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## Survey of Trap Contents of Four Species of Bladderworts (Utricularia) from Harlow Lake in Marquette, MI.

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## ABSTRACT

Bladderwort (*Utricularia* spp.) is a carnivorous macrophyte, which utilizes a bag-like trap to capture prey. There are many species of *Utricularia*, including *U. resupinata*, which is unique in that it is completely buried in the sediment, except for the tips of its leaves. There is a lack of information pertaining to the trap content among different species of *Utricularia* from the same habitat, including in the Great Lakes bladderworts. The trap contents of four species of *Utricularia* species from one location in the Upper Peninsula of Michigan were surveyed. *U. vulgaris*, *U. intermedia*, *U. minor*, and *U. resupinata* were analyzed for a difference in diversity and abundance of prey. Five specimens of four *Utricularia* species were randomly collected from Harlow Lake, Marquette, MI. Traps were removed, fixed, measured, and contents were examined and identified to order. There was no significant difference in species diversity between trap contents; however, there were unique organisms found only in certain bladderwort species. Only *U. intermedia* contained prey from the order Ephemeroptera, *U. minor* and *U. intermedia* contained Trichoptera, and only *U. vulgaris* contained individuals in Unionoida, Basommatophora, and Copepoda. *U. intermedia* had the highest Shannon diversity index of 1.26, followed by *U. vulgaris* (1.13), *U. minor* (0.848), and *U. resupinata* (0.41). The most abundant order was Cladocera in all species except *U. minor*, which was dominated by Ostracoda. Although a more extensive survey of trap contents, including consideration of invertebrate community structure and bladder morphology and phenology, is needed to gain more accurate analysis results, this project has demonstrated fewer differences in prey in different *Utricularia* species than was expected, suggesting less niche separation than might be expected given their shared environment.

## INTRODUCTION

Botanical carnivory is a fascinating adaptation where flora attract, capture, and digest animal prey using modified leaf structures (Pavlovič & Saganová, 2015). It is characteristically found in low nutrient (particularly nitrogen), waterlogged environments, with the primary benefit of enhanced photosynthesis and growth (Friday, 1992).

Bladderwort (*Utricularia* spp.) is a carnivorous macrophyte, which utilizes a bag-like structure to capture prey. This complex trap contains less liquid than could be held in the trap, resulting in a hydrostatic pressure difference. When one of the trigger hairs on the outside of the watertight lid is disturbed, the trap-door flies open and the negative interior pressure sucks water and the prey into the bladder (Friday, 1991; Schnell, 2002). Prey consists of mostly zooplankton and phytoplankton. (Alkhalaf et al., 2009; Kurbatova & Yu. Yershov, 2009; Harms 1999).

Previous studies of *Utricularia* include its investment in carnivory, size and shape of traps (Friday, 1991), cost-benefit analysis (Pavlovič & Saganová, 2015), biomechanics (Poppinga et al., 2015), and prey spectra. A majority of literature examines the trap content in multiple or a single *Utricularia* species between different locations (Alkhalaf et al., 2009; Kurbatova & Yu. Yershov, 2009; Harms 1999). However, Guiral & Rougier (2007) looked at the trap contents among different species of *Utricularia* from the same habitat. They concluded that the adaptive differences in organization and size class distributions among the Bladderwort species limited feeding competition in a tropical pond environment, but it is unclear if this pattern is common across regions and habitats.

One unique species of bladderwort is *Utricularia resupinata*, which resides as vegetative mats at the bottom of the waterbody, buried in the sediment. This life trait raises the question of

its feeding habits given that its traps are completely or partially buried (Michigan Flora Online, 2017). There is little information available on the prey spectra of *U. resupinata*.

This study aims to describe the trap content of several *Utricularia* species, including *U. resupinata*, from one location in the Upper Peninsula of Michigan, and compare to the prey spectra cited in previous literature.

This project hypothesized there would be a significant difference in trapped organisms between *Utricularia* species. This research could contribute to interspecific competition knowledge among bladderworts, and act as a preliminary description of *U. resupinata* prey selectivity and feeding habits. In addition, it would be one of the first descriptions of *Utricularia* prey content in the Great Lakes Region.

## MATERIALS AND METHODS

Bladderwort specimens were randomly collected from within approximately 100 m along the west shore of Harlow Lake, Marquette, MI 49855 (46.63066, -87.487855). At each plant, pH and water temperature was measured for the physical description of the area. Five individuals of four *Utricularia* species were collected and separated by species into individual gallon plastic bags with lake water. To retard digestion inside the traps, specimens were kept on ice or in a refrigerator and stored in the dark until they could be examined.

