Effects of 2, 4-D Herbicide Treatments Used to Control Eurasian Watermilfoil on Fish and Zooplankton in Northern Wisconsin Lakes

By

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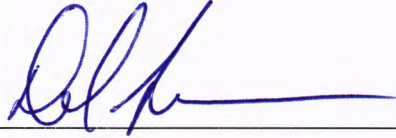
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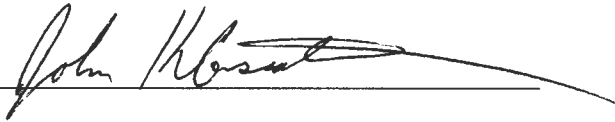
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EXECUTIVE SUMMARY

Eurasian Watermilfoil (EWM; *Myriophyllum spicatum*) is one of the most prolific aquatic invasive plants in North America. Since the 1950s, the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) has been used to control EWM. Little was known regarding the effect of 2, 4-D treatments on zooplankton and fishes outside of a few laboratory studies. One of these laboratory studies reported a 15.6% reduction in larval Fathead Minnow *Pimephales promelas* survival at 2, 4-D concentrations of 0.05 parts per million (ppm). This could be a concern because the United States Environmental Protection Agency allows spot treatments with concentrations of 4 ppm and whole-lake treatments of 2 ppm. Increasing demand for whole-lake 2, 4-D treatments to control EWM in Wisconsin lakes warranted additional examination of fish and zooplankton responses to these treatments. The objectives of this study were to determine if whole-lake 2, 4-D herbicide treatments used to control EWM affected: 1) abundance, diversity, and size of zooplankton; 2) feeding, growth and size structure of larval fishes, and 3) abundance, diversity, and survival of fishes at different life history stages.

Sampling occurred over three years (2015-2017) on six lakes in northern Wisconsin. No herbicide treatment occurred on any lake in 2015 (pre-treatment) or 2017 (post-treatment). In 2016, whole-lake treatments using the DMA® 4 IVM formulation of 2, 4-D occurred on three lakes between May 24th and June 7th; the remaining three lakes served as reference systems. Sampling took place from May through August of each year and included collection of limnological data, aquatic plant surveys, zooplankton collection, sampling of larval fish using quatrefoil light traps and ichthyoplankton tows, seining, net pen trials and collection of water samples to determine 2, 4-D concentrations. In the laboratory, all crustacean zooplankton were counted and identified to determine density (i.e., number/L), and body length was measured for

Daphnia spp.*,* and calanoid and cyclopoid copepods. Zooplankton density and body length data were compared using mixed-effects models by the main effects of lake type (i.e., reference or treatment) and year, along with the interaction of lake type and year. All larval fishes from both gears were identified. Cyprinid and Largemouth Bass *Micropterus salmoides* peak relative abundance from quatrefoil light traps and Yellow Perch *Perca flavescens*, Black Crappie *Pomoxis nigromaculatus* and Bluegill *Lepomis macrochirus* peak relative abundance from ichthyoplankton tows were compared using mixed-effects models. From ichthyoplankton tows, Yellow Perch, Black Crappie and Bluegill were measured to total length. Yellow Perch and Black Crappie hatch dates indicated that Yellow Perch hatching occurred well before herbicide application, so only Black Crappie diets, foraging success, and mean daily growth rates were analyzed. As a metric of growth, linear regressions of Yellow Perch and Bluegill total length in relation to day of year were compared among lake type-year combinations using analysis of variance.

Peak concentrations of 2, 4-D were lower than (0.152 to 0.257 ppm) than the target concentration of 0.3 ppm and degradation of 2, 4-D occurred fastest in Kathan Lake and was slowest in Manson Lake. No EWM was detected in treatment lakes after herbicide treatments in 2016. In 2017, EWM was sampled in Kathan Lake (4% vegetative coverage) and Manson Lake (9.4% vegetative coverage), but was not detected in Silver Lake. No statistically significant responses to the herbicide treatments were detected in any of the zooplankton or larval fish metrics I measured. However, different trends were observed for some zooplankton taxa in treatment lakes during 2017, the year after the herbicide applications occurred. Specifically, *Daphnia* spp. densities in Kathan and Silver lakes during 2017 were low during May when peak densities had been observed in 2015 and 2016 and were high during mid-summer when low

abundances had been observed in the two previous years. This trend was also observed for *Bosmina* spp. in Kathan Lake. Additionally, cyclopoid copepod densities remained low in Kathan and Manson lakes in 2017 when compared to 2015 and 2016. While these zooplankton trends may reflect delayed responses to the herbicide treatments, the trends were not consistent among treatment lakes and no statistical differences between treatment and reference lakes were detected.

No significant differences in larval abundance of Largemouth Bass, cyprinids, Yellow Perch, Black Crappie, and Bluegill were detected between treatment and reference lakes. Peak relative abundance of larval Yellow Perch from ichthyoplankton tows appeared to be lower in treatment lakes in 2017 (the year after herbicide was applied), a trend that was not observed in reference lakes, but the differences between lake types was not statistically significant. Slopes of larval Yellow Perch and Black Crappie total length in relation to day of year were not significantly different among lake types (reference vs. treatment) or years. Larval Black Crappie showed no detectable response to herbicide application in terms of diet, feeding success, or mean daily growth rates. Net pen trials for juvenile Bluegill and Yellow perch indicated no significant change in mortality resulting from herbicide treatments, and no treatment effect on catch-per-effort of juvenile Bluegill and Yellow Perch in August seine hauls was detected.

My findings suggest that 2, 4-D herbicide treatments had little effect on the metrics I measured. However, the lack of statistically significant responses to 2, 4-D herbicide treatments observed in this evaluation does not necessarily mean that herbicide application has no effects on these or other metrics. Potential effects may not be detectable in a lake setting given the inherent variation in many of the metrics measured and the number of lakes included in my study. Observed declines in Yellow Perch abundance and changes in zooplankton trends for treatment

lakes in the year after herbicide application occurred may be a result of changes in aquatic plant communities and not a direct effect of the herbicide. These observations warrant further investigation and this work suggests that additional laboratory assessments might focus on Yellow Perch, along with zooplankton such as *Daphnia* spp., cyclopoid copepods, and *Bosmina* spp. Additionally, this assessment did not address the effects of repeated herbicide applications on the same lake over time, which remains an important question, because EWM coverage in some lakes may return to levels where there is public interest in subsequent herbicide applications.

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INTRODUCTION

Eurasian Watermilfoil (*Myriophyllum spicatum*; EWM) is a dicotyledonous perennial plant with finely dissected leaves that represents one of the most problematic and aggressive submerged aquatic macrophytes in the United States (Sorsa et al. 1988; Smith and Barko 1990; Madsen et al. 1991; Parsons et al. 2001). Eurasian Watermilfoil is native to Europe, Asia and northern Africa, and while the exact time of introduction is not clear, EWM is now widespread throughout much of North America (Smith and Barko 1990; Madsen 1991).

Eurasian Watermilfoil begins growing early in spring, before most native plants emerge, and upon reaching the water surface, EWM branches profusely to form dense mats (Parsons et al. 2001). These dense mats shade out native vegetation and alter community composition (Madsen et al. 1991). Eurasian Watermilfoil can propagate and spread by both sexual reproduction and vegetative propagules; though vegetative propagules are considered the most effective mechanism for dispersal (Madsen et al. 1988; Smith and Barko 1990; Madsen and Smith 1997). Vegetative expansion of EWM can occur via stolons (typically local expansion), and by fragmentation, which allows for long distance dispersal both within and among water bodies (Madsen at al. 1988; Smith and Barko 1990; Madsen and Smith 1997). Fragmentation can be further divided into auto-fragmentation and allo-fragmentation. Auto-fragmentation is self-induced separation of shoot apices from the plant, usually occurring after peak biomass is attained (i.e., the apical tip develops roots and separates). Allo-fragmentation is the mechanical, involuntary separation of the plant usually caused by boats, mechanical removal, or wave action. While both means are viable, auto-fragments have been shown to be more successful at establishing. In one study, 46% of all EWM auto-fragments which settled on substrate successfully established (Madsen and Smith 1997).

Introduction of EWM in aquatic ecosystems can lead to a variety of detrimental effects. For example, the adaptability, and prolific growth rates and spreading capability of EWM allow it to readily displace native macrophytes (Sorsa et al. 1988; Madsen et al. 1991; Harrahy et al. 2014). Further, abundant EWM growth has many economic, recreational, and ecological effects such as interfering with boating, increased nutrient loading, changes in nutrient cycling, and altering habitat for invertebrates, fish and waterfowl (Smith and Barko 1990; Madsen 1991; Parsons et al. 2001; Kovaleno et al. 2010; Harrahy et al. 2014).

Several methods have been used in an attempt to control EWM, including manual and mechanical harvest, and biological methods such as the Milfoil Weevil *Euhrychiopsis lecontei*. However, these methods are costly and not likely to result in eradication (Sorsa et al. 1988; Helsel et al. 1996; Parsons et al. 2001; Harrahy et al. 2014). For example, in a 1,912-ha New York lake, labor costs associated with hand pulling of EWM ranged from $146,475 to $351,748 per year and annual pulling was needed to maintain low EWM densities (Kelting and Laxson 2010). Introduction of Milfoil Weevils have been associated with EWM declines in some Wisconsin and Minnesota waters, but predation by *Lepomis* spp. may cause declines in weevil densities below levels needed for effective control (Sutter and Newman 1997; Ward and Newman 2006). Alternatively, a variety of herbicides such as 2, 4-dichlorophenoxyacetic acid (2, 4-D), fluridone, endothall, and diquat, have provided relatively effective control for EWM (Sorsa et al. 1988; Green and Westerdahl 1990; Wagner et al. 2007; Gettys et al. 2014).

The herbicide 2, 4-D has been used to control EWM in the United States since the 1950s (Nault et al. 2014). Two types of 2, 4-D are used for aquatic applications, dimethylamine salt (DMA) and butoxyethyl ester (BEE), which are both available in liquid and slow-release granular forms (WDNR 2012; Harrahy et al. 2014). These formulations are marketed under the

trade names Aqua-Kleen®, Weedar 64® and Navigate®, among others. This herbicide works by mimicking the natural plant hormone auxin, which affects respiration, decreases food reserves by producing insufficient chlorophyll, causes excessive growth and cell division, and ultimately results in death (Parsons et al 2001; Kovalenko et al. 2010; Harrahy et al. 2014; Nault et al. 2014). After application, 2, 4-D tends to dissipate relatively rapidly with dissipation rates depending on lake stratification, water chemistry, movement and temperature, and substrate composition (Parsons et al. 2001; Nault et al. 2012). Decomposition of 2, 4-D is primarily accomplished by microorganisms, along with ultraviolet light, which break down 2, 4-D into carbon dioxide, water, chlorine, and a variety of chemicals depending on formulation (Mullison 1970; Parsons et el. 2001; WDNR 2012; Harrahy et al. 2014).

Many studies have reported effective use of 2, 4-D for selective control of EWM without significant effects on native aquatic plants (Hesel et al. 1996; Parsons et al. 2001; Kovalenko et al. 2010). The chemical 2, 4-D is not selective for EWM and affects all dicotyledonous plants. However, it is the timing of application that allows for EWM control with minimal effects to native aquatic plants. Whole-lake treatments typically take place in the spring after lakes have stratified and EWM has begun growing, but most native macrophytes remain dormant. Selectivity of 2, 4-D towards EWM has been shown to decrease during higher exposure times in whole-lake treatments (Nault et al. 2012; Nault et al. 2014) and spot treatments with high concentrations (Getsinger 1982). The ability to treat entire lakes with the possibility of long-term EWM control has led to greater use of whole-lake treatments across several states, including Wisconsin. While the effects of 2, 4-D and other herbicide treatments on aquatic macrophytes have been well studied, little is known regarding the effects of these herbicides on other aquatic organisms outside of laboratory setting (DeQuattro and Karasov 2015).

Zooplankton play a significant role in aquatic ecosystems, acting as grazers, predators, and serving as prey for many aquatic organisms, including larval fishes (Balcer et al. 1984). If zooplankton are negatively affected by 2, 4-D, this could influence organisms at both higher and lower trophic levels. Specifically, most fish species depend on zooplankton during early life stages of development, with gape limitations determining the size and type of zooplankton that are consumed (Schael et al. 1991; Devries et al. 1998). Consequently, changes in zooplankton communities could have negative effects on fish growth, survival and recruitment (Welker et al. 1994; Graeb et al. 2004; Kaemingk et al. 2014). For example, Kaemingk et al. (2014) reported that mortality of larval Yellow Perch *Perca flavescens* in a Nebraska lake was strongly correlated to total available zooplankton biomass, and growth at older larval stages was influenced by zooplankton abundance. Welker et al. (1994) reported that larval Bluegill *Lepomis macrochirus* growth was positively correlated with zooplankton abundance in both a mesocosm and an Illinois lake. Furthermore, a laboratory study conducted by Graeb et al. (2004) indicated Yellow Perch growth and survival was significantly influenced by zooplankton size and species.

Previous laboratory and mesocosm experiments have reported varying results regarding the effects of 2, 4-D on zooplankton (Sanders 1970; Boyle 1980; Releyea 2005). Sanders (1970) described immobilization of *Daphnia magna* under certain 2, 4-D formulations and concentrations, while two other mesocosm studies reported no significant change in crustacean zooplankton densities after treatment (Boyle 1980; Relyea 2005). Little research regarding the effects of 2, 4-D on zooplankton has been conducted in a lake setting. However, Couch and Nelson (1982) reported that zooplankton density and diversity did not decrease following spot treatments with the butoxyethyl ester (BEE) formulation of 2, 4-D. Conversely, Harrahy (2014) reported negative correlations between both taxa richness and copepod abundance and the

number of days following a granular treatment of 2, 4-D, but had no reference system for comparison.

Fish are vulnerable to pollution and environmental degradation in all stages of development, but especially during early life (Hiltibran 1967; Meehan et al. 1973). Fairchild et al. (2009) exposed juvenile and swim-up larvae of Rainbow Trout *Onchorynchus mykiss* to varying concentrations of the free acid form of 2, 4-D in a laboratory setting and reported no significant mortalities for either larval or juvenile fish after 30-d exposure at varying concentrations (0, 7, 14, 27, 54 and 108 mg/L). However, larval fish did show a significant decrease in growth and weight at the 108 mg/L concentration level, but juveniles did not. In a similar study, Hiltibran (2011) used the DMA salt formulation of 2, 4-D and reported no effects on the survival of Bluegill, Green Sunfish *Lepomis cyanellus,* Smallmouth Bass *Micropterus dolomieu* and Lake Chubsucker *Erimyzon sucetta* at 25 mg/L for 8 d. Conversely, Cope et al. (1970) reported that 2, 4-D concentrations of 10 mg/L caused significant mortality in Bluegills, and that concentrations of 5 and 10 mg/L delayed spawning by two weeks in ponds treated with the propylene glycol butyl ester of 2, 4-D. However, this and other ester formulations of 2, 4-D have been reported to be more toxic than other formulations of 2, 4-D (Cope et al. 1970; Meehan et al. 1974; WDNR 2012; DeQuattro and Karasov 2015).

There is a general lack of research regarding the effects of 2, 4-D herbicide treatments used for EWM control on zooplankton and fish communities in natural systems. Most of the previous research has focused on laboratory, mesocosm, or spot-treatment experiments, and not whole-lake herbicide treatments. For example, a recent laboratory study observed declines in larval Fathead Minnow *Pimephales promelas* survival with DMA® 4 IVM formulation of 2, 4-D when exposed for a period of 30 days at constant concentrations (DeQuattro and Karasov 2015).

Larval Fathead Minnow survival was significantly lower in the 0.05 parts per million (ppm) treatment group (82.5 ± 4.29% survival) when compared to the control (98 ± 1.12% survival), and while statistical trends (i.e., $0.05 \leq p \leq 0.10$) of decreased larval survival were observed at concentrations of 0.5 ppm (90.83 ± 2.24% survival) and 2.0 ppm (90 ± 2.75% survival) they were not significant. This same study also stated that both Weedestroy® AM40 and DMA® 4 IVM formulation of 2, 4-D had no significant effects on fecundity, fertilization, hatchability or embryonic development of Fathead Minnows. However, male Fathead Minnow tubercle presence decreased significantly (up to 24%) at 2.0 ppm for DMA® 4 IVM and at all concentrations for Weedestroy® AM40, suggesting that the chemical may be an endocrine disruptor. These findings are of concern due to the fact that the DMA® 4 IVM formulation is currently being used in Wisconsin for whole-lake treatments, with the United States Environmental Protection Agency permitting whole-lake treatment concentrations of 2, 4-D up to 2 ppm and spot treatment concentrations up to 4 ppm (DeQuattro and Karasov 2015).

Mesocosm and laboratory studies may not accurately represent fish responses that may occur following herbicide treatments in a lake setting, as environmental conditions cannot be entirely replicated within controlled settings. Specifically, laboratory studies do not replicate the natural degradation of 2, 4-D in the environment, as laboratory studies are typically conducted at a constant concentration. Existing field studies of 2, 4-D treatments have not reported significant effects of 2, 4-D on fishes (Paul et al. 2006; Kovalenko et al. 2010; Webb et al. 2016). In a Minnesota study, two lakes were treated with a mix of endothall and 2, 4-D, with no significant treatment effects observed on the number of prey items, stomach content mass, diet composition or abundance of major diet items in adult Bluegill when compared to the two reference lakes (Webb et al. 2016). Paul et al. (2006) used AquaKleen® (BEE formulation of 2, 4-D) to spot treat

EWM in New York lakes and held juvenile Walleye *Sander vitreus,* Brook Trout *Salvelinus fontinalis* and Fathead Minnows in cages at the surface of treatment sites. No significant mortality was observed for any of the species. However, in this same study, a laboratory experiment determined 96-hr LC5Os (lethal concentration for 50% of population) for Brook Trout, Walleye, and Fathead Minnow were 0.76, 0.66, and 2.22 mg/L, respectively (Paul et al. 2006). In another study, two Minnesota lakes were spot-treated with 2, 4-D and endothall, but no differences in biomass or diversity of fishes were observed when compared to reference lakes (Kovalenko et al. 2010). These previous studies were not focused on whole-lake treatments of 2, 4-D herbicides. However, following spot treatments, 2, 4-D can dissipate through the water rapidly and spot treatments essentially become low dose, whole-lake treatments (Nault et al. 2012).

There has been increasing demand for 2, 4-D treatments of EWM in Wisconsin, with over 20,000 acres of EWM being treated from 2000 to 2015; over 50% of those treatments occurred in those last 5 years (WDNR unpublished data). This increase in use of 2, 4-D, and the recent implementation of whole-lake treatments in Wisconsin, have led scientists and managers to question what effects these treatments may have on aquatic communities. DeQuattro and Karasov (2015) showed that two similar amine formulations of 2, 4-D can have varying effects on Fathead Minnows, and that specific 2, 4-D product effects may not be comparable. These differences may be due to inert ingredients, with 2, 4-D accounting for less than 50% of ingredients for both formulations used in this study. Given the lack of published information, evaluations of whole-lake treatments are needed to determine the effects of 2, 4-D on zooplankton and early life history stages of fish. The objectives of my study were to determine if whole-lake 2, 4-D herbicide treatments used to control EWM affected: 1) abundance, diversity,

and size of zooplankton; 2) feeding, growth and size structure of larval fishes, and 3) the abundance, diversity, and survival of fishes at different life history stages.

METHODS

Study Area and Experimental Design

This study was conducted during 2015-2017 on six lakes in northern Wisconsin, all of which contained EWM (Table 1, Figure 1). Lakes were selected based on similarities in surface area, water chemistry, bathymetry, and both plant and fish communities. None of the lakes had been treated with 2, 4-D since at least 2010. Three lakes (i.e., Brandy, Little Bearskin, and Upper Gresham) were considered reference lakes and received no 2, 4-D treatment during the study, while the remaining three lakes (i.e., Kathan, Silver, and Manson) were considered treatment lakes and received a whole-lake treatment of 2, 4-D using the DMA® 4 IVM formulation of 2, 4- during 2016. Pre-treatment data were collected on all lakes during 2015 and data were collected from all lakes in 2017, which represented the year after herbicide applications occurred. Herbicide treatments did not occur on any lake in 2015 and 2017.

Water Quality, Temperature, and Productivity

Secchi depth readings and temperature (°C) and dissolved oxygen (mg/L) profiles were recorded at 0.5 m depth intervals using a YSI™ 556 MPS at 7- to 14-d intervals from May to August at the deepest part of each lake. A temperature logger (Onset® Hobo Pro V2 or Tidbit V2) was attached to a cinder block and deployed at a depth of < 1 m in each lake from May to August each year to record hourly temperatures. Additionally, algal and chlorophyll-*a* samples were collected at four randomly-selected locations on each lake; sites remained fixed throughout the study. Samples were collected using a 2-m integrated tube sampler at 7- to 14-d intervals,

from which a composite sample on each date was collected and delivered to Wisconsin

Department of Natural Resources (WDNR) personnel. Chlorophyll-*a* samples were filtered using

Millipore® SM 5 µm membrane filters (SMWP 04700, 47 mm diameter) and a vacuum source by

WDNR personnel. Filters were then placed into a test tube, wrapped in aluminum foil, packed on

ice and shipped to the Wisconsin State Laboratory of Hygiene for analysis.

Secchi depth and chlorophyll-*a* concentrations were analyzed using mixed-effects models

with lake type (2, 4-D herbicide application in 2016 vs. reference) and year as main effects and a

lake type and year interaction term. Prior to analysis, data were tested for normality and

homogeneity of variance using Shapiro-Wilks tests and Levene's tests, respectively. Mixed-

effects models were conducted in SAS 9.4® using the PROC GLIMMIX procedure; lake and

lake within year (i.e., year/ sub=lake) were included as random effects. Secchi depth and

chlorophyll-*a* concentrations within each sampling period were considered independent

observations within lakes, but sampling period was not considered a main effect (essentially a

blocking variable). If main effects or interactions were significant ($P < 0.05$), post-hoc *t*-tests

using PROC LSMEANS were used for comparison of factor- or treatment-level means. Alpha

was not corrected for multiple comparisons because of the large variation in metrics and the low

number of lakes within lake type (N = 3), resulting in low power (i.e., difficult to detect a

significant difference if one existed).

Aquatic Plants

To determine the effects of 2, 4-D on EWM and native plants, point-intercept surveys

were conducted on each lake following WDNR protocols (Madsen 1999; Hauxwell et al. 2010;

Nault et al. 2014). Sampling sites were established by WDNR, and sampling was completed each

year between mid-July and mid-August. Within lakes, aquatic plant surveys took place in the

same week each year to allow for comparability with previous surveys. Sampling sites were located using a point-intercept grid uploaded to a hand-held GPS unit. At each site, water depth was recorded, and one sample of aquatic plants was collected. In water ≤ 4.6 m deep, a double-headed rake attached to a telescoping pole was used to collect the plant sample. The rake was lowered until it came into contact with the bottom substrate, at which point the rake was twisted to make two complete rotations before being retrieved. At sample sites > 4.6 m deep, a 2.27-kg weighted rake attached to a rope was deployed and dragged along the lake bottom for approximately 0.3 m and then pulled to the surface. In addition to depth, dominant sediment type, collection method (pole vs. rope), rake fullness, and plant species present were recorded at each site. Dominant sediment type was qualitatively reported as muck, sand, or rock based on sediment on plant roots or texture when the rake was in contact with the lake bottom. Rake fullness was rated as: 1) few plants, single layer across tines; 2) plants cover rake in single layer, but tines are visible and 3) rake is completely covered and tines are not visible (Hauxwell et al. 2010; Figure 2). When possible, plants were identified to species using Borman et al. (2014) and Skawinski (2014); unknown plants were vouchered for later identification by University of Wisconsin-Madison Center for Limnology personnel. Aquatic plant density maps were created to illustrate changes in relative abundance and distribution of EWM and all other aquatic plants.

Zooplankton

To determine if 2, 4-D herbicides affected zooplankton, sampling occurred from mid-May to mid-August of each year at 7- to 14-d intervals. Zooplankton were sampled at four randomly-selected locations on each lake and sites remained fixed throughout the study. Zooplankton were collected using Sea-Gear® model 9000 plankton nets (30-cm opening, 3:1

length-to-diameter ratio, 80-µm mesh, 80-µm cod end bucket). The net was lowered to within 1 m of the lake bottom and retrieved. Depth of each tow was measured to the nearest 0.5 m to estimate zooplankton densities (i.e., number/L). Zooplankton samples were preserved in 95% ethanol.

Zooplankton were identified following Balcer et al. (1984) and enumerated by diluting each sample to the nearest 25 ml, to a minimum of 100 ml total sample volume. For every 25 ml, a 1-ml subsample was randomly taken using a Hensen-Stempel pipette and placed into a zooplankton counting wheel. Using a dissecting microscope from 20 to 50x magnification, all crustacean zooplankton were identified and counted. Cladocerans were identified to genus when possible, while copepods were identified to order (Calanoida or Cyclopoida). In each sample, up to 10 *Daphnia* spp., 10 calanoid and 10 cyclopoid copepods were measured (nearest µm) using Leica Application Suite V4.10.0® software or an ocular micrometer. *Daphnia* spp. were measured from the anterior base of the carapace to the base of the tail-spine (body length), and from the tip of the helmet to the tip of the tail-spine (total length); copepods were measured from the anterior portion of the carapace to both the base and end of the caudal rami (Devries et al. 1988; Welker et al. 1994). To estimate zooplankton diversity, Shannon's diversity index (*H′*) was calculated for each lake in each year (i.e., data pooled across all samples for a specific year) using the equation

$$H' = - \sum_{i=1}^{S}(p_i)(\log_e p_i),$$

where *s* is the number of species and p_i is the proportion of the total sample represented by the *i*th species (Kwak and Peterson 2007).

Using a similar approach to those described in the analysis of Secchi depths and chlorophyll-*a* concentrations, total zooplankton, *Daphnia* spp., calanoid copepod, cyclopoid

copepod, *Bosmina* spp. and nauplii densities (number/L), along with body lengths of *Daphnia*

spp., calanoid and cyclopoids, were analyzed using mixed-effects models. Main effects of lake

type (treatment vs. reference) and year, along with the interaction between lake type and year

were used in all models. Total zooplankton, *Daphnia* spp. and calanoid copepod densities, along

with calanoid and cyclopoid copepods body lengths were $\log_e$ transformed before analysis

because of unequal variances among some treatments. Before transformation, 1 was added to

Daphnia spp. and calanoid copepod densities because a density of zero was recorded on at least

one lake during a single sampling period for each taxa. Shannon's diversity index for

zooplankton was analyzed with mixed-effects models including the main effects of lake type

(treatment vs. reference) and year, and the interaction between lake type and year. Random

effects of lake nested within lake type [i.e., lake (lake type)] and year within lake with an

autoregressive covariance structure [i.e., random year/ sub=lake (lake type) type=ar(1)] were

used for this analysis. Sampling period 1 was removed from all zooplankton analyses because no

zooplankton samples were collected during this period in 2017.

Larval Fish

Larval fish were collected each year using quatrefoil light traps (4-mm opening, 250-µm

mesh capture bags) and Sea-Gear® Model 9000 ichthyoplankton nets (1,000-µm mesh, 75-cm

diameter net mouth, 5:1 net length-to-diameter ratio). Sampling began in mid-May and continued

through mid-July of each year at 7- to 10-d intervals, with both gears fished within 24 h of each

other. Light traps were set at dusk and fished overnight at four randomly-selected locations at

depths ≤ 2.4 m on each lake; these sites remained fixed during the course of the study. Light trap

catch per effort (CPE; fish/trap night) was estimated for the period encompassing 30 min after

sunset to 30 min before sunrise. Ichthyoplankton nets were towed behind a boat for 3 to 5 min

just below the surface during daylight hours. Tows were conducted at six randomly-selected locations on all lakes except for Silver Lake (N = 4); these sites remained fixed throughout the study. A General Oceanics® Model 2030R flow meter was mounted in the mouth of the ichthyoplankton net to allow calculation of total volume of water filtered (m^3) and larval fish CPE (fish/100 m^3). Larval fish were preserved in 95% ethanol for subsequent processing.

Larval fish were identified to species using Auer (1982), with cyprinids identified to family. To determine changes in diversity, Shannon's diversity index was calculated for ichthyoplankton tows and quatrefoil light traps. To determine if relationships between mean TL and day of year were different among years and lakes, up to 50 randomly-selected Yellow Perch, Black Crappie *Pomoxis nigromaculatus*, and Bluegill from ichthyoplankton tows and light traps were measured for total length (TL) per site on each sampling date. Larval fish were placed into a petri dish placed under a dissecting microscope at magnifications of 8 to 20x. Fish were measured using Leica Application Suite V4.10.0® software or an ocular micrometer to the nearest µm. When fish were too large for measurement under a microscope (> 12 mm), they were measured using digital calipers to the nearest 0.1 mm.

Sagittal otoliths were removed from up to 30 randomly-selected Yellow Perch and Black Crappie to estimate hatch timing and daily growth. Otoliths were removed from larvae collected on the date of peak larval abundance for each species. Larval Yellow Perch were initially selected as they were expected to be present in all lakes during typical herbicide treatment periods (early to mid-May; Schael et al. 1991; Isermann and Willis 2008). Black Crappies were selected because they should have been spawning or incubation of eggs would be occurring when herbicide treatments occurred (Schael et al. 1991). Otolith daily ring counts were used to estimate hatch dates and average daily growth rates. Daily growth increments have not been

validated for Black Crappie, but have been validated for age-0 White Crappie *Pomoxis annularis* (Sweatman and Kohler 1991) and have been used previously to estimate age of larval Yellow Perch (Fitzgerald et al. 2001; Isermann and Willis 2008).

After larval fish were measured for TL using an ocular micrometer, otoliths were removed and placed on a glass slide and viewed at 400x magnification; immersion oil was used to improved otolith clarity. An image was taken of the single best otolith using ImagePro® software and a Nikon DS-Fi1 camera. Two separate readers estimated the age (count of the number of daily rings) of each otolith independently, and the ages from each reader were averaged for each fish.

Daily growth increments in White Crappie otoliths appear at hatch, so no correction was used to calculate hatch date (Sweatman and Kohler 1991). Black Crappie hatch dates were calculated by subtracting date at capture from mean otolith daily counts. Mean larval Black Crappie average daily growth rate (DGR) was calculated using methods similar to Pine and Allen (2001) in which the mean TL at hatch (4.3 mm) was subtracted from the TL at capture (TL_c) and divided by age in d using the equation:

$$DGR = (TL_c - 4.3)/age.$$

Most Yellow Perch exhibit daily growth increments 1 day after hatch, so hatch dates were corrected by adding 1 day to the mean growth increment count and subtracted from day of capture (Isermann and Willis 2008). Daily growth rates for larval Yellow Perch were estimated using the methods described by Kaemingk et al. (2014) where TL_c is the length at capture, 4.7 is the mean TL at hatch, and age is the number of days post hatch using the equation:

$$DGR = (TL_c - 4.7)/age.$$

When possible, diet items were also removed from the entire digestive tract of larval Black Crappie. Copepods were identified to order and carapace length was measured. Cladocerans were identified to genus when possible and total length was measured. To determine if herbicide treatments affected foraging success, the number of zooplankton per larval fish diet was calculated. Foraging success was then compared among lakes and among years within lakes (e.g., pre-treatment vs. treatment year in Silver Lake). To determine if diet composition changed due to herbicide application, percent composition by number (Chipps and Garvey 2007) was also calculated for Black Crappie diets for calanoid copepods, cyclopoid copepods, cladocerans, and nauplii.

Peak relative abundance of larval cyprinids and age-0 Largemouth Bass from quatrefoil light traps, and peak relative abundance of larval Yellow Perch, Black Crappie and Bluegill CPE from ichthyoplankton tows were compared using mixed-effects models with lake type (treatment vs. reference) and year as main effects and a lake type and year interaction. Larval cyprinid, Yellow Perch and Black Crappie CPE were $\log_e$ transformed before analysis. Larval Black Crappie mean daily growth rate and Shannon's diversity index for larval fish captured in quatrefoil light traps and ichthyoplankton tows were also analyzed in this manner. Sampling period was not used in these analyses because of observed variation in hatch timing and timing of peak catch rate of larval species among lakes and years that could affect conclusions regarding the effects of the herbicide treatments, as CPE of larvae typically declines rapidly after peak abundance is detected. Consequently, if peak abundance of larval Black Crappies was detected before herbicide application dates on some lakes, but after application dates on other lakes, I might erroneously conclude that herbicide application affected larval crappie abundance when the difference merely reflected natural variation or the dates selected for sampling each lake.

Mean daily growth rates were not calculated for larval Yellow Perch or Bluegill, so growth was described using slopes from linear regressions of TLs in relation to day of year (January 1st = day 1). Slopes were estimated for each lake type in each year and were compared using PROC GLM in SAS $9.4^{®}$. Only Yellow Perch TLs from sampling periods 2 and 3 and Bluegill TLs from sampling period 5 to 8 were used because of low sample sizes during other sampling periods. Larval Black Crappie foraging success was analyzed among lake type and year combinations (i.e., reference 2016, treatment 2016, reference 2017, treatment 2017) using a Kruskal-Wallis test, while the effects of year and lake type were analyzed using a Wilcoxon rank sum test because the assumption of normality was not met. Both nonparametric tests were conducted in SAS $9.4^{®}$ using PROC NPAR1WAY.

Juvenile Fish Abundance and Mortality

To assess trends in juvenile fish abundance, seines (0.32-cm mesh, 30.48-m long, 1.83-m high) were pulled in an arc perpendicular to the shoreline at six randomly-selected locations on each lake. These sites remained fixed during the course of the study. All fish captured in seines were identified to species and counted. The first 50 fish of each species collected at each site were measured (TL; mm) on each sampling date. Shannon's diversity index values for seine data, along with juvenile Yellow Perch ($\leq$ 70 mm TL) and Bluegill ($\leq$ 100 mm TL) seine CPE from August seining events were analyzed using mixed-effects models. Yellow Perch of these TLs were selected for analysis because they should incorporate the age-0 fish that were larvae during ichthyoplankton tows in the same year.

To evaluate immediate effects of herbicide applications on juvenile fish survival, up to 30 Bluegill and 30 Yellow Perch $\leq$ 125 mm TL captured during May to June seining and electrofishing events were held in aerated tanks for at least 10 min, then transferred into two 1.0-

m^3 net pens (0.64-cm mesh) and held for 48 h. Net pens were stationed at randomly-selected locations on each date. After 48 h, fish were removed and categorized as dead or alive, and external symptoms that may have resulted in mortality were noted (e.g., wounds from pen, etc.). Net pen trials were conducted on each lake between late May and early June of each year, which included trials conducted before and after herbicide treatments occurred in 2016. Net pen trials occurring in 2015 and 2017 and before herbicide application dates in 2016 were considered reference observations (i.e., no 2, 4-D present); net pen trials occurring up to 1 week after herbicide application on treatment lakes were considered treatment observations. A Wilcoxon rank sum test was used to determine if survival of juvenile Yellow Perch and Bluegill from net pen trials differed between treatment and reference conditions.

2, 4-D Degradation Rates

To quantify 2, 4-D degradation rates following treatments, water samples were collected using a Van Dorn horizontal sampler at a depth of 1.5 m from four randomly selected locations on each lake; sampling locations remained fixed during the study. After collection, water samples were placed in 250-ml glass bottles, put on ice and preserved using 1:1 H_2SO_4 (10 drops per 250 ml sample). A depth of 1.5 m was selected for collecting water samples because it was representative of water depths from which fish were collected. Water samples were collected once during the week before herbicide applications occurred, on the day each application was completed, 24 h after each application was completed, at 2-d intervals until 7-d post-application, 7-d intervals until 42 d after application, and 10-d intervals until 62-d post-application. In non-treatment years, water samples were collected once in June and again in July to establish a base concentration within all lakes. Additional samples were collected below the thermocline from Manson Lake (6 m) and Silver Lake (5 m) to determine if 2, 4-D was present, as it was not

believed to disperse into the hypolimnion. All water samples were analyzed at the Water and

Environmental Analysis Laboratory at the University of Wisconsin-Stevens Point using high

performance liquid chromatography coupled with a triple-stage quadrupole mass spectrometer to

determine concentrations of 2, 4-D to the nearest 0.5 µg/L.

RESULTS

Water Quality, Temperature, and Productivity

Mean Secchi depth was lower in 2017 than previous years in all lakes, but this change

appeared greater in treatment lakes (Figure 3). Mixed-effects models results indicated no

significant interaction between lake type and year when comparing mean Secchi depth and

Secchi depth did not differ between treatment and reference lakes (Table 2; Figure 4). Mean

Secchi depth did differ among years and was lowest in 2017 and highest in 2015. Mean

chlorophyll-*a* levels were highest in all lakes in 2017, except for Kathan Lake, which had its

lowest mean chlorophyll-*a* levels in 2017 (Figure 5). Mixed-effects models results indicated no

significant interaction between lake type and year when comparing mean chlorophyll-*a* levels

and levels did not differ between treatment and reference lakes or among years (Table 2; Figure

6). Water temperatures within each lake were generally similar among years, except that

temperatures between mid-May and early June were lower in 2017 than in the previous two

years (Figure 7). Water temperatures in Manson Lake during mid-May to early June appeared

lower in 2015, but the temperature logger had slid from its original position into deeper water

where it had sunk into the sediment. The temperature logger was recovered on June 16th and

placed back in its original position. Additionally, in 2017 water temperatures were only recorded

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until June 18th in Brandy Lake because the memory of the temperature logger was filled and it was no longer recording.

Aquatic Plants

All lakes contained EWM in 2015, ranging from 1.7 to 12.9 percent of vegetative coverage (Table 3). In 2016, no EWM was sampled in any of the three lakes where herbicide was applied and the percent of vegetated area containing EWM increased or remained steady between 2015 and 2016 in reference lakes (Table 3). In 2017, the percent of EWM occurrence increased in two reference systems (i.e., Little Bearskin and Upper Gresham lakes), but decreased in Brandy Lake (Figures 8-10). In 2017, EWM was sampled in two lakes where herbicide was applied in 2016 (Kathan and Manson lakes), but was not observed on Silver Lake (Figures 11-13). While EWM was not detected on Silver Lake during point-intercept surveys, wandering surveys conducted in June 2017 did detect a small bed of EWM on the east side of the lake (E. Heath, Onterra LLC., personal communication).

Zooplankton

Taxa collected in my 768 zooplankton samples included *Daphnia* spp., calanoid copepods, cyclopoid copepods, copepod nauplii*, Bosmina* spp.*, Diaphanosoma* spp.*, Holopedium* spp.*, Leptidora kindtii, Chydorus* spp.*, Eurycercus* spp.*, and Ceriodaphnia* spp. Mean total zooplankton densities ranged from a minimum of 8.6 ± 4.5/L in Upper Gresham Lake to a maximum of 922.8 ± 366.1/L in Little Bearskin Lake. Within lakes, $\log_e$ transformed total zooplankton densities followed similar trends among years (Figure 14). Mixed-effects models comparing $\log_e$ total zooplankton density and Shannon's diversity index of zooplankton taxa indicated no significant interactions between lake type and year and the main effects of lake type and year were not significant (Table 2; Figures 15 and 16).

The mixed-effects model for $\log_e +1$ transformed density of *Daphnia* spp. indicated no significant interaction between lake type and year, densities did not differ between treatment and reference lakes, but did differ among years (Table 2; Figure 17). *Daphnia* spp. densities were significantly greater in 2017 than in 2015 and 2016. Despite the lack of statistical differences between treatment and reference lakes, trends in *Daphnia* spp. abundance within Kathan and Silver lakes appeared to be different in 2017 compared to the previous two years, while all other lakes followed similar trends among years (Figure 18). I detected a significant interaction between lake type and year when comparing *Daphnia* spp. body lengths (Table 2; Figure 19). *Daphnia* spp. body length was significantly different among all years in references lakes with smallest average body length observed in 2016 and largest average lengths observed in 2017. *Daphnia* spp. body length in treatment lakes during 2016 was significantly lower than body lengths in treatment lakes during 2015 and 2017.

