (Bojko et al. 2021a). Largely due to their tolerances, many Ponto-Caspian amphipods have become high-impact invaders across Europe, with high rates of dispersal and establishment, and commonly carry with them an onslaught of co-invasive parasites. Recent efforts to catalogue the expanding diversity of co-invasive parasites have been supported by (histo)pathological (Bojko et al., 2013; Warren et al., 2022; Ovcharenko et al., 2010) and molecular studies (Wattier et al. 2007; Grabner et al. 2015, Bacela-Spychalska et al. 2018). In this study, >1000 invasive *P. robustoides* from Poland were histologically screened for parasites (from viruses to large Metazoa) to better understand the potential for parasite-carrying-capacity, parasite diversity in this host, and the likelihood of what future invasions may bring to naïve environments and ecosystems, such as the United Kingdom.

4.1. Parasites of *Pontogammarus robustoides*

Existing parasitological associations for *P. robustoides* consist of viruses (putative nudiviruses) (Bojko, 2017; Bojko and Ovcharenko, 2019), pathogenic bacteria (*Pseudomonas* sp.; *Vibrio* sp.) and hyperparasitic bacteria (rickettsia-like), microsporidians (*Nosema pontogammari*; *Dictyocoela muelleri*; *Dictyocoela berillonum*; *Dictyocoela* sp.) (Ovcharenko et al. 2009; Wilkinson et al. 2011; Bacela-Spychalska et al. 2018), haplosporidians (*Haplosporidium orchestiae* and *Haplosporidium echinogammari*) (Urrutia et al. 2019), gregarines (*Cephaloidophora mucronata* and *Uradiophora ramosa*) (Ovcharenko et al. 2009), trematodes (*Caudostes skrjabini*) (Chernogorenko et al. 1978), cestodes (*Amphiline foliaceae*) (Bauer et al. 2002), and rotifers (*Encentrum gammari*; *Proales gammari*; *Cephalodella jakubskii*) (Bojko and Ovcharenko, 2019). We have provided greater evidence in this study for the presence of nucleus-infecting viruses, using electron microscopy and histology to reveal their pathology and virion structure, suggesting the bacilliform virus we see in *P. robustoides* may be similar to 'Dikerogammarus haemobaphes nudivirus' (DhNV) or 'Dikerogammarus haemobaphes Bacilliform Virus' (DhBV) (Allain et al., 2020; Bojko et al., 2019), pending further genomic confirmation.

Bacterial pathogens from *P. robustoides* include the intriguing discovery of a hyperparasitised microsporidian parasite (Ovcharenko and Wroblewski, 2016), *Pseudomonas* sp., and *Vibrio* sp. (causing 'black spot') (Austin and Alderman, 1987). In this study, we evidence a systemic bacterial infection in a single animal (Fig. 4 D-E); however, the taxonomy of the bacterial pathogen is undetermined.

The protozoan(-like) parasite diversity (haplosporidians, gregarines, ciliates, and microsporidians) consisted of *Haplosporidium* spp. (Urrutia et al. 2019), *Cephaloidophora*/*Uradiophora* spp. (Ovcharenko et al. 2009), putative *Dictyocoela* sp. (Microsporidia: Glugeida), and yet taxonomically undetermined ciliated protozoans (putative Ciliophora).

