A Spiny Water Flea Invasion and Effects on the Zooplankton Community in Southern Green Bay, Lake Michigan

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By Casey Ann Merkle

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Abstract

The spiny water flea (Bythotrephes longimanus) is an invertebrate aquatic invasive species (AIS) in the Great Lakes that competes with native fish species for zooplankton, perhaps contributing to a decline of fish populations or changes in zooplankton communities in Lake Michigan. Bythotrephes produce two types of eggs, immediately hatching versus resting eggs which are tolerant to harsh conditions and allow for rapid dispersal. We determined Bythotrephes population density and population dynamics in Green Bay during the summer months of 2015 and 2016. Population dynamics were similar at both sites in Green Bay in each year, with peak population abundances in September of 2015 and late July in 2016. Resting eggs were produced by July 8 in 2015, and by June 17 in 2016; production continued into at least early October in both years, after which sampling ceased. Production rates for zooplankton and consumption rates for Bythotrephes were estimated in Green Bay and showed that consumption exceeded production at times. Zooplankton composition changed and abundance generally declined with an increase in Bythotrephes abundance. Sampling conducted by the Fox River Navigational System Authority did not observe Bythotrephes in 2015 and only on a single date at one site in 2016 in the Lower Fox River. Due to the negative effects Bythotrephes cause on zooplankton communities in Green Bay, population dynamics in Green Bay should be considered when managing the lock system along the Fox River to prevent further invasion upstream of the Lower Fox River and into Lake Winnebago.
Introduction

For a long period of time, humans have been responsible for causing extensive damage and expensive casualties to crucial ecological services, or ecosystem services. Despite some public recognition of the importance of ecosystem services, there is still a lack of political support to enforce management laws for environmental protection. This is in large part due to the imbalance between human benefits on a short-term versus long-term scale. In northeastern Wisconsin, the Fox-Wolf watershed has dramatically changed due to rapid industrialization and shows examples of human-mediated losses of ecosystem services from physical, chemical, and biological changes. The essential Great Lakes house many non-native species that pose a threat to river systems and inland lakes within the Fox-Wolf watershed, which can sometimes lead to harmful biological changes within local ecosystems. This study is focused on an invasive species, commonly known as the spiny water flea, to determine population dynamics and its effect on zooplankton communities in Green Bay which has been shown to lead to ecological and economic damage (Lehman 1988; Branstrator 1995, 2005; Yan et al. 2001; Vanderploeg 2002; Barbiero and Tuchman 2004; Strecker et al. 2006; Fernandez 2009; Walsh et al. 2016).

Location of Study

Due to the scouring and depositional action of vast continental mountains of glacial ice 10,000-15,000 years ago, we have a rich geographic landscape in North America that is spotted with thousands of inland lakes, complex river systems, and the magnificent Laurentian Great Lakes (Schindler and Vallentyne 2008). The Laurentian Great Lakes make up the largest freshwater resource in the world. Wisconsin’s economy flourished by using the Great Lakes waterways to provide access for international and national trade. One of the most well-known
areas of primarily industrial and agricultural use is the Fox-Wolf River Basin (Fig. 1). It drains over 15,500 square kilometers (6,000 square miles) and provides transportation routes throughout northeastern Wisconsin (Ball et al. 1985). Within this area is located the largest inland lake in Wisconsin, Lake Winnebago. It has a vast surface area of 557 square kilometers (215 square miles) and a mean depth of 4.7 meters. The water quality is low, with a Secchi depth measurement of about 1 meter (chlorophyll $a$ averages 59 mg/m$^3$). Nearshore is rocky and there are some rocky reefs offshore, while most of the bottom is fine sediment (Stelzer et al. 2008). The lake supports a thriving and diverse fish community, including lake sturgeon, freshwater drum, walleye, sauger, yellow perch, white bass, trout-perch, gizzard shad, and emerald shiner (Stelzer et al. 2008). Activities on and around Lake Winnebago include commercial and recreational fishing, recreational boating, swimming, and other water sports. The lake is also well known for its popular fishing and hosts an annual fishing tournament. Lake Winnebago’s high production and premier fisheries are due to a major input of nitrogen (N) and phosphorous (P) nutrients from residential and agricultural run-off. Lucky people will catch the largest walleye and sturgeon fishes in the country in this highly productive, inland lake.
From the northern outlet of Lake Winnebago, the Lower Fox River flows for over 62 kilometers (39 miles) before emptying into southern Green Bay (Fig. 2; Ball et al. 1985). The Lower Fox River is the major tributary to Lake Michigan and Green Bay. The river has an average flow of 125 m$^3$/sec and makes up less than 7% of the Fox-Wolf Watershed. Though it is a small percentage of the drainage basin, it is the area where the most severe deterioration of water quality exists (Sager and Wiersma 1972; Qualls et al. 2013). The river drains an area of 17,197 square kilometers (6,640 square miles) and enters the southern tip of the bay (Qualls et al. 2013). At the point where bay meets river, the mouth, there has historically been an extremely
low abundance of life indicated by the lack of bottom-dwelling organisms and low dissolved oxygen levels, which is due to the heavy sediment flowing from the Lower Fox River (Balch et al. 1956). In the mid 1800s (Fig. 3), the river was mainly used for transportation, water supply, and had an abundant supply of fish and game. Through the 1800s, a major player in boosting the economy around the Great Lakes was industrialization along the river. The Lower Fox River provided industrial water supply, commercial shipping, recreational boating, fishing, and swimming. Since the early 1900s through the 1970s it maintained its industrial and commercial uses, including a booming paper company, foundries, and waste water treatment plants. This led to catastrophic pollution issues that affected the entire watershed. Recreational activities disappeared due to the river’s overuse and poor condition, which compromised the river’s thriving tourist economy (Smith and Snell 1891). The Fox-Wolf Basin pollution was widespread and negatively impacted ecosystem health and the economy. Pollution that was created by anthropogenic mistreatment was carried downstream by the Lower Fox River from Lake Winnebago to dump contaminants straight into Green Bay.
Figure 2. Winnebago to Green Bay along the Fox River (FRNSA at http://www.foxlocks.org/the-locks/lock-system-overview/).
Figure 3. Timeline of environmental degradation and recovery in the Lower Fox River and Green Bay. Adapted from Qualls et al. (2013). BOD waste load allocation refers to demand of dissolved oxygen. Non-point source pollution includes agriculture and urban run-off.

