Knowl. Manag. Aquat. Ecosyst. 2018, 419, 16
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https://doi.org/10.1051/kmae/2018012

www.kmae-journal.org

Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Onema

Research Paper Open Access

Effects of water bugs on crustacean zooplankton in a shallow littoral zone

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Abstract – Interactions within the food chain are essential to ecosystem ecology. At the same time, very little is known of the trophic relationships between Heteroptera and zooplankton. The aim of this study was to examine the community structure of water bugs in the littoral zone of shallow lakes and their impact on crustacean zooplankton communities. A study on the effect of Heteroptera on zooplankton communities was carried out in the conditions of a laboratory experiment with two treatments: (1) aquariums in which the Heteroptera species *Ilyocoris cimicoides* and *Plea minutissima* were present (control treatment) and (2) aquariums from which Heteroptera were removed (removal treatment). The decisive factor in reducing the number of crustacean zooplankton was most likely the presence of predatory Heteroptera in a composition typical of the shallow littoral of eutrophic lakes. Their impact is reflected in both a decrease in the species richness and abundance of crustaceans and a transformation of the size structure of the zooplankton. The results of the study are the first to suggest a substantial seasonal impact of water bugs on crustacean zooplankton in a shallow lake. The results of the experiments confirm that predatory Heteroptera exert considerable pressure on crustacean zooplankton during the spring and summer peaks in abundance.

Keywords: Water bugs / crustacean zooplankton / shallow lake / land-water contact zone

Résumé – Effets des punaises d'eau sur le zooplancton crustacéen dans une zone littorale peu profonde. Les interactions au sein de la chaîne alimentaire sont essentielles à l'écologie des écosystèmes. En même temps, on sait très peu de choses sur les relations trophiques entre les hétéroptères et le zooplancton. L'objectif de cette étude était d'examiner la structure des communautés des punaises d'eau dans la zone littorale de lacs peu profonds et leur impact sur les communautés de zooplancton crustacéen. L'étude sur l'effet des hétéroptères sur les communautés zooplanctoniques a été réalisée dans les conditions d'une expérimentation en laboratoire avec deux traitements: (1) aquariums dans lesquels étaient présentes les espèces d'hétéroptères Ilyocoris cimicoides et Plea minutissima (traitement témoin) et (2) aquariums dont les hétéroptères ont été enlevés (traitement de retrait). Le facteur déterminant dans la réduction du nombre du zooplancton crustacéen a été très probablement la présence d'hétéroptères prédateurs dans un assemblage typique du littoral peu profond des lacs eutrophes. Leur impact se traduit à la fois par une diminution de la richesse et de l'abondance des crustacés et par une transformation de la structure en taille du zooplancton. Les résultats de l'étude sont les premiers à suggérer un impact saisonnier important des punaises d'eau sur le zooplancton crustacéen dans un lac peu profond. Les résultats des expériences confirment que les hétéroptères prédateurs exercent une pression considérable sur le zooplancton crustacéen pendant les pics d'abondance printanier et estival.

Mots-clés : Punaise d'eau / zooplancton crustacéen / lac peu profond / interface terre-eau

1 Introduction

The littoral zone in shallow lakes comprises a mosaic of vertical and horizontal microhabitats provided by macrophytes. These habitats are part of a highly complex structure, including submerged vegetation and the shape of the lake basin, which create spatial differentiation in the water/land contact zone. This zone provides habitats enabling a different species composition of aquatic invertebrates from that found in the lake, due to the occurrence of different habitats (water and land) side by side (Naiman and Decamps, 1997). A shallow littoral zone covered

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by aquatic vegetation is often the site of concentrations of plankton in shallow lakes (Burks *et al.*, 2002; Geraldes and Boavida, 2004; Bogacka-Kapusta, 2007; Basińska *et al.*, 2009). However, the coastal zone is also an attractive microsphere for predatory invertebrate macrofauna, which may exert considerable pressure on assemblages of other macroinvertebrates, zooplankton, and even juvenile fish (Louarn and Cloarec, 1997; Kopp *et al.*, 2006; Boda and Csabai, 2009; Klečka and Bourkal, 2012).

