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SPATIAL AND TEMPORAL RELATIONSHIPS BETWEEN THE INVASIVE SNAIL *BITHYNIA TENTACULATA* AND SUBMERSED AQUATIC VEGETATION IN POOL 8 OF THE UPPER MISSISSIPPI RIVER

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SPATIAL AND TEMPORAL RELATIONSHIPS BETWEEN THE INVASIVE SNAIL *BITHYNIA TENTACULATA* AND SUBMERSED AQUATIC VEGETATION IN POOL 8 OF THE UPPER MISSISSIPPI RIVER

By Alicia M. Weeks

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of M.S. in Biology, Aquatic Science.

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ABSTRACT

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Bithynia tentaculata is an invasive snail that was first reported in Lake Michigan in 1871 and has since been rapidly spreading through the waters of the U.S. This invasion has been extremely problematic in the Upper Mississippi River, specifically Navigation Pools 7 and 8, as this area serves as part of the major migratory flyway. As an intermediate host for several exotic trematode parasites, *B. tentaculata* is associated with severe regional waterfowl mortality. This study was designed to assess the abundance and distribution of *B. tentaculata* relative to submersed aquatic vegetation (SAV) as it provides nesting and food resources for migrating waterfowl. Temporal changes at specific locations were assessed from 2007 to 2015 using the rake score method of the Long-Term Resource Monitoring Program to survey vegetation and snail densities. Data suggest that *B. tentaculata* densities have nearly quadrupled since 2007 despite minor changes in vegetation abundance, distribution, and composition. Understanding the spatial distribution of *B. tentaculata* in relation to other habitat features, including submersed vegetation, and quantifying any further changes in the abundance and distribution of *B. tentaculata* over time will be important for understanding the potential locations and magnitude of risks of disease transmission to waterfowl.

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INTRODUCTION

Species invasion is a rapidly growing problem in the Upper Mississippi River (UMR). As one of the world's most productive ecosystems, the UMR supports diverse assemblages of macro-invertebrates, fish, waterfowl and macrophytes (Fremling, 2005). As human demand on this system continues to rise, anthropogenic stressors such as urban development, eutrophication, habitat loss and the spread of invasive species increasingly impinge on this ecosystem (Angradi *et al*., 2009; Houser and Richardson, 2010; Moore *et al*., 2012). Ecosystems that are stressed are more susceptible to invasive species as the native populations are often suppressed (Johnson *et al*., 2009). This creates a "window of opportunity" in which conditions such as flow regime, turbidity and light availability facilitate invasion (Tockner *et al*., 2010).

The most common source of invasive species transport is through the ballast water of cargo ships from overseas, as is the case for the invasive faucet snail, *Bithynia tentaculata* (Jokinen, 1992). *Bithynia tentaculata* (Linnaeus, 1758) was first reported in Lake Michigan in 1871 and has since rapidly spread through waters of the U.S. (Mills *et al*., 1993). This invasion has been problematic in the UMR, specifically in Navigation Pools 7 and 8, near La Crosse, Wisconsin. In addition to the introduction of this invasive snail, *B. tentaculata* is a host to at least four trematode parasites that have been associated with severe mortality in waterfowl each year. The UMR is extremely important for bird migrations, as approximately 40 percent of North America's waterfowl use its resources (The Nature Conservancy, 2016). Since the discovery of *B. tentaculata* in the UMR in

2002, more than 15 waterfowl species have been affected by trematodiasis during spring and fall migrations. Lesser scaup (*Aythya affinis*) and American coot (*Fulica americana*) are two species of waterfowl that have suffered the highest mortality rates (Sauer *et al*., 2007). With thousands of waterfowl dying every year, the total mortality is estimated to be greater than 70,000 individuals (U.S. Fish and Wildlife Service, 2012). Although waterfowl such as lesser scaup have been in constant decline due to habitat loss and poor water quality, epizootic disease has potentially exacerbated mortality rates (Herrmann and Sorensen, 2011). Aquatic macrophytes are a major food source for waterfowl and provide adequate nesting areas as well as protection from predators (Moore *et al*., 2010). However, these areas may also provide habitat for *B. tentaculata* and its parasites, playing a critical role in parasite transmission and subsequent waterfowl mortality.

Sphaeridiotrema globulus, Sphaeridiotrema pseudoglobulus and *Cyathocotyle bushiensis* are three of the trematode species that infect *B. tentaculata* in the UMR. These species have complex life-cycles involving two intermediate hosts and a definitive waterfowl host (Sandland *et al*., 2013). *Bithynia tentaculata* is the only known first intermediate host for these parasites while several snail species, both native and non-native, can serve as second intermediate hosts. Free-swimming miracidia locate and penetrate the first intermediate host and development begins (Figure 1). Asexual reproduction eventually generates numerous free-swimming cercariae, which are shed from *B. tentaculata* and then infect suitable second intermediate hosts (Herrmann and Sorensen, 2009). Cercariae encyst and become metacercariae within the second intermediate host where they reside until the host is eaten by a waterfowl. *Sphaeridiotrema globulus, S. pseudoglobulus* and *C. bushiensis* must survive within the intermediate hosts for prolonged periods as the potential for most transmission only occurs during waterfowl migrations during the spring and fall (Herrmann and Sorensen, 2009). Once in the bird's digestive tract, metacercariae develop into adult worms which undergo sexual reproduction. Trematode eggs are spread into the water via the feces of the waterfowl for up to 21 days, thus continuing the cycle (Cole, 2001). During infection, waterfowl may become lethargic, slow moving and lose the ability to fly due to high energetic costs associated with immune response. A lethal dose of parasitic infection can occur within as little as 24 hours after feeding leading to mortality after 3-8 days (Hoeve, 1988). Death of infected hosts is caused by intestinal inflammation, hemorrhaging and blood loss.

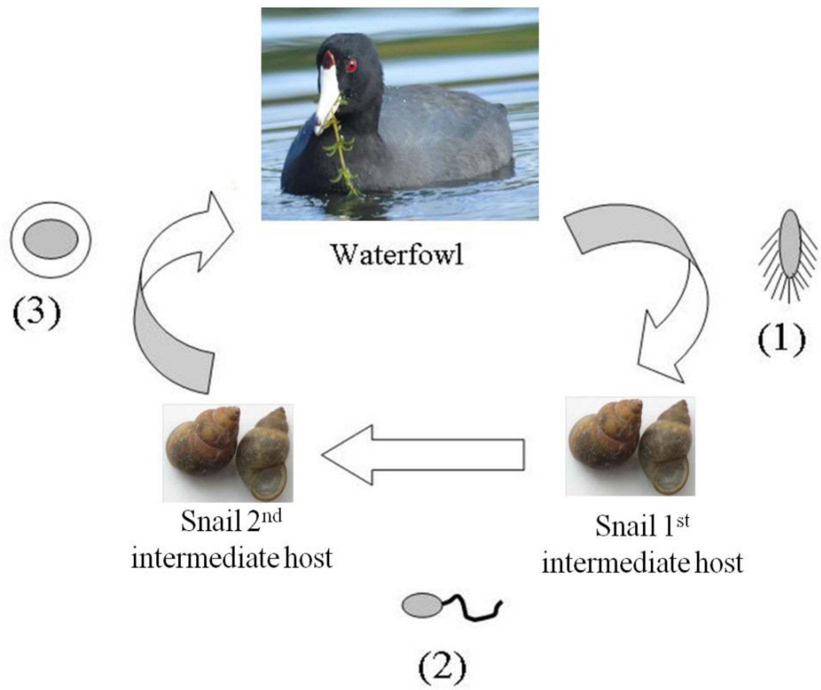

Figure 1. Life cycle of *Sphaeridiotrema globulus*, *Sphaeridiotrema pseudoglobulus* and *Cyathocotyle bushiensis.* (1) Miracidia infection of 1st intermediate snail host, (2) Cercariae infection of 2nd intermediate snail host and (3) Metacercariae transmission to definitive waterfowl host.