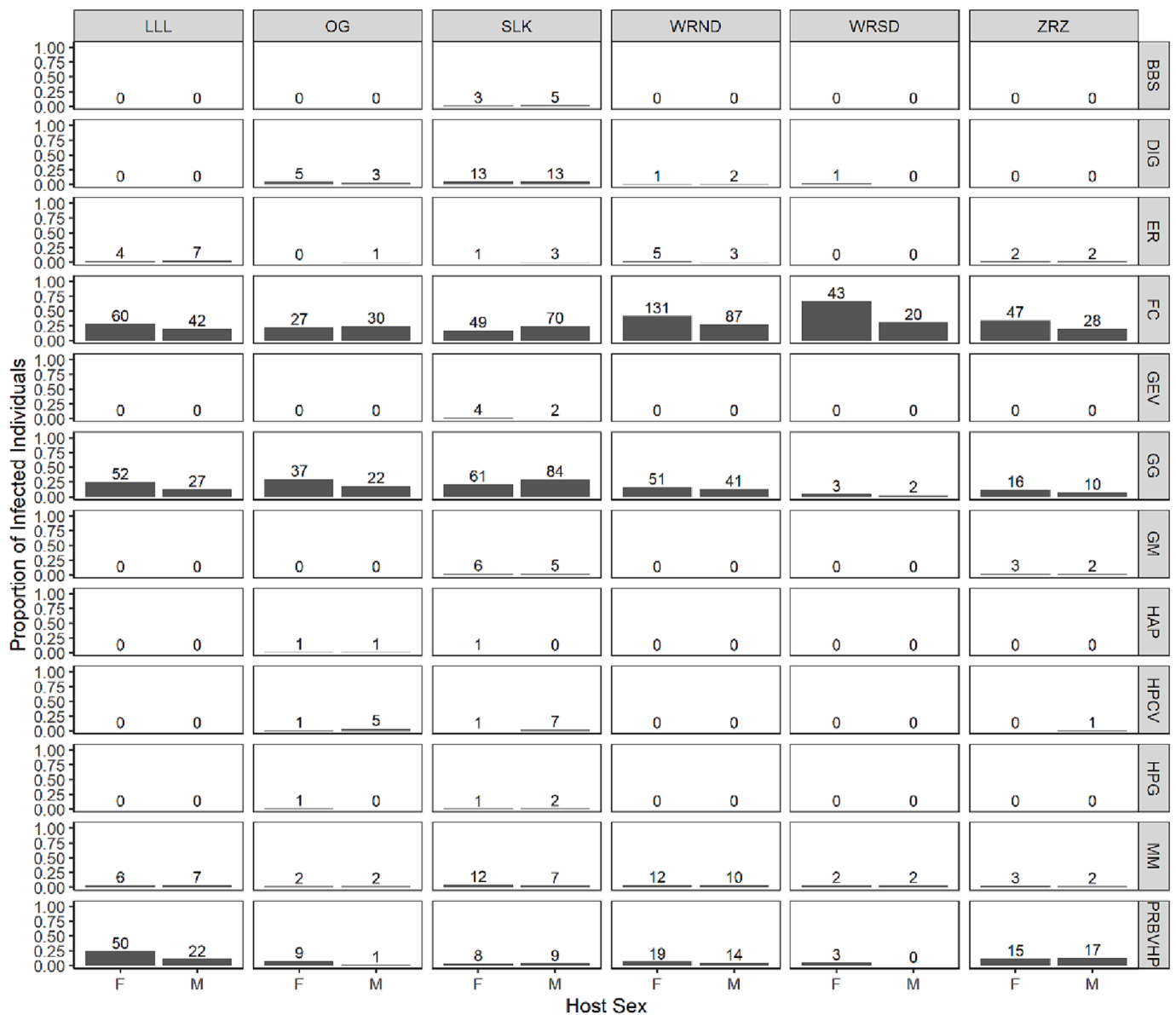


Fig. 7. The proportion of infected female and male *P. robustoides*, based on raw counts (number above each bar) for each parasite type and location. Proportion is calculated based on the total number of amphipods, sampled from each location (LLL = 182, OG = 104, SLK = 269, WRND = 265, WRSD = 65, ZRZ = 139 amphipods). Note: Codes presented within the figure are abbreviations of host sex, parasite type, and sampling site. For host sex F = female, M = male, U = unsexed. For parasite type BBS = systemic bacteria, DIG = digenea, ER = external rotifer, FC = fouling ciliates, GEV = gut epithelia virus, GG = gut gregarines, GM = gregarines with microsporidia, HAP = haplosporidium, HPCV = hepatopancreatic cytoplasm virus, HPG = hepatopancreatic gregarines, MM = muscle microsporidia, and PRBVHP = ‘Pontogammarus robustoides bacilliform virus’ in hepatopancreas. For sampling site LLL = Lucień Lake in Lucień, OG = Oder in Gryfino, SLK = Szczecin Lagoon in Kopice, WRND = Włocławski Reservoir in Nowy Duninów, WRSD = Włocławski Reservoir in Stary Duninów, and ZRZ = Zegrzński Reservoir in Zegrze.