Within lakes, $\log_e +1$ transformed calanoid copepod densities were similar among years, except for lower densities observed in Little Bearskin Lake during 2017 (Figure 20). I detected no significant interaction between lake type and year and the main effects of lake type and year were not significant when comparing $\log_e +1$ transformed calanoid copepod densities (Table 2; Figure 21). The mixed-effects model used to compare $\log_e$ transformed calanoid copepod body lengths indicated no interaction between lake type and year, $\log_e$ body lengths did not differ between treatment and reference lakes, but there was a significant year effect (Table 2; Figure 22). $\log_e$ transformed calanoid copepod body lengths were significantly greater in 2017 than in 2015 and 2016.

I detected no significant interaction between lake type and year when comparing cyclopoid copepod density, and densities did not differ between treatment and reference lakes or

among years (Table 2; Figure 23). Despite the lack of statistical differences, cyclopoid copepod density in Kathan and Manson lakes appeared lower in 2017 (year after herbicide treatment) when compared to 2015 and 2016, while all other lakes followed similar trends among years (Figure 24). Results from the mixed-effects model used to compare $\log_e$ transformed body lengths of cyclopoid copepods indicated no significant interaction between lake type and year, $\log_e$ body length did not differ between treatment and reference lakes, but there was a significant year effect (Table 2; Figure 25). All years were significantly different, with highest $\log_e$ body lengths observed in 2016 and lowest values observed in 2017.

I detected no significant interaction between lake type and year when comparing densities of copepod nauplii and *Bosmina* spp., and the main effects of lake type and year were not significant (Table 2; Figures 26 and 27). Despite the lack of statistical difference between treatment and reference lakes, *Bosmina* spp. trends were different in Kathan Lake during 2017, peaking during mid-summer when low abundances had been observed in the previous two years (Figure 28). Within lakes, copepod nauplii densities were similar among years (Figure 29).

Larval Fish

Quatrefoil light traps

Across all six lakes, 525 quatrefoil light trap nights were completed in the three years of sampling collecting a total of 9,882 larval fish. The most common taxa captured in light traps were cyprinids, Yellow Perch, Mottled Sculpin *Cottus bairdii,* Largemouth Bass and Bluegill. All of these common taxa within each lake were caught in every year. Light traps caught a greater diversity of taxa than ichthyoplankton tows. I detected no significant interaction between lake type and year when comparing mean values of Shannon's diversity index based on CPE in

quatrefoil light traps (i.e., fish/trap night; Table 2; Figure 30). There was no difference between treatment and reference lakes, and the effect of year was not significant.

Within-lakes, $\log_e$ transformed peak CPE of larval cyprinids appeared to be higher in treatment lakes during 2017 than in the previous two years, while the opposite trend was observed in reference lakes (Figure 31). Results of the mixed-effects model comparing $\log_e$ transformed peak CPE of larval cyprinids indicated a significant interaction between lake type and year (Table 2; Figure 32). $\log_e$ transformed peak larval cyprinid CPE was significantly lower in reference lakes during 2017 than in reference lakes during 2015 and 2016. There were no within-year differences in $\log_e$ transformed peak CPE of larval cyprinids among treatment and reference lakes.

Peak CPE of age-0 Largemouth Bass in light traps exhibited large variation within lakes and among years being lowest in all treatment lakes in 2017, but this was not true of any reference lake (Figure 33). A mixed-effects model indicated no significant interaction between lake type and year when comparing peak CPE of age-0 Largemouth Bass in light traps, there was no difference between treatment and reference lakes, and the effect of year was not significant (Table 2; Figure 34).

Ichthyoplankton tows

Over the 3 years of sampling, 816 ichthyoplankton tows were completed, capturing a total of 18,763 larval fish. The most abundant species captured in ichthyoplankton tows were Yellow Perch, Black Crappie, and Bluegill. A mixed-effects model indicated there was no significant interaction between lake type and year when comparing Shannon's diversity index calculated from CPEs in ichthyoplankton tows (fish/100 m^3). Shannon's diversity index did not differ between treatment and reference lakes, but the effect of year was significant (Table 2;

Figure 35). Shannon's diversity index was significantly lower in 2015 than in 2017, but the 2016 index value was not significantly different from values calculated for 2015 or 2017.

I detected no significant interaction between lake type and year when comparing $\log_e$ transformed peak CPE of larval Yellow Perch in ichthyoplankton nets, there was no difference in peak CPE between treatment and reference lakes, and the effect of year was not significant (Table 2; Figure 36). Despite the lack of statistical difference between treatment and reference lakes, peak CPE of larval Yellow Perch appeared to be lower in all treatment lakes in 2017 compared to the previous two years, but this trend was not observed in reference lakes (Figure 37). There was no significant difference in slopes of larval Yellow Perch TL in relation to day of year among all lake type and year combinations (Table 2; Figure 38).

Larval Black Crappie CPE was variable among lakes and years and relatively low on all lakes except Kathan Lake in 2015; Kathan Lake had similar Black Crappie CPE in all three years (Figure 39). Results from the mixed-effects model indicated a significant interaction between lake type and year when comparing $\log_e$ transformed peak CPE of larval Black Crappie in ichthyoplankton tows (Table 2; Figure 40). In reference lakes, Peak CPE of larval Black Crappie was significantly lower in 2015 than in 2016 and 2017. However, there were no within-year differences in $\log_e$ transformed peak CPE of Black Crappies between treatment and reference lakes.

Larval Bluegill CPE was variable among lakes and years (Figure 41). Larval Bluegill CPE from ichthyoplankton net tows did not have a significant lake type by year interaction and was not different among treatment and reference lakes, and the year effect was not significant (Table 2; Figure 42). There was no significant difference in slopes of larval Bluegill TL in relation to day of year among all lake type and year combinations (Table 2; Figure 43).

Peak hatch dates of larval Yellow Perch were slightly later in 2016 than 2015, but in both years, peak hatch date occurred well before 2016 calendar dates when the herbicide applications occurred (Figure 44 and 45). In addition, larval Yellow Perch were no longer being caught in ichthyoplankton tows or CPE was very low on herbicide application dates. Therefore, larval Yellow Perch were excluded from hatch date and daily growth assessment in 2017. Furthermore, daily growth rate analysis was not performed on data from 2015 and 2016 because peak abundance of larval Yellow Perch from these years occurred before herbicide treatments took place (i.e., both observations were pre-treatment based on calendar dates of treatments).

Catch rates of larval Black Crappie in ichthyoplankton tows were low in 2015 on all lakes except Kathan Lake, and hatch dates, daily growth rates, and diets were not examined for that year. Black Crappie peak hatch dates were later in 2017 than 2016, but aligned with herbicide application dates in both years, indicating eggs were incubating or larvae were present at the time of application (Figure 46 and 47). Mixed-effects model results indicated there was no significant interaction between lake type and year when comparing larval Black Crappie daily growth rates, growth rates did not differ between treatment and reference lakes, and the effect of year on growth rate was not significant (Table 2; Figure 48).

Diets and foraging success

Mean percent composition of diet items from larval Black Crappie captured in ichthyoplankton tows on the date peak CPE was observed was variable among lakes and years (Figure 49). Cladocerans were the most common diet item and included *Bosmina* spp.*, Chydorus* spp.*, Daphnia* spp.*, Diaphanasoma* spp. *and Holopediu*m spp. (Figure 49). The next most common taxa were copepod nauplii and cyclopoid copepods, followed by a low occurrence of

calanoid copepods. Larval Black Crappie foraging success (i.e., number of crustacean zooplankton per diet) was highest in 2017 in all lakes except Silver Lake (Figure 50). Results from Kruskal-Wallis and Wilcoxon rank sum tests indicated larval Black Crappie foraging success was not significantly different among lake type and year combinations (H_1=4.6187, $P =$ 0.2019) lake type ($Z_1 = -0.8807$, $P = 0.3973$) or year ($Z_1 = -1.3611$, $P = 0.2007$; Figure 51). Diets and foraging success were not calculated for larval Yellow Perch in 2017 for the same reasons that daily growth rate analysis was not performed.

Net pen trials

There was no significant difference in percent survival of juvenile Yellow Perch between reference (95.4% ± 2.6%) and treatment net pen trials (100%; $Z_1 = -0.9824$, $P = 0.34$; Figure 52). There was also no significant difference in percent survival of juvenile Bluegill between reference (96.5 ± 2.5%) and treatment trials (96.8 ± 1.9%; $Z_1 = 1.8701$, $P = 0.11$; Figure 53).

Seining

Catch-per-effort of juvenile Yellow Perch in August seines was variable among lakes and years, but was generally lower in 2017 than in the previous two years for both reference and treatment lakes (Figure 54). Mixed-effects model results indicated there was no interaction between lake type and year and the main effects of lake type and year were not significant when comparing Shannon's diversity index calculated from seine CPE (Table 2; Figure 55). There was not a significant interaction between lake type and year, and the main effects of lake type and year were not significant when comparing juvenile Yellow Perch seine CPE (Table 2; Figure 56). Catch-per-effort of juvenile Bluegill in seines was variable among years and lakes (Figure

57). There was not a significant interaction between lake type and year and the main effects of lake type or year were not significant when comparing Bluegill CPE in seines using a mixed-effects model (Table 2; Figure 58).

2, 4-D degradation rates

Herbicide applications took place between late May and early June 2016. Peak 2, 4-D concentrations did not reach the target concentration of 0.3 ppm on any treatment lake. Kathan Lake was treated on May 24, reaching a peak concentration of 0.234 ($\pm$ 0.036) ppm (Figure 59). Manson Lake received a treatment on June 2, reaching a peaking concentration of 0.257 ($\pm$ 0.052) ppm (Figure 60). Silver Lake was treated June 7, with a peak concentration of 0.152 ($\pm$ 0.083) ppm (Figure 61). Concentrations of 2, 4-D from samples collected in non-treatment years and reference lakes were below the limit of detection, except for one instance from June 30, 2017 on Brandy Lake with a 2, 4-D concentration of 0.0024 ($\pm$0.0001) ppm. Samples collected below the thermocline on Manson and Silver lakes had low concentrations, but the chemical was detectable (Table 4).

DISCUSSION

Mean whole-lake concentrations of 2, 4-D did not reach the target concentration of 0.3 ppm on any lake, but still resulted in control of EWM in all lakes, while the percent occurrence of EWM remained constant or increased in reference lakes during 2016. In 2017, EWM was not sampled in Silver Lake, but was sampled in Kathan and Manson lakes at 64% and 74% of their pre-treatment abundance, respectively. This quick recolonization of EWM in Kathan and Manson lakes, but not in Silver Lake, does not appear to be an effect of lower than target concentrations because Silver Lake had the lowest peak 2, 4-D concentration of all treatment

lakes. However, percent occurrence of EWM was relatively low in Kathan and Silver lakes in 2015 and herbicide treatments would typically be delayed until EWM occurrence was higher (E. Heath, Onterra LLC., personal communication). Failure to reach the target concentration could have made it more difficult to detect an effect of herbicide application on the metrics I measured. However, 2, 4-D degradation rate is dependent on lake stratification, water chemistry, water movement, temperature, substrate composition, microbial presence, and whether the lake has been treated before, making 2, 4-D trends variable among treatments (Parsons et al. 2001; Nault et al. 2012; Nault et al. 2017). Efficacy and selectivity of 2, 4-D is dependent on concentration and exposure time, with long term exposure (> 14 days) at concentration as low as 0.1 ppm providing long-term EWM control (Nault et al. 2017). Target concentrations of 0.3 ppm may not be needed if exposure to 2, 4-D is of sufficient duration.

While no analysis of the effects of 2, 4-D treatment on native plant species was conducted, previous studies have reported significant reductions in relative abundance of native plants following 2, 4-D treatments (Getsinger 1982; Nault et al. 2012; Nault et al. 2014; Nault et al. 2017). Conversely, other studies have reported EWM control with no significant effects on native plants (Hesel et al. 1996; Parsons et al. 2001; Kovalenko et al. 2010). Changes in macrophyte communities can subsequently affect higher trophic levels, as aquatic plant communities provide refuge and foraging habitat for zooplankton, juvenile fish and other aquatic organisms (Van Donk and Van De Bund 2002; Weber and Brown 2012). Loss of habitat could result in an indirect effect of 2, 4-D treatments and not necessarily a direct effect of the chemical.

Based on my results, 2, 4-D herbicide application at the concentrations measured did not have any immediate effects on the zooplankton metrics I measured. In general, zooplankton densities are highest in spring, and undergo a mid-summer decline usually attributed to age-0

fish predation (e.g., Luecke et al. 1990; Jeppesen et al. 1998). Most of my study lakes showed this trend for total zooplankton densities, which were similar within lakes and between years, but annual trends differed in some treatment lakes for individual taxa. For example, D*aphnia* spp. densities in herbicide treatment lakes during 2017 (year after treatment) peaked during the period when lowest densities were observed in the previous two years of sampling; this discrepancy was most apparent on Kathan and Silver lakes. Additionally, cyclopoid copepod densities in Kathan and Manson lakes were relatively low throughout the entire sampling period in 2017, while densities in Silver Lake and all three references lakes appeared similar to previous years. *Bosmina* spp. densities did not follow a typical seasonal trend in Kathan Lake, but did in all other treatment lakes. Whether or not these observed trends in individual treatment lakes were a result of the herbicide applications cannot be determined, and may have been the result of other factors.

Cooler water temperatures in spring of 2017 could have led to changes in zooplankton cycles. Zooplankton growth and reproduction are dependent on water temperature and are reduced during prolonged periods of cooler water (Persaud and Williamson 2005). Cooler water temperatures in spring could therefore result in decreased or delayed abundance of zooplankton species, and this timing could vary among lakes with different temperature regimes. Additionally, many zooplankton species consume phytoplankton, and changes in nutrient dynamics or phytoplankton abundance could influence zooplankton trends (Schoenberg and Carlson 1984). If aquatic plant biomass was lower in 2017 treatment lakes, this could lead to an increase in available nutrients and an increase in algal production, leading to the mid-June peak observed in phytoplankton grazers such as *Daphnia* spp. While mean Secchi disk values were lowest in treatment lakes in 2017, this was also true of all reference lakes. In addition, chlorophyll-*a* concentrations were highest in 2017 in all lakes, except Kathan Lake, and probably

a result of higher spring precipitation increasing nutrient inputs. Kathan Lake may have been more vulnerable to treatment effects because it is a shallow eutrophic lake that does not stratify. This would allow 2, 4-D to disperse across the entire water column and would not be hindered by the thermocline as in the other treatment lakes, making exposure to the chemical unavoidable. In Manson and Silver lakes 2, 4-D samples taken below the thermocline were detectable, but at very low concentrations.

Changes in plant communities by chemical or biological control have been shown to alter zooplankton populations (Richard et al. 1985; Jeppesen et al. 1998). Hence, reductions in plant biomass or preferred macrophytes may have changed zooplankton refuge during early sampling periods (May) in study lakes. This could in turn lead to increased zooplankton predation in some treatment lakes and explain the inverse trend to the normal spring or early summer zooplankton peak observed, but not the mid-summer peak. Macrophytes provide refuge for algal grazing zooplankton and reduce predation by zooplanktivores (Scheffer et al. 1993). *Bosmina* spp. are primarily found in open water habitats, while cyclopoid copepods tend to associate with dense vegetation and *Daphnia* spp. can be found in both areas (Jeppesen et al. 1998). Therefore, decreased aquatic vegetation due to herbicide treatments would be a plausible explanation for observed declines in *Daphnia* spp. and cyclopoid copepods early in the open-water season. In future studies, zooplankton sampling should continue beyond the year after herbicide application to assess annual variation in zooplankton trends and to assess zooplankton-macrophyte interactions.

In a laboratory setting, 2, 4-D herbicides significantly reduced larval Fathead Minnow survival by approximately 15% at relevant concentrations (Dequattro and Karasov 2015). However, CPE of larval cyprinids in quatrefoil light traps did not differ between treatment and

reference lakes. Dequattro and Karasov (2015) used flow through systems at constant 2, 4-D concentrations for 30 d, which does not replicate the natural degradation of 2, 4-D in whole-lake treatments. Additionally, while a 15% reduction in larval fish survival would be detectable in a controlled environment such as a laboratory experiments, it would be difficult to detect in a lake setting with large natural variation in CPE and higher natural mortality rates.

Cyprinids were only identified to family because of difficulty in identification, but this could have confounded the date of peak larval abundance, with different species hatching at different times. In addition, if some species of larval cyprinids were more tolerant to the treatments, while others were affected, it could reduce competition, increasing survival of the tolerant species masking any treatment effect. Identification of cyprinids to species may have been a better approach, but larval cyprinids guides are generally limited to genus because of difficulty in identification. Paul et al. (2006) indicated that Fathead Minnows had higher 96-hr LC50s for AquaKleen® than Brook Trout or Walleye, suggesting that Fathead Minnows may be less vulnerable to 2, 4-D than other native species.

Previous studies have not reported significant effects of 2, 4-D on centrarchids at concentrations relevant to whole-lake treatments. Age-0 Largemouth Bass CPE from quatrefoil light traps was not significantly different between treatment and reference lakes. A previous field study in Michigan lakes also observed no effects of habitat loss on age-0 Largemouth Bass abundance, size or diet following whole-lake treatments of EWM with fluridone (Valley and Bremigan 2002). Peak abundance of larval Bluegill was also not significantly different by treatment or year. Bluegill spawn several times throughout the summer, so peak abundance may not have been effectively captured by sampling methods of this study. Alternatively, concentrations of 2, 4-D would have been low and declining during Bluegill spawning and when

larvae were present. Hiltibran (1967) did not observe reduced survival of Bluegill fry exposed to a DMA salt formulation of 2, 4-D at concentrations higher than were present in this study. Additionally, Cope (1970) did observe significant mortality in juvenile (80-110 mm) Bluegills exposed to high concentrations (10 ppm) of 2, 4-D. However, Cope (1970) used a propylene glycol butyl ester version of 2, 4-D, which would not be directly comparable to the DMA® 4IVM formulation. Black Crappie larval abundance was not different by year or treatment, and no significant effects were observed on average daily growth rates, foraging success or diets of larval Black Crappie between 2016 and 2017.

Despite lack of statistical differences, Yellow Perch peak larval abundances did appear lower in treatment lakes during 2017 compared to previous years. This could be due to a variety of factors such as reduced fecundity, increased predation because of lack of refuge (i.e., macrophytes), change in forage base or abiotic differences between years. Genotoxic effects and DNA damage have been recorded on a neotropical fish species exposed to 2, 4-D, but at concentrations greater than observed during whole-lake treatments (Ruiz de Arcaute et al. 2016). There is also evidence to suggest the DMA® 4 IVM formulation of 2, 4-D may be an endocrine disruptor in Fathead Minnows (DeQuattro and Karasov 2015). The DMA® 4 IVM formulation of 2, 4-D could also act as an endocrine disruptor in Yellow Perch, resulting in reduced fecundity after treatment leading to reduced recruitment. Future laboratory studies should aim to determine if 2, 4-D concentrations used in whole-lake treatments can act as endocrine disruptors in native fishes such as Yellow Perch.

Additionally, Yellow Perch spawn in the littoral zone, laying a ribbon of eggs over rocky substrate or structure such as aquatic vegetation (Weber and Les 1982; Weber et al. 2011). If aquatic vegetation was limited in treatment lakes in 2017, potential spawning habitat may have

been reduced for Yellow Perch in the spring of 2017, which could lead to a decline in larval

production. Yellow Perch also use vegetation as nursery areas, especially as pre-larvae, and

Yellow Perch abundance has been reported to increase in a reservoir as EWM increased (Dibble

et al. 1997). Loss of EWM biomass or potentially other native macrophytes by 2, 4-D

applications in the post-treatment year may reduce refuge for age-0 Yellow Perch leading to

increased predation and ultimately decreases in relative abundance observed in treatment lakes.

Whole-lake treatments occur when EWM and other early emerging aquatic plants begin growth,

but most aquatic plants are still dormant. These macrophyte species would have already been

growing when Yellow Perch were spawning and larvae were present in the year of the 2, 4-D

treatment, but plants may have been affected enough that they were not present when Yellow

Perch were spawning or larval in the year post-treatment. If early emerging aquatic plant species

were affected, but recolonization by later emerging plants occurred, this would explain why peak

abundance of larval Yellow Perch appeared lower, but this was not observed for other species,

which spawn later. Sampling aquatic plants in both spring and late summer may have helped in

determining if spawning habitat or refuge was limited during this time. In future studies, diets of

larval Yellow Perch could also be examined in the year after herbicide applications to determine

if changes in aquatic plant communities are changing larval Yellow Perch foraging success, and

in turn leading to declines in Yellow Perch recruitment.

The overall species diversity of larval and juvenile fishes sampled from quatrefoil light

traps, ichthyoplankton tows, and seining did not appear to be significantly affected by 2, 4-D

treatments. Similar to previous research, no direct mortality associated with the chemical was

observed in juvenile fish during net pen trials (Paul et al. 2006; Kovalenko et al. 2010; Webb et

al. 2016). While other studies have reported fish avoidance of 2, 4-D herbicides, avoidance was

not probable in this study (Tierney 2016). Larval fish captured in quatrefoil light traps and ichthyoplankton tows have limited locomotion, and juvenile Yellow Perch and Bluegill used in net pen trials would not be able to avoid exposure. Conversely, juvenile fish sampled during shoreline seining would have been able to avoid 2, 4-D by taking refuge below the thermocline in Manson and Silver lakes, but this is not likely as it would be metabolically stressful and these fish were capture in the littoral zone, indicating that they were exposed to the chemical. In addition, 2, 4-D was detected below the thermocline, but at low concentrations. However, LC50 concentrations from a reregistration study by the United States Environmental Protection Agency on 2, 4-D were much greater than observed in lake concentrations during this study. This laboratory study reported LC50 values ranging from 80 to 2244 ppm for amine and salt formulations depending on fish species (DeQuattro and Karasov 2015). The concentration of 2, 4-D in lake settings, therefore, was not likely at high enough concentration to result in the direct mortality of juvenile fish.

While my study currently represents the most extensive assessment regarding the effects of whole-lake 2, 4-D treatments on fish and zooplankton, the ability to detect population level effects of the herbicide treatments on fish and zooplankton populations was complicated by the inherent natural variation in many of the metrics I measured. Extensive spatial and temporal variation could mask possible effects of the treatments, if these effects are subtle. In addition, while sampling was extensive, a sample size of three treatment lakes may not offer sufficient power to detect differences, if they exist. Consequently, failure to detect differences in metrics does not necessarily mean there are no effects on fish and zooplankton when applying 2, 4-D herbicides, but that effects were not detectable. In addition, any direct effects of herbicide application may only be relevant to the DMA$^{®}$ 4IVM formulation of 2, 4-D because registration

of 2, 4-D compounds is only for the active ingredient and not inert ingredients. In the case of the DMA® 4IVM formulation 2, 4-D makes up 46.3% of the ingredients, while remaining ingredients are not reported. DeQuattro and Karasov (2015) reported varying effects of two different 2, 4-D formulations, which may be due to varying effects of these inert ingredients.

In summary, whole-lake 2, 4-D treatments did not lead to detectable effects on zooplankton and larval or juvenile fishes. While zooplankton trends were different in some treatment lakes in the year after herbicide application, and larval Yellow Perch relative abundance appeared to be lower in treatment lakes during 2017, it seems unlikely that the actual herbicide treatment directly affected these metrics. These delayed trends may represent an indirect result of the herbicide application caused by loss of aquatic vegetation. Lack of field-based studies limits the comparability of my research with previous work, but the lack of previous research highlights the importance of my study. Both laboratory- and field-based studies should continue to determine the effects of 2, 4-D herbicides and indirect effects of aquatic plant loss on biotic communities. Additionally, my assessment did not address the effects of repeated herbicide treatments on the same lake over time. This remains an important question because following herbicide treatments, EWM coverage in some lakes may returns to levels where there is public interest in subsequent treatments.

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Table 1. Location (county), surface area (acres), trophic status, hydrologic lake type and treatment type for the six lakes used to determine the effects of 2, 4-D herbicide treatments on aquatic communities in northern Wisconsin.

Lake Name	County	Acres	Trophic Status	Hydrologic Lake Type	Treatment Type
Brandy	Vilas	113	Mesotrophic	Drainage	Reference
Kathan	Oneida	214	Eutrophic	Drainage	Whole-lake (2, 4-D)
Little Bearskin	Oneida	184	Mesotrophic	Drainage	Reference
Manson	Oneida	236	Mesotrophic	Drainage	Whole-lake (2, 4-D)
Silver	Vilas	57	Mesotrophic	Seepage	Whole-lake (2, 4-D)
Upper Gresham	Vilas	362	Mesotrophic	Drainage	Reference

Table 2. Statistical output for analyses of covariance and mixed-effects models used for comparisons of selected metrics including *F*-statistics, degrees of freedom (*df*), and *P* values for the main effects of lake type (reference vs. treatment) and interaction terms. Statistically significant effects ($P < 0.05$) are noted in bold.

Metric	df	F	P
Mean Secchi Depth			
Lake Type	1, 164	0.64	0.4244
Year	**2, 8**	**24.17**	**0.0004**
Lake Type*Year	2, 164	1.26	0.2870
Mean Chlorophyll-*a* Concentration			
Lake Type	1, 149	0.01	0.9095
Year	2, 8	0.94	0.4285
Lake Type*Year	2, 149	0.97	0.3800
Zooplankton Diversity			
Lake Type	1, 4	0.87	0.4043
Year	2, 8	2.07	0.1884
Lake Type*Year	2, 8	3.88	0.0662
Log$_e$ (Total Zooplankton Density)			
Lake Type	1, 162	0.01	0.9102
Year	2, 8	1.07	0.3864
Lake Type*Year	2, 162	2.58	0.0787
Log$_e$(*Daphnia* spp. Density +1)			
Lake Type	1, 162	3.61	0.0592
Year	**2, 8**	**16.94**	**0.0013**
Lake Type*Year	2, 162	2.89	0.0586
Daphnia spp. Body Length			
Lake Type	1, 170	0.16	0.6904
Year	**2, 8**	**14.97**	**0.0020**
Lake Type*Year	**2, 170**	**3.07**	**0.0489**
Log$_e$(Calanoid Density +1)			
Lake Type	1, 162	0.28	0.5990
Year	2, 8	0.45	0.6542
Lake Type*Year	2, 162	1.40	0.2503
Log$_e$(Calanoid Body Length)			
Lake Type	1, 172	0.91	0.3413
Year	**2, 8**	**23.67**	**0.0004**
Lake Type*Year	2, 172	1.76	0.1756
Cyclopoid Density			
Lake Type	1, 162	0.03	0.8736
Year	2, 8	1.06	0.3895
Lake Type*Year	2, 162	1.87	0.1573
Log$_e$(Cyclopoid Body Length)			
Lake Type	1, 174	2.44	0.1199
Year	**2, 8**	**14.68**	**0.0021**
Lake Type*Year	1, 174	0.09	0.9152
Bosmina spp. Density			
Lake Type	1, 162	0.07	0.7892
Year	2, 8	1.10	0.3789
Lake Type*Year	2, 162	1.42	0.2448
Nauplii Density			
Lake Type	1, 162	0.04	0.8503
Year	2, 8	3.67	0.0739
Lake Type*Year	2, 162	2.66	0.0727

Metric	df	F	P
Shannon Index Quatrefoil Light Traps			
Lake Type	1, 4	0.01	0.9240
Year	2, 8	1.63	0.2552
Lake Type*Year	2, 8	0.63	0.5562
Log$_e$(Larval Cyprinid CPE)			
Lake Type	1, 4	1.02	0.3694
Year	2, 8	4.32	0.0535
Lake Type*Year	**2, 8**	**8.78**	**0.0096**
Age-0 Largemouth Bass CPE			
Lake Type	1, 4	0.66	0.4618
Year	2, 8	0.13	0.8807
Lake Type*Year	2, 8	0.83	0.4720
Shannon Index Larval Tows			
Lake Type	1, 4	0.14	0.7307
Year	**2, 8**	**6.80**	**0.0188**
Lake Type*Year	2, 8	3.52	0.0801
Log$_e$(Larval Yellow Perch CPE)			
Lake Type	1, 4	0.62	0.4765
Year	2, 8	3.54	0.0792
Lake Type*Year	2, 8	1.06	0.3899
Larval Yellow Perch Total Length			
Lake Type	5	0.65	0.6627
Day of the Year	**1**	**12.13**	**0.0023**
Lake Type*Day of the Year	5	0.63	0.6793
Log$_e$(Larval Black Crappie CPE)			
Lake Type	1, 4	0.02	0.8990
Year	**2, 8**	**20.75**	**0.0007**
Lake Type*Year	**2, 8**	**7.16**	**0.0165**
Larval Bluegill CPE			
Lake Type	1, 4	0.76	0.4336
Year	2, 8	0.87	0.4556
Lake Type*Year	2, 8	0.50	0.6259
Larval Bluegill Total Length			
Lake Type	5	0.33	0.8901
Day of the Year	**1**	**26.47**	**<0.0001**
Lake Type*Day of the Year	5	0.35	0.8801
Larval Black Crappie Daily Growth Rate			
Lake Type	1, 4	0.11	0.7554
Year	1, 4	0.02	0.8928
Lake Type*Year	1, 4	1.77	0.2540
Shannon Index Seining			
Lake Type	1, 4	0.07	0.8096
Year	2, 8	0.92	0.4356
Lake Type*Year	2, 8	0.63	0.5562
Yellow Perch (< 70 mm) Seining CPE			
Lake Type	1, 4	0.45	0.5371
Year	2, 8	2.29	0.1633
Type*Year	2, 8	0.40	0.6846
Bluegill (< 100 mm) Seining CPE			
Lake Type	1, 4	0.08	0.7912
Year	2, 8	1.01	0.4060
Lake Type*Year	2, 8	1.68	0.2466

Table 3. Percent of vegetated area that contained Eurasian Watermilfoil during point-intercept surveys conducted between late July and early August of each year. Surveys took place within the same two-week period each year.

Lake Type	Lake	2015	2016	2017
Reference	Brandy	5.1	15.8	8.6
	Little Bearskin	4.9	4.1	9.9
	Upper Gresham	1.7	2.3	4.0
Treatment	Kathan	6.3	0	4
	Manson	12.8	0	9.4
	Silver	3.4	0	0

Table 4. Concentration of 2, 4-D (ppm) sampled below the thermocline up to 7 d after herbicide treatment (DAT) on Silver and Manson Lakes. Samples were collected at a depth of 5 m on Silver Lake and a depth of 6 m on Manson Lake.

Lake	DAT	Concentration (ppm)
Manson	0	0.008
	1	0.004
	3	0.007
	5	0.012
	7	0.000
Silver	0	0.002
	1	0.015
	3	0.019
	5	0.039
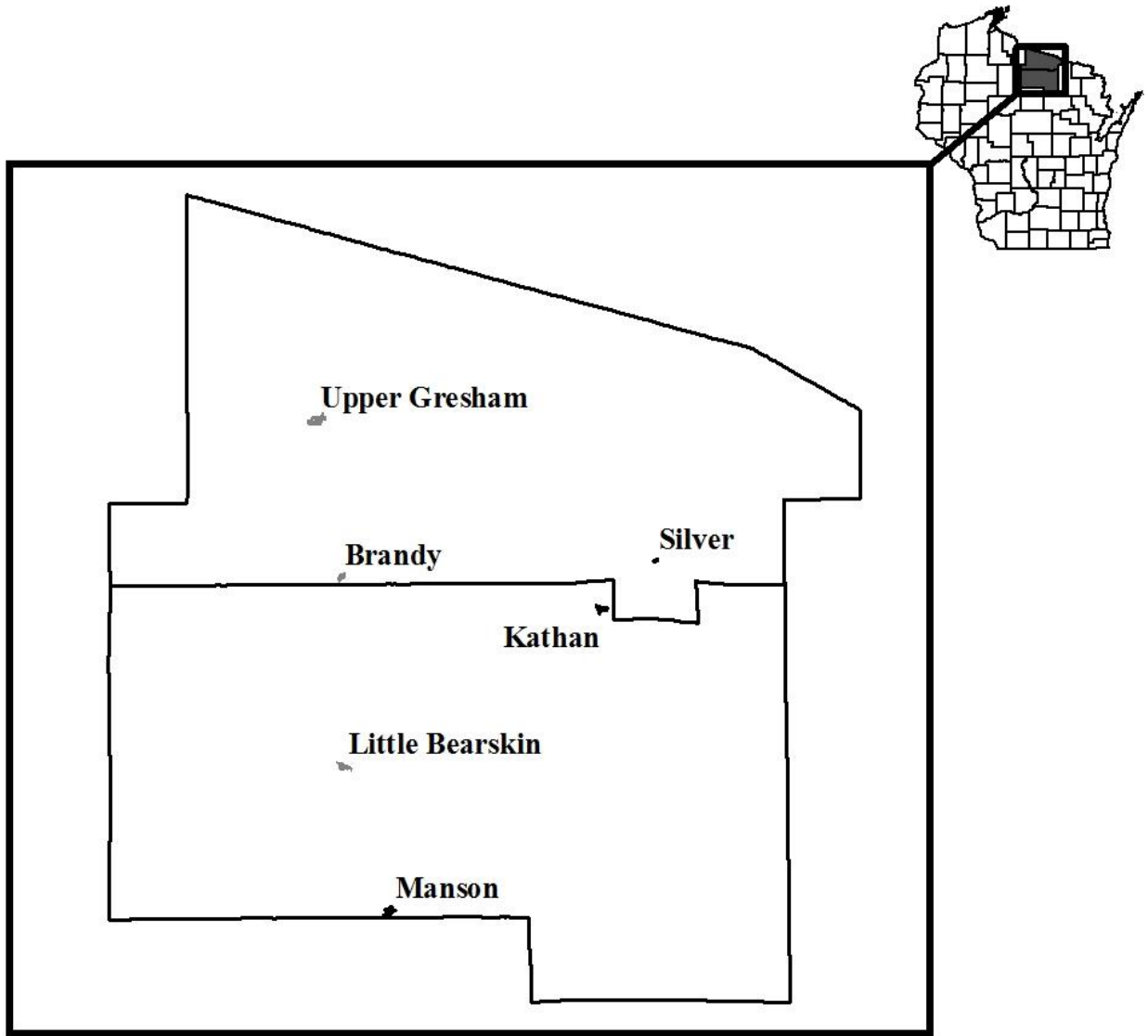
Figure 1. Location of study lakes in Vilas and Oneida counties, Wisconsin. Treatment lakes (Kathan, Manson and Silver) are black; reference lakes (Brandy, Little Bearskin and Upper Gresham) are gray.

Fullness Rating	Coverage	Description
1		Only few plants. There are not enough plants to entirely cover the length of the rake head in a single layer.
2		There are enough plants to cover the length of the rake head in a single layer, but not enough to fully cover the tines.
3		The rake is completely covered and tines are not visible.

