

AN ASSESSMENT OF THE LONG-TERM PHENOLOGY AND IMPACT OF  
*BYTHOTREPES LONGIMANUS* IN ISLAND LAKE RESERVOIR, MINNESOTA,  
USING SEDIMENT RECORDS.

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## Abstract

*Bythotrephes longimanus*, a predacious zooplankter, has invaded >130 inland lakes in North America, yet few studies have documented the long-term phenology and impact of an invasion. This study used *Bythotrephes* spines and *Daphnia ephippia* in <sup>210</sup>Pb-dated sediment cores from four sites in Island Lake Reservoir (Minnesota, United States) to document the establishment and growth of the *Bythotrephes* population and to examine corresponding changes in daphnid species composition and density during a 40-year period spanning the invasion. Based on sediment evidence, *Bythotrephes* invaded the reservoir in 1982.2, or about 8 years prior to what was its first detection in the water column in 1990. If accurate, this makes Island Lake Reservoir the first documented inland lake invaded in North America. Year to year population density of *Bythotrephes* followed a logistic growth model, and generally took about 10 years to reach annual carrying capacity. A notable impact of *Bythotrephes* on the daphnid community occurred within the first few years but did not intensify with increased *Bythotrephes* density over time. Daphnid species composition shifted in the western basin toward *D. mendotae* dominance, but did not shift in the eastern basin. The reservoir maintained a population of its largest-bodied daphnid, *D. pulex*, despite *Bythotrephes* presence. Coincident with *Bythotrephes* invasion, mean daphnid ephippium size decreased both within species and for the whole 3-species daphnid assemblage. This research suggests that lakes could harbor *Bythotrephes* for years before its first detection in the water column, that annual population build-up of *Bythotrephes* may require years before reaching peak annual densities, that the impact of *Bythotrephes* on food webs occurs relatively quickly, and that long-term impacts of *Bythotrephes* in a reservoir may be less severe than in natural lake ecosystems.

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## Introduction

Human mediated exchanges of invasive species are having a detrimental impact on global biodiversity. Invasive species have reduced native species populations and caused local extinctions (Pimentel et al. 2005), modified food webs (De Stasio et al. 2008), and possibly facilitated the establishment of other exotic species (Ricciardi 2001). Economically, it has been estimated that invasive species cost the United States \$120 billion in damages and control measures, annually (Pimentel et al. 2005).

Freshwater habitats, important to life for terrestrial and aquatic organisms alike, are particularly susceptible to invasive species (Sala et al. 2000). The Laurentian Great Lakes, for example, have received at least 182 invasive species since the 1800s (Mills et al. 1994, Holeck et al. 2004, Ricciardi 2006). The susceptibility of freshwater ecosystems to species invasions may be in part related to the widespread use of freshwater for recreational and economic activities that frequently puts humans and their transportation networks in close contact with freshwater ecosystems.

One aquatic invasive species of regional importance in Midwestern North America is *Bythotrephes longimanus* (Arthropoda:Crustacea). *Bythotrephes* is a large (1 cm total length) carnivorous zooplankter that was transported from Eurasia to the United States in the ballast water of cargo ships several decades ago (Sprules et al. 1990, Berg et al. 2002). After its first appearance in North America in Lake Ontario in 1982 (Johannsson et al. 1991), it spread quickly to all of the Laurentian Great Lakes (Lange and Cap 1986, Burr et al. 1986, Cullis and Johnson 1988, Evans 1988). In the early 1990s, *Bythotrephes* began to appear in numerous inland lakes in the Great Lakes

watershed, including Island Lake Reservoir (Minnesota, United States), which was the first inland Minnesota lake reported infested (Gravelle 1990) and is the location of this study. As of 2011, *Bythotrephes* had been detected in 20 inland lakes in Minnesota (Branstrator et al. 2006, unpublished data), and over 130 total inland lakes in North America from mainly southern Ontario, Canada, and Midwestern states of the United States (Yan et al. 1992, MacIsaac et al. 2000, Weisz and Yan 2010).

*Bythotrephes* is able to establish populations rapidly in newly invaded ecosystems because of its ability to reproduce clonally when growth conditions (temperature, food) are favorable. Dispersal of *Bythotrephes* between and within lakes, and its temporal escape from harsh winter conditions, is facilitated by the production of dormant eggs (resting eggs) that are resistant to environmental exposure to salinity, temperature variation, low oxygen, and high and low pH (Grigorovich et al. 1998, Brown 2008). Resting eggs are about 0.5 mm diameter and are produced by sexual reproduction with males, typically in the autumn. Once broadcast, resting eggs sink rapidly to the lake floor, but have been shown to resist gut passage in fish (Jarnagin et al. 2000) and waterfowl (Charalambidou et al. 2003). Range expansion through resting eggs is believed to be the primary means by which *Bythotrephes* disperses and establishes new populations (Panov et al. 2004). The resting egg stage is also the only life stage of *Bythotrephes* known to overwinter in North American lakes (Rivier 1998). Hence, the initiation of entire populations of free-swimming individuals each growing season is dependent on hatching from resting egg banks in the spring.

The available information on the density and growth of a *Bythotrephes* population during its establishment in a lake is limited, but indicates that there may be variation between lakes in the species early population buildup. For example, in Lake Michigan, after *Bythotrephes* first appeared in 1986, its seasonal abundance generally increased during the first six years (Evans 1988, Lehman and Cárceres 1993). By contrast, in Harp Lake, Ontario, Canada, the *Bythotrephes* population did not demonstrate a consistent increase in seasonal abundance during the early years of its invasion. After establishing an initial population in Harp Lake in 1993, *Bythotrephes* numbers and biomass grew in 1994 and 1995, with annual peaks of about 13 animals  $\text{m}^{-3}$  each year (Yan and Pawson 1997, Yan et al. 2001). In the following two years, however, the population was scarce, averaging only 0.26 animals  $\text{m}^{-3}$  in 1996, but then rebounded in 1998 with a seasonal peak of about 25 animals  $\text{m}^{-3}$  (Yan et al. 2001). These data from Lake Michigan and Harp Lake suggest that early population growth of *Bythotrephes* may not conform to a single growth model (e.g., exponential or logistic). However, these two data sets are too short in duration to evaluate their fit to any particular model.

A well-documented outcome of *Bythotrephes* invasion is its effect on native zooplankton ecology. As a carnivore, *Bythotrephes* has been shown to decrease native zooplankton species richness and abundance through predation and in some instances through competition with other carnivores (Yan et al. 2002, Strecker and Arnott 2008, Lehman 1991, Branstrator 2005). For example, Strecker et al. (2006) found a fourfold decrease in the abundances of certain zooplankton species post invasion. In Lake Huron, Lake Michigan, and the central and eastern basins of Lake Erie there was a marked drop

in native zooplankton species diversity during the first year post invasion and the lowered species diversity persisted over time in Lake Huron and Lake Michigan (Barbiero and Tuchman 2004). After invasion of Harp Lake, at least six native zooplankton species disappeared, all of which were vulnerable to *Bythotrephes* predation (Yan et al. 2001).

Daphnids, a preferred food type of *Bythotrephes*, have also been shown to shift in species composition and body size post invasion. In several of the Great Lakes the daphnid species composition shifted from a multiple species assemblage including *D. mendotae*, *D. pulicaria*, and *D. retrocurva*, to dominance by *D. mendotae* (Lehman 1991, Barbiero et al. 2001, Barbiero and Tuchman 2004). This same shift in daphnid species composition was observed in some of the small, inland lakes studied by Strecker et al. (2006). In the Great Lakes, size shifts within the zooplankton community were due in part to this change in species composition, but also within a species in response to size-selective removal by *Bythotrephes* that left three of five daphnid species smaller in average post-invasion body size (Barbiero and Tuchman 2004).

Despite the available information on *Bythotrephes* ecology, the majority of studies on this invader are short term and were begun post invasion. Studies on invasive species often have this dilemma because usually the invasion is unplanned and the invader is not detected until it has established a sizable population. As a result, the date of establishment may be different from the date of first detection, the rate of early population growth is rarely known, and prior knowledge of the prey assemblage is rarely documented.

Lake sediments provide an expansive, long-term fossil archive of past environmental conditions and a possible avenue of research to address some of these historical, time-sensitive questions. Sediment archives include remains of nutrients, phytoplankton, and zooplankton and have been used to address questions about eutrophication, acidification, human disturbance of lakes, invasive species, rapid evolution, and climate change (Jeppesen et al. 2001). Of the zooplankton species common to freshwater lakes, cladocerans are some of the best preserved in sediments. They are represented in the fossil record by durable chitinous pieces such as head shields, carapaces, post-abdomens, ephippial eggs, claws, and spines that are preserved (Frey 1986, Korhola and Rautio 2001). The age of these fossils is commonly determined through  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ , or other elemental dating of the surrounding sediment (Olsson 1986). Sediment studies have been used to track other invasive species in lakes over time. Suchy and Hann (2007) used sediment cores from Lake of the Woods (Ontario, Canada) to document the establishment of the invasive *Eubosmina coregoni*. Hairston et al. (1999) tracked the failed invasion of *Daphnia exilis* in Onondaga Lake (New York, United States) using resting eggs.

*Bythotrephes* represents a unique opportunity to study invasion patterns over a long period of time using lake sediment. The long caudal, laterally barbed spine of a *Bythotrephes* individual is made of a durable chitin that is relatively well preserved in sediment (Keilty 1988). In Gulspettvann Lake, Norway, *Bythotrephes* spines were recovered from 22 cm down in sediment cores (Nilssen and Sandoy 1990). A unique characteristic of *Bythotrephes* is that their spines do not undergo a loss of exoskeleton

during the molting process even though the spine lengthens during the first two molts (Rivier 1998). For other cladoceran species, the remains in the sediment are the product of the molted exoskeletons of sequential instars as well as the exoskeleton present at death. In these populations a species' density is *indirectly* related to the accumulation rates of their remains (Kerfoot 1974). For *Bythotrephes*, however, the lack of exoskeleton loss during molting means that one spine found in the sediment represents one individual. Moreover, the spines are easily distinguishable from all native zooplankton. As a result, their accumulation in the sediment can be used to establish an invasion year (Forman 1998, Keilty 1998, Branstrator et al. 2006) and can be used to track abundances over time (Keilty 1988, Hall and Yan 1997).

Using paleolimnological records from Island Lake Reservoir, this study had three objectives. The first objective was to establish the year of first appearance of *Bythotrephes* in Island Lake Reservoir. The second objective was to describe the population density of *Bythotrephes* from that time until present. The third objective was to determine the effect of *Bythotrephes* establishment on the ecology of the daphnid species assemblage. Spine remains in the sediment were used to assess the date of first appearance of *Bythotrephes* and to estimate densities through time. Inter-basin densities were examined to explore reservoir heterogeneity. Ehippium remains were used to infer whether or not species composition, density or size of daphnids occurred coincident with invasion by *Bythotrephes*.

## **Materials and Methods**

### *Study Site*

Island Lake Reservoir (ISLR) is located in St. Louis County, Minnesota, approximately 30 km north of the city of Duluth (Fig. 1). As described by Niemela (1991), the formation of ISLR began in the 1890s when a low-rise dam of the Cloquet River was built to retain 10 ft of water for logging purposes. A higher, current dam structure was built between 1913-1915 to retain 35 ft of water. The structure was raised again in the 1920s to retain 40 ft of water, which is the dam's current capacity. Until 1924, the water was used for log driving, but is now used for hydroelectric generation downstream of the outlet. The creation of ISLR flooded part of the Cloquet Rivershed, and the floor of the lake is still heavily laden with dead wood (Niemela 1991).

The reservoir has two main basins that are bisected by a roadway (Fig. 1). Water flow is primarily east to west through a narrow (approximately 65 m wide) channel under the roadway. One inlet of the reservoir is the Cloquet River located at the eastern end of the eastern basin (Fig. 2). A second inlet is located on an arm of the western basin and receives water from Boulder Lake Reservoir (Fig. 3). A dam controls the outlet of water at the southwestern end of the western basin.

The bathymetry of ISLR is complex (Figs. 2 and 3). The eastern basin covers two historical, glacial lakes, Orchard Lake and Island Lake. The western basin may cover one or more historical oxbow lakes. The surface area of ISLR is approximately 32 km<sup>2</sup> with the eastern basin being about half the area of the western basin (MNDNRa 2011). Of the surface area, 44% is littoral which is defined as the area that is shallower than 4.6



m (Lindgren 2006). The maximum depth is approximately 29 m and is in the eastern basin located under historical Orchard Lake (MNDNRa 2011). The bathymetry of ISLR is highly irregular, which is not surprising given its reservoir status.

As a reservoir, the water level within both basins is influenced by winter drawdown by Minnesota Power, Inc. Prior to 1996, the seasonal water level fluctuations were between 1.8–5.9 m. After 1996, the winter drawdown has been less than 3.3 m (Lindgren 2006). During the summer, a thermocline often forms over deeper water but can be disrupted periodically.

The chemistry of ISLR has been described periodically. Conductivity is 70–94  $\mu\text{S cm}^{-1}$  (Lindgren 2006, Sorensen et al. 2005). Total alkalinity and pH in 1994 were 30 ppm and 7.0, respectively. Average secchi disk readings from 1991–2003 ranged from 1.2–3.4 m and were influenced by algal abundance. ISLR water is tannin stained giving it a brown color, which also influences secchi depth. Tannin staining varies between years depending on the amount of water that is flushed through the bogs in the reservoir's watershed (Lindgren 2006). ISLR has moderate fertility with total phosphorus of about 19  $\mu\text{g L}^{-1}$  and chlorophyll *a* of 8.5  $\mu\text{g L}^{-1}$  (Lindgren 2006, Branstrator et al. 2006).

