


Article

Bladderwort Relationship to Zooplankton in Two Northern Michigan Lakes

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Abstract: Traditionally, most of our understanding of lake zooplankton dynamics is from data collected in the pelagic, open water zone. However, the littoral and benthic zones of lakes can play a role in zooplankton community interactions and relationships. *Utricularia* spp., or the bladderworts, inhabit littoral and shallow waters of lakes and consume zooplankton. Limited information exists on the dietary habits of bladderworts and how diet varies across lakes and seasonally. Bladderwort dietary assemblage in two Michigan, USA, lakes was quantified during the thermally stratified period of 2020, and was compared to zooplankton in the adjacent water column. Bladderwort trap size was positively related to zooplankton prey size, but not number of prey consumed or taxa richness. Bladderworts consumed a variety of prey, the majority dominated by littoral cladoceran and ostracod taxa. Seasonal change was observed, with significantly higher ostracod dominance earlier in the season, shifting into cladoceran dominance later in the season. Ostracods were more commonly found in traps than the water column, whereas Cladocera were more commonly found in the water column than the traps. These results shed light into the dietary habits of bladderworts, which can have consequences for food web dynamics and energy transfer in small lake systems.

Keywords: cladocera; copepod; food web interactions; ostracod; *Utricularia*; water mite



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1. Introduction

Zooplankton interactions within and across trophic levels in lakes are a vital part of energy flows and ecosystem function. Ecosystem processes are being altered in many lakes because of global changes [1–3], and such changes can influence zooplankton community dynamics [4–6]. For example, ecosystem changes could result in zooplankton inhabiting different parts of a lake, such as if the hypolimnion becomes hypoxic, zooplankton may reside in the more well-oxygenated littoral or shallower waters versus the hypolimnion [7,8], which could influence food web interactions. Changes to zooplankton behavior and habitat preference can also affect the information we collect and our interpretation of the zooplankton community depending on when and how we sample [9–11].

Research on how environmental factors affect food webs and zooplankton communities typically has been performed in the pelagic zone of lakes [12–14]. Planktivorous fish and invertebrate predators are often identified as main predators of zooplankton, and they affect zooplankton density, composition, and size structure [15–17]. However, zooplankton taxa also inhabit more littoral or benthic regions versus the pelagic zone, which can have different predators than the pelagic zone [7,11]. More information is needed to better understand predator effects on zooplankton in littoral regions in order to gain a more comprehensive understanding on lake-wide zooplankton density, size, and diversity characteristics.

The bladderwort *Utricularia* is a genus of carnivorous plant that is a predator of zooplankton in littoral zones of lakes and in shallower lake systems [18,19]. *Utricularia* is a cosmopolitan group and their distribution ranges from tropical to arctic regions,

and they are present in many different lake types such as in oligotrophic to eutrophic systems [20]. Bladderworts have unique traps that they use to capture prey triggered by guard hairs, antennae, and bristles, and then the organisms are digested by enzymes within the trap [21]. Bladderworts can consume a variety of prey items, and the presence of bladderworts may even affect growth rates of some vertebrate predators via competition of similar prey items [22]. Limited research suggests that bladderworts commonly eat rotifers, Cladocera, and copepods [20,23], many of which are benthic and littoral taxa. Therefore, the community of zooplankton found in the traps and in the water column may be different if bladderwort more commonly eat littoral or benthic taxa, reflecting the habitat the bladderworts are typically observed in.

However, data on the amount and type of zooplankton consumed by bladderwort plants is limited. Of the current studies on aquatic bladderworts, many have focused on genetic and environmental factors affecting the trapping mechanisms and growth of bladderworts [24–26]. Studies of bladderwort predation on zooplankton in lakes in situ have typically focused on just one or a few sampling events [20,27], or have been from experiments [22,28,29]. The seasonal relations between bladderwort diet and zooplankton have received very little attention, as has the relationship between diet composition in the trap versus the zooplankton composition in the water column. Further research on these topics is important to better understand relations between trap and zooplankton characteristics, bladderwort diet composition over space and time, and relations between bladderwort diet composition versus the open water zooplankton community. Information on these relations could also inform us on how much secondary productivity may enter the littoral (i.e., carnivorous plants) versus the pelagic (i.e., fish) energy cycle in shallow lakes, especially with global change and altered lake conditions.

To address some of this knowledge gap, we collected zooplankton and bladderwort traps in two similar-sized lakes in Michigan, USA, during the thermally stratified period in 2020. We tested for relations between trap size and zooplankton prey size, number of prey items, taxa dominance and the taxa richness in traps, and how these relations changed across the thermally stratified period. We also examined what zooplankton taxa were most commonly found in the traps and if a difference existed between the composition of zooplankton in the water column compared to the composition of zooplankton found in traps. The results from our study can help shed light into bladderwort predation dynamics on zooplankton and how such relations change over time.