In the laboratory, traps were removed from foliage using forceps, rinsed with dH<sub>2</sub>O to remove surface debris, and fixed in 70% OH to preserve organisms. Bladders were then separated by species, and stored in 5mL plastic vials with 2.5 mL 70% OH and 2mL dH<sub>2</sub>O.

To examine trap content, plastic vials were emptied into dishes. Traps from each species were randomly selected from the dishes, length was measured (cm), and contents were examined. Traps were opened and manipulated under dissecting microscope using fine needles

and forceps. Total prey per trap were counted and separated into major taxa using visual keys (Haney, J.F. *et al* 2013). 39 traps were examined (*U. vulgaris*=10, *U. intermedia*=10, *U. minor*=9, *U. resupinata*=10).

Shannon diversity index of each trap was calculated, which takes into account both the number of species present and the dominance of species in relation to another. In addition, abundance of organisms was calculated to characterize trap contents in means of percentages.

#### *Data Analysis*

An Independent Chi-square was conducted on the diversity of trap content between *Utricularia* species. To compare average trap size between species, a Kruskal-Wallace analysis was conducted. Average Shannon diversity indexes were compared by a Kruskal-Wallace analysis.

## RESULTS

At the site of specimen collection, the average water temperature was  $17.93 \text{ }^{\circ}\text{C} \pm 0.38$  and the average pH of water was  $6.78 \pm 0.67$ . A total of 97 individuals from eight Orders were described in the traps contents. Cladocera was the most abundant order and made up 55.67% of the total samples (Figure 1). All traps contained some algae.

#### *Diversity and Trap Size*

Prey community of traps did not differ between species (Independent Chi-square,  $P=0.093$   $df=26$ ) (Figure 1). This lack of difference is driven by the dependence of all species on Cladocera; however, unique prey species were found in several different *Utricularia* species. Average Shannon diversity index between bladderwort species was not significant (Kruskal-Wallace,  $P=0.436$ ,  $df=3$ ) (Figure 2). Nevertheless, *Utricularia* trap size differed greatly between

species (Kruskal-Wallis,  $P=0.00$ ,  $df=3$ ). On average, *U. vulgaris* had the largest traps ( $0.355 \text{ cm} \pm 0.043$ ) followed by *U. intermedia* ( $0.239 \text{ cm} \pm 0.0378$ ), *U. minor* ( $0.161 \text{ cm} \pm 0.034$ ), and *U. resupinata* ( $0.062 \text{ cm} \pm 0.04$ ) (Figure 3).

#### *Utricularia intermedia*

Traps contained 28 individuals in five orders, yielding the highest Shannon index of all four species (0.180) (Figure 1). Cladocera was the most abundant prey at 57.14% of *U. intermedia* trap contents, followed by Trichoptera (14.29%), Ostracoda (10.71%), and Ephemeroptera (7.14%) (Figure 1). Approximately 10% of the contents were unidentifiable. *U. intermedia* contained one unique species: a prey from the order Ephemeroptera (Table 1).

#### *Utricularia vulgaris*

Thirty-seven individuals from five orders were found in this species, yielding the second highest Shannon index of the species (0.115) (Figure 2). Cladocera was the most abundant prey at 59.45%. The second most abundant prey was unidentifiable due to digestion (21.61%), followed by Unionoida (10.81%), Basommatophora (5.41%) and Copepoda (2.70%) (Figure 1). *U. vulgaris* contained unique organisms found only in their traps including Unionoida, Basommatophora, and Copepoda (Table 1). It is noteworthy that *U. vulgaris* traps contained sediment particles, in addition to more algae than other *Utricularia* species.

#### *Utricularia minor*

Traps contained 18 individuals from three orders, yielding the third highest Shannon index (0.186) (Figure 2). Ostracoda was the most abundant prey creating 66.67% of the trap

contents. This was the only species that Cladocera (22.22%) did not make up a majority of the individuals (Figure 1). *U. minor* was the only species other than *U. intermedia* to contain organisms from Trichoptera.

### *U. resupinata*

Fourteen individuals from two orders were found in *U. resupinata* traps. This species had the lowest Shannon index (0.187) (Figure 2). Similar to other species, Cladocera (85.21%) was the most abundant order found in these traps, with Ostracoda making up the other 14.28% (Figure 1). This species contained some sediment particles in their traps.

## DISCUSSION

Amongst species, traps had significantly different lengths, which appears to correlate to the size of captured prey organisms. For example, *U. vulgaris* had the largest average trap length and was the only species that included large organisms such as Basommatophora (freshwater snail) in their traps (Figure 3). These results relate to findings by L. E. Friday, (1991) who predicted that larger traps accommodate more and larger prey.