The diversity of fish in ISLR includes 17 species. The assemblage is dominated by walleye (*Stizostedion zander*), yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*), and white sucker (*Catostomus commersoni*). Fish are well known to prey on *Bythotrephes*, and in Island Lake, Olson (2009) showed that yellow perch and bluegill (*Lepomis macrochirus*) consume *Bythotrephes*.

### *Sediment Collections*

Site selection for the collection of sediment cores was based on ISLR bathymetry and the pre-impoundment lacustrine basins. At each of these sites, a flat uniform surface was selected in order to avoid obtaining cores with slanted sediment layers. A slanted surface is more likely to have sediment sloughing and focusing that would make analysis more difficult. The first three sites were selected based on their position overlying the historical lakes. The two eastern basin sites were chosen to correlate with the historical Island Lake and Orchard Lake (Fig. 2). One of the western basin sites was chosen to correlate with an original oxbow lake. The other western basin site was chosen based on the flat plain available and our ability to successfully retrieve a core (Fig. 3).

Sediments were retrieved with a push piston-coring device (modified Bolivia design) on loan from the Limnological Research Center at the University of Minnesota, St. Paul. The coring device was outfitted with clear polycarbonate coring tubes with an internal diameter of 6.67 cm. Generally speaking, the coring device was used as follows. The coring device was assembled according to the instructions provided by Myrbo and Wright (2008). The length of polycarbonate tube used ranged between 1.5–2 m. To push the tube into the sediments several sequential connected drive rods were used, each measuring 2 m length. A minimum of three people operated the coring device. Immediately after the sediment core was brought back to the surface, the bottom was capped to prevent sediment slippage or loss.

The cores varied in length between 1–1.5 m. Cores that were not extruded in the field were sealed at the top with Zorbitrol<sup>®</sup> to preserve the water-sediment interface, and

capped. Zorbitrol<sup>®</sup> solidifies the water at the surface to prevent homogenization of the first few centimeters of the core materials. Sediment extrusion was done using a Glaser Griffith extruder. The extruded cores were sectioned in 0.5-cm increments from the surface down to 15 cm, 1-cm increments down to 40 cm, and 2-cm increments thereafter to the base of the core. Each increment was stored in an airtight sealable plastic container. All extruded material was stored in a Percival environmental chamber (3°C) at the University of Minnesota Duluth. Non-extruded material was stored in the archive cooler facility of the Limnological Research Center.

At site 1, 3 cores were collected on March 16, 2009, designated A, B, and C (Table 1). At site 2, 3 cores were collected on March 17, 2009, designated A, B, and C. At site 3, 2 cores were collected on March 17, 2009 designated A and B. At site 4, 1 core was collected June 23, 2010 designated B. Cores labeled A were used for maintaining an archive sample. Cores labeled B were utilized for loss on ignition (LOI) and <sup>210</sup>Pb dating of the sediment. Cores labeled C were used for zooplankton fossil recovery. At sites 3 and 4 where a C core was not collected, the material from core B was also utilized for fossil recovery.

### *Laboratory Analyses*

Sediment Dating - All B cores were processed for LOI in preparation for <sup>210</sup>Pb dating based on the procedure described by Dean (1974). For each core, all available sections were processed. This resulted in  $n = 105$  samples (core 1B),  $n = 85$  samples (core 2B),  $n = 103$  samples (core 3B), and  $n = 51$  samples (core 4B). For each core slice

a homogenized sub-sample of between 1-5 gm was taken and placed into a pre-weighed, acid washed crucible. The sub-samples were processed through subsequent burns at 105° and 550°. The sub-samples were weighed on an analytical balance down to 0.0001 gm before and after each burn. The same Mettler-Toledo AG 204 balance was used for all measurements. Burning at 105°C was in a Thermo Scientific Precision drying oven overnight to remove all water content. Burning at 550°C was for 4 h in a Thermolyne muffle furnace to remove organic carbon. Samples were fully cooled between burnings and placed in a desiccator to prevent water uptake if extended cooling occurred (e.g., overnight). Crucibles were never touched with bare hands in order to reduce contact with skin oils, which can influence weight estimates.

From each sample, a second homogenized sub-sample was freeze-dried for  $^{210}\text{Pb}$  dating using a Labconco Freezone 6 Freeze Dry System. All samples were delivered to Dr. Daniel Engstrom at the St. Croix Watershed Research Station of the Science Museum of Minnesota, whose research staff were contracted to perform both the analytical and interpretive work associated with  $^{210}\text{Pb}$  core dating. Briefly, the  $^{210}\text{Pb}$  method of sediment dating is based upon the radioactive decay of radon that is released from the Earth into the atmosphere. Products of radon decay become embedded into the sediment. With a half-life of  $22.26 \pm 0.22$  yr, the amount of  $^{210}\text{Pb}$  as a function of depth can be used to determine sedimentation rate and sediment age. Lake sediments have minerals that contain a significant amount of uranium, which supplies a constant rate of  $^{210}\text{Pb}$ , which interferes with dating. This is considered the supported  $^{210}\text{Pb}$  activity and is subtracted from total activity to find the amount relevant to dating, or unsupported  $^{210}\text{Pb}$  (Olsson

1986). For the ISLR sedimentation rates, the c.r.s. (constant rate of supply) model was used with confidence intervals calculated by first-order error analysis of counting uncertainty (Appleby 2001).

Cladoceran fossils - From cores 1C, 2C, 3B, and 4B, *Bythotrephes* spines and *Daphnia* ephippia were recovered from material dated as 1970–2009. This resulted in  $n = 19$  (core 1C),  $n = 46$  (core 2C),  $n = 18$  (core 3B), and  $n = 10$  (core 4B) separate sections of material. To maintain the integrity of both the spines and the ephippia, the samples were not passed through a mesh, except for 3B samples, which were passed through a 53- $\mu\text{m}$  mesh. The samples were instead diluted with distilled water and manually searched under a Leica MZ125 dissecting microscope. *Bythotrephes* caudal spine fossils were removed and stored in 95% Ethanol at room temperature.

To reconstruct estimates of the total number of *Bythotrephes* individuals per stratum, a modification of the Hall and Yan (1997) calculation was used. Their original equation used a small number of fragment types to calculate *Bythotrephes* density. The ISLR sediment samples revealed a large array of fragment types (Fig. 4). In order to obtain a conservative estimate of density, fragments distinctive of only a single individual were used. For example, single laterally paired barbs were not used in the calculations because this fragment could be part of an intact 1-paired barb or 2-paired barb caudal spine. The characteristic kink of the caudal spine was the diagnostic element typically used in the calculation of an individual.

To identify and count *Daphnia* ephippia, material was examined under a Nikon Eclipse E400 compound microscope. Distinguishing features of the ephippium cases

were used to identify three species including *Daphnia mendotae*, *D. pulex*, and *D. retrocurva*. The ehippia were distinguished based on their shape, number of resting eggs, and condition of the dorsal ridge (Fig. 5). The *D. mendotae* ehippium has a smooth dorsal ridge while the *D. pulex* ehippium has a 'hairy' dorsal ridge. The *D. retrocurva* ehippium is smaller with a rounder ventral side than the other two species (Kerfoot et al. 2004). Ehippia were transferred to 95% Ethanol and stored at room temperature. Measurements were made along the dorsal ridge for each ehippium (Fig. 5).

With recovered *Bythotrephes* caudal spines and *Daphnia* ehippia, the densities per slice were extrapolated to  $n\ m^{-2}\ yr^{-1}$  using the diameter of the core and the number of years represented by the sediment slice. These extrapolations resulted in the possible computation of a fraction of an individual. At sites 1, 3, and 4, there were always 1 or more years represented per slice. At site 2, where sediment accumulation rates were considerably faster than at other sites, several slices represented 1 year (Table 2). We assumed that this was the result of slippage or sloughing, and not increased water column production. This assumption was supported by a lack of significant changes in the average summer temperature or total snowfall specific to the time period studied (MN DNR (b) 2011). There were also no drastic changes in the water flow or drawdown procedures through Minnesota Power, Inc (Minnesota Power 2008). *Bythotrephes* densities ( $n\ m^{-2}\ yr^{-1}$ ) at site 2 were therefore corrected (reduced) based on an average sedimentation rate at sites 1, 3, and 4 per decade. The same correction was used for the accumulation rate of *Daphnia* ehippia. Also, in reporting results, when there were

several core slices per year at site 2, an average was taken to produce a single density estimate per year.

At sites 1 and 2, sediment dating used material from the B cores, but zooplankton analyses used material from the C cores. Dates for C cores were therefore estimated by associating equivalent horizons by depth. For example, the date of 5 cm in 1C was assumed to be the same as 1B. The cores were taken in close proximity to one another, around 2-3 meters apart, which allows for this assumption to be relatively sound. In order to confirm this assumption, however, core material from 1B and 2B, near the invasion horizons of *Bythotrephes*, were examined for *Bythotrephes* presence and abundance.

To quantify the degree of spine fragmentation, the percent of calculated *Bythotrephes* individuals that were reconstructed or pieced together from fragments (i.e., broken) was determined. To do this, the designations from Fig. 4 were used. Any piece that was not designated a whole spine (a<sup>1</sup>) was considered a fragment. The calculation for each sample was:

$$\%Broken = (1 - (\#whole / total\#)) * 100 \quad (1),$$

where total# is the total number of *Bythotrephes* represented in the sample (Fig. 4). Calculations were done only on slices with more than three calculated individuals. Fragmentation at site 3 was not calculated because 3B core slices had been processed differently by passing them through a sieve (see above).

*Bythotrephes* population density was modeled with a nonlinear least squares approach and Gauss-Newton algorithm using a logistic (sigmoidal) growth model:

$$dN/dt = rN \times (1-N/K) \quad (2),$$

where  $r$  is the population growth rate under ideal conditions,  $N$  is the population size, and  $K$  is the carrying capacity. The integrated form of the differential (Eq. 2) is:

$$N_t = K/(1+be^{-rt}) \quad (3),$$

where  $b$  is a constant and  $N_t$  is the population density at a given time  $t$ . Both  $N_t$  and  $t$  were known.  $K$  was estimated based on the raw data. Eq. 3 was then used to estimate  $b$  and  $r$ . For model input,  $N_t$  was based on an overlapping, three-year average of *Bythotrephes* density. For example, in one year  $N_t$  was the average of densities for 2007, 2008, and 2009, and the subsequent  $N_t$  was the average of densities for 2008, 2009, and 2010. This was done in order to overcome variability in sediment dating and to account for anticipated slippage and homogenization due to natural bioturbation and possible anthropogenic artifacts introduced during core collection and processing. For model input, the first year was assumed to be 1980 because there were no *Bythotrephes* in ISLR based on the majority of samples. At this time,  $t_0=0$  and  $N_t=0$ . For each site,  $K$  was incremented by units of 25 (site 4) or 100 (sites 1-3) until the best-fit model, based on  $R^2$ , was found.

To assess spatial, inter-basin differences in mean daphnid ephippium densities, samples were pooled by basin and compared by a two sample (separate variance)  $t$ -test.

To assess temporal changes in the mean daphnid ephippium densities and lengths, two sample (separate variance)  $t$ -tests were conducted that compared pooled samples before and after 1990 and 2000. The 1990 date reflected the assumed date of *Bythotrephes* invasion in ISLR (but see Results). The 2000 date reflected the general



date when the highest annual densities of *Bythotrephes* were first achieved in ISLR based on this study.

Multiple linear regression was used to explore how daphnid ephippium densities in the sediments, and various abiotic factors associated with the reservoir's limnology, predicted *Bythotrephes* densities in the sediments. The abiotic factors included average summer air temperature (MNDNRb 2011) and total annual precipitation (MNDNRb 2011). Average summer air temperature was computed as the average of daily average air temperatures reported for May to October.

Simple linear regression was used to assess how *Bythotrephes* density could be predicted by daphnid ephippium density during the periods before and after 1990 and 2000.

Time series analysis, an autocorrelation function, was used to determine the correlation between *Bythotrephes* densities achieved through time on a roughly annual time step.

All statistics were done in SYSTAT V10.0.

## **Results**

### *Sedimentation and Dating*

There was variation in the water content and the organic content of the sediment within each core and among the cores from the four sites (Fig. 6). The 105°C burn showed that for all four sites the top, most recent, sediment had the highest water content

at 91-98%. For sites 1, 2, and 3, the sediment water content decreased gradually with depth, reaching 73-79% at 25 cm. The down-core decrease in water content in core 4 was more extreme, reaching 35% by 14.5 cm. Regarding the 550°C burn, which measured organic content, at site 2 the loss values were relatively stable at about 20% for all material down to 25 cm. At sites 1 and 3, loss began at 23% and 25%, respectively, then decreased gradually with depth to 14 cm where loss was 18% in both cores. Loss then diverged below 14 cm at sites 1 and 3. Site 4 was again the most different and erratic with a surface loss of 28% (the highest of all four sites) followed by a sharp decrease to 16% at 1 cm, which persisted to 6 cm. Between 6 and 8 cm, loss was about 22% and below this loss sharply decreased to 4% by 14.5 cm.

Measurable, unsupported  $^{210}\text{Pb}$  activity was found in the upper portions of each core (Fig. 7). This unsupported activity generally decreased gradually with depth indicating minimal mixing during and prior to collection. Some mixing is suggested to have occurred from 2 to 10 cm in the core from site 2 in the western basin (Fig. 7).