Some of these observations have been identified in previous studies; however, the taxonomy of the ciliated protozoan requires further clarification. Both gregarines and ciliates were common throughout the dataset and noted across sites and sexes.

The muscle-infecting microsporidian parasite observed across sites is considered to be either the unofficial *Nosema pontogammari*, or one of several associated *Dictyocoela* spp., based on its development and muscle-associated pathology (Bacela-Spychalska et al. 2018). *Nosema pontogammari* was described using transmission electron microscopy and wet-prepared material, but its classification lacks the genetic data necessary for official microsporidian taxonomic acceptance (Bojko et al., 2022; Ovcharenko and Wróblewski, 2016). It is possible that the muscle-infecting microsporidian observed is a *Nosema*, but equally possible that this is a *Dictyocoela* or *Cucumispora* from the Glugeida, which commonly infect the musculature of amphipods (Ovcharenko et al. 2010; Bojko

et al. 2015; Bojko et al. 2017; Bacela-Spychalska et al. 2018). *Dictyocoela* spp. are thought to have the potential to undergo vertical transmission (Wilkinson et al. 2011), but we did not note infection in the gonad of males or females. Microsporidian infections were present across sites, and invading *P. robustoides* may carry microsporidians with them to new locations.

An intriguing association made during this study is the presence of microsporidian-like inclusions in some gregarine infections of *P. robustoides*. Microsporidia are known to infect protozoa – most recently, *Hyperspora aquatica* was described from a protozoan parasite (*Marteilia cochillia*) of cockles (Stentiford et al. 2017). Metchnikovellids are common microsporidia-like hyperparasites of parasitic gregarines (Mikhailov et al. 2022; Frolova et al. 2023). Microsporidia have also been noted in gregarines infecting crustacean host gut systems (Clotilde-Ba and Toguebaye, 1995). It may be possible that the gregarines

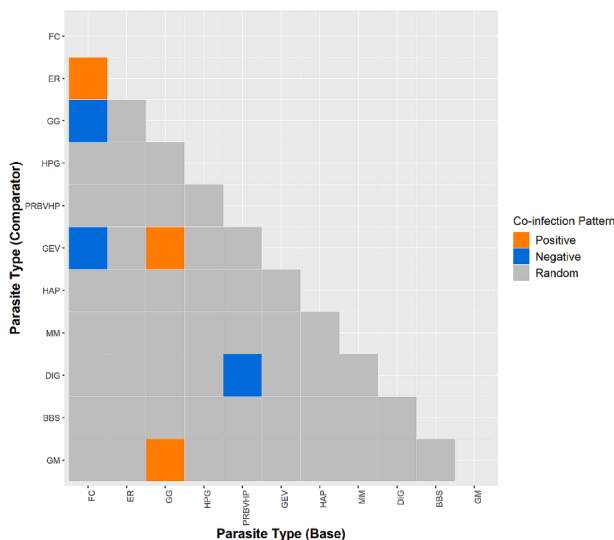


Fig. 8. A heatmap showing the results of the probabilistic co-occurrence model, assessing the probability of co-infection by 11 different parasite types, recorded in sexed *P. robustoides* hosts sampled from six locations in Poland. Cells highlighted in blue indicate positive co-infections (i.e., parasite pairs which co-occur significantly more frequently than expected by chance), whilst cells highlighted in orange indicate negative co-infections (i.e., parasite pairs which co-occur significantly less frequently than expected by chance). Cells highlighted in grey denote random co-infections. Note: Codes presented within the figure are abbreviations of parasite type; BBS = systemic bacteria, DIG = digenea, ER = external rotifer, FC = fouling ciliates, GEV = gut epithelia virus, GG = gut gregarines, GM = gregarines with microsporidia, HAP = haplosporidium, HPCV = hepatopancreatic cytoplasm virus, HPG = hepatopancreatic gregarines, MM = muscle microsporidia, and PRBVHP = ‘Pontogammarus robustoides bacilliform virus’ in hepatopancreas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can carry the same microsporidians that infect the amphipod host. A follow-up study to explore this would be of great interest.