2. Materials and Methods

2.1. Study Sites

The lakes sampled for this study were Arbutus Lake and Spider Lake in the northwest corner of the Lower Peninsula of Michigan, USA (Figure 1). These lakes are in the northern temperate region, with snowy winters and moderately humid and warm summers. Data were collected in 2020 from late May through early August during the daytime (close to the solar noon), approximately every 1.5 weeks for each lake. This time period in this region is during the thermally stratified period (spring and summer calendar months, when the lakes exhibit thermal stratification) [30,31], and air and surface water temperatures are commonly between about 15–25 °C. The lakes were chosen due to their close geographic proximity and similarity in size (surface area and depth), as well as having previously observed bladderwort populations. Two similar lakes were sampled in order to compare bladderwort populations and prey relations across similar systems in the same geographic region. Arbutus and Spider Lake have a surface area and maximum depth of 1.60 km² and 13 m and 1.82 km² and 11 m, respectively. Each lake has similar fish communities; each lake contains piscivores and planktivores such as Northern Pike (*Esox lucius*), Largemouth (*Micropterus salmoides*) and Smallmouth Bass (*Micropterus dolomieu*), Black Crappie (*Pomoxis nigromaculatus*), Yellow Perch (*Perca flavescens*), Bluegill (*Lepomis macrochirus*), and multiple minnow species. Both lakes possess shallow bays, which contain bladderworts. Both lakes are dimictic and oligotrophic.

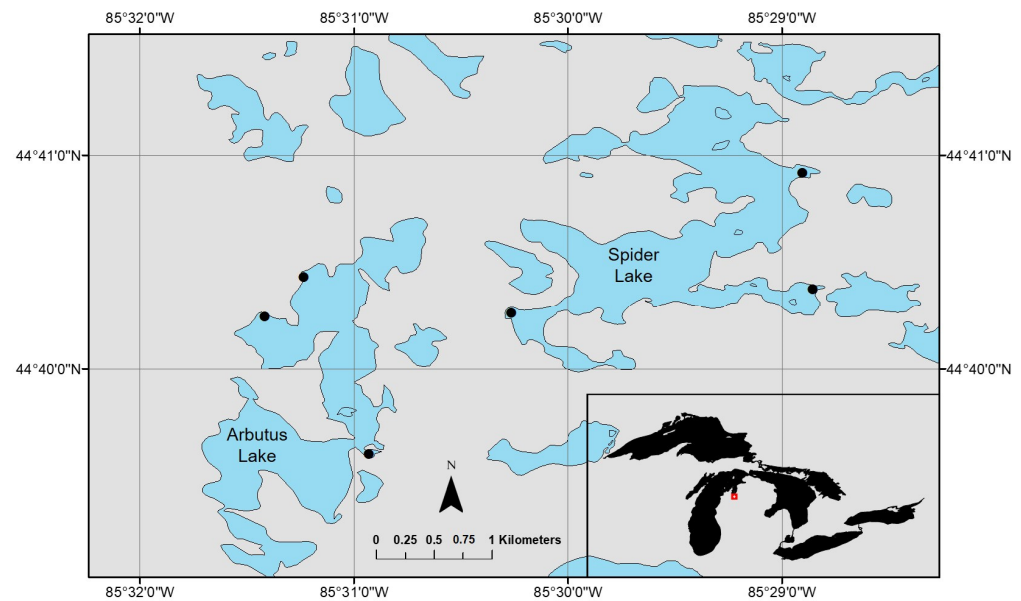


Figure 1. Locations of Arbutus and Spider Lakes in Michigan, USA. The black circles on the map indicate each bay that was sampled in each lake during the study period. Each sampling location consists of both where bladderwort traps were collected in the littoral zone and where zooplankton were collected by tow nets just adjacent to the plants in the pelagic zone. The red circle in the inset map indicates the specific location of these lakes in Michigan's Lower Peninsula.

2.2. Sampling Protocol

Three bays in Arbutus Lake (sampling coordinates: 44.673864° N, 85.520551° W; 44.670712° N, 85.523579° W; and 44.660016° N, 85.515453° W) and three bays in Spider Lake (sampling coordinates: 44.671019° N, 85.504375° W; 44.672912° N, 85.480881° W; and 44.681951° N, 85.481700° W) were targeted to be sampled on each sampling event for each lake (Figure 1). Each bay was defined as a partially enclosed, shallower area that contained bladderworts. Three separate bays of similar size and depth were chosen to increase the replication per sampling event and per lake, and to capture any inter-lake variation in bladderwort trap and zooplankton dynamics. All bays in each lake were not able to always be sampled each time because of weather conditions (samples were collected from a kayak). Bladderwort traps were collected from the live bladderwort plants in the littoral zone of these bays, and zooplankton were collected just adjacent to the bladderworts in each bay in the pelagic zone, just outside the littoral zone (Figure 1). A total of 29 bladderwort (but bladderworts in two bays did not have diet items across the sampling period (see Results), and so 27 bladderwort samples were used in analyses) and 27 zooplankton samples (54 total samples) were collected across bays and lakes during the sampling period.