The supported and unsupported  $^{210}\text{Pb}$  (Fig. 7) and loss on ignition data (Fig. 6) were used to model age at depth (Fig. 8). Sites 1 and 3 had similar long-term age at depth profiles, attaining about 170 yr by 27 cm depth. The long-term age at depth profile at site 2 was steeper, indicating faster sedimentation and an age of 161 yr at 48 cm depth. At site 4, the long-term age at depth profile was somewhat shallower, indicating slower sedimentation and an age of 126 yr at 13 cm depth. Accuracy of the dating decreased with depth from about  $\pm 1.3$  yr at the top of the cores to  $\pm 24.4$  yr at the bottom of the

cores. The standard error values became much larger as dates neared or surpassed 150 yr.

Sediment accumulation rates for the period between 1965 to 2010, modeled with  $^{210}\text{Pb}$  activity, were similar among sites 1, 3, and 4, but substantially different compared to site 2 (Fig. 9). At sites 1, 3, and 4, sediment accumulation rates were relatively stable during this time period and ranged from 0.02-0.03  $\text{g cm}^{-2} \text{yr}^{-1}$ . By contrast, at site 2, rates were about 4 to 6  $\times$  greater (0.08-0.12), and increased progressively by decade from later to recent (Table 2).

#### *Bythotrephes Densities*

At all four sites, either full *Bythotrephes* caudal spines or diagnostic spine fragments, and sometimes both, were recovered from sediment dated prior to 1990 (Fig. 10). At site 1, the first occurrence in the non-dated core (C) was 1977.6 and the first occurrence in the corresponding dated core (B) was 1983.7. At site 2, the first occurrence in both the non-dated core (C) and corresponding dated core (B) was 1982.2. At site 3, the first occurrence in the dated core (B) was 1986.5. At site 4, the first occurrence in the dated core (B) was 1982.2.

After the first appearance in the sediment, *Bythotrephes* spine density generally increased through time (Fig. 10). At site 1, notable peak densities occurred in 1992.0 (2421.6  $n \text{ m}^{-2}$ ) and 2005.1 (2926.5  $n \text{ m}^{-2}$ ). At site 2, notable peak densities occurred in 2002.4 (3229.2  $n \text{ m}^{-2}$ ) and 2009.1 (3644.3  $n \text{ m}^{-2}$ ). At site 3, notable peak densities occurred in 1996.8 (2131.3  $n \text{ m}^{-2}$ ) and during 2002-4 (3988.5  $n \text{ m}^{-2}$ ). At each of these

three sites, the latter peak densities were larger peaks. At site 4, near peak densities were achieved at the time of first occurrence in 1982.2 ( $484.4 \text{ } n \text{ m}^{-2}$ ). Latter peaks in 1999.4 and 2005.7 were only slightly larger.

A logistic growth curve provided a good model fit to population density trajectories at all four sites (Fig. 11, Table 3). The  $K$  for the best-fit curve varied between 2000-4000 for sites 1, 2, and 3. At site 4, the  $K$  for the best-fit curve was 725. Intrinsic growth rates under ideal conditions ( $r$ ) at the two eastern basin sites (1 and 3) were about 2-3  $\times$  estimates of  $r$  at the two western basin sites (2 and 4) (Table 3).

Modeling the average densities of *Bythotrephes* at the three deep sites (1-3) indicated that densities progressively increased through time (Fig. 12). Densities reached a peak in 2004.5 with  $3136.7 \text{ } Bythotrephes \text{ m}^{-2} \text{ yr}^{-1}$ . The standard error was greatest around 1990 and in the 2000s. The best-fit logistic growth model had an assigned carrying capacity of  $3700 \text{ } Bythotrephes \text{ m}^{-2} \text{ yr}^{-1}$  ( $R^2=0.96$ ).

There was substantial variability in the percent fragmentation of *Bythotrephes* spines within and among sites (Fig. 13). The percent of fragmented spines was generally greater at the two western basin sites 2 and 4 (range of about 40-100%) than at the single eastern basin site 1 (range of about 10-60%) where fragmentation was estimated.

#### *Daphnia ephippia*

The total average density of *Daphnia ephippia* was greater ( $t$ -test,  $t = 12.4$ ,  $df = 54.2$ ,  $P < 0.01$ ) in the eastern basin than the western basin (Fig. 14). In the eastern basin, the highest densities occurred in the 1970s and steadily declined thereafter but the decline

was not fully coincident with the *Bythotrephes* invasion. In the eastern basin, there was no significant difference in ephippium densities before and after 1990 or before and after 2000 (Table 4). In the western basin, ephippium densities appeared to increase steadily through time from the 1970s to present, and the increase was somewhat coincident with *Bythotrephes* invasion. Specifically, there was a significant increase in ephippium density after 1990 in the two western basin sites and a significant increase after 2000 in one of the two western basin sites (Table 4).

Proportional species composition of the *Daphnia* ephippium egg bank at site 1 remained relatively constant from 1960–2008 (Fig. 15). There were no statistically significant differences in ephippium densities for any of the three species before and after 1990 or before and after 2000 (Table 5). The egg bank was numerically dominated by *D. mendotae*. *D. retrocurva* was rare, reaching a peak density in 1996.7 of  $155.3 \text{ n m}^{-2} \text{ yr}^{-1}$ .

Proportional species composition of the *Daphnia* ephippium egg bank at site 2 was not as consistent as at site 1. At site 2, *Daphnia* ephippium density was significantly different before and after 1990 for all three species, and before and after 2000 for two of the three species (Table 5). Where ephippium density was different, it always increased through time. At site 2, the egg bank was historically co-dominated by *D. mendotae* and *D. pulex* (Fig. 15). After 1990, *D. mendotae* persistently dominated the assemblage and the gains in total daphnid ephippium density after 1995 were contributed largely by *D. mendotae*.

Shifts in ephippium length occurred within each species population over time (Fig. 16). The species with the longest average ephippium length was consistently *D.*

*pulex* followed by *D. mendotae*, and *D. retrocurva*. The average ephippium lengths of *D. mendotae* at both sites and of *D. pulex* at site 1 were significantly smaller after 1990 compared to before 1990 (Table 6). The average ephippium lengths of *D. mendotae* and *D. retrocurva* were significantly smaller at site 2 after 2000 compared to before 2000 (Table 6).

Multiple linear regression indicated that of the variables tested, *Daphnia* ephippium density ( $P < 0.01$ ) and total precipitation ( $P = 0.05$ ) significantly explained 78% of the variation in *Bythotrephes* density in the sediments at site 2 (Table 7). At sites 1, 3, and 4 there were no detectable significant effects of ephippium density, temperature, or precipitation on *Bythotrephes* density. Further modeling with site 2 only, that took only daphnid species ephippium density into consideration, showed that *D. mendotae* was the only species to have a significant predictive effect ( $P < 0.01$ ) on *Bythotrephes* density (Table 7). Exploration of this trend using simple linear regression revealed that over the full time period 1982-2008, *D. mendotae* density most strongly predicted *Bythotrephes* density (Fig. 17 A-C). However, during the earlier years, 1982-1990, *Bythotrephes* density was more strongly predicted by *D. pulex* than by *D. mendotae* density (Fig. 17 D-F). By contrast, during the latter years, 1990-2008, *Bythotrephes* density was more strongly predicted by *D. mendotae* than *D. pulex* density (Fig. 17 G-I) coincident with the increased percentage of *D. mendotae* in the daphnid assemblage (Fig. 15).

Time series analysis indicated that the average *Bythotrephes* density in a given year was highly positively correlated with its density of the prior year ( $r = 0.8$ ).

## Discussion

Island Lake Reservoir can be considered to have worked well as a site to study the phenology and impact of a *Bythotrephes* establishment. The sedimentation rates in the lake were high enough to resolve changes in sediment composition and fossil density on the order of years and sometimes months. Also, because the  $^{210}\text{Pb}$  profiles were clean, this led to date profiles with low margins of error over the time period studied. One complication in the accuracy of the data, however, concerned estimates of fossil densities in core 3B (Table 1). On the one hand, because core 3B was dated, the dates of fossils recovered at certain sediment depths can be considered accurate. On the other hand, because there were handling and processing errors that occurred during analysis, quantification of the total numbers of fossils was questionable. To correct this, absolute values of fossil densities for core 3B had to be approximated using the fractions of samples quantified from another core that was accurately processed. The relative trends and dates for the *Bythotrephes* and *Daphnia ephippia* densities in core 3B are therefore reliable, but the absolute densities are less dependable.

### *Date of Establishment*

Sediment evidence in ISLR indicated that *Bythotrephes* was present in the lake several years prior to its first detection in the water column, which was in 1990 (Gravelle 1990). At all four sites, *Bythotrephes* spines or spine fragments were recovered from sediment that dated older than 1990 (Fig. 10). The oldest *dated* sediment in which *Bythotrephes* remains were recovered was 1982.2 (cores 2B and 4B). Factoring in  $\pm 1$

standard deviation of the  $^{210}\text{Pb}$  dating for these two cores, the earliest establishment date is 1979.9 and the latest is 1984.5, or 10.1-5.5 years prior to 1990. Even at site 3, where *Bythotrephes* remains were not first found until sediment dated to 1986.5, the latest date of establishment is calculated to be 1988.4, or 1.6 years prior to its first detection in the water column.

Lake Superior is the most likely source population for *Bythotrephes* in ISLR. Lake Superior was first documented invaded in August 1987 (Cullis and Johnson 1988), but based on recent ballast water studies (Colautti et al. 2003, Rup et al. 2010) it is highly probable that a population had established earlier. Lake Superior receives a disproportionate amount of ballast water, about 52% of the transoceanic (saline) ballast and 75% of Great Lakes (freshwater, potentially with residual saline) ballast (Colautti et al. 2003). The Duluth-Superior Harbor in particular received the highest annual tonnage of ballast discharge (19.6 million tonnes) and annual number of discharge events (677) of any Great Lakes port in a study between 2003-2005 (Rup et al. 2010). Moreover, the total number of ballast on board ships entering the Great Lakes was the highest in the late 1970s and early 1980s (Sprules et al. 1990, NOAA 2011) making this a particularly likely era for invasive species inoculation. With the intensity of shipping during this time, in addition to the high amount of ballast discharged into the Duluth Harbor, it is highly probable that Lake Superior supported populations of *Bythotrephes* and could have actively served as a dispersal hub to ISLR prior to 1987.

The intensity of recreational fishing activity on the reservoir and its proximity to Lake Superior likely facilitated *Bythotrephes*' range expansion to ISLR. The Duluth-



Superior Harbor on Lake Superior is approximately 40 km, by road, from ISLR and the two bodies of water share local recreational boater activity. The reservoir has historically been known for walleye fishing and a 2006-7 angling survey found that ISLR was one of the most intensely fished inland lakes in the area (Frohnauer 2008). The means of transport is unclear but there are many possibilities. *Bythotrephes*' clump on nets and fishing lines (MacIsaac et al. 2004), and are time consuming to remove making these vectors some of the most likely modes of transport between lakes. Bait buckets, live well water, or bilge water could also be implicated but would probably carry lower densities of propagules. Also, it is noteworthy that the resting eggs of *Bythotrephes* can withstand limited desiccation (up to 4 hr, unpublished data) and extreme chemical conditions of hypoxia and pH (Brown 2008) making this life stage particularly resistant to short-term exposure that could be experienced during dispersal between lakes.

The results of this study suggest that *Bythotrephes* first invaded inland lakes in North America much earlier than previously thought. To date, the earliest published occurrence of *Bythotrephes* in an inland lake was in 1989 when it was detected in Lakes Muskoka, Roseau, and Joseph in southwest Ontario, Canada (Yan et al. 1992). It is thought to have invaded those lakes from nearby Lake Huron (MacIsaac et al. 2004). The current study indicates that ISLR was most likely invaded in 1982.2 (Fig. 10), almost a decade earlier than the three inland Canadian lakes. The results of this study also suggest that there was an almost 8-yr gap between its year of invasion in 1982.2 and its record of first detection in 1990 (Gravelle 1990). From a management perspective this raises considerable concern because it suggests that a lake could serve as a dispersal hub

for years before it is recognized invaded and treated as such through management protocols. It would be interesting to recover cores from the Great Lakes, including the Duluth-Superior Harbor of Lake Superior, to document when *Bythotrephes* actually established populations within the United States to better understand their past and future range expansion.

### *Rate of Establishment*

Modeling indicated that the logistic growth equation effectively described the inter-annual density pattern of *Bythotrephes* from 1980 to 2009 or 2010. Logistic growth is characterized by a sigmoid curve on a density vs. time plot. It is commonly associated with populations in a habitat that show increasing response to self-limitation (density-dependence) as their numbers increase. Strong density dependence is generally considered to reflect resource limitation or other external influences (e.g., predation, parasites) that reduce fecundity and increase mortality to the point where net population growth is ultimately zero, symbolized by  $K$ . In ISLR, previous work established that annual death rates and diel vertical migration behavior of *Bythotrephes* is under strong bottom-up control by food abundance (Brown et al. 2011), which is consistent with density-dependence as the major form of its population regulation in the lake.