Figure 2. Illustration of rake fullness categories used in Wisconsin Department of Natural Resources plant surveys (Hauxwell et al. 2010). At sites less than 4.6 m deep double headed rake on a telescoping pole was lowered to the bottom and two complete rotations were made. At sample sites greater than 4.6 m deep, a 2.27-kg weighted rake attached to a rope was deployed and dragged along the lake bottom for approximately 0.3 m and then pulled to the surface. All plant species were identified and given a rake fullness rating pictured above.
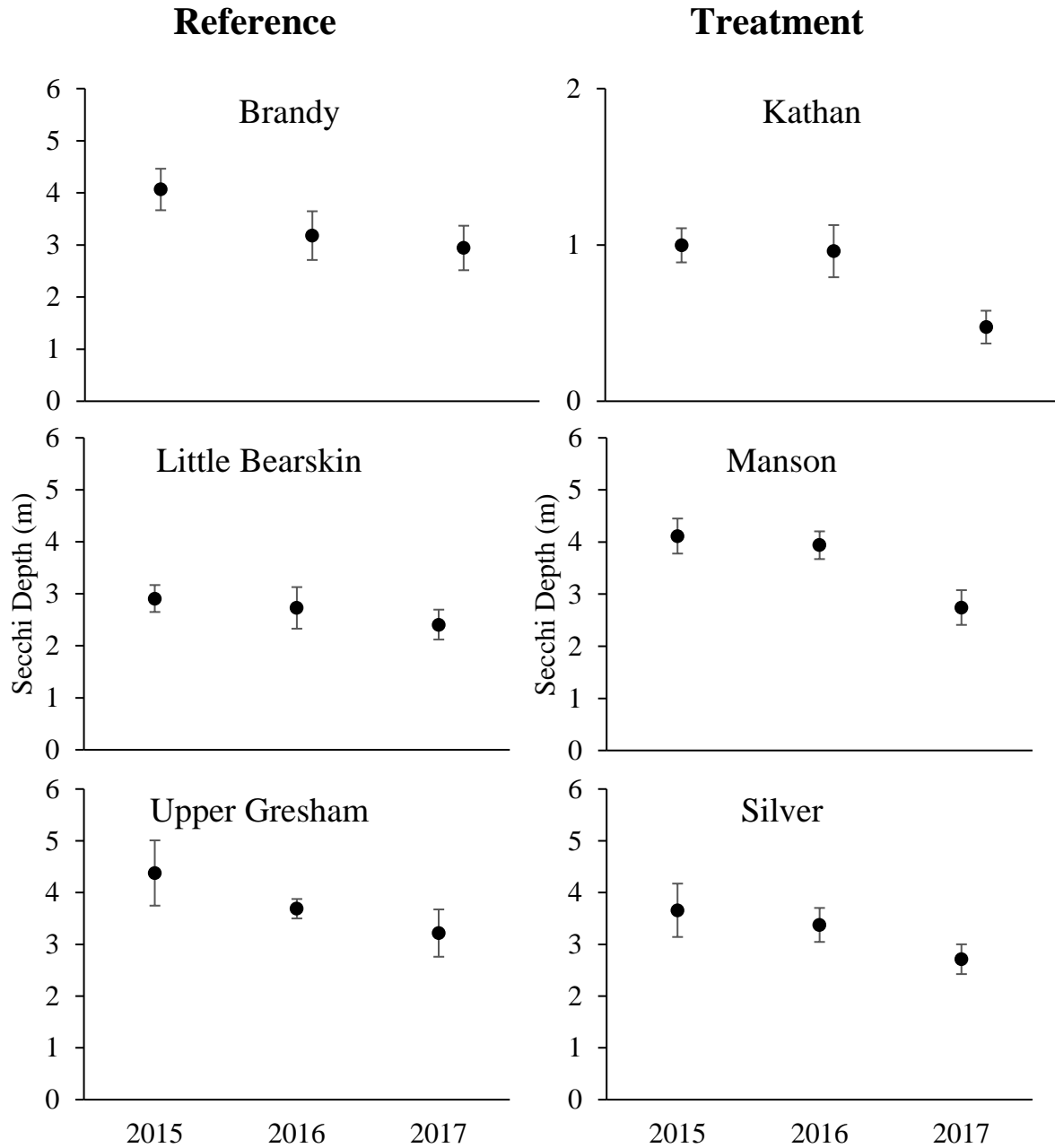
Figure 3. Mean Secchi depth (m) from May through August for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals. Range of *y*-axis varies among lakes because of relatively low Secchi depths on Kathan Lake.
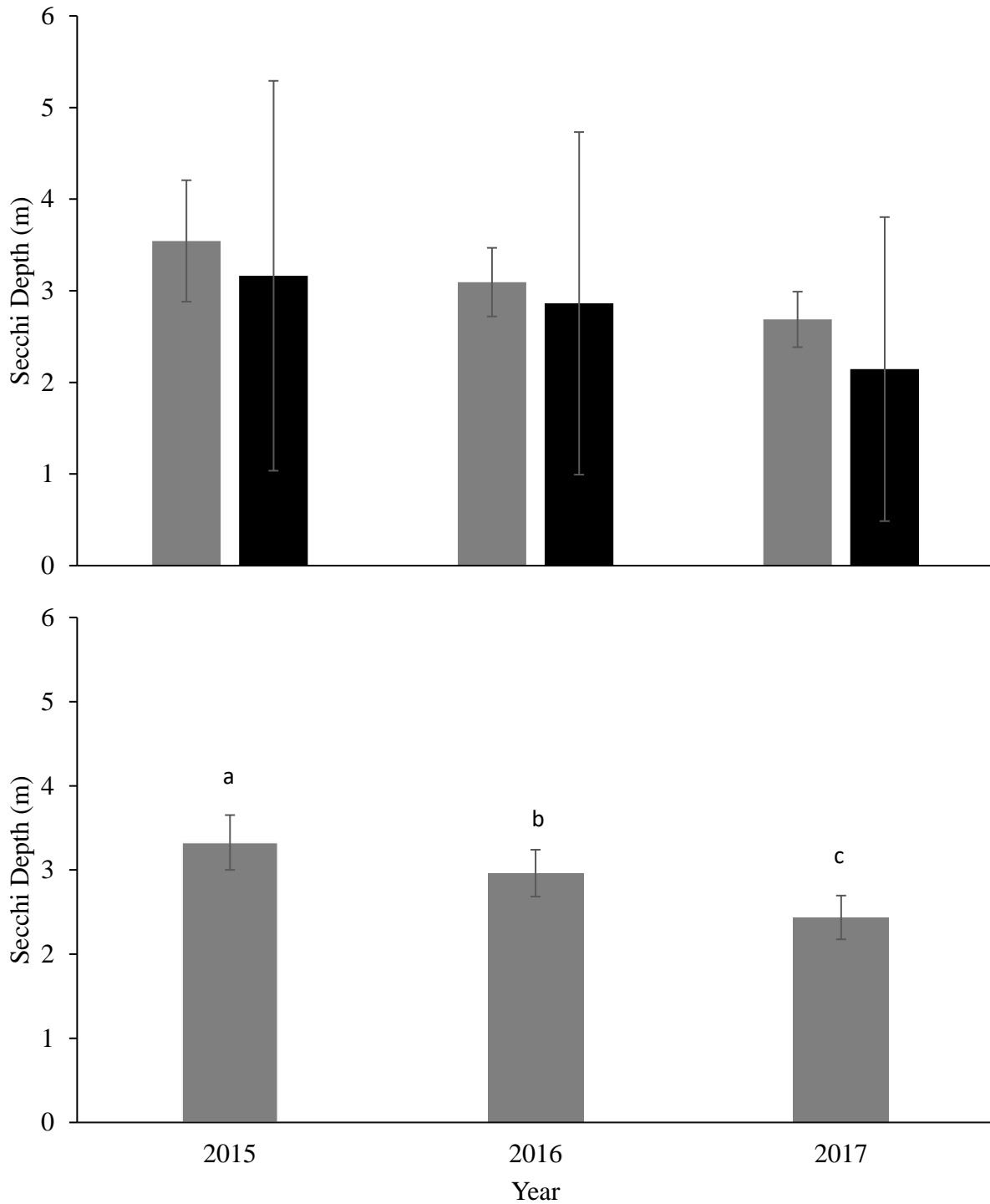
Figure 4. Mean Secchi depth (m) from May through August each year for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017 (top panel) and mean Secchi depth for all lakes during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017; bottom panel). Error bars are 95% confidence intervals. Columns denoted with different letters were significantly different ($P < 0.05$; Table 2).
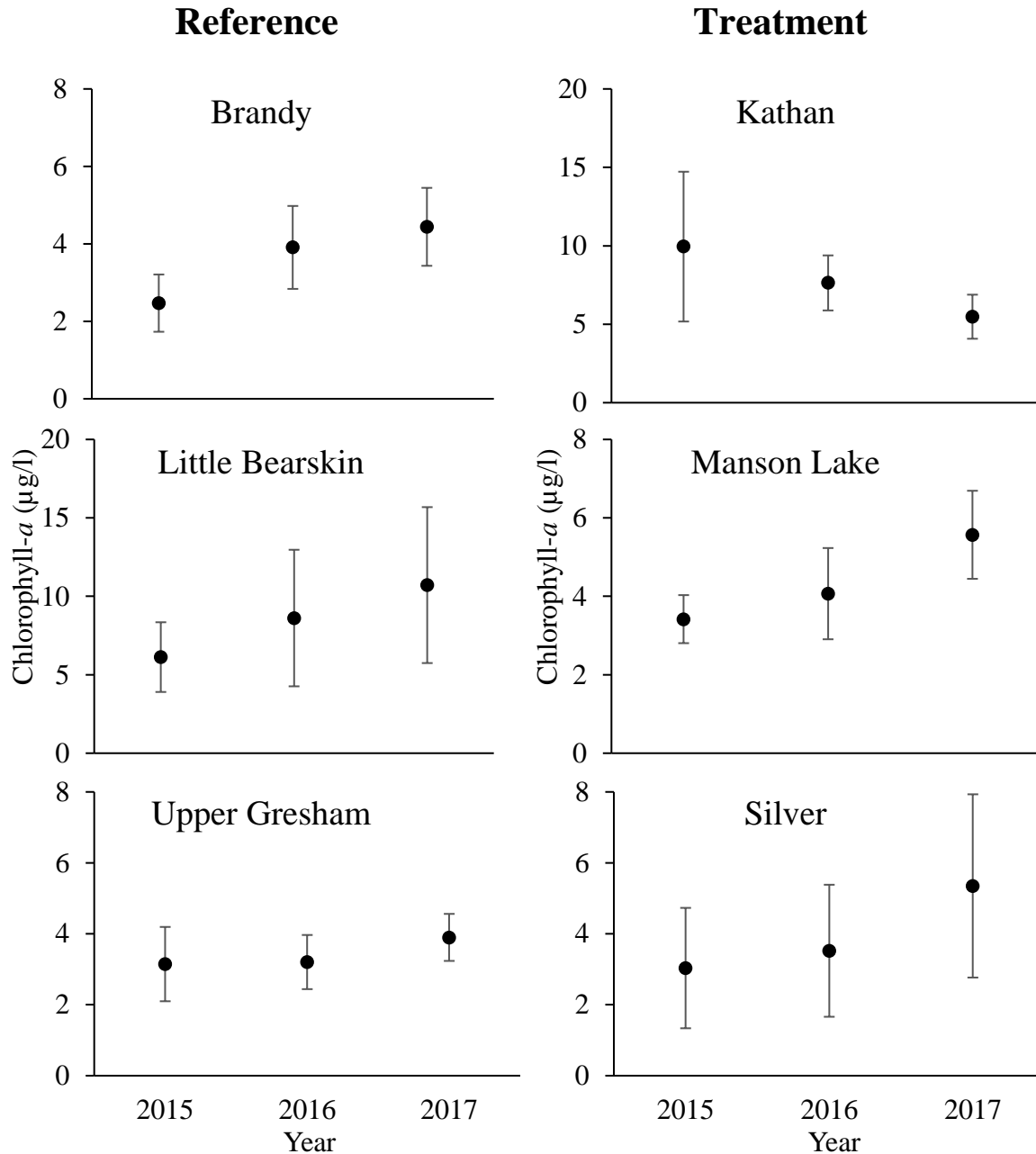
Figure 5. Mean chlorophyll-*a* concentrations (µg/l) from reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Samples were collected using an intergrated tube sampler and combined on each sampling date for a composite sample. Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in chlorophyll-*a* levels among lakes.
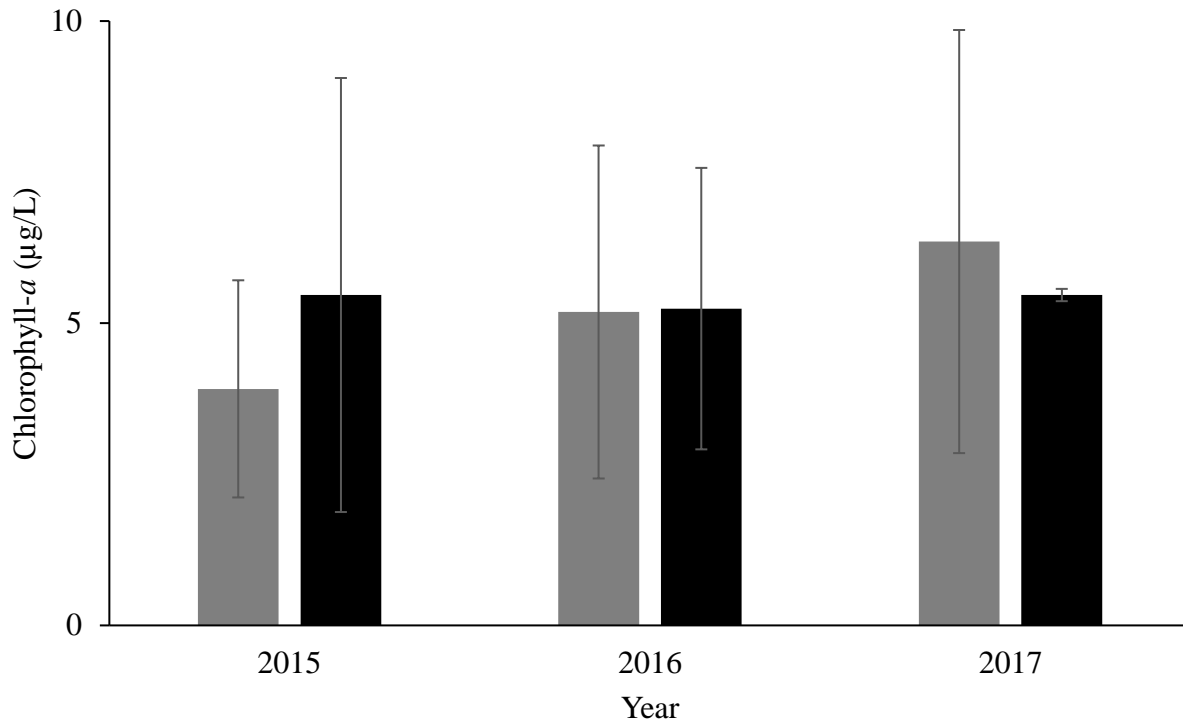
Figure 6. Mean chlorophyll-*a* concentrations (µg/l) from May through August for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Sampling took place at approximately 7- to 14-d intervals each year. Error bars are 95% confidence intervals.
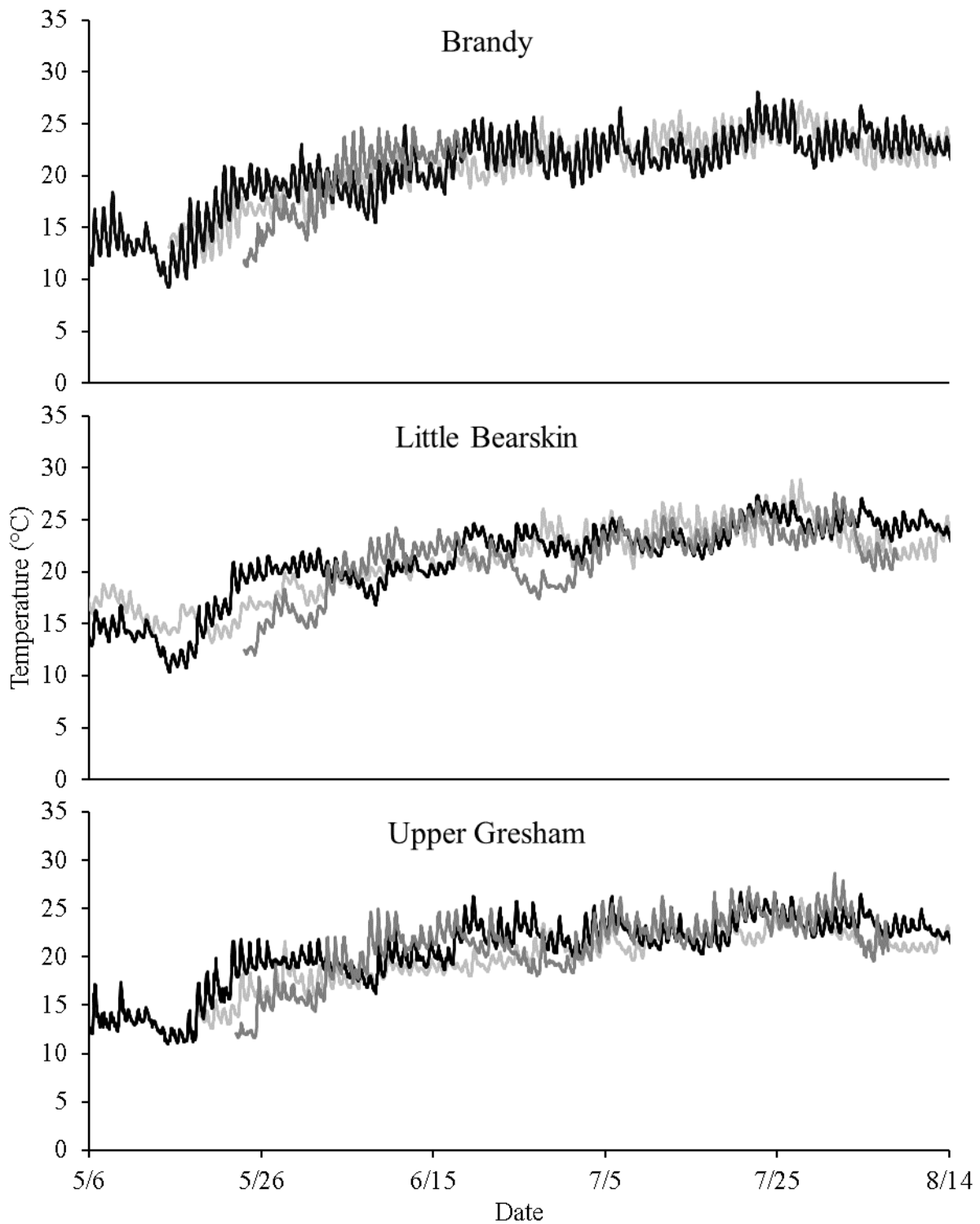
Figure 7. Hourly water temperatures (°C) collected from May to August in each lake using an Onset® Hobo Pro V2 or Tidbit V2 temperature logger for 2015 (light gray), 2016 (black) and 2017 (dark gray). Temperature loggers were attached to a cinder block and deployed at a depth of less than 1 m.
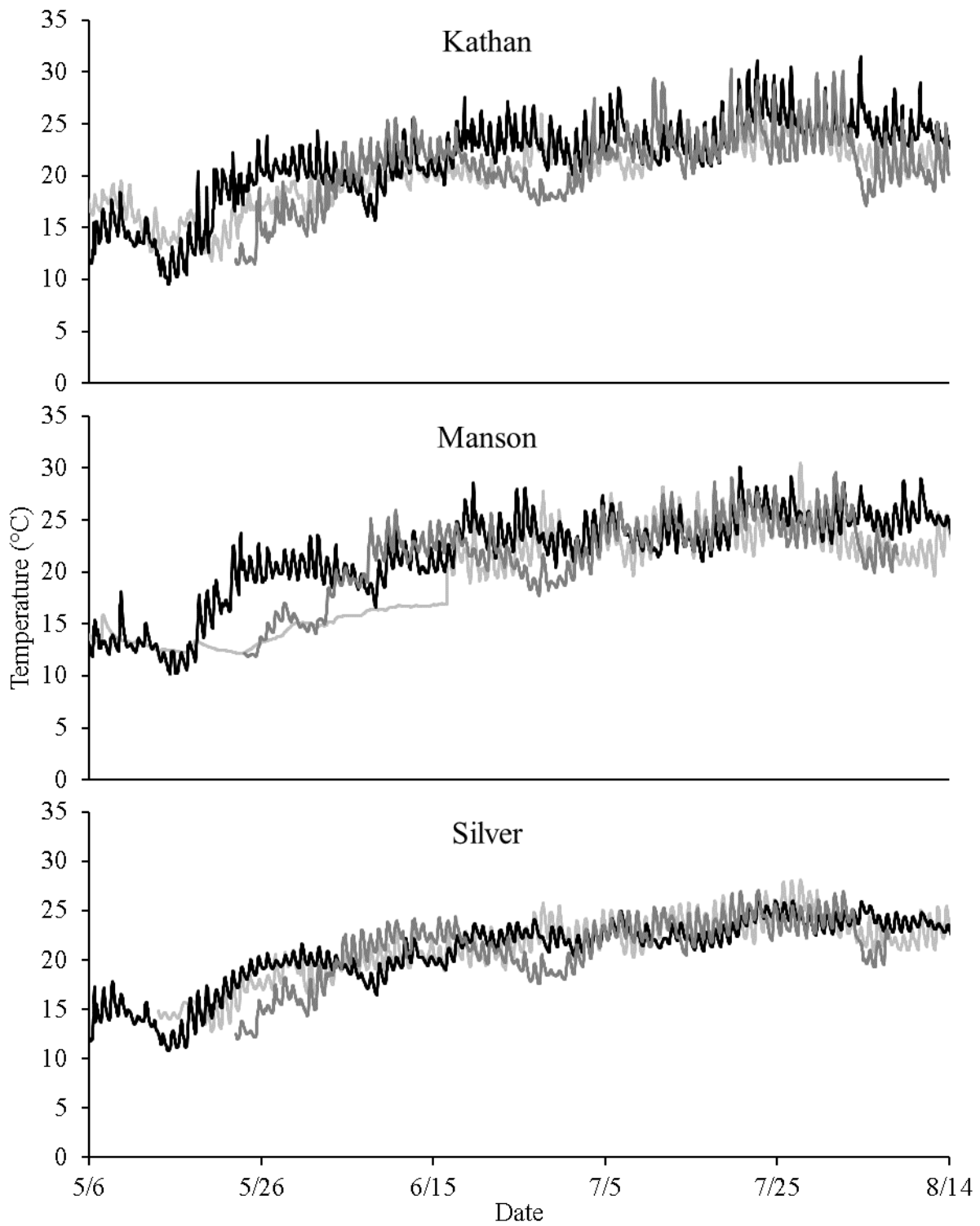
Figure 7. (continued).
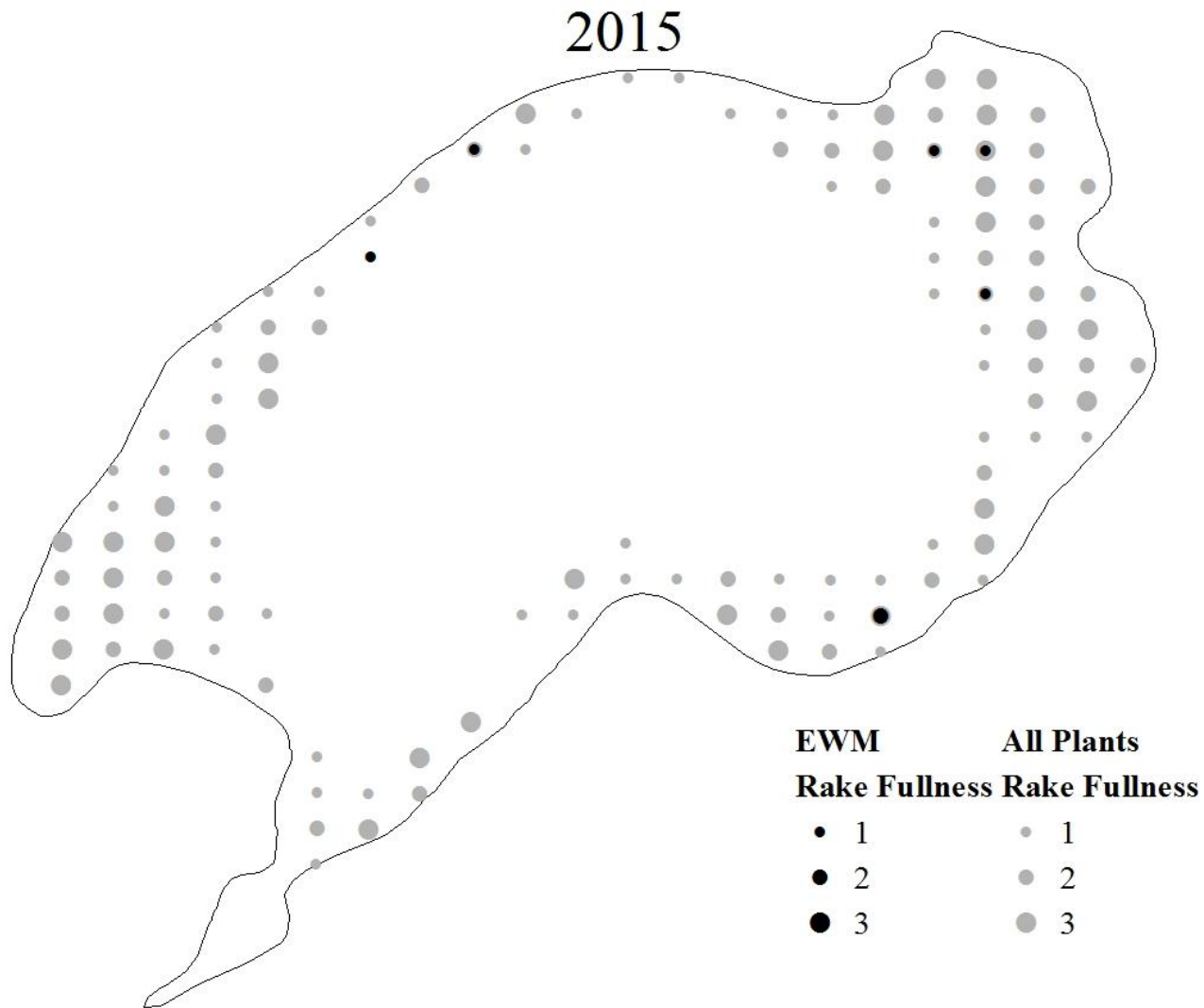
Figure 8. Distribution and rake fullness ratings for Eurasian Watermilfoil (EWM) and all aquatic plants in Brandy Lake (reference lake), Vilas County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.

2016

EWM
Rake Fullness

- 1
- 2
- 3

All Plants
Rake Fullness

- 1
- 2
- 3

2017

EWM
Rake Fullness

- 1
- 2
- 3

All Plants
Rake Fullness

- 1
- 2
- 3
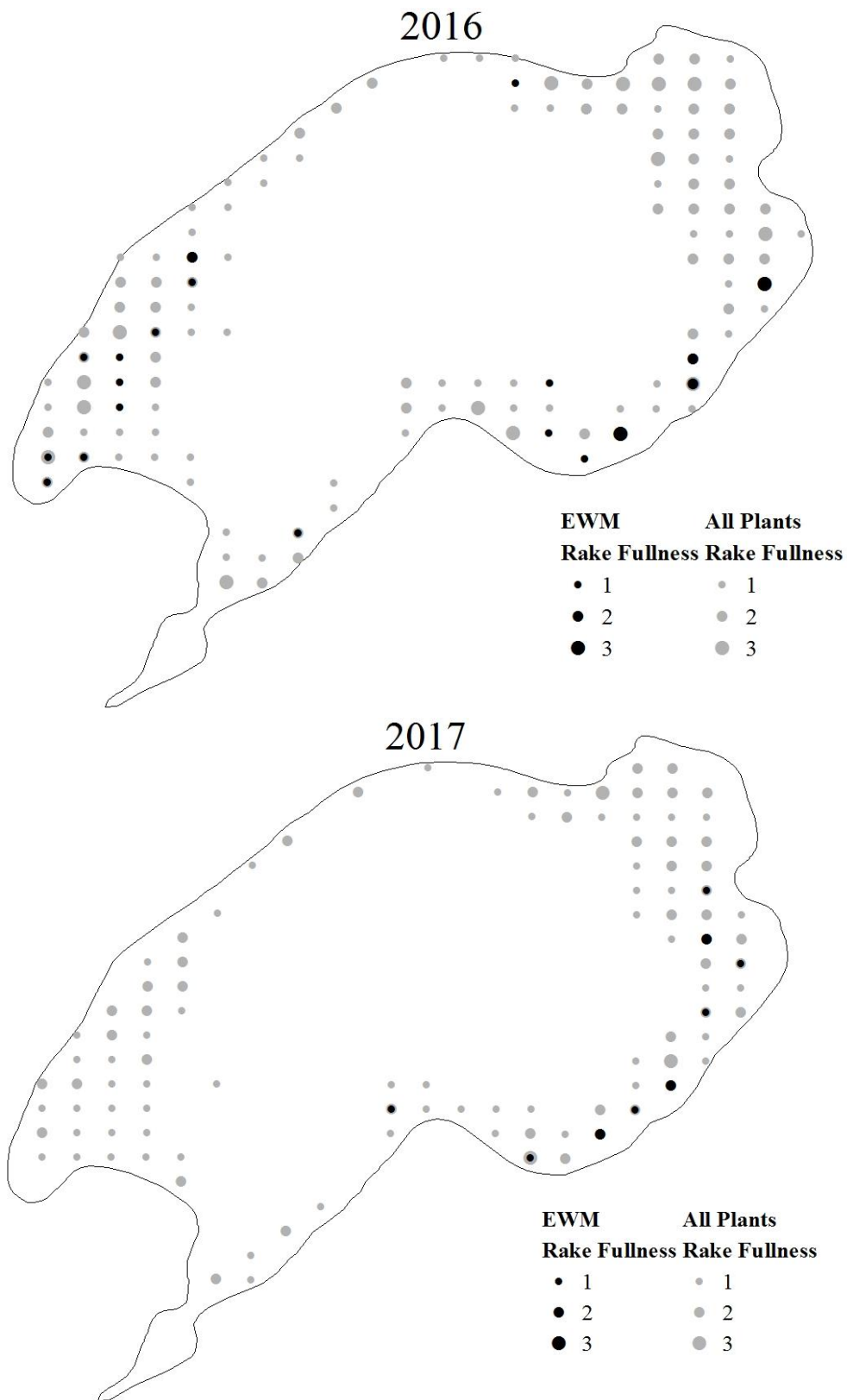
Figure 8. (continued).
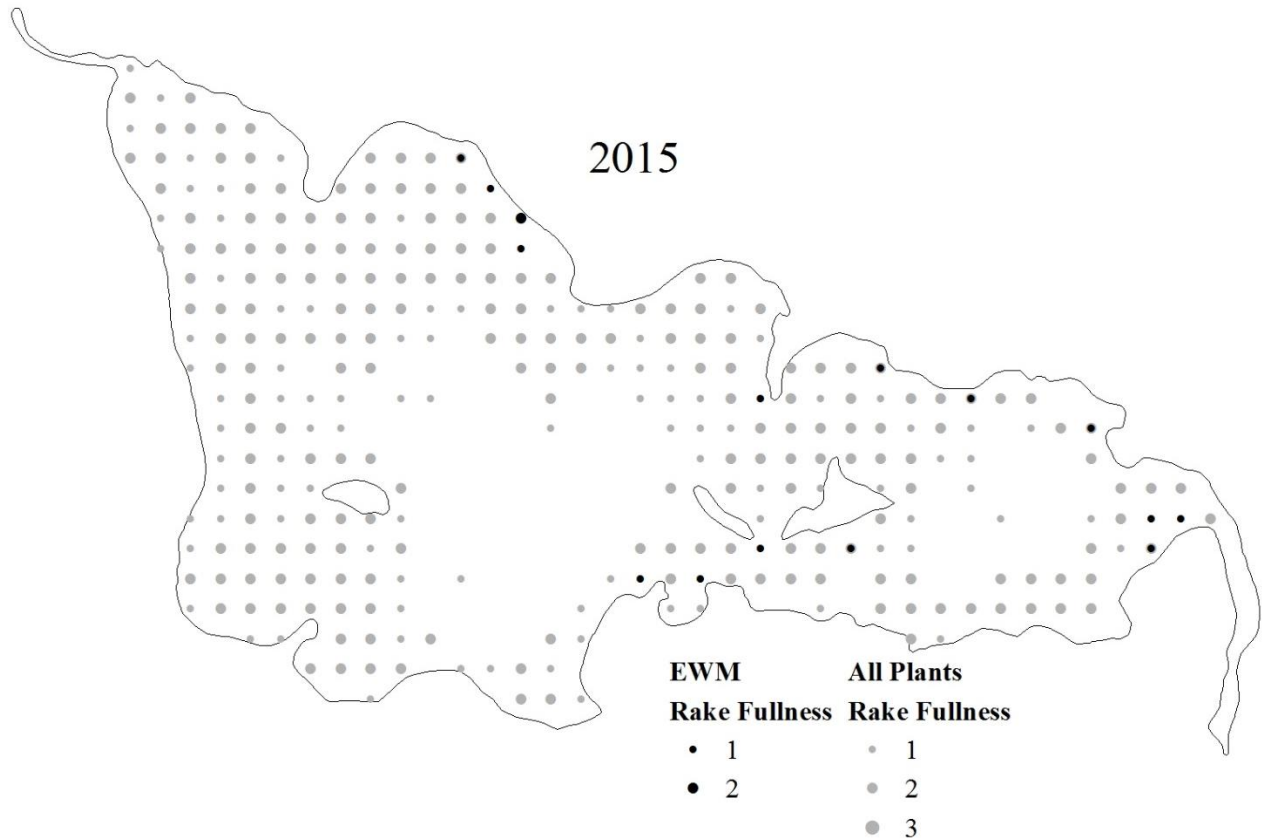
Figure 9. Distribution and rake fullness rating for Eurasian Watermilfoil (EWM) and all aquatic plants in Little Bearskin Lake (reference lake), Oneida County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.

2016

EWM
Rake Fullness

- 1
- 2

All Plants
Rake Fullness

- 1
- 2
- 3

2017

EWM
Rake Fullness

- 1
- 2

All Plants
Rake Fullness

- 1
- 2
- 3
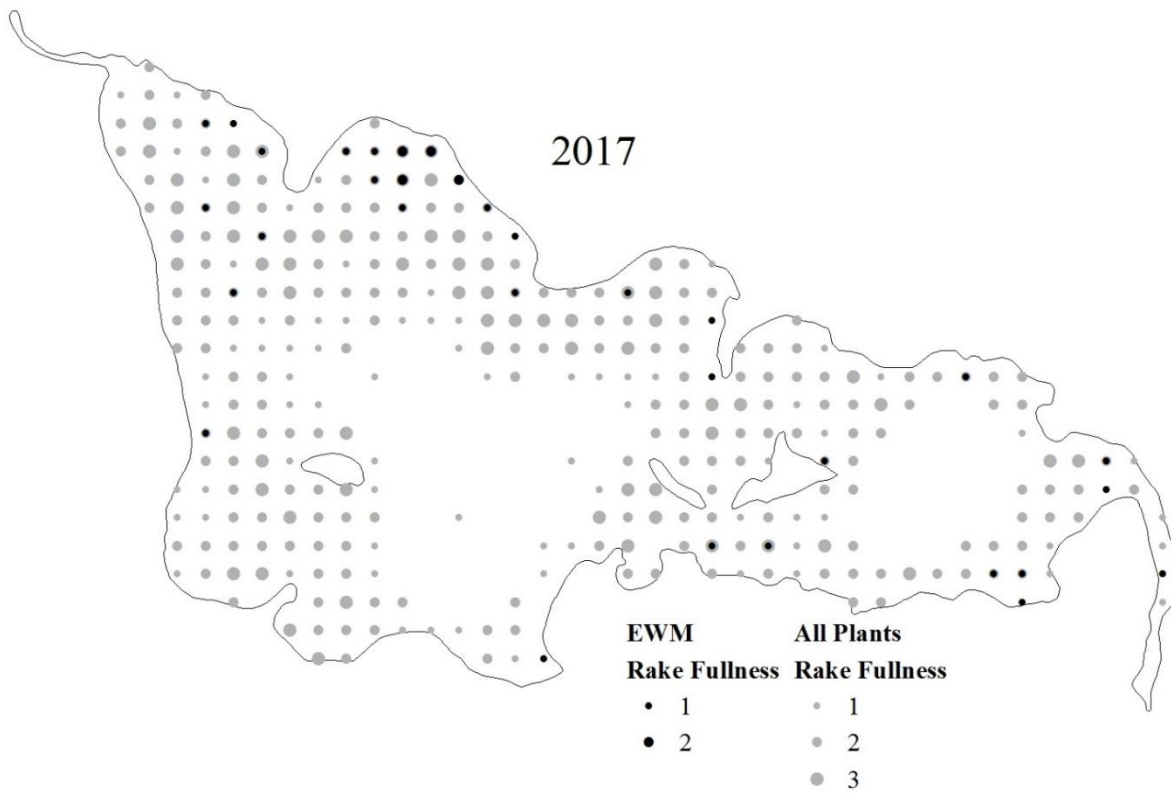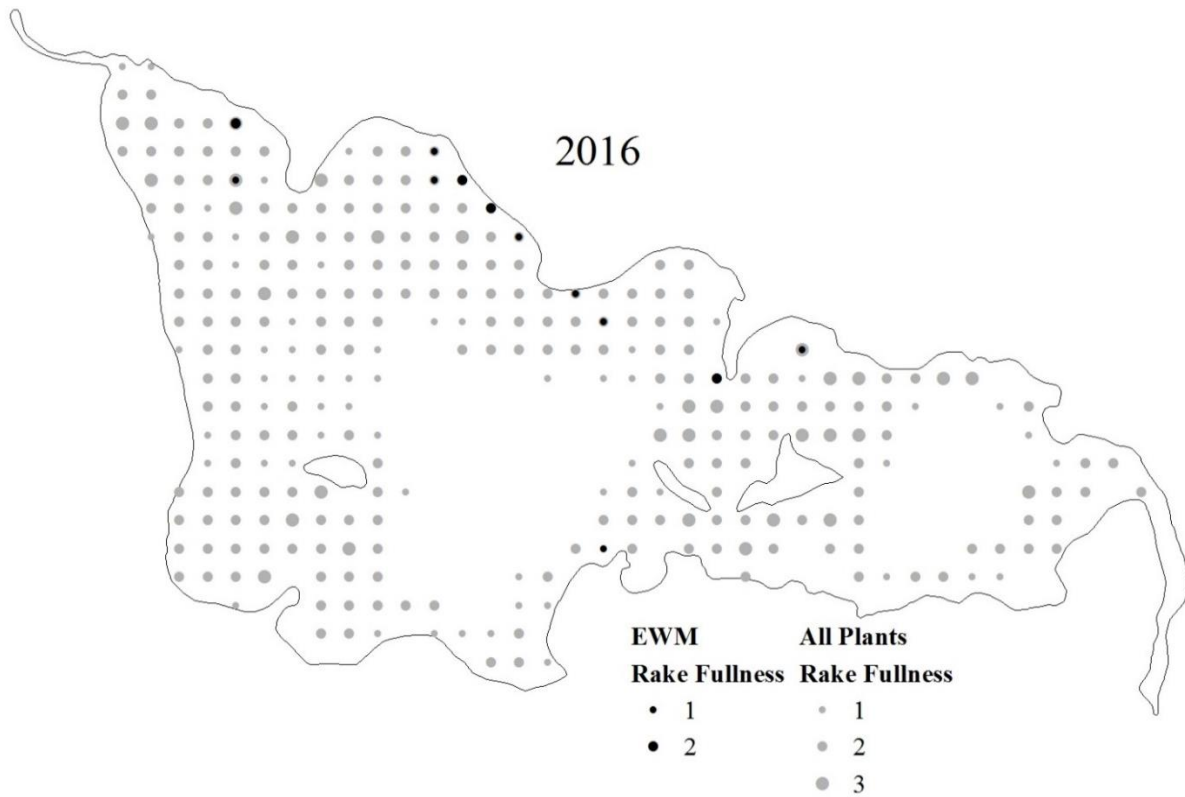
Figure 9. (continued).

Figure 10. Distribution and rake fullness rating for Eurasian Watermilfoil (EWM) and all aquatic plants in Upper Gresham Lake (reference lake), Vilas County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.

2016
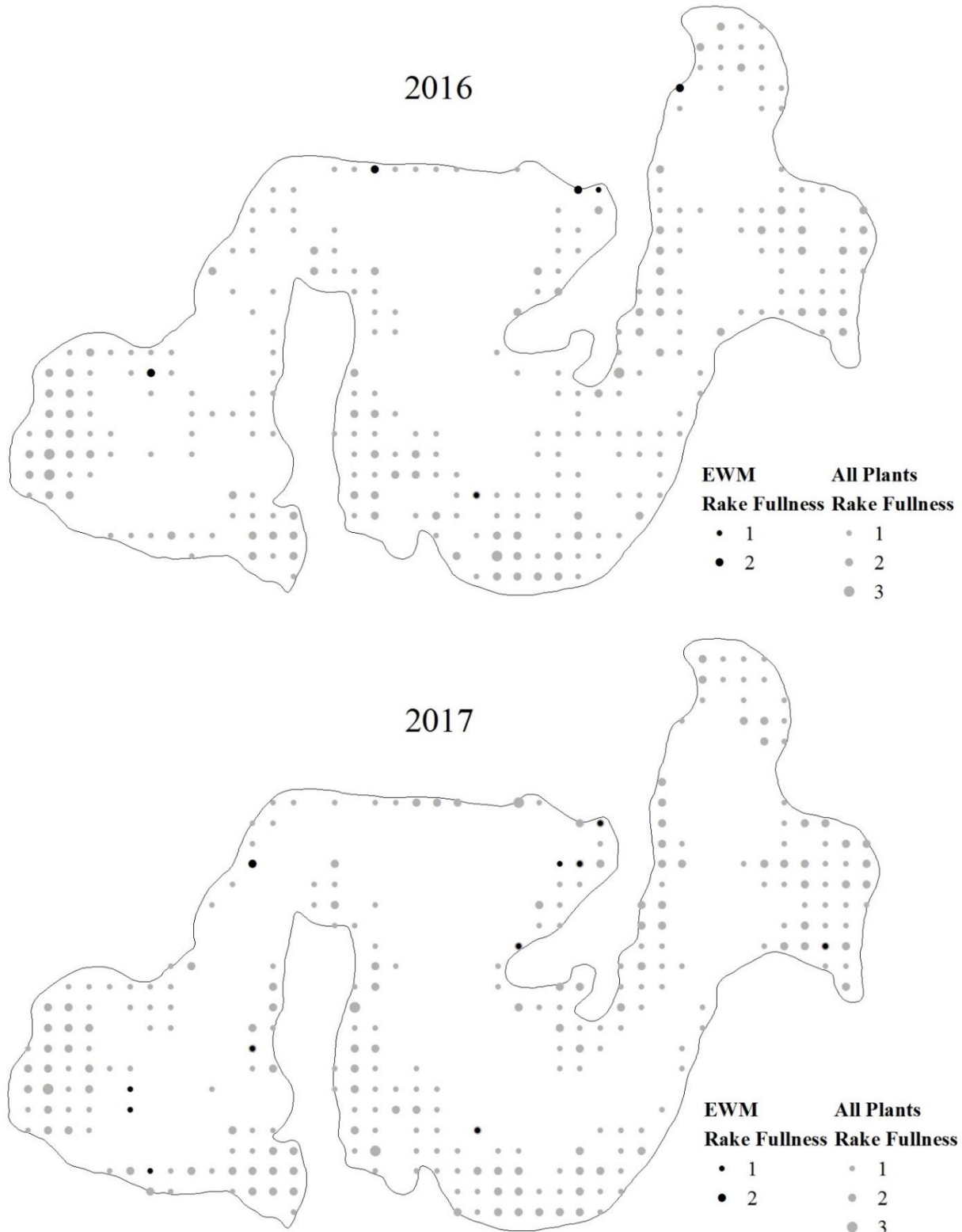
2017

Figure 10. (continued).
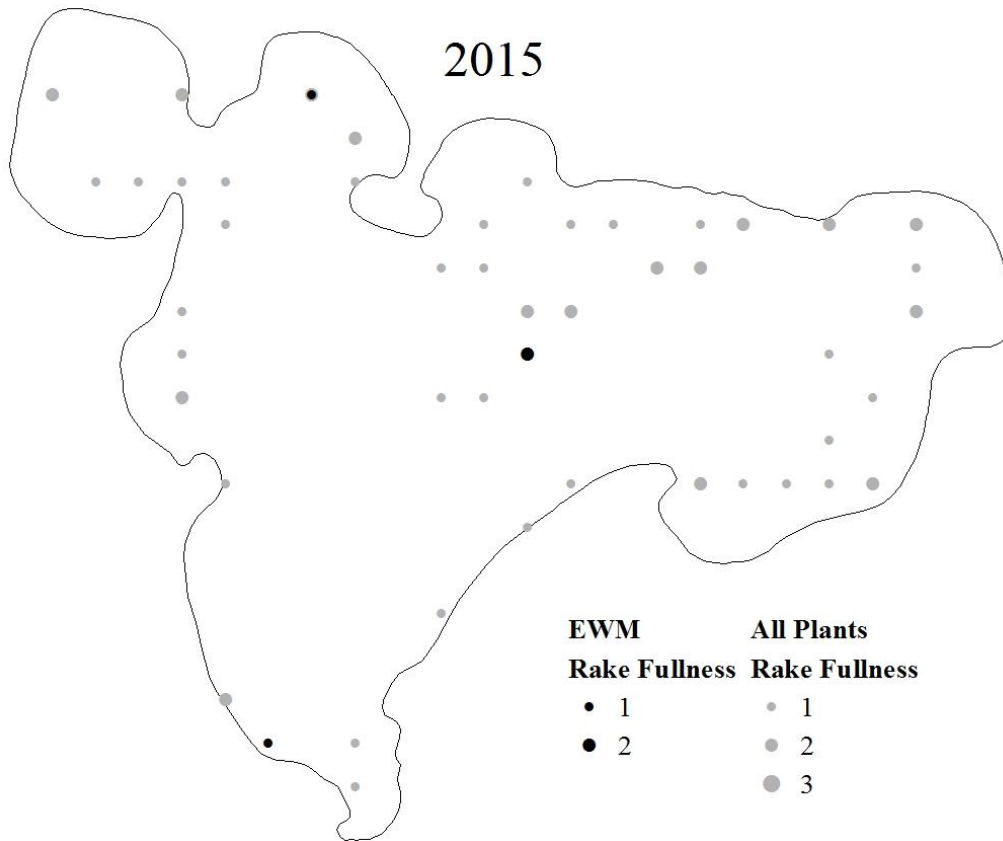
Figure 11. Distribution and rake fullness rating for Eurasian Watermilfoil (EWM) and all aquatic plants in Kathan Lake (treatment lake), Oneida County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.