The collection of bladderwort traps was adapted to procedures of those outlined by [32]. Plants were selected randomly from each site, and then at least five individual traps that were at least 1 mm long at each site were collected at random and preserved in 70% ethanol by volume for later analysis. Within each bay, zooplankton were also collected, but in the pelagic zone (just outside the littoral zone) adjacent to where bladderwort traps were collected. Zooplankton were collected in the open water using vertical net tows (80 µm mesh size, 5" mouth diameter, 15" height, LaMotte™, Chestertown, USA) from just above the lake bottom to the surface. Zooplankton samples were collected adjacent to the bladderwort traps in order to sample the zooplankton community close to the traps, but in the open water zone. Zooplankton samples were also preserved in 70% ethanol by volume for later laboratory processing. Bladderwort and zooplankton samples were counted within six months of collection. Depth of the zooplankton vertical tows was similar across lakes and bays, ranging from ~1–2.5 m depth.

Back in the lab, each bladderwort trap, bladderwort diet items, and zooplankton from the water column were counted and measured at $40\times$ magnification under a Nikon 1000 SMZ dissecting scope with a calibrated digital camera attached. Collected bladderworts were measured for total length and width. Bladderwort traps were then individually dissected to count and measure zooplankton and other diet items. Diet items were identified to the lowest possible taxonomic resolution, which was typically the genus resolution [33–35]. All diet items in bladderwort traps were identified to obtain the number of diet items per trap. Zooplankton samples from the vertical tows were subsampled and counted until three aliquots were counted, five percent of the volume dilution was counted, or until 100 individual organisms were counted, whichever came first [35]. The zooplankton taxa observed per sample (bladderwort and vertical tow) were measured for length, density was calculated, and biomass was calculated by established length–mass regressions [36–38].

Rotifers were not counted from the vertical net tows because the mesh size used, although appropriate for crustacean zooplankton and other larger organisms, would have allowed smaller rotifers to escape through the mesh [39].

2.3. Data Analysis

2.3.1. Analysis 1: Relations between Bladderworts and Zooplankton Prey Items

First, we tested whether certain zooplankton characteristics were related to bladderwort trap size dimensions and if certain zooplankton taxa were found more commonly than others in bladderwort traps. Simple linear regressions were used to test for significant relations between bladderwort trap length (bladderwort trap length and width were strongly related: $R^2 = 0.92$) and mean zooplankton prey length, mean number of prey per trap, and the mean number of zooplankton taxa per trap (measure for diversity).

Our main zooplankton response variable was percent dominance of density. Zooplankton percent dominance of density (hereafter referred to as PDD) was used instead of percent dominance of biomass because the two were related for different zooplankton taxa (R^2 : 0.30–0.91; p : 0.01–<0.0001) and because some organisms in the traps were partially digested by the traps, which affects the length to mass conversion for biomass for those individuals. PDD was used for the zooplankton variables, instead of absolute density, to standardize for any environmental factors that may have affected the absolute densities differently across each lake and bay and across the season. PDD was used also to account for inherent differences between the amount of zooplankton in each trap versus the water column. We tested whether a significant effect existed for lake, bay sampled, and their two-way interaction with the bladderwort trap or zooplankton (either prey items in traps or in the water column) response variables. Because no individual lake ($p \geq 0.09$), bay ($p \geq 0.38$), or interaction ($p \geq 0.17$) effect existed on any zooplankton or trap size variable, we pooled all bays and lakes together as separate observations.

We tested whether a significant difference existed in the PDD of zooplankton prey groups in bladderwort traps. A Kruskal–Wallis with a post hoc Dunn test [40] for pairwise comparisons was used to test if the PDD of broad groups (cladoceran, copepods, mites, and ostracods) and more specific genera/groups that constituted $\geq 5\%$ of prey diet item composition: chydorids (this group contained multiple genera, but the genera were grouped together for this analysis because some genera in this group were rarely observed), *Bosmina*, *Daphnia*, *Eurycercus*, and *Ophryoxus*, were significantly different from one another in their PDD in the traps. The non-parametric Kruskal–Wallis test was used for these two tests because the homogeneity of variance assumption failed with the parametric one-way ANOVA test. However, results were essentially the same whether a parametric or non-parametric test was used for analyses. Further, an independent t -test was used to test whether pelagic versus littoral/benthic taxa PDD were significantly different in traps. Littoral/benthic versus pelagic taxa were categorized according to their typical habitat preferences [11,34,41]. Table S1 contains a list of the taxa observed in our study and a description on whether they were categorized as littoral or pelagic for analyses. We acknowledge that the distribution of zooplankton taxa can change from day to night and

across the year, and that the same taxon can be found in both the littoral and pelagic zones (i.e., some taxa in our study were found both in bladderwort traps and the pelagic zone tow net samples). However, this analysis was still useful to test whether taxa consumed by bladderworts were typically littoral or pelagic taxa.