Finally, the metazoan parasite diversity that we observed histologically included a series of trematodes, and an endoparasitic organism, thought to be a crustacean (putatively an isopod). The trematode *Caudostes skrjabini* has been identified in *P. robustoides* collected from the River Volga, Russia (Chernogorenko et al. 1978). It is possible that the same trematode has a natural or invasive range extending into Poland, but it is equally possible that *P. robustoides* host several similarly looking trematodes. Accordingly, amphipods may host multiple cryptic trematode species (Shaw et al. 2020). Trematodes are common members of biological invasions and have been noted in several studies (Blakeslee et al. 2013). Digenea were most common at Szczecin Lagoon in Kopice, but were either rare or missing from other sample sites (Fig. 7). No cestodes were found in the current study.

The putative endoparasitic isopod was noted only once (despite one isopod being noted externally to an amphipod), and there was a high amount of organ disruption in its host. The parasite altered the position of the hepatopancreas and gut (Fig. 2A). The flattened and elongated body structure, with nerve ganglia distributed along the underside of the parasite, as well as its own gut and hepatopancreatic organs, suggest that this is likely an isopod. This parasite group has not been noted in *P. robustoides* previously, and most isopod parasites are noted from decapod hosts, but Podasconidae are known to parasitize amphipods (Williams and Boyko, 2012).

4.2. Parasite invasion risk

Lots of parasitic taxa have been associated with biological invasions involving Crustacea - in particular, the parasites of invasive amphipods are a recently well documented group (Bojko et al., 2021a; Bojko and Ovcharenko, 2019; Foster et al., 2021; Wood et al., 2021). For example, microsporidians have been found in invasive hosts on multiple occasions, some in populations far away from their host’s native range (Wattier et al. 2007; Burgess and Bojko, 2022). In other cases, microsporidians have been completely lost during amphipod invasions, via factors likely associated with enemy release (Bojko et al. 2013). In this study, we identified several freshwater locations in Poland housing the invasive amphipod *P. robustoides*. The parasite communities hosted by this amphipod varied across sites. Rare taxa, such as the putative viral pathologies, systemic bacteria, haplosporidians, and the endoparasitic isopod, were only present at one or two locations. It is likely that their range is, at the moment, constrained to those areas. However, given the invasion potential of *P. robustoides*, we can expect a concomitant spread of the host and its parasites into Europe, and further to the UK, as it was the case for the other Ponto-Caspian amphipods: *Dikerogammarus villosus* and *Dikerogammarus heamobaphes* (Hatcher et al. 2019). The data we provide may help in the risk assessment associated with these taxa and determine the origin of the invading populations, facilitating the exchange of biosecurity information between regions and countries.

Identifying parasites in *P. robustoides* is important in establishing a baseline for this species. This is particularly relevant in light of the different degrees of host specialization displayed by parasites, such as microsporidians. Amphipod-infecting microsporidians are often host generalists, as seen in several studies across Europe (Bacela-Spychalska et al. 2018, Quiles et al. 2020, Prati et al. 2022). Spillover or spillback events between native/invasive amphipods are to be expected following the invasion of *P. robustoides*. For instance, *Dictyocoela* spp. (known parasites of *P. robustoides*), have a wide range of hosts, and most amphipod species screened for microsporidians tend to be associated with one or more *Dictyocoela* spp. (Bojko and Ovcharenko, 2019; Quiles et al. 2020). This broad host range may enhance the invasion potential. Accordingly, *Dictyocoela* spp. are some of the more common invasive microsporidia – most recently documented to have made it past York in the United Kingdom (Burgess and Bojko, 2022). However, when assessing invasion risks for these parasites, it is important to note that *Dictyocoela* are already common throughout Europe, and many of these species are already in invaded sites, including the UK. Knowledge of the microsporidian species and strain, derived from PCR or metagenomic tools, would be an important next step in monitoring these parasites, given their significant pathological impact on *P. robustoides* musculature and the potential spillover to native amphipods.