2016

EWM
Rake Fullness
• 1
● 2

All Plants
Rake Fullness
· 1
• 2
● 3

2017

EWM
Rake Fullness
• 1
● 2

All Plants
Rake Fullness
· 1
• 2
● 3
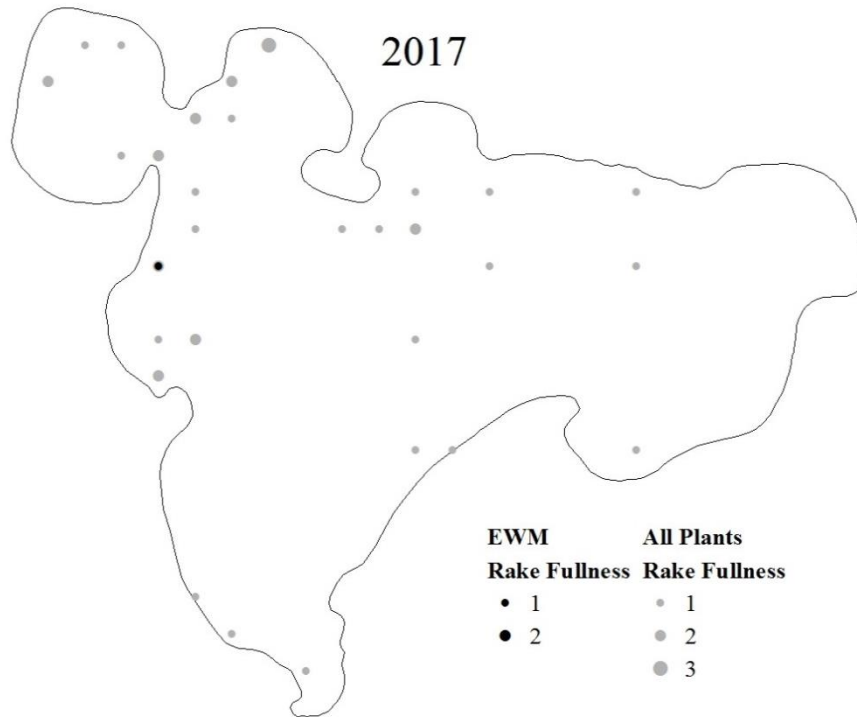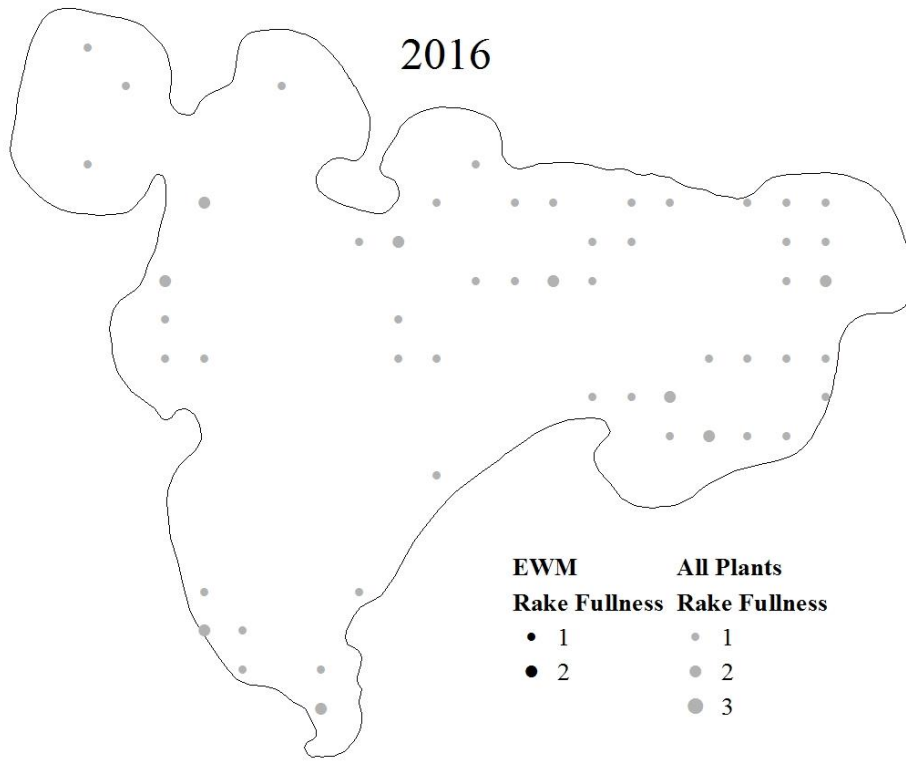
Figure 11. (continued).

2015
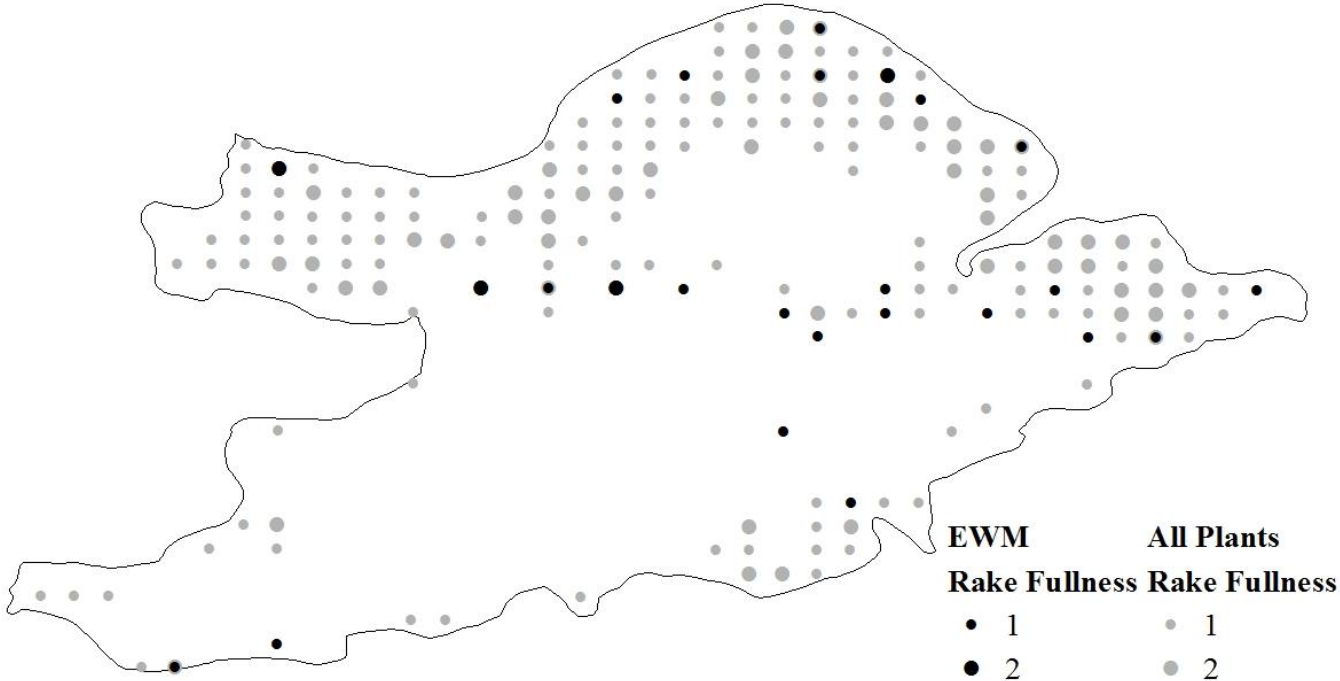
Figure 12. Distribution and rake fullness rating for Eurasian Watermilfoil (EWM) and all aquatic plants in Manson Lake (treatment lake), Oneida County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.

2016
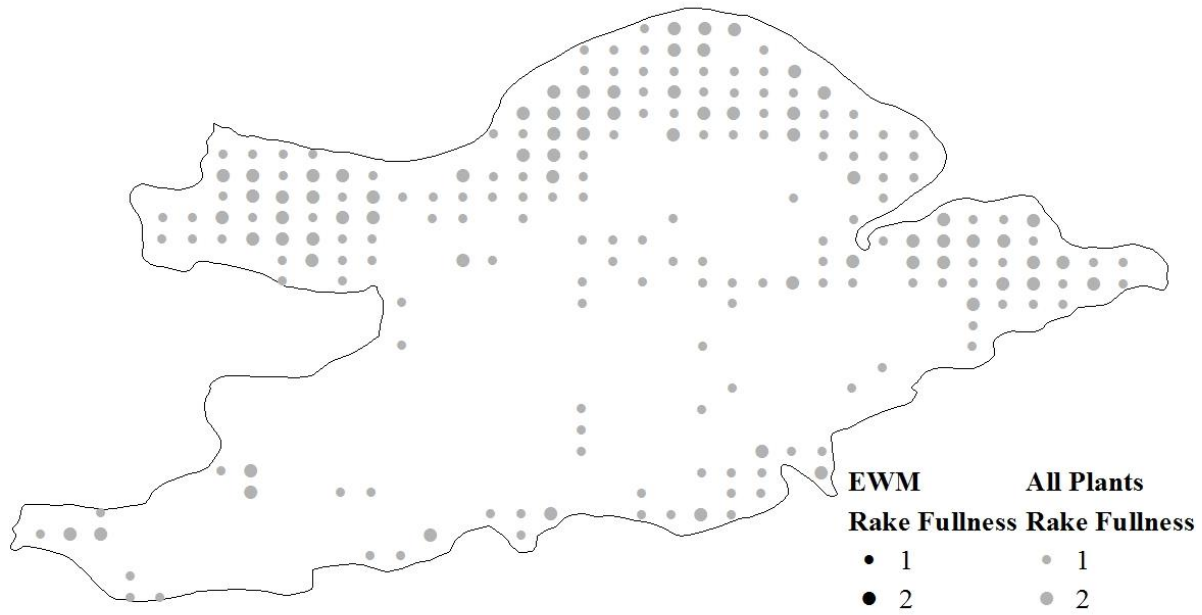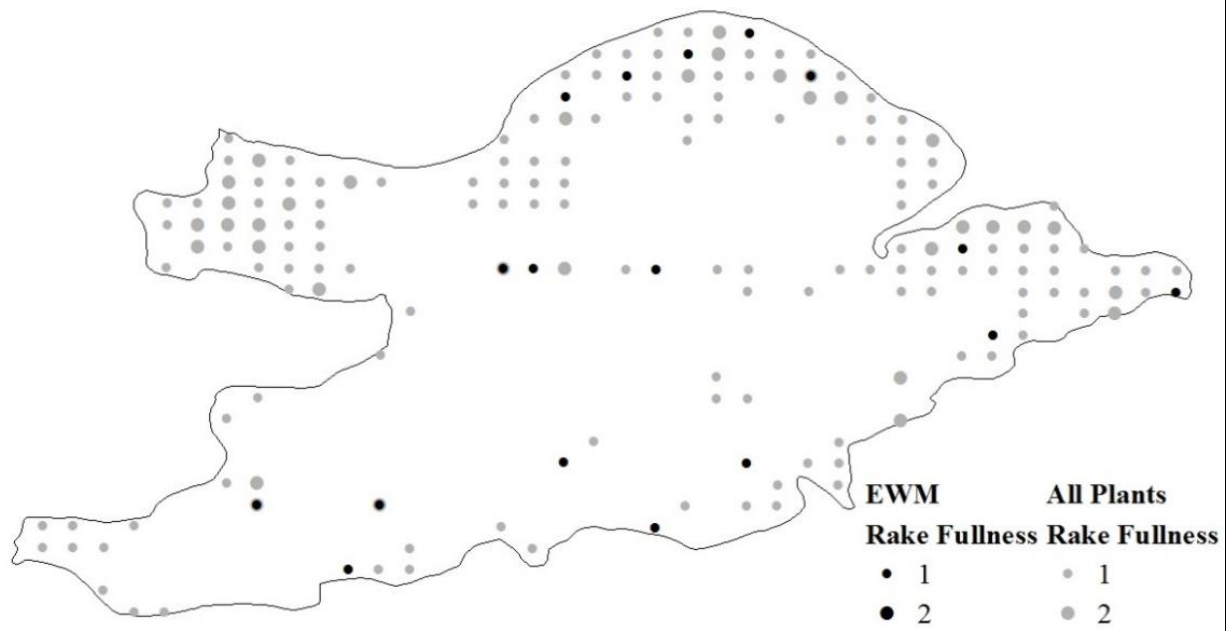
2017

Figure 12. (continued).
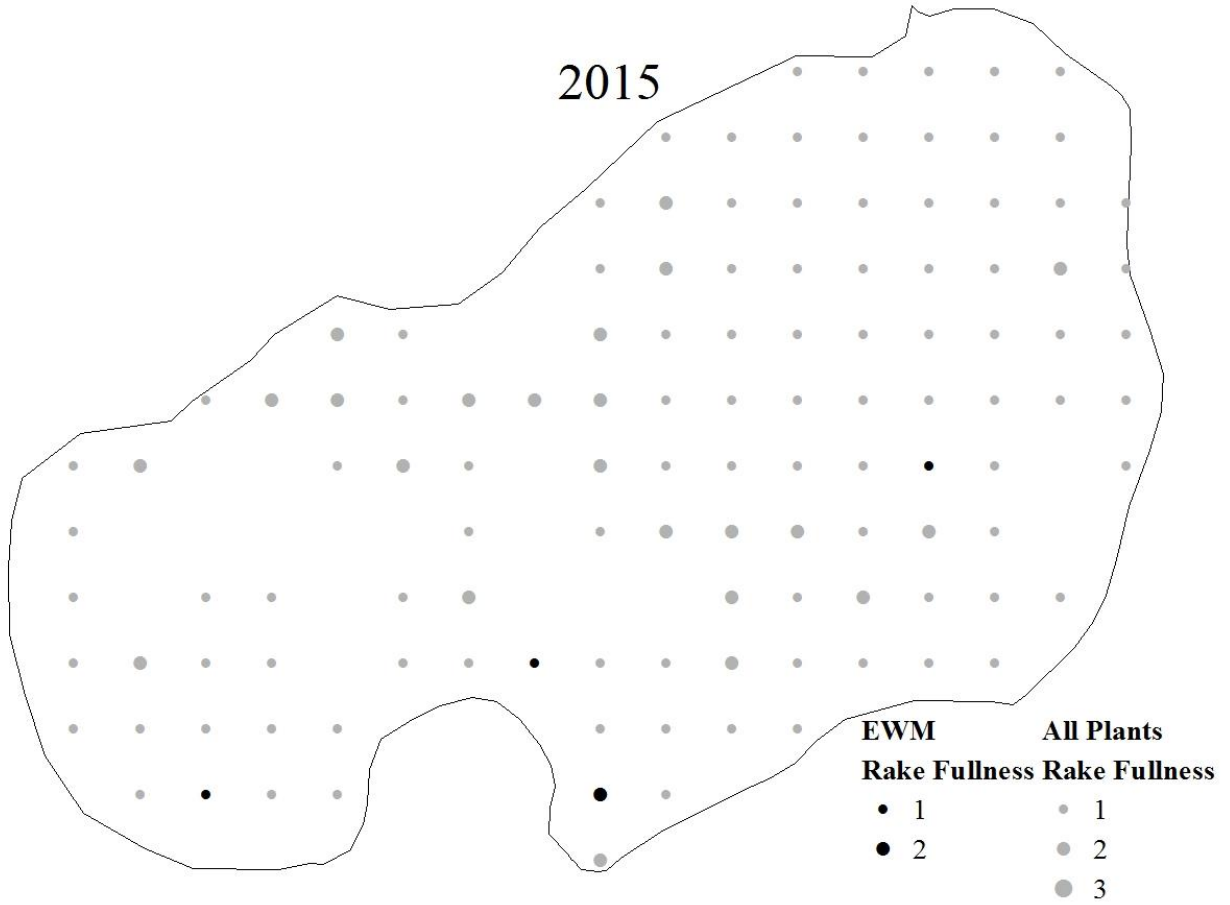
Figure 13. Distribution and rake fullness rating for Eurasian Watermilfoil (EWM) and all aquatic plants in Silver Lake (treatment lake), Vilas County, Wisconsin from 2015 to 2017. Size of circles correspond to rake fullness ratings with EWM in black and all plants combined in gray.
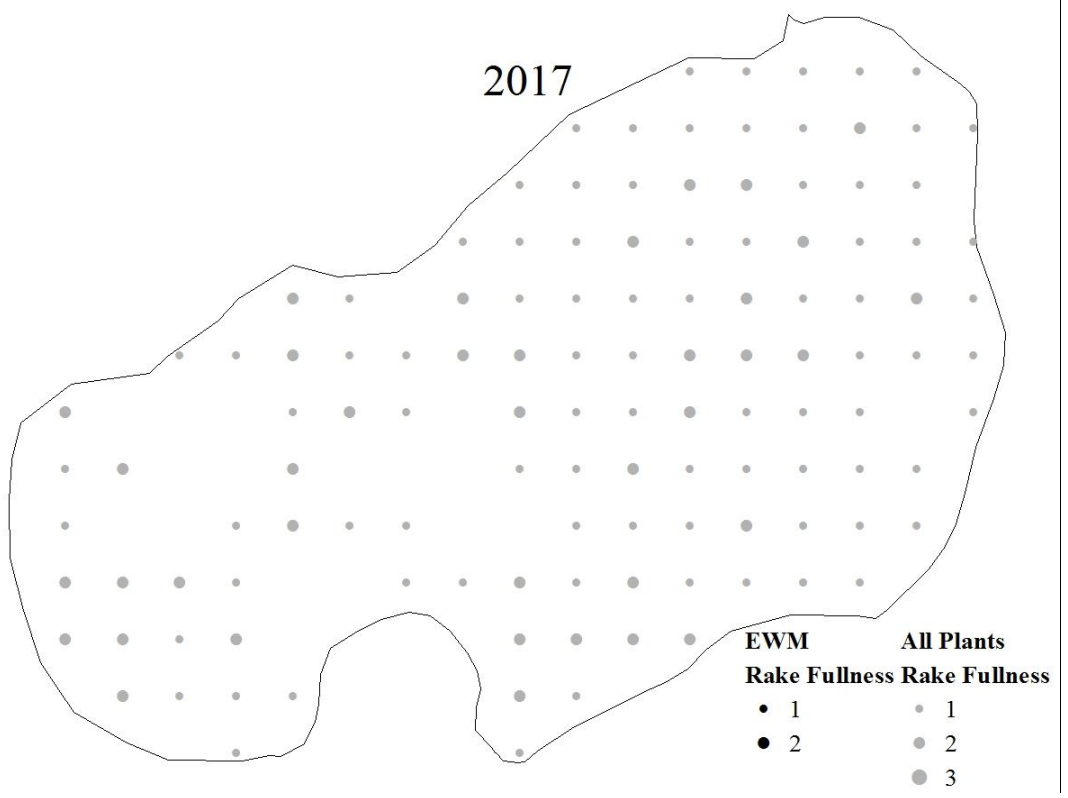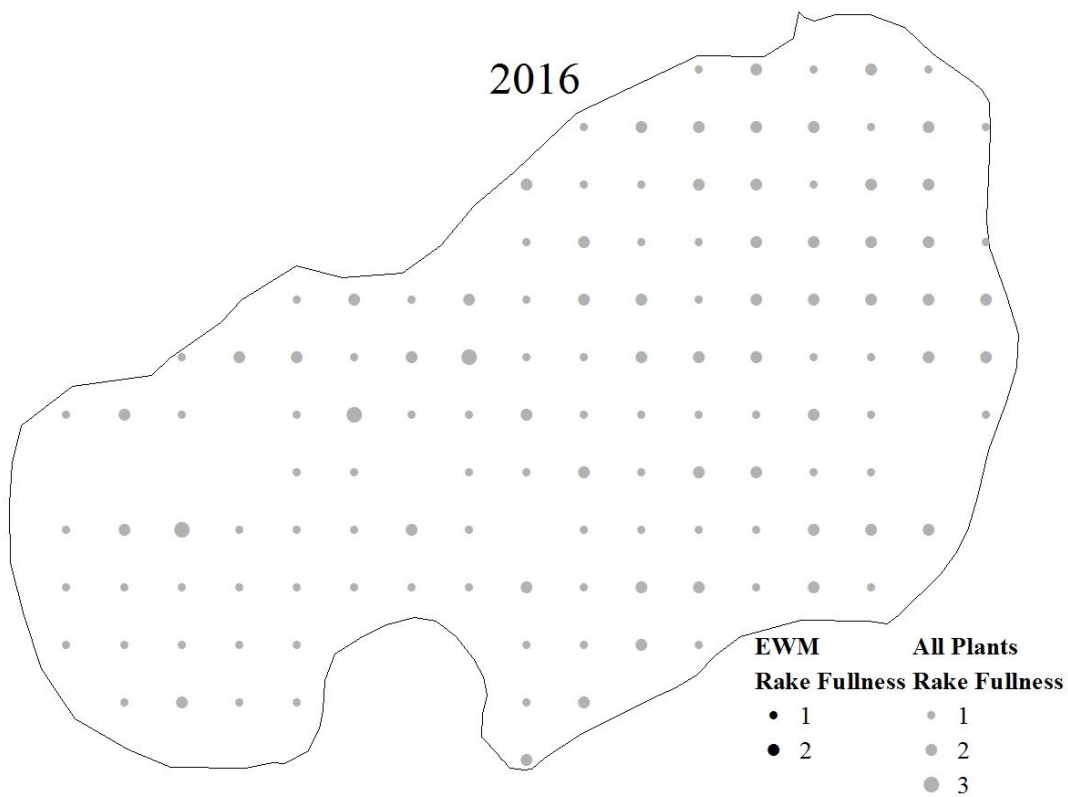
Figure 13. (continued).
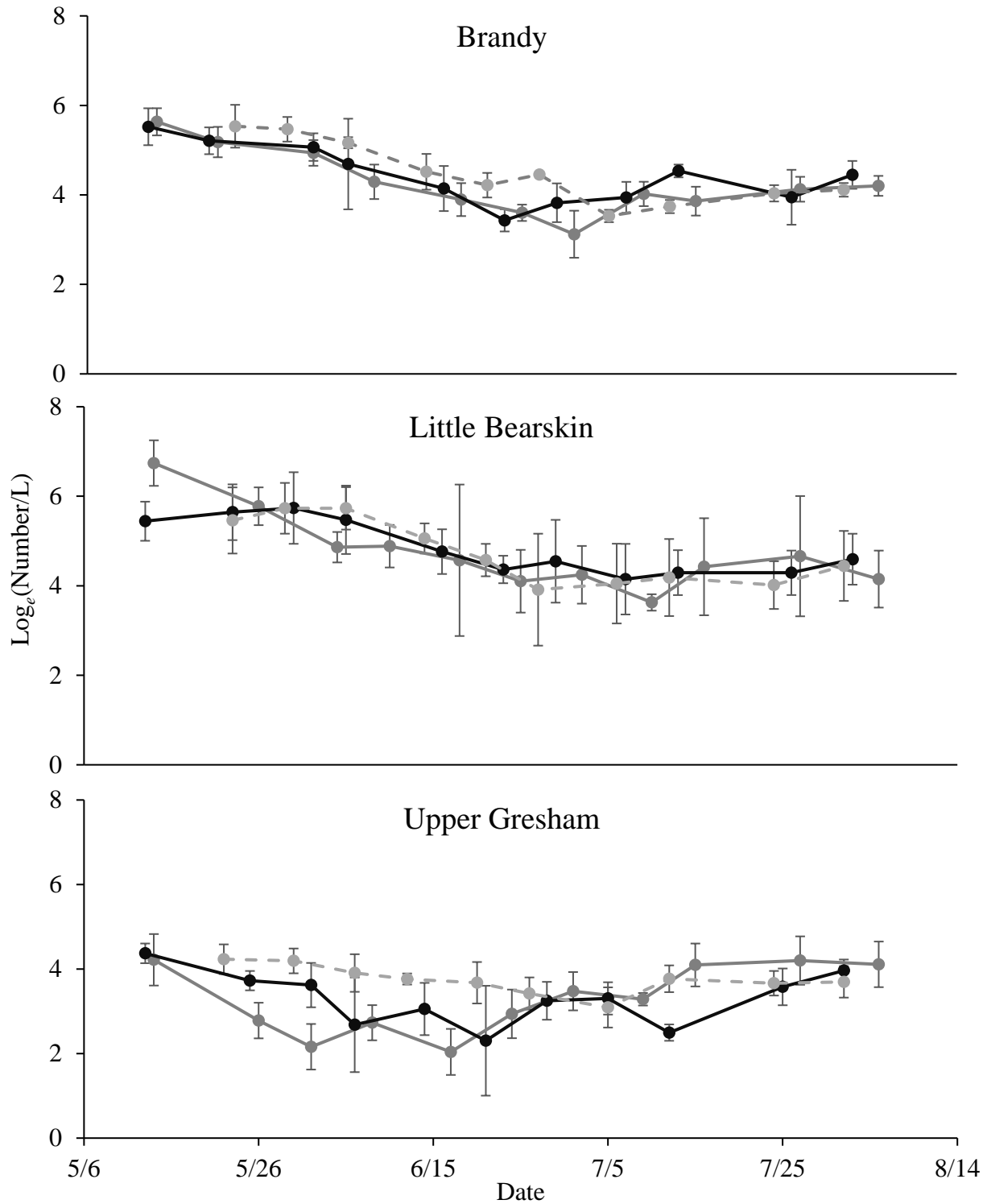
Figure 14. Log*e* transformed total zooplankton densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide treatment dates for the three lakes treated with 2, 4-D herbicide in 2016. Error bars are 95% confidence intervals.
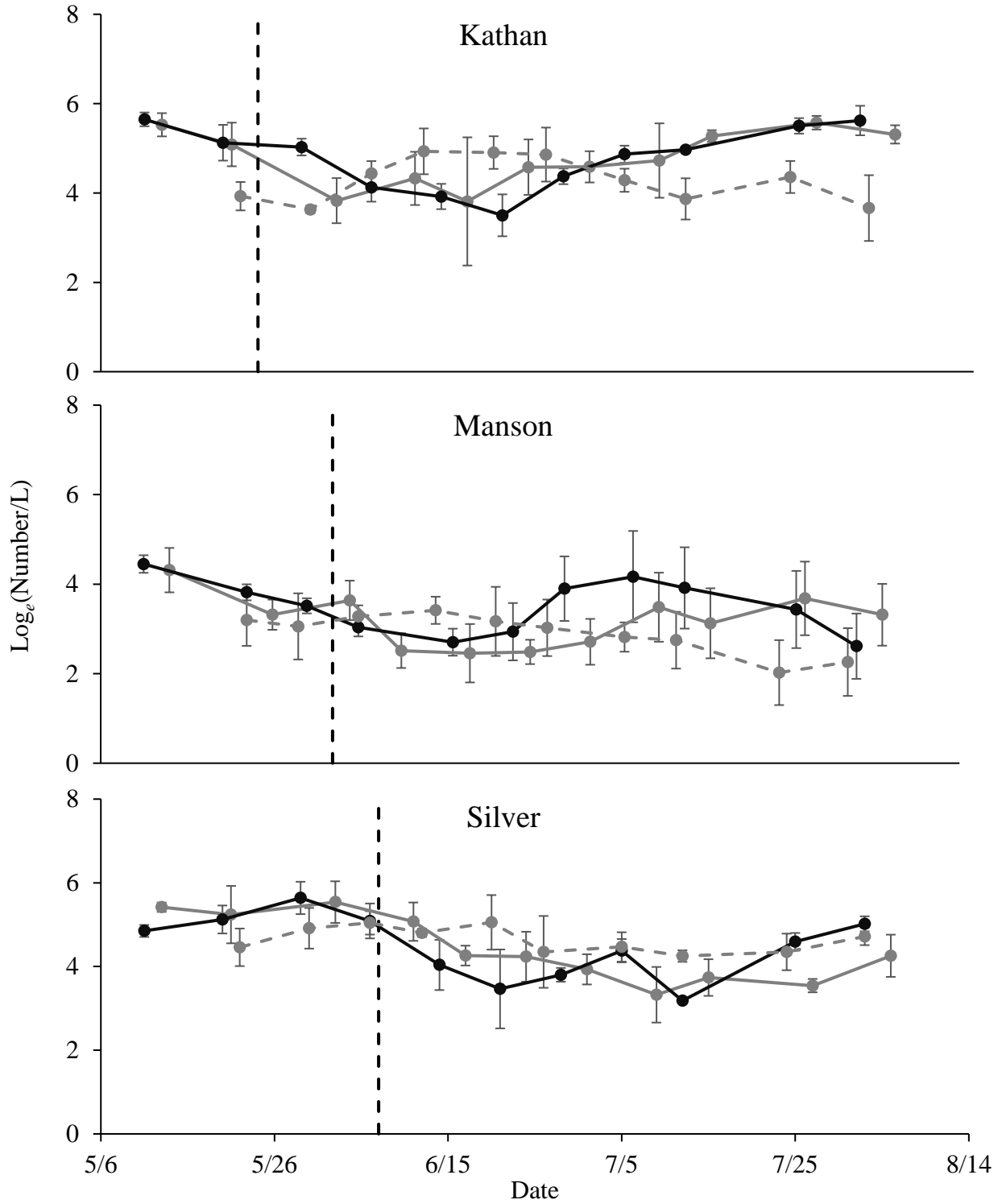
Figure 14. (continued).
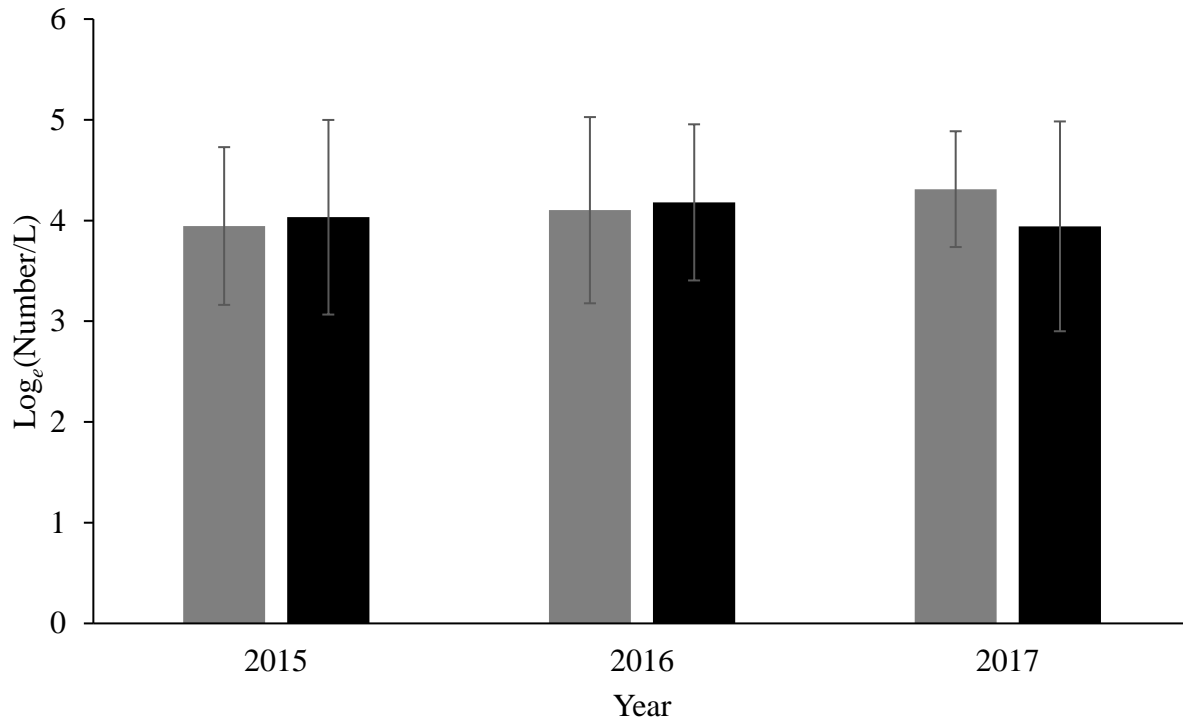
Figure 15. Mean log$_e$ transformed total zooplankton densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
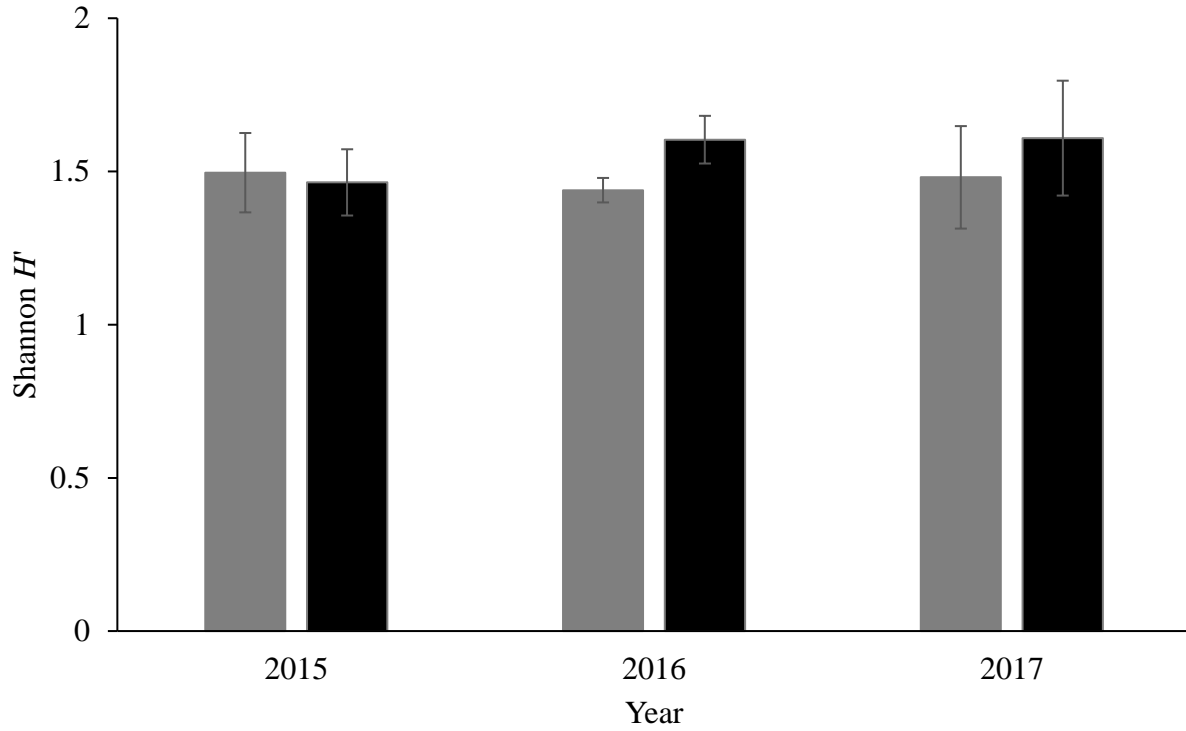
Figure 16. Mean Shannon's diversity index (H') calculated using densities (number/L) for zooplankton taxa collected in reference (gray bars) and treatment lakes (black bars) from May to August sampling each year. Error bars are 95% confidence intervals.
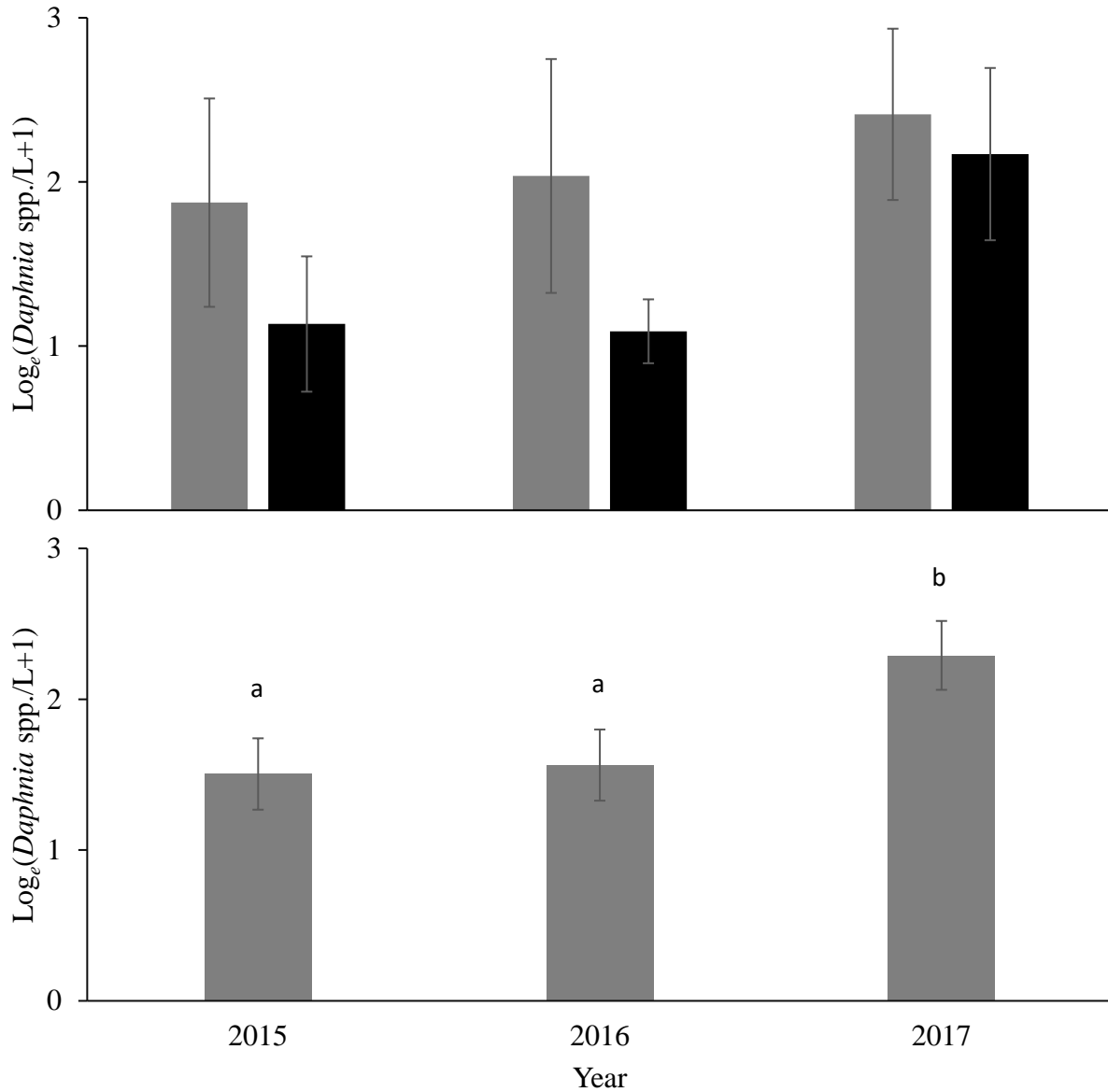
Figure 17. Log$_e$ +1 transformed *Daphnia* spp. densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017 (top panel) and log$_e$ +1 transformed *Daphnia* spp. densities for all lakes during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017; bottom panel). Error bars are 95% confidence intervals. In the bottom panel, columns denoted with different letters represent significant differences ($P < 0.05$; Table 2).
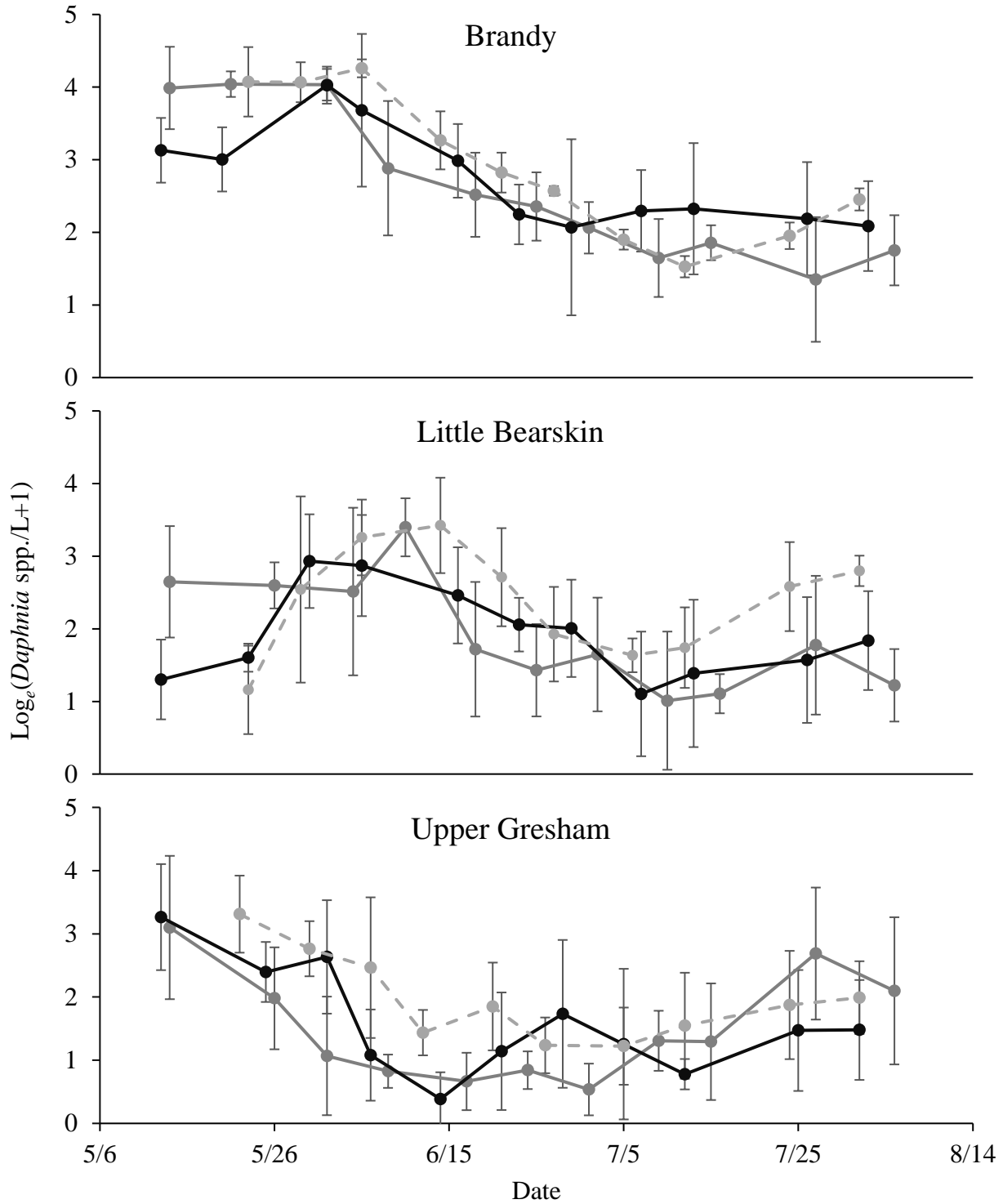
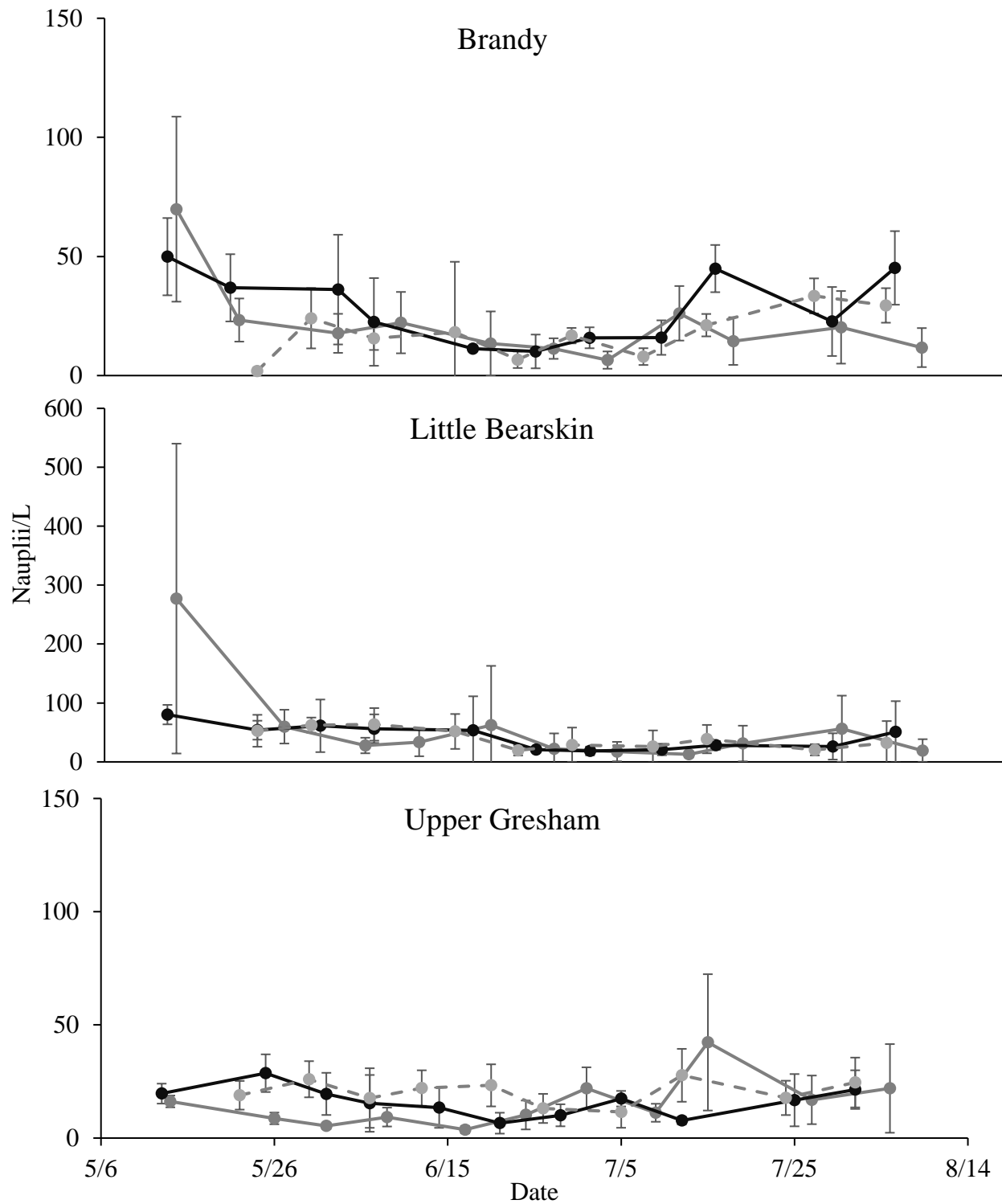Figure 18. Log$_e$ +1 transformed *Daphnia* spp. densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide treatment dates for the three lakes treated with 2, 4-D herbicide in 2016.
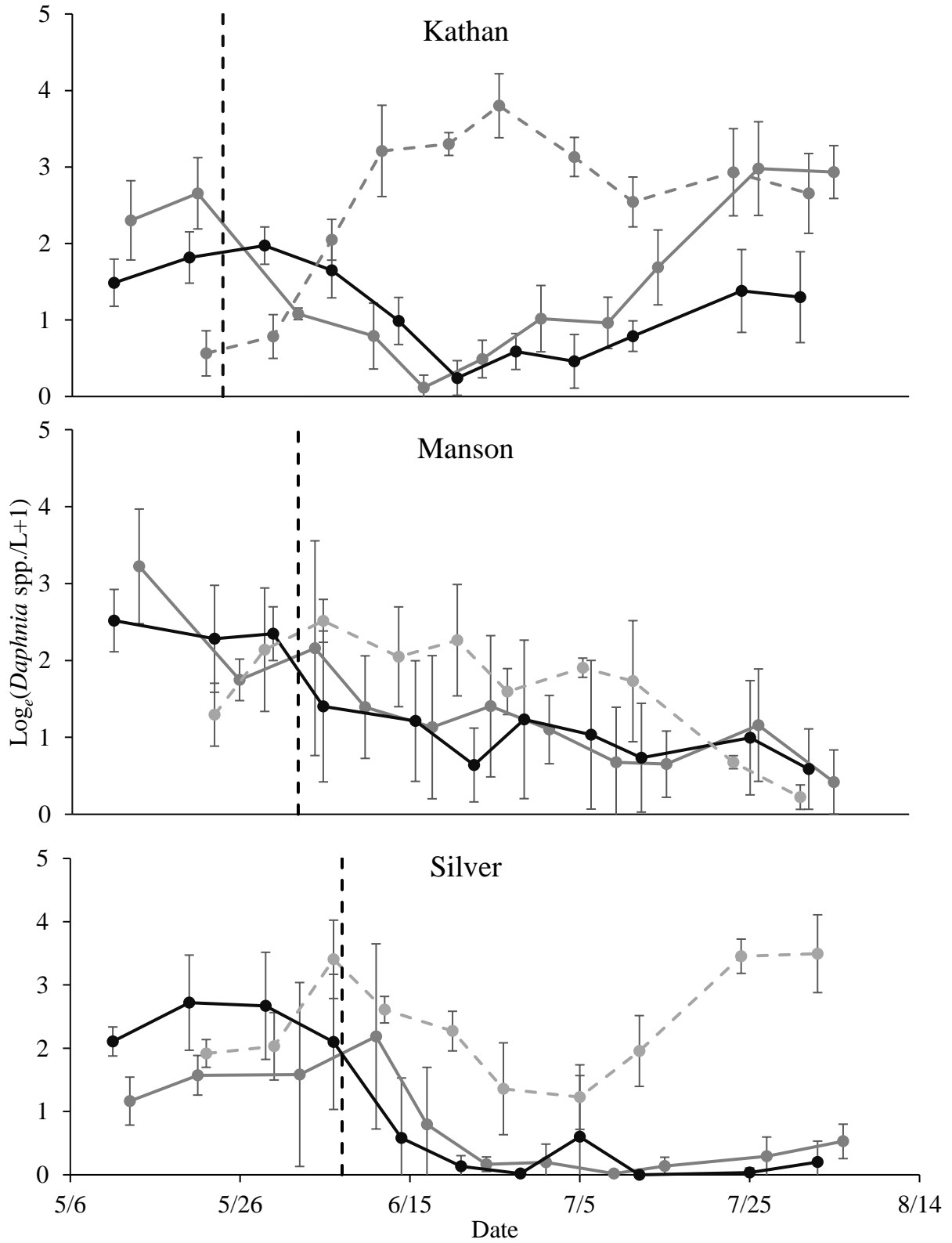
Figure 18. (continued).
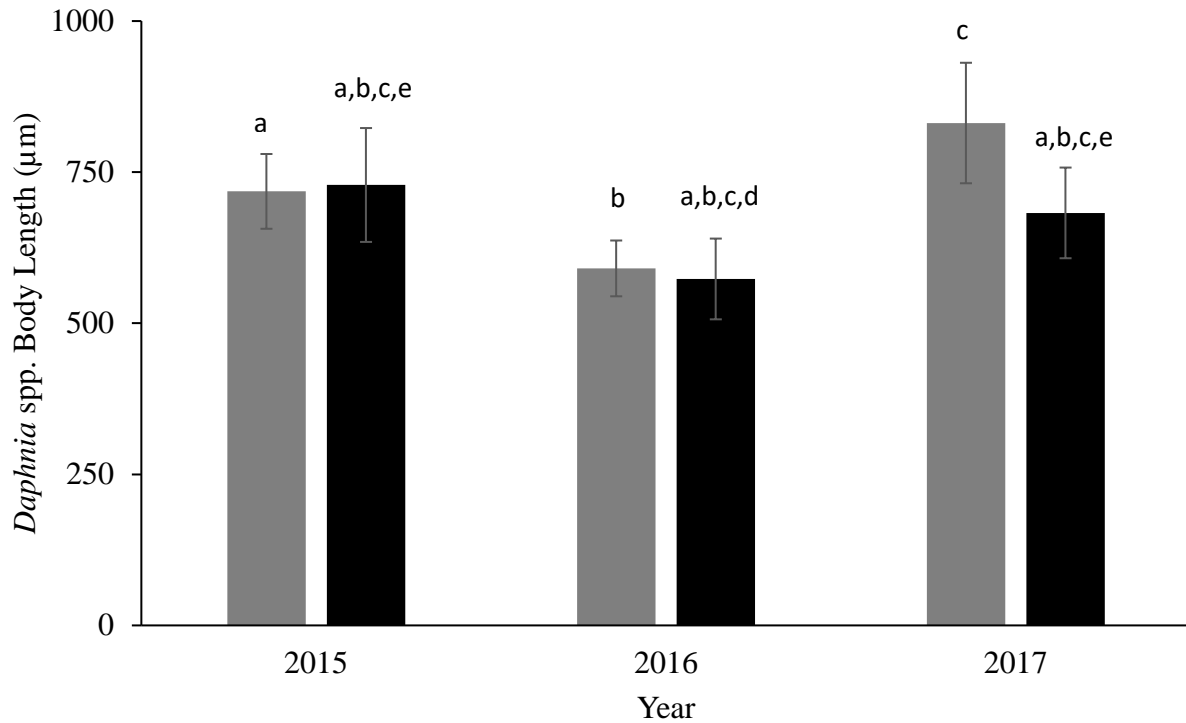
Figure 19. Mean *Daphnia* spp. body lengths (µm) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals. Columns denoted with different letters were significantly different ($P < 0.05$; Table 2).
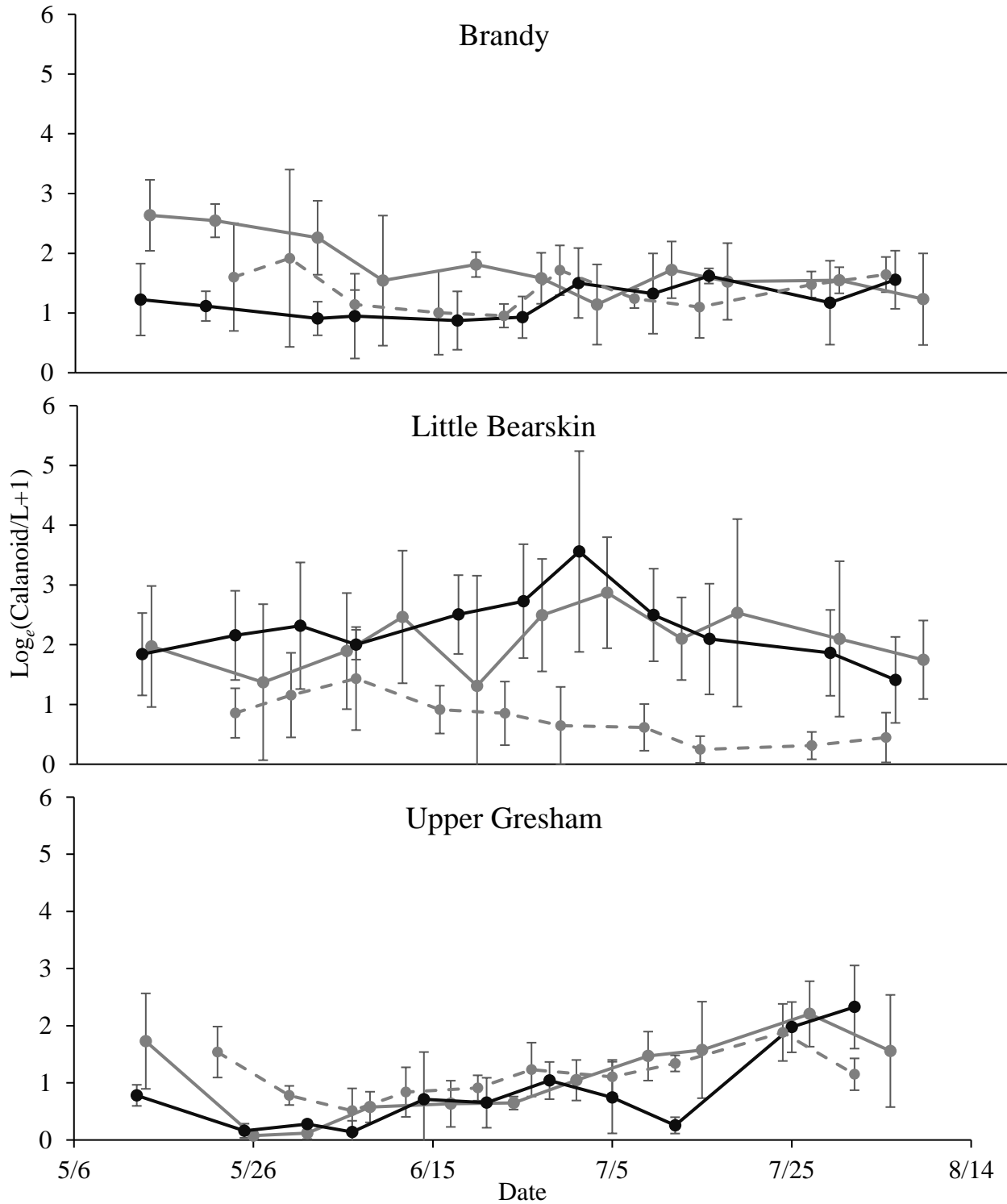
Figure 20. Log$_e$ +1 transformed calanoid copepod densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide application dates for the three lakes treated with 2, 4-D herbicide in 2016. Error bars are 95% confidence intervals.
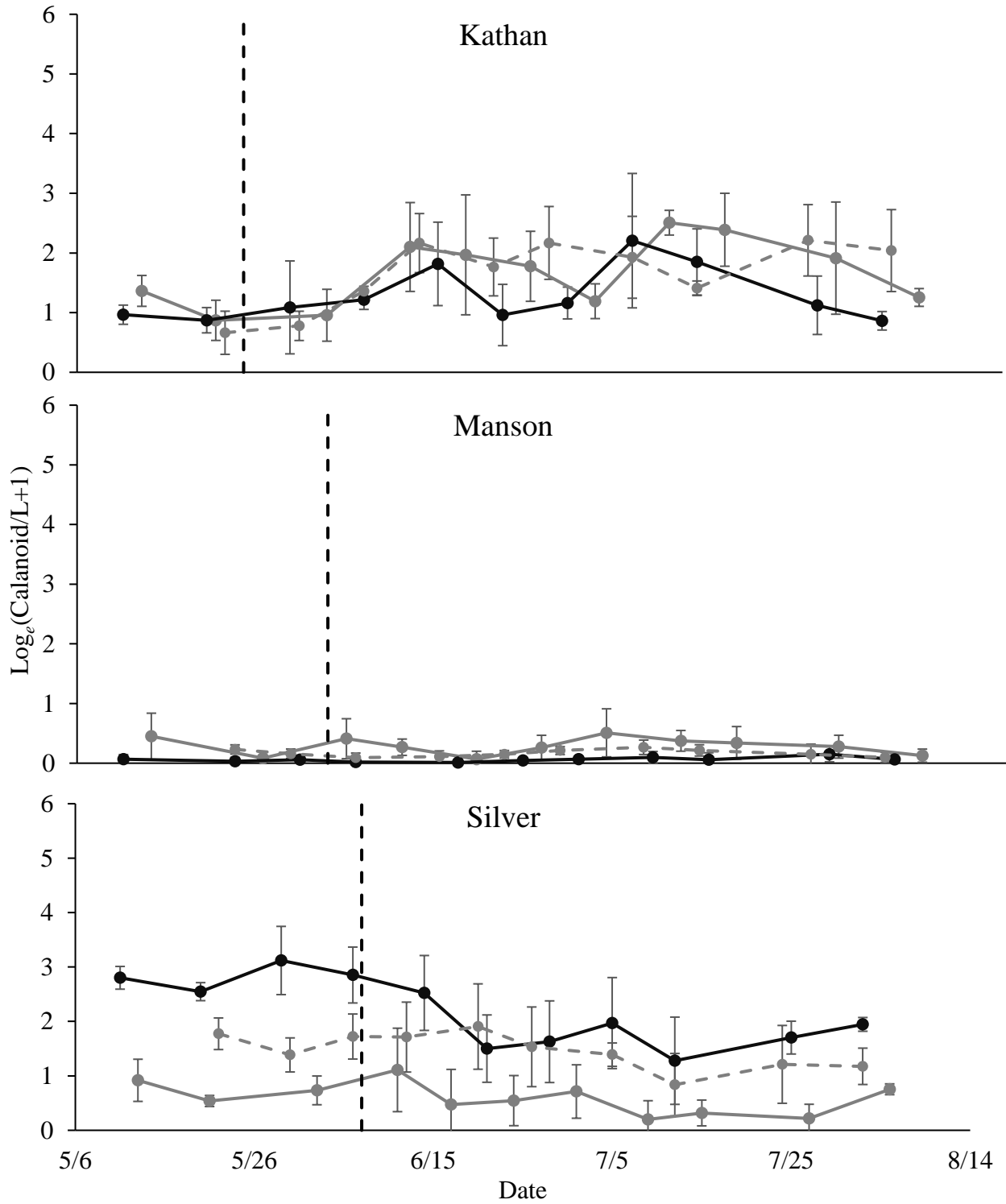
Figure 20. (continued).
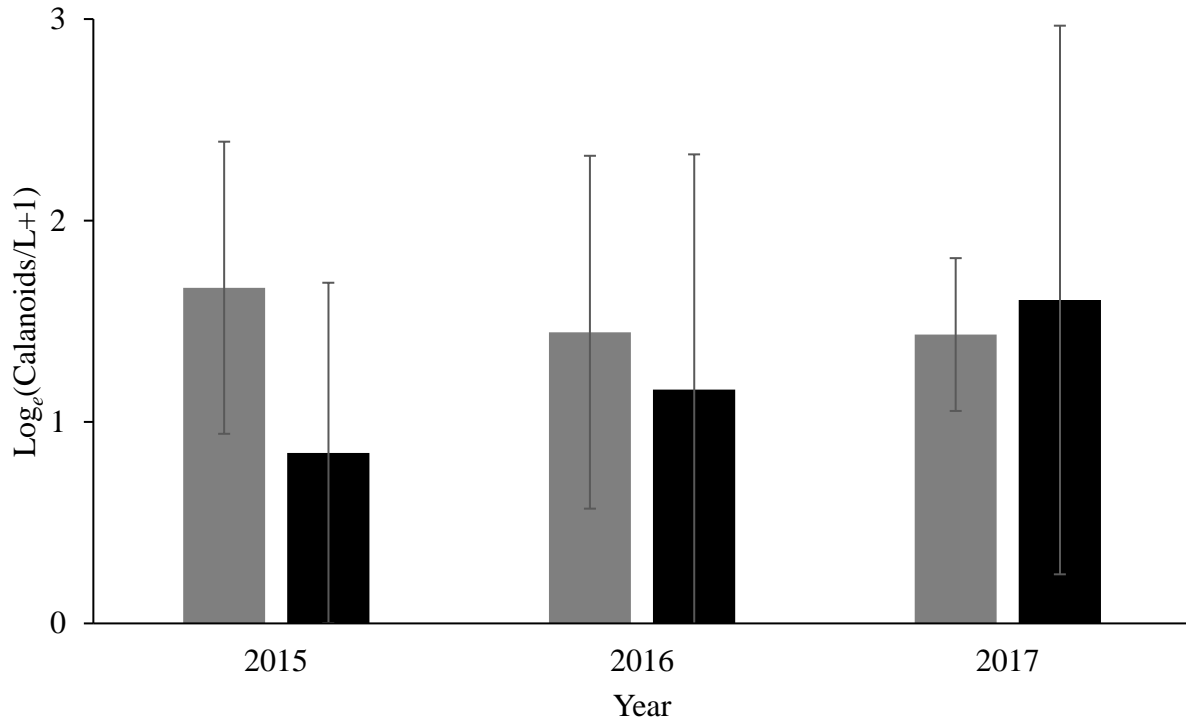
75