There are many factors that affect the temporal and spatial distribution of *B. tentaculata* and its parasites (Peirce *et al*., 2016). The greatest concern is that these parasites can be easily spread via migration in waterfowl (Cole, 2001). Seasonal variation and changes in temperature can affect the behavior and abundance of both waterfowl and *B. tentaculata* populations as well as the available habitat (Herrmann and Sorensen, 2009). Increased rip-rap such as rocky and woody debris may create habitat for snails, waterfowl and other organisms. These areas are often enhanced with the restoration of islands, specifically in lower Pool 8 (Langrehr *et al*., 2007). The re-construction of islands that had been previously lost due to increased water levels associated with lock and dam operations allows for plants to re-colonize in areas that would otherwise be too deep (De Jager and Yin, 2010). These islands create conditions that are ideal for plant growth such as shelter from wind and current as well as increased water clarity due to the settling of suspended sediments (Johnson and Hagerty, 2008). Such features also create habitat for *B. tentaculata* and waterfowl as vegetation patch size increases and water velocity decreases overtime (Moore *et al*., 2010).

Gastropods often reside in low velocity habitats where they are able to successfully attach to substrates such as rock, silt and submersed aquatic vegetation (Richter, 2001). *Bithynia tentaculata* can be found at high densities in these areas as they are extremely tolerant of varying physical and chemical conditions (Mitchell and Cole, 2008). Although snails are primarily known as grazers, it has been suggested that the preferred foraging method for *B. tentaculata* is filter feeding (Brendelberger *et al*., 1993). This offers an energetic advantage to *B. tentaculata* as it is able to feed via grazing and filter feeding (Brendelberger, 1995). The presence of an operculum also allows

B. tentaculata to seal its shell during unfavorable conditions (Richter, 2001). These advantages not only allow *B. tentaculata* to potentially outcompete native snails but also gain the potential to become a dominant species overtime (Wood *et al*., 2011).

Often temporal changes in habitat facilitate rapid growth of invasive species as they are able to quickly colonize new areas. Past studies have shown that submersed aquatic vegetation (SAV) has increased dramatically in Pool 8 since 2005 as a result of decreased discharge levels (De Jager and Yin, 2010; DeLain and Popp, 2014). It is possible that snail abundances have increased as well although, to date, no study has quantified whether *B. tentaculata* abundances have increased over time. The objective of this study was to assess temporal and spatial relationships of *B. tentaculata* and submersed vegetation in 2007 and 2015 in Pool 8 of the UMR. Understanding the spatial distribution of *B. tentaculata* in relation to other habitat features, including submersed vegetation, and quantifying any further changes in the abundance and distribution of *B. tentaculata* over time will be important for understanding the potential risks of disease transmission to waterfowl.

METHODS

Study Sites

This study was conducted in Pool 8 of the Upper Mississippi River. Pool 8 is approximately 37 kilometers long extending from Lock and Dam 7 near Dresbach, Minnesota to Lock and Dam 8 near Genoa, Wisconsin. There are three major ecological zones within this navigational pool. The upper portion of the pool consists of braided channels and forested islands while the central portion of the pool consists of backwater marshes and side channels. The lower half of the pool is an impounded area that contains open water with mostly silt substrate. This lower area contains numerous low lying islands, many of which are habitat rehabilitation projects (Figure 2). In 1989 a habitat rehabilitation project, consisting of a series of islands, was implemented in lower Pool 8 in order to restore potential habitat that had been lost to high current velocity and wind fetch (Janvrin, 2011). The project was completed in 2011 and the islands are now inhabited by macrophytes, waterfowl, invertebrate and fish communities.

Pool 8 sampling locations were selected using a stratified random sampling design. Using geographic information system software (ArcMap 10.2), 450 sampling sites were randomly generated based on the Universal Transverse Mercator coordinate system. Of the 450 sites selected for Pool 8, 115 were found to be vegetated and inhabited by *B. tentaculata* in 2007. I chose to re-sample these specific locations using the same procedures in 2015 in order to assess changes in macrophtye and gastropod distribution and density overtime (Figure 2). Sampling sites were divided by strata:

backwater (n=16), impounded (n=93), main channel (n=2), and side channel (n=4). This study utilized data from the vegetation monitoring component of the Long-Term Resource Monitoring Program (LTRMP) (Yin *et al*., 2000) for 2007. This year is the only one in which LTRM procedures were used to collect and identify snails in addition to SAV.

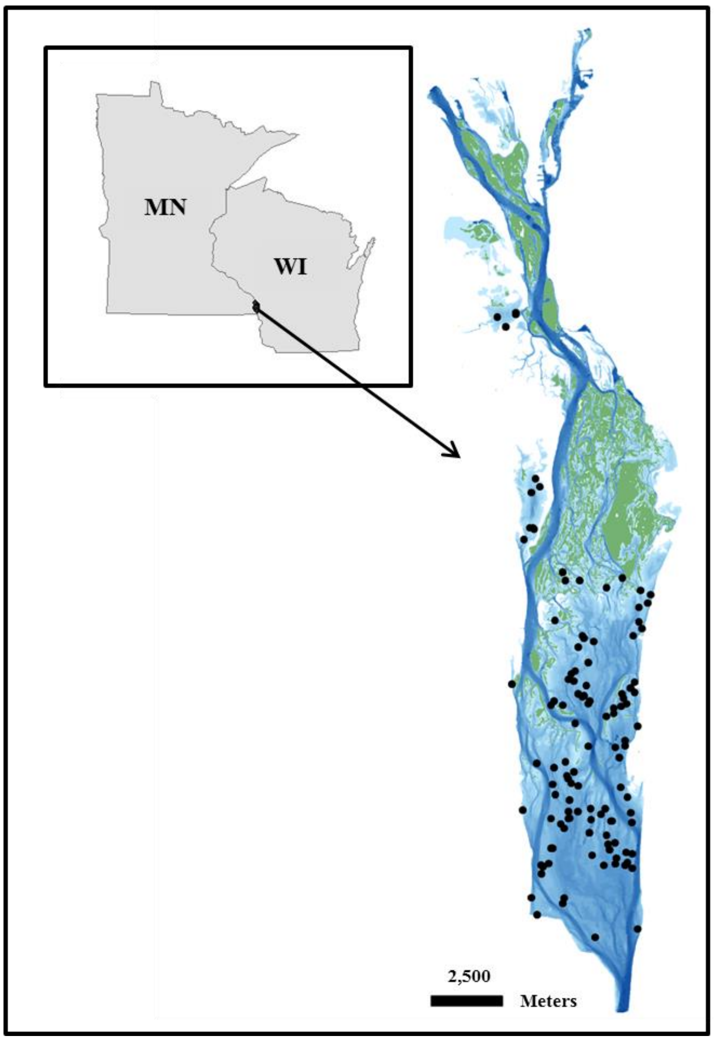

Figure 2. Sampling locations (n=115) in Pool 8 of the Upper Mississippi River in both 2007 and 2015.

Collection Methods

Sampling was conducted from June 25, 2007 to July 17, 2007 and from June 12, 2015 to July 23, 2015. Samples were taken within 10 meters of selected GPS coordinates, with each site consisting of two sub-sampling locations around the boat. Upon arrival, the boat was anchored at the bow and stern to prevent movement during sampling. The sampling technique involved visual examination of the macrophyte species present using a vegetation collecting rake (Kenow *et al*., 2007). Percent cover of rooted floating, non-rooted floating, emergent species and filamentous algae were visually inspected and recorded at each site. Each sub-sample was recorded separately and consisted of a rectangular quadrat, approximately 1.5 meters long by 0.36 meters wide (which approximated rake size). The collecting rake was used to measure the density of submersed aquatic vegetation. The rake was extended to the distal end of the quadrat, lowered to the sediment and dragged toward the boat. Once perpendicular with the boat, the rake was then rotated 180 degrees to minimize loss of vegetation. The collecting rake is scaled using 10 cm increments to record water depth at each location around the boat. The rake score or vegetation density was determined by analyzing the amount of SAV on the rake using the pre-marked 20 percent increments, ranking 1-5 with a five indicating 81-100% of the rake filled with SAV. Plants were identified to species level using an experienced field crew as well as a taxonomic key by Crow and Hellquist (2000). All snails, native and non-native, were collected in addition to vegetation at each site.