Why *Bythotrephes* would require between 10-20 yr in ISLR to reach what appears to be its annual  $K$  (Fig. 11) is unclear. In considering this observation, it is important to keep in mind that the  $K$  symbolized in Eq. 2, and depicted in Fig. 11, reflects the annual, not instantaneous,  $K$  for the population. Hence, in this application,  $K$  is the sum of water

column production from early June to early November (Brown and Branstrator, in press). One explanation for the lag of 10-20 yr between year of first invasion and attainment of annual  $K$  relates to the role of the resting egg. In ISLR and most other temperate lakes, *Bythotrephes* parthenogenic females do not overwinter. Each spring the entire population of free swimming individuals must be rebuilt from the resting egg bank (Yurista 1997, Rivier 1998). Thus, it is possible that during initial years post invasion the egg bank for recruitment was limited in size, which limited the population from attaining peak densities during the growing season (Fig. 18A). I hypothesize that once resting egg densities in the sediments reached a critical number, spring inoculation was sufficient to maximize production of the population over the entire season as defined by the local resource base (Fig. 18B).

A variety of circumstantial evidence is consistent with the hypothesis that resting egg production limited early population growth rates of *Bythotrephes* in ISLR. First, Brown and Branstrator (in press) estimated that 85% of resting eggs in the sediment fail to recruit to the second instar, indicating a potential bottleneck in this phase of the life cycle and the need to build a sufficiently large resting egg bank each fall. Second, because resting egg production in the fall requires the production of male offspring, and subsequent sexual reproduction with those males, Allee effects could become important during early colonization years and limit resting egg production. Third, autocorrelation detected a strong positive correlation between the current population density and the population density one year prior ( $r = 0.8$ ) suggesting strong inter-year dependence in density of *Bythotrephes*. This could be related to population momentum associated with

resting egg production. Finally, Sarnelle and Knapp (2004) demonstrated that it can take years for founding populations of another cladoceran, *Daphnia middendorffiana*, to reach densities in natural settings that are detectable by standard sampling methods. This implies that parthenogenesis as a life history trait, despite its potential to fuel fast population growth in cladocerans, may not always ensure immediate colonization success.

An alternative to the hypothesis that resting egg production limited early population growth rates of *Bythotrephes* in the reservoir, is the hypothesis that early population growth was limited by poorly adapted morphs. In other words, year-to-year directional selection for genotypes with faster population growth might account for the pattern of persistent inter-annual increases in total seasonal density. Faster population growth could be accomplished by selection favoring individuals with faster somatic growth, quicker maturation, or better overall survival. This hypothesis could be tested in part by examining morphological traits such as spine length and body size of *Bythotrephes* collected during early versus late phases in the invasion timeline. Despite its potential merit, this hypothesis is inconsistent with patterns of population growth at site 4 (see below) where increases in overall seasonal density did not change through time.

Although at each site the *Bythotrephes* population appeared to have reached or nearly reached  $K$  by 2009-2010, the absolute values of  $K$  and the growth trajectories differed among sites (Fig. 11). As  $K$  likely depends on local food availability,

temperature, predation and other biotic and abiotic factors, the data suggest that the sum effect of these factors differs among sites.

Western basin data suggests that food availability is likely the most important determinant of *Bythotrephes* population  $K$  in this system. In making this argument, I made the assumption based on other studies that densities of *Daphnia* ephippia in the sediments were a strong measure of past food availability in the lake (Jankowski and Straile 2003, Jeppesen et al. 2003). Multiple linear regressions for site 2 showed a positive relationship between *Bythotrephes* density and *Daphnia* ephippium density, particularly with *D. mendotae* (Table 7, Fig. 17).

There was also a time-sensitive shift in the association between *Bythotrephes* densities and the densities of particular species of *Daphnia* ephippia (Fig. 17). Over the entire timeframe of analysis, 1982-2008, *D. mendotae* was a stronger predictor than *D. pulex*. However, during the early years of invasion (1982-1990), *D. pulex* was a stronger predictor and during the later years (1990-2008) *D. mendotae* was a stronger predictor of *Bythotrephes* densities. Although these shifts suggest that *Bythotrephes* density was limited by available food resources, the relationships between *Bythotrephes* and *Daphnia* ephippia densities were only seen at site 2. This points to the possibility that other cladoceran species, including *Bosmina* and *Diaphanosoma*, likely contributed to *Bythotrephes*' food base and influenced the local  $K$ . Evidence of strong bottom up control (Brown et al. 2011) in ISLR is consistent with the hypothesis of a food driven  $K$ . Finally, I also found that annual precipitation values had a significant, positive effect on *Bythotrephes* density at site 2 (Table 7). This might be explained by increased habitat

(taller water column) and more overall production during those years of heavy rain and higher water levels in the reservoir, but the explanation will require further consideration as water level did not appear to affect densities of *Bythotrephes* in the eastern basin.

Brown et al. (2011) demonstrated that reservoirs foster higher maximum densities of *Bythotrephes* than natural lakes and that ISLR provides exceptional habitat for the establishment and growth of this invader. In particular, ISLR has low zooplanktivorous fish abundances, a well oxygenated water column, and increased Chl *a* concentrations that may contribute to the local success of this invader. If *Bythotrephes* densities in ISLR took at minimum 5 yr to be detected and then 10-20 yr to reach its annual *K*, it is highly probable that these colonization benchmarks required similar if not longer periods to be achieved in other lakes where growth potential for the invader is not as strong. In the case of Lake Superior, ecosystem size may compromise sampling frequency and the spatial extent of sample collection, and as a result lakes of this size may harbor unnoticed invasive species even longer. Hence, it is not a stretch to propose that Lake Superior could have supported *Bythotrephes* as early as the late 1970s.

The spatial component of this study permitted me to assess the phenology and density of the invading *Bythotrephes* population at four separate locations in the reservoir. This showed definitively that at the three deep locations (sites 1, 2, and 3) there was repetition in the general trend of population build up over time. The spatial component also showed that site 4 was a clear outlier (Fig. 10). Lower overall densities achieved by *Bythotrephes* at site 4 may be the result of local abiotic and biotic factors. The LOI analyses showed that the water content and organic content of the sediment at

site 4 was much lower than at other sites (Fig. 6), which indicates that there may be greater water flow and less material settling out of the water column. The lower sediment volume per year and  $^{210}\text{Pb}$  flux at site 4 supports this (Fig. 8). Site 4 was also shallower, so we should expect less sediment fallout. Also, because *Bythotrephes* is a pelagic species its overall densities may be lower at shallower, nearshore sites such as site 4 where food and refuge may be in less supply and fish vulnerability more intense compared to deeper sites 1, 2, and 3.

#### *Spine Recovery and Fragmentation*

Calculations of the number of *Bythotrephes*  $\text{m}^{-2} \text{yr}^{-1}$  did not appear to be influenced by decomposition or breakage of the spine remains. There was no consistent trend of increased fragmentation with depth that would indicate that the spines were decomposing (Fig. 13). Hall and Yan (1997) found no dissolution or decomposition after incubating spines in lake conditions for 1 year. Spine fragments were recovered as deep as 22 cm in a Norwegian lake (Nilssen and Sandoy 1990), several centimeters deeper and years older than the remains I recovered in ISLR. This evidence indicates that the increasing trends seen in ISLR *Bythotrephes* densities were not an artifact of older spines decomposing.

That said, there appeared to be greater fragmentation of spines in the western basin than the eastern basin. This might indicate increased consumption by fish in the western basin (Fig. 13). Olson (2009) demonstrated that yellow perch in ISLR consume *Bythotrephes* and that spines in their stomachs exhibit some fragmentation. In a

laboratory fish feeding experiment Jarnagin (2004) found that 87% of *Bythotrephes* spines that passed through fish were damaged. Field yellow perch collection by Jarnagin (2008) found that 95.4% of spines consumed were damaged. Spines that pass through fish are more fragmented than ones that settle out of the water column unperturbed. A hypothesis that implicates higher fish predation in the western basin is also consistent with lower intrinsic growth rates ( $r$ ) predicted for subpopulations in the western basin sites compared to the eastern basin sites (Table 3). Another explanation for increased spine breakage in the western basin sites, however, is that more benthic or abiotic activity was occurring there to agitate and break up the spines post-settlement. This could be in the form of increased densities of zoobenthos or increased water column mixing. Data indicate that surface sediment mixing at least could have been more significant at site 2 than at the other sites (Fig. 7). If increased mixing was occurring, summer benthic temperatures could also be higher at these sites leading to higher zoobenthic activity and consumption.

#### *Daphnia ephippia* density

It was expected that the density and species richness of the daphnid assemblage in ISLR would decrease after *Bythotrephes* invasion as has been seen in free swimming populations in several other lakes (Yan et al. 2001, Yan et al. 2002, Barbiero and Tuchman 2004, Strecker et al. 2006). However, the ephippium data in ISLR indicated that the daphnid assemblage size and species composition did not change as drastically as in other studied lakes coincident with the establishment of a *Bythotrephes* population.



Historically, the eastern basin had more ephippia (Fig. 15), and the introduction of *Bythotrephes* affected no change in this east-west difference. The eastern basin also demonstrated no change in the mean ephippium density and none of the species disappeared. The western basin, by contrast, showed increases in ephippium density associated with *Bythotrephes* establishment (Table 4). The majority of the increase was attributed to *D. mendotae* (Table 5). An increase in the density of *D. mendotae* was also observed in Lake Michigan, Lake Huron, and Harp Lake in response to *Bythotrephes* invasion (Barbiero and Tuchman 2004, Yan et al. 2001).

One surprising result was the observation that *D. pulex* continued to thrive post invasion of *Bythotrephes* (Fig. 15, Table 5). This was not expected based on previous work in Lakes Michigan, Huron, and Erie (Barbiero and Tuchman 2004) that showed that the large daphnid *Daphnia pulicaria*, which is an equivalent in size and shape to *D. pulex*, is highly vulnerable to predation by *Bythotrephes*. Despite its size, size alone does not act as a refuge from *Bythotrephes* predation because the feeding mechanism utilized by *Bythotrephes* is to handle the prey head first, shred the body with mandibles, and consume the soft interior (Schulz and Yurista 1999, Riviera 1998). One explanation is that because *Bythotrephes* use visual cues to find prey (Muirhead and Sprules 2003) the high tannin content in ISLR could have provided some degree of visual refuge for *D. pulex* from *Bythotrephes*.

It was also surprising that *D. retrocurva* continued to persist in ISLR post invasion. Previous work on natural lakes both larger and smaller than ISLR have shown

this species to be highly vulnerable to *Bythotrephes* predation (Lehman 1991, Yan and Pawson 1997).

Contrasts in the response of the eastern and western basin daphnid assemblages to invasion may be related to the historical condition. The eastern basin had a historical assemblage dominated by *D. mendotae* and saw no density-related changes in response to *Bythotrephes*. The species composition there both before and after invasion resembled that of post-invasion Lake Michigan, Lake Huron, and Harp Lake where dominance had shifted to *D. mendotae* (Yan and Pawson 1997, Barbiero and Tuchman 2004). In the western basin, by comparison, the historical dominance by *D. pulex* was replaced by *D. mendotae*. This more closely reflected changes that transpired in the Laurentian Great Lakes post invasion, but not as drastic (Makarewicz et al. 1995, Barbiero and Tuchman 2004). The overall lack of response by *D. retrocurva* to invasion in ISLR is perhaps related to its diminished contribution pre-invasion (Fig. 15).

#### *Daphnia ephippium* size

The most consistent change in the daphnid assemblage post *Bythotrephes* establishment was a downward shift in mean ephippium size (Fig. 16, Table 6). Multiple studies have found that the length of the ephippium and the daphnid mother are positively correlated (Jeppesen et al. 2002, Jankowski and Straile 2003). Specifically, Jankowski and Straile (2003) recovered ephippia from sediment and compared them with historical records of parthenogenic female length and found a strong correlation. Therefore, I assume that the downward shift in ephippium size post-establishment signals a shift in

the size of sexually reproducing female daphnids. Shifts in cladoceran size in Harp Lake post invasion were attributed to a shift in species composition (Yan et al. 2001), but in ISLR this cannot entirely be the case. At site 1, there was no shift in species composition yet the mean ephippium size significantly decreased (Figs 15 and 16, Table 6). Post invasion, both *D. mendotae* and *D. pulex* significantly decreased in size. At site 2, the decrease in the total mean ephippium size of the assemblage appears to be influenced by both a decrease in *D. mendotae* size and a decrease in the relative density of *D. pulex*. Lake Michigan mean cladoceran size also decreased post invasion due to shifts in species composition and species-specific shifts, however *D. mendotae* was one of the few species whose mean size did not change (Barbiero and Tuchman 2004). In ISLR it seems that daphnid individuals are maturing and reproducing at a smaller size, shifting energy allocation to reproduction rather than growth. A similar response was seen in *D. retrocurva* in Lake Michigan during the 1960s in response to increased predation by zooplanktivorous fish (Wells 1970). Increased size, helmets, or longer tail spines among daphnids do not hinder *Bythotrephes*' ability to consume them (Schulz and Yurista 1999). As with fish, however, *Bythotrephes* also relies on vision to detect prey so we might expect its impact on prey size to parallel the effect of increased fish predation in this instance. Accelerated maturation in daphnids may also partly explain the increase in *D. mendotae* and *D. pulex* ephippium densities in the sediment at site 2 post invasion (Fig. 15).

An alternative explanation for reduced daphnid ephippium size is that fish predation became stronger over time and just happened to be coincident with

*Bythotrephes* invasion. This seems unlikely for two reasons. First, overall densities of daphnids, which are a preferred prey of fish, remained high throughout the invasion period. Second, previous work has shown that *Bythotrephes* population dynamics, and presumably *Daphnia* dynamics, in ISLR are not strongly controlled by top-down fish predation (Brown et al. 2011).

