Aquatic Invasive Species Monitoring Project

Year 2018 Report

To the

Fox River Navigational System Authority

By

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Objectives

The Aquatic Invasive Species (AIS) Control and Monitoring Plan of the Fox River Navigational System Authority (FRNSA, 2009 update) has the stated objective to "Monitor the presence and map the distribution of fish and invertebrate AIS in the Fox River three navigation pools immediately up and downstream of the Rapide Croche Lock." Under the supervision of Dr. Bart De Stasio, Ph.D., Lawrence University, two students (Scotia Dettweiler and Callie Ochs) were employed during the summer of 2018 to carry out the investigations.

Sampling Design

Monitoring occurred at five of the standard six sites along the lower Fox River, WI during the summer of 2018 (Table 1, Figure 1). Each sampling site designated a general area for sampling efforts, and was further separated into mid-channel versus near-shore sampling locations, depending on the type of sampling performed. We conducted 17 different sampling trips during the summer (Table 2). We could not sample site FR-3 due to inability to gain access to a boat ramp. Sites were sampled three or four times over the course of the summer. Separate boats were employed upstream and downstream of the Rapide Croche dam site on each date, and all nets and equipment were sanitized thoroughly using bleach prior to the next sampling event according to the protocols established by the WI DNR to prevent the spread of AIS (http://dnr.wi.gov/topic/fishing/documents/vhs/disinfection_protocols.pdf).

Table 1. Latitude and Longitude coordinates of the sites sampled along the lower Fox River, WI during summers 2008-2018.

Location	Latitude	Longitude
Upstream of Rapide Croche		
FR-A (above Cedar lock)	N 44° 16.562	W 88° 20.541
FR-B (above Kaukauna Guard lock)	N 44° 16.665	W 88° 17.042
FR-3 (above Rapid Croche lock)	N 44° 19.077	W 88° 11.962
Downstream of Rapide Croche		
FR-4 (below Rapid Croche lock)	N 44° 18.947	W 88° 11.413
FR-C (above DePere dam)	N 44° 25.813	W 88° 04.273
FR-D (below DePere dam)	N 44° 27.742	W 88° 03.354

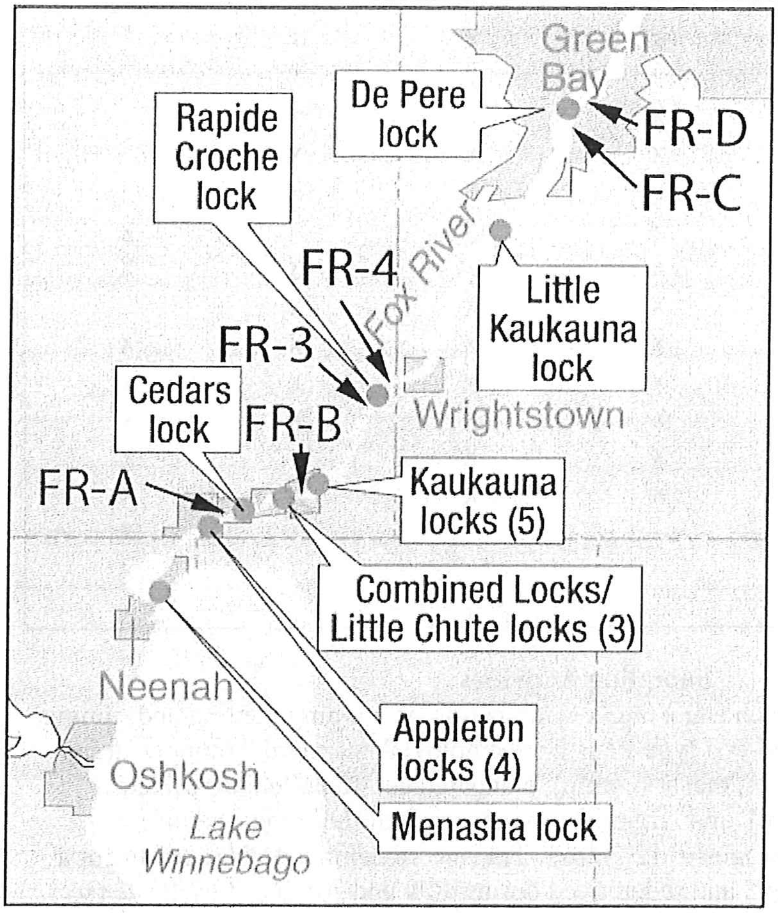

Figure 1. Map of sampling locations along the lower Fox River, WI.

Table 2. Sampling effort upstream and downstream of the Rapide Croche dam on the lower Fox River, WI during summer 2018. Dates on which sampling was performed at each site are indicated for each type of sampling effort.

Site and Date	Dip Net	Plankton Tow	Benthic Grab	Seine Netting	Fish Trap
FR-A 06/21/18	X	X		X	
FR-C 06/25/18	X	X	X		
FR-D 06/25/18	X	X	X	X	
FR-B 06/26/18	X	X	X	X	
FR-4 07/09/18	X	X	X	X	
FR-A 07/11/18	X	X	X	X	
FR-B 07/12/18	X	X	X	X	
FR-C 07/17/18	X	X	X		X
FR-D 07/17/18	X	X	X	X	X
FR-4 07/23/18	X	X	X	X	
FR-B 07/25/18	X	X	X	X	X
FR-A 07/26/18	X	X	X	X	X
FR-D 08/1/18	X	X	X	X	X
FR-C 08/1/18	X	X	X		X
FR-4 08/6/18	X	X	X	X	X
FR-A 08/8/18					X
FR-B 08/8/18					X

Sampling Activities

Fish: Fish were sampled at each site using a combination of trapping, netting and seining techniques. Three sizes of cod-end type traps were employed; standard "minnow" traps (length=0.42m, opening=22mm, mesh=6.4mm), elongated eel traps (length=0.78m, opening=40mm, mesh=6.4mm), and larger hand-made traps of the same design (length=2m, opening=125mm, mesh= 12.5mm). Traps were deployed without bait for a maximum of 24 hours, emptied, and redeployed during July and August at each site (see Table 2). Netting included mid-channel as well as shoreline locations at each site using standard hoop nets (2ft diameter, 10ft length, 1in square mesh) either unbaited or using frozen cod as bait. We also conducted at least three (and up to five) beach seine hauls at each shoreline location on each sampling day (1/4 inch mesh, 4 foot height, 20 foot length). If possible, fish were identified in the field to the species level and then released. Specimens of new species compared to existing records, non-native species, or specimens difficult to identify in the field were saved live for later identification in the laboratory. Specimens were transported to Lawrence University in accordance with WI Administrative Code NR 40 and all applicable permitting requirements under a WI Scientific Collector's permit (SCP-NER-148). Upon return to the laboratory specimens were frozen for disposal or transferred to ethyl alcohol (70%) for long-term preservation. Specimens were identified to the species level when possible, using Hubbs and Lagler (2004), Lyons *et al.* (2000), and the Wisconsin Fish ID software (2005).

Benthic invertebrates: Mid-channel areas were sampled using a standard Ekman grab sampler (0.15m X 0.15m box size). Replicate grab samples were collected at each site and filtered through a wash bucket with mesh bottom (mesh size=500um). Shoreline areas at each site were sampled with a combination of dip netting and beach seining techniques (generally until no new taxa were obtained). Animals captured were washed into sorting trays, picked into sealed containers and later preserved with 80% ethyl alcohol. Specimens were identified in the laboratory to the genus or species level, when possible, using the references listed above for plankton identifications as well as Pecharsky *et al.* (1990), Merritt *et al.* (2008) and Hilsenhoff (1995).

Plankton: On each sampling date oblique tows were performed at the mid-channel location of each site using a Wisconsin-type plankton net with retaining collar (mouth diameter=0.13m, mesh size=63 um). Samples were preserved in 80% ethyl alcohol and examined in the laboratory using 10X to 400X magnification. All zooplankton in the samples were identified to the species level, when possible, using Edmonson (1965), Balcer *et al.* (1984), Pennak (1989), Hopkins (1990), and Thorp and Covich (1991). Abundances in samples were not enumerated, but entire samples were examined to determine presence of each species.

Results

Fish:

A total of 25 species of fish were collected from the five sites during the summer of 2018 (Table 3). Twenty species of fish were observed downstream of the Rapide Croche barrier, while 15 of the total 25 species were found upstream of Rapide Croche. As in the previous year, only one invasive fish species, the round goby (*Neogobius melanostomus*), was documented during the summer. Round goby was found at sites sampled below Rapide Croche, and was not observed at any of the sites above the barrier (which extends upstream to the pool above the Cedar Lock).

Table 3. Fish species presence documented in the lower Fox River, WI upstream and downstream of the Rapide Croche dam during summer 2018. A value of one indicates presence. Sites FR-A, and FR-B are upstream, with FR-4, FR-C and FR–D downstream of Rapide Croche dam. The round goby (highlighted) was the only invasive fish species observed.

North of Rapid Croche

Fish	FR-A	FR-B	FR-4	FR-C	FR-D
Ambloplites rupestris (Rock Bass)		1	1		1
Ameiurus natalis (Yellow Bullhead)	1				
Ameiurus sp.		1			
Couesius plumbeus (Lake chub)	1	1	1		
Dorosoma cepedianum (Gizzard Shad)					1
Etheostoma nigrum (Johnny darter)	1				
Lepisosteus osseus (Longnose Gar)	1				
Lepomis gibbosus (Pumpkin seed)	1	1			1
Lepomis gulosus (Warmouth)					
Lepomis macrochirus (Bluegill)			1		
Luxilus cornutus (Common Shiner)			1		
Micropterus dolomieu (Smallmouth bass)	1				1
Micropterus salmoides (Largemouth bass)		1	1		
Neogobius melanostomus (Round goby)			1		1
Notemigonus crysoleucas (Golden Shiner)			1		
Notropis atherinoides (Emerald Shiner)					1
Notropis dorsalis (Bigmouth Shiner)					1
Notropis volucellus (Mimic Shiner)	1		1		1
Notropis wickliffi (Channel Shiner)			1		1
Perca flavescens (Yellow Perch)	1	1	1		1
Percina caprodes (Log Perch)					1
Pimphales notatus (Bluntnose minnow)	1		1		1
Pimphales vigilax (Bullhead minnow)	1	1			1
Pomoxis annularis (White Crappie)	1	1			1
Pomoxis nigromaculatus (Black Crappie)					
TOTAL	**10**	**7**	**10**	**0**	**13**

Benthic Invertebrates:

There were 66 groups of benthic invertebrates observed during the summer of 2018 (Table 4). Zebra mussels were observed at all sites both above and below Rapide Croche. Rusty crayfish were found at one site above the Rapide Croche. More invertebrates were collected from sites above the Rapide Croche barrier (50) compared to sites below the barrier (25).

Table 4. Benthic invertebrate taxa documented upstream and downstream of the Rapide Croche dam during summer 2018 (value of 1 indicates presence). Highlighted groups are considered "invasive" species.