The diversity and abundance results in this study are not consistent with previous literature, and therefore did not support the hypothesis. While there was no difference in diversity of trap content between species in this study, Alkhalaf et al., (2009) cites variation of prey spectra between *Utricularia* species and study sites. However, a definite conclusion cannot be drawn because this study included multiple species from one site, rather than multiple species from multiple sites. Samples were collected within a 100 m stretch along the shoreline of Harlow Lake. As a result, they have the same available resources and the possibility of direct

competition within each other, which could explain the lack of difference in prey community between bladderwort species. The small sample size in this study makes it hard to ensure statistical certainty. Further studies with a larger sample size for each species could draw conclusive results, particularly because unique organisms were found in several *Utricularia* species.

The dominant trap content of Cladocera in this study does not correspond to previous studies surveying trap contents. Almost all previous literature cites Copepods as the most abundant organisms between *Utricularia* species examined, including *U. vulgaris* (Alkhalaf et al., 2009; Andirkovics et al., 1998; and Harms, 1999). It is interesting to compare the results from this study to work done by Guiral & Rougier (2007) who examined two *Utricularia* species coexisting in the tropical pond French Guiana. The most abundant trap content varied seasonally between species, a factor not considered in this study. They found that *U.sp2* prey consisted of mostly Copepods and Cyclopoids and *U. gibba* had Rotifers and Cladocerans in late August. Although direct comparisons of these results and Guiral & Rougier (2007) cannot be made due to the vast difference in study site characteristics, it does allow for further considerations of data and highlight future research opportunities.

The result of *U. resupinata* lacking a different prey content diversity from other species was surprising. Because they are buried in the sediment, one could expect that their prey spectra would be different from bladderworts whose foliage is aboveground. In addition, there were no unique organisms found only in these traps compared to the other three species. It is interesting that the unique life history of this species did not result in a difference of prey community, which suggests they are not exploiting resources unavailable to aboveground *Utricularia* species. The presence of sediment particles was understandable due to its submersion in the substrate.



Curiously, the only other species that had sediment grains in its trap was *U. vulgaris*, whose foliage is above the sediment.

Moving forward, a more extensive survey of trap contents, with a larger sample size, is needed to gain analysis that is more accurate and understand trap contents between these species. In the future, a survey modeled after Guiral & Rougier (2007) would be suggested. This could allow researchers to look into a possible shift of aquatic invertebrate structure with lake turnover in one location. This could be useful in exploring a relationship of prey contents and invertebrate community structure in addition to interspecific competition. Also, attention to species-specific bladder morphology and phenology is needed to give a more detailed description of the plants.

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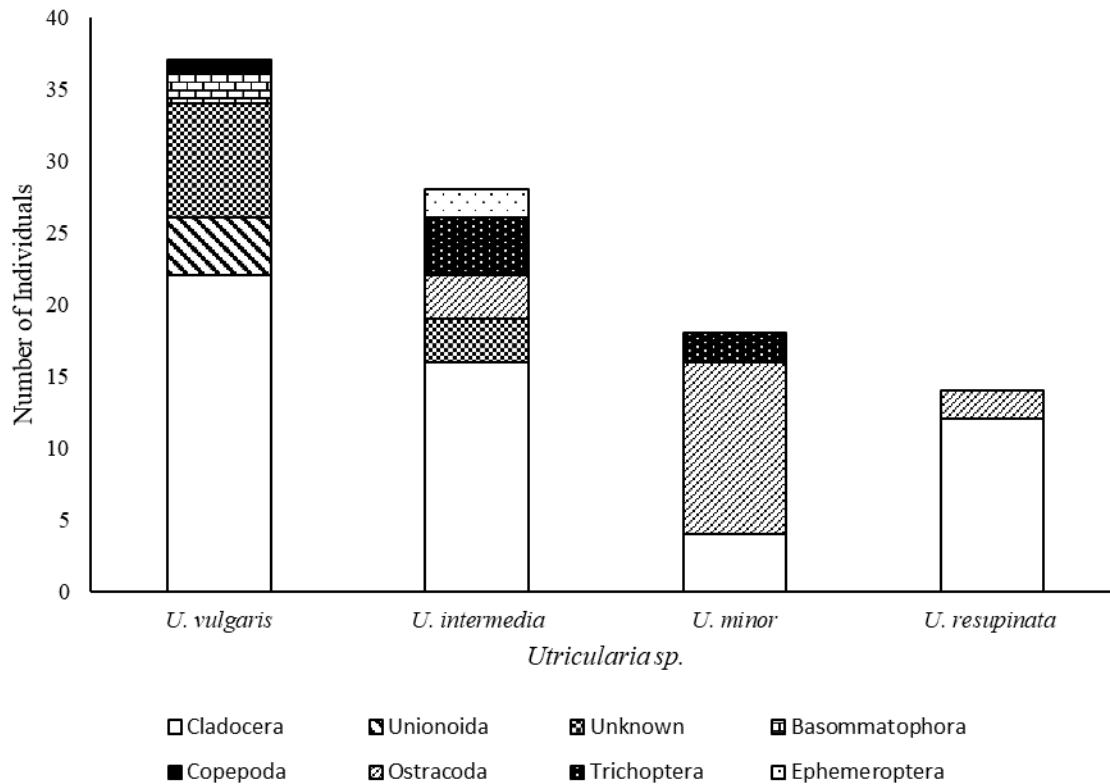