Figure 21. Mean log$_e$ transformed calanoid copepod densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
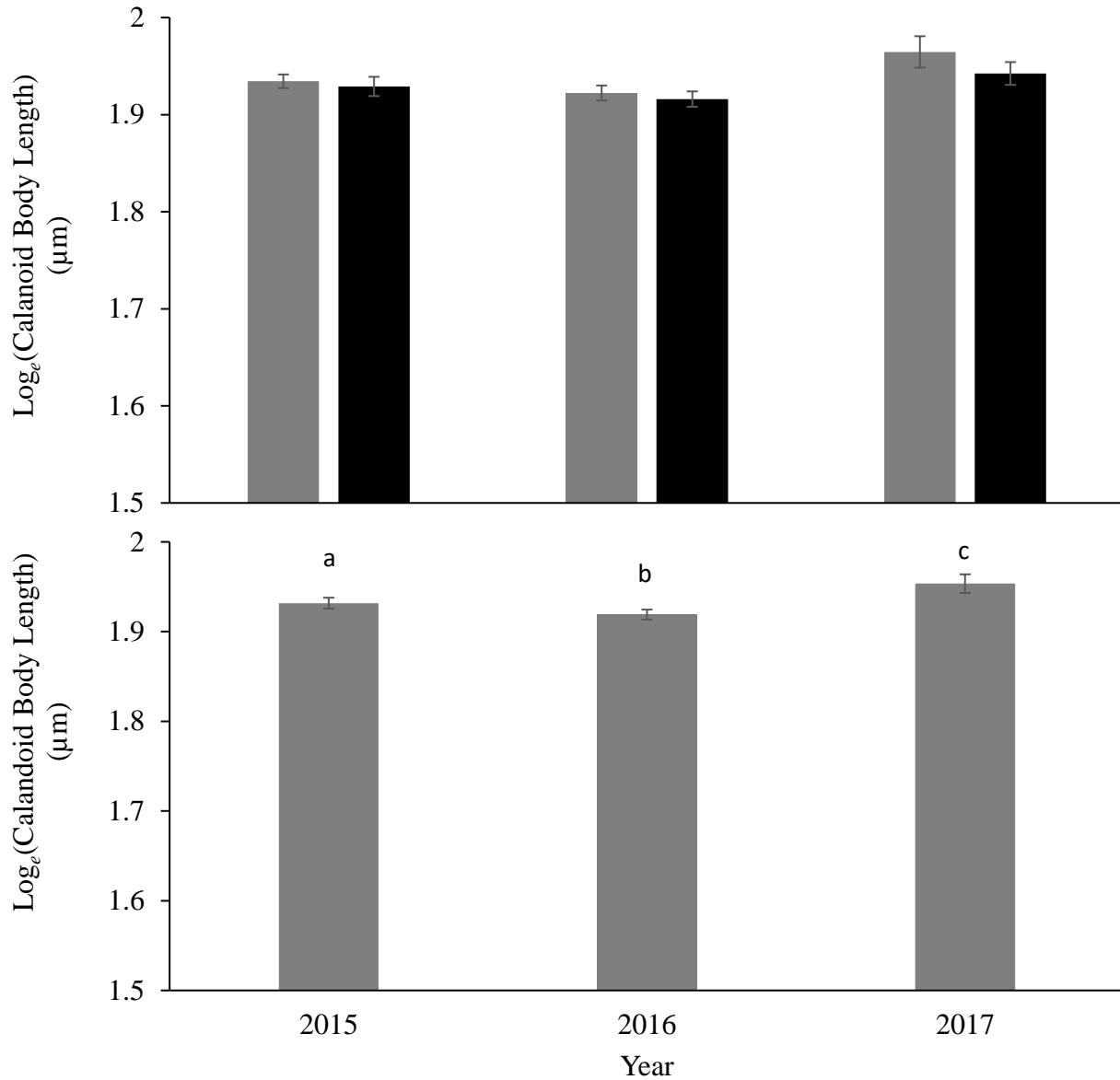
Figure 22. Mean $\log_e$ transformed calanoid copepod body lengths (µm) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017 (top panel) and $\log_e$ transformed calanoid copepod densities for all lakes during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017; 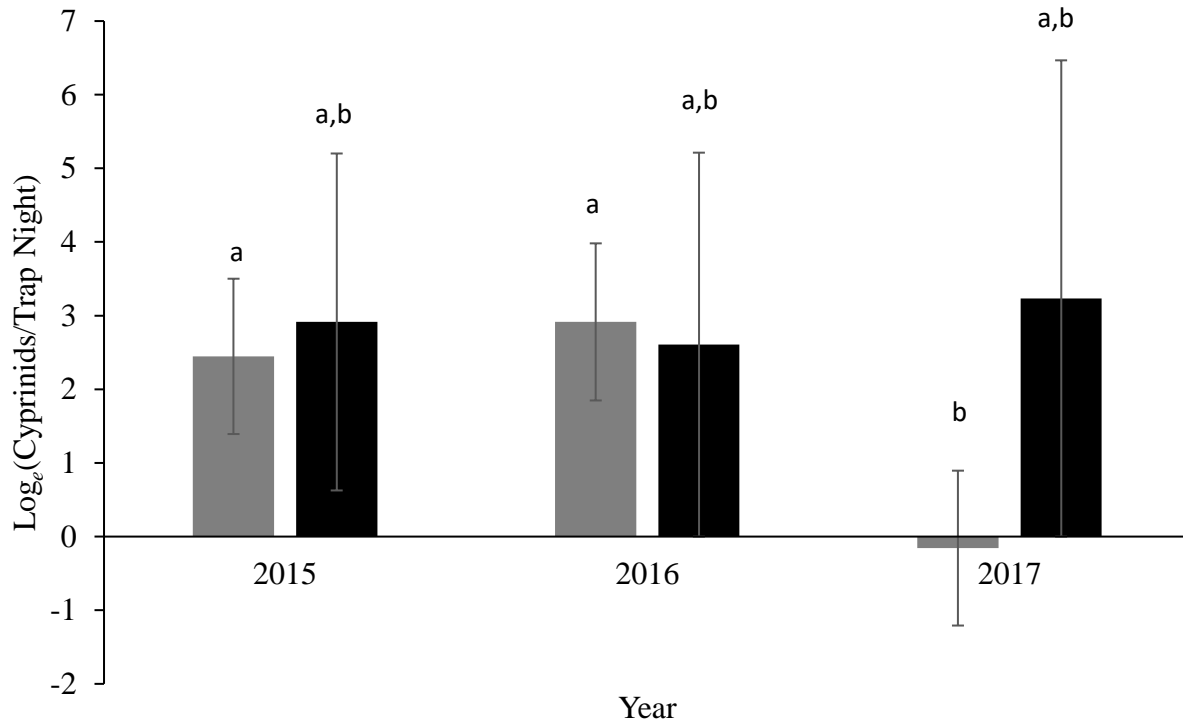bottom panel). Error bars are 95% confidence intervals. In the bottom panel, columns denoted with different letters were significantly different ($P < 0.05$; Table 2).
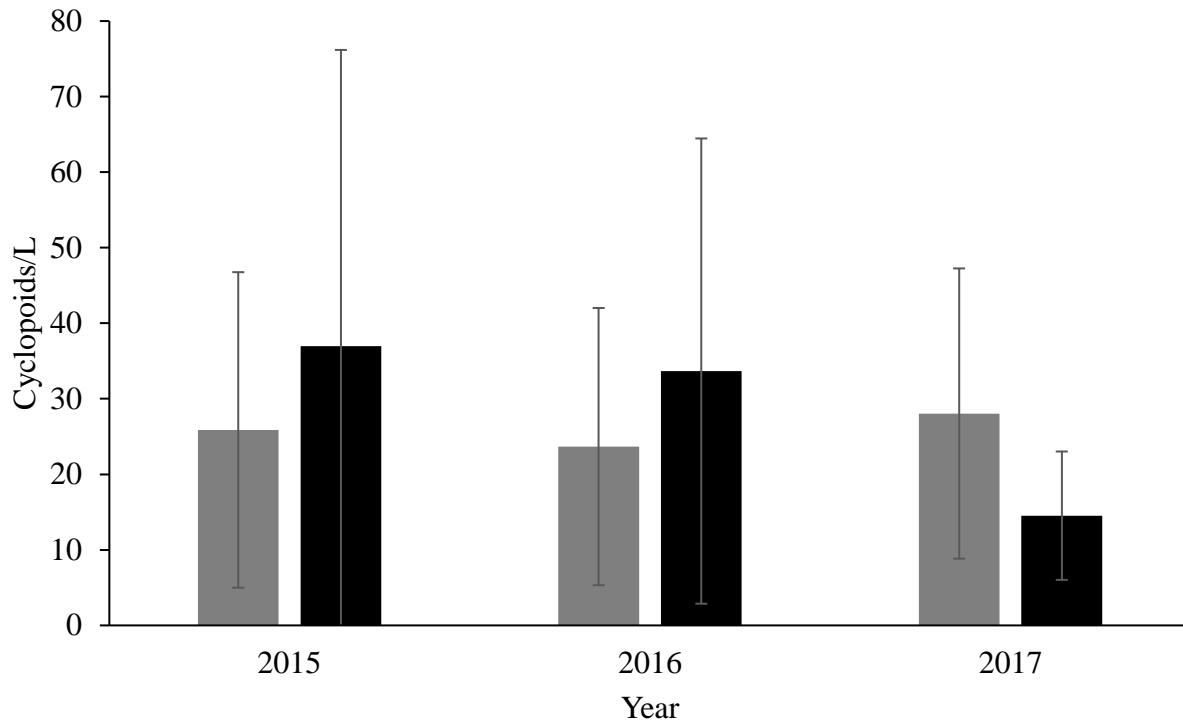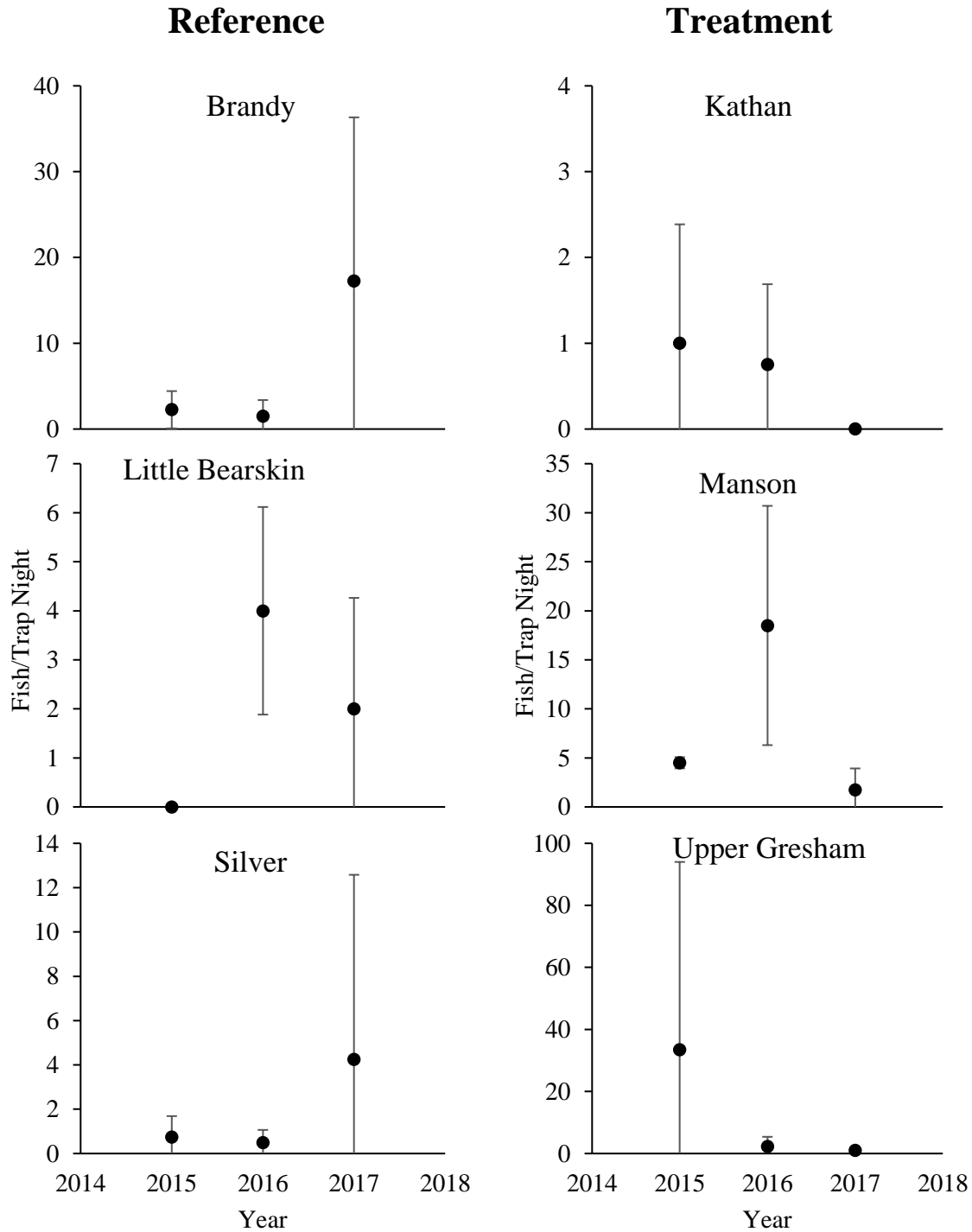
Figure 23. Mean cyclopoid copepod densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
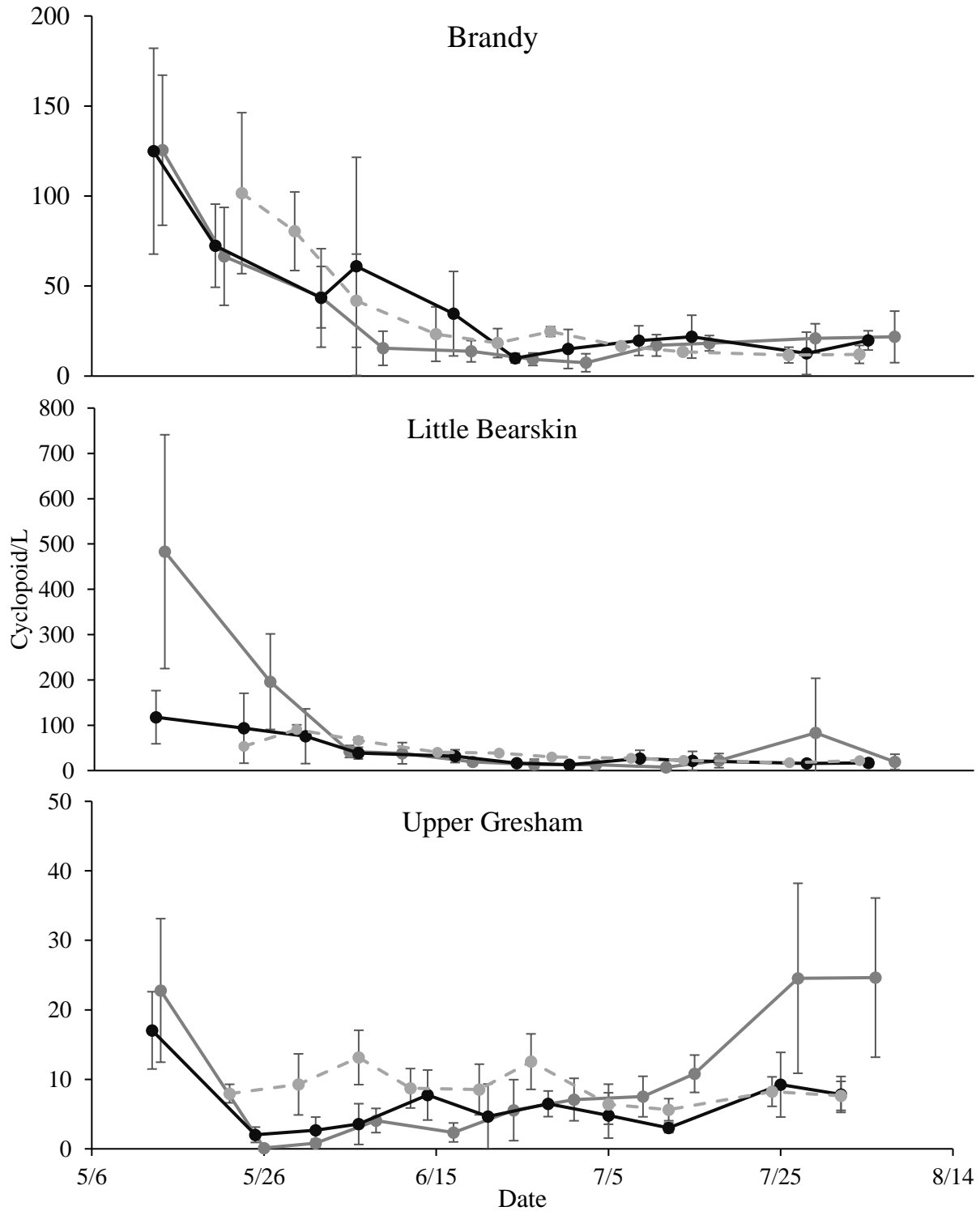
Figure 24. Cyclopoid copepod densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide treatment application dates for the three lakes treated with 2, 4-D herbicide in 2016. Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in cyclopoid copepod densities among lakes.
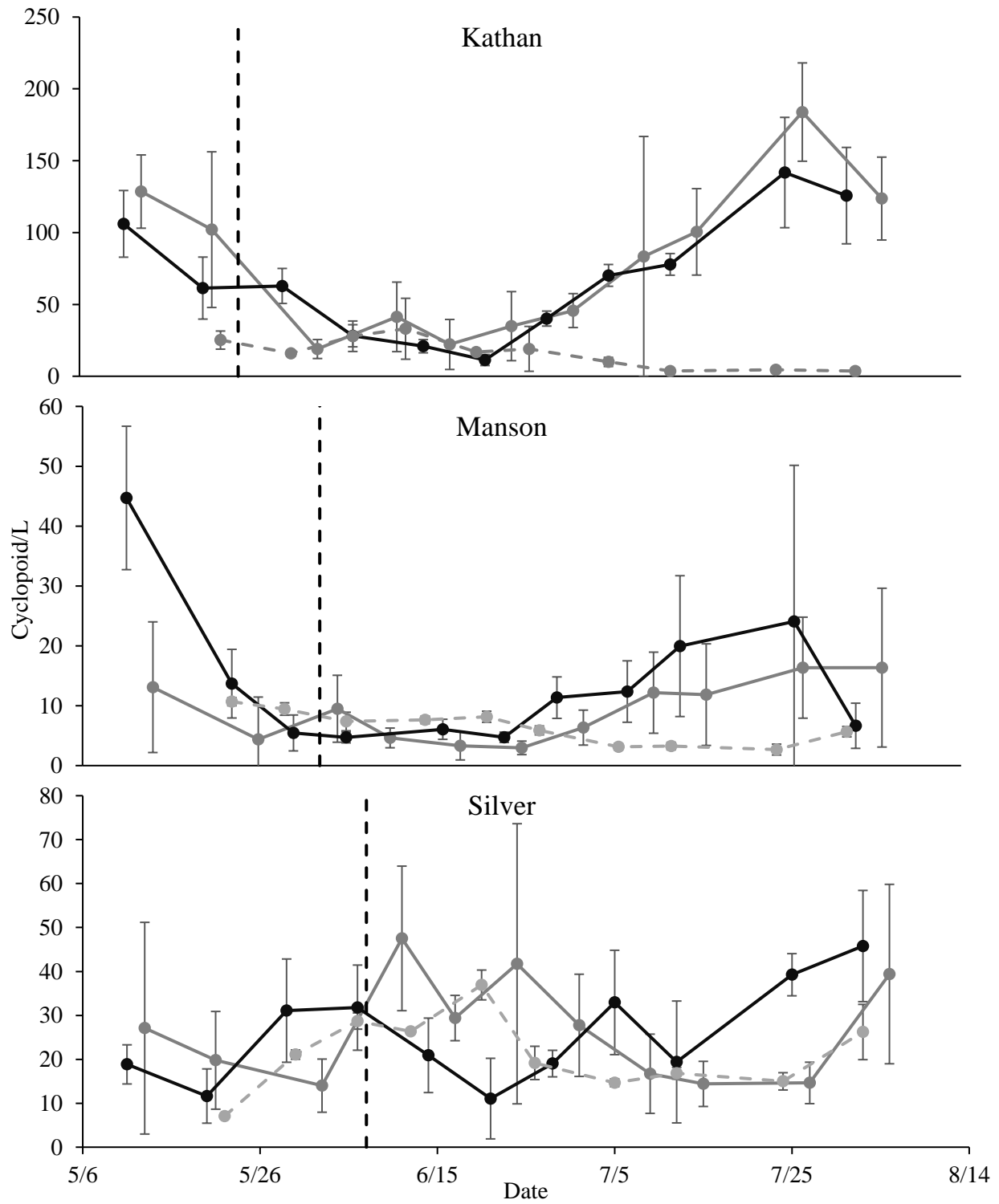
Figure 24. (continued)

Figure 25. Mean log$_e$ transformed cyclopoid copepod body length (µm) for reference (gray) and treatment lakes (black) from 2015 to 2017 (top panel) and log$_e$ transformed cyclopoid copepod densities for all lakes during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017; bottom panel). Error bars are 95% confidence intervals. In the bottom panel, columns denoted with different letters were significantly different ($P < 0.05$; Table 2).