Macroinvertebrates	**FR-A**	**FR-B**	**FR-4**	**FR-C**	**FR-D**
Amphipods, Sideswimmers					
(g) Caecidotea	1				
(g) Crangonyx	1				
(g) Gammarus				1	
(sp) Hyallela azteca	1	1	1	1	
(g) Monoporeia sp.	1				
Annelids, Worms					
Enchytraeidae (F)					1
Hirudinea, Leeches (SubC)					
(g) Cystobranchus			1		
(g) Erpobdella		1			
(g) Haemopis	1				
(g) Placobdella		1			
Crustacea					
Crayfish					
(sp) Orconectes rusticus (Rusty crayfish)		1			
(sp) Orconectes virilis (Virile crayfish)	1	1			
Isopoda, Aquatic Sow Bugs					
(g) Asellus	1				
Hydrachnidia, Water mites					
(g) Limnesia		1			1
(g) Limnochares				1	
(g) Oxus		1			
(g) Protzia				1	
(g) Tyrellia			1		
Insects					
Coleoptera, Beetles					
(g) Berosus	1				
(g) Celina	1				
(g) Haliplus				1	
(g) Laccophilus			1		
(g) Peltodytes		1			
(g) Stenelmis			1		
Diptera, Flies					
Chironomidae (F)					
(SubF) Chironominae	1		1	1	1
(SubF) Orthocladiinae				1	
(SubF) Podonominae		1		1	
(SubF) Tanypodinae			1		
Muscidae (F)		1			

Table 4 (continued)

Macroinvertebrates	**FR-A**	**FR-B**	**FR-4**	**FR-C**	**FR-D**
Emphemeroptera, Mayflies					
(g) Caenis	1	1	1	1	1
(g) Centroptilum	1				
Hemiptera, Bugs					
Corixidae, waterboatmen (F)					
(g) Palmacorixia	1	1			
(g) Sigara				1	
(g) Trichocorixia	1	1			
Gerridae, waterstrider (F)					
(g) Gerris	1				
(g) Neogerris	1				
(g) Rheumatobates			1		
Nepidae, water scorpion (F)					
(g) Ranatra	1	1	1		
Odonata, Dragonflies/Damselflies					
Anisoptera, Dragonflies (SubO)					
(g) Aeshna	1				
(g) Arigomphus					1
(g) Epicodulia	1				
Zygoptera, Damselflies (SubO)					
(g) Argia	1				
(g) Enallagma	1	1			
(g) Lestes	1				
(g) Nehalennia	1	1			
Trichoptera, Caddisflies					
Hydropsychoidea (SuperF)					
(g) Arctopsyche	1				
(g) Diplectrona		1			
(g) Lype		1			
(g) Neureclipsis		1			
(g) Parapsyche	1				
Limnephiloidea (SuperF)					
(F) Leptoceridae		1			
Snails					
(g) Amnicola	1				
(g) Bulimus	1				
(g) Fossaria	1				
(g) Goniobasis	1				
(g) Gyraulus	1	1		1	
(g) Helisoma		1			
(g) Lymnaea			1		
(g) Menetus		1			
(g) Physella	1	1		1	
(g) Pleurocera	1	1	1		
(g) Stagnicola	1				
(g) Valvata	1				
(sp) Dreissena polymorpha (Zebra Mussels)	1	1	1	1	1
(g) Sphaerium	1				
TOTAL	36	26	13	13	6

Plankton:

A total of 18 taxa of zooplankton were recorded in 2018, with 15 occurring upstream of the barrier and 16 below (Table 5). The invasive spiny water flea, *Bythotrephes longimanus,* was not collected at any sites.

Table 5. Zooplankton documented from sites upstream and downstream of the Rapide Croche dam during Summer 2018. A value of one indicates presence. The spiny water flea *Bythotrephes longimanus* (highlighted) was the only invasive species observed.

Zooplankton	**FR-A**	**FR-B**	**FR-4**	**FR-C**	**FR-D**
Acanthocyclops vernalis	1	1	1	1	1
Alona Sp.	1		1	1	
Bosmina longirostris				1	1
Calanoid order				1	
Ceriodaphnia		1	1		1
Chydorus sp.	1	1	1	1	1
Cyclopoid order	1		1	1	
Daphnia pulicaria	1	1	1	1	1
Diacyclops thomasi	1	1		1	1
Diaphanosoma birgei	1		1		
Dreissena polymorpha (veliger)		1	1		1
Eubosmina coregoni	1	1			
Leptodiaptomus sicilis		1	1	1	1
Leptodiaptomus siciloides	1	1	1	1	1
Leptodora kindti	1	1	1		
Mesocyclops edax	1	1	1	1	1
Skistodiaptomus oregonesis	1	1	1	1	1
TOTAL	**12**	**12**	**13**	**12**	**11**

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Spiny Water Flea (*Bythotrephes longimanus*) and Round Goby (*Neogobius melanostomus*) Monitoring in Southern Green Bay, Lower Fox River, and Lake Winnebago During 2018

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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 smaller crustacean prey. The species can lay two types of eggs, resting eggs and immediately hatching eggs. Resting eggs are tolerant to harsh conditions, allowing them to be rapidly distributed when attached to fishing and other recreational gear and boats. The goal of this research was to determine spiny water flea population dynamics in southern Green Bay, the lower Fox River and Lake Winnebago, including when they produce resting eggs. Six sites along the lower Fox River, two sites in southern Green Bay and two sites in Lake Winnebago were sampled with oblique net tows approximately biweekly from early June until late September 2018 to determine the abundance of juvenile and adult stages of the spiny water flea, including the type of eggs being produced by adult females. No spiny water fleas were collected from Lake Winnebago or the lower Fox River during 2018. In southern Green Bay juvenile and female spiny water fleas were first observed 20 June and individuals were observed continuously until at least 30 September when sampling ceased. Peak population abundances occurred during the middle of August at both sites in southern Green Bay. Females began producing both kinds of eggs as early as 3 July. Females produced resting eggs until the last sampling on 30 September.

Fish sampling was conducted along shorelines near the outflows of Lake Winnebago and at the six locations established along the lower Fox River for monitoring by the Fox River Navigational System Authority. No round goby were collected from Lake Winnebago. Round goby were observed only at the three sites below the Rapide Croche barrier. The results of this study will help provide important data that can be used to guide management decisions related to boat use along the lower Fox River. The ultimate goal is to determine the current distribution of spiny water flea and round goby in the Winnebago-Fox River-Green Bay system, and help delay the spread of these and other AIS from Green Bay through the Fox River into upstream lakes of Wisconsin.

Introduction

The spiny water flea, *Bythotrephes longimanus* (formerly known as *B. cederstroemii),* is an aquatic invasive species (AIS) of the zooplankton that has spread throughout the Great Lakes, and was first observed in Lake Michigan in 1986 (Lehman 1987). This invasion has resulted in changes to the food web dynamics in Lake Michigan (Lehman & Caceres 1993) and also Green Bay (De Stasio et al. 2018). It is a predatory cladoceran that competes with small fish and other native invertebrates for food such as smaller zooplankton like the water flea *Daphnia* (Lehman 1991, Pothoven *et al.* 2003, Pothoven & Hook 2014, Merkle & De Stasio 2018). The rapid spread and success of this species throughout the Great Lakes and inland lakes in the region is due in large part to the production of a resistant stage that can survive harsh conditions during transport to new areas. The species can produce two types of offspring, either immediately hatching eggs or the resistant embryos called resting eggs. Resting eggs are in a dormant state and range from 0.4-0.5 mm in diameter with a golden brown color. The 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).

This resistant resting stage and the fact that the tail spines of this species causes it to get caught on fishing line, fabric, anchor ropes, etc. has allowed the species to spread rapidly to new areas and become a successful invasive species. Spiny water fleas have been recorded in Green Bay since at least the late 1980s (Jin & Sprules 1990) and there is concern that it may spread up the lower Fox River and into the Lake Winnebago, Upper Fox River and Wolf River systems if boats and gear are allowed to navigate upstream without being properly cleaned (Merkle & De Stasio 2018). Some cleaning procedures will kill juveniles, adults and immediately hatching eggs but not resting eggs. Concern for this possibility is warranted based on the data on spiny water flea population dynamics collected in 2015 from southern Green Bay. Although spiny water fleas are not established in the lower Fox River, adult females were observed by late June and produced resting eggs from early July until at least early October during both 2015 and 2016. (Merkle & De Stasio 2018). Monitoring from 2015-2017 has shown that peak population abundances can occur in both August and September (De Stasio 2017). Understanding the population dynamics and when the various stages occur in Green Bay and the lower Fox River is important for planning effective cleaning protocols and practices.

Another AIS of concern in northeastern Wisconsin is the round goby (*Neogobius melanostomus*). It is known to be established in Green Bay and the lower Fox River as far upstream as the invasive species barrier at Rapide Croche (Kornis & Vander Zanden 2010). Round goby have been shown to have important impacts on food webs and fisheries (Lederer *et al.* 2008, Kornis *et al.* 2012), and there is concern that the inland spread of this AIS would do damage to the important sport fishery in Lake Winnebago, known to drive at least $234 million of the local economy (Cook & Neiswender 2007).

The goal of this study was to determine the spatial and temporal distribution of spiny water flea and round goby in Lake Winnebago, the lower Fox River and southern Green Bay during the main boating season in 2018. In addition, the project was to provide documentation of the abundance of each life stage of the spiny water flea during the study period, with special attention paid to when resting eggs were being produced.

Methods

Field Collections - Samples for spiny water flea were collected from the lower Green Bay area from 7 June through 30 September, 2018. Two sampling sites established during previous research on lower Green Bay were sampled (Table 1 & Fig. 1; De Stasio & Richman 1998, De Stasio *et al.* 2008, 2014, 2018, Merkle & De Stasio 2018). Oblique plankton tows from just above the sediment to near the surface were collected using a standard conical plankton net (0.50 m diameter opening, 200 cm length, 250 um mesh; Aquatic Research Instruments, Hope, ID) fitted with a flow meter (Oceanics, Corp). The net was towed at a constant speed of 2 mph for 3 min. Duplicate samples were collected to allow estimation of variability among samples. On the lower Fox River, samples were collected from mid-channel areas at six locations established and monitored since 2006 (Fig. 1; De Stasio 2016). Samples on Lake Winnebago were obtained approximately biweekly from 19 June – 25 September with duplicate plankton tows conducted the same as for Green Bay but at two sites at the northern end of the lake (Fig. 1). Samples were held live in closed 2-L 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, decontaminated and prevented from release into natural waterways or public water treatment system at all times.

Round goby were sampled along shorelines and shallow rocky reef areas in the outflow regions of Lake Winnebago during summer 2018 (Fig. 2). Shallow areas at 10 sites were sampled using a combination of trapping and angling techniques, procedures we have found to be effective for many years for catching round goby in the lower Fox River. Three sizes of cod-end type traps were employed; standard "minnow" traps (length=0.42m, opening=22mm, mesh=6.4mm), elongated eel traps (length=0.78m, opening=40mm, mesh=6.4mm), and larger hand-made traps of the same design (length=2m, opening=125mm, mesh= 12.5mm). Traps were deployed with bait (e.g. previously frozen fish, cheese) for a maximum of 24 hours, emptied, and redeployed multiple times. Hook & line angling was conducted in the sampling areas utilizing standard fishing gear as recommended by the WI Department of Natural Resources; size #14 hooks, Berkley Gulp! Maggots bait, and no bobber.

In the lower Fox River fish were sampled at each site using a combination of trapping, netting and seining techniques. Three sizes of cod-end type traps were employed, as described above for Lake Winnebago sampling. Traps were deployed without bait for a maximum of 24 hours, emptied, and redeployed during June, July and August at each site. Netting included mid-channel as well as shoreline locations at each site using standard hoop nets (2ft diameter, 10ft length, 1in square mesh) either unbaited or using cheese or frozen cod as bait. We also conducted at least three (and up to five) beach seine hauls at each shoreline location on each sampling day (1/4 inch mesh, 4 foot height, 20 foot length).

If possible, fish were identified in the field to the species level and then released. Specimens of new species compared to existing records, non-native species, or specimens difficult to identify in the field were saved live for later identification in the laboratory. Specimens were transported to Lawrence University in accordance with WI Administrative Code NR 40 and all applicable permitting requirements under a WI Scientific Collector's permit (SCP-NER-148). Upon return to the laboratory specimens were frozen for disposal or transferred to ethyl alcohol (70%) for long-term preservation. Specimens were identified to the species level when possible, using Hubbs and Lagler (2004), Lyons *et al.* (2000), and the Wisconsin Fish ID software (2005).