Water bugs are a group of organisms characteristic of the land/water zone. They are present in large numbers in the shallow littoral of eutrophic lakes and small water bodies (Savage, 1989). This zone is usually difficult to access for fish, and then species of predatory Heteroptera often constitute a group of consumers. Hence it seems that by feeding on small invertebrates, Heteroptera can exert a significant effect on the abundance of crustacean zooplankton. Literature data indicate that crustaceans may use this zone as a potential refuge offering protection against pressure from fish. Heteropteran predators are active swimmers and are able to penetrate even very shallow water (depth less than 1 cm). They are able to exert considerable pressure on other aquatic invertebrates, macroinvertebrates and small fish, because they are present in large numbers and relatively large biomass. They are unique predators that are able to reach planktonic crustaceans assembled in the small depths in the littoral (Louarn and Cloarec, 1997; Blaustein, 1998; Eitam and Blaustein, 2004, 2010). Previous research has mainly focused on the effect of the physical and chemical properties of water on the occurrence of water bugs (Dias-Sylvia *et al.*, 2010; Mieczan *et al.*, 2014; Płaska and Tarkowska-Kukuryk, 2014; Płaska *et al.*, 2016). There is still little information, however, on the effect of food abundance on the occurrence of Heteroptera, or on the trophic relationships in the predator-prey (water bugs and zooplankton) system. Such information can be found in only a few works (Dieguez and John, 2003; Klečka and Bourkal, 2012, 2013). These, however, have focused on predation by only one Heteroptera family, the Notonectidae, mainly in the context of predation on Culicidae larvae (Dieguez and John, 2003; Eitam and Blaustein, 2004; Saha *et al.*, 2008; Eitam and Blaustein, 2010). As water bugs are abundant in the shallow littoral of lakes (Płaska, 2012), it therefore seems that they may play an important role in shaping the qualitative and quantitative structure of zooplankton. On the other hand, as Heteroptera exhibit pronounced seasonal changes in abundance and taxonomic composition (literatura), it seems that their pressure may vary between seasons. Literature data indicate that this group usually has two peaks in numbers – in spring and in early autumn (Wróblewski, 1980; Płaska, 2012). The main objective of the study was to determine the following: (i) the structure of Heteroptera in the water/land contact zone of shallow eutrophic lakes, taking into account seasonal variation in the species composition of predatory Heteroptera; (ii) the effect of this structure on the abundance of planktonic crustaceans (top-down regulation) in the conditions of a laboratory experiment during the peaks of Heteroptera abundance (in spring and autumn).

2 Materials and methods

2.1 Study area

Water bugs and zooplankton communities were examined in the shallow littoral zone of two eutrophic lakes, Miejskie and Skomielno (Łęczna-Włodawa Lakeland, eastern Poland). The area of Miejskie Lake is 45.4 ha and its maximum depth is 2.2 m (51° 30' 18" N, 22° 52' 50" E). The vegetation of the land/water contact zone is dominated by *Ceratophyllum demersum*, *Hydrocharis morsus-ranae*, *Lemna minor* and *Carex* sp. At a depth of 0.5 m, *Phragmites australis* and *Potamogeton crispus* were dominant. The area of Skomielno Lake is 74.3 ha and its maximum depth is 5.5 m (51° 29' 28" N, 23° 1' 3" E) (Harasimiuk *et al.*, 1998). *C. demersum*, *H. morsus-ranae* and *Carex* sp. were dominant in the contact zone. At a depth of 0.5 m, *Sparganium ramosum* and *Hottonia palustris* were dominant.

2.2 Sampling and identification of Heteroptera and zooplankton communities

Sampling was done four times in 2016, once every two months from May to November. Samples were taken from two microhabitats: the land/water contact zone (max. depth 0.05 m) and among emergent macrophytes (max. depth 0.5 m) (Fig. 1). Each time, 3 samples were collected from each site. In each type of micro-habitat and on each sampling day water bugs were sampled using a metal frame (0.5 m × 0.5 m – 0.25 m^2) and their abundance was calculated per m^2 of bottom surface. One sample was analysed alive and two samples were preserved immediately with 80% alcohol. The abundance of crustacean zooplankton and their community composition were determined by the Utermöhl method (Utermöhl, 1958). Three samples 10 L were filtered through a plankton net with 0.25 mm mesh size. Crustacean samples (whole one sample = 100 ml) were sedimented for 24 h in a cylinder stoppered with Parafilm, and then the upper volume of 20 ml was gently removed. Morphological identification of water bugs and zooplankton was mainly based on works by Wróblewski (1980); Savage (1989); Błędzki and Rybak (2016).