The State of Wisconsin refers to Green Bay as an important vast water resource, and specifically states that water quality standards for rivers emptying into it should be as high as practicable (Ball et al. 1985). The International Joint Commission reported Green Bay the largest embayment of Lake Michigan and largest freshwater estuary in the world, 189 kilometers (118 miles) long and with a mean width of 37 kilometers (23 miles) (Richman et al. 1990). Green Bay is an elongated arm of Lake Michigan, separated by the Door County Peninsula (Ball et al. 1985). A man-made dredged channel that is 6 to 9 meters deep and was created for easy boat access runs for almost 18 kilometers (11 miles) from the mouth of the Lower Fox River (Ball et al. 1985). The bottom material is a mixture of silt, sand, gravel and rock. Silt and sand make up the primary material of the variable depth dredged (navigational) channel. Elsewhere in the bay, depths range from 3 to 4.5 meters deep (Ball et al. 1985). Green Bay has one of the most productive Great Lakes fisheries, home to many species of fish including yellow perch and
walleye. This is due to nutrients flowing from Lake Winnebago through the Lower Fox River and collecting into the bay (Fig. 4). Groups that benefit from the bay include sport and commercial fishermen, industries, farmers, municipal sewage, energy utilities, commercial shippers, shoreline residents, recreational boaters, swimmers, land developers, and land fillers. In some cases, these beneficiaries (especially industrial corporations) have taken advantage of this resource and deteriorated the condition of the bay (Ball et al. 1985). Historical evidence shows that during the 19th and 20th centuries, exacerbated by municipal and industrial wastewater discharges, Green Bay was in a highly over productive condition and contaminated with toxins (Ball et al. 1985). Since the early 1960s, this condition has led to oxygen depletion and harmful algal blooms in the lower Fox River and southern Green Bay (Sager and Wiersma 1972). In the 1990s environmental policy actions started a ripple of change through regulating pollutants and cleaning up the river and bay. Phosphorous loading from municipalities was cut by 50% (Fig. 5; Baumgart 2005). This brought some revival to bottom fauna, plankton, and fish in the river and the mouth of the bay again (Qualls et al. 2013). However, since the 1980s, yellow perch recruitment has decreased leading to smaller populations in succeeding years (Wisconsin Department of Natural Resources 2015). A report on the current status of the Fox-Wolf Basin states that the levels of ammonia and dissolved oxygen (DO) have improved since the 1970’s (Qualls et al. 2013). Dredging solids from the river continues because there is an excess of sediment loading, which causes poor DO conditions for aquatic life. Excess sediment suggests that better land use practices need to be implemented to reduce the amount of run-off such as limiting fertilizers, loose dirt, and construction. Other concerns include toxic algal-blooms and increased levels of toxins from anthropogenic sources (e.g. PCBs, dioxins, DDT, arsenic, and mercury). Warnings are in place that caution people to avoid consumption of fish from Green
Bay due to the high levels of toxins found in the bay. Finally, biological invasions from non-native organisms in Green Bay present a major issue to the local ecosystems of Lake Winnebago, the Lower Fox River, and southern Green Bay (Qualls et al. 2013).

Figure 4. Aerial photo of the Lower Fox River mouth on April 12, 2011. Adapted from Qualls et al. (2013).
Aquatic Invasive Species

Aquatic Invasive Species (AIS) are a global environmental problem, and highly concentrated in the Laurentian Great Lakes basin with at least 182 non-indigenous species recorded since 1840 (Mills et al. 1994; Holeck et al. 2004; Ricciardi 2006). Invasions from non-native species into the Fox River from Green Bay are a challenging problem for northeastern Wisconsin aquatic ecosystems (Cochran 1993; 1994). Due to Wisconsin’s abundance of freshwater inland lakes and river systems, immediate attention must be aimed at preventing any secondary spread from Lake Michigan, a major source of invasive species. Secondary spread is when non-native species that have invaded a major area (e.g. the Great Lakes) spread into
smaller inland lakes (e.g. Lake Winnebago) and streams (e.g. the Lower Fox River) in the surrounding region (Vander Zanden and Olden 2008).

There are many challenges that come with preventing secondary spread of an invasive species. But, it is important to critically assess the options for prevention, because the consequences of AIS expansions range from catastrophic to having no effect. Determining what sort of impacts result from AIS has been a continuous area of interest. One negative impact includes AIS influencing trophic structure and dynamics within a local ecosystem (MacIsaac 2001; Vanderploeg et al. 2002; Mills et al. 2003; Yurista 2010). It has also been shown that freshwater ecosystems are vulnerable to impacts from AIS due to their isolation and nearby human activities (Ricciardi and Rasmussen 1999). Shifts in trophic structure and dynamics lead to a manipulation of predator-prey interactions and can increase competition for food among and within native and non-native species. This can lead to serious negative ecological disturbance of ecosystem structure.

In the early 1990s the zebra mussel invaded Green Bay and began changing trophic level interactions through an increased dominance of blue-green algae, cyanobacteria (De Stasio et al. 2014). In other locations in the Great Lakes this biological invasion led to increased water clarity due to their high filtering rates of water by zebra mussels (Higgins and Vander Zanden 2010). However, an increase in water clarity has not been observed in Green Bay following the invasion (De Stasio et al. 2008, 2014). Since the introduction of another AIS, the spiny water flea, in Lake Michigan in 1986, the expected increases in water clarity following invasion by zebra mussels has not occurred in Green Bay which may be due to the spiny water flea disrupting trophic level interactions. High levels of nutrient run-off (P and N) also have effects on driving water clarity by increasing algal growth (Schindler and Vallentyne 2008), so the lack of increases in water
clarity in Green Bay following invasion by zebra mussels could also have been affected by the high nutrient loading of this system.

Like the zebra mussel invasion in Green Bay, there is a long list of invasive species that have successfully established populations in Lake Michigan, Green Bay, and the Lower Fox River. Invasive species that end up in Lake Michigan from oceanic vessels via ballast water are susceptible to secondary spread across Wisconsin (Ricciardi 2006). The historical industrialization of the Fox-Wolf watershed had high levels of human activity, which typically is positively correlated with invasions (Ricciardi 2001). There is a record of notable invasive species already reported in lower Green Bay, including the sea lamprey, zebra mussel, round goby, common carp, and spiny water flea. Zebra mussels, round goby, and the common carp have already established themselves in the Lower Fox River, and many invasive species which have yet to establish themselves pose a high invasion risk (De Stasio 2013; Kiehnau 2015). Because the Fox River flows into Green Bay, it acts as a corridor facilitating the spread of invasive species to other areas of the state.

*Fox River Navigational System Authority*

To prevent the spread of invasive species upstream of the Lower Fox River, US Army Corps of Engineers built a permanent barrier along the river in the 1980s. This was at the request of the Department of Natural Resources (DNR) so the sea lamprey, a parasitic fish, would not be able to disperse upstream to Lake Winnebago from Green Bay and bring catastrophe to native fish species (Wisconsin State Database 2008). The Fox River Navigational System Authority (FRNSA) was created in 2001 by the State of Wisconsin, to oversee locks, harbors, real property, structures, and facilities related to navigation that are located on or near the Fox River. This
navigational system was under the control and ownership of the federal government from 1 April 1984, until management was transferred to the state of Wisconsin in 2004 (Wisconsin State Database 2008; FRNSA 2016). One major goal of the navigational system is to prevent the spread of the sea lamprey by maintaining the sea lamprey barrier at the Rapide Croche lock. This action was recommended by the DNR which oversees the authority and ultimately makes approvals of management decisions (Wisconsin State Database 2008). Another concern is to restore the thriving tourist economy that existed before the 1970s, when recreational boaters could pass through the entire Fox River from Green Bay to Lake Winnebago. Overall, the goal of the managers is to preserve the public rights in the Fox River, ensure public safety, and protect life, health, and property (FRNSA 2016).

Currently, one of the greatest threats leading to dispersal of invasive species lies within the fishing community. Boats are a possible vector for propagate dispersal by transporting water that can easily house AIS. Boaters and policy makers that essentially use the rivers as roads, are making poor management decisions that could open pathways allowing flow of invasive species from Lake Michigan up the Fox River into inland lakes of Wisconsin. The spiny water flea, a predatory zooplankton in Green Bay is potentially one of the fore mentioned invasive species, which could spread upstream in the Fox River from Green Bay to Lake Winnebago. This study is mainly concerned with risks of invasion by spiny water flea in Lake Winnebago and other inland lakes upstream of the lower Fox River. This body of freshwater is at risk from invasion by spiny water flea, along with many other invasive species such as the sea lamprey (*Petromyzon marinus*) and quagga mussels (*Dreissena bugensis*), due to its proximity to Lake Michigan and direct pathway through the Fox River leading from southern Green Bay (Fig. 2). As of 2016, all locks on the lower Fox River except for Rapide Croche and Menasha remain open. In 2015, the
Menasha lock closed at the request of the DNR due to reported sightings of the invasive fish species the round goby in the Fox River at Little Lake Butte Des Morts (Ebert 2015). Rapide Croche lock is and will remain closed at the request of the DNR to maintain a barrier against sea lamprey. Finally, the Little Chute locks are closed currently awaiting repairs (FRNSA 2017). Boaters can get from Kaukana Lock #1 to Menasha and Little Lake Butte Des Morts (Fig. 2). If spiny water flea becomes established in Little Lake Butte Des Morts, the probability for it to successfully colonize Lake Winnebago increases and makes this “stepping-stone” an immediate concern.