Spiny Water Flea Laboratory Procedures – Samples were preserved in 70% denatured alcohol upon return to the laboratory. Either entire samples were enumerated, or samples were subsampled using a Folsom plankton splitter (Wildco Inc., Yulee, FL) using liquid dishwashing soap to eliminate surface trapping of specimens. Subsamples were counted at 10X – 40X using five categories for life stages of spiny water fleas: juveniles, males, females with no eggs, females with immediately hatching eggs, and females with resting eggs. Resting eggs are a golden brown color (Jarnagin *et al.* 2000); this trait and lack of hatching over a 2-week observation period in the laboratory were used to determine the production of resting eggs by females. Samples from the lower Fox River were not enumerated, but presence/absence for all plankton species was determined.

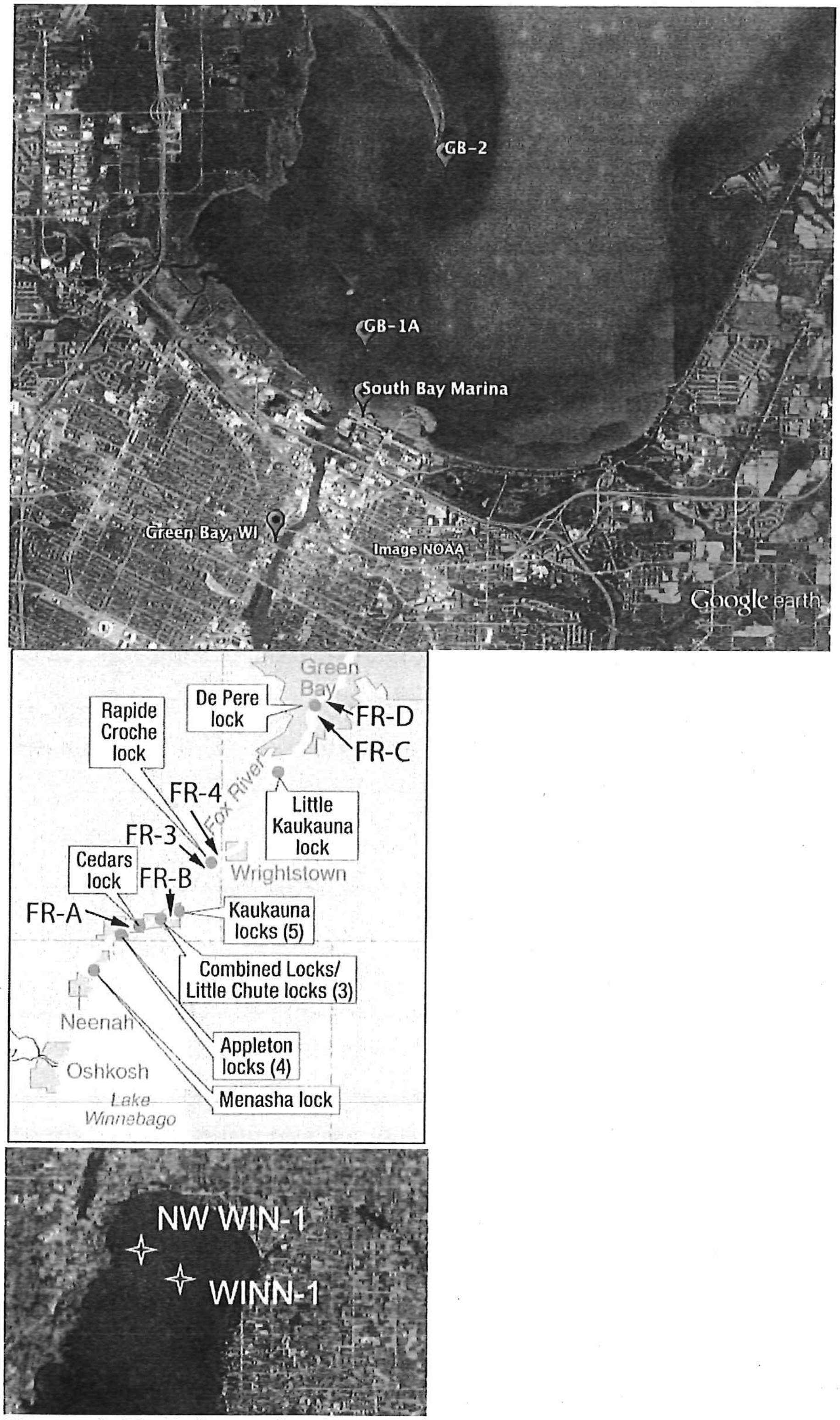

Figure 1. Map of sampling locations in 2018 in lower Green Bay (top panel), lower Fox River, WI (middle panel), and Lake Winnebago (bottom panel).

Table 1. Latitude and Longitude coordinates of established sampling sites along the lower Fox River, WI (top section), lower Green Bay (middle section) and Lake Winnebago (bottom section). Sites GB-1A and GB-2 are established sampling locations from studies by De Stasio and colleagues (De Stasio *et al.* 2008, 2014, 2018).

Lower Fox River Locations	Latitude	Longitude
Upstream of Rapide Croche		
FR-A (above Cedar lock)	N 44° 16.562	W 88° 20.541
FR-B (above Kaukauna Guard lock)	N 44° 16.665	W 88° 17.042
Downstream of Rapide Croche		
FR-4 (below Rapid Croche lock)	N 44° 18.947	W 88° 11.413
FR-C (above DePere dam)	N 44° 25.813	W 88° 04.273
FR-D (below DePere dam)	N 44° 27.742	W 88° 03.354

Lower Green Bay Locations	Latitude	Longitude
GB-1A	N 44° 32.952	W 87° 59.890
GB-2	N 44° 34.817	W 87° 58.733

Lake Winnebago Locations	Latitude	Longitude
WINN-1	N 44° 11.22	W 88° 23.63
NW WIN-1	N 44° 11.82	W 88° 25.33

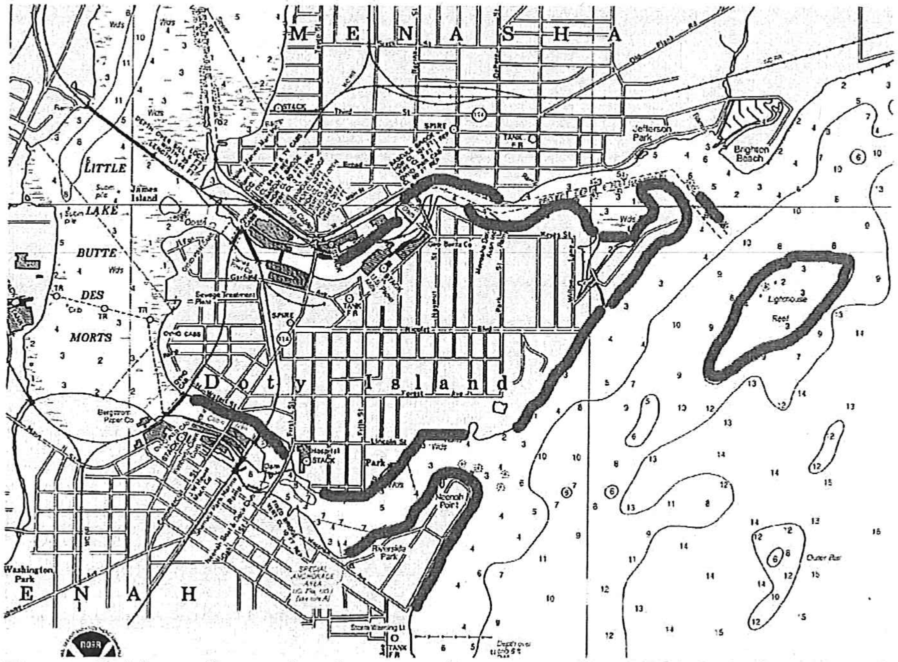

Figure 2. Map of round goby sampling areas for 2018 in Lake Winnebago, WI. Red highlighted areas are where round goby are most expected to occur (e.g. shallow, rocky areas, pipes).

Results

The overall abundance and dynamics of spiny water fleas were similar at the two Green Bay sites from June through September (Fig. 3). The population was not present on the first sampling date (3 June 2018), but was found at both sites as juveniles as well as adults by 20 June (Fig. 4). Abundances at both locations increased slightly during early July, decreased during July, and then increased again during August. These dynamics were similar to those in 2015, except that overall abundances were generally lower in 2016 (Fig. 3, top). In both years there were population increases in July, followed by decreases in August and second population increases in September.

Seasonal changes in the composition of spiny water flea at the two sampling sites followed similar patterns as well (Fig. 4). No individuals were collected on 7 June 2018 at GB-1A (GB-2 could not be sampled on that date due to rough weather). By 20 June samples at both sites consisted of a mixture of juveniles and females without eggs, and at GB-2 females with immediately hatching eggs were also observed. Females with resting eggs were observed at GB-2 on 3 July, and were collected again on 30 September. No females with resting eggs were collected at GB-1A. Juveniles typically comprised from 50% to 60% of the total population abundance, and no males were observed in samples during 2018.

No spiny water fleas were collected from Lake Winnebago or the lower Fox River during 2018 (19 June – 25 September).

On the lower Fox River round goby was found at sites below the Rapide Croche invasive species barrier (Table 2) but was not observed at any of the sites above the barrier (which extends upstream to the pool above the Cedar Lock). Round goby was not caught in Lake Winnebago or areas along the outflow channels during 2018 (Table 3). Sampling was effective for catching other species (e.g. yellow perch, largemouth bass) using both traps and angling.

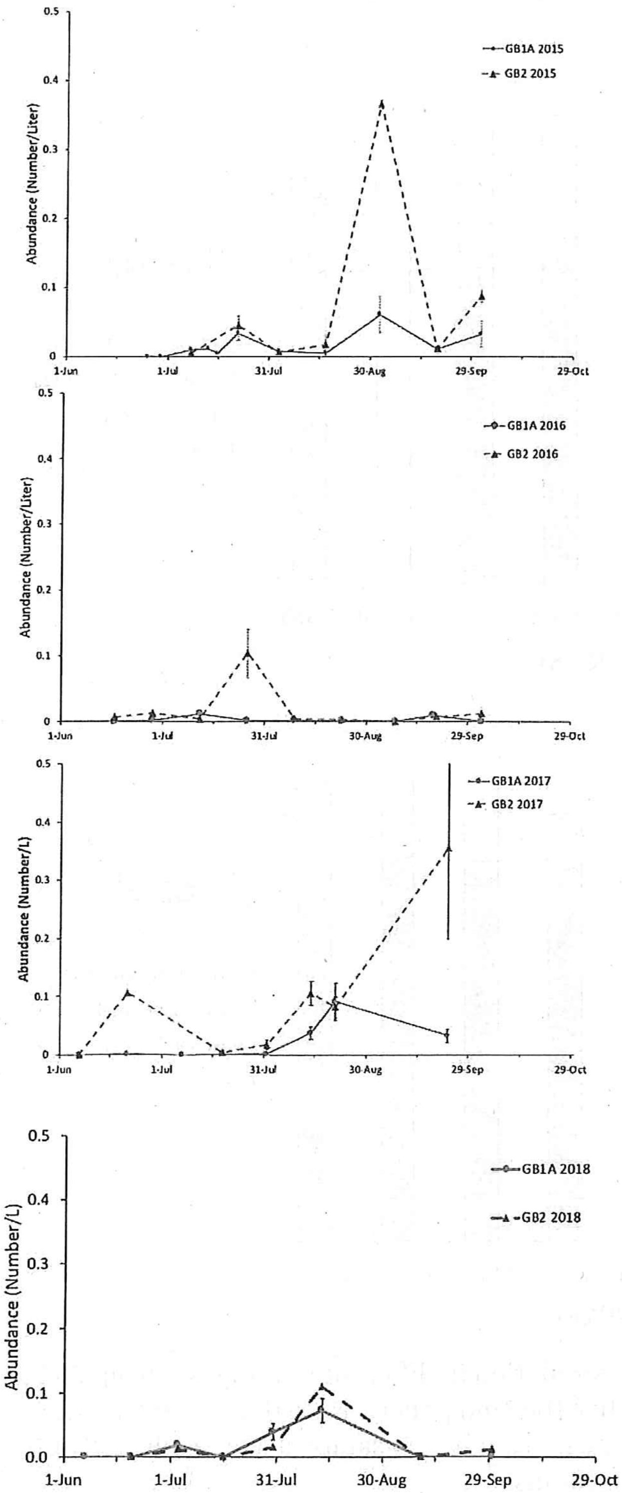

Figure 3. Total population abundance (mean +/- 1 standard error) of spiny water flea in southern Green Bay at GB-1A (solid blue line) and GB-2 (dashed red line) during 2015, 2016, 2017, and 2018.