2.3 Laboratory experiments

A laboratory experiment using intact natural communities can help to identify the various effects of water bugs on crustacean communities. The experiment was performed in two periods: spring (May) and autumn (September) 2016. The water from the land/water contact zone containing zooplankton, suspended solids and phytoplankton was transported to the laboratory. The study on the trophic relationships between water bugs and planktonic crustaceans included the following experimental treatments: (1) Aquariums containing zooplankton and predatory water bugs (Heteroptera present) – control treatment; (2) Aquariums containing only zooplankton, from which Heteroptera were removed – removal treatment.

For each experimental treatment, the study was conducted in three aquariums. The aquariums were 0.25 m^2 in area. They

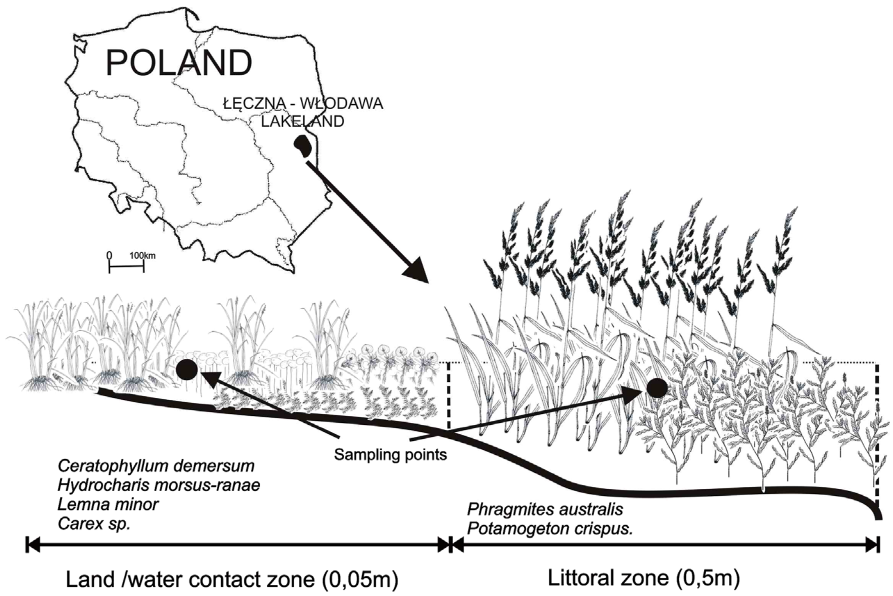

Fig. 1. Localization of the study sites in the investigated lakes.

were filled with water containing plankton to a depth of 10 cm. Aquatic vegetation of a composition similar to that of the land/water littoral zone was placed in them as well. Both the water with the plankton, water bugs and vegetation (*H. morsus-ranae* and *C. demersum*) and the bottom sediment were collected from the water/land zone of the lakes. Predatory water bugs caught in the lake were separated live from the samples and carefully moved to an aquarium with a habitat corresponding to the natural conditions in the littoral zone (*Ilyocoris cimicoides* – 4 ind. and *Plea minutissima* – 3 ind. in each experimental aquarium (Heteroptera Present). All the aquariums were set up for one day to stabilize the habitat conditions before the experiment was begun. Throughout the duration of the laboratory experiment, *i.e.* for 7 days, 100-ml samples were taken from all aquariums and preserved with Lugol's solution for zooplankton analysis. Samples were taken in 3 replicates from each aquarium. For all sampling times combined, a total of 63 samples from the control treatment and 63 samples from the removal treatment were taken. After 24-hour sedimentation, quantitative analysis of the material was performed using a Nikon SMZ800 stereomicroscope at maximum magnification of 120×. Crustaceans were further analysed in terms of size classes: small – up to 0.5 mm, medium – from 0.6 to 0.9 mm, and large – 1 mm and larger.

2.4 Data analyses

Statistical analyses were performed using Statistica 6.0 software. First, the normality of the data distribution was checked by the Kolmogorov-Smirnov test. The influence of sampling dates was checked by analysis of variance (ANOVA). Pearson's correlations were used to examine the relationship between zooplankton communities and potential grazing pressure (abundance of water bugs).