Procedures to remove invasive species from boats and gear have been exhaustively tested and continue to be improved upon, but management issues arise when these tests are not implemented appropriately (Beyer 2009). Thus, transferring boats over the locks along the Lower Fox River is feasible and preventive measures are available to consider for managers to reach a resolution. Mainly, if locks are opened there has to be a decontamination process implemented that will not allow invasive species to cross the barrier. A project proposed by FRNSA is the Rapide Croche Lift Construction, which was approved and considered “dependant on funding”, will permit boats to be transferred over the lock that is permanently sealed to prevent invasive aquatic species from Lake Michigan passing upstream to Lake Winnebago. At this lock, there will be constructed a boat lift/transfer and cleaning station using a 43°C hot water cleansing chamber and flushing system, which meets the requirements for decontaminating aquatic invasive species (Beyer 2009; FRNSA 2016). However, management has considered constructing a boat lift over the Menasha lock as well, but without a cleaning station, which would focus on keeping the round goby and other future invasive fish species out of Lake Winnebago (FRNSA 2016). Since, spiny water flea resting eggs are tolerant to desiccation, a
simple transfer of boats without cleaning would facilitate their dispersal over the barrier at Menasha. Methods that implement boat trailer and equipment decontamination are strongly recommended to ensure that this invasive species does not become established in Lake Winnebago.

Ecological Consequences

The issue with opening the locks on the Lower Fox River is that it would allow a dispersal corridor to open for invasive species from Green Bay to Lake Winnebago. This will have major negative impacts on both the ecology and economics of the region. Aquatic ecosystems are fragile. Environmental and biological factors play crucial roles in such a fragile system stable. The complexity of aquatic systems is an impressive network of species relationships (Fig. 6) within the community. The network is set up by a system of trophic levels, primary producers, primary consumers, secondary consumers, and so on. Aquatic ecosystems are also dynamic in many ways. Turnover rates, which are the replacement of nutrients or the time it takes for energy to move through trophic levels back to primary producers, are subject to changes in any trophic level. The basic web of an aquatic ecosystem starts with photosynthesis by algae, the primary producers. Algae provide energy and nutrients for the herbivores, primarily microcrustacean zooplankton like *Daphnia*, which is an important genus of zooplankton because they are efficient algae grazers. Tiny zooplankton are consumed by the small and juvenile fish, which are then consumed by larger fish. In Lake Winnebago and Lower Green Bay, the larger fish and major players important to sport fishing are lake sturgeon, white bass, walleye and yellow perch (Ball et al. 1985; Probst and Cooper 2011).
Figure 6. Trophic interactions typically seen in Green Bay or Lake Winnebago. Modified from Kitchell (1993).

*Trophic Level and Eutrophication*

Trophic status is used to categorize lakes based on their productivity, which include low, medium, high, and extreme productivity defined as oligotrophic, mesotrophic, eutrophic, and hypereutrophic, respectively. Many factors contribute to cultural eutrophication, which is the over-fertilization of lakes and rivers with P and N caused by humans. Anthropogenic developments causing this phenomenon range from conversion to water mains, flush toilets, and sewage drains to land conversion destroying wetlands, riparian zones, and native vegetation. Also, increasing fertilizer production and use, as well as expanded livestock holding areas, and
waste processing have all contributed to freshwater eutrophication (Schindler and Vallentyne 2008). Invasive species are also included in this concept and can negatively affect trophic interactions in a food web through a trophic cascade that leads to eutrophication. If a step in the trophic web is removed or added (i.e. addition of invasive predator), the lake will become eutrophic without modifying nutrient loading (Schindler and Vallentyne 2008). Nutrient loading can occur with high levels of P, which often is the main nutrient driving algae production. This nutrient is required for algal growth but in large amounts, often caused by land run-off and wastewater discharges, leads to the overproduction of algae, known as eutrophication. The excessive algae growth creates a thick mat on the surface of the lake and blocks sunlight from supporting life under the surface. As algae settle to the bottom of the lake, oxygen is consumed for the process of decomposition. This causes low dissolved oxygen levels and threatens fish and invertebrates in the bottom waters and sediments, as has been documented in lower Green Bay (Wisconsin Department of Natural Resources 1993; Qualls et al. 2013).

The interactions between the upper trophic levels play a role in determining algal abundance and diversity in Green Bay, Lake Michigan (Porter 1977; Richman et al. 1984, 1990; Lehman and Sandgren 1990; Sager and Richman 1991). Bottom-Up control greatly influences the interactions between the trophic levels (Lampert and Sommer 1997; Dodson 2005). This hypothesis explains that effects are seen moving up the ladder of the trophic web. For example, an increase in nutrient input would lead to an increase in algal abundance, thus facilitating an increase in zooplankton, then planktivorous fish, and upwards. Adversely, the Top-Down effect hypothesizes that adjacent trophic levels are negatively correlated and effects can cascade down through food webs (Lampert and Sommer 1997; Dodson 2005). As an example, recreational fishing often decreases the abundance of planktivorous fish. This relieves predation pressure on
zooplankton, and leads to an increase in zooplankton with a decrease in algal abundance. Both affect the trophic web in an aquatic system, as seen by the two theoretical examples given (Dodson 2005). If an invasive predator disrupts a given trophic level it can create complex interactions among trophic levels above and below its newly attained trophic position.

**Ecosystem Services**

The reason governments and managers should be concerned about this issue is because eutrophication affects a myriad of services from which humans benefit. These natural services are termed ecosystem services, or ecological services. Freshwater lakes offer ecosystem services such as clarifying water and providing space for agricultural run-off, recreational boaters and commercial and recreational fishing, all of which greatly benefit humans. Cultural eutrophication affects ecosystem services because it increases algal growth while decreasing water clarity and oxygen levels in the bottom-sediment which are necessary for bottom-dwelling life like lake sturgeon, a big game fish (Fig. 10).

In the Fox River and Lower Green Bay oxygen in the water was at an all-time low during a 35-year period before 1972 (Ball et al. 1985). This was mainly due to the increase in industrial waste from the Fox River paper mills which resulted in consumption of oxygen by bacteria in the water (Ball et al. 1985). For a while waste disposal was modified to lessen its contribution to depleted oxygen levels (Ball et al. 1985). However, recent studies show that depletion of bottom oxygen is a problem in southern Green Bay, perhaps due to the interaction of warmer temperatures, changes in wind patterns and ecological changes following the invasion by an invasive species, the zebra mussel (Qualls et al. 2013).
This race has not stopped for the environment. It seems that once one problem is fixed or shows improvements, another is knocking on the door. Aquatic ecosystems are at risk by the increasing threat of some invasive species that are a contributing factor to eutrophication through a trophic cascade (Walsh et al. 2016). Recently spiny water flea invasions have been studied to determine their ecological and economic impact. A study by Walsh et al. (2016) determined that post-invasion of the spiny water flea, water clarity decreased rapidly in Lake Mendota. This provides an example of how spiny water fleas impact inland freshwater ecosystems. The more information we collect on ecosystem impacts caused by the spiny water flea and other AIS, the clearer it becomes that we must prevent their spread from southern Green Bay, and possible subsequent decimation of the Lower Fox River and Lake Winnebago.