#### *Post invasion versus high density*

Although it took several years for *Bythotrephes* to reach peak population densities in ISLR, the impact of the invasion appeared to occur soon after establishment. For example, ephippium densities at site 4 were significantly higher post invasion, but not after high *Bythotrephes* densities were established (Table 4). Also, species-specific size shifts were greatest post invasion, but were not statistically significant before and after high *Bythotrephes* densities were reached (Table 6). Swift and permanent changes in the cladoceran communities were seen in Harp Lake (Yan et al. 2001) and Lake Michigan (Barbiero and Tuchman 2004) following invasion. The sediment evidence from ISLR further supports the notion that the impact of *Bythotrephes* on native plankton assemblages occurs within the first few years of introduction. Future studies should include fossil recovery of other cladoceran species such as *Bosmina longirostris* and *Chydorus sphaericus* to further assess the impact of *Bythotrephes* in this system.

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**Table 1:** Core collection dates, lake surface status, and corresponding analyses done for each core. Core name indicates the site (1, 2, 3, or 4) and the type (A, B, or C). Processing indicates whether the cores were left intact or extruded in the field.

Core	Collection Date	Surface Type	Processing	Analyses
1A	3/16/09	Ice	Whole	Archive.
1B			Extruded	Loss on ignition, $^{210}\text{Pb}$ dating.
1C			Extruded	Zooplankton fossil retrieval.
2A	3/17/09	Ice	Whole	Archive.
2B			Extruded	Loss on ignition, $^{210}\text{Pb}$ dating.
2C			Extruded	Zooplankton fossil retrieval.
3A	3/17/09	Ice	Whole	Archive.
3B			Extruded	Loss on ignition, $^{210}\text{Pb}$ dating, Zooplankton fossil retrieval.
4B	6/23/10	Open	Extruded	$^{210}\text{Pb}$ dating, Zooplankton fossil retrieval.

**Table 2:** Average sediment accumulation rate ( $\text{g cm}^{-2} \text{yr}^{-1}$ ) by decade by site.

Decade	Core			
	1B	3B	4B	2B
00s	0.030	0.031	0.025	0.126
90s	0.029	0.025	0.025	0.109
80s	0.030	0.024	0.026	0.098
70s	0.028	0.021	0.028	0.084



**Table 3:** The assigned  $K$  used to obtain the best fit growth model and estimated parameters  $r$  and  $b$  and the coefficient of determination ( $R^2$ ) based on nonlinear least squares modeling of Eq. 3 using data presented in Fig. 11.

Site	Parameters			
	$K$	$r$	$b$	$R^2$
1	2250	0.37	21	0.91
2	3400	0.12	17	0.96
3	4000	0.25	89	0.97
4	725	0.14	3	0.79

**Table 4:** Two sample *t*-test (separate variance) results for ephippium densities by site between the indicated years of comparison including *t*-statistic, degrees of freedom (df), and *P*-value. Where  $P < 0.05$ , shift in density always increased over time.

Years of Comparison	Site	<i>t</i>	df	<i>P</i>
1970-1990 vs. 1991-2008	1 (eastern)	0.772	9.9	0.46
	3 (eastern)	1.103	10.3	0.30
	2 (western)	-6.112	29.7	<0.01
	4 (western)	-3.605	5.7	0.01
1970-2000 vs. 2001-2008	1 (eastern)	-3.134	14.2	0.99
	3 (eastern)	0.831	15.9	0.42
	2 (western)	-6.911	12.0	<0.01
	4 (western)	-0.849	5.5	0.43

**Table 5:** Two sample *t*-test (separate variance) results for ephippium densities by species between the indicated years of comparison including *t*-statistic, degrees of freedom (df), and *P*-value. Where  $P < 0.05$ , shift in density always increased over time.

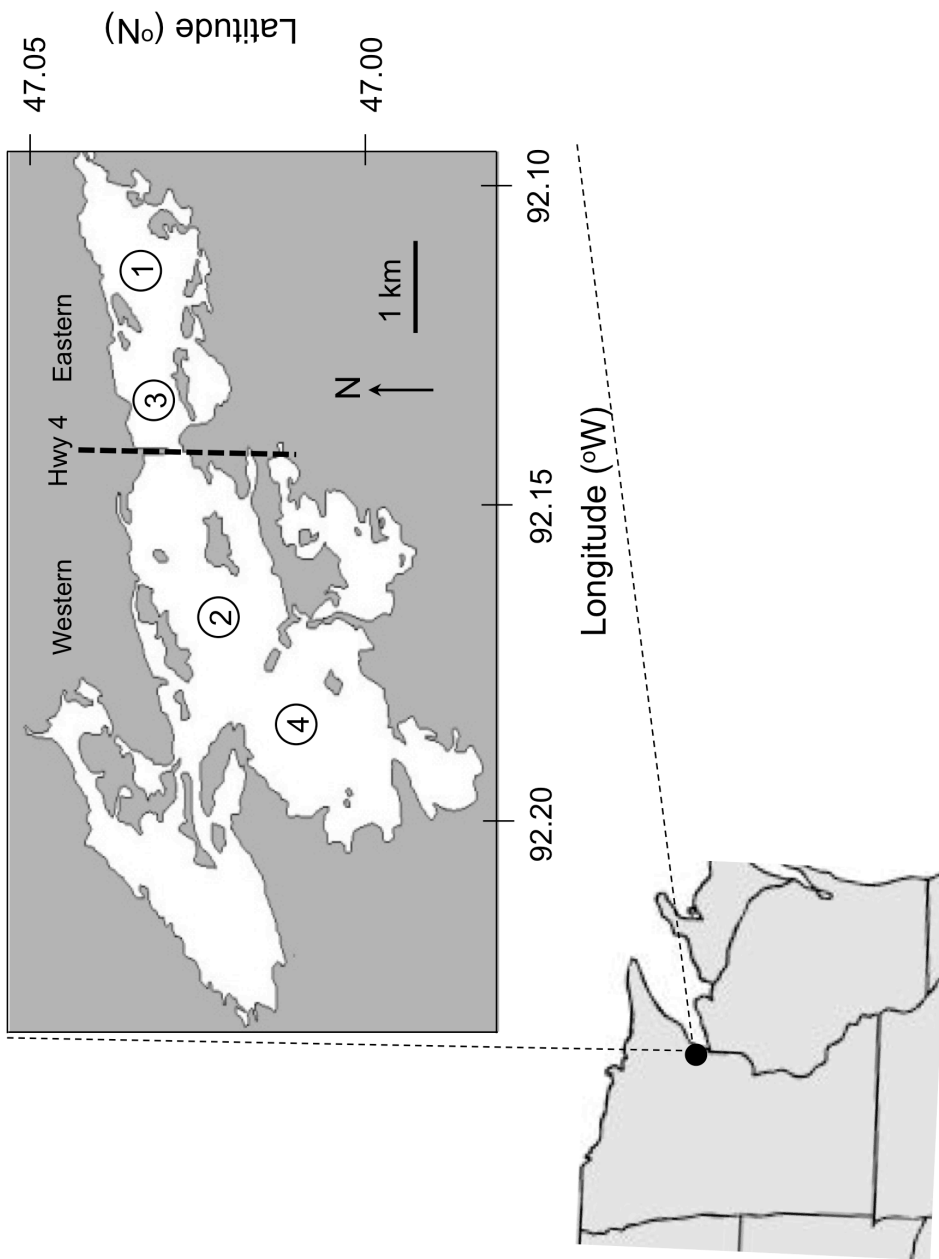
Years of Comparison	Site	Species	<i>t</i>	df	<i>P</i>
1970-1990 vs. 1991-2008	1 (eastern)	<i>D. mendotae</i>	0.61	15.8	0.55
		<i>D. pulex</i>	0.715	18.3	0.48
		<i>D. retrocurva</i>	0.345	19.0	0.73
	2 (western)	<i>D. mendotae</i>	-5.468	25.4	<0.01
		<i>D. pulex</i>	-2.361	33.0	0.02
		<i>D. retrocurva</i>	-2.303	27.7	0.03
1970-2000 vs. 2001-2008	1 (eastern)	<i>D. mendotae</i>	-0.098	12.8	0.92
		<i>D. pulex</i>	1.867	16.0	0.08
		<i>D. retrocurva</i>	---	---	---
	2 (western)	<i>D. mendotae</i>	-6.217	12.3	<0.01
		<i>D. pulex</i>	-2.610	16.8	0.02
		<i>D. retrocurva</i>	-2.160	9.6	0.06

**Table 6:** Two sample t-test (separate variance) results for ephippium length by species between the indicated years of comparison including *t*-statistic, degrees of freedom (df), and *P*-value. Where  $P < 0.05$ , shift in length always decreased over time. Dashes indicate insufficient data for analysis.

Years of Comparison	Site	Species	<i>t</i>	df	<i>P</i>	
1970-1990 vs. 1991-2008	1	<i>D. mendotae</i>	1.961	506.9	0.05	
		<i>D. pulex</i>	3.696	149.7	<0.01	
		<i>D. retrocurva</i>	---	---	---	
		All	2.478	693.7	0.01	
	2	<i>D. mendotae</i>	3.647	181.0	<0.01	
		<i>D. pulex</i>	0.484	465.9	0.63	
		<i>D. retrocurva</i>	0.913	23.4	0.37	
		All	6.517	687.1	<0.01	
	1970-2000 vs. 2001-2008	1	<i>D. mendotae</i>	1.739	233.2	0.08
			<i>D. pulex</i>	1.836	43.0	0.07
			<i>D. retrocurva</i>	---	---	---
			All	3.161	293.3	<0.01
2		<i>D. mendotae</i>	2.426	562.8	0.02	
		<i>D. pulex</i>	-1.319	286.3	0.18	
		<i>D. retrocurva</i>	2.284	34.5	0.03	
		All	5.698	950.5	<0.01	

**Table 7:** Multiple linear regression results for *Bythotrephes* densities including the  $R^2$  and each variable's coefficient,  $t$ -statistic, and  $P$ -value. Data used encompasses 1982-2009.

Predictor Variable	Site	Coefficient	$t$	$P$	$R^2$
Ehippia	1	-0.02	-0.14	0.89	0.18
Temperature		271.72	1.58	0.14	
Precipitation		53.67	1.01	0.33	
Ehippia	2	0.78	10.70	<0.01	0.78
Temperature		40.94	1.04	0.31	
Precipitation		34.275	2.06	0.05	
Ehippia	3	-0.13	0.92	0.37	0.10
Temperature		62.84	0.26	0.80	
Precipitation		47.71	0.55	0.59	
Ehippia	4	0.09	0.78	0.49	0.58
Temperature		-16.13	-0.42	0.70	
Precipitation		27.05	0.77	0.49	
<i>D. mendotae</i> ehippia	2	0.89	7.40	<0.01	0.74
<i>D. pulex</i> ehippia		0.21	0.74	0.43	
<i>D. retrocurva</i> ehippia		0.31	0.27	0.79	
Precipitation		29.14	1.51	0.14	