Figure 26. Mean *Bosmina* spp. densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
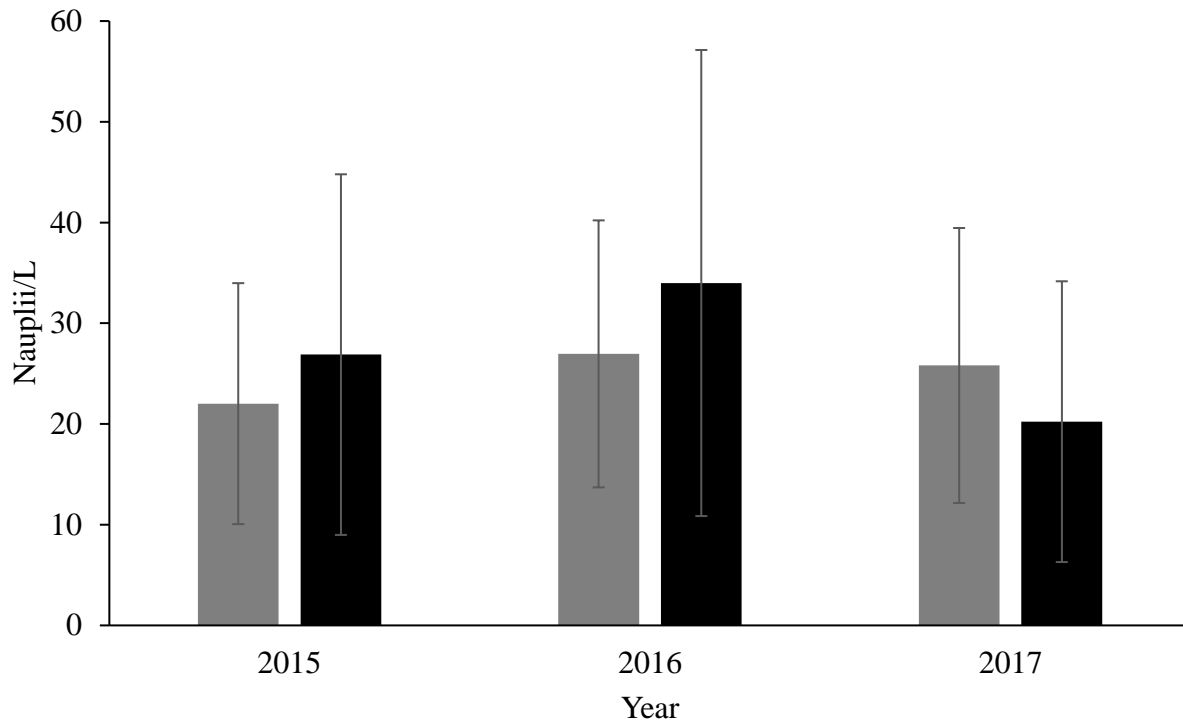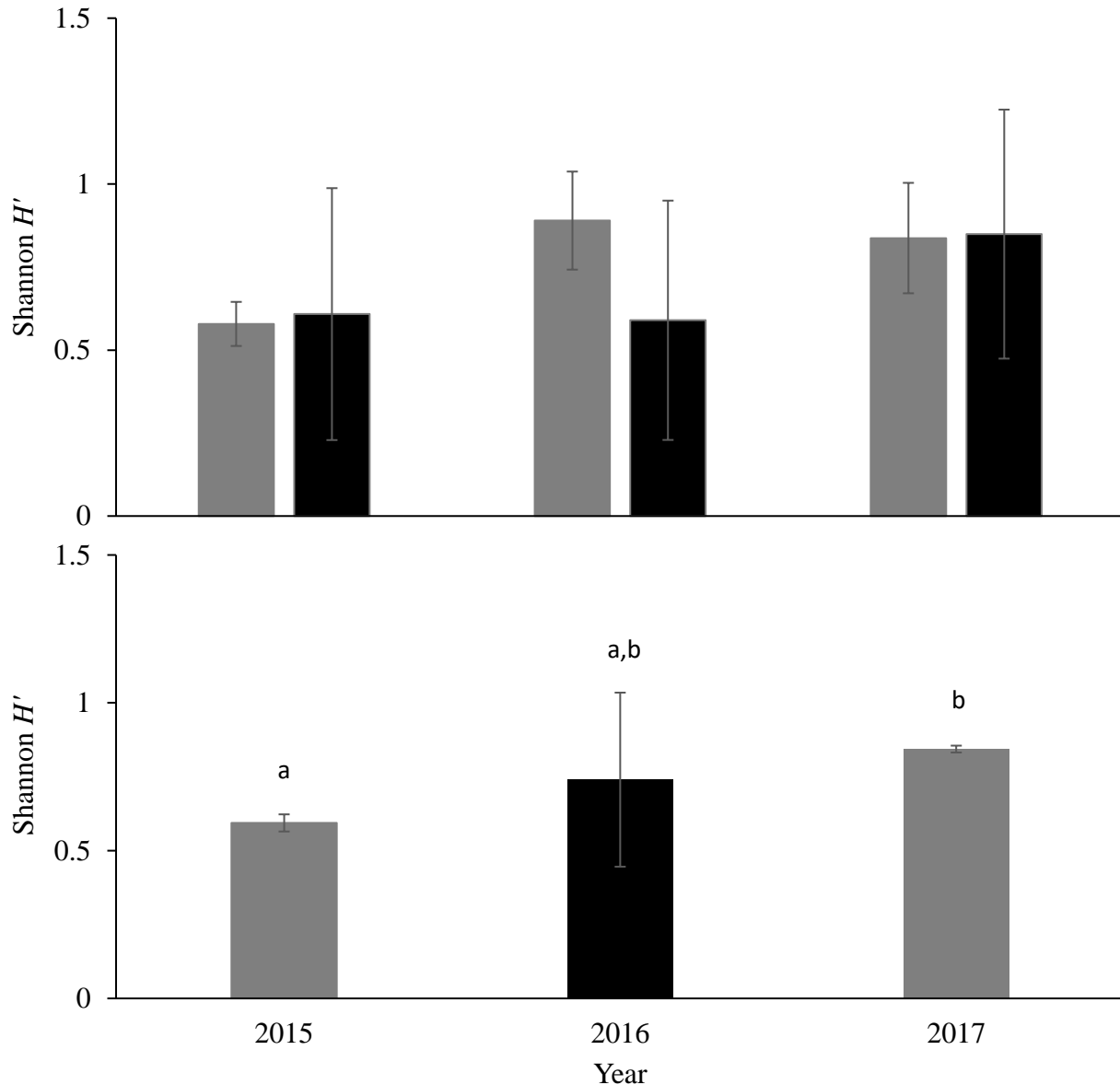
Figure 27. Mean copepod nauplii densities (number/L) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
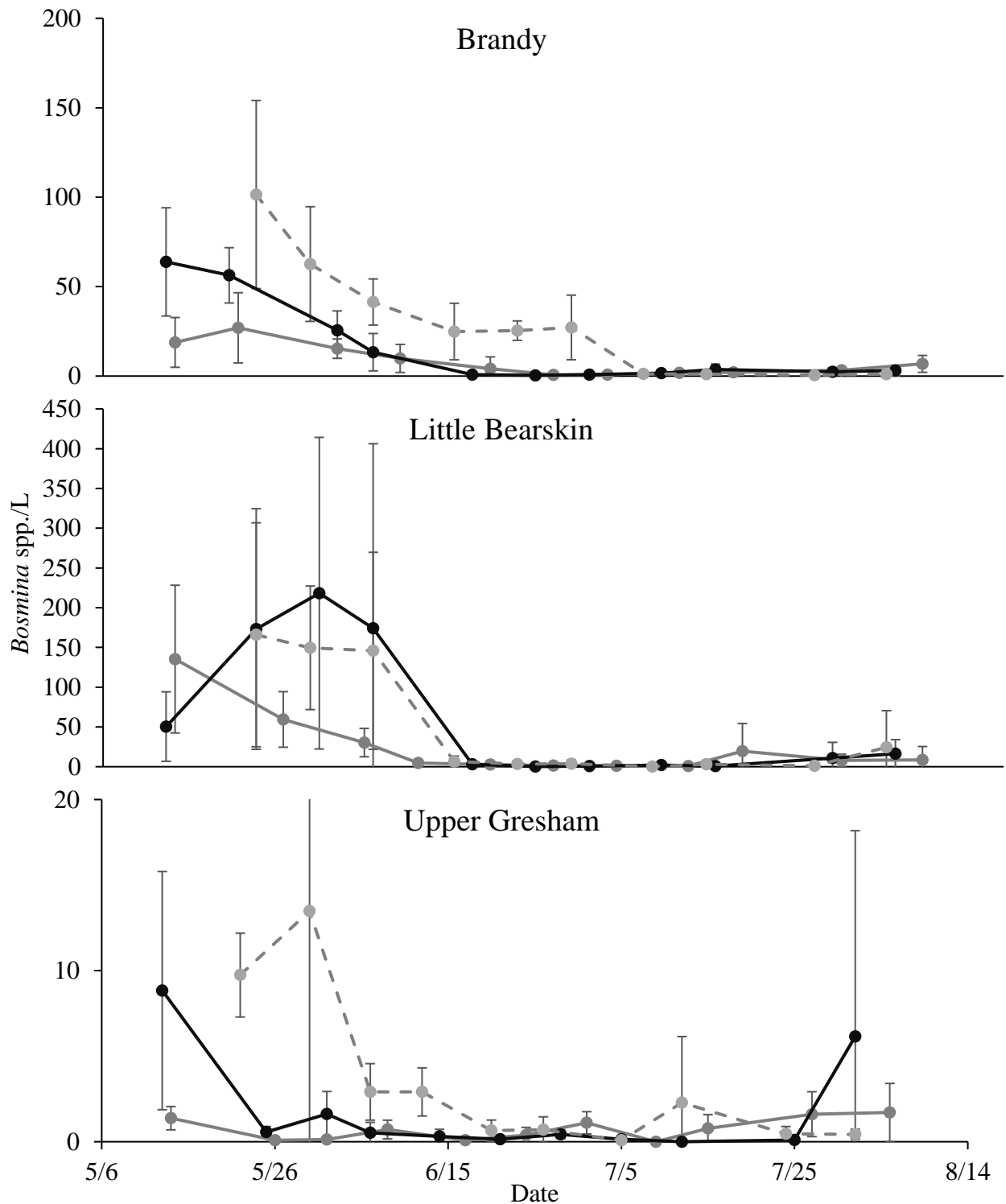
Figure 28. *Bosmina* spp. densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide treatment application dates for the three lakes treated with 2, 4-D herbicide in 2016. Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in *Bosmina* spp. densities among lakes.
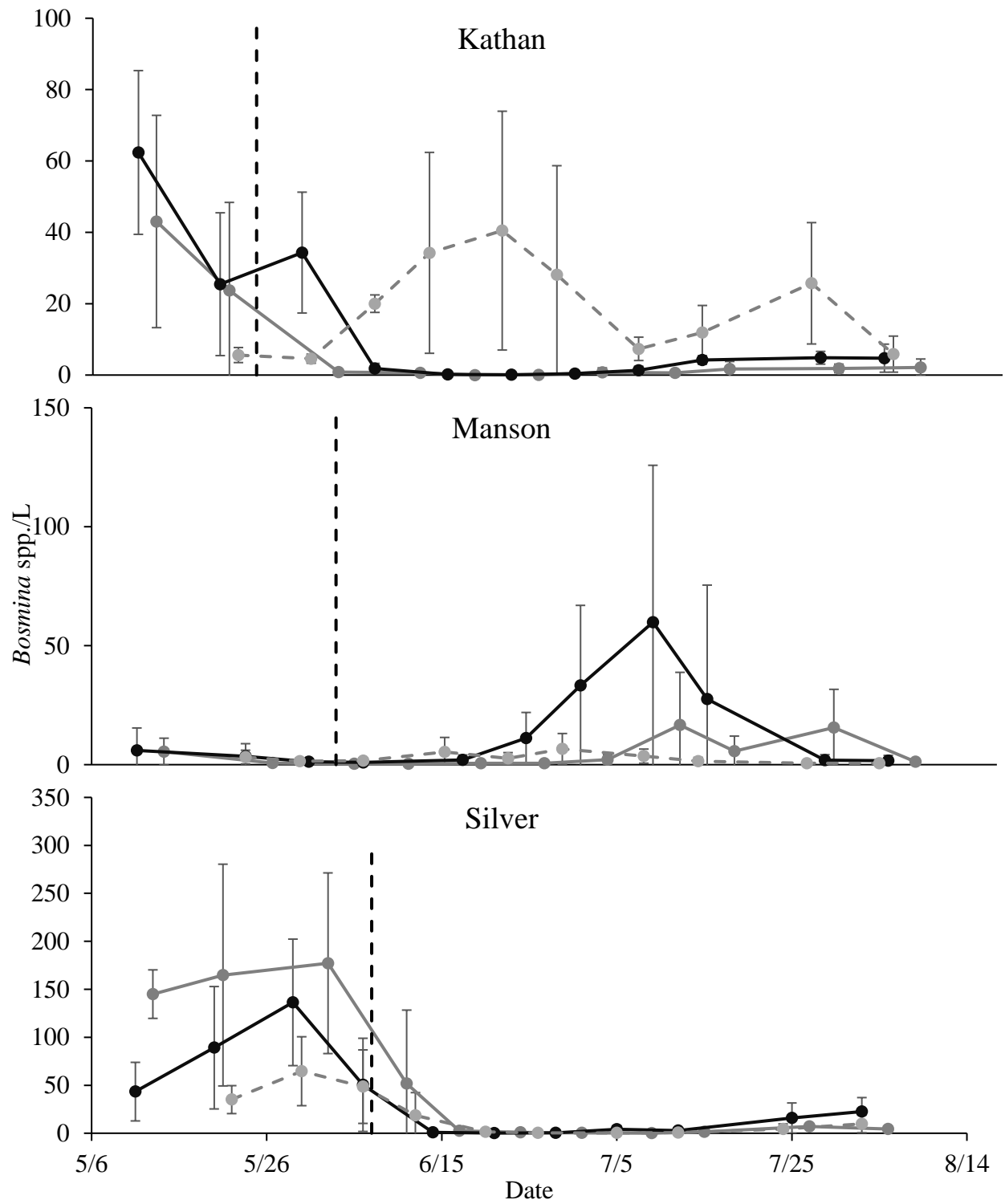
Figure 28 (continued).

Figure 29. Copepod nauplii densities (number/L) for 2015 (gray dashed line), 2016 (black solid line) and 2017 (gray solid line). Vertical dashed lines represent herbicide treatment dates for the three lakes treated with 2, 4-D herbicide in 2016. Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in nauplii densities among lakes.
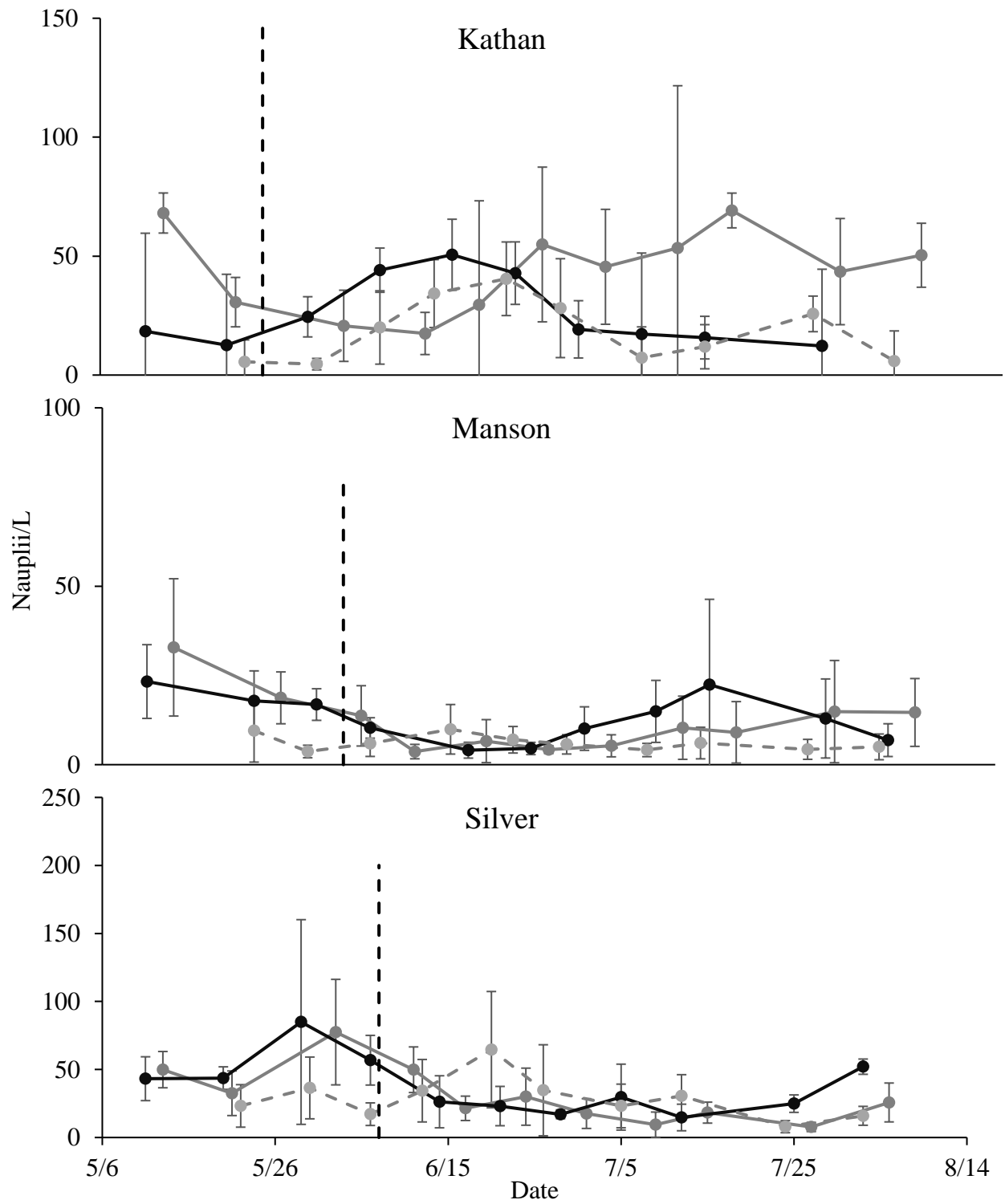
Figure 29 (continued).
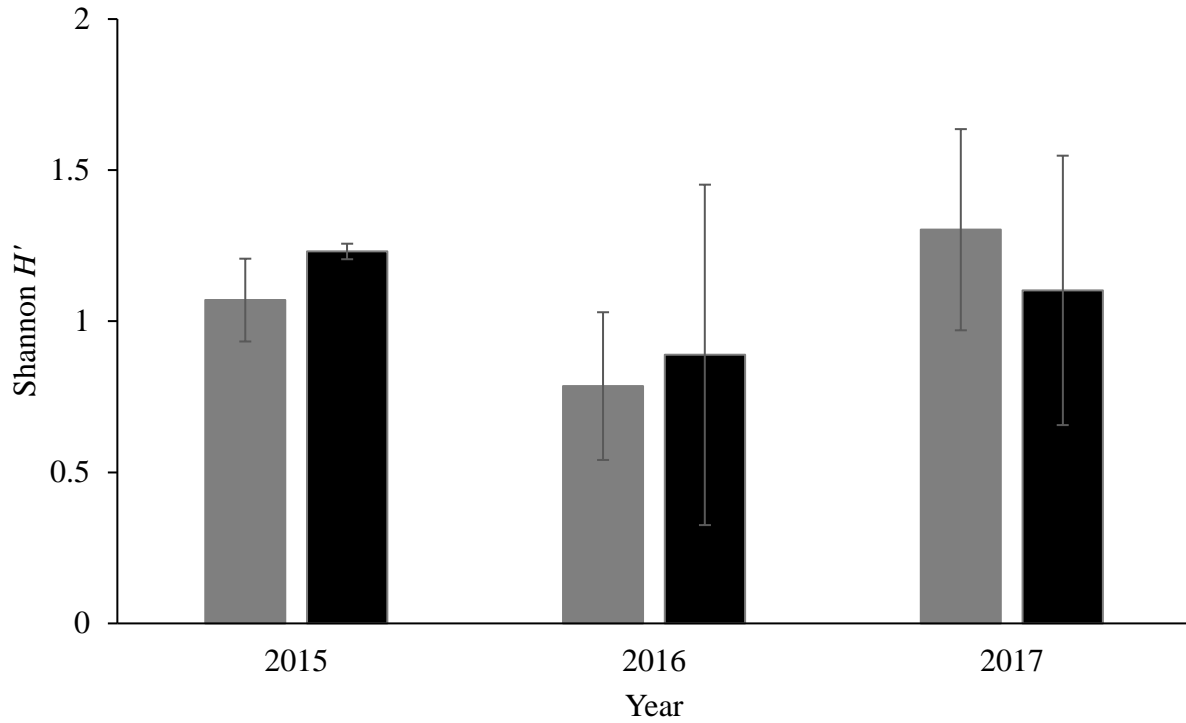
Figure 30. Mean Shannon's diversity index (*H'*) calculated from quatrefoil light trap CPE (fish/trap night) of larval fish species for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
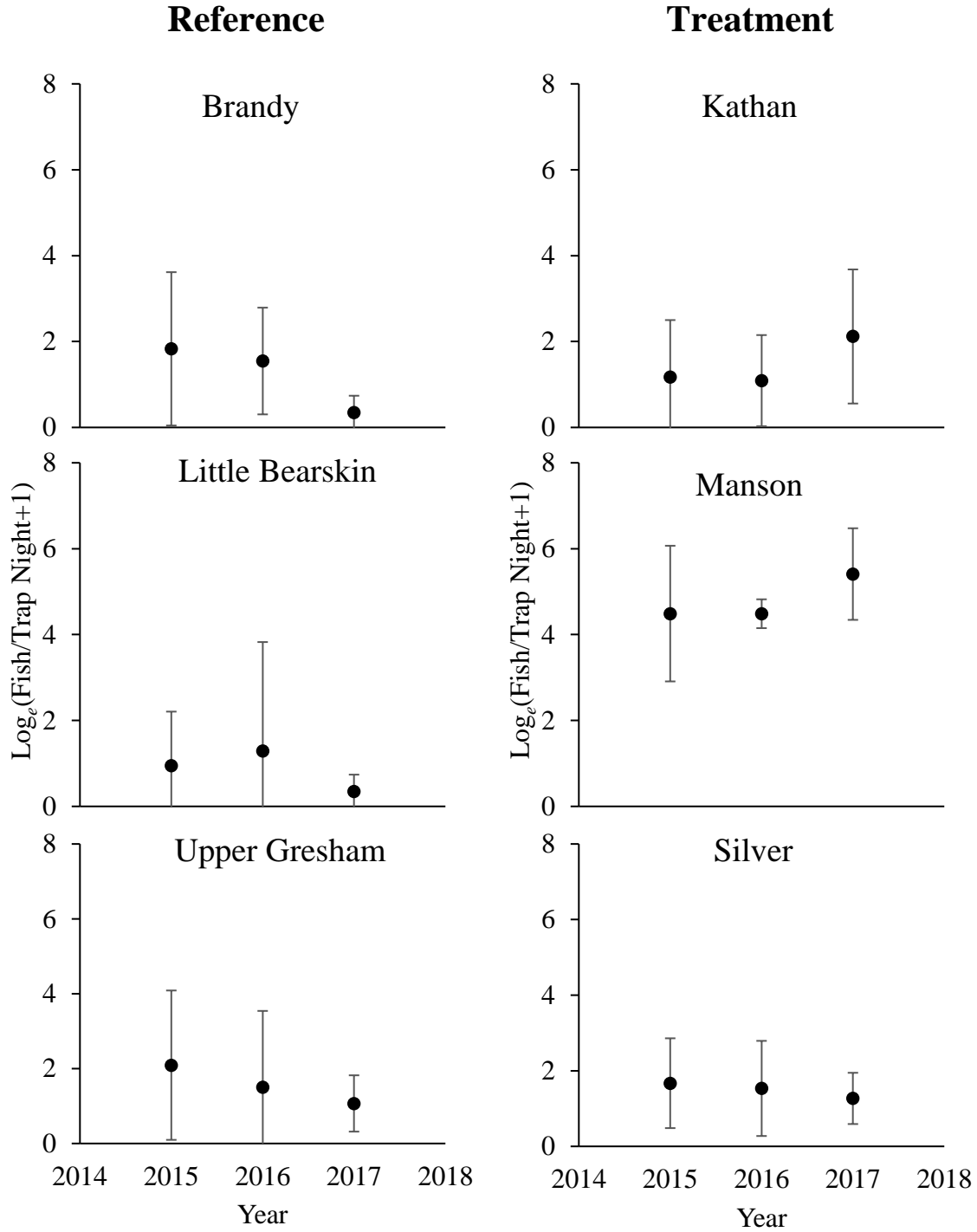
Figure 31. Peak catch-per-effort of $\log_e +1$ transformed larval cyprinids in quatrefoil light traps (fish/trap night) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals.

Figure 32. Peak log$_e$ catch-per-effort of larval cyprinids in quatrefoil light traps (fish/trap night) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals. Columns denoted with different letters were significantly different ($P < 0.05$; Table 2).

Figure 33. Peak catch-per-effort of larval Largemouth Bass in quatrefoil light traps (fish/trap night) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide tre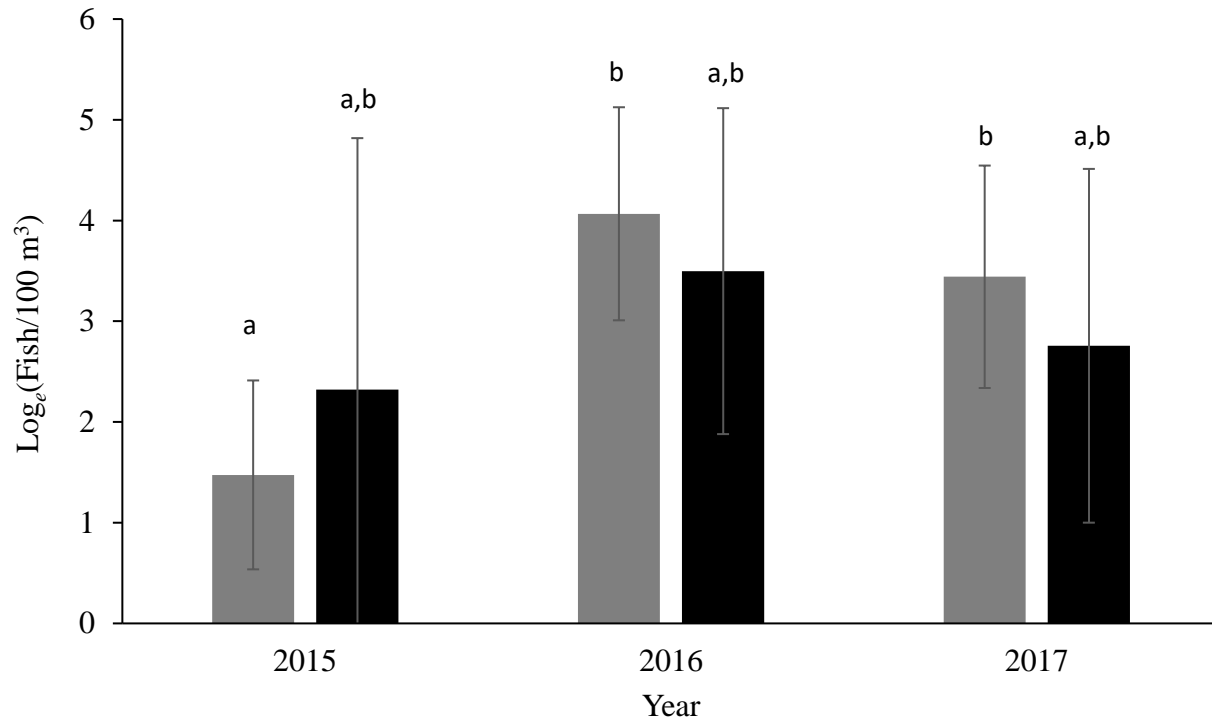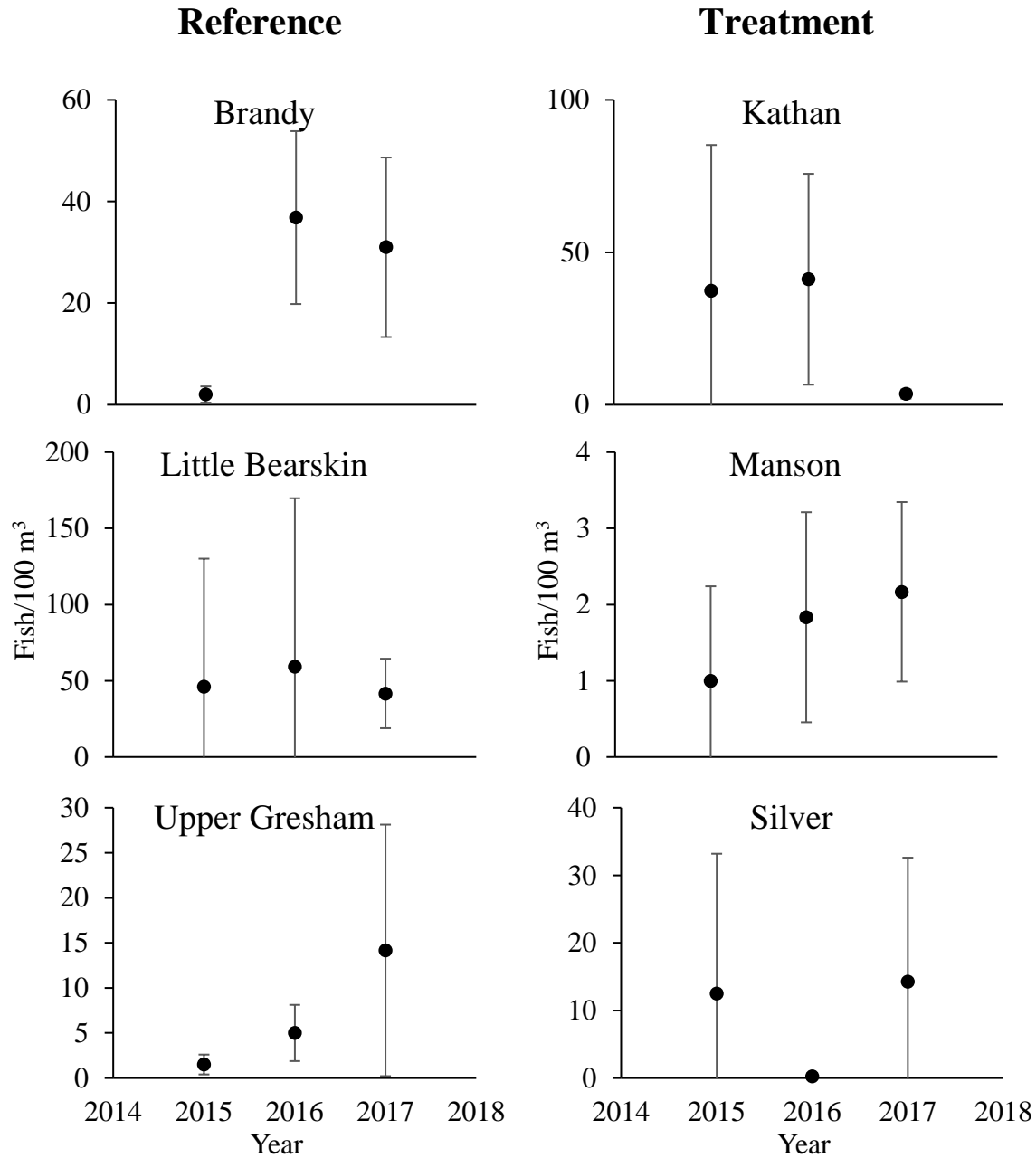atments (2017). Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in fish/trap night among lakes.
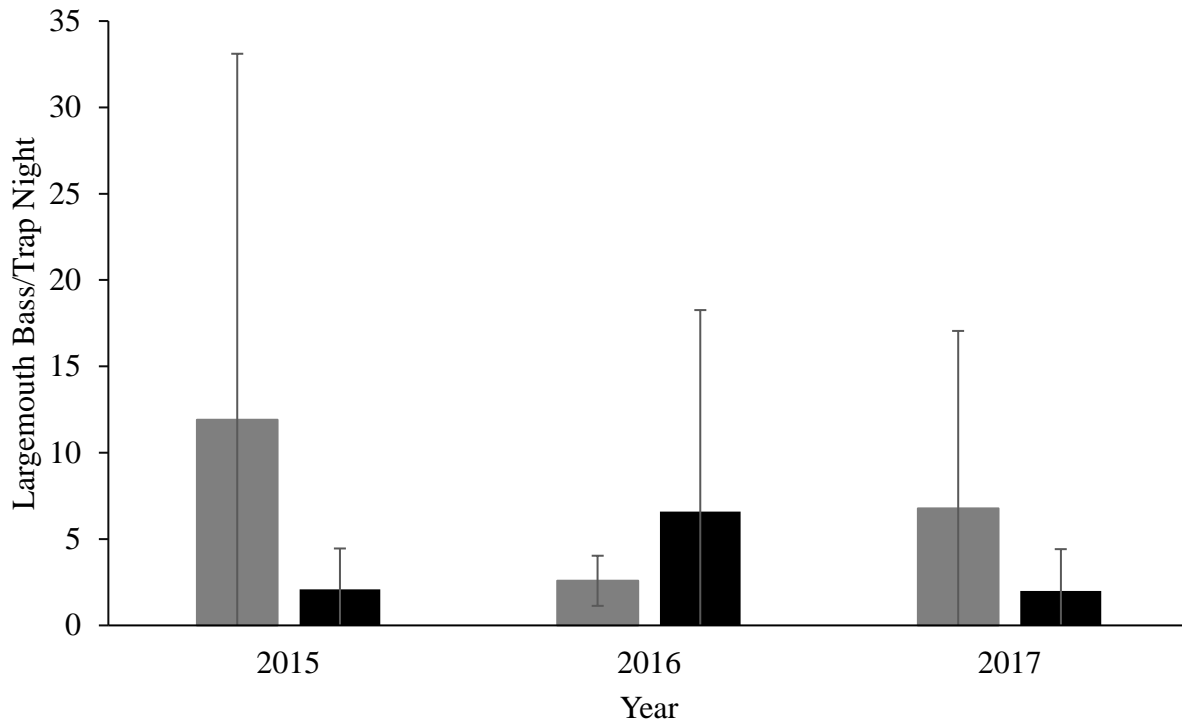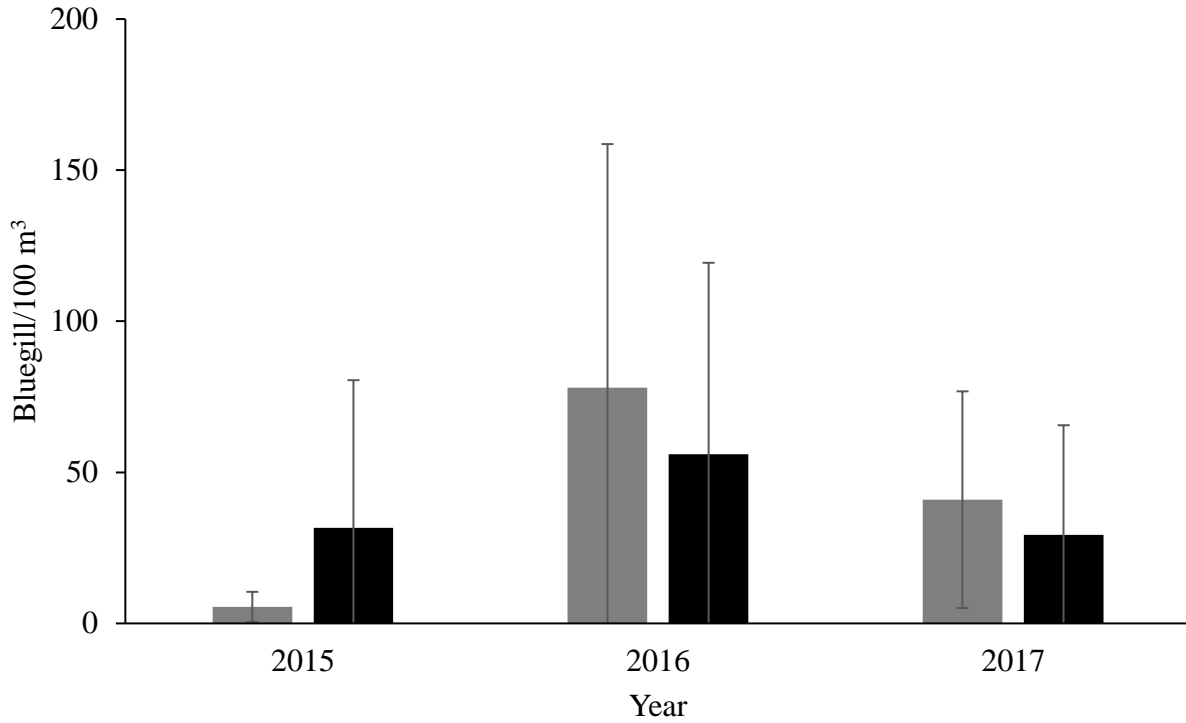
Figure 34. Mean peak larval Largemouth Bass catch-per-effort in quatrefoil light traps (fish/trap night) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.

