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SEASONALITY OF MICROSPORIDIAN AND GREGARINE PARASITISM IN *GAMMARUS PULEX* (CRUSTACEA: AMPHIPODA) INHABITING THE TRIBUTARY OF THE SŁUPIA RIVER

Piotr Wróblewski[1], Mykola Ovcharenko[2], Jolanta Eichenlaub[2], Volodymyr Yuryshynets[3]

[1] *Witold Stefanski Institute of Parasitology of the Polish Academy of Sciences, ul. Twarda 51/55, 00-818 Warszawa, Poland*
[2] *Pomeranian University in Słupsk, Institute of Biology and Science of Earth, ul. Arciszewskiego 22b, Słupsk,76-200, Poland*
e-mail: mykola.ovcharenko@apsl.edu.pl
[3] *Institute of Hydrobiology National Academy of Sciences of Ukraine, Prospect Geroiv Stalingradu 12, 04210 Kyiv, Ukraine*

Abstract

Seasonal observations of prevalence and intensity of microsporidians and gregarines infecting the native amphipod *Gammarus pulex*, in the tributary of small Baltic coastal river were analyzed. Using aggregate data from 12 date-by-site comparisons, we found that both prevalence and intensity of Apicomplexa were seasonally unimodal. Parasitism first increased and then declined seasonally after peaking midseason. In the case of infections caused by Microsporidia, the highest increase falls in the early autumn period, reaching the value from 48 to 56%. The minimum share of infected hosts was recorded in March 2016 and January 2017. In the early spring period, there was a slight decrease in microsporidian infection with subsequent minor fluctuations in the summer. We showed that this parasite can decreases the survival of *G. pulex* in the late stages of infection development in autumn. However, the host reproduction in the spring and the summer could potentially reduce the negative impact of the parasite on host populations.

Key words: parasitism, seasonality, *Gammarus pulex*, Microsporidia, Apicomplexa, small coastal river

INTRODUCTION

Presence of pathogens is a key factor that can alter population size, behavior, survival and physiology of amphipod hosts. Seasonal changes in parasitism are important to the extent that the parasites or pathogens under study act as agents of selection (Altizer et al. 2006). Our study was carried out on original material, collected from a stream that flows into the Słupia River. Previous studies provided in this locality shown that pathogens and other symbionts was accounted for nearly 90% of the total sample. The detected *Gammarus pulex* parasites belong to seven different phyla, the most common about them were gregarines, microsporidians, and parasitic rotifers (Ovcharenko et al. 2019). The seasonal dynamic of Microsporidia and Apicomplexa and their possible impact on the *G. pulex* population inhabiting the tributary of the small coastal river were analyzed in this study.

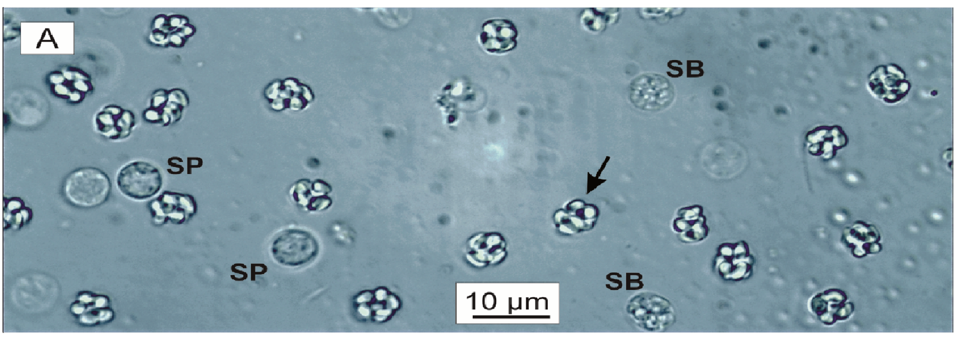
MATERIAL AND METHODS

Our study was carried out on original material, collected from a stream that flows into the Słupia River (54°28′30,79″N 17°02′37,20″E). Amphipods were caught using a standard kick sampling procedure. Samples were taken every month at approximately the same time from the same habitat. A total of 12 samples were taken between February 2016 and January 2017. For parasitological analysis 50 individuals from every sample were taken. A total of 600 specimens of *G. pulex* were dissected. Fresh preparations of infected tissues and stained smears were studied. Smears were air dried, fixed in methanol and stained with Giemsa stain solution. Live and stained spores were observed and measured under an Olympus BX50F4 microscope. For measurements, the software „Analysis Pro 2.11" in combination with Olympus BX50F4 microscope was used.