4.3. Conclusions

We provide a histopathological screening study for *Pontogammarus robustoides* populations distributed throughout Poland, while also providing an up-to-date list of parasitic and commensal taxa known to be associated with this invasive host. Our screening data have identified a range of new taxonomic associations, such as several viral-like pathologies, and the distribution of these symbiotic taxa across freshwater sites in Poland.

We highlight that co-infections of these symbionts are largely unlinked, but some associations, e.g., ciliates and gregarine infections, are more likely. Males and females appear equally capable of hosting the same complement of parasites. As the hosted microsporidians are likely *Dictyocoela* spp., the associated risks for native amphipods may be

reduced since this parasite group is already widespread. The greatest pathogenic risk associated with the invasion of *P. robustoides* seems to lay with the possible introduction of viral, bacterial, and haplosporidian species. However, more studies are necessary to unravel their host range and impacts on amphipods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jip.2023.107970>.

References

- Allain, T.W., Stentiford, G.D., Bass, D., Behringer, D.C., Bojko, J., 2020. A novel nudivirus infecting the invasive demon shrimp *Dikerogammarus haemobaphes* (Amphipoda). *Sci. Rep.* 10 (1), 1–13.
- Austin, B., & Alderman, D. J. 1987. Bacterial shell disease of crustaceans. International council for the exploration of the sea. ISBN 978-87-7482-673-6.
- Bacela-Spychalska, K., Wróblewski, P., Mamos, T., Grabowski, M., Rigaud, T., Wattier, R., Ovcharenko, M., 2018. Europe-wide reassessment of *Dityocoelea* (Microsporidia) infecting native and invasive amphipods (Crustacea): molecular versus ultrastructural traits. *Sci. Rep.* 8 (1), 1–16.
- Bauer, O.N., Pugachev, O.N., Voronin, V.N., 2002. Study of parasites and diseases of sturgeons in Russia: a review. *J. Appl. Ichthyol.* 18 (4–6), 420–429.
- Bij de Vaate, A., Jazdzewski, K., Ketelaars, H.A., Gollasch, S., Van der Velde, G., 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can. J. Fish. Aquat. Sci.* 59 (7), 1159–1174.
- Blakeslee, A.M., Fowler, A.E., Keogh, C.L., 2013. Marine invasions and parasite escape: updates and new perspectives. In: *Adv. marine biol.*, Vol. 66. Academic Press, pp. 87–169.
- Bojko, J., 2017. Parasites of invasive Crustacea: risks and opportunities for control. University of Leeds. Doctoral dissertation.
- Bojko, J., Stebbing, P.D., Bateman, K.S., Meatyrd, J.E., Bacela-Spychalska, K., Dunn, A.M., Stentiford, G.D., 2013. Baseline histopathological survey of a recently invading island population of 'killer shrimp'. *Dikerogammarus villosus*. *Dis. Aquatic Organisms* 106 (3), 241–253.
- Bojko, J., Dunn, A.M., Stebbing, P.D., Ross, S.H., Kerr, R.C., Stentiford, G.D., 2015. *Cucumispora ornata* n. sp. (Fungi: Microsporidia) infecting invasive 'demon shrimp' (*Dikerogammarus haemobaphes*) in the United Kingdom. *J. Invertebr. Pathol.* 128, 22–30.
- Bojko, J., Bacela-Spychalska, K., Stebbing, P.D., Dunn, A.M., Grabowski, M., Rachalewski, M., Stentiford, G.D., 2017. Parasites, pathogens and commensals in the "low-impact" non-native amphipod host *Gammarus roeselii*. *Parasit. Vectors* 10 (1), 1–15.