3 Results

3.1 Water bugs and crustacean communities – field study

Altogether 18 species of Heteroptera aquatica were found in the lakes. Differences in the numbers of water bugs between zones of the lakes were statistically significant (Skomielno Lake ANOVA, $F = 9.9$, $P < 0.005$, Miejskie Lake ANOVA, $F = 17.77$, $P < 0.001$). The Heteroptera community structure in the two lakes was similar and generally dominated by *I. cimicoides* (L.) (more than 40% of total abundance) and *P. minutissima* Leach, which accounted for more than 20% of the total abundance (Fig. 2). Water bug abundance was characterized by spring-autumn maxima. The average abundance of dominant bugs in the land/water zones was 22 ind. m^{-2} for *I. cimicoides* and 8 ind. m^{-2} for *P. minutissima* in Skomielno Lake, and 16 ind. m^{-2} for *I. cimicoides* and 7 ind. m^{-2} for *P. minutissima* in Miejskie Lake (Fig. 3).

Differences in the numbers of zooplankton were significantly higher in the land/water zone (0.05 m) than in the shallow littoral zone (0.5 m) (Skomielno Lake ANOVA, $F = 16.6$, $P < 0.001$, Miejskie Lake ANOVA, $F = 48.1$, $P < 0.001$). The crustacean zooplankton community in both lakes was dominated by nauplii (29%), *Alona* sp. (12%), *Ceriodaphnia* sp. (10%) and *Macrocyclops albidus* (9%), which contributed 60% of the total zooplankton numbers. Higher abundance of crustacean communities (270–330 ind. dm^{-3}) was noted in the land/water zone than in the greater depths in Skomielno Lake.

The highest abundance of water bugs in both lakes was observed in the early summer (May) in the water/land contact zone, and ranged from 63 to 80 ind. m^{-2}. In the zooplankton community, maximum abundance was noted in the spring in the water/land zone, with 270–330 ind. dm^{-3}. In subsequent months,

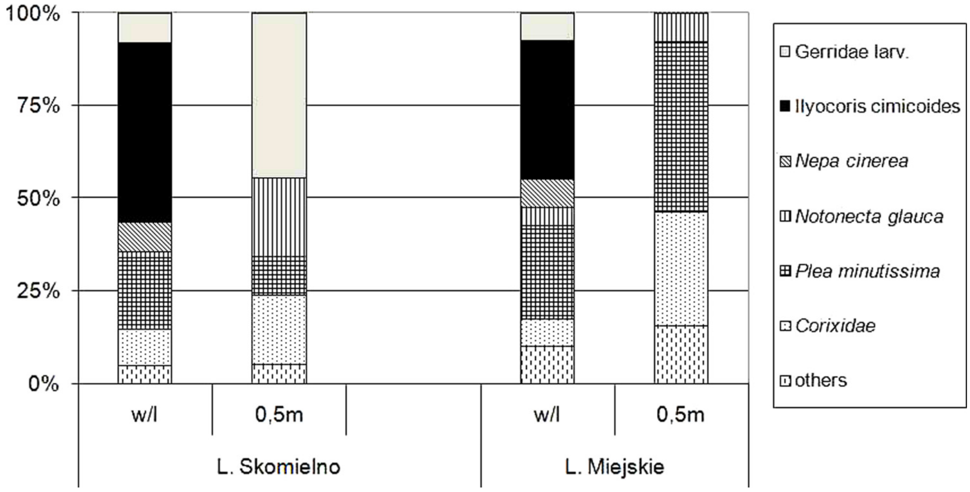

Fig. 2. Percentage abundance of water bugs in the investigated lakes.

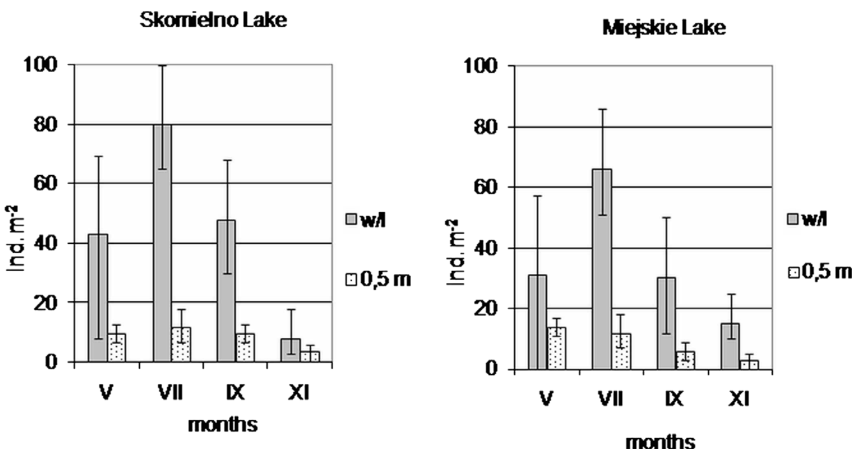

Fig. 3. Density of water bugs in the investigated lakes (I ± standard deviation).