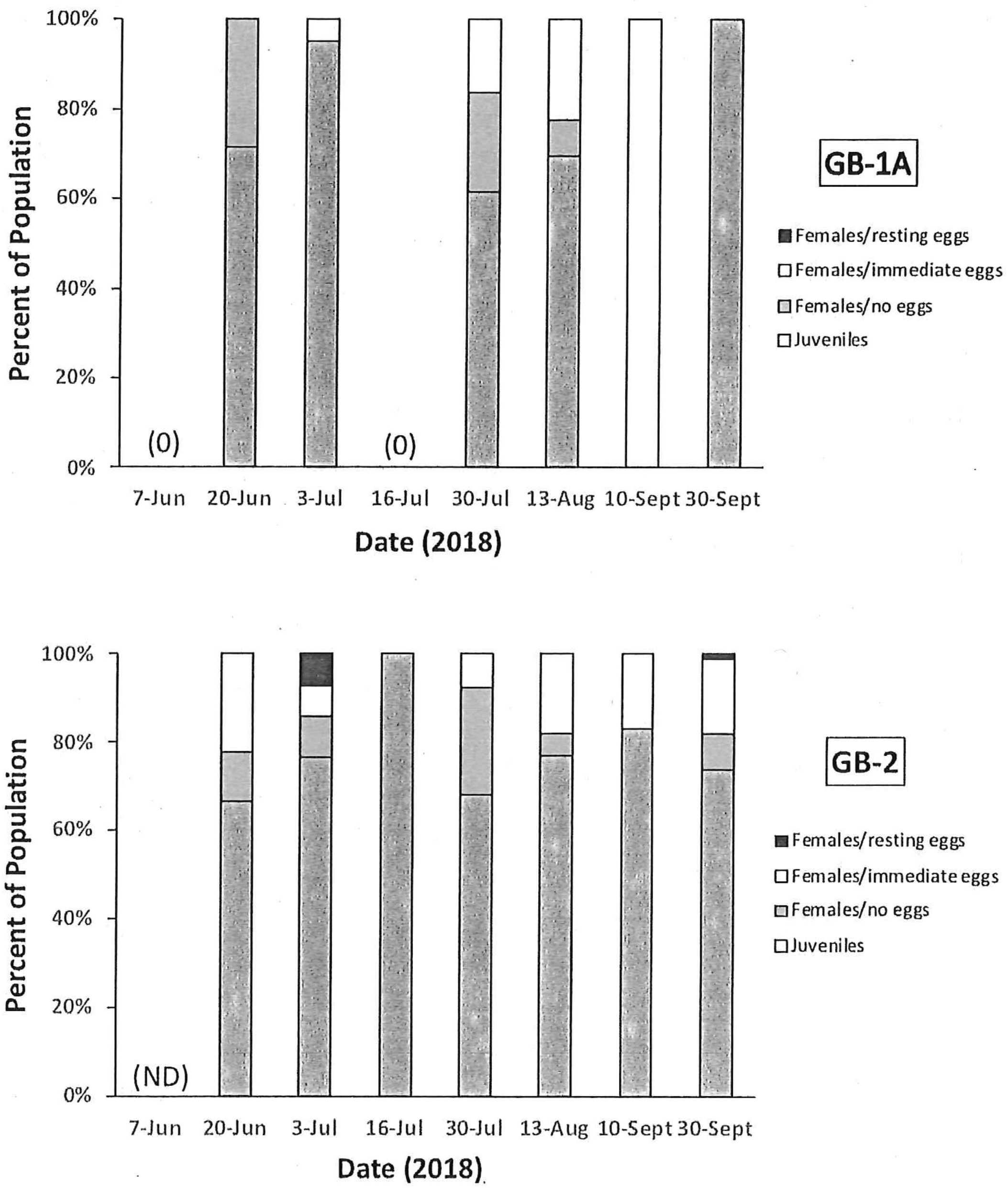

Figure 4. Percent of spiny water flea population in different life stages during 2018 for location GB-1A (top panel) and GB-2 (bottom panel). Note that no spiny water fleas were observed at GB-1A on 7 June or 16 July. No samples were collected at GB-2 on 7 June due to rough weather conditions.

Table 2. Lower Fox River fish sampling effort (dip net sweeps, seine net trawls, 24-h trap sets) and number of round goby captured at established sampling sites during 2018. Sampling occurred approximately monthly June – August 2018.

Site	Dip Net Effort	Seine Effort	Trap Effort	Number Caught
FR-A	22	9	6	0
FR-B	24	12	6	0
FR-4	21	11	3	5
FR-C	17	0	6	0
FR-D	18	12	6	16

Table 3. Fish sampling data for Lake Winnebago, WI during summer 2018.

Date	Locations	Sample Type	Sampling Effort	Round Goby	Other Fish
6/21/18	1,9	Angling	2.5 h	0	3
6/28/18	7, 10	Angling	2 h	0	2
7/2/18	1,2	Angling	2.5 h	0	0
7/10/18	1,3	Traps (7 traps)	21 h each	0	12
7/11/18	2,4	Traps (6 traps)	22 h each	0	1
7/18/18	5,6	Traps (6 traps)	23 h each	0	2
7/30/18	6	Angling	1.5 h	0	0
7/30/18	6,8	Traps (6 traps)	24 h each	0	1
8/7/18	10,2	Angling	2.5 h	0	2
8/21/18	3, 9	Angling	1.5 h	0	1

Locations:
1-Smith Park Dock Association, Menasha
2-End of Nicolet Blvd, Neenah
3-Jefferson Park, Menasha
4-Riverside Park, Neenah
5-Behind Bergstrom Mahler Art Museum, Neenah
6-Kimberly Park
7-N. Water Street, Neenah
8-End of Lincoln St, Neenah
9-Lighthouse Reef, Lake Winnebago
10-Menasha Lock/Whiting Paper Company Channel

Discussion

No spiny water fleas were observed in Lake Winnebago or in the lower Fox River. This indicates that spiny water flea was not established in the lower Fox River or Lake Winnebago. Spiny water fleas are well established in southern Green Bay and in 2018 were observed from 7 June through 30 September. Similar to previous years studied population size of spiny water fleas at both locations in southern Green Bay generally increased from June to early July, and then decreased in late July.

Our first sampling on 7 June did not detect any individuals, again supporting the conclusion that the population was started in early June by individuals hatching from resting eggs that had overwintered in the sediments. Juveniles had matured by late June and females started producing immediately hatching eggs by 20 June and resting eggs by 3 July. This pattern of population dynamics (resting egg hatching, juvenile maturation and subsequent production of eggs) is consistent with previous studies in the Laurentian Great Lakes (e.g. Yurista 1992, 1997), inland lakes in North America (Yan *et al.* 2001, Brown & Branstrator 2005, 2011) as well as European lakes where the species is native (Herzig 1985, Rivier 1998, Straile & Hälbich 2000). Typically the newly hatched individuals from the resting eggs will mature and reproduce asexually (using parthenogenesis) to produce the next generation quickly. Some females switch to sexual reproduction at some point to produce resting eggs that are resistant to harsh conditions. This sexual reproduction requires males, so normally males will be observed in the population when females carrying resting eggs are found. During our sampling in 2018 no males were observed. Males are often very rare so this is not unexpected. The presence of females carrying resting eggs indicates that resistant resting eggs could be spread by boaters and other recreational users to new locations as early late June or early July. This same pattern has been observed each year since our sampling began in 2015 (Merkle & De Stasio 2018).

In Fall 2015 reports were obtained by the Wisconsin Department of Natural Resources that round goby had been caught in Little Lake Butte des Morts just below the Menasha dam (Ebert 2015). This resulted in concern that this AIS might be able to disperse through the boat lock at Menasha that was functioning at that time. The WI DNR decided to force the closure of the lock in Fall 2015 and increased sampling efforts in Lake Winnebago and encouraged the public to report any round goby caught in the area. During the sampling conducted for our study in 2018 round goby were observed only in the lower Fox River, at sampling locations below the Rapide Croche invasive species barrier. No round goby were caught in Lake Winnebago, despite sampling throughout the summer in shallow, rocky habitats known to be preferred by the fish. We used multiple methods that successfully catch round goby in the lower Fox River. These data support the conclusion that the round goby has not successfully established a population in Lake Winnebago at this time.

One of the main objectives of this research was to determine the population dynamics of spiny water fleas and the timing of resting egg production in southern Green Bay and the lower Fox River. The results show that spiny water fleas have not been established in Lake Winnebago or the river, but in southern Green Bay there is resting egg production from late June until at least late September (and likely longer based on data from 2015-2017). The proportion of females producing resting eggs was small compared to the rest of the population during this entire time period, but even this small proportion has the potential to allow secondary invasion of inland waters if proper preventative procedures are not followed when moving boats or gear to new locations or allowing boats to move upstream. The fact that spiny water fleas are not established in the lower Fox River even though a thriving population occurs right at the mouth of the river indicates that the river may act as a natural barrier to upstream dispersal of this invasive species. Given the variation observed between the 4 years sampled it will be important to be conservative in any given year when considering how best to reduce the spread of this AIS into his important ecological and recreational system.

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Bythotrephes longimanus in shallow, nearshore waters: Interactions with *Leptodora kindtii*, impacts on zooplankton, and implications for secondary dispersal from southern Green Bay, Lake Michigan

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ABSTRACT

The spiny water flea *Bythotrephes longimanus* is a predatory cladoceran that invaded Green Bay, Lake Michigan by 1988 and has been shown to negatively affect zooplankton prey. *Bythotrephes* is thought to occur where a deep-water daytime refuge from fish predation is available. Information from shallow, nearshore environments is relatively sparse, yet risk of secondary dispersal from these areas to inland waters is high. The production of desiccation-tolerant resting eggs, coupled with recreational boating activities, can facilitate spread inland. We determined *Bythotrephes* population demographics and dynamics at two sites in southern Green Bay during 2015 and 2016 to examine interactions with zooplankton and timing of resting egg production. Estimates of prey consumption rates by *Bythotrephes* were compared to those for a native predatory zooplankter, *Leptodora kindtii*, and against productivity estimates for potential crustacean prey. *Bythotrephes* population dynamics were similar at both sites in each year, with biomass peaks in September 2015 and July 2016. Earliest resting egg production occurred by 8 July 2015 and 17 June 2016; resting eggs occurred until at least October each year, when sampling ceased. Consumption by *Bythotrephes* generally exceeded that by *Leptodora*. Zooplankton productivity rates were lower than consumption rates on all dates in 2015 but approximated or exceeded consumption rates in 2016. *Bythotrephes* has become a major predator in the Green Bay lower food web, changing energy transfer through this major Great Lakes ecosystem. Its success has increased potential dispersal to inland lakes, especially from shallow, nearshore habitats such as occur in southern Green Bay.