Other site conditions were also recorded including substrate type and presence of detritus. Substrate type was categorized into silt/clay, mostly silt with sand, mostly sand with silt, hard clay, gravel/rock and sand. Water temperature and weather conditions were recorded along with additional site comments. These constituents are important in order to assess possible causation for high vegetation or snail abundances.

Sample Processing

Collected snails and vegetation were stored in 92 oz. Whirl-Pak bags while in the field. Samples were then brought back to the lab for further identification and analysis. All samples were frozen upon arrival in order to ensure preservation of the snails. Each sample was allowed adequate time to thaw prior to analysis. Macrophytes were separated from the snails and plants were weighed using a digital scale (± 0.01 grams) for each site. Snails were identified to species (Perez and Sandland, 2014) counted and measured using digital calipers (± 0.01 mm). Shell length of a snail is often used in the aging process to determine juveniles from mature reproductive adults (Sandland and Haro, 2008).

Statistical Analysis

All data were assessed for parametric assumptions of normality and homogeneity of variance using R-Studio software version 3.1.1. Because these assumptions were often violated, non-parametric tests (paired Wilcoxon signed-rank test) were used to determine whether *B. tentaculata* abundance and distribution, as well as relationships with SAV, had changed over time. All snails that were collected in 2015 were measured for total shell length; however only a subset of snails from each site were measured in 2007. As a result, relative length frequencies of *B. tentaculata* were calculated in order to assess possible changes in size structure within the population from 2007 to 2015. Bin ranges were standardized to 5mm, incorporating snail lengths from 1 to 15mm.

To determine if *B. tentaculata* associate or prefer particular SAV species, the electivity of *B. tentaculata* for different SAV species was calculated using an electivity index (E) (Jenkins 1979):

$$E_i = \ln\left[\frac{r_{i(1-p_i)}}{p_{i(1-r_i)}}\right]$$

Where r_i is the proportion of sites with SAV species *i* and *B. tentaculata* present, relative to the number of sites with *B. tentaculata*, regardless of SAV species. P_i is the proportion of sites with SAV species *i*, relative to all sites, regardless of SAV species. An electivity value of greater than zero suggests a preference for that species whereas a negative value indicates potential avoidance. A Chi-square test was used to determine the significance of the electivity using the following equation:

$$X^2 = \frac{{E_i}^2}{\frac{1}{x_i} + \left(\frac{1}{m} - x_i\right) + \frac{1}{y_i} + \left(\frac{1}{n} - y_i\right)}$$

Where E_i is electivity, x_i is number of sites with *B. tentaculata* and SAV species *i*, y_i is the number of sites with SAV species *i* present, m is the total number of sites with *B. tentaculata*, and n is the total number of sites. A critical value of 3.84 was used based on one degree of freedom and a Type 1 probability value of p=0.05.

The relative abundance of each species was calculated to determine if there were any major changes in SAV composition in 2007 compared to 2015. To further examine the relationship between *B. tentaculata* and SAV densities, quantile regression models were developed using the Quantreg package (Koenker and Bassett, 1978) in the statistical program R- Studio. This method can be used to determine trends in the density of *B. tentaculata* with known densities of SAV. Typically, there are numerous factors affecting the distribution of organisms, however measuring and incorporating every factor may not be possible. Quantile regression accounts for this issue by estimating multiple rates of change based on a single predictor variable and hence, creating a more complete assessment of the bivariate relationship (Cade and Noon, 2003). Quantile estimates (0.05, 0.25, 0.5, 0.75, 0.95) were used to assess changes in *Bithynia tentaculata* density (y) as a function of SAV density (x). For example, the 95th quantile (0.95) indicates that 95% of the values of *Bithynia tentaculata* density are less than or equal to the specified SAV density (Cade and Noon, 2003). For the 50th or median quantile (0.50), 50% of the data points are at or below the regression line and 50% of the data points fall above the line. Generally, the largest sample variations will exist at quantile values near 0 and 1. Larger quantile values (0.95) more efficiently estimate the upper limits of the data whereas smaller quantile values (0.05) better estimate the lower limits (Lancaster and Belyea, 2006). Increased variance in *B. tentaculata* density along the SAV axis would indicate that other abiotic and biotic factors may be influencing their distribution in different ways across the full range of SAV density. Two data points from the 2015 sampling period were not incorporated into the model as they were determined outliers via visual inspection.

To assess possible spatial relationships, distribution maps of total SAV and *B. tentaculata* densities within Pool 8 of the UMR were created using ArcMap Version 10.2. In addition, spatial patterns of *B. tentaculata* and select vegetation species (*Ceratophyllum demersum, Vallisneria americana, Elodea canadensis*) were quantified using Moran's I spatial autocorrelation statistics with GS+ (Gamma Design Software, 2004):

$$I(h)=\sum (\sum Z_i Z_{i+h})/(\sum Z_{i+h}^2)$$

Where I(h) is the autocorrelation for interval distance class *h*, Z_i is the measured sample value at point *i* and Z_{i+h} is the measured sample value at point *i+h.*

Moran's values range from -1 (negative correlation) to 1 (positive correlation) with a value of zero indicating the absence of autocorrelation. Positive correlation can be inferred when high values at one location are associated with high values at neighboring sampling locations whereas negative correlation refers to high values associated with low values (Kraan *et al*., 2009). Moran's I results were plotted against separation distance at 325 meter intervals within a 0 to 5,500 meter range in order to better understand the relationship of spatial correlation and distance between sampling locations. This was the smallest distance interval in which there was a sufficient number of sampling site pairs within each class to accurately compute the Moran's Index. Olea (2006) found it to be difficult to accurately determine spatial patterns with fewer than 50 measurements per class. The nugget (degree of correlation at 1^{st} sampling interval) and the range (measure of scale of correlation) were reported in the results. The estimated range for each species was recorded as the distance when the correlation value leveled off or became constant. Small range values indicate a fine-scale patchy pattern or localized aggregation whereas large ranges suggest patches exist with large diameters. Large nugget values indicate a strong similarity between sites at the specified sampling distance, and hence a more strongly 'patchy' pattern whereas nugget values approaching zero would suggest a random pattern with no correlation between sites.

A cross correlogram was also generated to further assess the spatial patterns between *B. tenaculata* and SAV. These analyses helped to determine the overall degree of aggregation, or patchiness, in the relationship between SAV and *B. tentaculata*, as well as compare the location and size of patches of one relative to the other.

RESULTS

There was a significant increase in mean *B. tentaculata* abundance between 2007 and 2015 in Pool 8 (Wilcoxon signed-rank test, V= 3296.5, df=229, P < 0.001) (Figure 3). The abundance of *B. tentaculata* was 3.7 times greater in 2015 compared to 2007. There was also a significant difference in mean SAV density between years (Wilcoxon signed-rank test, V=6586.5, df=229, P = 0.03). The mean density of SAV decreased between 2007 and 2015 at these specific locations in Pool 8 (Figure 3). In addition, size distribution of *B. tentaculata* shifted between sampling periods (Figure 4). In 2007, there was a unimodal size distribution with the majority of snails measuring between 5 and 10 mm in length. Relative frequency was negatively correlated with length in 2015 as most snails measured between 1 and 5 mm. However, the majority of the snails were between 1 and 10 mm for both sampling years.