the abundance of planktonic crustaceans sharply decreased, and in November it did not exceed 40 ind. dm^{-3}(Fig. 4).

3.2 Laboratory experiments

During the experiments, two consecutive stages in zooplankton were observed. The start of the first experiment (May) was characterized by the dominance of copepods, with a large contribution of nauplii (60%) and *Eucyclops macruroides* (21%). In the second experiment (September), the zooplankton community was dominated by nauplii (34%), *Macrocyclops albidus* (18%), *M. fuscus* (15%), *Ceriodaphnia* sp. (14%) and *Alona* sp. (13%).

During the experiments in May and September a significant decrease in the total number of planktonic crustaceans was recorded in all control treatment aquariums. At the beginning of the experiment in May, the abundance of zooplankton was significantly higher than in September (ANOVA $F = 12.6$, $P < 0.001$). The average decline in numbers during the 7 days of the experiment was 60% in May (initial abundance 353 ind. dm^{-3}, final abundance 140 ind. dm^{-3}) and 75%. In September (initial abundance 230 ind. dm^{-3}, final abundance 47 ind. dm^{-3}) (Fig. 5A, B, Fig. 6A, B). On the seventh day of the experiment in September, the decline in abundance was 62% (to 82 ind. dm^{-3}), which was almost the same as in May over the same period. In the removal treatment aquariums, the average zooplankton abundance decreased by 32% in May (from 300 to 205) while in September it did not change significantly during the experiment (from 235 on day 1 to 220 ind. m^{-3} on day 7) (Figs. 5B, B). Irrespective of the study period, the data analysis indicated a strong positive relationship between the abundance of water bugs and zooplankton. In the control treatment, abundance of Heteroptera showed a strong positive correlation with the abundance of zooplankton ($r = 0.48$, $P < 0.01$).

In terms of the size class structure of the planktonic crustaceans in the control treatment aquariums, the greatest average decline was noted for the class of large crustaceans, amounting to 74% on average, while the decline among small crustaceans was 51% and the average number of medium-sized individuals remained stable. In the removal treatment aquariums, the average abundance of small crustaceans decreased by 44% and that of medium-sized crustaceans by 17%, while large ones remained relatively stable (decline of 5%).

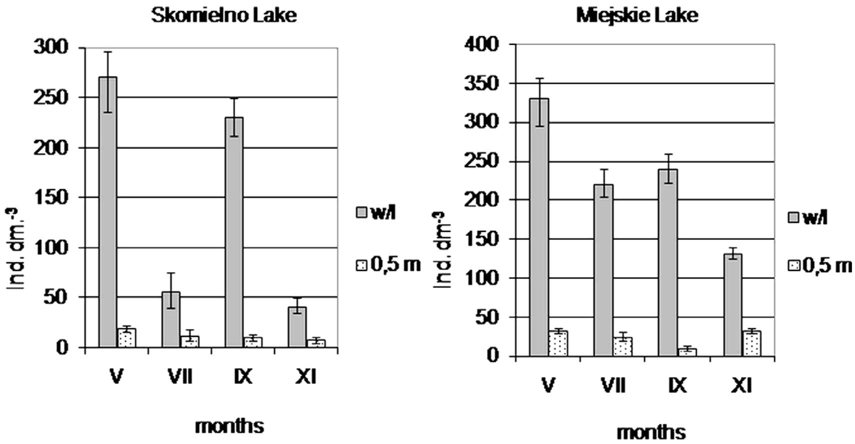

Fig. 4. Density of crustacean zooplankton in the investigated lakes (I ± standard deviation).