**Figure 1**

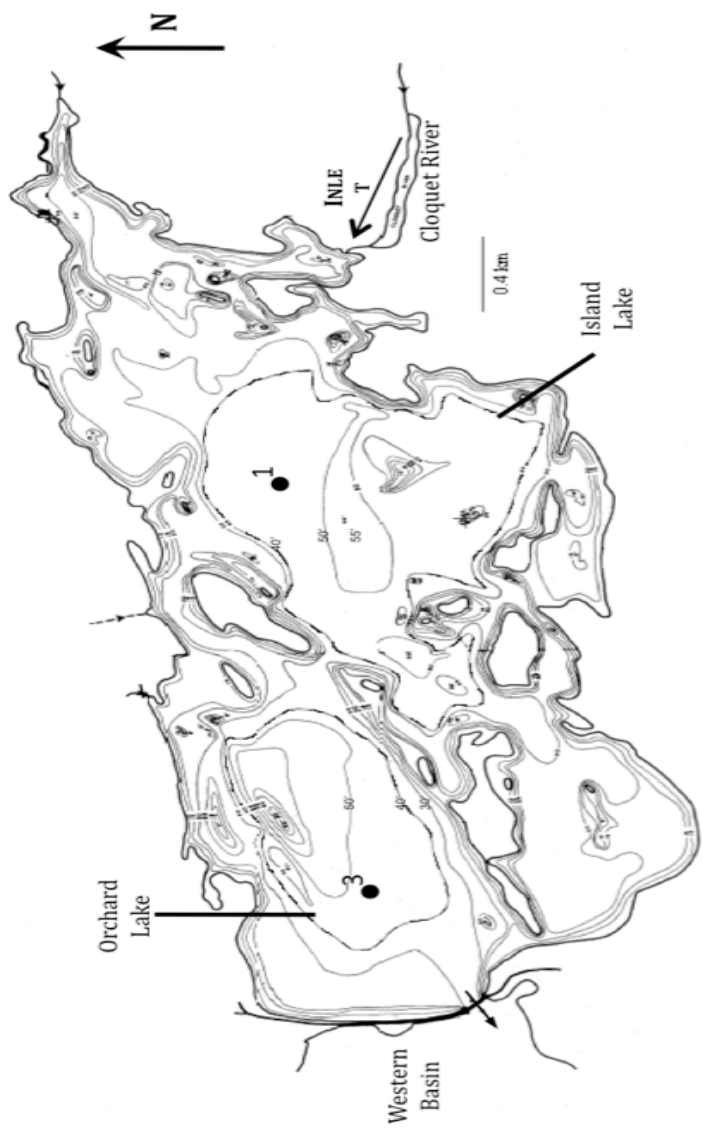
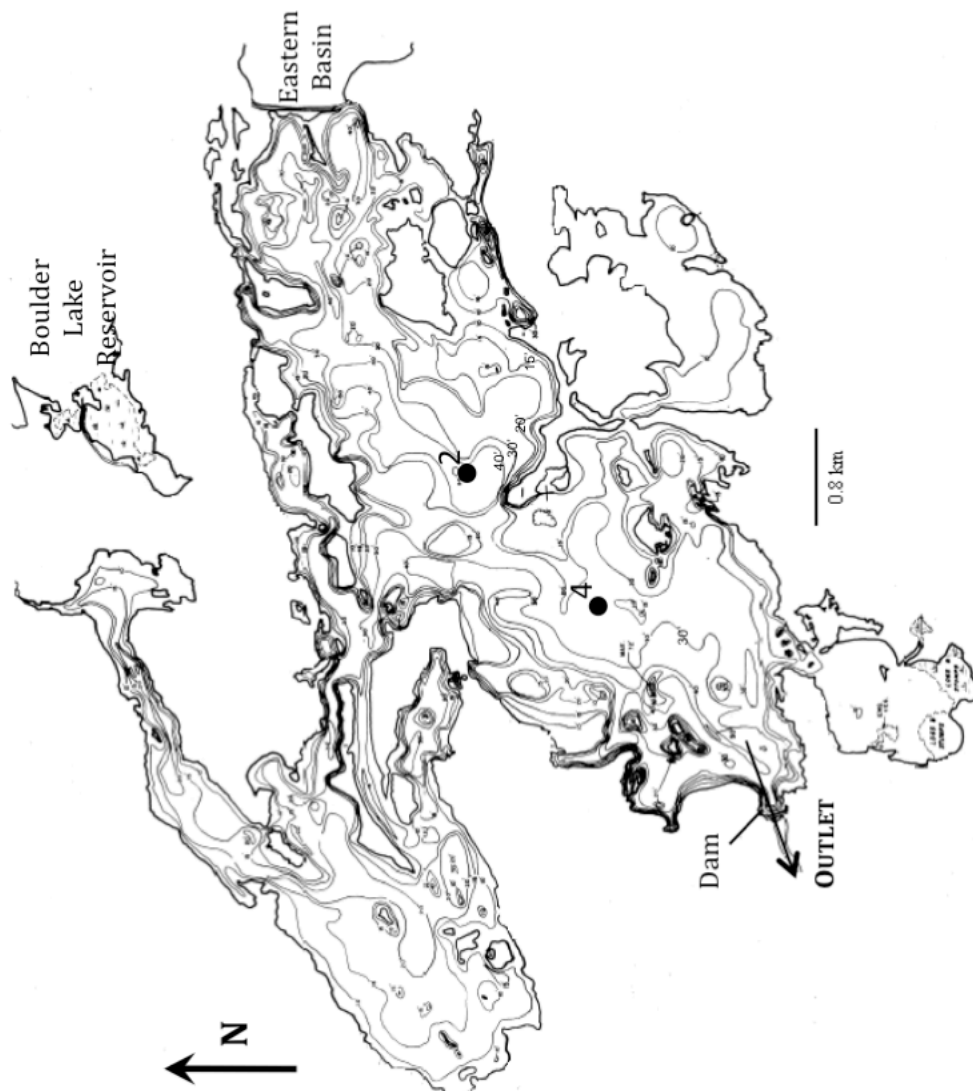


Figure 2



**Figure 3**
















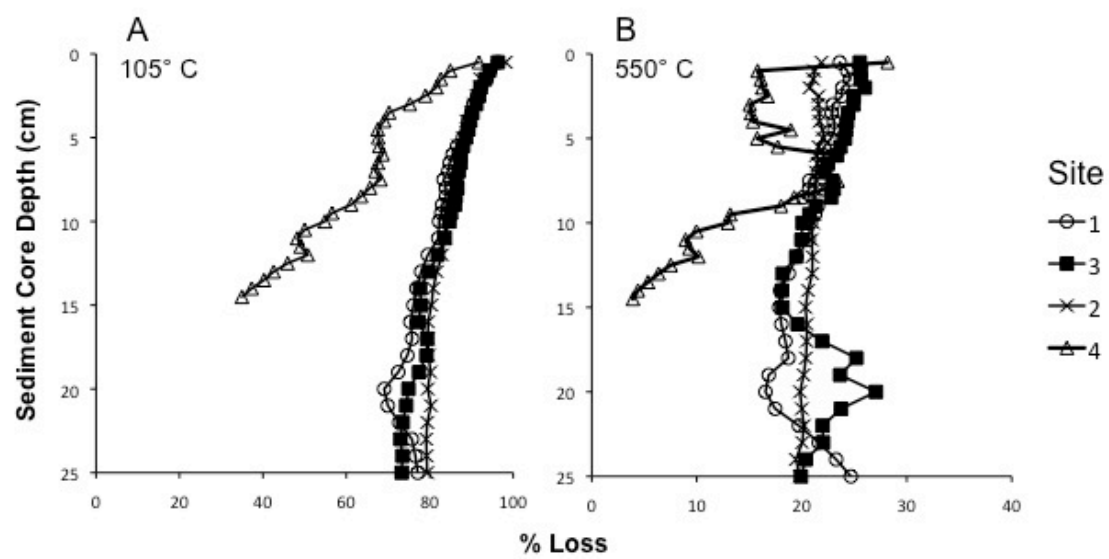
<i>Bythotrephes</i> Fragment	Designation	Description	<i>Bythotrephes</i> Fragment	Designation	Description
		Full <i>Bythotrephes</i> individual.			
	<b>a<sup>1</sup></b>	(a <sup>1</sup> ) Full spines (1, 2, or 3 barb)		<b>c</b>	(c) Fragment - mid-kink and distal
	<b>a<sup>2</sup></b>	(a <sup>2</sup> ) 1, 2, or 3 barbed spine lacking end distal to kink		<b>d</b>	(d) Fragment - long distal end
	<b>a<sup>3</sup></b>	(a <sup>3</sup> ) Spine lacking barbs		<b>e</b>	(e) Fragment - single barb
	<b>a<sup>4</sup></b>	(a <sup>4</sup> ) Spine lacking end proximal to kink		<b>f<sup>1</sup></b>	(f <sup>1</sup> ) Fragment - short tip
	<b>a<sup>5</sup></b>	(a <sup>5</sup> ) Spine lacking ends distal and proximal to kink		<b>f<sup>2</sup></b>	(f <sup>2</sup> ) Fragment - mid-piece without kink
	<b>a<sup>6</sup></b>	(a <sup>6</sup> ) Spine lacking part of kink and distal			
	<b>b</b>	(b) 1, 2, or 3 barb set			
			<b>Calculations</b>		
			$A = a^1 + a^2 + a^3 + a^4 + a^5 + a^6$		
			$C = c - a^6$		
			$D = d - (a^2 + a^5)$		
			Total # <i>Bythotrephes</i> represented = A + C (if C > 0) + D (if D > 0)		