RESULTS AND DISCUSSION

Microsporidia belonging to the genus *Dictyocoela* were detected in every from inspected samples (Fig. 1A).

The pathogenicity of *Dictyocoela* spp. is associated with their localization in sarcoplasm of muscle cells. Heavily infected specimens have been documented as whitish in color due to the occurrence of numerous spores filling the infested tissue of the host, and it was also noted that infection was not present in the gut wall. Sporogonial stages and spores occurred within the cytoplasm of host cells, and specifically the sporogonial stages could also be found floating in the haemolymph or adhering to the haemocytes. The highest increase in the prevalence falls in the early autumn period, reaching the value



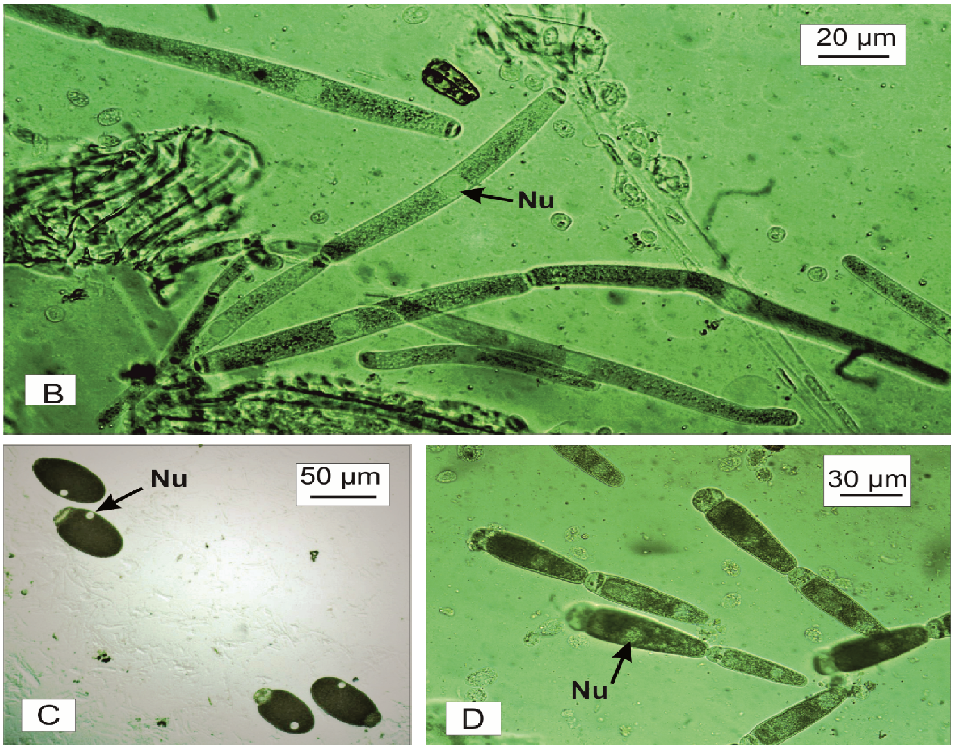


Fig. 1. Morphology of living microsporidians and gregarines parasitizing investigated gammarid host *Gammarus pulex*. A: octospores of *Dictyocoela* sp. (Microsporidia); B-D: adult trophozoites and syzygies of apicomplexan parasites *Heliospora longissima* (B), *Cephaloidophora gammari*, (C) and *Cephaloidophora* sp. (D)

to 56% (Fig. 2). The minimum share of infected hosts was recorded in March (28%), April (26%) 2016 and January 2017 (26%). In the spring period, there was a slight decrease in microsporidian infection with subsequent minor fluctuations in the summer.