Figure 35. Mean Shannon's diversity index (H') calculated using catch-per-effort (CPE) of larval fish taxa in ichthyoplankton tows (fish/100 m^3) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017 (top panel). The bottom panel shows H' for all lakes during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals. In the bottom panel, columns denoted with different letters were significantly different ($P < 0.05$; Table 2).
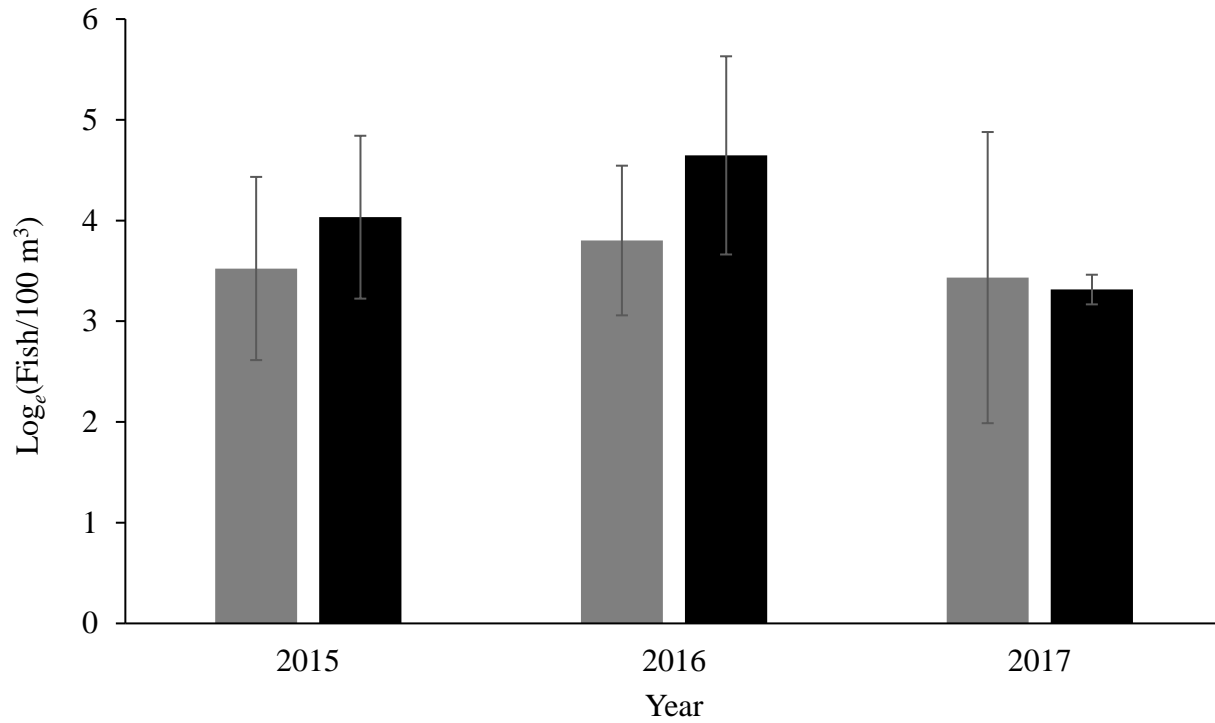
Figure 36. Mean peak abundance of $\log_e$ transformed larval Yellow Perch catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.
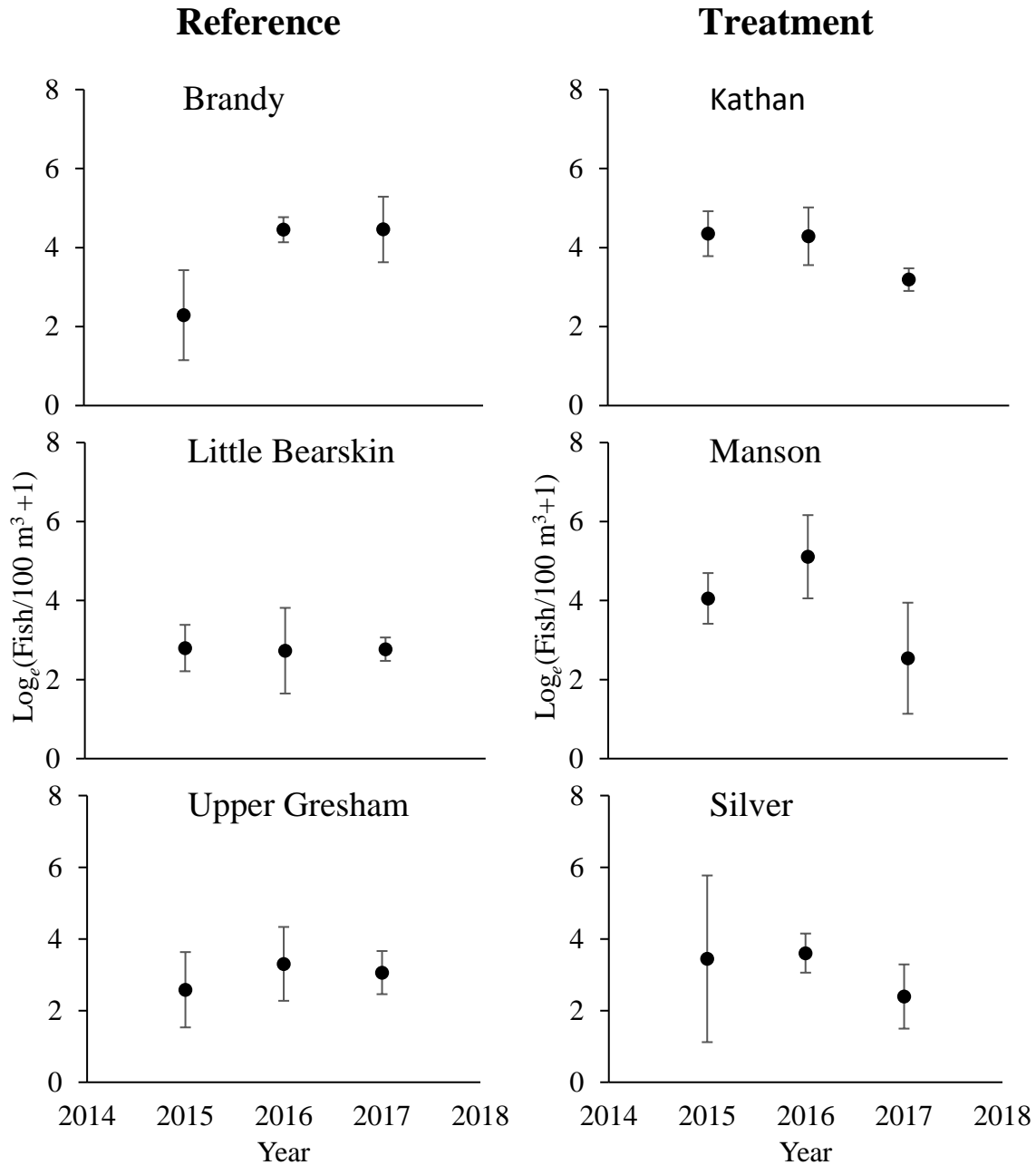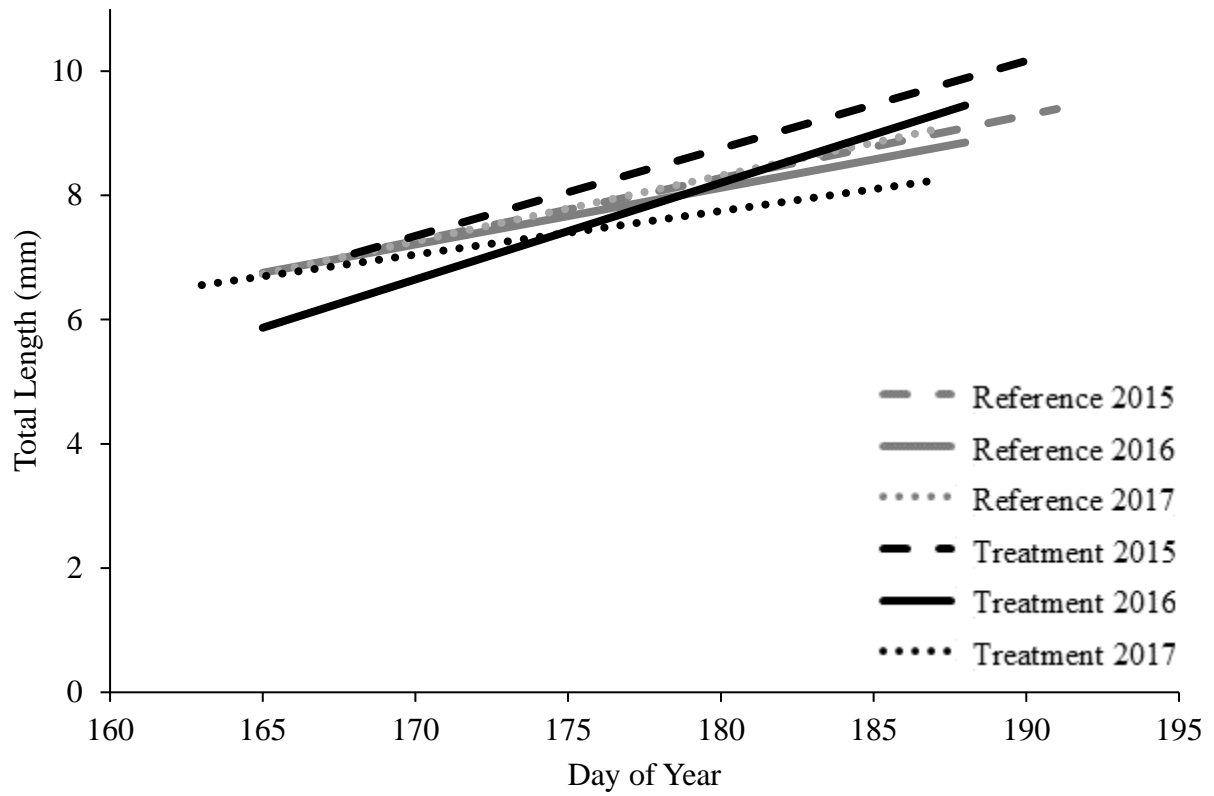
Figure 37. Peak abundance of $\log_e$ +1 transformed larval Yellow Perch catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals.
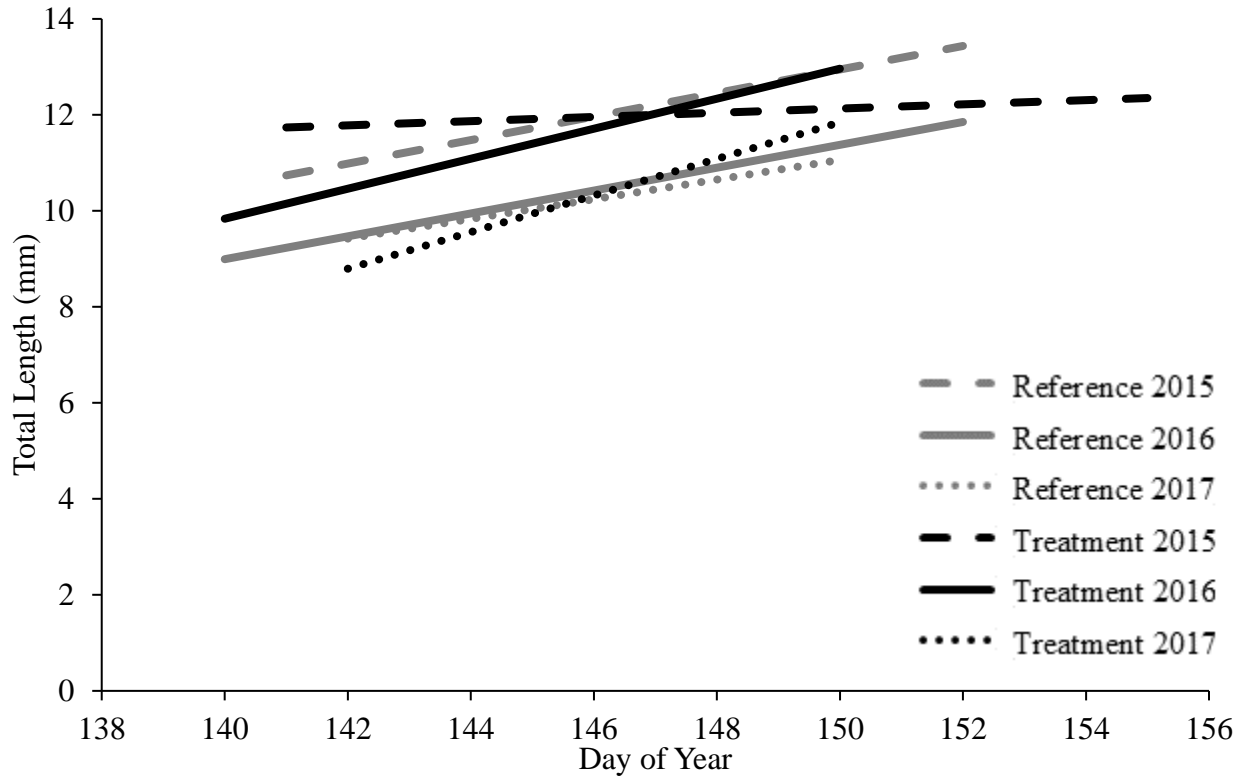
Figure 38. Mean Yellow Perch total length in relation day of year for reference and treatment lakes during 2015 to 2017 combinations.
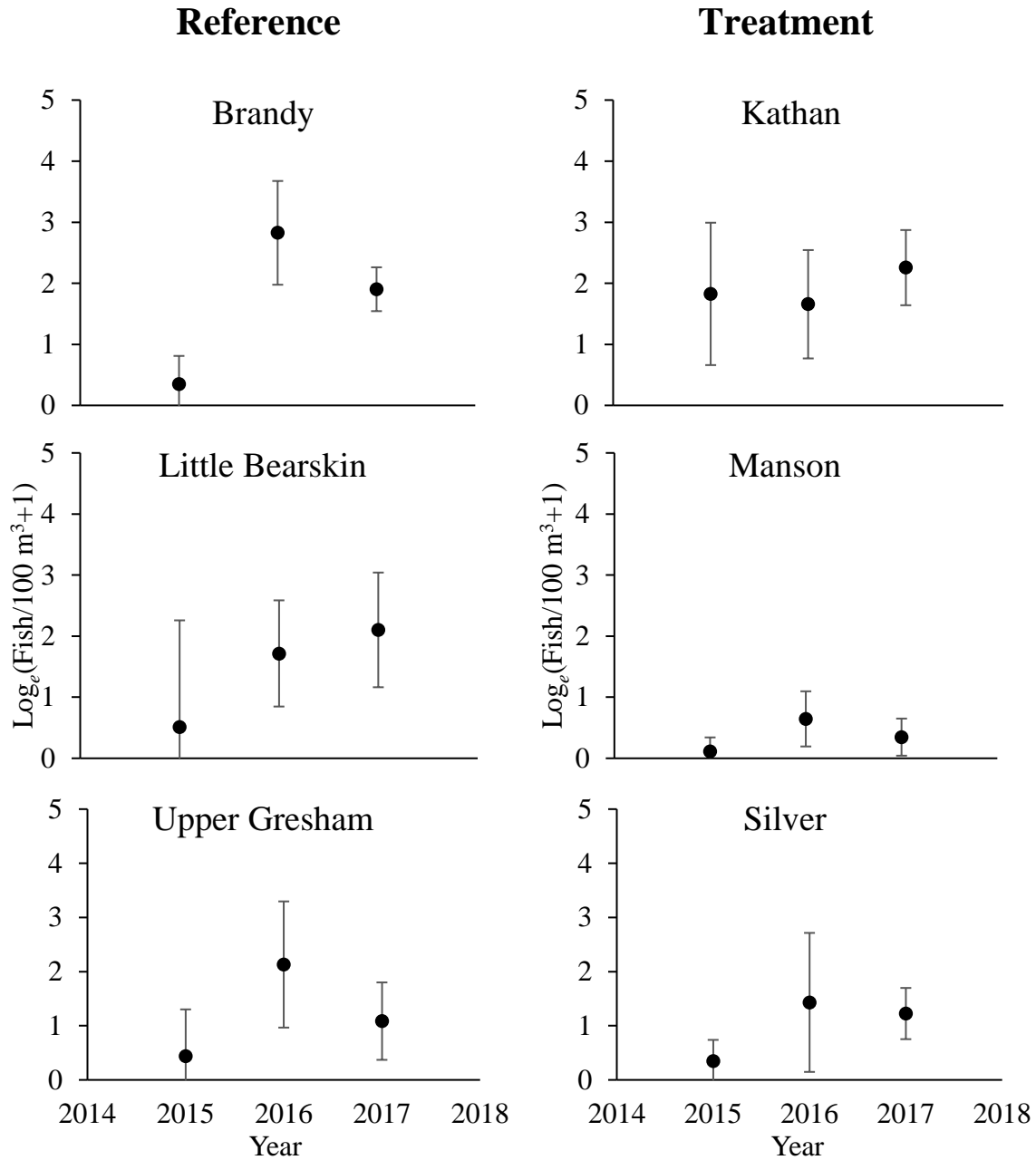
Figure 39. Peak abundance of $\log_e +1$ transformed larval Black Crappie catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals.

Figure 40. Mean peak abundance of log$_e$ transformed larval Black Crappie catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals. Columns denoted with different letters were significantly different ($P < 0.05$; Table 2).

Figure 41. Peak abundance of Bluegill catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals. Range of y-axis varies to account for differences in fish/100 m^3 among lakes.

Figure 42. Mean peak larval Bluegill catch-per-effort in ichthyoplankton nets (fish/100 m^3) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars are 95% confidence intervals.