Figure 4



**Figure 5**



**Figure 6**

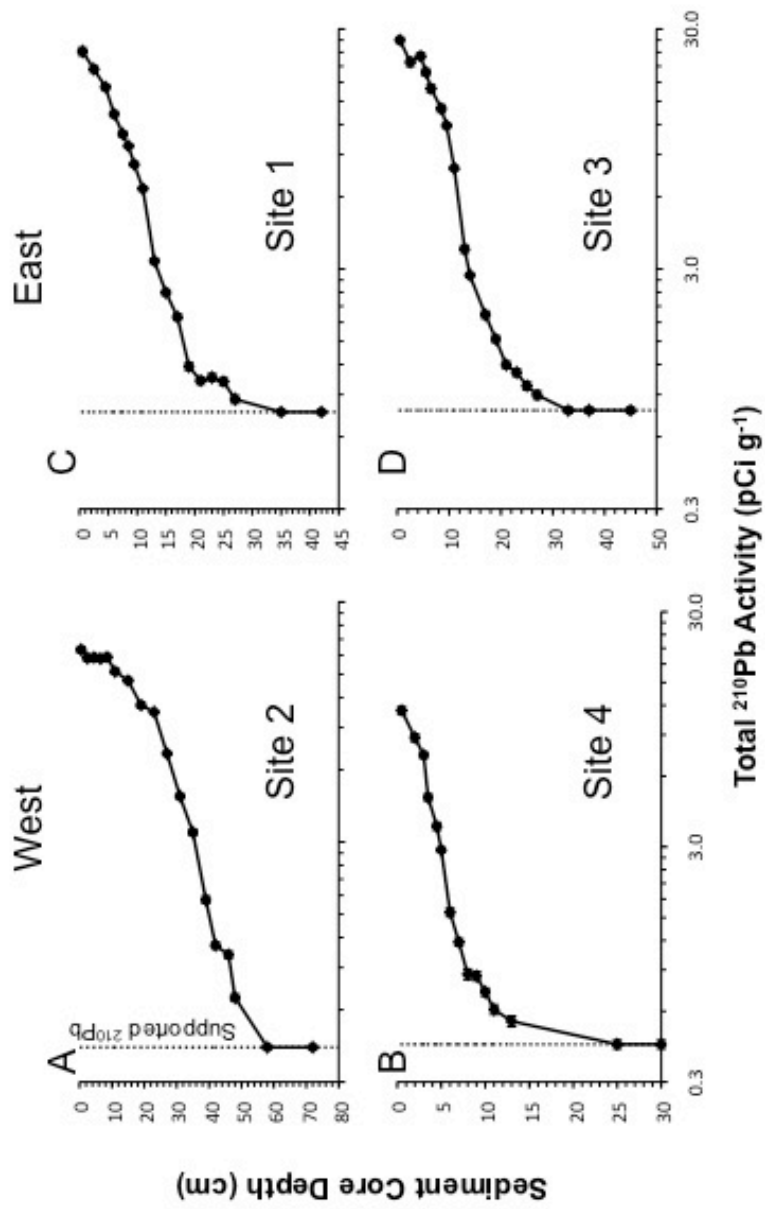


Figure 7

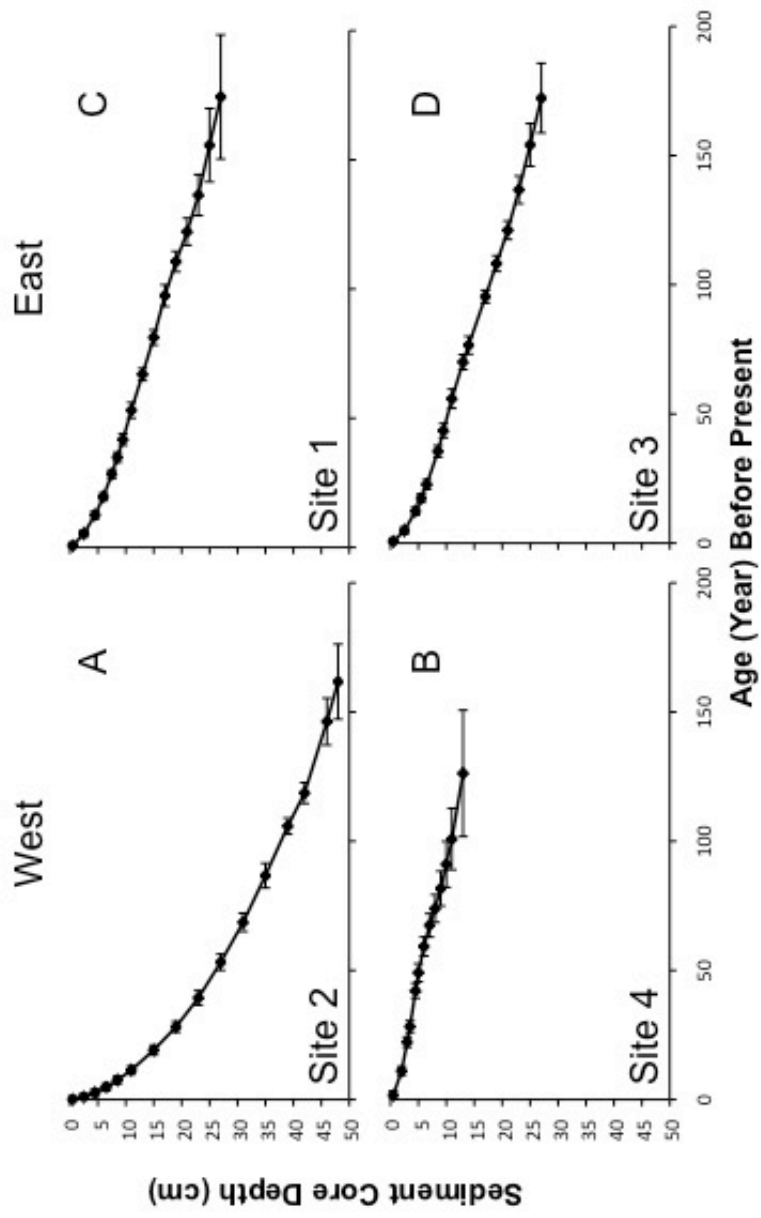


Figure 8

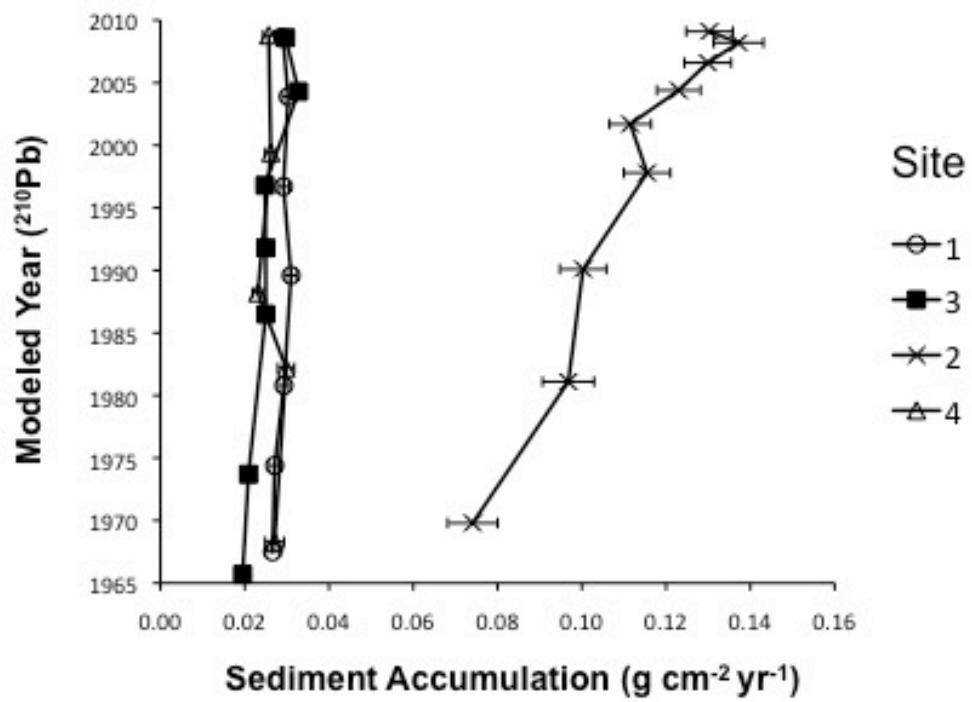


Figure 9

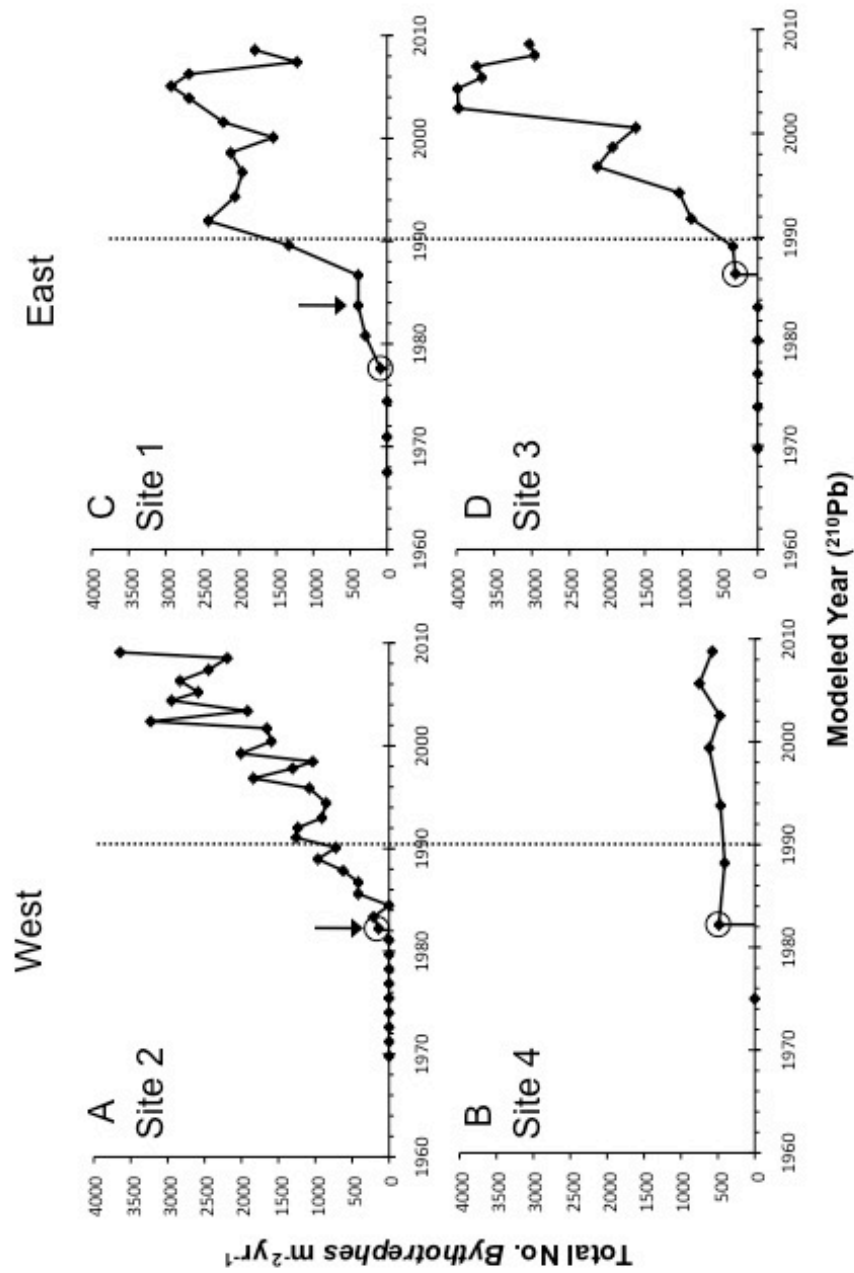


Figure 10

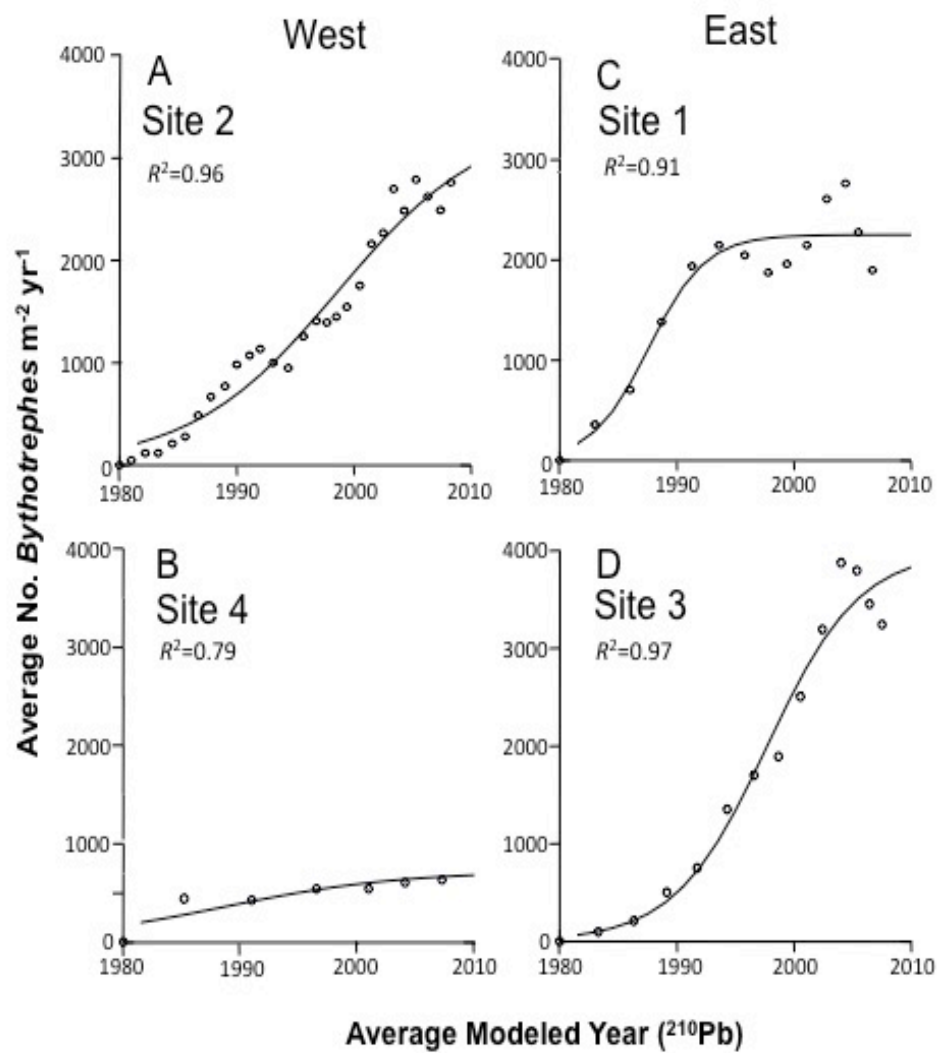


Figure 11



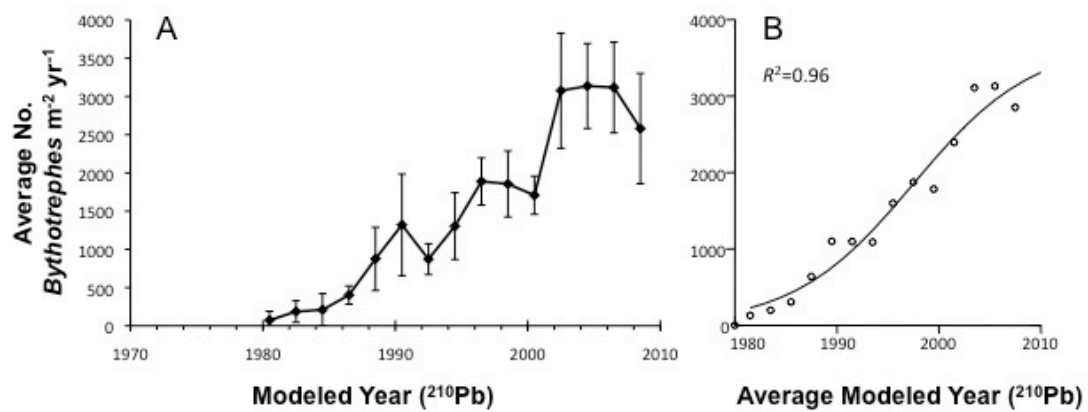