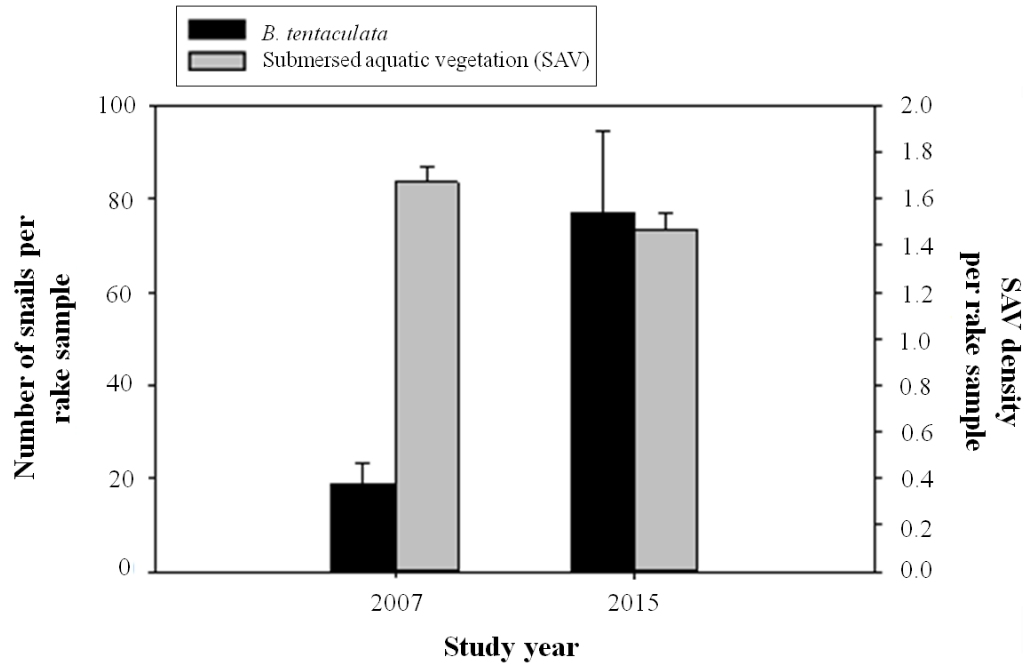

Figure 3. Mean density (± SE) of *B. tentaculata* and submersed aquatic vegetation (SAV) in 2007 and 2015 in Pool 8 of the Upper Mississippi River.

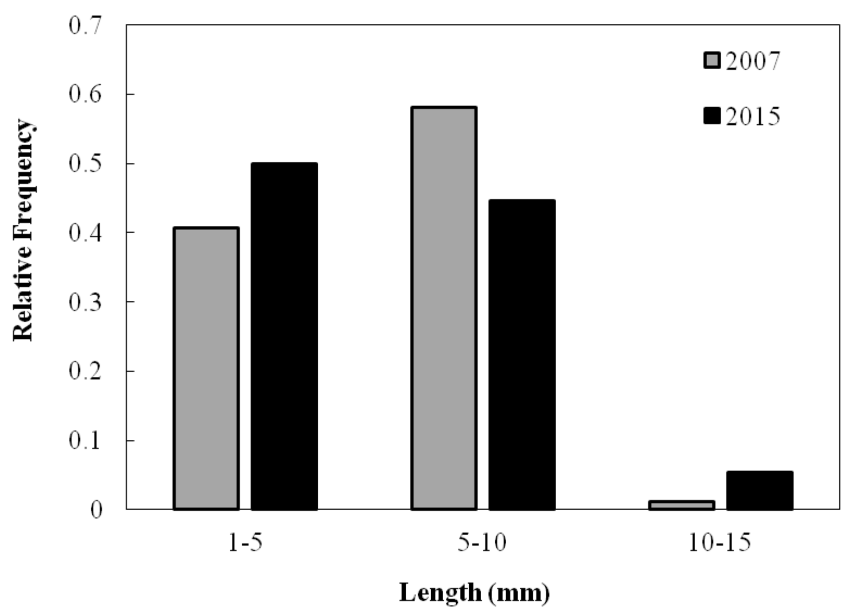

Figure 4. Relative length distribution of *B. tentaculata* in 2007 and 2015 in Pool 8 of the Upper Mississippi River.

Temporal changes in electivity were found for *Vallisneria americana* (Table 1). In 2015, *B. tentaculata* was positively associated with *V. americana*, suggesting that this particular species could play a role in the distribution of *B. tentaculata.* All other vegetation species were neutral in association with *B. tentaculata*. In 2007, no species were found to have significant electivity indices when using a critical value of 3.84.

There were minor temporal changes in vegetation composition between 2007 and 2015 (Figure 5). Three additional species (*Najas flexilis*, *Potamogeton richardsonii* and *Ranunculus longirostris*) were collected in 2007 compared to 2015; however, it should be noted that these species accounted for < 2% of the total abundance. Of the most common vegetation species, *Vallisneria americana*, *Elodea canadensis* and *Myriophyllum spicatum* increased in percent occurrence between 2007 and 2015, whereas *Ceratophyllum demersum* was obtained less often.

Table 1. Electivity of *Bithynia tentaculata* for species of submersed aquatic vegetation in 2007 and 2015 in Pool 8 of the Upper Mississippi River.

	2007		2015	
Species	E_i	X^2	E_i	X^2
Vallisneria americana	0.44	3.54	0.85	12.16*
Elodea canadensis	-0.08	0.13	0.11	0.23
Ceratophyllum demersum	-0.24	1.01	-0.10	0.16
Potamogeton crispus	-0.71	3.47	-0.16	0.14
Potamogeton zosteriformis	-0.34	0.86	-0.19	0.27
Heteranthera dubia	-0.05	0.07	0.47	1.21
Myriophyllum spicatum	-0.14	0.35	0.41	1.92
Potamogeton foliosus	0.17	0.69	-0.21	0.26
Stuckenia pectinatus	0.06	0.05	-1.02	0.86
Potamogeton nodosus	-1.60	2.31	-0.80	1.01

*Significance was calculated using a p-value of 0.05 and 1 degree of freedom.
E_i refers to the calculated electivity index.

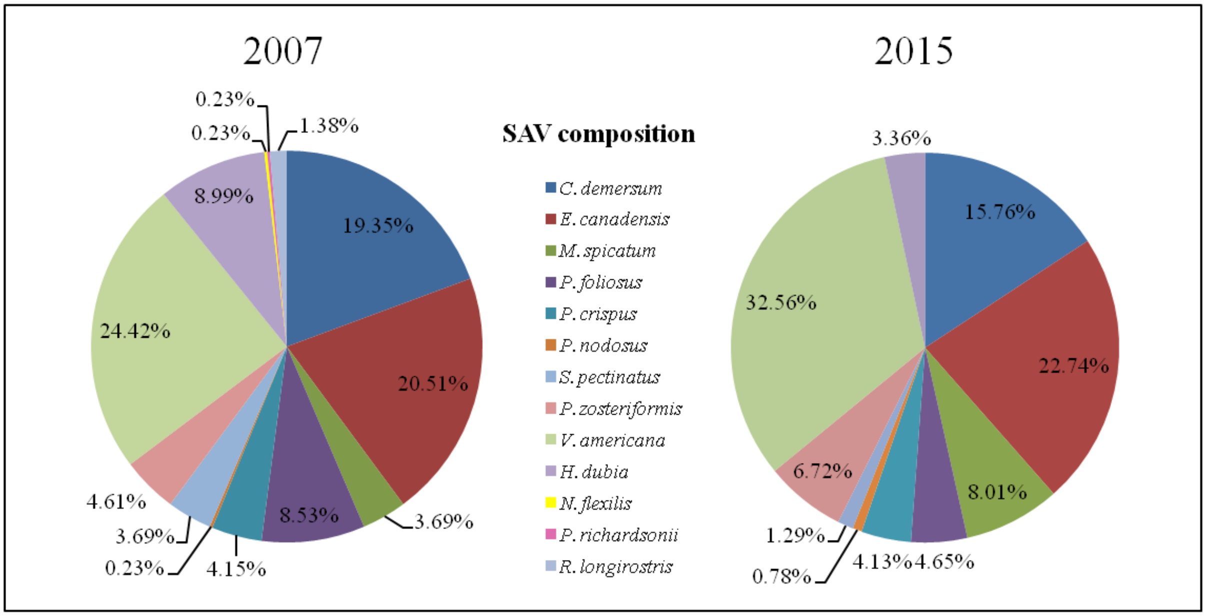

Figure 5. Composition of submersed aquatic vegetation species in Pool 8 of the Upper Mississippi River in 2007 and 2015.