Figure 1. Number of individuals of Order in traps of four *Utricularia* species. There was not a significant difference in diversity of trap contents between species (Independent Chi-square,  $p=0.093$ ,  $df=26$ ). *U. vulgaris* has the highest Shannon diversity index of 1.136 and includes unique organisms not found in other Bladderwort species: Unionoida, Basommatophora, and Copepoda. All species included algae/cyanobacteria in trap contents.

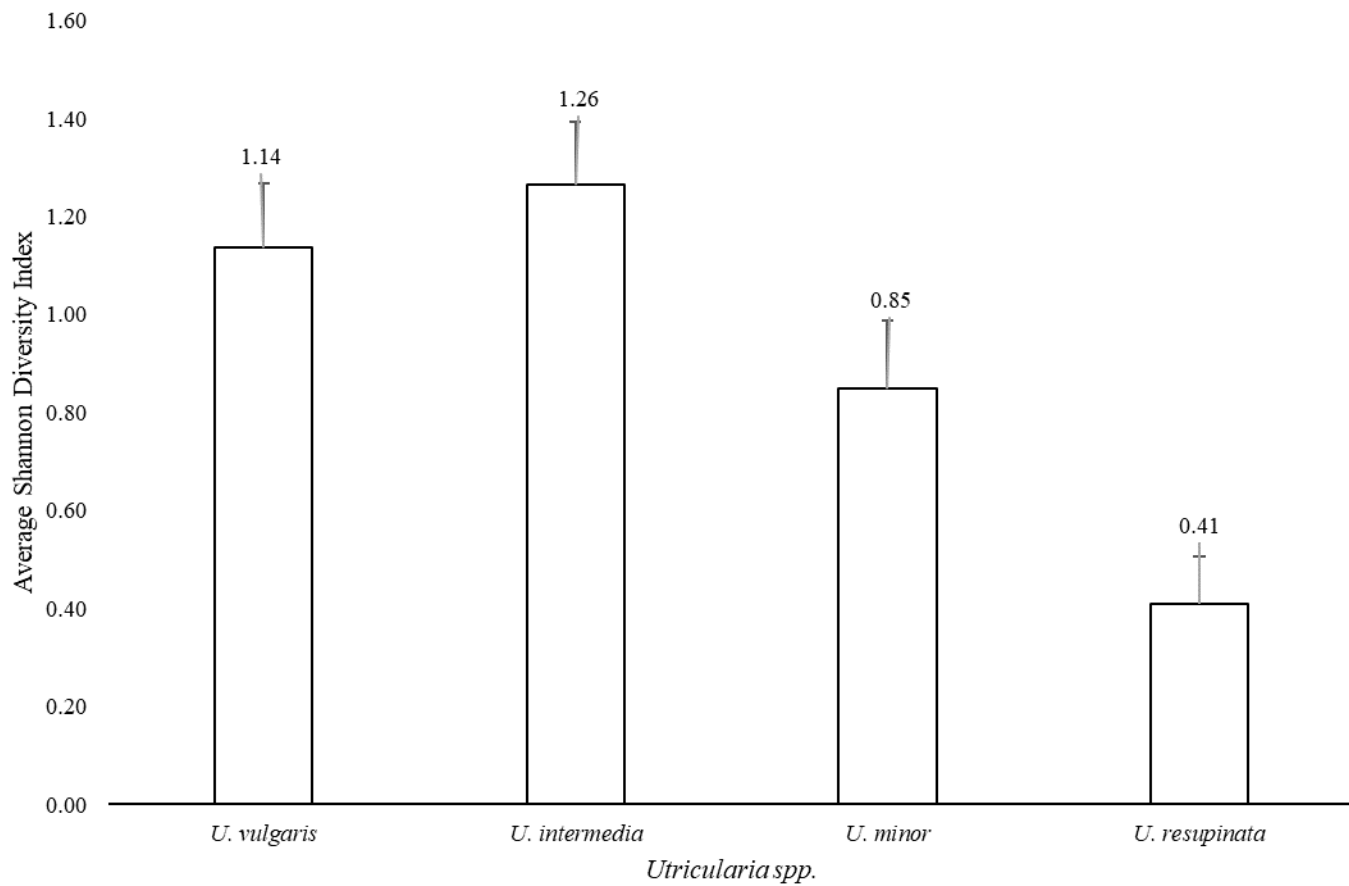


Figure 2. Average Shannon Diversity index between four species of *Utricularia* species. Average diversity index was  $1.14 \pm 0.13$  in *U. vulgaris*,  $1.26 \pm 1.28$  for *U. intermedia*,  $0.85 \pm 0.14$  in *U. minor*, and  $0.41 \pm 0.10$  for *U. resupinata*.