*Bythotrephes longimanus*

*Bythotrephes longimanus*, commonly known as the spiny water flea, is a large voracious predator relative to its prey zooplankton, which includes *Daphnia* (Fig. 7; Lehman 1987, 1988; Evans 1988; Lehman and Branstrator 1995; Lehman and Cáceres 1993). Formerly it had been known as *B. cederstroemii*, but further molecular analysis confirmed that all North American specimens are *Bythotrephes longimanus* (Therriault et al. 2002). The US Geological Survey in 2017 reported that this originally Eurasian cladoceran is successfully established in North America in the Laurentian Great Lakes and in basins across Wisconsin, Minnesota, Michigan, Ohio, and Illinois (Bur et al. 1986; Lehman 1987; Mills et al. 1994; Yan and Pawson 1997; US Geological Survey 2017). *Bythotrephes* reproduces primarily via parthenogenesis, and its generation time is 11-14 days at temperatures from 13-16.5 °C (Fig. 8; Mordukhai-Boltovskaya 1957; Lehman 1987). An important life cycle adaptation for *Bythotrephes* is their ability to
switch between sexual and asexual reproduction during certain times of the year. Sexual reproduction creates resting eggs that range from 0.4-0.5 mm in diameter with a golden-brown color. Resting eggs are known to tolerate a wide range of conditions, including temporary drying and even passage through the guts of fish (Jarnagin et al. 2000; Branstrator et al. 2013). Since they are desiccation-resistant, they have a higher chance of dispersal via fishing boats, gear, and buckets. Furthermore, there need only be one resting egg to establish a population due to subsequent asexual reproduction following hatching (Brown and Branstrator 2011). This is a key attribute that drives the successful range expansion of *Bythotrephes*.

Figure 7. *Bythotrephes* adult female carrying resting eggs.
Figure 8. *Bythotrephes* in instar stages a) Instar I, b) Instar II, c) Instar III. Adapted from Yurista (1992).

Distinguishing characteristics of *Bythotrephes* are the conspicuous cyclops feature of its eye structure and the unusually long tail-spine. It has been identified to be a preferred food item of whitefish (*Coregonus*), trout (*Salmo*), arctic char (*Salvelinus*), and perch (*Perca*), which is possibly due to the obvious eye spot (Fitzmaurice 1979; Langeland 1978). The long tail-spine may explain why small juvenile fish tend to avoid it as a food item (Barnhisel 1991). Preferred prey of *Bythotrephes* include *Daphnia* (Lehman 1991; Pothoven et al. 2003) and other large cladocerans (>2.0mm) (Shulz and Yurista 1999).

Since the invasion of *Bythotrephes* into the Laurentian Great Lakes in 1984, there have been documented populations in 90 inland lakes across the Midwest, USA and Ontario, Canada (Bur et al. 1986; Berg and Garton 1988; Strecker et al. 2006). *Bythotrephes* were first reported from Lake Michigan in 1986 (Lehman 1987). In Wisconsin, there had been documented
sightings of *Bythotrephes* in 13 inland lakes as of 2016 (WI DNR 2017). Investigations within aquatic systems have documented *Bythotrephes* impact on zooplankton community structure and dynamics (Yan et al. 2001; Vanderploeg et al. 2002; Foster and Sprules 2009). Other studies show their impacts on ecosystem services. Walsh et al. (2016) compared conditions in Lake Mendota (Madison, Wisconsin), between post-invasion and pre-invasion time periods and discovered that since the detection of *Bythotrephes* in 2009, average water clarity in Lake Mendota has declined along with a reduction in *Daphnia* biomass. This loss of herbivorous zooplankton creates unfavorable, eutrophic conditions which negatively impacts both the recreational and ecological functions of the lake.

Evidence has been collected on the effects of the *Bythotrephes* driving changes in important ecosystem functions such as predator-prey interactions, prey-availability, and zooplankton species composition which lead to changes in ecosystem health (Sprules et al. 1990; Lehman 1991; Dumitru et al. 2001; Yurista et al. 2010). Ecosystem functions have declined in Green Bay, but it is not yet fully understood whether *Bythotrephes* is a contributing factor to the decline. The present study is the first to examine the effects from *Bythotrephes* on zooplankton communities in southern Green Bay.

Zooplankton are the primary consumers in the trophic web of aquatic ecosystems. Most are herbivores, such as *Daphnia*, while much larger zooplankton, such as *Bythotrephes*, are planktivorous. In Green Bay, zooplankton communities play an important role in driving the decrease in algal abundance (Mackey 1982; Sager and Richman 1991). Species that are abundant in Green Bay include *Bosmina, Chydorus, Daphnidae, Cyclopoida* and *Calanoida* (Fig. 9; Balch et al. 1956; Mackey, 1982; Beranek 2007). However, *Bythotrephes* invasions are changing zooplankton community structure in freshwater lakes (Sprules et al. 1990; Dumitru et al. 2001;
Yan et al. 2001) and consequently we would expect the abundance of *Daphnia* in Green Bay to decrease over time since the invasion of *Bythotrephes*.

![Graph showing percent biovolume of different zooplankton groups from 1986 to 2006 at Lower Green Bay.](image)


**Study Goals**

This study focuses on the effects on the zooplankton community from predator-prey interactions driven by the introduction of *Bythotrephes* into southern Green Bay, Lake Michigan (Fig. 10). Invertebrate planktivores do not exclusively drive shifts in zooplankton community composition, but they have been shown to be one leading factor (Hall et al. 1976; Gannon and Stemberger 1978; Lehman 1987, 1988, 1991; Branstrator 2005; Pangle et al. 2007; Rennie et al. 2011; Kelley 2013; Pothoven and Höök 2014). Under conditions of nutrient enrichment, as seen in Green Bay and the Fox River, the average size of zooplankton will decrease to smaller species
with simpler life history traits and rapid reproduction (Gannon and Stemberger 1978). A combination of eutrophication and increased predation pressure from *Bythotrephes* will result in changes to zooplankton community structure, causing an ecological shift towards a less diverse array of zooplankton species in Green Bay.

![Trophic web interactions of a highly productive system with the introduction of Bythotrephes](image)

**Figure 10.** Trophic web interactions of a highly productive system with the introduction of *Bythotrephes*. Adapted from Walsh (2016).

Furthermore, we specifically determined when during the summer *Bythotrephes* produced resting eggs, due to the concern that resting eggs could be carried upstream via boat movement through the river. This information will be helpful to managers at the Wisconsin DNR and FRNSA when making decisions to prevent the dispersal of this invasive species. We also
compared zooplankton community structure pre- and post-invasion of *Bythotrephes* in Green Bay, and assessed the impact of *Bythotrephes* on potential zooplankton prey using a bioenergetics model of consumption and prey production. Due to similarities between Lake Winnebago and Green Bay, results from this study are representative of the potential effects on zooplankton communities following a future invasion in Lake Winnebago by *Bythotrephes*. As we gain more knowledge on the ecology of individual species, we will be better able to understand community ecology in aquatic ecosystems and the cascading effects of an invasive predator like the spiny water flea. Due to the negative effects *Bythotrephes* cause on zooplankton communities in Green Bay, these results should be considered when managing the lock system along the Fox River.