Introduction

The relative importance of fish and invertebrate predators in the Laurentian Great Lakes basin has been changing following the arrival of invasive species over the past few decades. Establishment of macroinvertebrate predators like *Bythotrephes longimanus* (hereafter *Bythotrephes*) has led to significant shifts in zooplankton composition and abundance in both the Great Lakes (Lehman and Caceres, 1993; Barbiero and Tuchman, 2004; Pothoven and Höök, 2014) and inland lakes (Strecker and Arnott, 2008; Dumitru et al., 2001; Brown et al., 2012; Kerfoot et al., 2016). Most aquatic invasive species first arrive in the Great Lakes, and then move into inland lakes by secondary dispersal; therefore, understanding the dynamics in nearshore environments of invasive species populations, as well as potential dispersal vectors, is critical. Shallow water areas are locations of high recreational boating activity that can lead to secondary dispersal, yet these habitats are not well studied by current monitoring programs or scientific studies (but see Yurista et al., 2016 for addressing these needs). Recent efforts focused on management of invasive species highlight the importance of examining the sequential steps of the invasion process, especially those that regulate secondary dispersal (Vander Zanden and Olden, 2008). A major driver of overall invasion success is the amount of propagule pressure of invasive species while moving into new areas (e.g. Colautti et al., 2006). For species like *Bythotrephes* a major determinant of propagule pressure is likely the movement of resting eggs via recreational boating activities. Females carry resting eggs in a brood chamber (Branstrator, 2005), and eggs can be transported along with females that become attached to fishing line or in water retained in the anchor compartment, bilge, or live wells of boats (Kerfoot et al., 2011; Kelly et al., 2013). Local abundance of females carrying resting eggs, along with the intensity of recreational activities, will influence the possibility for secondary dispersal from nearshore areas. Determining factors affecting population dynamics and demographics of *Bythotrephes* in these habitats is needed to understand both the

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ecological role of this invertebrate predator, as well as how best to manage the spread of this successful aquatic invasive species.

This study focused on interactions between *Bythotrephes*, the native cladoceran predator *Leptodora kindtii* (hereafter *Leptodora*), and their prey populations of zooplankton in southern Green Bay, Lake Michigan. The southern region of the bay is hypereutrophic, and historically has supported extremely high zooplankton productivity (Richman et al., 1984; De Stasio et al., 2018). Food availability for cladoceran predators like *Bythotrephes* and *Leptodora* should be abundant in this location. In order to better understand the role of *Bythotrephes* in this food web relative to other predators, we determined *Bythotrephes* consumption rates on zooplankton using the stage-structured bioenergetics model of Yurista et al. (2010) and compared them to *Leptodora* consumption estimated using a conversion efficiency model, and also to zooplankton production rates. In addition we documented the patterns of *Bythotrephes* resting egg production in order to provide information on timing of secondary dispersal risk to inland lakes.

Methods

Sample sites and dates

We obtained samples from two sites located in southern Green Bay, Lake Michigan, established during previous research programs (Fig. 1; 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 biweekly from June–October 2015 and May–October 2016. These stations are <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 between 0.5 m above the bottom and the surface with an ichthyoplankton net (0.5 m diameter, 2.0 m length, 250 μm mesh). The net was towed at 0.9 m/s for either 5 min or 3 min to adjust for differences across dates in plankton density and resulting net clogging conditions. Samples were transported live to the laboratory in accordance with WI Administrative Code NR 40 and all applicable permitting requirements. Animals and potentially contaminated water were maintained in the laboratory at Lawrence University and prevented from release into natural waterways or public water treatment system at all times.

Counting procedures

Live samples were examined in the laboratory to determine the number of females carrying resting eggs and also loose resting eggs. Animals carrying resting eggs were transferred to 12-well tissue culture plates and stored in a dark incubator at 18 °C to confirm dormant state of eggs. For preservation, sample volume was reduced the same day as collection by straining through a mesh cup (250 μm mesh) and preserving in 70% denatured alcohol or 4% buffered formaldehyde. Samples were counted separately for *Bythotrephes longimanus*, *Leptodora kindtii*, and other crustacean zooplankton. For *Bythotrephes*, entire samples were enumerated if density was low. If a sample contained >300 specimens it was subsampled using a Folsom plankton sample splitter. Subsamples were counted at 10×–40× magnification using five categories that correspond to life stages of *Bythotrephes* (juveniles, males, females with no eggs, females with immediately hatching eggs/embryos, females with resting eggs). Body size measurements were determined for each instar from samples for July, August and September 2015. Images were obtained at 40× and measured using ImageJ software (Ver. 2.0.0-rc-15/1.49m). Core body length and tail spine length were determined for 40–100 individuals, depending on availability in samples (Burkhardt, 1994).

Zooplankton species composition and abundance were determined by subsampling duplicate samples for each date and site. Aliquots were obtained with a wide-mouth pipette from samples diluted to a known volume. Subsamples were counted in a circular zooplankton counting tray, with individuals identified to the species level, or the lowest taxonomic unit possible, using Balcer et al. (1984). We applied a correction to our abundance data to account for reduced sampling efficiency due to possible net clogging. The correction factor was based on an assessment we performed in summer 2017 to provide information on the possible magnitude of this issue. We collected duplicate samples simultaneously with both a Clarke-Bumpus sampler (0.13 m diameter, 250μm mesh) and the ichthyoplankton net used in this study (0.5 m diameter, 250μm mesh) from GB1A and GB2 on multiple dates in summer 2017. Mean density of zooplankton from icthyoplankton net samples was 32.9% (SD = 1.12%) of those determined from Clarke-Bumpus

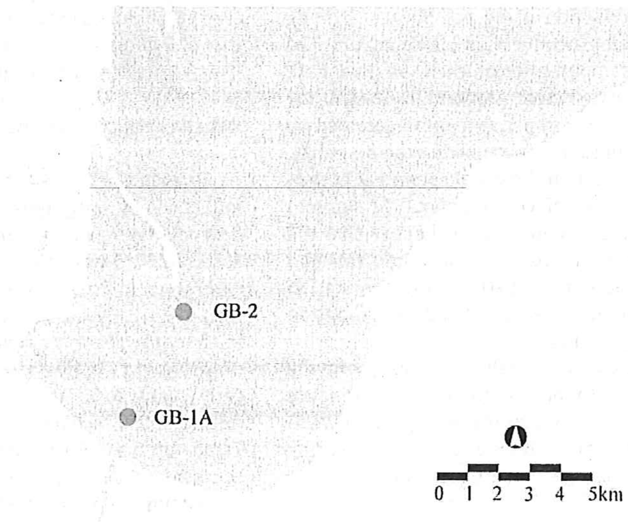

Fig. 1. Map of southern Green Bay showing locations GB-1A and GB-2 sampled during 2015 and 2016.

samples. There was no clear trend across dates in terms of variation in sampling efficiencies, and no significant differences in collection efficiencies between the various taxonomic groups (ANOVA, $F_{10,66} = 1.35$, $p = 0.221$) Based on these findings we decided to multiply each abundance estimate in both years by 3.04 to correct for reduced ichthyoplankton net filtering efficiency. Taxa-specific biomass was determined using biovolume and dry-weight conversions based on previous work on Green Bay zooplankton (Richman et al., 1984). The duplicate biomass estimates from each station were averaged for each date to obtain a single overall biomass density for inner Green Bay for each predator and prey group.

Production and consumption estimates

Production rates for zooplankton on each date were calculated for Cladocera, Cyclopoida, and Calanoida using production-to-biomass (P/B) relationships from Shuter and Ing (1997); $(P/B) = a + b \times T$ where a and b are group specific coefficients for each group and T is temperature (°C). Consumption rates for *Bythotrephes* were calculated using the bioenergetics model from Yurista et al. (2010), which combines water temperature and median biomass of each instar. Consumption is estimated as $\text{ug dry weight consumed/instar/day} = a + b \times T + c \times T^2 + d \times \text{median instar ug} + e \times \text{median instar ug} \times T$ where a, b, c, d, and e are instar specific coefficients and $T = \text{temperature}$ (°C). Number of animals per liter was converted to biomass separately for each instar using dry weights provided by Yurista et al. (2010) and then summed to estimate total *Bythotrephes* biomass. We also initially calculated *Bythotrephes* consumption rates using the conversion efficiency method as in Pothoven and Höök (2014), but decided that the bioenergetics method was more useful because of observed changes in demographics over seasons. Estimates using the conversion efficiency method resulted in lower consumption estimates than from the bioenergetics approach; conversion efficiency values were 49.8% (SD = 5.3) lower on average than bioenergetics estimates. This is consistent with the findings of Pothoven and Höök (2014). The bioenergetics approach should account for demographic changes better than the conversion efficiency method, but does assume that food limitation is not important. Given the high biomass of prey typically documented in the hypereutrophic inner bay this assumption does seem reasonable (De Stasio et al., 2018). Consumption rates for *Leptodora* were determined as in Pothoven and Höök (2014) by estimating production using the Shuter and Ing (1997) relationship and dividing by a conversion efficiency of 0.234 (Vijverberg et al., 2005). Water temperatures on each sampling date were obtained from a buoy located near station GB-1A (http://seatemperature.info/green-bay-water-temperature.html; accessed on 24 January 2017). Our two sampling stations are within the inner bay, and our previous studies have shown that the water column at both of these stations is continually uniformly mixed throughout the summer (B. De Stasio, unpublished data). Consequently, we believe that the use of surface water temperature is reasonable for the production and consumption estimates. Consumption estimates from each station were averaged for each date to obtain a single overall consumption rate for inner Green Bay for each predator.

Data were tested for normality and generally were non-normally distributed. Transformations failed to meet assumption of normality for comparisons, so non-parametric tests were employed (Kruskal-Wallis and Mann-Whitney tests). Statistical analyses were conducted using PAST (Paleontological Statistics Package, version 3.1; Hammer et al., 2001).

Results

Bythotrephes population dynamics

There were similar patterns in population dynamics of *Bythotrephes* in both years examined (Fig. 2). *Bythotrephes* was observed on all dates sampled except for the very earliest date in 2016 (31 May). Adult females were present by the end of June each year. In both years there was an increase in biomass by late July, reaching approximately 10–15 mg/m^3 in each year, followed by biomass decreases during August. During 2015 there was a large, second population increase in early September, reaching 74.9 mg/m^3 (Fig. 2A). In 2016 a smaller increase occurred in mid-September, peaking at only 2.4 mg/m^3. In both years, the overwhelming majority of biomass was comprised of females, with <8.6% (SE = 1.8) accounted for by juveniles and 6.7% (SE = 1.4) consisting of males. Once the first females reached maturity in June of both years, the majority began producing embryos asexually (Table 1). However, females carrying sexually produced resting eggs were observed by 8 July 2015, with approximately 1–2% of females producing resting eggs throughout the year until sampling ended in early October when over 4% of females carried sexual broods. In 2016, females with resting eggs appeared as early as 17 June, and about 1% of females were making resting eggs through June and July. No females with resting eggs were recorded during August–October in 2016 (Table 1).