- Bojko, J., Stentiford, G.D., Stebbing, P.D., Hassall, C., Deacon, A., Cargill, B., Dunn, A.M., 2019. Pathogens of *Dikerogammarus haemobaphes* regulate host activity and survival, but also threaten native amphipod populations in the UK. *Dis. Aquat. Organ.* 136 (1), 63–78.
- Bojko, J., Burgess, A.L., Baker, A.G., Orr, C.H., 2021a. Invasive non-native crustacean symbionts: diversity and impact. *J. Invertebr. Pathol.* 186, 107482.
- Bojko, J., Clark, K.F., Stewart-Clark, S., Stentiford, G.D., 2021b. *Panopeispora mellora* n. gen. n. sp. (microsporidia) infecting Say's crab (*Dyspanopeus sayi*) from the Atlantic shoreline of Canada. *J. Invertebr. Pathol.* 184, 107652.
- Bojko, J., Ovcharenko, M., 2019. Pathogens and other symbionts of the Amphipoda: taxonomic diversity and pathological significance. *Dis. Aquat. Organ.* 136 (1), 3–36.
- Bojko, J., Reinke, A.W., Stentiford, G.D., Williams, B., Rogers, M.S., Bass, D., 2022. Microsporidia: a new taxonomic, evolutionary, and ecological synthesis. *Trends Parasitol.* 8, 642–659.
- Boshko, E.G., 1994. A new rotifer species of the genus *Enentrum* (Rotifera, Dicranophoridae) from Amphipoda of the water bodies of Ukraine. *Vestnik Zoologii* 6, 74.
- Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R J.* 9 (2), 378–400.
- Burgess, A., Bojko, J., 2022. Microsporidia are coming: *cucumispora ornata* and *dityocoelea berillonum* invade northern britain. *BioInvasions Records* 11.
- Chernogorenko, M.L., Komarovova, T.L., Kurandina, D.P., 1978. Life cycle of the trematode, plagioporus skrjabini Kowal, 1951 (Allocreadiata, Opcoelidae). *Parazitologija* 12 (6), 479–486.
- Clotilde-Ba, F.L., Toguebaye, B.S., 1995. Occurrence of microsporidia and gregarines in the shrimp *Penaeus notialis* from Senegal (West Africa). *Bulletin of the European Association of Fish Pathologists* 15 (4), 122–124.
- Copilaș-Ciocianu, D., Sîdagytė-Copilaș, E., 2022. A substantial range expansion of alien Ponto-Caspian amphipods along the eastern Baltic Sea coast. *Oceanologia* 64 (1), 227–232.
- Copilaș-Ciocianu, D., Sidorov, D., Sîdagytė-Copilaș, E., 2023. Global distribution and diversity of alien Ponto-Caspian amphipods. *Biol. Invasions* 25 (1), 179–195.
- Cuthbert, R.N., Kotronaki, S.G., Dick, J.T., Briski, E., 2020. Salinity tolerance and geographical origin predict global alien amphipod invasions. *Biology Letters* 16 (9), 20200354.
- Diagne, C., Leroy, B., Gozlan, R.E., Vaissière, A.C., Assailly, C., Nuninger, L., Courchamp, F., 2020. InvaCost, a public database of the economic costs of biological invasions worldwide. *Sci. Data* 7 (1), 1–12.
- Foster, R., Peeler, E., Bojko, J., Clark, P.F., Morrill, D., Roy, H.E., Bass, D., 2021. Pathogens co-transported with invasive non-native aquatic species: Implications for risk analysis and legislation. *NeoBiota* 69, 79–102.
- Frolova, E.V., Paskerova, G.G., Smirnov, A.V., Nasonova, E.S., 2023. Diversity, distribution, and development of hyperparasitic microsporidia in gregarines within one super-host. *Microorganisms* 11 (1), 152.