**Methods**

*Sample Sites and Dates*

We obtained samples from two sites located in southern Green Bay, Lake Michigan, established during previous research programs (Fig. 11; De Stasio and Richman 1998; De Stasio et al. 2008, 2014). GB-1A is located at N 44° 32.95`, W 87° 59.89` and GB-2 is at N 44° 34.82`, W 87° 58.73`. Sampling took place from June-October 2015 and May-October 2016. We collected samples approximately bi-weekly at each site. These stations are less than 3 m deep and represent the shallow and well-mixed conditions that regularly occur in the inner-bay (De Stasio et al. 2014). Duplicate oblique plankton tows were performed beneath the surface to the point where it was not visible (50cm diameter opening, 200cm length, 250µm mesh; Aquatic Research Instruments, Hope, ID). The net was attached to the back of the boat and towed for a
set amount of time (5min or 3min) at a constant speed (2mph) using a timer and GPS unit to track speed and time. The sample dates for the summer of 2015 at GB-1A with 1 replicate were 25 June, 29 June, 8 July, 13 July, 16 July, 28 July. Sample dates with two replicates were 22 July, 3 August, 17 August, 2 September, 19 September, and 2 October. The same dates were sampled at GB-2 in 2015 as GB-1A with 2 replicates, except on 8 July with 1 replicate at GB-2. Sampling in 2016 took place at both sites on 31 May (GB-1A only), 17 June, 28 June, 12 July, 26 July, 9 August, 23 August, 8 September, 19 September (GB-1A only), 20 September (GB-2 only), and 3 October (all with 2 replicates). In addition, on a single date in 2015, tows were collected in the South Bay Marina in June to check for presence of spiny water fleas (De Stasio et al. 2015). Samples were held live in closed 2L containers and transported to the Lawrence University laboratory facility in accordance with WI Administrative Code NR 40 and all applicable permitting requirements. Animals and potentially contaminated water was maintained in the laboratory at Lawrence University and prevented from release into natural waterways or public water treatment system at all times (De Stasio et al. 2015). Surface temperatures on each sampling date were gathered for Green Bay (Sea Temperature from http://seatemperature.info/green-bay-water-temperature.html).
Figure 11. Green Bay, Lake Michigan with sampling locations GB-1A and GB-2 highlighted. Adapted from De Stasio et al. (2008).
Laboratory Observations

Upon return to the laboratory the same day sample volume was reduced by straining through a mesh cup (250μm mesh) and preserved in 70% denatured alcohol or 4% formaldehyde. For counting, samples were again rinsed through the mesh to remove preservative, transferred to a beaker and diluted with deionized water. Wetzel (1979) was referred to for assistance in procedural matters.

Bythotrephes Enumeration

Entire samples were enumerated if density of Bythotrephes was low. If the entire sample contained too many individuals to count reliably (>300 specimens) then it was subsampled using a Folsom Plankton Sample Splitter (Wildco Inc., Yulee, FL) using liquid dishwashing soap to eliminate surface tension. Subsamples were counted at 10X - 40X magnification using a dissecting microscope (Nikon Instruments Inc.) using five categories that correspond with life stages of Bythotrephes (juveniles, males, females with no eggs, females with immediately hatching eggs/embryos, females with resting eggs). Loose resting eggs were enumerated also. If an animal carried a resting egg in the brood sac it was transferred to a labeled 12-well tissue culture plate with forceps and stored in a dark incubator at 18°C to confirm dormant state of the eggs.

Zooplankton enumeration

The same samples for each date also were quantified to estimate zooplankton species composition and abundance. Samples were diluted with deionized water in a 1000mL glass
beaker. A small amount of dish soap was added and organisms were mixed thoroughly within the beaker to avoid nonrandom distribution. Subsamples were immediately obtained with a wide-mouth pipette (10mL total volume) to avoid settling of organisms. Subsamples were transferred to a circular zooplankton counting tray. Depending on the density of the sample, subsamples varied from 1-40 mL and were enumerated by hand tallies on a zooplankton counting sheet to facilitate counting and identification of different organisms. Individuals were identified to the species level when possible using Baler et al. (1984). Data were recorded for each species and for total zooplankton. Taxa-specific biomass was determined with dry-weight conversions from Mackey (1982) and Bottrell et al. (1976).

**Calculations**

Production rate for zooplankton were calculated for Cladocera, Cyclopoidea, and Calanoida using the zooplankton production model from Shuter and Ing (1997). Consumption rates for *Bythotrephes* were calculated using the bioenergetics model from Yurista et al. (2010). Measurement was calculated from number of animals per liter to biomass by multiplying number of animals by individual dry weight. *Bythotrephes* biomass was calculated with separate instar dry weights provided by Yurista et al. (2010) and then summed for total biomass. Student’s two sample t-test was performed to test the significance between variables for location and year.
Results

Resting Egg Density

Resting eggs were observed during both years and at both locations sampled in Green Bay (Fig. 12). On several dates in 2015, resting eggs were observed at GB-1A and GB-2 (Fig. 13). Total density of resting eggs peaked in October 2015 at GB-2 with just over 0.002 females carrying resting eggs L\(^{-1}\). No resting eggs were recorded at GB-1A in 2016, but eggs were found on four dates at GB-2 in 2016 (Fig. 14). Density of females carrying resting eggs peaked in July at GB-2 in 2016 just under 0.001 females carrying resting eggs L\(^{-1}\). It is evident from the aforementioned results that there is no clear pattern when resting eggs were being laid throughout the summer. Total mean densities of all sites combined in 2016 were significantly lower compared to 2015, by about 0.0005 females carrying resting eggs L\(^{-1}\) (Student’s two-sample t-test, \(P = 0.03\); Table 1). Overall, mean densities between site GB-1A and GB-2 with both years combined were not significantly different, but GB-2 showed a higher mean density than GB-1A by about 0.0004 females with resting eggs L\(^{-1}\) (Student’s two-sample t-test, \(P = 0.07\); Table 1).
Figure 12. Means of site GB-1A and GB-2 for *Bythotrephes* resting egg density on each date in A) 2015 and B) 2016 across summer months. Resting egg density measures number of females carrying resting eggs per liter. Error bars are 1 ± SE.
Figure 13. Mean *Bythotrephes* resting egg density of replicates on each date for site A) GB-1A in 2015 and B) GB-2 in 2015. Resting egg density measures number of females carrying resting eggs per liter. Error bars are 1 ± SE.
Figure 14. Mean *Bythotrephes* resting egg density of replicates for site GB-2 in 2016. Resting egg density measures number of females carrying resting eggs per liter. Error bars are 1 ± SE. Note that resting eggs were not observed at GB-1A in 2016.

Table 1. Student’s two-sample t-test for significance between locations and years with mean and standard error.

<table>
<thead>
<tr>
<th>Student's two-sample t-test</th>
<th>Mean</th>
<th>Standard Error</th>
<th>N</th>
<th>df</th>
<th>P-Value</th>
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<td>GB-1A 2015</td>
<td>1.91E-04</td>
<td>6.90E-05</td>
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<td>29</td>
<td>0.08</td>
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<tr>
<td>GB-2 2015</td>
<td>1.04E-03</td>
<td>5.50E-04</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>5.47E-04</td>
<td>1.34E-03</td>
<td>31</td>
<td>67</td>
<td>0.03</td>
</tr>
<tr>
<td>2016</td>
<td>5.58E-05</td>
<td>2.88E-04</td>
<td>38</td>
<td></td>
<td></td>
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<tr>
<td>GB-1A 2016</td>
<td>9.06E-05</td>
<td>3.58E-05</td>
<td>38</td>
<td>67</td>
<td>0.07</td>
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<tr>
<td>GB-2</td>
<td>5.05E-04</td>
<td>2.47E-04</td>
<td>31</td>
<td></td>
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</tr>
</tbody>
</table>
Bythotrephes Population Dynamics

There were changes in population dynamics of *Bythotrephes* across summer months in 2015 and 2016 (Fig. 15; Fig. 16). The overall abundance and dynamics of *Bythotrephes* were similar at GB-1A and GB-2 from June to October in 2015, with a peak abundance on 13 July at GB-1A with just above 2 *Bythotrephes* L⁻¹ (Fig. 15). Abundance was overall higher at GB-1A than GB-2, but both populations maintained similar dynamics. *Bythotrephes* was present on the first sampling date at GB-1A in 2015, but was at the lowest abundance observed during the entire sampling period with just above 0.002 *Bythotrephes* L⁻¹. In 2016, the population did not appear on the first sampling date 31 May 2016 at GB-1A (Fig. 16; GB-2 was not sampled on that date). By 17 June a population of *Bythotrephes* was observed and consisted of 30% juveniles (just above 0.00007 *Bythotrephes* L⁻¹) at GB-1A. On the same date, there were about 50% females with immediately hatching eggs at GB-2 (Fig. 16). In general, *Bythotrephes* was higher in abundance at both sites in 2015 as compared to 2016 (note the difference in scale on the y-axes; Fig. 15; Fig. 16). Results for both years and at both sites indicated a decline in population abundance by October (the last date sampled).
Figure 15. Mean *Bythotrephes* abundance measured on each date at A) GB-1A and B) GB-2 in 2015. Key indicates the different life stages of *Bythotrephes*. Error bars represent ± 1 SE.
Figure 16. Mean *Bythotrephes* abundance measured on each date at A) GB-1A and B) GB-2 in 2016. Key indicates the different life stages of *Bythotrephes*. Error bars represent ± 1 SE.
Zooplankton Community Trends