Quantile regression revealed a unimodal association between total SAV abundance and the abundance of *B. tentaculata* for sites in the 95th quantile in both sampling years (Figure 6). Hence, *B. tentaculata* tended to be most abundant at sites with intermediate amounts of SAV and least abundant in areas of very low or very high SAV densities. The regression model indicated that *B. tentaculata* density was near zero at quantile values less than 0.95 in 2007 and less than 0.75 in 2015 (Table 2, Figure 6). Hence, intermediate densities of SAV were associated with the highest variance of *B. tentaculata* density suggesting that intermediate levels of SAV may set an upper bound on the abundance of *B. tentaculata*, and that other factors may prevent *B. tentaculata* from reaching high densities in such areas. Nevertheless, both sampling years showed that SAV density was an important factor for predicting *B. tentaculata* density at the upper and lower bounds of SAV.

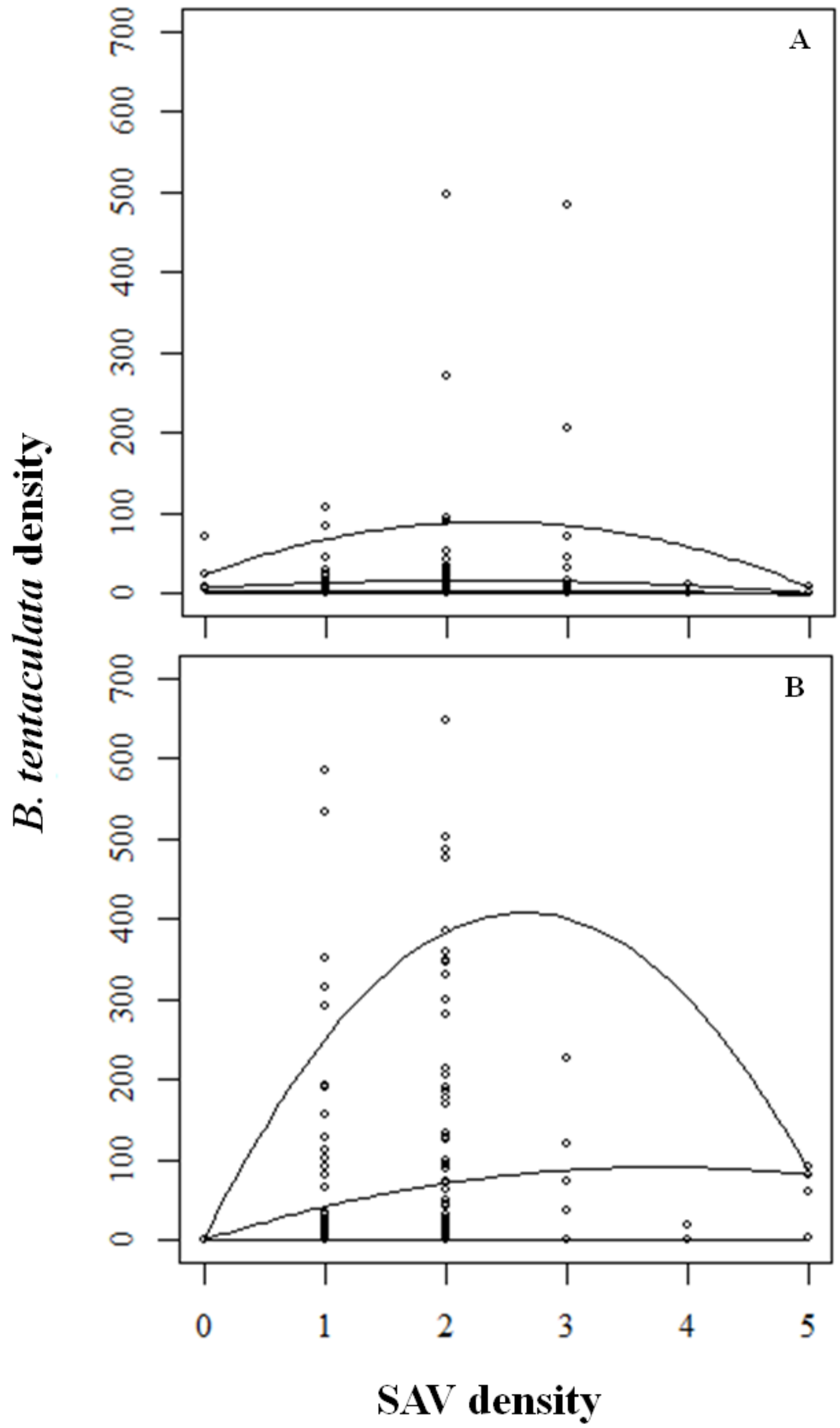

Figure 6. Quantile regression model between total submersed aquatic vegetation (SAV) density and *B. tentaculata* density in 2007 (A) and 2015 (B). Regression lines represent quantile values of 0.05, 0.25, 0.5, 0.75 and 0.95.

Table 2. Quantile regression model statistics for B. tentaculata density vs. submersed aquatic vegetation (SAV) density in 2007 and 2015 in Pool 8 of the Upper Mississippi River, n=115.

Year	Tau	Intercept	Slope 1	Slope 2
2007	0.05	0.0	0.0	0.0
	0.25	0.7	0.5	-0.2
	0.50	3.3	1.0	-0.3
	0.75	7.0	8.6	-2.0
	0.95	24.0	55.5	-11.7
2015	0.05	0.0	0.0	0.0
	0.25	0.0	0.0	0.0
	0.50	0.0	0.8	2.2
	0.75	0.0	48.2	-6.4
	0.95	0.0	309	-58.1

Spatial distribution maps indicate increases in *B. tentaculata* abundance in 2015 compared to 2007 (Figure 7). The highest snail densities were collected in the impounded region of Pool 8 where SAV densities were also the highest. Few *B. tentaculata* individuals were collected in upper Pool 8 in 2007 and none were found in 2015. Visual temporal changes in SAV densities were localized with differences occurring from site to site rather than on a pool-wide scale (Figure 8). The largest change in vegetation density occurred at Target Lake in upper Pool 8 as density declined from 2007 to 2015. This may correlate with the reduction in *B. tentaculata* that was observed in Target Lake (TL) in 2015.