Figure 43. Bluegill total length across day of year for all lake type-year combinations. Sampled were collected using ichthyoplankton tows from sampling periods 3 through 5.
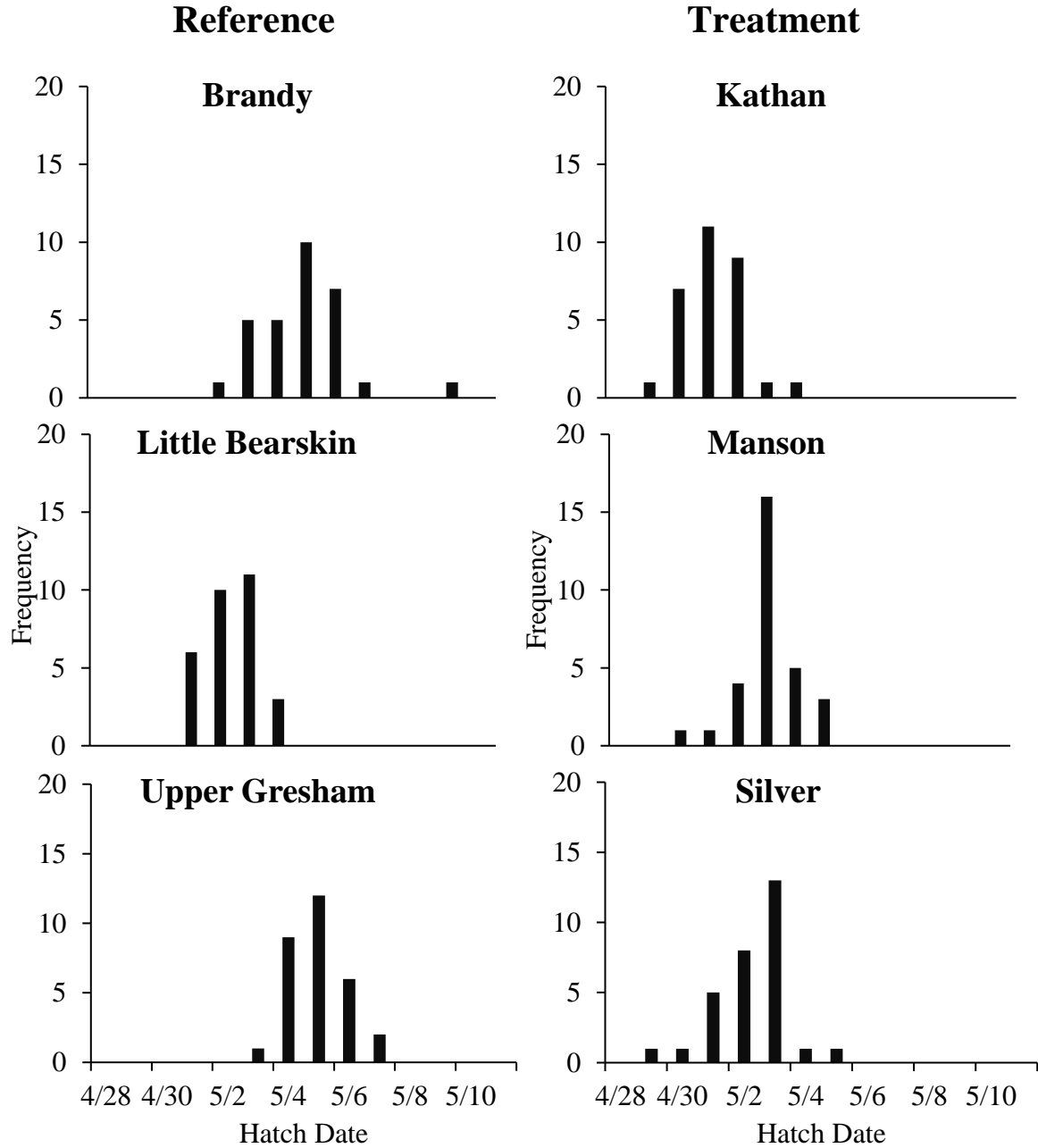
Figure 44. Hatch dates of larval Yellow Perch sampled from reference (left column) and treatment lakes (right column) on the date peak catch-per-effort in ichthyoplankton nets was observed in 2015. Hatch dates were estimated using otolith daily ring counts.
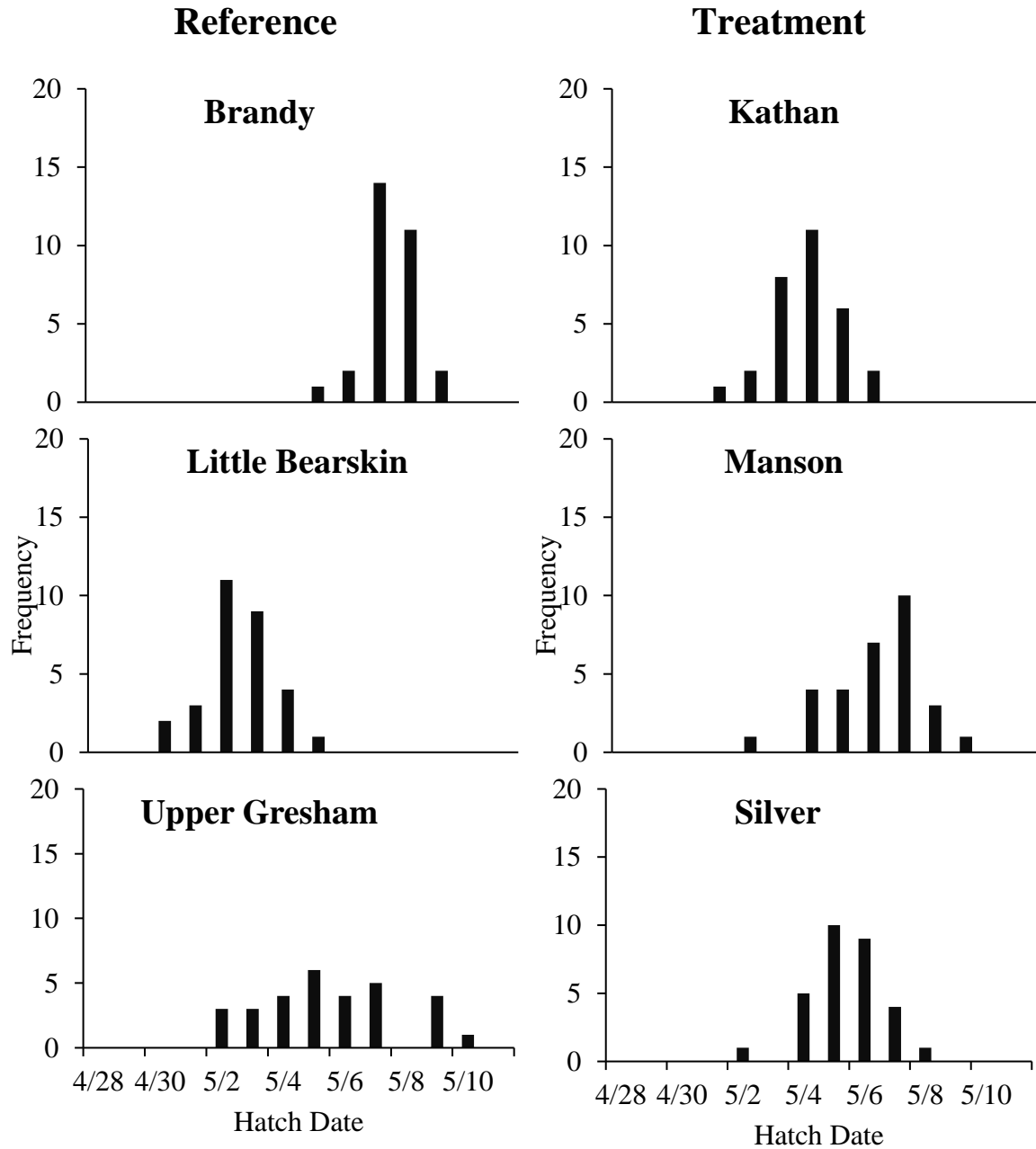
Figure 45. Hatch dates of larval Yellow Perch sampled from reference (left column) and treatment lakes (right column) on the date peak catch-per-effort in ichthyoplankton nets was observed in 2016. Hatch dates were estimated using otolith daily ring counts.
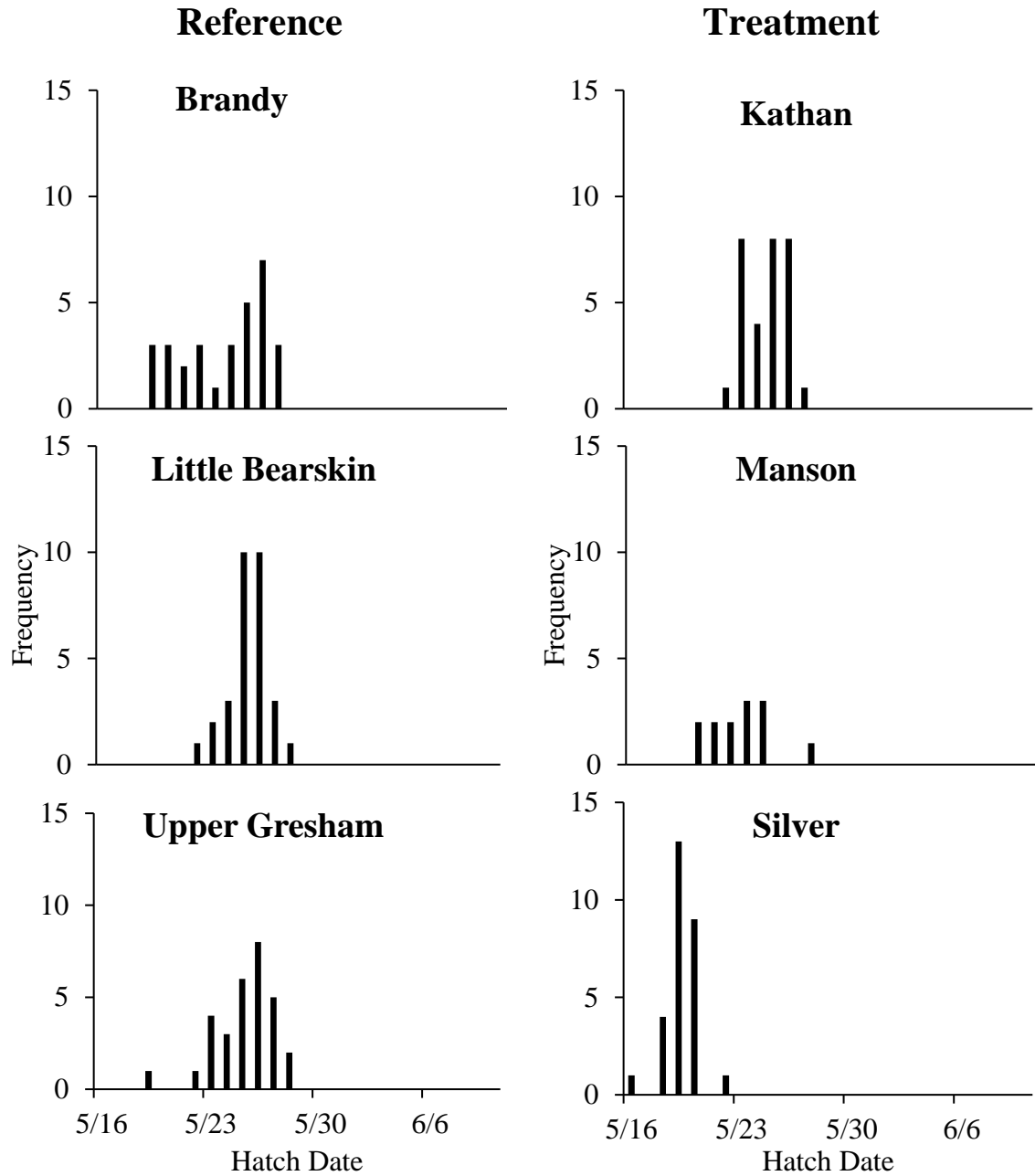
Figure 46. Hatch dates of larval Black Crappie sampled from reference (left column) and treatment lakes (right column) on the date peak catch-per-effort in ichthyoplankton nets was observed in 2016. Hatch dates were estimated using otolith daily ring counts.
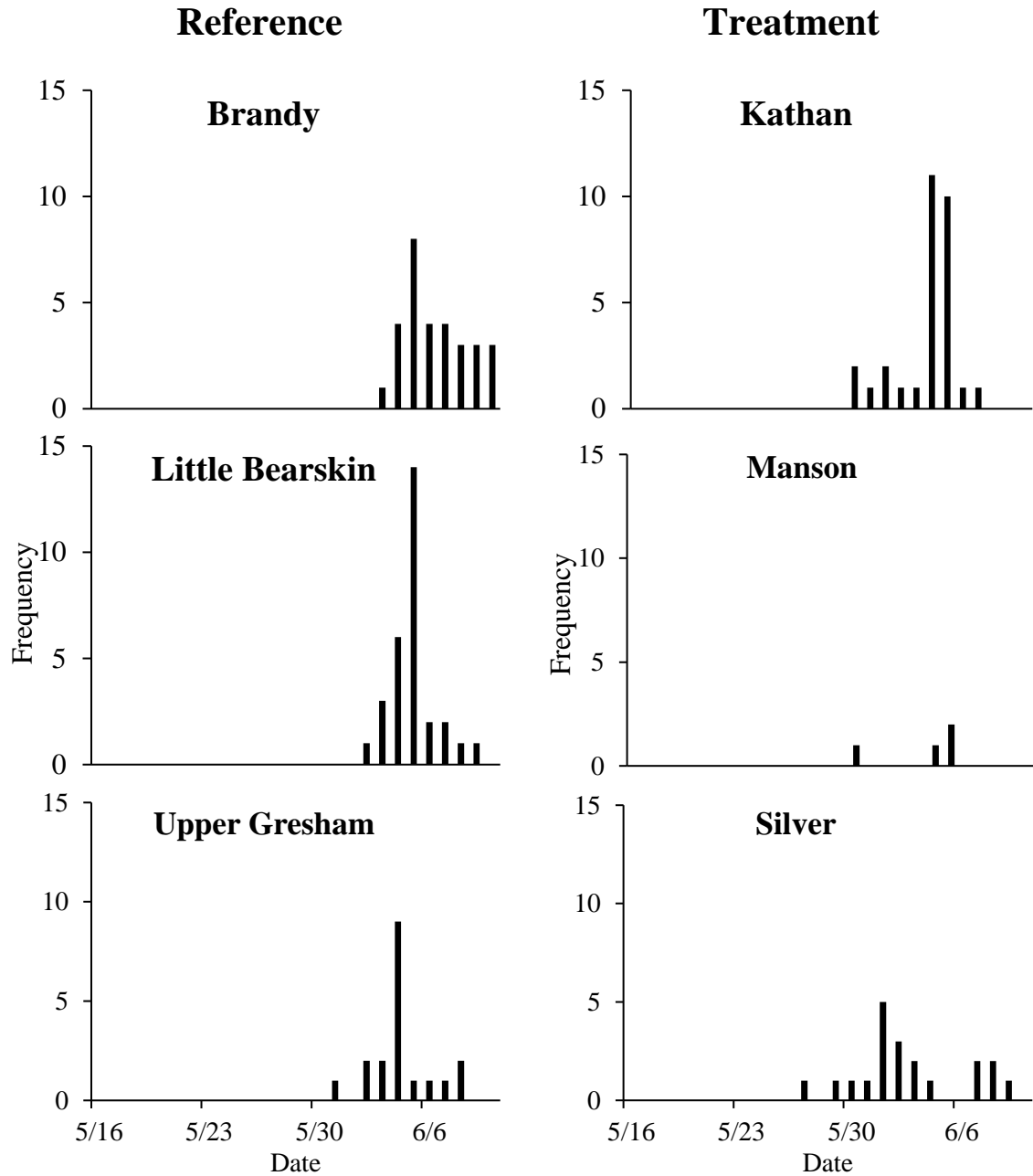
Figure 47. Hatch dates of larval Black Crappie sampled from reference (left column) and treatment lakes (right column) on the date peak catch-per-effort in ichthyoplankton nets was observed in 2017. Hatch dates were estimated using otolith daily ring counts.
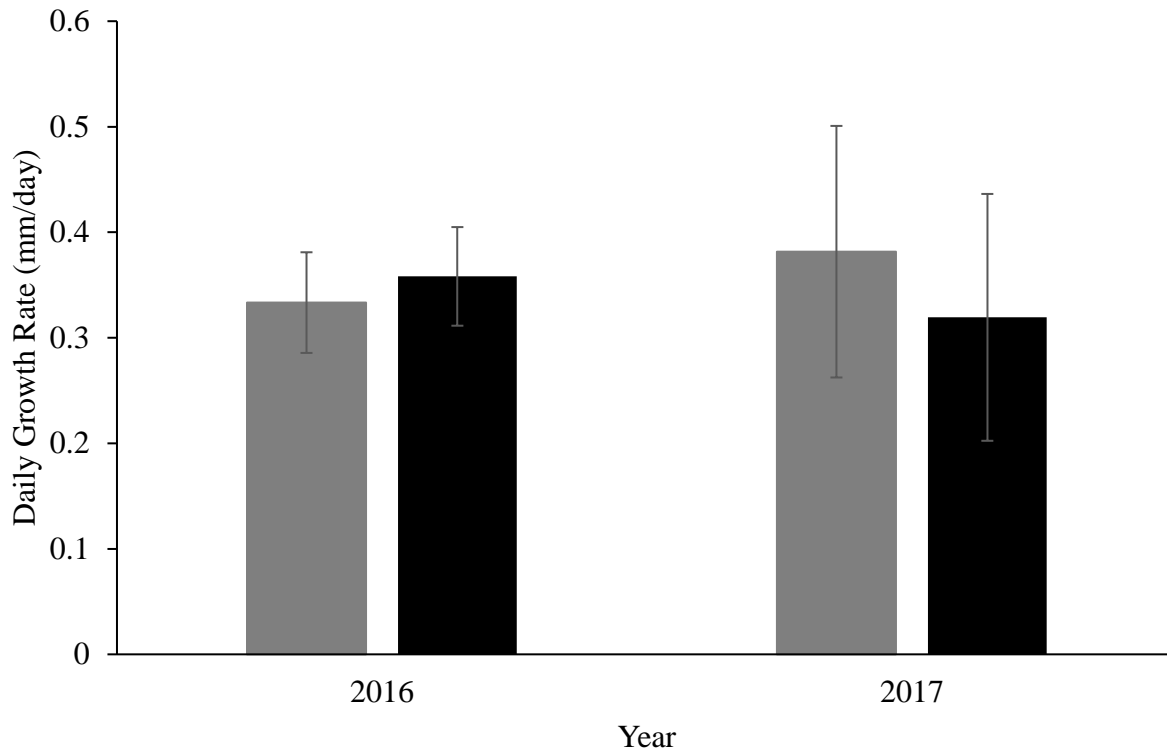
Figure 48. Mean larval Black Crappie daily growth rate (mm/day) for reference (gray bars) and treatment lakes (black bars) in 2016 and 2017. Error bars represent 95% confidence intervals.
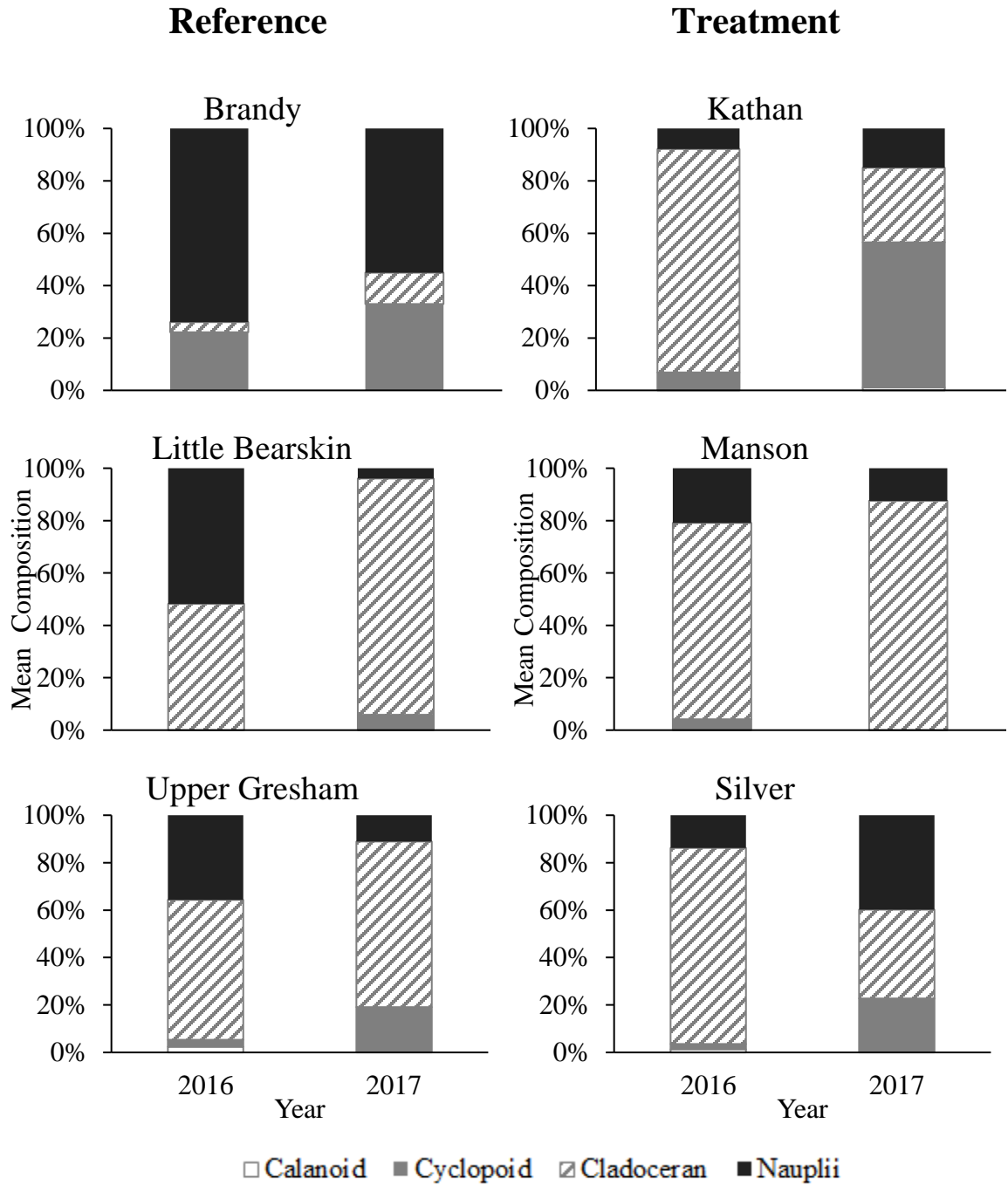
Figure 49. Larval Black Crappie percent diet composition by number of calanoid copepods, cyclopoid copepods, cladocerans and copepod nauplii for reference (left column) and treatment lakes (right column) in 2016 and 2017.
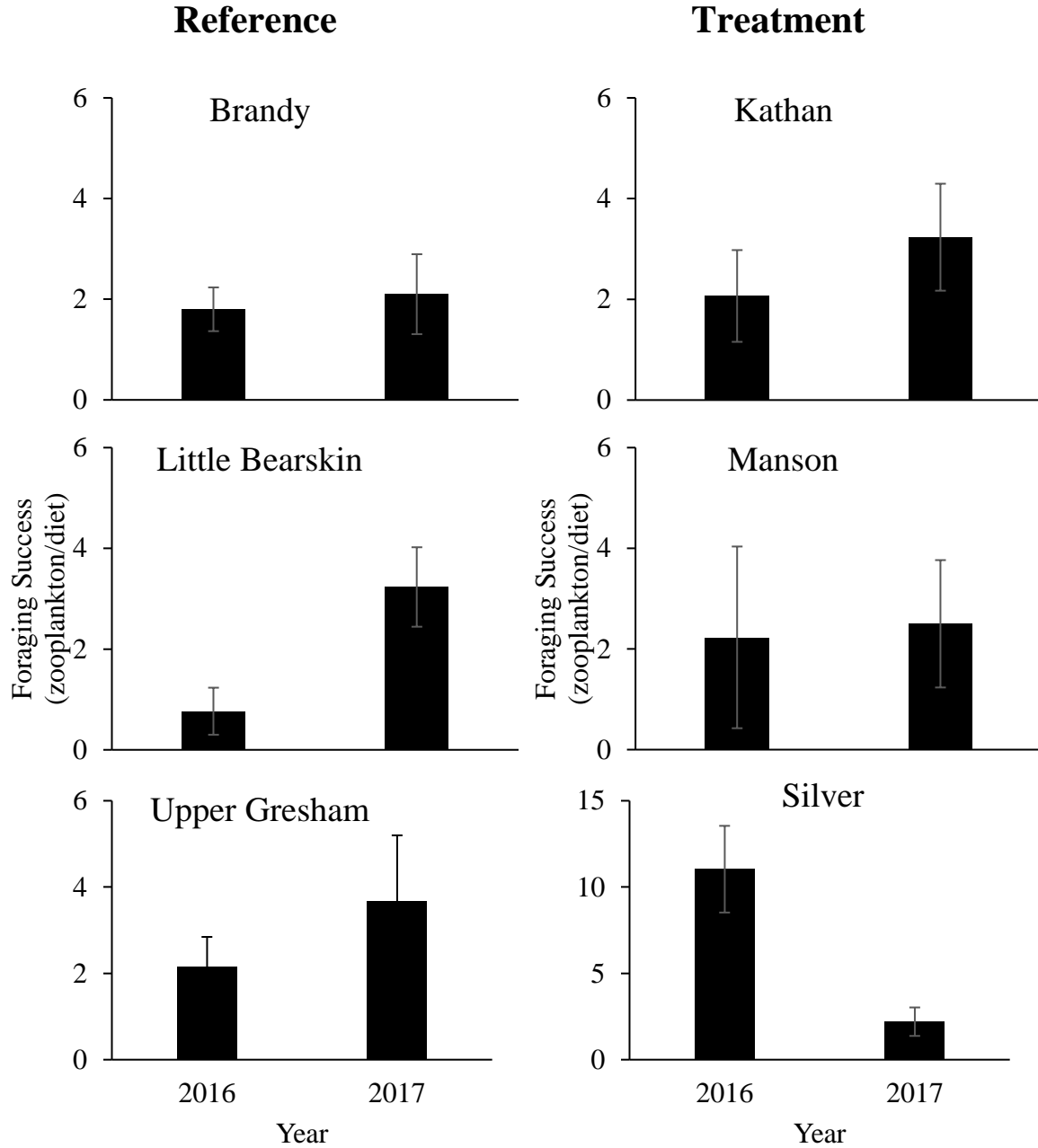
Figure 50. Mean larval Black Crappie daily foraging success as measured by zooplankton in the diets of individual crappie (*y*-axis) for reference (left column) and treatment lakes (right column) from 2016 and 2017. Error bars represent 95% confidence intervals. Range of *y*-axis varies to allow for differences in foraging success observed among lakes.
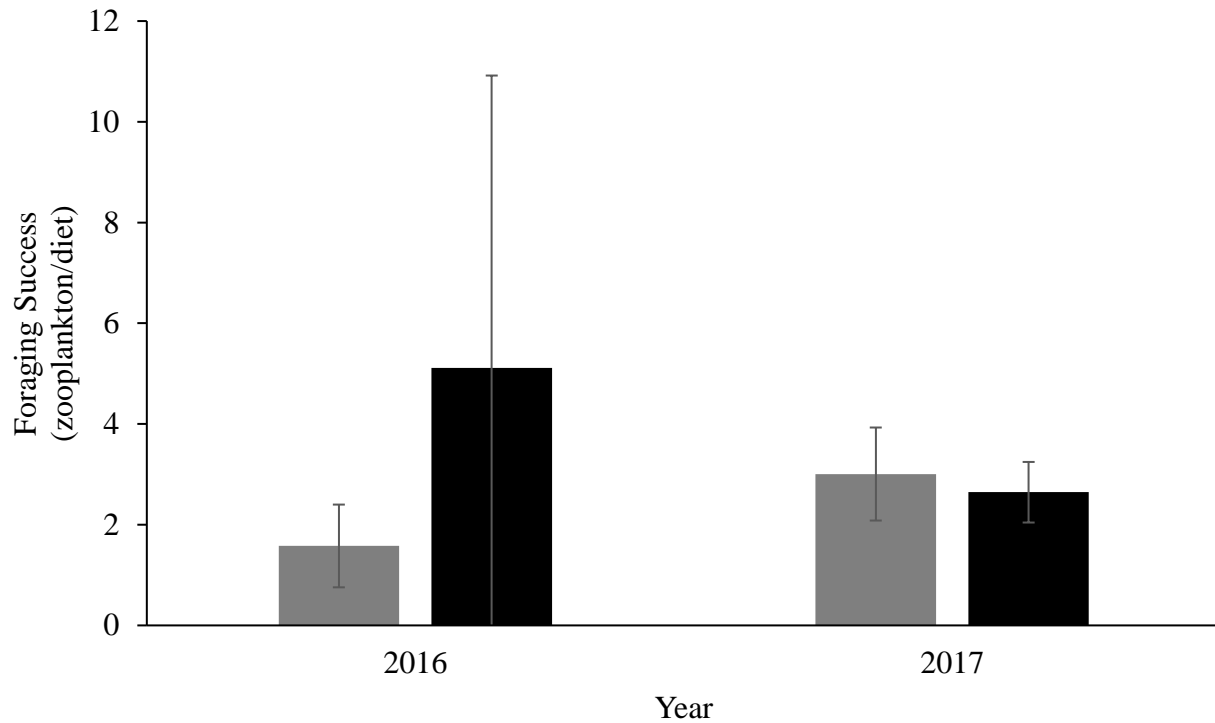
Figure 51. Mean larval Black Crappie foraging success (zooplankton/diet) for reference (gray bars) and treatment lakes (black bars) in 2016 and 2017. Error bars represent 95% confidence intervals.
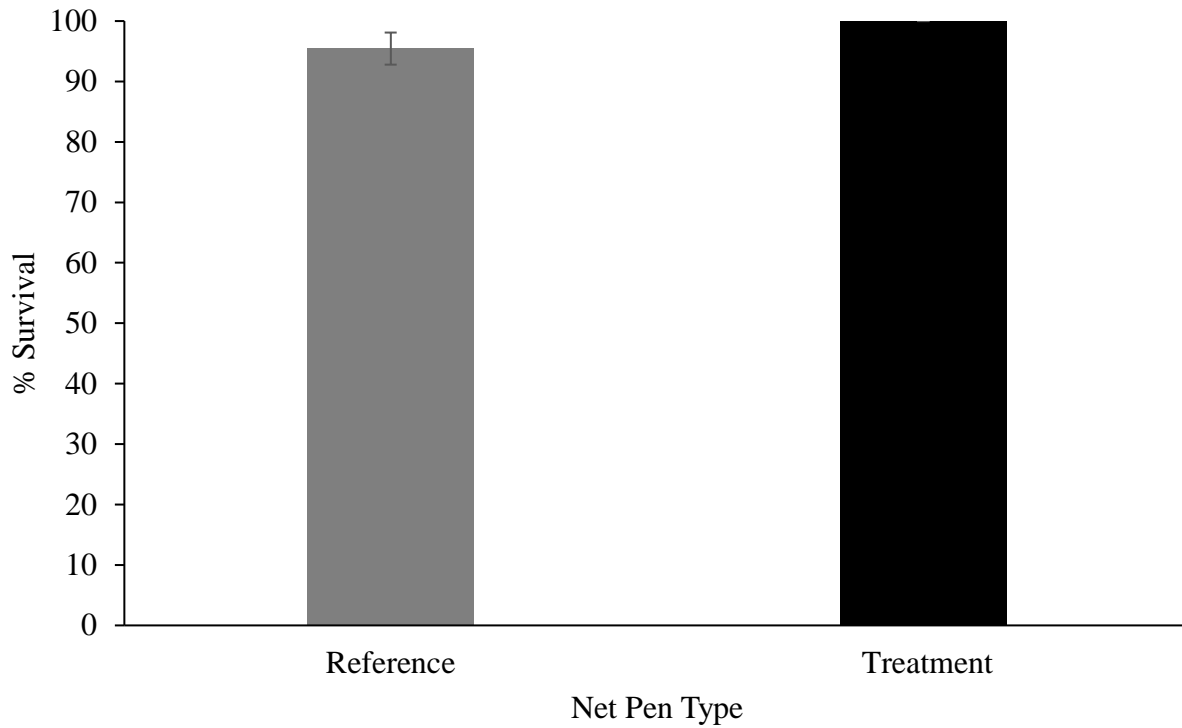
Figure 52. Percent 48-h survival of juvenile (< 125 mm total length) Yellow Perch during net pen trials. Net pen trials were considered reference (gray bars; N=15) when no 2, 4-D herbicide was present. Treatment trials (black bars; N=3) were conducted in 2016 when the herbicide was present in the three treatment lakes. Error bars represent 95% confidence intervals.
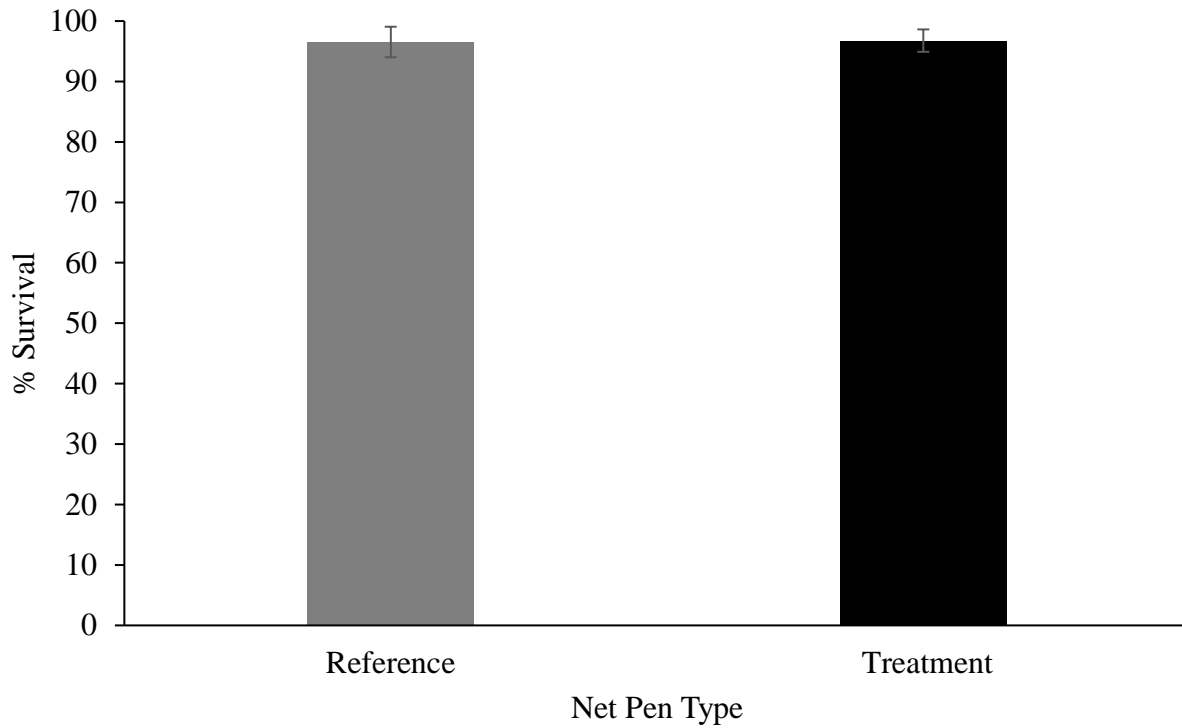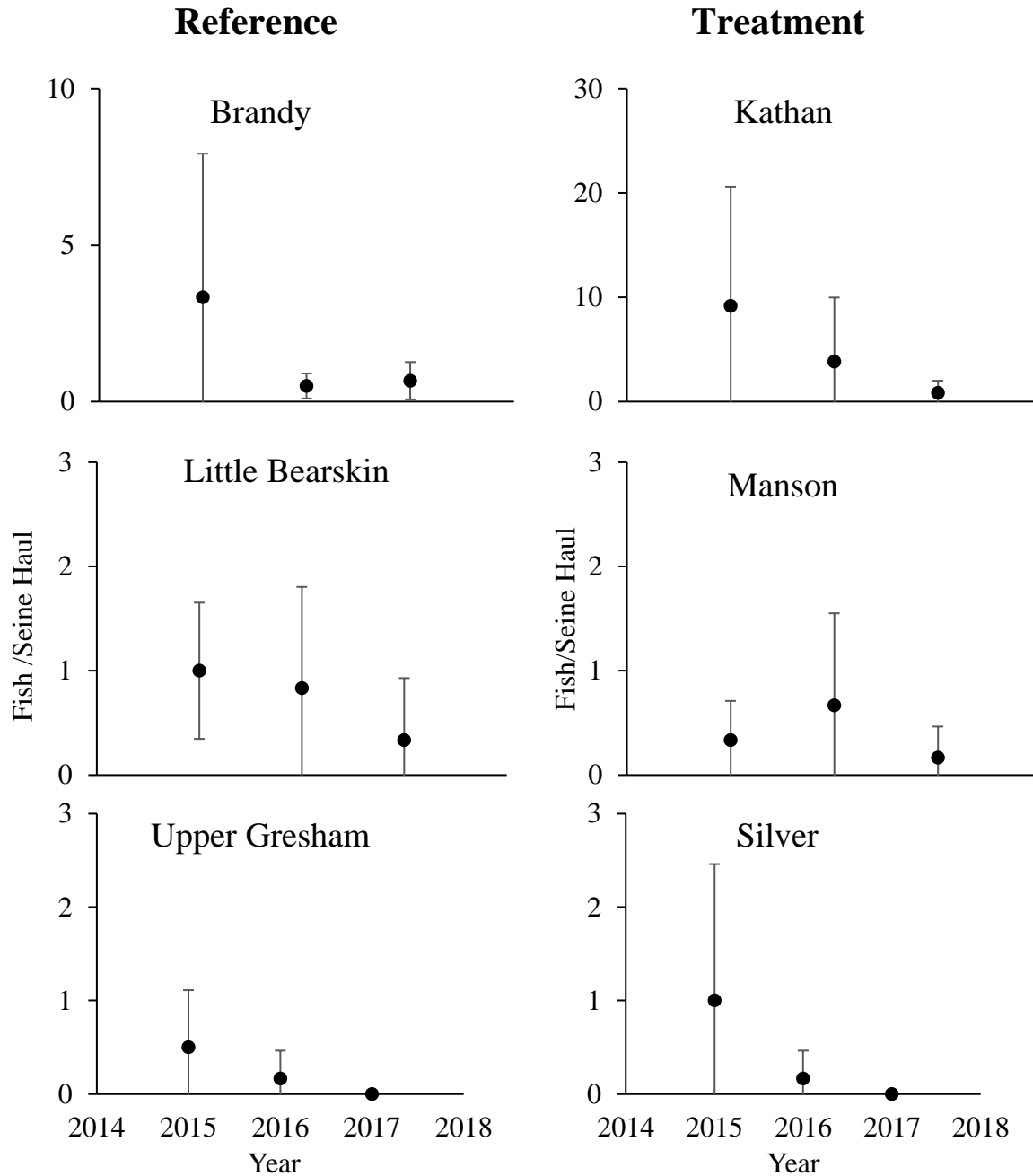
Figure 53. Percent 48-h survival of juvenile (< 125 mm total length) Bluegill during net pen trials. Net pen trials were considered reference (gray bars; N=24) when no 2, 4-D herbicide was present. Treatment trials (black bars; N=12) were conducted in 2016 when the herbicide was present in the three treatment lakes. Error bars represent 95% confidence intervals.

Figure 54. Juvenile Yellow Perch (< 70 mm total length) seine catch-per-effort (fish/seine haul) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals. Range of *y*-axis varies to account for differences in juvenile Yellow Perch seine catch-per-effort among lakes.
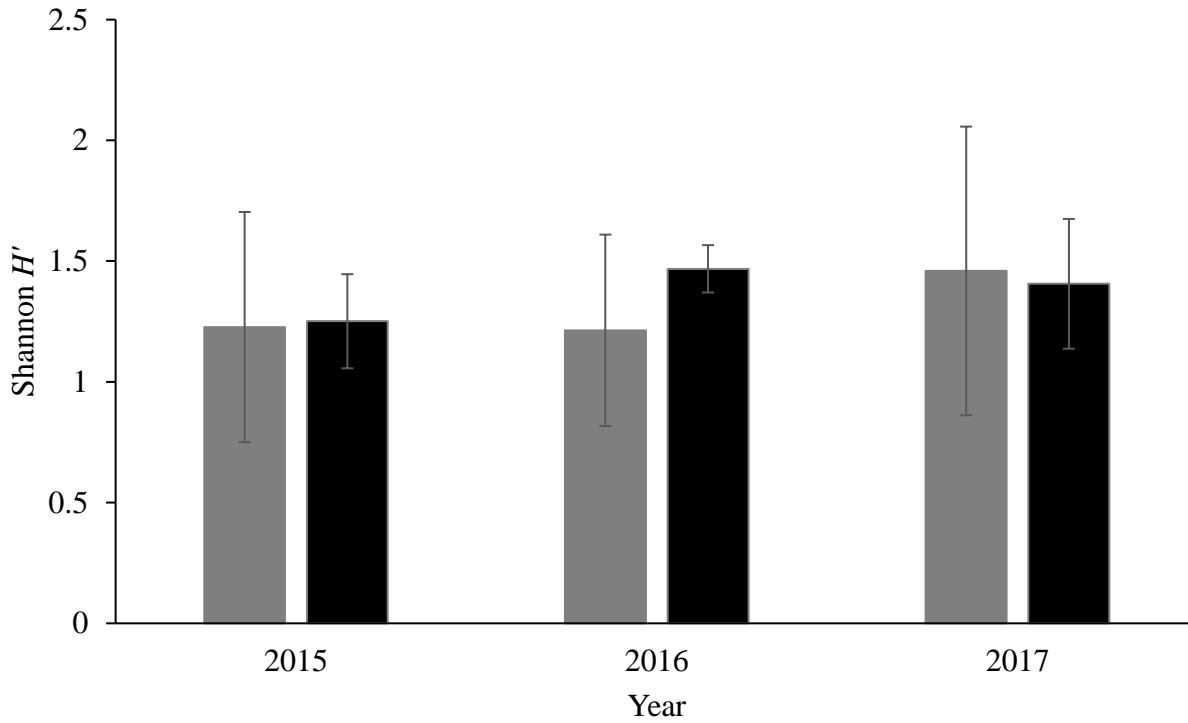
Figure 55. Mean Shannon's diversity index (H') calculated using catch-per-effort of fish species in seine hauls (fish/seine haul) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Samples were collected using shoreline seining from May through August of each year. Error bars are 95% confidence intervals.
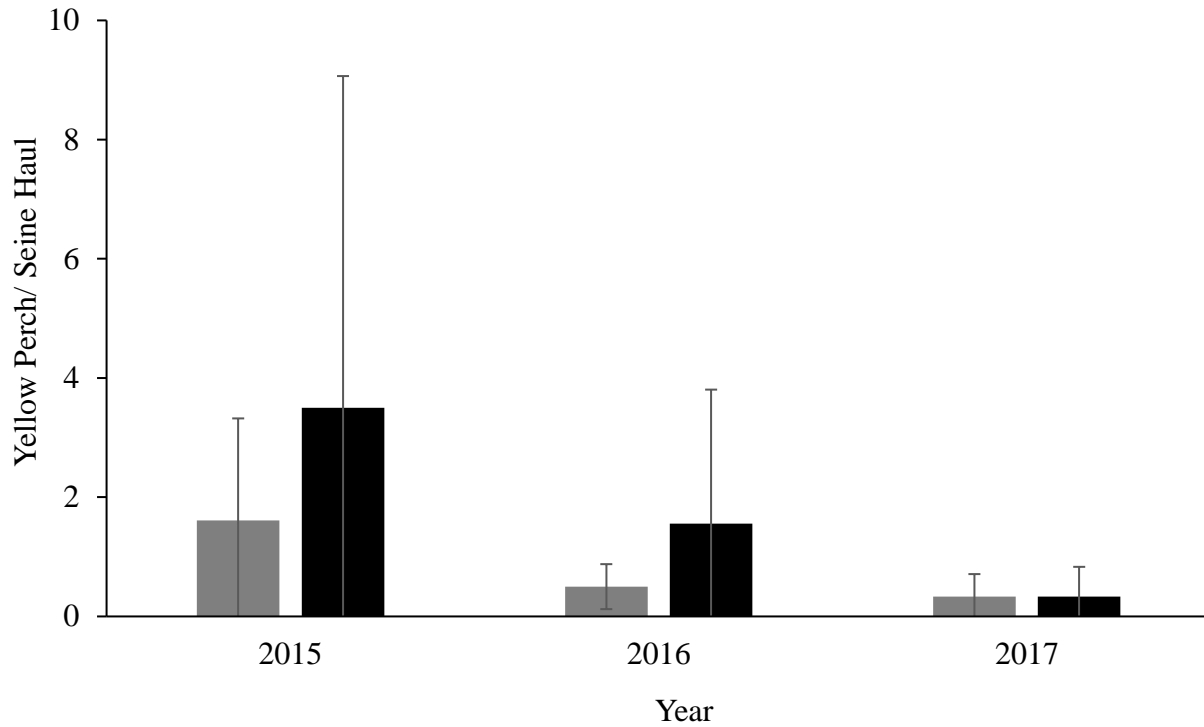
Figure 56. Mean juvenile Yellow Perch (< 70 mm total length) catch-per-effort in seine hauls (fish/seine haul) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars represent 95% confidence intervals.
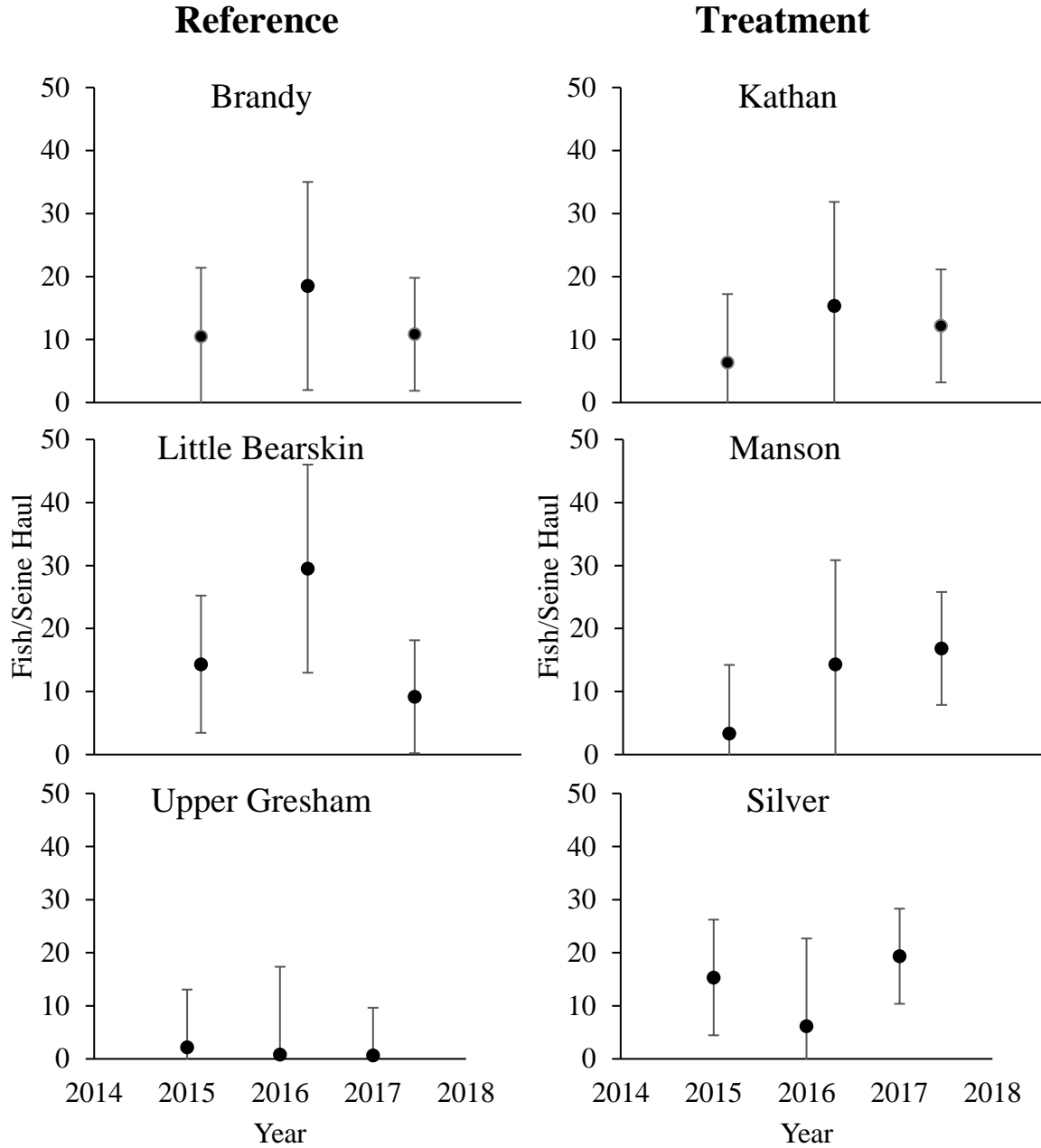
Figure 57. Bluegill (< 100 mm total length) seine catch-per-effort (fish/seine haul) for reference (left column) and treatment lakes (right column) during the year before herbicide treatments occurred (2015), the year of the herbicide treatments (2016), and the year after herbicide treatments (2017). Error bars are 95% confidence intervals.
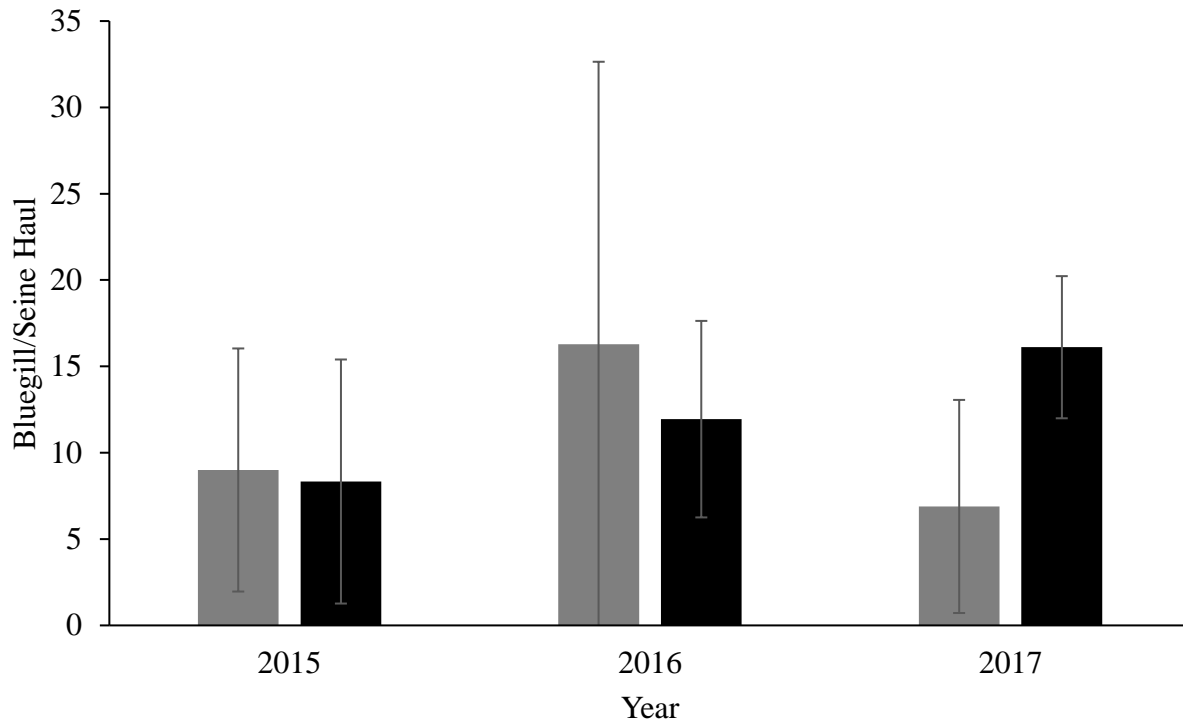
Figure 58. Mean Bluegill (< 100 mm total length) catch-per-effort in seine hauls (fish/seine haul) for reference (gray bars) and treatment lakes (black bars) from 2015 to 2017. Error bars represent 95% confidence intervals.
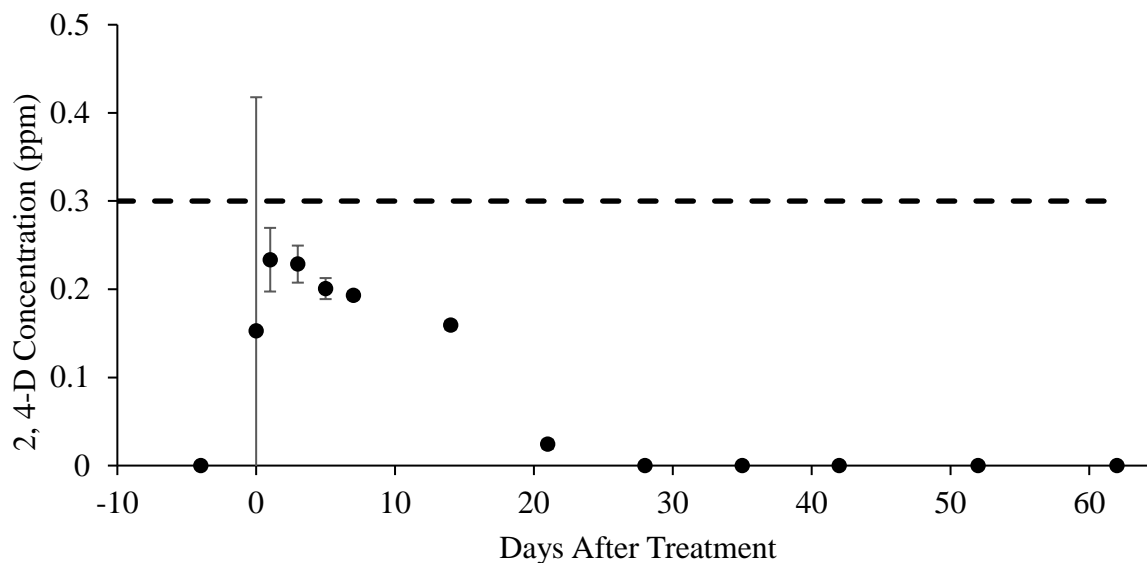
Figure 59. Mean concentration of 2, 4-D (ppm) for Kathan Lake. Whole-lake 2, 4-D application with the DMA® 4 IVM formulation took place May 24, 2016. Samples were collected at a depth of 1.5 m using a Van Dorn horizontal sampler and analyzed using high performance liquid chromatography coupled with a triple-stage quadrupole mass spectrometer. Dashed line represents target concentration (0.3 ppm). Error bars are 95% confidence intervals.
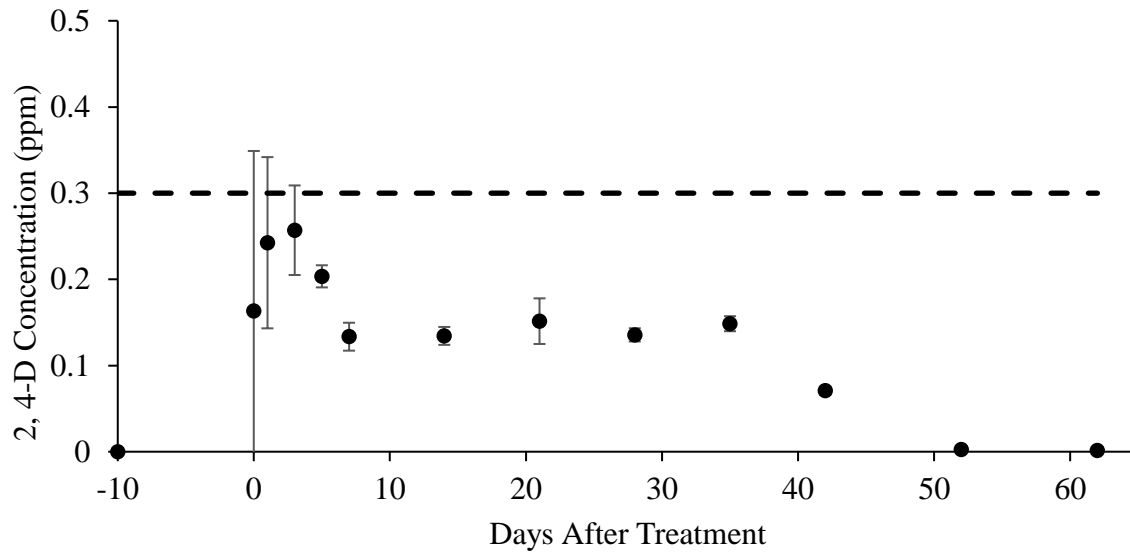
Figure 60. Mean concentration of 2, 4-D (ppm) for Manson Lake. Whole-lake, 2, 4-D application with the DMA® 4 IVM formulation took place June 2, 2016. Samples were collected at a depth of 1.5 m using a Van Dorn horizontal sampler and analyzed using high performance liquid chromatography coupled with a triple-stage quadrupole mass spectrometer. Dashed line represents target concentration (0.3 ppm). Error bars are 95% confidence intervals.
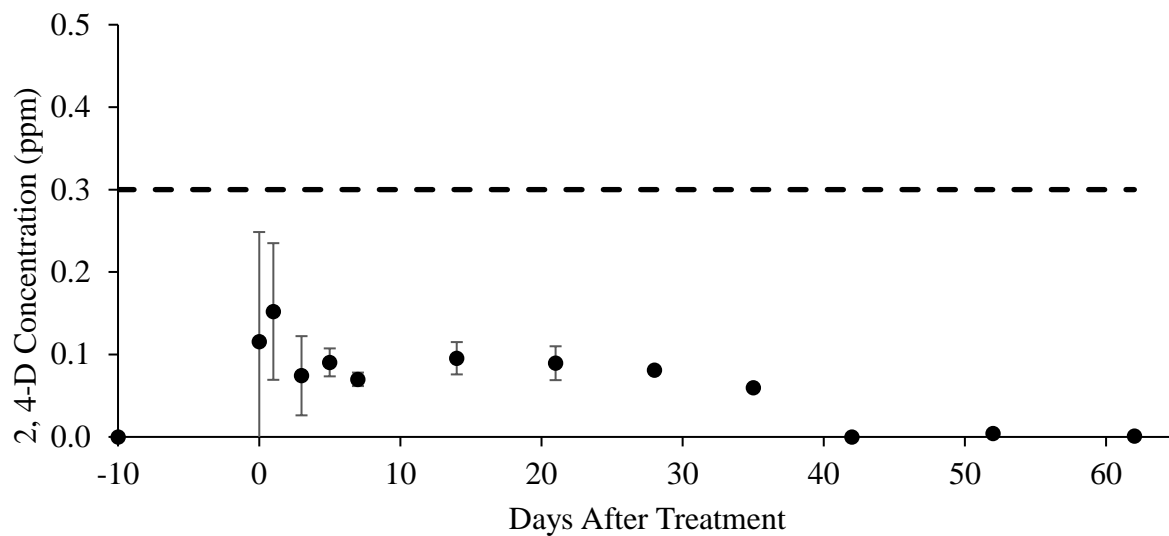
Figure 61. Mean concentration of 2, 4-D (ppm) for Silver Lake. Whole-lake 2, 4-D application with the DMA® 4 IVM formulation took place June 7, 2016. Samples were collected at a depth of 1.5 m using a Van Dorn horizontal sampler and analyzed using high performance liquid chromatography coupled with a triple-stage quadrupole mass spectrometer Dashed line represents target concentration (0.3 ppm). Error bars are 95% confidence intervals.