Bythotrephes core body length of adults changed across time in 2015, but did not result in changes in total body length (Fig. 3). Median core body length did not differ across time for younger instars (Mann-Whitney tests, $p > 0.05$), with a median length of 1.59 mm for instar 1, and 2.00 mm for instar 2. Core body length of instar 3 individuals did decrease significantly across the summer, from a July median of 2.57 mm to 2.05 mm in September (Fig. 3B; Mann-Whitney test, $U = 42$, $p = 0.0046$). However, slight increases across time in tail spine length of instar 3 offset decreases in core body length (Fig. 3B), resulting in non-significant changes in total lengths (Fig. 3C; Kruskal-Wallis, $H = 0.55$, $p = 0.759$). There were no significant changes across time for any of the other instars in either tail length or body length (all Mann-Whitney tests, $p > 0.05$).

Leptodora population dynamics

Biomass of *Leptodora* varied between the two years studied, exhibiting an inverse relationship to *Bythotrephes* biomass (Fig. 2B & D). During 2015 *Leptodora* biomass peaked in late July at 0.82 mg/m^3, compared to the *Bythotrephes* biomass peak of 14.20 mg/m^3 at the same time. *Leptodora* biomass decreased in August and remained in the range of 0.2–0.4 mg/m^3 for the remainder of the sampling season (Fig. 2B). Biomass of *Leptodora* was higher in 2016, and often exceeded that of *Bythotrephes*. *Leptodora* biomass fluctuated in the range of 2–8 mg/m^3, with a peak in late August of 14.17 mg/m^3 (Fig. 2D).

Predatory cladoceran consumption estimates

Estimates of consumption by predatory cladocerans can be heavily influenced by water temperature, and surface water temperatures in Green Bay were warmer in 2016 than 2015 (Fig. 4). Early June temperatures in 2015 were 15–16 °C and warmed to just lower than 20 °C by early June. In 2016 temperatures were approximately 3 °C warmer than in 2015. By late July, temperatures in both years reached 24 °C. In 2015 temperatures then decreased throughout the rest of the season, dropping to 17 °C by early October. Temperatures in 2016 increased in early August to 25 °C, and remained at this level until the last week of August, after which they decreased until reaching 17 °C in early October. Temperatures in August and early September 2016 were typically 4–5 °C warmer than the corresponding period in 2015 (Fig. 4).

Consumption estimate trends for *Bythotrephes* essentially followed those observed for biomass (Fig. 5A & C). In both years there was a late July peak of 20 mg/m^3/day, followed by decreases in early August. In 2015, consumption decreased to a low of 3.0 mg/m^3/day and then increased again in late August and early September to a peak of 76.8 mg/m^3/day. Consumption rates in 2016 continually decreased through August to a low in early September of 0.004 mg/m^3/day. Rates in late September 2016 increased again to 2.7 mg/m^3/day (Fig. 5C).

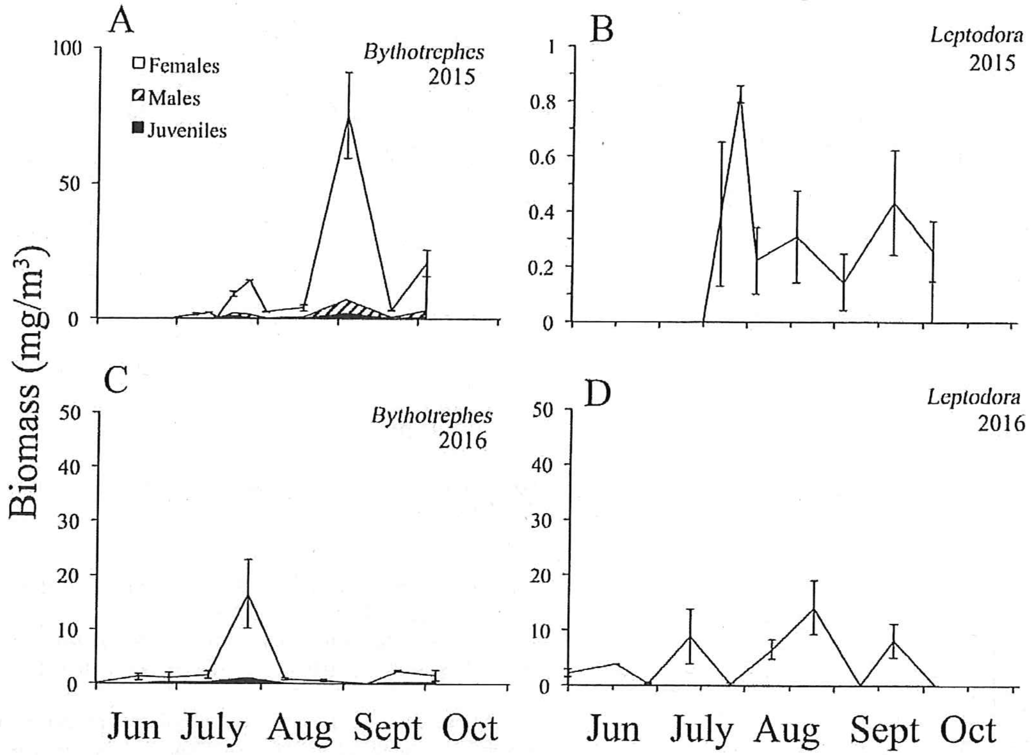

Fig. 2. Biomass of predatory cladocerans (mean ± 1 SE) in southern Green Bay during 2015 (A & B) and 2016 (C & D). Biomass for *Bythotrephes longimanus* are shown in A & C, and biomass for *Leptodora kindtii* are given in B & D.

Leptodora consumption estimates were significantly lower in 2015 than in 2016 (Fig. 5B & D; Kruskal-Wallis, $H = 4.167$, $p = 0.040$). Rates reached a maximum of 0.75 mg/m^3/day in late July 2015 and then decreased to <0.2 mg/m^3/day for the remainder of the year. In 2016, rates fluctuated around 1 mg/m^3/day during June, exhibited peaks in July of 7.3 mg/m^3/day, a late August peak of 11.7 mg/m^3/day, and a final peak in mid-September of 5.0 mg/m^3/day (Fig. 5D). *Leptodora* consumption rates were significantly lower than those estimated for *Bythotrephes* in 2015 (Kruskal-Wallis, $H = 8.08$, $p = 0.0040$), but not in 2016 (Kruskal-Wallis, $H = 0.006$, $p = 0.940$).

Table 1

Abundance of female *Bythotrephes longimanus* (mean with 1 SD in parentheses) and percentages of females with asexual or sexual broods in Green Bay on all sampling dates for 2015 and 2016. On some dates only a single sample was available, therefore no estimates of SD are reported.

Date	Females (number/m^3)		Percent asexual broods	Percent sexual broods
2015				
25-Jun	0		0	0
29-Jun	0.02		100	0
8-Jul	3.52	(1.09)	49.9	1.8
13-Jul	4.99		56.3	2.3
16-Jul	1.84		15.5	0
22-Jul	15.96	(1.19)	76.9	0.7
28-Jul	27.91		74.6	1.0
3-Aug	5.07	(0.19)	83.8	0.4
17-Aug	7.34	(3.17)	86.6	1.4
2-Sep	160.14	(116.72)	75.1	1.3
19-Sep	5.28	(0.05)	74.2	0.9
2-Oct	41.19	(18.28)	63.1	4.4
2016				
31-May	0.00	(0.00)	0	0
17-Jun	2.79	(2.62)	68.5	0.3
28-Jun	1.99	(1.24)	59.1	1.5
12-Jul	3.27	(0.80)	61.0	1.0
26-Jul	35.97	(35.81)	80.3	0.6
9-Aug	2.09	(0.51)	47.3	0
23-Aug	1.15	(0.74)	75.4	0
8-Sep	0.01	(0.01)	0	0
19-Sep	4.87	(0.25)	68.0	0
3-Oct	3.20	(3.20)	0	0

Zooplankton population dynamics

We found the lowest zooplankton biomass in southern Green Bay in mid-summer, followed by increases in September in both years studied (Fig. 6). Biomass dry weight estimates in 2015 are available starting in mid-July, with total biomass of 0.11 mg/m^3 and dominated by cladocerans. Biomass during late July and August was 0.03–0.05 mg/m^3, followed by an increase to 0.17 mg/m^3 in late September. Cladocerans accounted for over 95% of the biomass on all dates in 2015, due almost exclusively to *Daphnia* throughout the year.

Zooplankton biomass in 2016 was significantly greater than in 2015 (Kruskal-Wallis, $H = 14.14$, $p = 0.0002$), with approximately 300 mg/m^3 at the beginning of June (Fig. 6B). Biomass decreased to a low of 1.44 mg/m^3 in late June, but then increased through July and August, reaching a peak biomass of 341.1 mg/m^3 in mid-September. During June, cladocerans comprised over 95% of the biomass, then decreased to approximately 60% throughout July and August (Fig. 6B). Cyclopoid copepods increased in relative biomass through August and September, reaching 40–45% of total biomass at times. Calanoid copepods exhibited low biomass, never reaching >18.6 mg/m^3.

Consumption relative to zooplankton production

Differences between zooplankton production and consumption by predatory cladocerans varied between years, mainly reflecting predator biomass changes. Consumption exceeded production by zooplankton prey on all dates in 2015 (Fig. 7). Differences between prey production and *Bythotrephes* consumption estimates ranged from

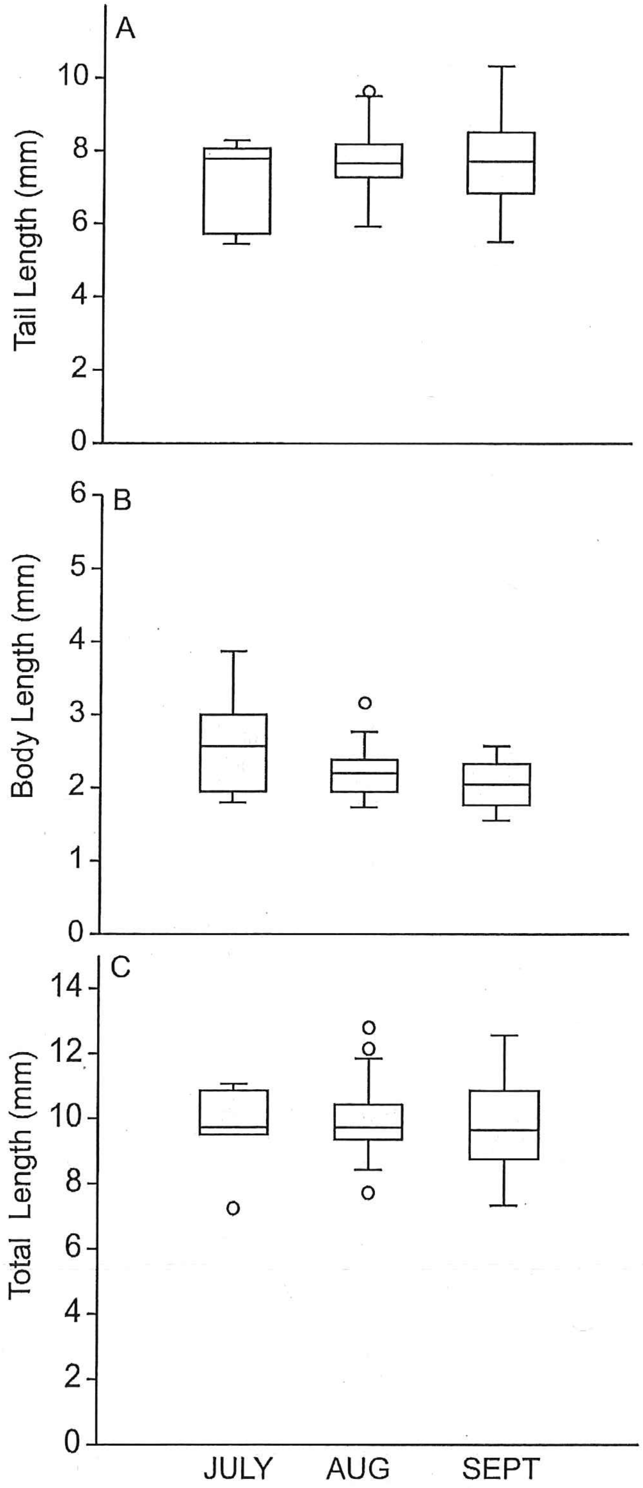

Fig. 3. Box and whisker plots of *Bythotrephes longimanus* A) tail length, B) core body length and C) total length from Green Bay for three months in 2015. Outliers are shown as circles beyond whiskers.