- Galen, S.C., Speer, K.A., Perkins, S.L., 2019. Evolutionary lability of host associations promotes phylogenetic overdispersion of co-infecting blood parasites. *J. Anim. Ecol.* 88 (12), 1936–1949.
- Getsevichyute, S., Simonene, G., 1974. The investigations of the parasitic fauna of (Amphipoda) *Pontogammarus robustoides* Grimm and *P. crassus* Grimm acclimatized in the lagoon of Kursiu Marios. *Acta Parasitologica Lithuanica* 12, 77–85.
- Getsevichyute, S. (1970). On the parasite fauna of *Pontogammarus robustoides* and *P. crassus* acclimatized in the Kurshian Mares Bay. *Eesti Pollumajanduse Akadeemia Teaduslike Toode Kogumik (Sbornik Nauchnykh Trudov Estonskoi)*, 70), 138-139.
- Grabner, D.S., Weigand, A.M., Leese, F., Winking, C., Hering, D., Tollrian, R., Sures, B., 2015. Invaders, natives and their enemies: distribution patterns of amphipods and their microsporidian parasites in the Ruhr Metropolis. *Germany. Parasites & vectors* 8 (1), 1–15.
- Grabowski, M., Bacela, K., Konopacka, A., 2007. How to be an invasive gammarid (Amphipoda: Gammaroidea) – comparison of life history traits. *Hydrobiologia* 590 (1), 75–84.
- Griffith, D., Veech, J.A., Marsh, C.J., 2014. R 'coccur' package: probabilistic species occurrence analysis in R. *J. Stat. Softw.*
- Gumuliauskaitė, S., Arbaciauskas, K., 2008. The impact of the invasive Ponto-Caspian amphipod *Pontogammarus robustoides* on littoral communities in Lithuanian lakes. *Eur. Large Lakes Ecosystem changes and their ecological and socioeconomic impacts* 127–134.
- Hatcher, M. J., Dick, J. T., Bojko, J., Stentiford, G. D., Stebbing, P., & Dunn, A. M. (2019). Infection and invasion: study cases from aquatic communities. *Wildlife Disease Ecology*; Wilson, K., Fenton, A., Tompkins, D., Eds, 262-295.
- Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S., Kats, L.B., 2015. Aquatic invasive species: challenges for the future. *Hydrobiologia* 750 (1), 147–170.
- Jazdzewski, K., Konopacka, A., Grabowski, M., 2004. Recent drastic changes in the gammarid fauna (Crustacea, Amphipoda) of the Vistula River deltaic system in Poland caused by alien invaders. *Divers. Distrib.* 10 (2), 81–87.
- Karatayev, A.Y., Burlakova, L.E., Padilla, D.K., Mastitsky, S.E., Olenin, S., 2009. Invaders are not a random selection of species. *Biol. Invasions* 11 (9).
- Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2022. Emmeans: estimated marginal means, aka least-squares means. R Package Version 1 (2018).
- Mamos, T., Grabowski, M., Rewicz, T., Bojko, J., Strapagiel, D., Burzyński, A., 2021. Mitochondrial genomes, phylogenetic associations, and SNP recovery for the key invasive Ponto-Caspian amphipods in Europe. *Int. J. Mol. Sci.* 22 (19), 10300.
- Mikhailov, K.V., Nasonova, E.S., Shishkin, Y.A., Paskerova, G.G., Simdyanov, T.G., Yudina, V.A., Aleoshin, V.V., 2022. Ribosomal RNA of metchnikovellids in gregarine transcripts and rDNA of microsporidia sensu lato in metagenomes. *Biol. Bull. Rev.* 12 (3), 213–239.
- Ovcharenko, M., Codreanu-Balcescu, D., Grabowski, M., Konopacka, A., Wita, I., Czaplinska, U., 2009. Gregarines (Apicomplexa) and microsporidians (Microsporidia) of native and invasive gammarids (Amphipoda, Gammaroidea), occurring in Poland. *Wiad Parazytol.* 55 (2), 237–247.