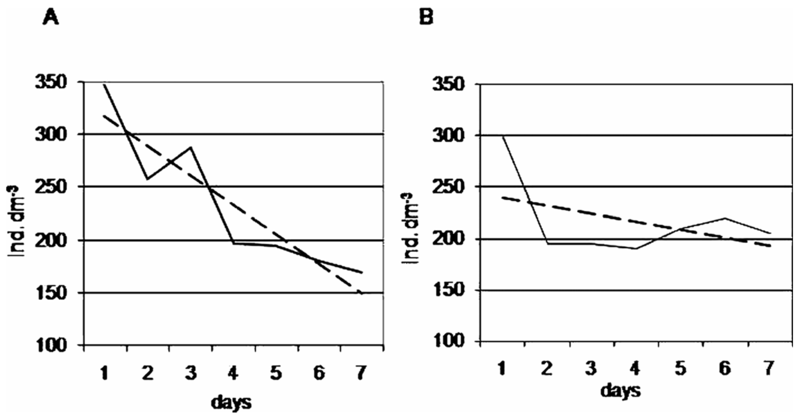

Fig. 5. Mean density of crustacean zooplankton in control (A) and removal (B) treatments in May.

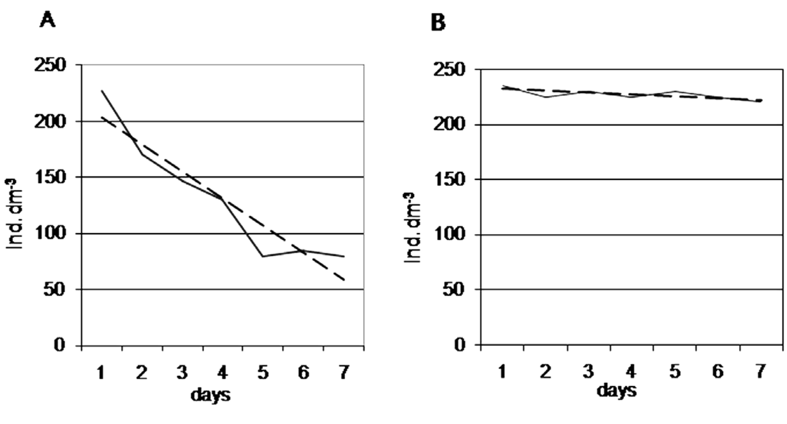

Fig. 6. Mean density of crustacean zooplankton in control (A) and removal (B) treatments in September.

4 Discussion

The initial stage preceding the present study was a multi-year analysis of the taxonomic composition, density and distribution of aquatic Heteroptera in various trophic types of water bodies (Płaska, 2012; Mieczan *et al.*, 2014; Płaska and Tarkowska-Kukuryk, 2014). That research showed that predatory species of Heteroptera are abundant in eutrophic lakes with shallows overgrown with vegetation. The number of identified taxa of water bugs and planktonic crustaceans is comparable with other studies examining the shallow littoral zone in eutrophic lakes (Lauridsen and Buenk, 1996; Burks *et al.*, 2002; Tolonen *et al.*, 2003). The number of planktonic crustacean species is generally higher in the littoral of the lake rather than in the pelagic zone, as confirmed by research (Kuczyńska-Kippen and Nagengast, 2006; Bogacka-Kapusta, 2007). The higher number of planktonic crustaceans is linked to the presence of refuges created by macrophytes. The highest number of species and the highest abundance occur in the emerged plants zone, and are slightly lower among submerged plants. Among emerged macrophytes, the number of planktonic crustaceans has been found to increase with plant density (Geraldes and Boavida, 2004; Basińska *et al.*, 2009; Horpilla *et al.*, 2009). Thus for our study we selected eutrophic lakes with a well-developed shallow littoral zone. In the present study, Heteroptera species characteristic of habitats with rich vegetation were observed, which is consistent with other studies (Burks et al., 2001; Bloech *et al.*, 2010; Płaska, 2012; Turić *et al.*, 2012, 2015). Also significant were the seasonal changes in the qualitative and quantitative structure of this group. The development cycle of Heteroptera usually involves two peaks in abundance, occurring in the spring and early autumn, as shown in the present study. Predatory pressure from Heteroptera on small invertebrates is greatest during these periods, which was reflected in the decrease in numbers of crustacean zooplankton. This pattern was also confirmed by the laboratory experiment. The Heteroptera used in the experiment are a crucial and typical element of littoral macrofauna, and hence the results of the experiment are indicative of the important role of all predatory insects in the shallow water of the littoral, especially in the water/land contact zone, in shaping the zooplankton population (Murdoch et al., 1984; Kopp *et al.*, 2006; Klečka and Bourkal, 2012, 2013).