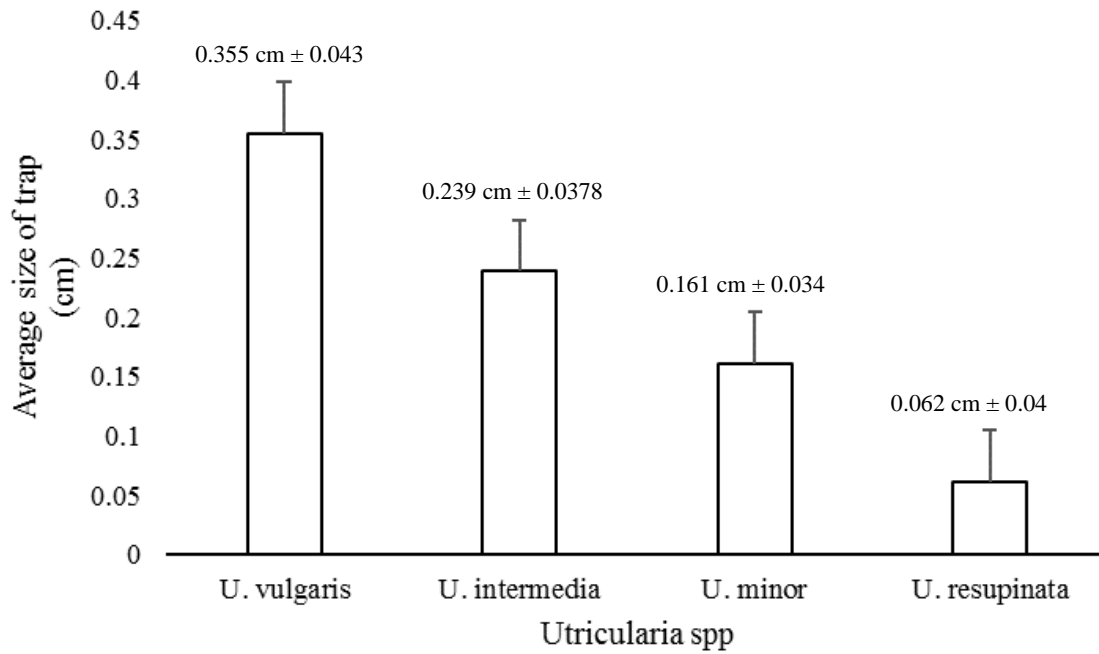


Figure 3. Average size of traps on *Utricularia* species. There is a significant difference in trap sizes (Kruskal-Wallis,  $P=0.00$ ,  $df=3$ ). On average, *U. vulgaris* had the largest traps ( $0.355 \text{ cm} \pm 0.043$ ) followed by *U. intermedia* ( $0.239 \text{ cm} \pm 0.0378$ ), *U. minor* ( $0.161 \text{ cm} \pm 0.034$ ), and *U. resupinata* ( $0.062 \text{ cm} \pm 0.04$ ).

Table 1. Prey

Order found in traps of *U. vulgaris*, *U. intermedia*, *U. minor*, and *U. resupinata*.

Order	Species			
	<i>U. vulgaris</i>	<i>U. intermedia</i>	<i>U. minor</i>	<i>U. resupinata</i>
Cladocera	X	X	X	X
Unionoida	X			
Basommatophora	X			
Copepoda	X			
Ostracoda		X	X	X
Trichoptera		X	X	
Ephemeroptera		X		