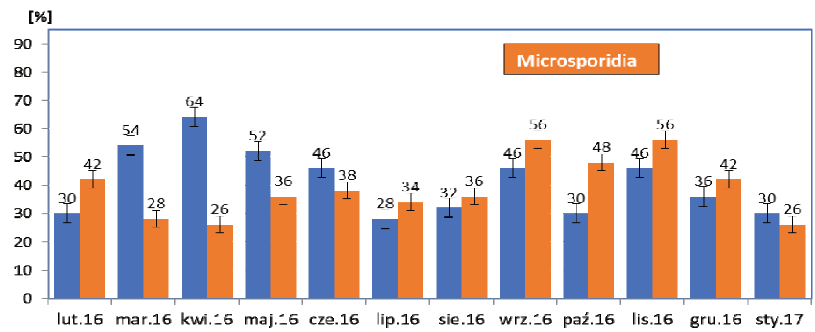


Fig. 2. Seasonality of prevalence (%) of Apicomplexa and Microsporidia in the tributary of the Słupia River from February 2016 to January 2017. Date abbreviations: Lut-16: 2016-02-27; mar-16: 2016-03-18; kwi-16: 2016-04-23; maj-16: 2016-05-23; cze-16: 2016-16-06; lip-16: 2016-07-25; sie-16: 2016-08-16; wrz-16: 2016-09-22; paź-16: 2016-10-22; lis-16: 2016-11-25; gru-16: 2016-16-12; sty-17: 2017-01-27.

Presence of three species of gregarinid parasites in the gut volume of *G. pulex* were confirmed in this study (Fig. 1). Occurrence of *Heliospora* (=*Uradiophora*) *longissima* was firstly registered in native gammarid host in Polish waters (Fig. 1B). This is widespread gregarine species which was described infected *G. pulex* in France and found also in: *Orchestia littorea* and *Caprella aequilibra* in France; *G. pulex* and *Gammaru sroeselii* in Germany; *Gammarus balcanicus* in Romania, and invasive *Dikerogammarus villosus* in Poland (Codreanu-Bălcescu 1996; Ovcharenko et al. 2009; Bojko and Ovcharenko 2019). *Cephaloidophora gammari* (Fig. 1C) was early recorded in *G. pulex* inhabiting South Baltic coastal stream in 2005 (Ovcharenko et al. 2006). Establishing the taxonomic position of *Cephaloidophora* sp. (Fig. 1D) requires additional research.
We found that prevalence of Apicomplexa was seasonally unimodal (Fig. 2). The highest increase in the prevalence falls in the early spring period, reaching the value to 64% in April 2016 (Fig. 2). The minimum share of infected hosts was recorded in July (28%), February and October 2016 (30% each) and January 2017 (30%). Infection significantly increases in the spring, then gradually decreases with mid-summer, almost reaching winter rates. In autumn, the infection rate rises again, although it does not reach spring indices, then it gradually decreases by winter. The rates of infection intensity change following prevalence fluctuations with a delay of one to two months (Fig. 3). The number of trophozoites in the sample significantly decreases by the end of the winter period, then increases rapidly by summer, reaching a maximum in June. Infection gradually decreases from mid-summer to autumn, reaching the minimum rates in the winter.

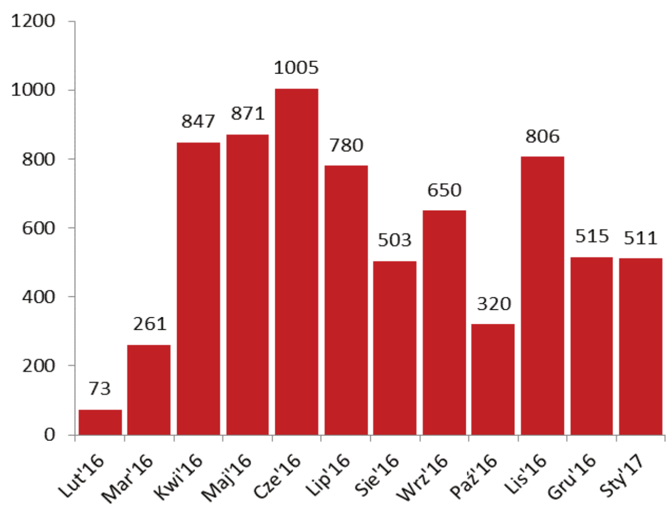


Fig. 3. Seasonal change in the number of gregarine trophozoites in the sample (50 host individuals). Date abbreviations are shown on Fig. 1 caption