The PDD of different broad and more specific zooplankton groups found in bladderwort traps were also tested for significant relations with Julian day using simple linear regressions. These tests were performed to examine if the bladderwort diet composition changed across the thermally stratified period.

2.3.2. Analysis 2: Trap and Water Column Relations of Zooplankton

For the next analysis, the relations between the PDD of different zooplankton groups in bladderwort traps and the PDD of different zooplankton groups found in the water column were tested for significant differences using independent *t*-tests. This analysis was performed just for the broad zooplankton groups of Cladocera, copepods, mites, and ostracods, because some taxa were either rare or absent in the water column or in the traps.

Bladderwort diet contents were also compared to the availability of different zooplankton taxa in the adjacent pelagic zone from the vertical net tows to determine if selectivity existed in bladderwort diets. We calculated Chesson's index of selectivity (α) [42] for each more specific zooplankton taxon to determine if bladderwort selectivity existed:

$$\alpha = \frac{r_i}{n_i} / \sum_{i=1}^m r_i / n_i$$

where r_i is the number of prey type i in the predator diet, n_i is the number of prey type i in the environment, and m is the number of prey types ($m = 10$ more specific taxa). Mean α values ($\pm 95\%$ confidence intervals (CI)) were compared to random feeding ($1/m = 0.1$) to assess selectivity for (lower bound of 95% CI > 0.1), neutrality towards (95% CI overlapping 0.1), or avoidance of (upper bound of 95% CI < 0.1) certain zooplankton taxa.

All analyses were performed in R v. 4.1.1 [43]; *p*-values were considered significant at $\alpha = 0.05$.

3. Results

All lakes had at least one trap with a zooplankton prey item on each sampling event. Two occurrences existed where no zooplankton were found in traps in a single bay during a single sampling event; therefore, across the sampling period, 27 bays had bladderwort traps with zooplankton diet items. Of the 960 individual traps dissected, just 16% of the traps ($n = 160$) had no diet items. Mean trap length of bladderworts sampled was 2.2 ± 0.5 (mean ± 1 S.D.) mm, and ranged from 1.6 to 3.4 mm. The mean zooplankton prey length found in traps was 0.5 ± 0.1 mm, ranging from an individual minimum and maximum of 0.1 (a water mite) to 1.5 mm (a *Daphnia*). Traps had a mean of 1.9 ± 1.2 diet items present, ranging from a mean of 0.1 to 4.3 diet items per site. Traps within a bay had a mean of 6.0 ± 2.5 different number of taxa present between them, ranging from one to ten different numbers of taxa.

Surprisingly, rotifers were not observed in any trap, and aquatic insects comprised less than 3% of taxa found in the traps; therefore, the focus for our analyses was on crustacean zooplankton. Only ~5% of the specimens found in traps were not identifiable, being too digested by the trap to identify the individuals.

3.1. Analysis 1: Relations between Bladderworts and Zooplankton Prey Items

Mean trap size was positively related to mean zooplankton prey size ($p < 0.0001$; $R^2 = 0.51$; Figure 2a). However, mean trap size was not significantly related to the mean number of zooplankton per trap ($p = 0.23$; Figure 2b), and therefore was not significantly related to the mean biomass of zooplankton per trap ($p = 0.14$). Mean trap size was also not significantly related to the different zooplankton taxa present per trap ($p = 0.47$; Figure 2c).