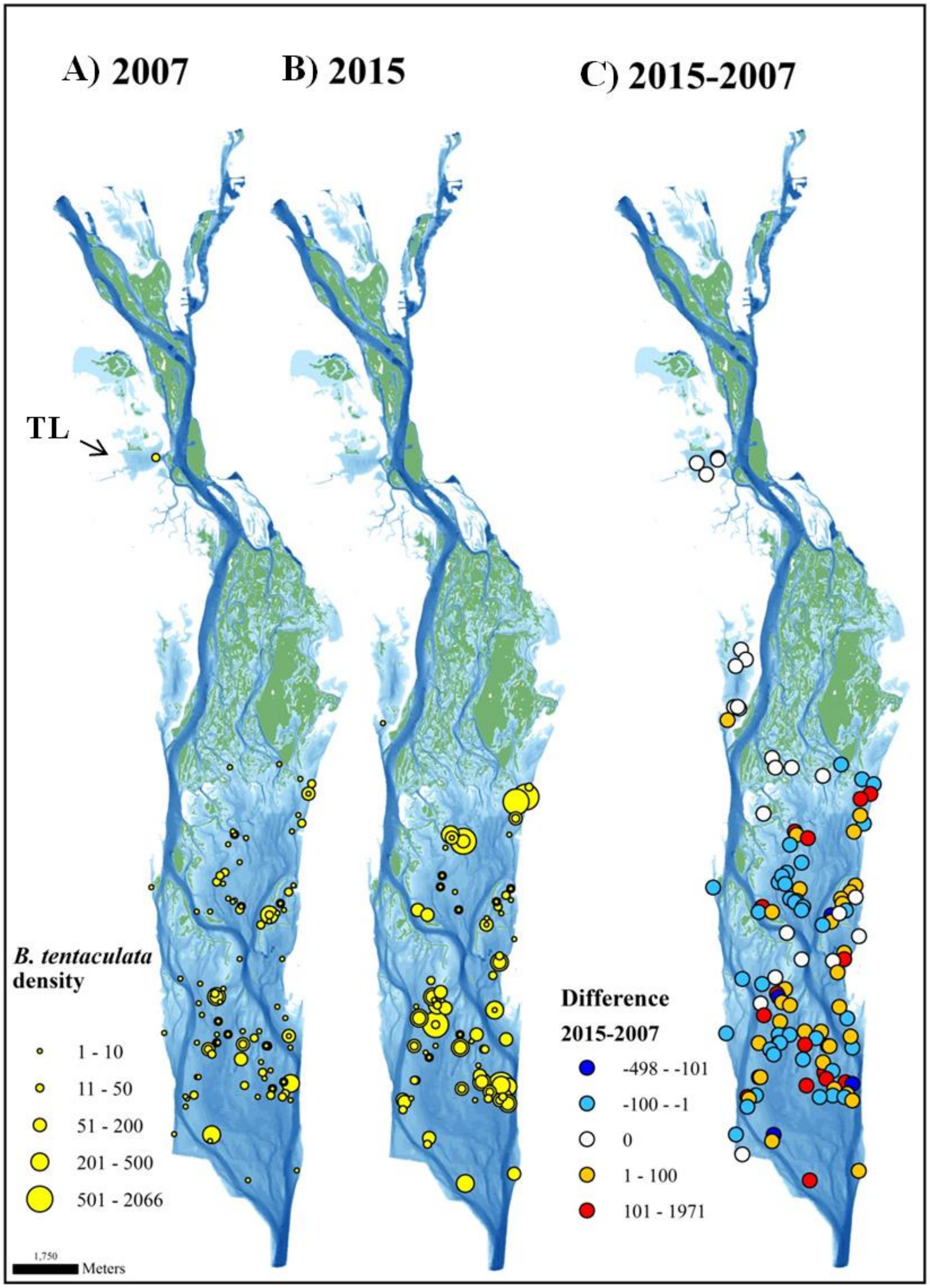

Figure 7. Mapped density of *B. tentaculata* in Pool 8 of the Upper Mississippi River in (A) 2007, (B) 2015 and (C) change in *B. tentaculata* density from 2007-2015.

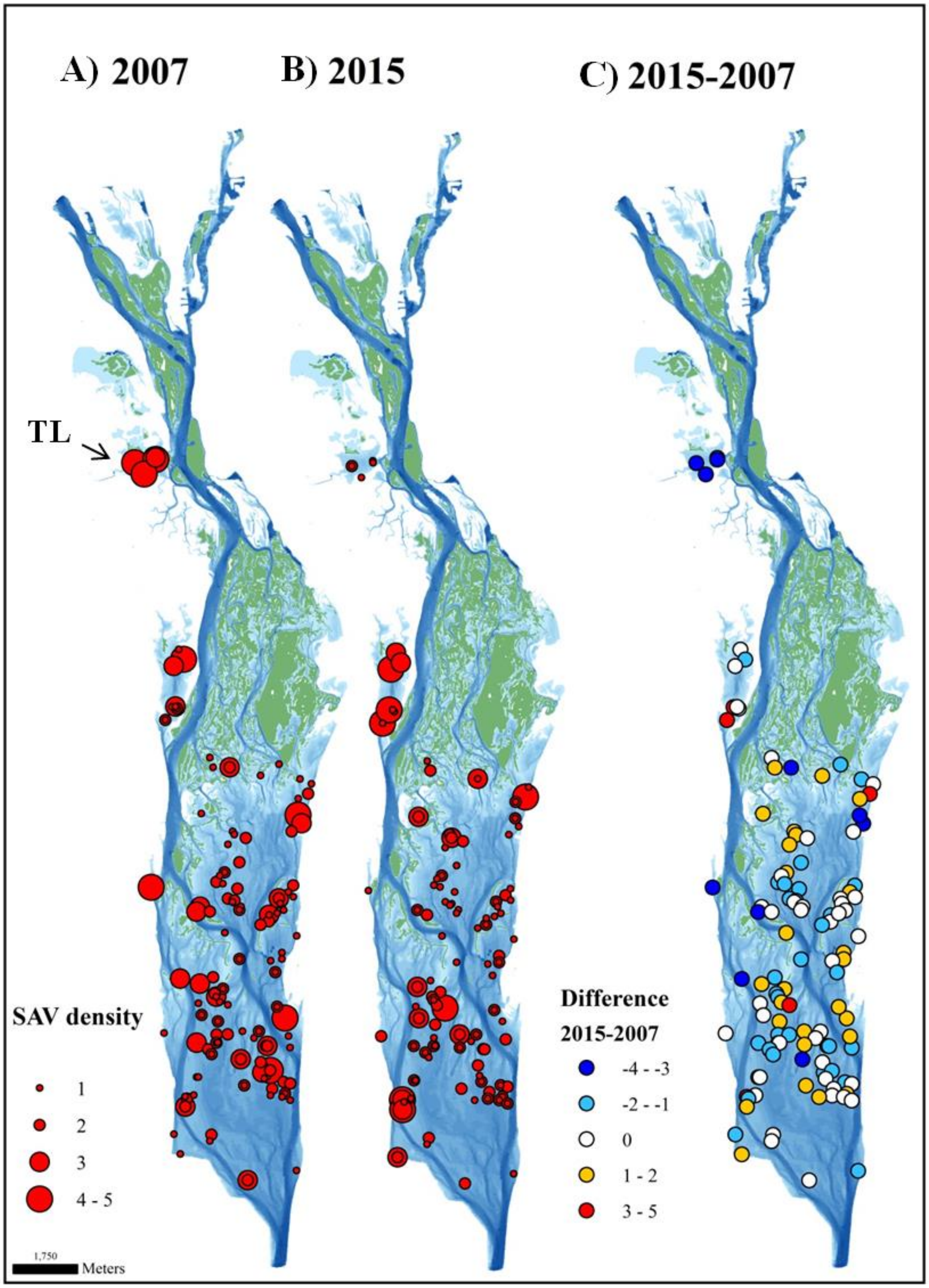

Figure 8. Mapped density of submersed aquatic vegetation (SAV) in Pool 8 of the Upper Mississippi River in (A) 2007, (B) 2015 and (C) change in SAV density from 2007-2015.