- Ovcharenko, M. O., Bacela, K., Wilkinson, T., Ironside, J. E., Rigaud, T., & Wattier, R. A. 2010. *Cucumispora dikerogammari* n. gen. (Fungi: Microsporidia) infecting the invasive amphipod *Dikerogammarus villosus*: a potential emerging disease in European rivers. *Parasitology*, 137(2), 191-204.
- Ovcharenko, M., Wróblewski, P.P., 2016. An intracellular prokaryotic microorganism associated with microsporidiosis of invasive amphipods *Pontogammarus robustoides*. *Baltic Coastal Zone. J. Ecol. Protection of the Coastline* 20.
- Perova, S.N., 2022. First find of *pontogammarus robustoides* in the upper volga river basin (Russia). *Inland Water Biol.* 15 (5), 697–700.
- Prati, S., Grabner, D.S., Pfeifer, S.M., Lorenz, A.W., Sures, B., 2022. Generalist parasites persist in degraded environments: a lesson learned from microsporidian diversity in amphipods. *Parasitology* 149, 973–982.
- Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Richardson, D.M., 2020. Scientists' warning on invasive alien species. *Biological Reviews* 95 (6), 1511–1534.
- Quiles, A., Wattier, R.A., Bacela-Spychalska, K., Grabowski, M., Rigaud, T., 2020. *Dictyocoela* microsporidia diversity and co-diversification with their host, a gammarid species complex (Crustacea, Amphipoda) with an old history of divergence and high endemic diversity. *BMC Evol. Biol.* 20 (1), 1–17.
- Roy, H.E., Hesketh, H., Purse, B.V., Eilenberg, J., Santini, A., Scalera, R., Dunn, A.M., 2017. Alien pathogens on the horizon: opportunities for predicting their threat to wildlife. *Conserv. Lett.* 10 (4), 477–484.
- Shaw, J.C., Henriksen, E.H., Knudsen, R., Kuhn, J.A., Kuris, A.M., Lafferty, K.D., Amundsen, P.A., 2020. High parasite diversity in the amphipod *Gammarus lacustris* in a subarctic lake. *Ecol. Evol.* 10 (21), 12385–12394.
- Stentiford, G. D., Ramilo, A., Abollo, E., Kerr, R., Bateman, K. S., Feist, S. W., ... & Villalba, A. 2017. *Hyperspora aquatica* n. gn., n. sp. (Microsporidia), hyperparasitic in *Marteilia cochillia* (Paramyxida), is closely related to crustacean-infecting microsporidian taxa. *Parasitology*, 144(2), 186-199.
- Urrutia, A., Bass, D., Ward, G., Ross, S., Bojko, J., Marigomez, I., Feist, S.W., 2019. Ultrastructure, phylogeny, and histopathology of two novel haplosporidians parasitising amphipods, and importance of crustaceans as hosts. *Dis. Aquat. Organ.* 136 (1), 87–103.
- Veech, J.A., 2013. A probabilistic model for analysing species co-occurrence. *Glob. Ecol. Biogeogr.* 22 (2), 252–260.
- Vilà, M., Hulme, P.E. (Eds.), 2017. *Impact of Biological Invasions on Ecosystem Services* (Vol. 12). Springer International Publishing, Cham.
- Warren, D.A., Burgess, A.L., Karemera, F., Bacela-Spychalska, K., Stentiford, G.D., Bojko, J., 2022. Histopathological survey for parasite groups in *Gammarus varsoviensis* (Amphipoda). *Diseases of Aquatic Organisms* 149, 47–51.
- Wattier, R.A., Haine, E.R., Beguet, J., Martin, G., Bollache, L., Muskó, I.B., Rigaud, T., 2007. No genetic bottleneck or associated microparasite loss in invasive populations of a freshwater amphipod. *Oikos* 116 (11), 1941–1953.
- Wilkinson, T.J., Rock, J., Whiteley, N.M., Ovcharenko, M.O., Ironside, J.E., 2011. Genetic diversity of the feminising microsporidian parasite *Dictyocoela*: new insights into host-specificity, sex and phylogeography. *Int. J. Parasitol.* 41 (9), 959–966.
- Williams, J.D., Boyko, C.B., 2012. The global diversity of parasitic isopods associated with crustacean hosts (isopoda: bopyroidea and cryptoniscoidea). *PLoS One* 7 (4), e35350.
- Wood, L., Smith, E., Bojko, J., Stebbing, P., 2021. Options for the control of *Dikerogammarus villosus* (killer shrimp) and other invasive amphipods: Invasive Amphipod Control. *Management of Biological Invasions* 12.