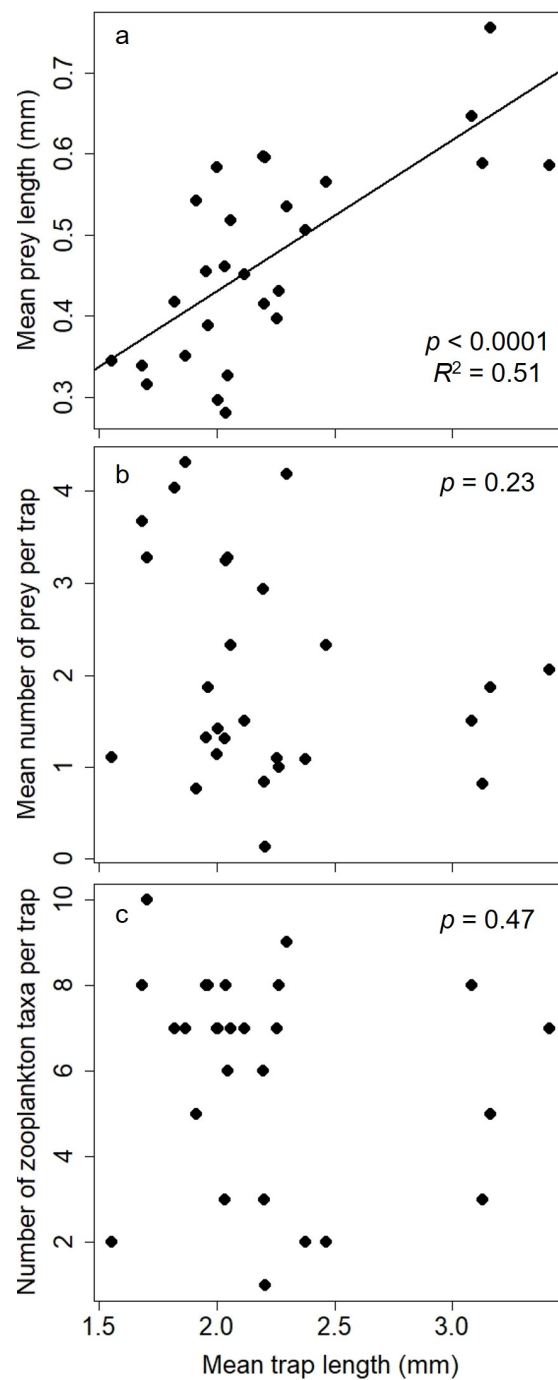


Figure 2. Relations between mean trap length and (a) mean prey length, (b) mean number of prey per trap, and (c) number of zooplankton taxa per trap; $n = 27$ for each analysis.

A significant difference existed in the PDD of the broad zooplankton groups in traps (Kruskal–Wallis: $H = 44.79$; $p < 0.0001$; Figure 3a). Dominance by Cladocera in traps was greater than dominance by copepods, mites, or ostracods ($p \leq 0.0003$); cladoceran taxa comprised $55 \pm 23\%$ of the broad zooplankton community dominance across traps. Ostracods ($p = 0.02$) and copepods ($p = 0.04$) were more dominant in traps compared to mites, but no difference existed in the PDD of ostracods or copepods in traps ($p = 1.00$). Ostracods comprised $22 \pm 25\%$ of the diet items in traps (Figure 3a).

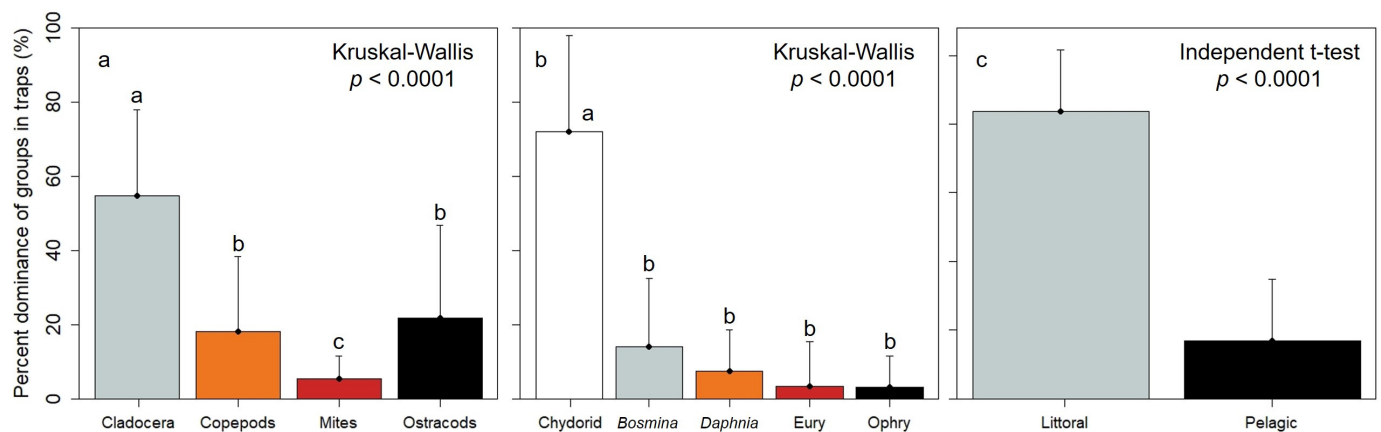


Figure 3. Percent density dominance in bladderwort traps of (a) major, broad taxonomic groups, (b) different taxa within Cladocera, and (c) littoral versus pelagic taxa. In panel (b) Eury = *Eurycercus* and Ophry = *Ophryoxus*. Letters above bars in (a,b) denote statistical differences; $n = 27$ for each analysis.

A significant difference also existed across traps between more specific/genera group PDD (Kruskal–Wallis: $H = 63.00$; $p < 0.0001$; Figure 3b). Chydorid taxa specifically dominated traps, comprising $67 \pm 32\%$ of the dominance of prey items, and being significantly more common in traps than the other specific taxa in the analysis ($p \leq 0.0001$; Figure 3b). No other more specific taxa were significantly different from each other in PDD in the traps ($p \geq 0.07$; Figure 3b). Similarly, because chydorid taxa are typically littoral/benthic genera, the PDD by littoral zooplankton taxa was significantly greater than that of pelagic taxa (independent t -test: $t = 13.59$; $p < 0.0001$). Littoral zooplankton taxa ($84 \pm 18\%$) dominated the bladderwort trap items (Figure 3c).