In both sampling years, spatial correlation declined as the distance between sampling locations increased for all vegetation species indicating that each species was distributed as patches (Figure 9). The spatial autocorrelation was positive for all vegetation species at distances less than 1000 meters. In 2007, *V. americana* and *C. demersum* were most highly correlated as indicated by the large nugget values and hence displayed the strongest degree of patchiness (Table 3). *Elodea canadensis* and total SAV also had relatively high nugget values, however the degree of patchiness was not as strong as for *V. americana* and *C. demersum*. The results for 2015 were similar to 2007 in regards to correlation and degree of patchiness for all vegetation species, however the scale of aggregation of *E. canadensis* declined. The degree of patchiness for total SAV declined from 2007 to 2015 as indicated by the net change of -0.06 for the nugget (Table 3). Conversely, the degree of patchiness increased for *V. americana* and *E. canadensis* in 2015. The spatial pattern of *B. tentaculata* more closely approximated the pattern of SAV (all species) as compared to any individual species for both sampling years. There was no spatial autocorrelation of *B. tentaculata* detected suggesting that these snails tended to be more randomly distributed throughout the study area. However, the cross correlogram revealed a positive correlation between *B. tentaculata* and SAV density at smaller lag distances although, as distance increased, the correlation became more random in 2015 (Figure 10). There was no clear spatial correlation between *B. tentaculata* and SAV density in 2007. The correlation values were random at both fine and large scales.

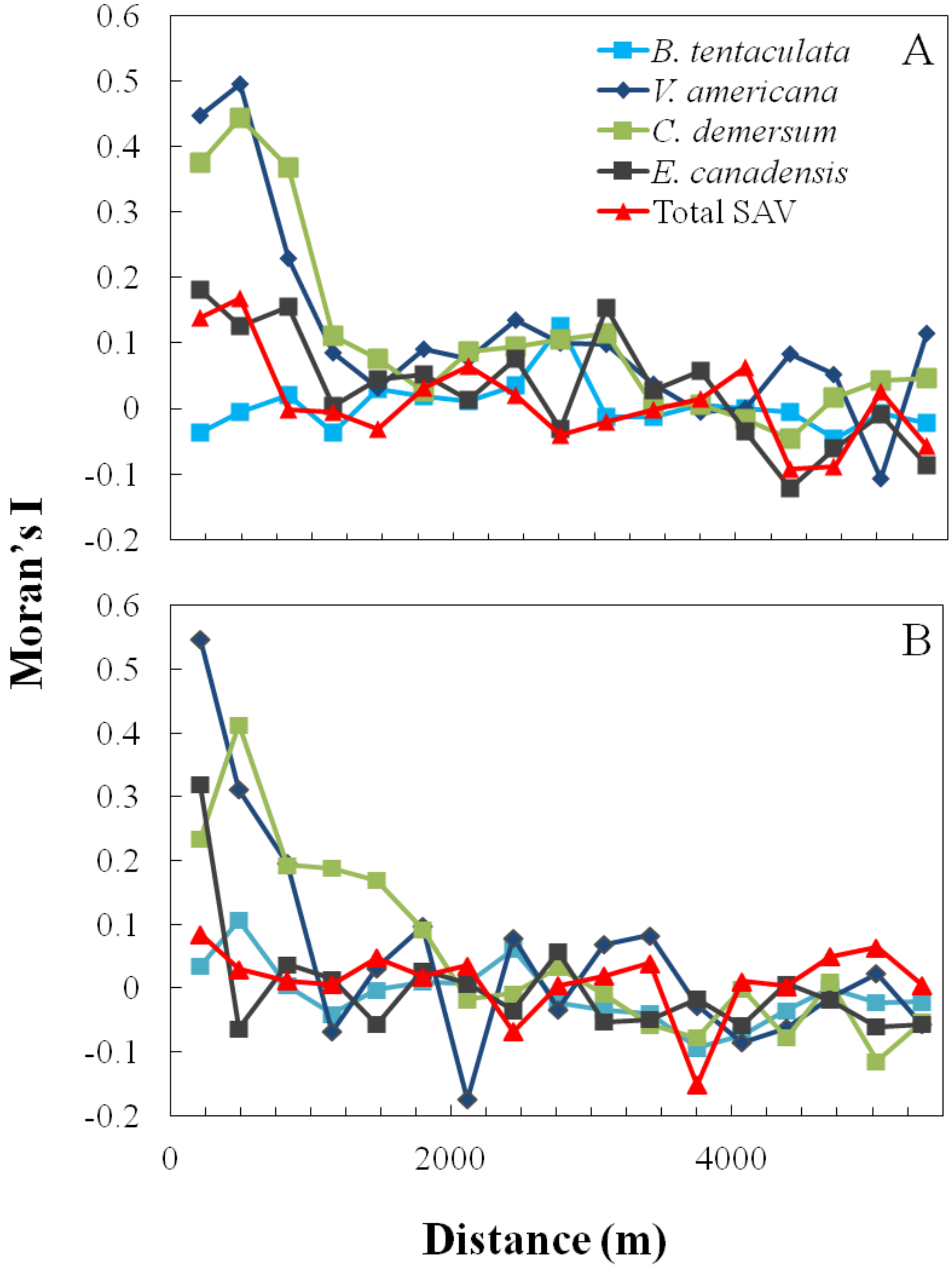

Figure 9. Spatial autocorrelation for vegetation species and *B. tentaculata* in (A) 2007 and (B) 2015 in Pool 8 of the Upper Mississippi River.

Table 3. Moran's I spatial autocorrelation for *B. tentaculata* and species of submersed aquatic vegetation in 2007 and 2015 in Pool 8 of the Upper Mississippi River.

	2007		2015	
Species	Nugget	Range	Nugget	Range
B. tentaculata	-0.04	212	0.03	831
Total SAV	0.14	831	0.08	831
C. demersum	0.38	1152	0.23	2111
E. canadensis	0.18	1152	0.32	489
M. spicatum	0.34	831	0.17	1466
P. foliosus	0.40	489	0.03	831
P. crispus	0.57	831	0.22	831
P. zosteriformes	0.20	1152	-0.02	831
P. nodosus	-0.09	831	-0.01	489
S. pectinatus	0.17	831	0.01	489
V. americana	0.45	1466	0.55	1152
H. dubia	0.13	1152	0.07	831

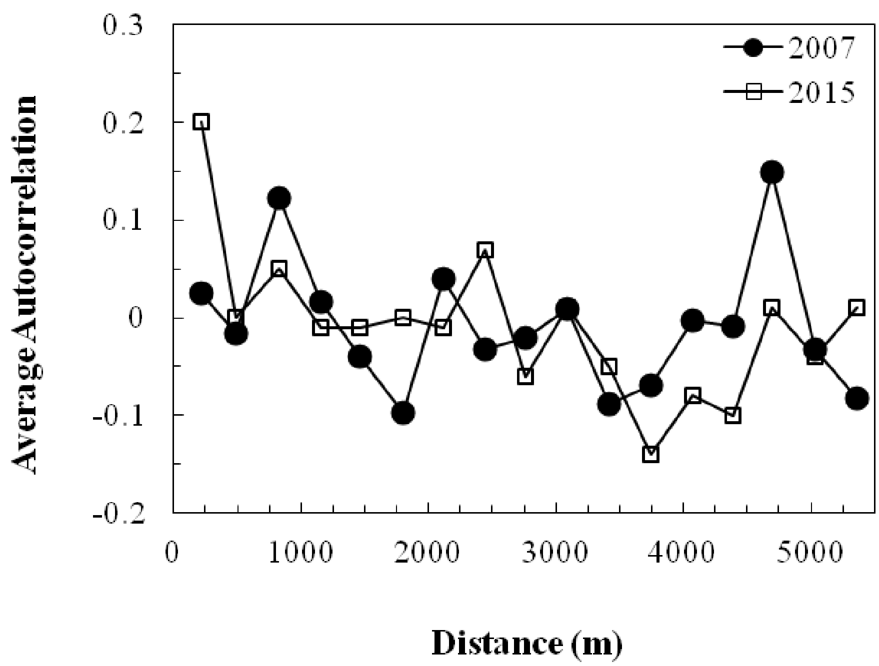

Figure 10. Cross correlogram of *B. tentaculata* and submersed aquatic vegetation in 2007 and 2015 in Pool 8 of the Upper Mississippi River.