In case of microsporidian infection we suggested that this parasite can decreases the survival of *G. pulex* in the late stages of infection development in autumn. However, the host reproduction in the spring and the summer could potentially reduce the negative impact of the parasite on host populations. In case of gregarines infection parasitism usually does not affect largely on population size due to the low pathogenicity. Seasonal infection of gregarines in the amphipod *Gammarus fasciatus* was studied by Grunberg and Sukhdeo (2017). They found that increasing host population density did not alter the abundance of *Heliospora longissima* and *Rotundula gammari*; however, the abundance of *H. longissima* was correlated with increased host size. It can be assumed that the intensity of infection increases proportionally to the age of the infected individuals. Seasonal changes in population size structure of *G. pulex* in a Cotswold stream appeared to indicate a growth check in late summer (Gee 2006). Perhaps this phenomenon is one of the reasons for the reduction of gregarines prevalence in July-August. On the other hand, Welton (2006) noted an increase of the population density of *G. pulex* until the beginning of autumn. In this case, a prevalence decrease in summer may indicate an increase in the population of low infected young animals.

CONCLUSION

The obtained data demonstrate that both prevalence and intensity of microsporidian and gregarine parasitism were seasonally unimodal. The infection rates first increased and then declined seasonally after peaking midseason. The host reproduction in the spring and the summer could potentially reduce the negative impact of the parasite on host populations.

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SEZONOWOŚĆ PASOŻYTNICTWA MIKROSPORYDIÓW I GREGARYN KIEŁŻA ZDROJOWEGO (CRUSTACEA: AMPHIPODA) ZAMIESZKUJĄCEGO DOPŁYW SŁUPI

Streszczenie

Przeanalizowano zmiany sezonowe występowania mikrosporydiów (Microsporidia) i sporowców (Apicomplexa) zakażających kiełża zdrojowego *Gammarus pulex* w dopływie małej przymorskiej rzeki polskiej strefy Bałtyku Południowego. Na podstawie wyników badań parazytologicznych 12 próbek zawierających po 50 osobników kiełży pobieranych comiesięcznie na tym samym stanowisku (54°28′30,79″N 17°02′37,20″E) wykazano, że zarówno prewalencja, jak i intensywność zainfekowania kiełży przez mikrosporydia i gregaryny były sezonowo niejednoznaczne. W przypadku zakażeń wywołanych przez Microsporidia najwyższy stopień zainfekowania przypada na wczesną jesień, osiągając wartość do 56%. Minimalny odsetek zainfekowanych żywicieli odnotowano w marcu 2016 r. i styczniu 2017 r. W okresie wiosennym zaobserwowano niewielki spadek prewalencji z nieznacznymi wahaniami latem. Odnotowano, że mikrosporydia z rodzaju *Dictyocoela* mogą doprowadzić do zmniejszenia liczebności populacji *G. pulex* w okresie jesiennym. Jednak wzrost tempo reprodukcji żywicieli wiosną i latem może potencjalnie zmniejszyć negatywny wpływ pasożyta na populacje kiełży. Najwyższy wzrost zainfekowania kiełży gregarynami przypada na wczesną wiosnę, osiągając wartość do 64%. Minimalny odsetek zainfekowanych żywicieli odnotowano w lipcu 2016 r. (28%), grudniu 2016 r. (30%) i styczniu 2017 r. (30%). Zakażenie znacznie wzrasta wiosną, a następnie stopniowo zmniejsza się w połowie lata, prawie osiągając wskaźniki zimowe. Jesienią zainfekowanie ponownie rośnie, chociaż nie osiąga poziomów wiosennych, następnie stopniowo zmniejsza się w okresie zimy.