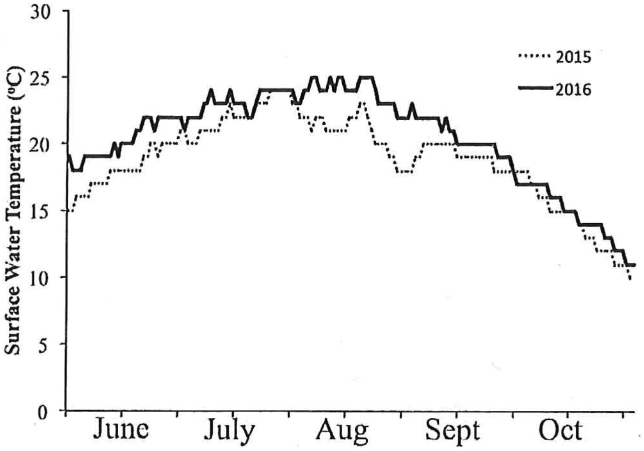

Fig. 4. Surface water temperature in southern Green Bay during 2015 (dotted line) and 2016 (solid line).

−1.1 to −76.79 mg/m³/day, with the largest difference occurring in early September (Fig. 7A), the time of the maximum biomass peak for the *Bythotrephes* population (Fig. 2A). During 2016, zooplankton production exceeded *Bythotrephes* consumption on all dates except for single dates in late June and late July. Differences between production and consumption in early June were 36.4 mg/m³/day, and decreased during June and July, until reaching −18.0 mg/m³/day in late July. Differences increased after that point and became positive in early August, reaching the highest value of 2016 at 39.1 mg/m³/day in mid-September (Fig. 7A).

The estimated contribution of *Leptodora* to predation in 2015 was lower than that by *Bythotrephes*. *Leptodora* consumption approximately equaled production by zooplankton on all dates, resulting in difference values ranging from 0.02 mg/m³/day in early July to −0.75 mg/m³/day in late July (Fig. 7B). Differences during August - October 2015 varied from −0.08 to −0.23 mg/m³/day. Consumption rates by *Leptodora* were much higher in 2016, but so was zooplankton production, producing positive differences throughout the summer (Fig. 7B). Difference values dropped from an early June value of 35.3 mg/m³/day to values close to zero through June and July. Difference values increased through late July and August, obtaining the largest difference in mid-September at 36.85 mg/m³/day.

Examining the combined estimates of consumption for both *Bythotrephes* and *Leptodora* demonstrated similar patterns in the two years for early summer (Fig. 7C). Production exceeded consumption until the end of June, and then was less than consumption until early August. In 2015, difference values stayed negative for the remainder of the year, reaching the lowest value of −76.9 mg/m³/day in early September. Difference values for 2016 became positive by mid-August and continued to increase until a high of 34.1 mg/m³/day in mid-September, after which values decreased to 5.6 mg/m³/day by early October (Fig. 7C).

Discussion

Our study demonstrates that *Bythotrephes* occurs in southern Green Bay from June through at least early October, and that sexual production of resting eggs occurred by early July at the latest. The population underwent a decline in August each year studied, and had variable fall dynamics depending on the year examined. Estimated prey consumption rates for *Bythotrephes* were greater than for *Leptodora* in both years studied, but there was high interannual variability in late summer effects on the zooplankton community. *Bythotrephes* has a clear potential to reduce zooplankton populations, and now plays an important role in lower food web dynamics of southern Green Bay.

This is the first study to determine the population dynamics of *Bythotrephes* and its effects on the zooplankton community in the productive southern end of Green Bay. Although *Bythotrephes* was previously reported from a single site north of Sturgeon Bay in 1987 (Jin and Sprules, 1990) and one collection from Sturgeon Bay in 2000

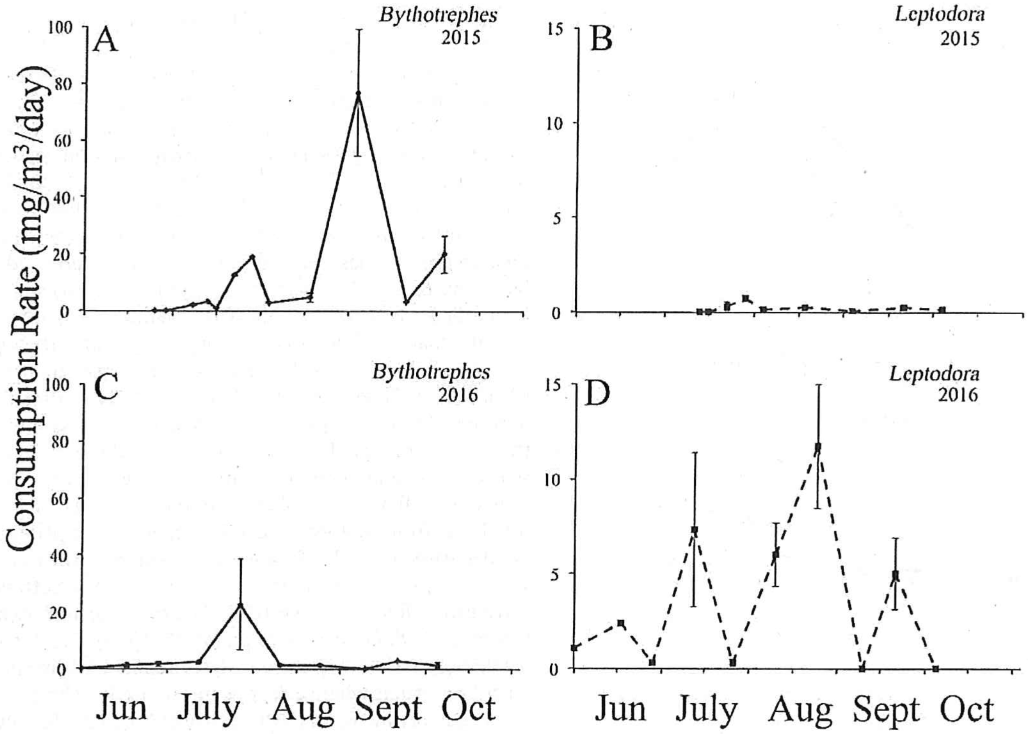

Fig. 5. Consumption rates for predatory cladocerans (mean ± 1 SE) in southern Green Bay during 2015 (A & B) and 2016 (C & D). Rates for *Bythotrephes longimanus* are shown in A & C, and rates for *Leptodora kindtii* are given in B & D.

(Pothoven et al., 2003), it has been unclear if this invasive species could thrive in the shallow, warm waters of the inner bay region. *Bythotrephes* often is considered a cool water stenotherm species with high mortality above 25 °C (Yurista, 1999), and that it typically needs a dark/deep water refuge to survive in the presence of visual fish predators (MacIsaac et al., 2000; Yan et al., 2011). However, it has also been reported to occur in shallow water habitats in Europe and Asia (MacIsaac et al., 2000). As observed in the inner bay of Green Bay during 2016, water temperatures reach at least 25 °C for extended periods of time (Fig. 4). Biomass of *Bythotrephes* during and after that time period was lower in 2016 compared to the same period in 2015 when temperatures were 4–5 °C cooler, suggesting that temperature stress may have contributed to the lack of a large population increase during late summer and fall of 2016. However, previous studies have found populations of *Bythotrephes* occurring in waters that reach as high as 30 °C (Grigorovich et al., 1998), consistent with our previous demonstration that *Bythotrephes* was found in the southern bay from 2004 to 2007, years when water temperatures routinely ranged from 26 to 28 °C during late July and August in the inner bay (De Stasio et al., 2018; B. De Stasio, unpublished data). These data indicate that extreme temperatures are not likely preventing *Bythotrephes* from thriving in this habitat. The inner bay is a shallow, constantly mixed environment with low water clarity due to high concentrations of suspended solids and chlorophyll (Secchi depths typically average 0.5 m or less during late summer; Qualls et al., 2013). The low light environment likely limits visual predation pressure on *Bythotrephes*, allowing them to survive in these shallow sites without a deep-water refuge. These data, along with observations of high concentrations of animals along the immediate shorelines of southern Green Bay (B. De Stasio, unpublished data), suggests that *Bythotrephes* could do well in productive, shallower, and warmer inland lakes in Wisconsin.

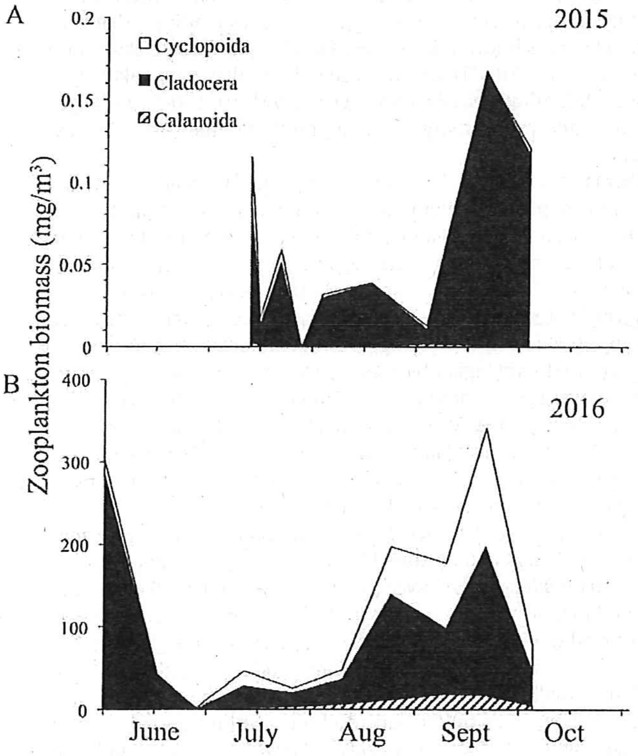

Fig. 6. Mean zooplankton biomass (mg/m³) in southern Green Bay for three major taxonomic groups during A) 2015 and B) 2016. No samples were available for counting from June 2015.