DISCUSSION

My results suggest that *B. tentaculata* abundance has nearly quadrupled at the sites that were sampled in 2007 despite minor changes in vegetation abundance, distribution, and composition. Of all gastropod species collected, *B. tentaculata* abundance was the highest and it now appears to be a dominant species within the UMR gastropod assemblage. Since this invasive snail was discovered in the UMR in 2002, *B. tentaculata* has successfully found its niche among several other native species. Invasive species tend to be more tolerant of varying environmental conditions leading to the high success rate of these organisms. Wood *et al*. (2011) monitored the response of *B. tentaculata* and a native snail (*Physa gyrina*) to desiccation. They found that mature *B. tentaculata* were able to resist desiccation for longer periods of time compared to *P. gyrina*. A water-level drawdown issued in 2002 in Pool 8 may help explain why this recently discovered invasive snail has become the dominant gastropod species. While the purpose of the drawdown was to increase vegetation within the pool, a possible consequence was a disruption of native gastropod populations.

A shift in size distribution of *B. tentaculata* from a dominant adult size class in 2007 to a dominant juvenile size class in 2015 suggests enhanced recruitment within this population. This could be biologically important as snails less than 5 mm are considered juveniles and snails larger than approximately 5 mm are considered sexually mature adults (Richter, 2001). An increase in juvenile snails could indicate a growing population. Moreover, adult snails are typically infected by trematode parasites at a higher rate compared to juveniles. In addition, larger adult snails typically have higher infection intensities compared to smaller adult snails (Sandland *et al*., 2013). With mature adults typically developing within 1-2 years, future parasite populations will have the opportunity to thrive with a growing adult snail population. As juvenile snails transition into mature adults, the population size of these opportunistic parasites will likely grow. Another potential explanation for an increase of juvenile snails in 2015 may be seasonal delay due to significant flooding that occurred in Pool 8 in May, 2014. Rapid change in water levels due to flooding can have significant effects on water temperature and turbidity levels. Therefore, female *B. tentaculata* may have postponed laying their eggs due to unfavorable conditions.

Bithynia tentaculata tended to be most abundant and yet highly variable at sites with intermediate amounts of SAV suggesting that intermediate abundances may be necessary, but not sufficient for high abundances of *B. tentaculata*, other environmental variables may limit their abundance even in areas SAV is at intermediate levels. Areas without SAV are unlikely to support *B. tentaculata* due to our sampling method (we sampled snails that were attached to vegetation). However, it is less clear why dense SAV beds did not support high densities of *B. tentaculata*. One explanation for this could be that dense vegetation beds slow local velocity and therefore may not be ideal for filter feeding organisms such as *B. tentaculata* (Brendelberger *et al*., 1993; Madsen *et al*., 2001). These areas may also experience extremely low concentrations of dissolved oxygen at night, making it difficult for many macroinvertebrates to survive. These environmental factors may also negatively affect parasitic density and distribution as the free-swimming larval stages tend to be more vulnerable to extreme conditions. On the other hand, intermediate densities of aquatic vegetation may create ideal physical and chemical conditions which allow high densities of *B. tentaculata* and its parasites to establish. Several other studies have reported a positive relationship between vegetation density and macroinvertebrate density (Collier *et al*., 1999; Strayer and Malcom, 2007). The non-linear relationship between SAV and *B. tentaculata* and the other unmeasured confounding variables may explain why the spatial patterns of the two organisms differed and why electivity associations were often weak.

While changes in mean SAV density were found to be statistically significant between 2007 and 2015, biological significance does not seem likely. Changes in vegetation densities were localized with minor fluctuations occurring at a pool-wide scale. The change in composition of species was limited to minor fluctuations as well. In both years, *V. americana*, *C. demersum* and *E. canadensis* were the dominant vegetation species collected. These three species made up over 60% of the total vegetation species collected in Pool 8. These results are consistent with data collected through the U.S. Army Corps of Engineers' Upper Mississippi River Restoration (UMRR) Program Long Term Resource Monitoring (LTRM) element, as distributed by the U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. The vegetation component of the LTRM element also surveyed the impounded region of Pool 8 in 2007 and 2015 and found that *V. americana, C. demersum* and *E. canadensis* were the most common SAV species collected with little variation between the two sampling years.

The positive association between *V. americana* and *B. tentaculata* suggests that this SAV species could play a role in the distribution of *B. tentaculata* within Pool 8.

Vallisneria americana was the most abundant vegetation species collected in 2015 and also happens to be a preferred vegetation species for both *B. tentaculata* and waterfowl such as American coot and lesser scaup. Because of this, transmission of trematode parasites may be enhanced. Interestingly, American coot primarily feed on aquatic vegetation such as *V. americana* and incidentally ingest snails that reside on those plants while Lesser scaup are molluscivores and feed directly on these snails (Herrmann and Sorensen, 2009). Despite differences in feeding strategies, parasite transmission from snails to waterfowl is extremely successful in the UMR. American coot and Lesser scaup suffer the highest mortality rates in Pool 8.

Although electivity suggested a correlation with *V. americana*, the spatial pattern of *B. tentaculata* more closely approximated the pattern of all vegetation species (SAV) as compared to any individual macrophyte species. However, the fine-scale spatial distribution of *B. tentaculata* suggests that *B. tentaculata* may be more locally concentrated than the distribution of potential habitats. This indicates that other environmental variables may limit the distribution of *B. tentaculata*. It is possible that we did not sample at a scale that would properly determine the pattern of *B. tentaculata*. A similar study assessed variation of macroinvertebrates in various stream orders and found that small scale variation of abundance and patch size was common (Downes *et al*., 1993). A fine-scale assessment may be necessary to more effectively predict the distribution of *B. tentaculata* in this region.

Another possible explanation for fine-scale spatial distribution of *B. tentaculata* is parasite infection. This could result in behavioral changes which could lead to *B. tentaculata* aggregating at smaller scales thereby enhancing transmission. Alternatively, parasite dilution could occur in areas of high intermediate host abundance, resulting in fewer metacercariae per snail (Sandland *et al*., 2014; Lagrue and Poulin, 2015). This may indirectly benefit definitive hosts because waterfowl will have to forage on more snails in order to ingest the equivalent parasite dose as when feeding in low snail density areas.

Bithynia tentaculata were found in association with vegetation most often in the impounded region of Pool 8. This supports the findings of earlier work which reported that *B. tentaculata* prefer areas of low velocity (Richter, 2001). Impounded regions typically have low velocity and are ideal locations for filter feeding organisms. Increased surface area of plants can increase the tolerance or resistance to flow. For example, the leaves of *V. americana* are long and thin with little surface area, while *C. demersum* has a larger surface area with multiple leaflets per leaf (Eggers and Reed, 1997). Large vegetation patches create protective hummocks that can locally slow water velocity, allowing for more sensitive plants to establish (Haslam, 1978). Several islands were restored in lower Pool 8 allowing for vegetation to establish at high densities (Yin *et al*., 2008). As these areas are often sheltered from wind and current in addition to having substantial plant growth, they are typically inhabited by a variety of organisms seeking food and shelter including macroinvertebrates such as snails and waterfowl. These areas could be problematic as they have potential to increase transmission of parasites between intermediate snail hosts and definitive waterfowl hosts.

While there are numerous potential hosts that aid in the transmission of parasites to waterfowl, *B. tentaculata* is the only known first-intermediate host and it is therefore a necessary player in waterfowl disease on the UMR. It is essential to understand the environmental factors that determine *B. tentaculata* distribution as this information could potentially lead to improved management strategies in the future. Understanding the spatial distribution of *B. tentaculata* in relation to other habitat features, including submersed vegetation, and quantifying any further changes in the abundance and distribution of *B. tentaculata* and its parasites over time will be important for understanding the potential risks of disease transmission to waterfowl.

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