For broad taxonomic groups, PDD by cladoceran taxa in bladderwort traps increased significantly over the season ($p = 0.01$; $R^2 = 0.23$; Figure 4a; Table 1). *Bosmina* PDD also increased significantly in traps with Julian day, driving the cladoceran relationship ($p = 0.047$; $R^2 = 0.15$; Figure 4b). Conversely, PDD by ostracods in traps significantly decreased with Julian day ($p = 0.02$; $R^2 = 0.21$; Figure 4c). No significant relationship existed between Julian day and PDD in traps by copepods or mites ($p \geq 0.053$; Table 1). No other more specific group/genera PDD was significantly related to Julian day ($p \geq 0.13$; Table 1).

Table 1. Simple linear regression model statistics between the percent dominance in density of different zooplankton groups in traps and Julian day; $n = 27$ for all analyses.

Taxa	Intercept Value ± 1 S.E.	Slope Value ± 1 S.E.	p -Value	R^2
Broad groups				
Cladocera	-44.7 ± 36.8	0.5 ± 0.2	0.01	0.23
Copepoda	36.5 ± 36.3	-0.1 ± 0.2	0.62	0.01
Mite	-15.4 ± 10.3	0.1 ± 0.1	0.053	0.14
Ostracoda	124 ± 40.1	-0.6 ± 0.2	0.02	0.21
Specific groups				
<i>Bosmina</i>	-49.4 ± 30.1	0.3 ± 0.2	0.047	0.15
<i>Chydorids</i>	101 ± 56.2	-0.2 ± 0.3	0.54	0.02
<i>Daphnia</i>	-22.4 ± 18.6	0.2 ± 0.1	0.13	0.09
<i>Eurycercus</i>	7.6 ± 20.9	-0.02 ± 0.1	0.83	0.00
<i>Ophryoxus</i>	-7.5 ± 14.5	0.1 ± 0.1	0.47	0.02
Other				
Pelagic taxa	-53.0 ± 30.6	0.4 ± 0.2	0.03	0.18
Taxa richness	-7.1 ± 3.6	0.07 ± 0.02	0.001	0.34

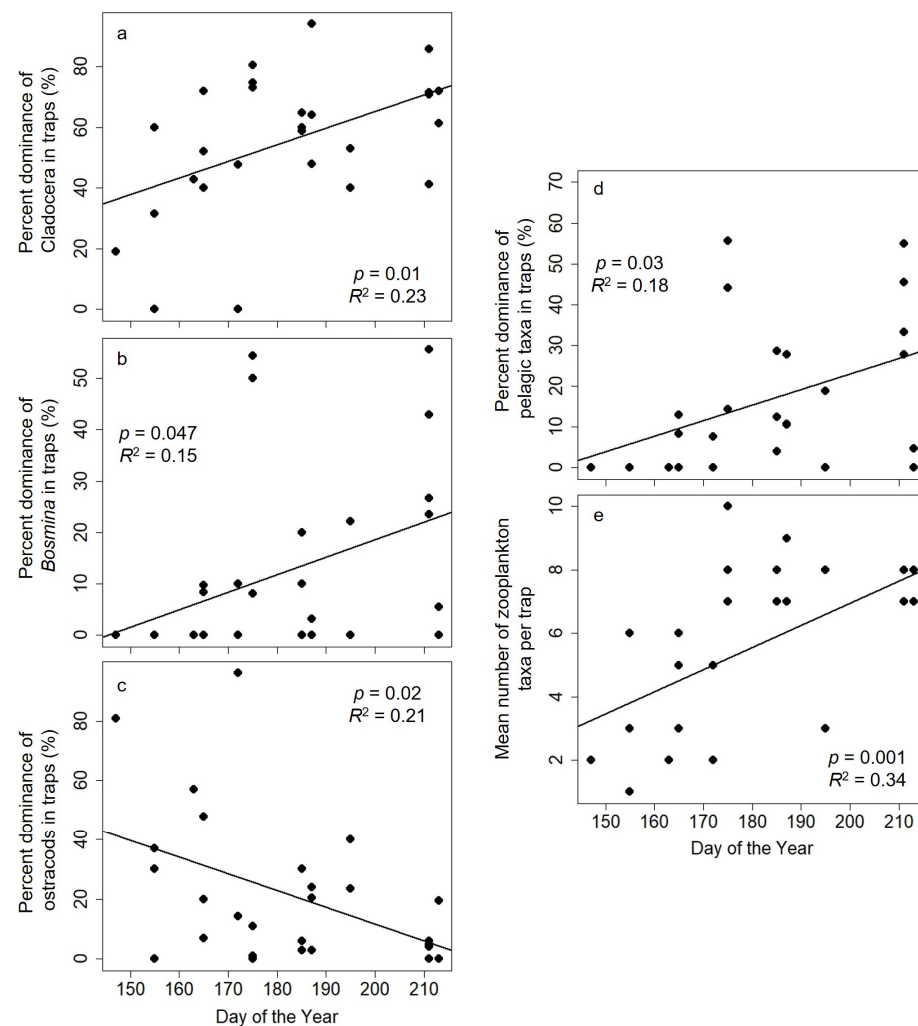


Figure 4. Relations between the percent density dominance in traps of (a) Cladocera, (b) *Bosmina*, (c) ostracods, (d) pelagic taxa, and (e) mean number of zooplankton taxa and the day of the year.