There were shifts in zooplankton community composition across summer months in 2015 and 2016 (Fig. 17; Fig. 18). For 2015, *Bythotrephes* biomass was noticeably greater than zooplankton biomass (Fig. 17). At peak biomass of *Bythotrephes* on 13 July 2015 for GB-1A, the composition of zooplankton included all groups except for *Leptodora* and biomass ranged from 0.0009 μg dry weight L⁻¹ for Calanoida to just above 0.02 μg dry weight L⁻¹ for *Daphnia*. Biomass for zooplankton at GB-2 on 22 July 2015 ranged from 0.0005 μg dry weight L⁻¹ for cyclopoids to just above 0.007 μg dry weight L⁻¹ for *Leptodora, Bythotrephes* accounted for the highest biomass at more than 2 μg dry weight L⁻¹. Generally, zooplankton biomass was higher at GB-1A compared to GB-2, with GB-1A having a peak biomass on 22 July 2015 just above 0.27 μg dry weight L⁻¹. At GB-1A *Eubosmina, Bosmina, Chydorus* and *Cyclopoida* became dominant towards the end of summer with a range from 14 to 18 μg dry weight L⁻¹. No zooplankton species were observed except for *Bythotrephes* on 2 October (the last date of sampling).

In 2016, zooplankton community composition changed across summer months and showed a higher biomass compared to 2015 (Fig. 18). Biomass of zooplankton peaked at GB-1A on 20 September 2016 at just above 98 μg dry weight L⁻¹. There was an inverse relationship between *Bythotrephes* and overall zooplankton abundance, as well as with individual groups of zooplankton. For instance, at GB-1A on 31 May 2016 the dominating zooplankton species was *Daphnia* at about 63 μg dry weight L⁻¹ when *Bythotrephes* were absent. On 17 June 2016 at GB-2, *Daphnia* dominated zooplankton community composition with biomass at about 24 μg dry weight L⁻¹ when *Bythotrephes* was present. At both sites, *Eubosmina, Bosmina, Chydorus* and *Cyclopoida* became dominant towards the end of summer with a range from 14 to 18 μg dry weight L⁻¹.
weight L$^{-1}$. There were large differences in biomass between 2015 and 2016, ranging from 0.006 to 0.279 $\mu$g dry weight L$^{-1}$ in 2015 and from 0.09 to 116 $\mu$g dry weight L$^{-1}$ in 2016 (note the difference in the y-axis scales between 2015 and 2016; Fig. 17; Fig. 18).
Figure 17. Biomass of each zooplankton species on each date across summer months in 2015 for A) GB-1A and B) GB-2. Note the different scale on the right side of the chart indicates *Bythotrephes* biomass. Two replicates were counted for other zooplankton species on each date at A) GB-1A for July 13 through October 2 and at B) GB-2 for July 22 through October 2.
Figure 18. Biomass of each zooplankton species collected from two replicates on each date in 2016 at A) GB-1A and B) GB-2. Note the different scale on the right side of the chart indicates *Bythotrephes* biomass.
Figure 19. Percent biomass for *Daphnia*, calanoid, cyclopoid, *Chydorus/Bosmina/Eubosmina* for 2015 and 2016. The mean biomass of each species for each date at both sites was summed to get total biomass for each year.
Bythotrephes Consumption

Surface water temperature of Green Bay from 2015 and 2016 shared similar changes across summer months, however showed slight differences between years (Fig. 20). Generally, temperature ranged from 15°C to 24°C in 2015 and 17°C to 25°C in 2016. Temperature varied by several degrees, especially from July to August between 2015 and 2016. Bythotrephes were not present until 17 June 2016 when temperature was 20°C. Temperature was 18°C on 31 May 2016 and Bythotrephes were undetected that day (Fig. 22). In 2015, the first sampling date on 25 June 2015 detected individuals when temperature was 19°C (Fig. 21). Towards the end of the summer, temperature dropped to 18°C in 2015 and 17°C in 2016 when Bythotrephes Instar 3 showed a slight increase in consumption rates (Fig. 22). In 2015, consumption rates ranged from 0.003 on 25 June to 22.2 µg L⁻¹ day⁻¹ on 2 September at GB-1A and 1.41 on 8 July to 131 µg L⁻¹ day⁻¹ on 2 September at GB-2 (Fig. 21). As for GB-1A in 2016, consumption was lowest on 8 September, 0.009 µg L⁻¹ day⁻¹, and highest on 12 July, 3.27 µg L⁻¹ day⁻¹ (Fig. 22). At GB-2 for 2016, consumption rates were highest on 26 July and lowest on 23 August (Fig. 22). GB-1A and GB-2 in 2015 had maximum Bythotrephes consumption rates consisting of mostly Instar 3. For 2016, consumption rates reached a maximum 45 µg L⁻¹ day⁻¹ at GB-2 and, also showed mostly Instar 3. The consumption model increases consumption as temperature increases, due to the physiological needs of Instar 1, Instar 2, and Instar 3 and is reflected in our results for Bythotrephes consumption rates.
Figure 20. Temperature of Green Bay’s surface water measured in degrees Celsius across summer months from May to October in 2015 and 2016.
Figure 21. Consumption rates in 2015 for *Bythotrephes* categorized by instar at A) GB-1A and B) GB-2. Note differences in y-axis scale between locations.
Figure 22. Consumption rates in 2016 for *Bythotrephes* categorized by instar at A) GB-1A and B) GB-2 across summer months. Note differences in y-axis scale between locations.
Production and Consumption Rates

There was an obvious difference in the production and consumption rates of zooplankton and *Bythotrephes* in 2015 compared to 2016 (Fig. 23; Fig. 24). In 2015, the year with the higher abundance of *Bythotrephes*, consumption exceeded production throughout the summer at both sites (Fig. 23). The largest difference was at GB-2 between August and September with about 130 mg m\(^3\) day\(^{-1}\) difference. In 2016, production generally exceeded consumption by *Bythotrephes* (Fig. 24). At GB-1A production was higher than consumption by up to 10 mg m\(^3\) day\(^{-1}\), and only on two dates during June and July did consumption exceed production (by about 1 mg m\(^3\) day\(^{-1}\)). At GB-2, production also typically equaled or exceeded consumption except for a single date in mid-July where consumption exceeded production by about 40 mg m\(^3\) day\(^{-1}\). After July 2016 production was greater than consumption by *Bythotrephes* at both sites through the remainder of the year, by as much as 20 mg m\(^3\) day\(^{-1}\) (Fig. 24).
Figure 23. Difference between mean production and consumption rates at sites A) GB-1A and B) GB-2 for 2015 across summer months.
Figure 24. Difference between mean production and consumption rates at sites A) GB-1A and B) GB-2 for 2016 across summer months.
Daphnia and Bythotrephes Interactions

*Daphnia* and *Bythotrephes* showed a generally reciprocal relationship between periods of increase and decrease for both years (Fig. 25; Fig. 26). In 2015 at GB-1A, *Bythotrephes* abundance ranged from 0.1 to just below 2.5 *Bythotrephes* L\(^{-1}\) and *Daphnia* ranged from 0.001 to 0.025 *Daphnia* L\(^{-1}\) (Fig. 25; top). At the same site in 2016, *Bythotrephes* abundance ranged from 0.0001 to just above 0.01 *Bythotrephes* L\(^{-1}\) and *Daphnia* ranged from 0.01 to 0.9 *Daphnia* L\(^{-1}\) (Fig. 25; bottom). A similar pattern is shown for 2015 and 2016 at GB-2 (Fig. 26). Generally, for both sites and both years, an increase in *Bythotrephes* abundance leads to a subsequence decline in *Daphnia* abundance.
Figure 25. Mean density of *Daphnia* and *Bythotrephes* for GB-1A on each date. Note the difference in scale for *Bythotrephes* compared to *Daphnia* between axes and between A) 2015 and B) 2016. Error bars are ± 1 SE.
Figure 26. Mean density of *Daphnia* and *Bythotrephes* for GB-2 on each date. Note the difference in scale for *Bythotrephes* compared to *Daphnia* between axes and between A) 2015 and B) 2016. Error bars are ± 1 SE.