Figure 12

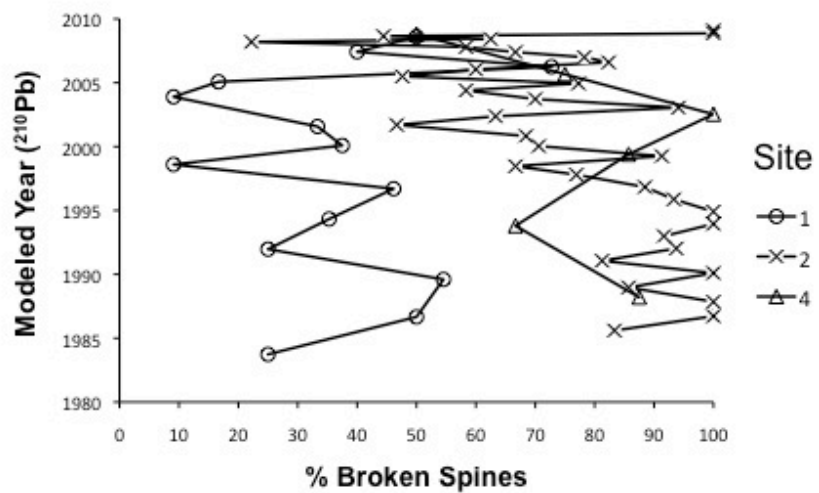


Figure 13

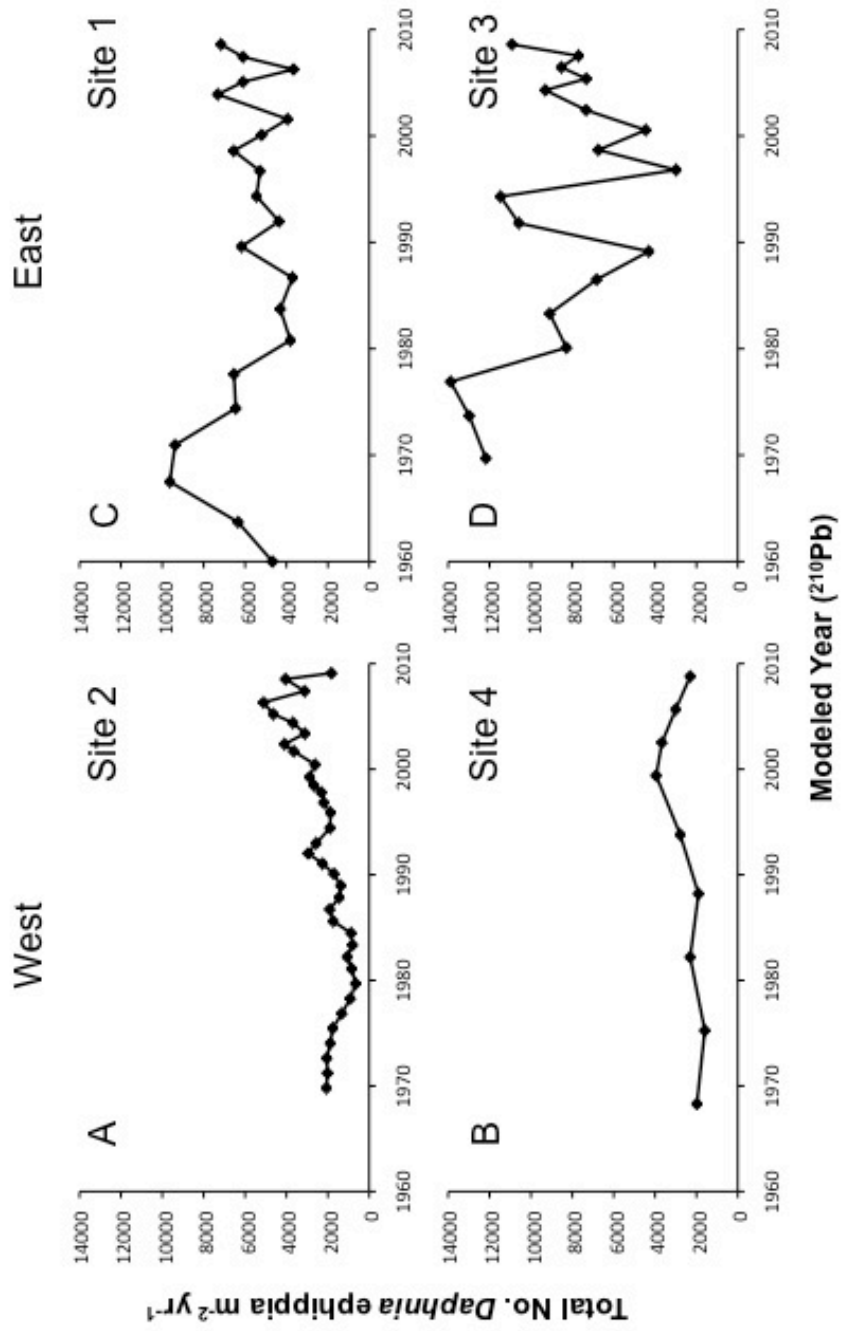


Figure 14

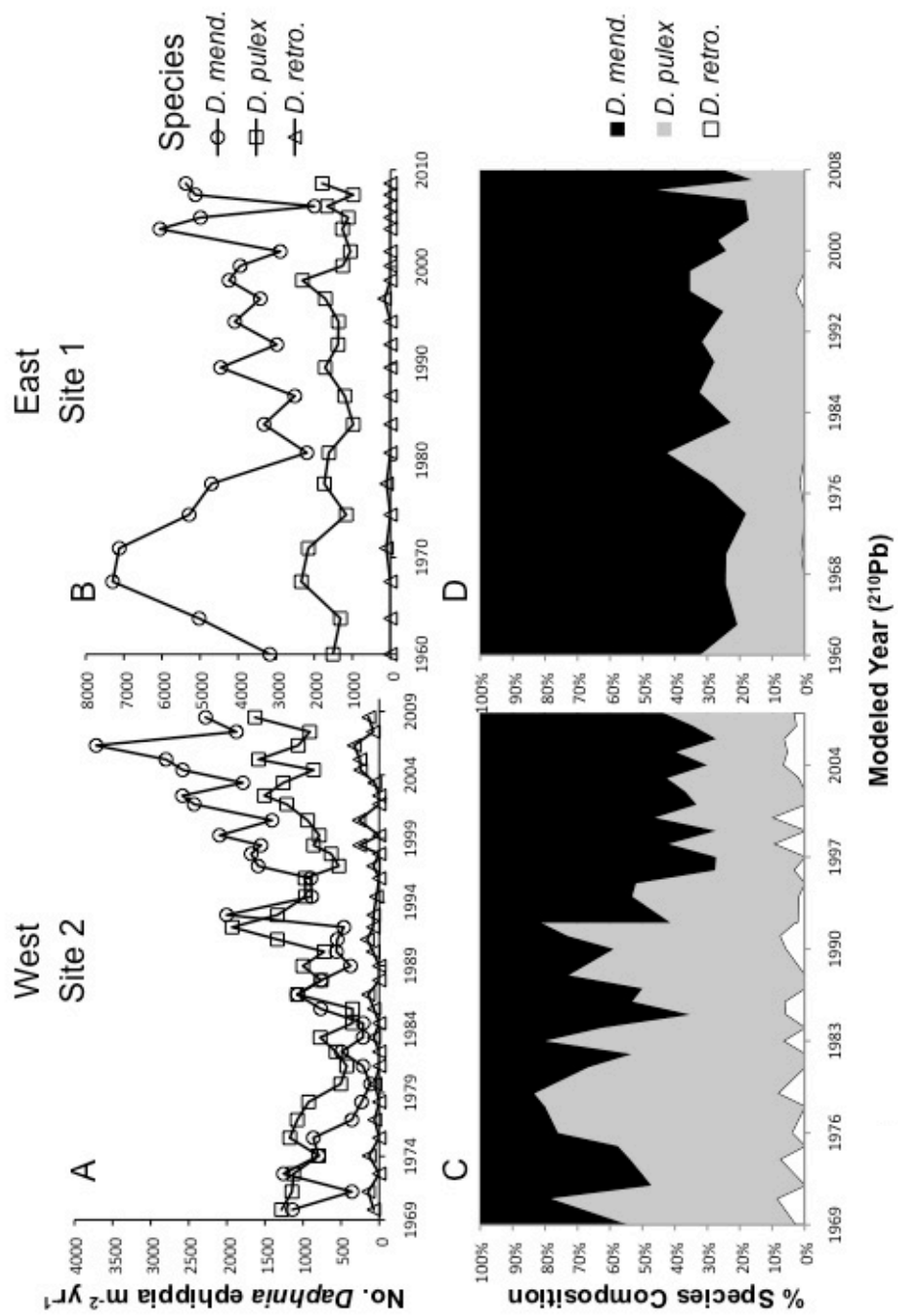
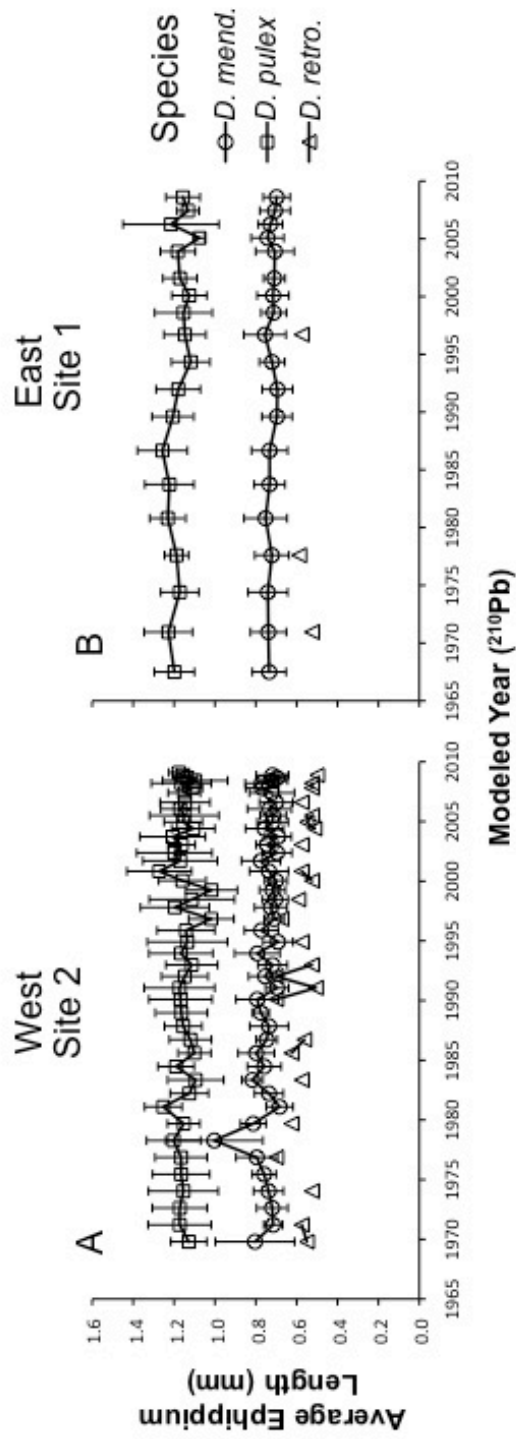


Figure 15



**Figure 16**

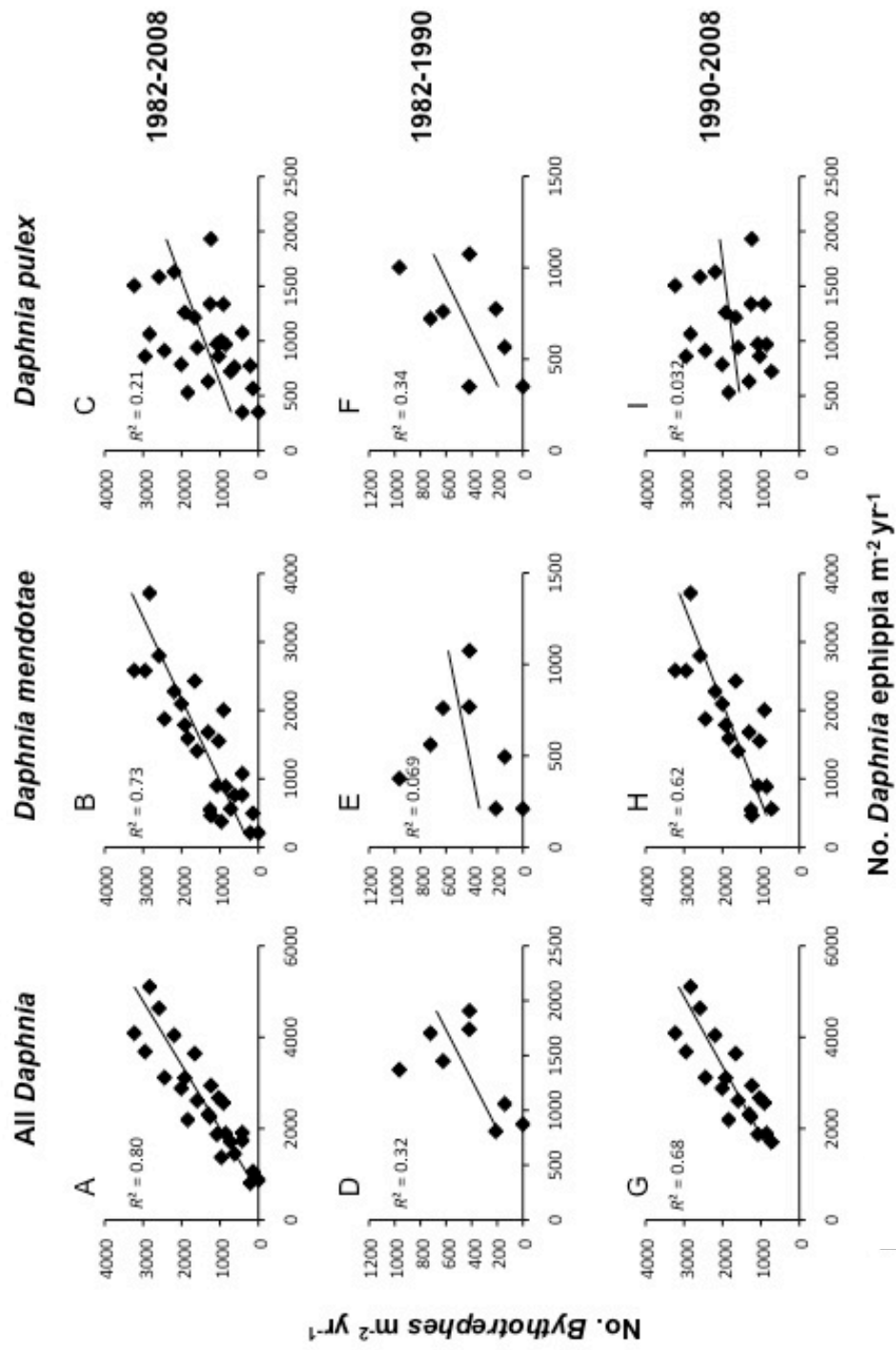
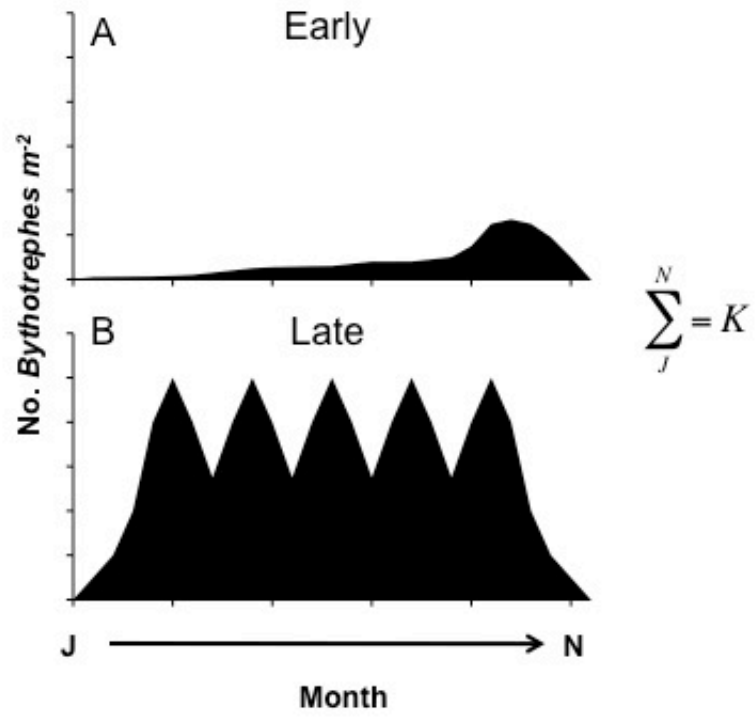


Figure 17



**Figure 18**