The results of the experiment clearly show that the number of zooplankton was significantly reduced in the aquariums with predatory water bugs as compared to the control aquariums with no predators. The reduction in the number of zooplankton was minimal in the removal treatment aquariums in both experimental cycles and should therefore be treated as the natural death rate in such conditions. The natural death rate in nature may be determined by diverse factors, such as food accessibility. In this experiment, some water was taken with the Heteroptera from the littoral zone of the lake in order to ensure a supply of natural feed (Savage, 1989; Błędzki and Rybak, 2016).

It should be noted that the literature contains little information on the importance of planktonic crustaceans as an element of the diet of predatory Heteroptera, while it is known that they can be their food. For this reason it was very difficult to determine the level of predation pressure exerted by Heteroptera on planktonic crustaceans in the shallow littoral. Previous observations have devoted much attention to the Notonectidae, which have a significant influence on the regulation of water invertebrates and even young fish and amphibians (Louarn and Cloarec, 1997; Kopp *et al.*, 2006; Vandekerkhove *et al.*, 2012). It has been demonstrated that they can be effective regulators of mosquitoes and help to control them, and that, if necessary, they can also feed on Chironomidae (Saha *et al.*, 2008, 2010). As shown in small water bodies, they can regulate the abundance and diversity of the entire invertebrate population (including plankton), as confirmed by experiments similar to those presented in our study (Eitam and Blaustein, 2010). It is likely, however, that larger individuals are chosen first (Blaustein, 1998). A study conducted by Louarn and Cloarec (1997) also seems to confirm the hypothesis regarding the preference of Notonectidae for larger taxa, showing that Notonectidae were more effective than *I. cimicoides* at feeding on pike fry. Although *I. cimicoides* chose smaller prey, vertical migrations of crustaceans were observed as a response to predation by Notonectidae (Gilbert and Hampton, 2001), or migration into clumps of vegetation as a response to predation by Odonata (Burks *et al.*, 2002).

The size of the prey as well as that of the predator is also a crucial factor. The predator must take into account the size of its prey, which largely determines its ability to defeat it. It also significantly influences the predator's choice of prey. This is described by the theory of optimal feeding (Schelby, 2014), according to which the predator chooses the largest possible prey from a wide size range because the energy required to hunt it will be less than the energy derived from eating it. The results of our experiment have confirmed this theory. Increased pressure on small and large planktonic crustaceans, with little pressure on the average size class, indicates that bugs consume the food available to them in natural littoral habitats. *I. cimicoides*, as a large taxon (about 40 mm), chooses the largest victims available among the plankton, while the co-occurring *P. minutissima* attains a size of 2 mm, so only small planktonic crustaceans remain within its range (Savage, 1989; Wróblewski, 1980).

Thus the functioning of the food web in the water/land contact zone seems to be distinctly different from that found in the pelagic zone. The numerous aquatic plants and low water depth here impede the access of fish to plankton, and for this reason crustaceans often concentrate in this microhabitat (Burks *et al.*, 2001, 2002; Geraldes and Boavida, 2004), where invertebrate predators such as water bugs can effectively reduce the abundance of zooplankton. Other authors have confirmed that aquatic invertebrates can regulate the abundance of plankton in the case of limited feeding by fish (low depth, high macrophytes density or poor visibility) (Horpilla *et al.*, 2009; Cobbaret *et al.*, 2010). Hence zooplankton present in large numbers in the water/land zone may be a source of food for water bugs (Eitam and Blaustein, 2010).

Our study has shown that that not only the larvae of dragonflies (Burks *et al.*, 2002) or Notonectidae (Eitam and Blaustein, 2010) but also other predatory Heteroptera species may influence zooplankton abundance and size structure in shallow, eutrophic reservoirs with abundant plants. To more comprehensively investigate the trophic relationship between water bugs and zooplankton, future research should take into account the potential food resources of both groups of organisms (algae, bacteria and protozoans).

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Cite this article as: Płaska W, Mieczan T. 2018. Effects of water bugs on crustacean zooplankton in a shallow littoral zone. *Knowl. Manag. Aquat. Ecosyst.*, 419, 16.