**Discussion**

The main goal of this study was to document the population dynamics of *Bythotrephes* and its effects on zooplankton community composition and dynamics in Green Bay. *Bythotrephes* have not been officially documented in southern Green Bay until recently (Jin and Sprules 1990; Beranek 2007; Kiehnau 2015; De Stasio and Merkle 2015, 2016). Furthermore, there has been sparse documentation on the presence or absence of resting eggs in Green Bay, Lake Michigan. Here, we show that resting eggs are produced throughout the summer at both sites in Green Bay in 2015 and at one site in 2016 (Fig. 12). A presence of resting eggs in Green Bay throughout the summer increases the chance of secondary spread from boats traveling from the bay to other inland lakes in Wisconsin. If even one resting egg passes from Green Bay to Lake Winnebago, there is a chance for a population to establish. In order to prevent the spread of *Bythotrephes* it is necessary to monitor the population in southern Green Bay and to prohibit the Lower Fox River from becoming a dispersal corridor for this invasive species. It is also important to understand the dynamic aspects of both zooplankton communities and *Bythotrephes* in southern Green Bay to visualize interactions and how predation pressure from this invasive species impacts the ecology and economics of southern Green Bay. Providing these results will help predict how Lake Winnebago might be affected by *Bythotrephes* invasion, because of the similarities in food web, depth, mixing patterns, etc. between Winnebago and southern Green Bay.
Zooplankton and Bythotrephes General Trend

Zooplankton communities demonstrate changing population dynamics across years, as shown from our results and results from Beranek (2007). In 2006 zooplankton population dynamics were different from results taken in the 1980s. There was a decrease in total zooplankton biovolume from the 1980s to 2006 and a mid-summer decline in zooplankton was dominated during and after by smaller zooplankton (Fig. 9; Beranek 2007). Zooplankton trends for 2015 and 2016 showed an increase in Daphnia percentage of biomass in 2015 and a decrease in 2016, however total zooplankton biomass was much lower than 2006 (Fig. 19). Comparing present results of this study indicate that zooplankton communities are continuously changing with a decrease in overall abundance over time which may be caused by recent invasions that cause predation pressure, shifts in predator-prey interactions, and abiotic factors such as nutrient loading and temperature changes.

One of the major findings from this study is that there is a relationship between biomass of Bythotrephes and the dynamics of zooplankton communities. The trend shows that generally when an increase in biomass of Bythotrephes occurs, it is followed with a decrease in zooplankton biomass, based on results between more so in 2015 than 2016 (Fig. 17; Fig. 18). Similar trends were documented for Lake Michigan soon after invasion by Bythotrephes (Lehman 1991). Another apparent interaction that was unexpected was the impact on Leptodora biomass, especially in 2015. An increase in Bythotrephes biomass suppresses Leptodora biomass from August through September at GB-2 in 2015. Also, Bythotrephes showed an increase in biomass while Leptodora decreased in biomass during July 2016 at GB-2. There is an obvious increase in zooplankton biomass while Bythotrephes biomass decreases at GB-2 in 2016 (Fig. 18; bottom). A similar result was observed by Pothoven and Höök (2014) which indicated a large
direct predatory impact on zooplankton over a short time period (1-2 months) in another eutrophic system in the Great Lakes, Saginaw Bay of Lake Huron. They also showed that *Bythotrephes* is more likely than *Leptodora* to create a larger impact on zooplankton. Our results suggest that there may be even further evidence to support that *Bythotrephes*, especially in greater abundance, will have a large impact on zooplankton biomass, including that of *Leptodora*.

The predator-prey interaction between *Daphnia* and *Bythotrephes* has a negative impact on the abundance of *Daphnia*. This is an expected result as *Daphnia* are a preferred prey item of *Bythotrephes* (Lehman 1987, 1988; Lehman and Branstrator 1995; Lehman and Cáceres 1993; Evans 1988). An invasion of *Bythotrephes* into a productive, freshwater lake, is shown to decrease biomass of *Daphnia* (Lehman 1991; Lehman and Caceres 1993; Fernandez et al. 2009; Walsh et al. 2016). Our results confirm this hypothesis. Generally, as *Bythotrephes* abundance increased across summer months in both 2015 and 2016, *Daphnia* abundance showed a consequential decline (Fig. 25; Fig. 26). Furthermore, our use of the bioenergetics model, developed by Yurista et al. (2010) provided consumption estimates across the summer months and indicated that zooplankton production would be exceeded by *Bythotrephes* consumption at times (Fig. 23; Fig. 24). In 2015, *Bythotrephes* consumption exceeded zooplankton production through the summer. Results for 2016 did not show this pattern, and may be explained by the lower abundance overall of *Bythotrephes* in 2016 compared to 2015. When *Bythotrephes* consumption did exceed production in 2016, it was during early to mid-July, as compared to mid-August shown by Yurista (2010).

*Bythotrephes* total population size at both locations in southern Green Bay changed as the summer progressed, with a slight decrease observed in August to September for 2015. A similar
pattern was apparent for the total population of *Bythotrephes* in 2016, with an increase in population for July and September. Additionally, the population in 2015 began exclusively as juveniles in June, indicating that this is when the population started to hatch from resting eggs. In 2016, *Bythotrephes* were not observed at GB-1A on 31 May, but were observed at all stages on 17 June. The population clearly began hatching earlier in 2016 compared to 2015 and this may be due to an earlier increase in surface water temperature (Fig. 20). Climate change has already been proven to show effects on food webs that consist of *Bythotrephes* (Manca and DeMott 2009). An increase in temperature will increase the rates of physiological processes by indirectly changing habitat use and altering predator-prey interactions (Manca and DeMott 2009). Resting egg hatching is dependent on temperature as shown by Andrew and Herzig (1984) and Yursita (1997). With earlier warming of the surface water, the *Bythotrephes* populations can hatch earlier and increase duration of survival for the season. As temperature continues to increase, it becomes more important to monitor *Bythotrephes* and the duration of their population, their impacts on zooplankton communities, and the presence of resting eggs.

**Resting Egg Dispersal**

Abundance of resting eggs was highly variable during 2015 and 2016, with no clear seasonal pattern of occurrence. Females began producing resting eggs by the first week of July and continued to produce them until October in 2015. Resting eggs began to appear on 17 June in 2016, and were not found towards the end of summer in October (when sampling ended). This is surprising, because it is expected for resting eggs to increase as summer ends, as this increases chances of recruitment for the following summer. Due to the increase in resting egg density for late July at GB-2 in 2016 and the fluctuating resting egg density throughout the summer of 2015
for both sites, boaters should not be allowed to travel from southern Green to Lake Winnebago because they could spread resting eggs through the upstream corridor of the Lower Fox River. Typically, the newly hatched individuals from the resting eggs will mature and reproduce asexually (using parthenogenesis) for rapid population growth. Sexual reproduction produces resting eggs and requires males, so normally males will be observed in the population when females carrying resting eggs are found, but even a few males can fertilize many females so males are often in lower abundance than females. This is why only one resting egg is required to establish a new population. As early as June and likely past August, the resting eggs could be present and pose a risk for *Bythotrephes* dispersal to inland lakes of Wisconsin or upstream the Lower Fox River to Lake Winnebago.