With the increase in *Bosmina* and *Daphnia* (positive but not significant relationship) with Julian day seasonally, the PDD of pelagic zooplankton taxa found in traps significantly increased with Julian day ($p = 0.03$; $R^2 = 0.18$; Figure 4d). Similarly, the mean number of zooplankton taxa found in traps had a positive significant relation with Julian day ($p = 0.001$; $R^2 = 0.34$; Figure 4e; Table 1).

3.2. Analysis 2: Trap and Water Column Relations of Zooplankton

PDD by Cladocera and ostracods in traps versus PDD in the water column was significantly different (independent t -test: $t \geq 2.61$; $p \leq 0.01$; Figure 5). Cladoceran taxa were more dominant in the water column versus in traps: cladoceran PDD was $76 \pm 35\%$ in the water column versus $55 \pm 23\%$ in the traps. Ostracods were more dominant in the traps, with $22 \pm 25\%$ versus 0% in the tows. Copepods and mites were not significantly different in traps versus tows ($p \geq 0.83$).

Bladderworts selected for the more littoral taxa of *Camptocercus*, *Chydorus*, *Eurycercus*, mites, *Ophryoxus*, ostracods, and *Pleuroxus* (lower bound of the 95% CI ≥ 0.11 , greater than 0.1). Bladderworts also selected for copepods (1.6 ± 0.5), which did contain littoral harpacticoids. Bladderwort selectivity for the pelagic *Bosmina* (0.1 ± 0.4) and *Daphnia* (0.5 ± 0.7) was neutral.

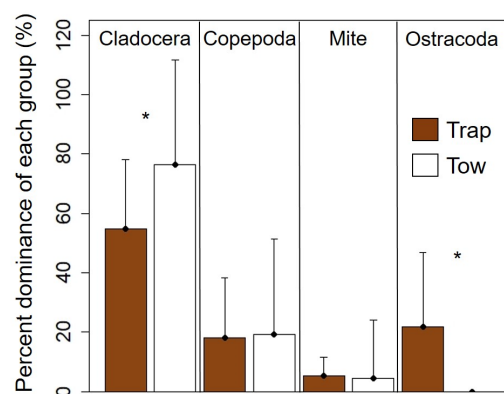


Figure 5. Percent density dominance of zooplankton groups collected in bladderwort traps in the littoral zone versus collected by tow nets just outside the bladderwort traps in the pelagic zone. Asterisks (*) above plots denote statistical difference for each analysis.

4. Discussion

The bladderworts in this study commonly consumed zooplankton and the zooplankton consumed consisted of a variety of taxa. Traps contained at least one prey item in ~84% of the 960 individual traps examined. Traps most commonly consumed Cladocera and ostracod taxa that are typical of more littoral environments [44]. However, pelagic taxa were also observed in the traps, such as *Daphnia* and *Bosmina*. This study provides insight on the diet composition of bladderworts in two lakes, across locations within a lake, and how bladderwort diet changes seasonally in relation to ambient, water column zooplankton conditions.

A significant, positive relation existed between mean prey length and mean trap length, indicating that larger traps can process and consume larger zooplankton. This finding suggests that bladderworts are a gape-limited predator [45], with larger traps consuming larger prey items. However, no difference was observed between mean number of prey items consumed and zooplankton taxa per trap and mean trap length, indicating that although larger traps often have larger prey, both large and small traps could have more or less zooplankton prey inside. The prey size range of 0.1 to 1.5 mm found in traps was similar to prey sizes observed in previous work [46,47], and likely reflects an adaptation to capturing zooplankton and other smaller prey items.

Cladocerans comprised the largest PDD of broad taxa found in traps. Much of the PDD Cladocera in the traps was of chydorid taxa, which are largely considered littoral and benthic, thereby having strong overlap in the habitat with bladderworts [48]. Therefore, the proportion of littoral/benthic taxa in traps was significantly greater than that of pelagic taxa. Some of the more common chydorid genera identified in the traps were *Chydorus* and *Pleuroxus*. Selectivity analysis complemented the observation that these littoral cladoceran taxa and others were selected for consumption. Some chydorids are strictly benthic and cling to surfaces and substrate. This life history and ecological strategy provides a contact point for bladderworts, increasing the chances of chydorids being consumed. However, pelagic taxa still comprised about 20 percent of the dominance in diet items in traps, indicating some predation on pelagic taxa despite less overlap in habitat between the two [46]. *Bosmina* and *Daphnia* were two pelagic taxa most commonly found in traps. Harpacticoid, cyclopoid, and calanoid copepods were recorded in the traps, though due to often being more digested than the other groups, identification was often difficult, and therefore we decided to be conservative and to group them all as copepods. However, we might predict that benthic/littoral copepods such as harpacticoids would be more commonly found in the traps, as they have more habitat overlap. Interestingly, although other studies have reported rotifers being present in traps [20,23], we did not find any rotifers in the traps in our study, even though they were observed, but not counted, in the water column tows. The authors of [23] reported some rotifers present in bladderwort

diets but found lower numbers; future work on bladderwort predation on rotifers versus crustacean zooplankton across lake types could provide more insight into factors affecting these predatory relationships. Overall, we found that traps were dominated by littoral and benthic zooplankton, indicative of having a stronger habitat overlap with these taxa, but pelagic taxa were still observed in the traps.