Bythotrephes interactions with zooplankton

Zooplankton biomass exhibited a negative relationship with *Bythotrephes* biomass dynamics (Figs. 2 and 6). During the year with high *Bythotrephes* biomass (2015), very low biomass of zooplankton was recorded, consisting primarily of large *Daphnia*. In 2016,

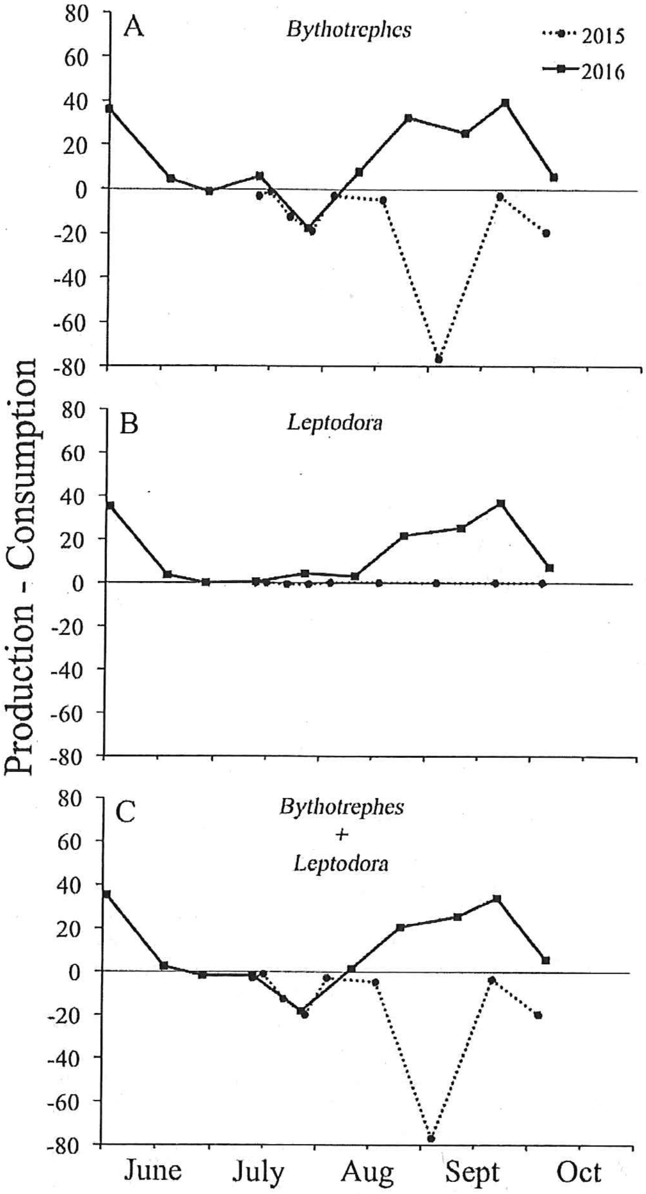

Fig. 7. Difference between mean total zooplankton production and consumption rates (mg/m^3/day) for A) *Bythotrephes longimanus*, B) *Leptodora kindtii*, and C) both predatory cladocerans combined in southern Green Bay during 2015 (dotted line) and 2016 (solid line).

Bythotrephes biomass was lower and zooplankton biomass was significantly higher, with a more diverse assemblage of cladocera and copepods than occurred in 2015. Although this relationship is based on only two years of data, this conclusion is also supported by significant decreases in total zooplankton biomass documented for these same sites following the invasion by *Bythotrephes* compared to pre-invasion years (De Stasio et al., 2018). Mean zooplankton biomass in the inner bay before the invasion approximated 4000 mg/m^3, and decreased to 600–1500 mg/m^3 after the invasion (2004–2007). Zooplankton biomass values in 2016 peaked at 341 mg/m^3, and were much lower in 2015 (Fig. 6), indicating that zooplankton biomass has continued to decrease recently. Similar negative relationships between zooplankton and *Bythotrephes* were documented for Lake Michigan soon after invasion by *Bythotrephes* (Lehman, 1991), as well as for inland lakes (Dumitru et al., 2001; Strecker and Arnott, 2008). Likewise, there was a large, direct predatory impact on zooplankton over a short time period (1–2 months) in Saginaw Bay, Lake Huron, a similarly shallow and productive bay of the Great Lakes (Pothoven and Höök, 2014).

Many studies have shown that *Daphnia* are a preferred prey item of *Bythotrephes* (Lehman, 1987; Lehman and Caceres, 1993; Lehman and Branstrator, 1995; Schulz and Yurista, 1998) and invasion of productive, freshwater lakes by *Bythotrephes* typically results in decreased biomass of *Daphnia* (e.g. Fernandez et al., 2009; Walsh et al., 2016). Our results are not entirely consistent with these other studies because *Daphnia* were the dominant zooplankton group in the year with the higher *Bythotrephes* biomass. In addition, *Daphnia pulicaria* and *D. mendotae* both have occurred regularly in southern Green Bay since invasion by *Bythotrephes* (De Stasio et al., 2018). While *D. mendotae* is the main daphnid that typically thrives in the presence of *Bythotrephes* (Yan et al., 2011), likely due to faster escape responses than other species (Pichlová -Ptáčníková and Vanderploeg, 2011), our results show that in some lakes larger species of *Daphnia* can successfully coexist with this invertebrate predator. Coexistence also depends on reduced size-selective predation pressure from fish, or availability of a deep-water refuge that allows survival of *Bythotrephes* (Walsh et al., 2016). Fish trawl data from southern Green Bay indicate that planktivory by fish has decreased recently (T. Paoli, Wisconsin Department of Natural Resources, *personal communication*). Trawl catch of both yellow perch and alewife after 2000 were 10% to 20% of abundances typically caught before the *Bythotrephes* invasion (1986–1988). The decreases in fish planktivory have likely allowed *Bythotrephes* to thrive in the shallow inner bay regions where a deep-water refuge is lacking.

Planktivory by fish in the inner bay of Green Bay has also been variable in recent years, which likely contributed to the large differences in zooplankton prey density between 2015 and 2016 (T. Paoli, Wisconsin Department of Natural Resources, *personal communication*). Trawl data for the inner bay in 2015 indicated extremely high densities of planktivores, especially gizzard shad, compared to 2016 when catch per effort levels were only 60.6% of those in 2015. Higher abundance of planktivores in 2015 is consistent with the observed lower zooplankton density. There are data on zooplankton biomass in the lower bay for only a few other recent years, but these years also indicate that zooplankton biomass is lower during years of high planktivore abundance (De Stasio et al., 2018). Those data also indicate that zooplankton biomass can differ dramatically among years, with 10–100-fold changes in biomass and productivity occurring multiple times over the past 20 years.

Other factors may also be contributing to the observed interannual changes in zooplankton biomass. For example, inflows from the Fox River have been viewed as a major driver of plankton productivity in the lower bay by creating a strong nutrient and trophic gradient (Richman et al., 1984; Sager and Richman, 1991; De Stasio et al., 2018). Those previous studies have documented persistent differences between GB1A and GB2, with higher zooplankton abundance at GB2. Our study also found higher biomass at GB2 (data not shown), but we employed averages of these two sites to better represent overall dynamics in the inner bay. Wind also can affect zooplankton patchiness in this shallow, well-mixed inner bay region, but given lower zooplankton biomass was recorded in 2015 throughout the entire summer it is unlikely that wind forcing caused the lower biomass.

Our comparison of *Bythotrephes* consumption estimates with zooplankton production demonstrated high variability in the potential impact of *Bythotrephes* on the lower food web in Green Bay (Fig. 7). Early summer dynamics were similar in both years, with consumption exceeding production as the *Bythotrephes* population increased. In 2015 consumption continued to be higher than production throughout the rest of the sampling season. In contrast, during late summer 2016 consumption was less than production, resulting in positive net production from mid-August into October. A study of *Bythotrephes* consumption in Saginaw Bay also documented differences among two years studied, but not a large as our changes among years (Pothoven and Höök, 2014). Consumption estimates for Green Bay (20–80 mg/m^3/day) are greater than those estimated for Saginaw Bay (maximum 8 mg/m^3/day), and during 2015 resulted in greater differences between production and consumption than observed in Saginaw Bay. However, the Saginaw Bay study showed that *Bythotrephes* consumption exceeded prey production by 200–300% in mid-summer of both years examined, similar to the pattern observed in Green Bay during 2015 (Fig. 7). Apparently production rates of zooplankton prey are fast enough to sustain high consumption rates for short periods of time.

Predation by *Leptodora* did not compensate for changes in *Bythotrephes* consumption, and estimates of consumption by both predatory cladocerans combined did not differ noticeably from those based on *Bythotrephes* alone (Fig. 7C). *Bythotrephes* consumption rates in Saginaw Bay were also much greater than those from *Leptodora* throughout the year (Pothoven and Höök, 2014), similar to those we observed in 2015. Our results in 2016 were more similar to those observed in Lake Erie where consumption was due mainly to *Bythotrephes* during the early summer and then switched to predation dominated by *Leptodora* later in the year (Pothoven et al., 2012). Consumption of *Leptodora* in Green Bay was generally low, with only slightly negative differences between prey production and consumption in 2015 and positive differences in 2016 (Fig. 7B). These consumption rates are similar to those observed in Saginaw Bay where typically <10% of production is consumed by *Leptodora*, with maximum rates of 13–45% depending on the year examined (Pothoven and Höök, 2014). Other studies have found *Leptodora* consumption to equal 15–43% of cladoceran prey production (Hillbricht-Ilkowska and Karabin, 1970) and 25–35% of *Daphnia* production (Hall, 1964; Wright, 1965). The study by Cummins et al. (1969) also showed high variability between years in percent of prey production consumed, ranging from 0.3–8.5% in one year, followed by 2.5–383.1% in the next. These interannual changes are similar to our results for Green Bay. In addition, in Green Bay there was a negative relationship between *Leptodora* and *Bythotrephes* biomass, with approximately 10-fold higher biomass of *Leptodora* during 2016 when *Bythotrephes* biomass was lower (Fig. 2). *Bythotrephes* may feed directly on *Leptodora* (Branstrator, 1995), but it also is likely a better competitor for similar prey (Schulz and Yurista, 1998; Foster and Sprules, 2009). In Green Bay it appears that the effect of *Leptodora* on lower food web interactions is driven by *Bythotrephes* dynamics, indicating a need for further research on these two important predatory cladocerans.

Impacts from *Bythotrephes* may also extend beyond effects on zooplankton, cascading down to affect water quality factors as well (Hoffman et al., 2001; Walsh et al., 2016). The dramatic differences in zooplankton biomass between 2015 and 2016 correspond to significant differences in chlorophyll levels, with average concentration of chlorophyll in the high zooplankton biomass year (2016) less than half the 2015 level (E. Erin Houghton, Green Bay Metropolitan Sewerage District, unpublished data). Unfortunately, we do not have quantitative data on phytoplankton species composition for 2015 and 2016. Previous studies have shown that mean summer chlorophyll concentrations in southern Green Bay significantly increased following invasion by *Bythotrephes* and zebra mussel, *Dreissena polymorpha* (De Stasio et al., 2008). The same was true for total phytoplankton biomass and net population growth rates (De Stasio et al., 2014; 2018), consistent with the conclusion that effects of *Bythotrephes* can be transmitted through multiple connections in food webs.

Resting egg production

A critical factor in the success of *Bythotrephes* as an invasive species is the production of resting eggs. Resting eggs are known to tolerate a wide range of conditions, including temporary drying and even passage through fish digestive systems (Jarnagin et al., 2000; Branstrator et al., 2013). Because they are desiccation-resistant, resting eggs have a higher chance of dispersal via fishing boats, gear, and buckets. Furthermore, a single resting egg can establish a population due to subsequent asexual reproduction following hatching (Brown and Branstrator, 2011). Consequently, the production of resting eggs has been a key attribute driving the successful range expansion of *Bythotrephes*.

Until now, there has been little information on production of resting eggs in Green Bay (Pothoven et al., 2003). Our study demonstrates that resting eggs are produced throughout the summer in southern Green Bay (Table 1). This constant presence of resting eggs increases the chance of secondary spread by boats traveling from the bay to other inland lakes in Wisconsin, especially those traveling up the Fox River. There are 17 locks between Green Bay and the next upstream lake, Lake Winnebago. The locks are currently operated by the Fox River Navigational System Authority (Fox River Navigational System Authority Statute, 2001). An invasive species barrier at the third upstream lock (Rapide Croche) currently also prevents boat transit further upriver. However, a boatlift and cleaning station at this location is planned and has the potential to open a new corridor for upstream dispersal into the larger Fox-Wolf Basin, a watershed encompassing about 40,000 km^2 in Wisconsin (Bertrand et al., 1976). Understanding the dynamics and demographics of *Bythotrephes* in southern Green Bay is critical for determining the risk for secondary dispersal inland beyond the Great Lakes proper.

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