*Results in a Broader Context*

The Fox-Wolf drains over 15,500 square kilometers (6,000 square miles) in northeastern Wisconsin and is a popular retreat for residents bringing in visitors each summer. The Fox River flows for 62 kilometers (39 miles) from Lake Winnebago to Green Bay, Lake Michigan (Ball et al. 1985). The locks along the Fox River are open, closed, or permanently closed to allow passengers access through the River. It is a privilege to enjoy the Fox River via water transportation, but has caused some negative effects on our freshwater systems within the area. Historically, the Fox River is a polluted water way littered with run-off from widespread agricultural and industrial use. Today, it still carries pollutants directly to the Green Bay and degrades water quality of Southern Green Bay (Sager and Wiersma 1972). The mouth of the Fox River may act as a natural barrier to some invasive species, because there is an established population of *Bythotrephes* in Green Bay that has not spread upstream, yet. However, at this
location there is a popular boat marina and high boat traffic during the summer, which could spread resting eggs from Green Bay to other inland lakes in Wisconsin if gear and boats are not properly decontaminated (Beyer 2009). Furthermore, FRNSA aims to boost economic activity and recreation by opening the locks and installing boat lifts (with and without cleaning stations) along the Lower Fox River and this would open a dispersal corridor for invasive species from Green Bay to Lake Winnebago.

Lake Winnebago is shallow and the largest inland lake in Wisconsin with an astounding surface area. Water clarity is poor and the shore is rocky. Green Bay and Lake Winnebago are similar in lake mean depth, sediment, and trophic status. Since Green Bay can support a population of *Bythotrephes* then Lake Winnebago may also support an invasion. Having shared characteristics may support the reasoning of whether *Bythotrephes* are able to establish a population in Lake Winnebago.

Fortunately, *Bythotrephes* have not invaded Lake Winnebago as of 2016 (De Stasio and Merkle 2016). However, there were 3 individuals observed during FRNSA sampling on the Lower Fox River during the summer of 2016 on one date at one site (De Stasio and Merkle 2016). Although the pollution and poor habitat quality at the mouth of the river acts as a natural barrier, some migrating fish may pass through and resting eggs can survive gut passage of fish. Thus, fish that travel from Green Bay through the Lower Fox River may be able to facilitate resting egg dispersal upstream as far as the invasive species barrier at Rapide Croche.

**Ecological Implications**

Changes in the dynamics of zooplankton communities by *Bythotrephes* has major ecological and economic consequences for southern Green Bay. Our results prove invasion by
*Bythotrephes* can cause zooplankton abundance to change throughout the season and between 2015 and 2016. A decrease in zooplankton, especially *Daphnia*, has a Top-Down effect on algal composition and abundance in Green Bay. As predation-pressure increases on grazing zooplankton, that relieves predation on algae and leads to eutrophication. The case in southern Green Bay is cultural eutrophication, because of the input of high P and N into the bay and the introduction of invasive, predacious zooplankton, both of which are human-mediated. Zebra mussels can filter out toxic blue-green algae while feeding, so algal abundance should have decreased after their invasion. It could be the predation pressure from *Bythotrephes* on *Daphnia* causing this adverse result. Additionally, it could be related to the high N and P inputs that increase nutrient availability for algae, thus facilitating cultural eutrophication. Increased cultural eutrophication, causes water clarity to decrease. This is a loss of an ecosystem service and can have economic consequences such as reduced visitation and recreation, as well as a reduction in housing prices around Green Bay (Walsh 2016). These consequences could also result in Lake Winnebago with invasion by *Bythotrephes*.

*Bythotrephes* may be complicating the network of species relationships in southern Green Bay and causing a Top-Down effect on algal abundance. In addition, their position in the trophic web also could have a Bottom-Up effect on the fishery in Green Bay. Predation by *Bythotrephes* decreases food availability for larval and small fish, perhaps reducing recruitment for succeeding years. The Wisconsin Department of Natural Resources (2015) has shown since the 1980s that yellow perch recruitment has decreased leading to smaller populations. This result may be a cause of *Bythotrephes* altering zooplankton composition and abundance. Thus, *Bythotrephes* may be threatening one of the Great Lakes most productive fisheries. Fisheries for yellow perch, walleye, sturgeon in Lake Winnebago may be affected if invasion occurs. Ecosystem services,
such as productive fisheries, are extremely important for the community around Green Bay and Lake Winnebago as it brings in visitors and income which drives economic activity.

**Recommendations and Future Research**

Although FRNSA is attempting to restore the tourist economy that existed along the river, during a healthier ecological and economical state, barriers should be kept in place to prevent further spread of invasive species upstream of the Lower Fox River dispersal corridor to Lake Winnebago. The negative impacts on fisheries and water clarity may come from *Bythotrephes* predation on zooplankton communities, which is why *Bythotrephes* should be prevented from invading Lake Winnebago. Even if a boat lift is installed at Menasha lock, it could be facilitating the dispersal of *Bythotrephes* resting eggs, since resting eggs have been shown to tolerate up to 4 hours of desiccation (Branstrator et al. 2013).

Boat traffic seems to be variable throughout the summer at separate locks. Table 2 shows how often boaters and visitors go through the locks along the Lower Fox River. With increasing visitors utilizing the Lower Fox River as a transport route, more chances of resting egg dispersal upstream will increase the likelihood of invasion in Lake Winnebago for *Bythotrephes*, among other invasive species.

Heavy use of the Lower Fox River has negative impacts on the ecological health of the river and southern Green bay. It may seem favorable to open the Fox Locks to increase economic activity for the community, but long term affects should be considered for a sustainable environment and economy. These long-term effects include major losses of ecosystem services, resulting in negative impacts on the ecological and economic state of Green Bay, the Lower Fox
River, and Lake Winnebago. It would be a mistake to mistreat the Lower Fox River and repeat history.

Given the variation observed between 2015 and 2016 future monitoring will be important to confirm these findings with additional study of this important ecological and economic system. For future direction of this research, it will be important to start sampling earlier and to track changes in population dynamics closely as temperature increases, because this has been shown to affect *Bythotrephes* populations (Manca and DeMott 2009). It will also be important to monitor the Lower Fox River and Lake Winnebago for any presence of *Bythotrephes* in future years, so that if caught early, managers on the river can allocate resources properly. Another consideration is to regulate monitoring for *Bythotrephes* in Little Lake Butte Des Morts, which is located right before Menasha lock, due to the habitat preferences of *Bythotrephes*, it is possible there is a presence of this invasive species brought in there by boaters.

One of the challenges in preventing the spread of invasive species is that there is a heavy reliance on boaters and users to follow the codes and regulations of AIS decontamination procedures. As a general recommendation, to prevent the mistreatment of natural waterways, and maintain healthy ecosystems, environmental programs should be implemented for early childhood education. Also, at locks and marinas around Green Bay, there should be outreach programs that educate about the importance of AIS prevention.
Table 2. Lock usage statistics for the 2016 season. Lock tenders are responsible for recording data (FRNSA at http://foxlocks.org/schedule-and-operations/lock-use-and-boat-traffic/).

<table>
<thead>
<tr>
<th>LOCK</th>
<th>Lockages</th>
<th>Craft</th>
<th>Passengers</th>
<th>Canoe/Kayak</th>
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<td>2,519</td>
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<tr>
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<td>1,453</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
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<td>124</td>
<td>449</td>
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<td>Cedars</td>
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<td>122</td>
<td>464</td>
<td>0</td>
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<tr>
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<td>3,069</td>
<td>4,218</td>
<td>18,337</td>
<td>167</td>
</tr>
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</table>
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I Hereby Reaffirm the Lawrence University Honor Code

Casey A Merkle