The bladderworts exhibited a seasonal change in their diets, which corresponded with a seasonal change in planktonic prey assemblage typical of northern temperate lake systems [49,50]. For example, *Bosmina*, *Daphnia*, and other cladoceran taxa often emerge from resting stages and become more numerous in inland lakes during the thermally stratified period in our study's region [50]. Arbutus and Spider Lakes in particular may be seasonally dominated by cladoceran taxa, which comprised most of the pelagic, water column composition of zooplankton (mean of about ~80% across sites) in this study (Figure 5). As the season progressed, a greater taxa richness as well as an increase in pelagic zooplankton consumption was observed. This trend was associated with an increase in taxa diversity in the adjacent water column. Conversely, ostracod PDD decreased in traps over time. We are not fully certain why this pattern was observed, as other studies have noted positive relations between water temperature and ostracod abundance [51,52], with ostracod abundance being higher in warmer temperature periods versus other periods of the year. Perhaps some other environmental variables or competition with certain zooplankton species in the lake resulted in PDD decrease in ostracods over the season, but more work would be needed to better understand this observation. Although our interest was to test linear relations with Julian day and bladderwort diet composition, some of the relations appear to plateau towards the end of our sampling period. The saturation of the PDD dominance of certain groups in bladderwort diet such as cladoceran taxa is likely a result of densities plateauing in the water column, after an increase earlier on in the season, indicative of seasonal succession of zooplankton in northern, temperate lakes [49,50].

Our findings indicate that the bladderwort diet is dependent both on the food available in the surrounding area and the size and density of the traps. The identification of a seasonal change in bladderwort diet indicates that bladderwort digestion of prey is quick enough to keep up with zooplankton seasonal change, as well as the ability for additional zooplankton to enter the bladderwort traps as the season continues [53]. Therefore, because zooplankton are consumed at a relatively rapid rate in bladderwort traps, the prey present in these traps may be a relatively accurate representation of the seasonal dietary change in these sites. As size of trap and number of prey were unrelated, we can also infer that the traps are not "sized" with specific prey in mind [20]. Some bladderwort species, such as the common bladderwort (*U. macrorhiza*), a common species in Michigan, USA, also have multiple size classes of traps [54]. Having a plant with traps of different sizes allows the plant to capture many different size classes of prey, increasing the predation potential over species that grow monotypic traps [46]. Therefore, the many different sizes of traps on a bladderwort trap permit capture of different zooplankton taxa that emerge seasonally.

The lakes in this study were both oligotrophic systems. Relations between bladderwort diet and the surrounding zooplankton community would benefit from future studies that span lake gradients, such as trophic state. Eutrophic systems often have different oxygen and fish predation conditions versus oligotrophic systems [8,55], which could affect bladderwort and zooplankton relations. For example, if a lake's hypolimnion is hypoxic or anoxic, perhaps zooplankton diel horizontal migration becomes greater, thereby increasing the habitat overlap between pelagic zooplankton and bladderwort traps [56]. If conditions force pelagic zooplankton to littoral regions of a lake, perhaps bladderworts would exhibit an increased dominance by pelagic zooplankton taxa in their diet during these conditions. As lake ecosystem function is being altered in many regions because of global change [57,58], studying bladderwort and zooplankton relations across lake types and gradients could be important in understanding how energy flows and pelagic–littoral coupling may change under different ecosystem stressors. Further research could also analyze bladderwort diets over multiple years, which has received little attention, and

across larger spatial scales [53]. Larger space or time studies would provide insight into inter-annual diet variations with environmental changes that may affect the plants and their diets. Long-term studies could provide important information into how ecosystem changes can affect bladderwort diet and water column zooplankton relations as well.

This study provides an analysis of bladderwort diet composition in relation to zooplankton characteristics, over the thermally stratified season, and in relation to ambient zooplankton concentrations across multiple bays and lakes. Bladderworts were found to consume zooplankton; although the majority of the diet contents in bladderwort were littoral/benthic zooplankton taxa, pelagic taxa were often commonly observed as well. A better understanding of the relations between bladderwort diet and water column zooplankton relations could provide important insight into energy flows and pelagic–littoral coupling under different lake conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15020171/s1>, Table S1: List of zooplankton taxa identified in bladderwort traps and zooplankton tow samples during the study period.

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