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Trophic Interactions and Regulators of Abundance of Largemouth Bass in Northeastern Minnesota

by

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Abstract — Largemouth Bass *Micropterus salmoides* abundance appears to be increasing in northeastern Minnesota. We sought to identify habitat metrics associated with Largemouth Bass to help managers identify lakes most likely to see increases in the species. We also sought to quantify trophic interactions between Largemouth Bass and other sport fish to determine the potential for competition or predation. We sampled nine lakes in northeastern Minnesota with no, recently detected, and historical populations of Largemouth Bass. We compared Largemouth Bass relative abundance (electrofishing catch per hour; CPH) to measures of aquatic vegetation abundance and water clarity and temperature. We also used carbon and nitrogen stable isotope analysis to compare trophic position of adult Largemouth Bass, Walleye *Sander vitreus*, Northern Pike *Esox lucius*, Yellow Perch *Perca flavescens* and Bluegill *Lepomis macrochirus*. We found plant biovolume to be the best predictor of Largemouth Bass CPH, although an additional model including water temperature was also significant in explaining variation in Largemouth Bass CPH. Trophic position of Largemouth Bass was lower than that of Northern Pike and Walleye and more littoral than all other fish species in most lakes sampled, indicating low diet overlap but our inference about trophic interactions was limited by our ability to adequately quantify littoral prey resources in most lakes. In lakes where aquatic vegetation is abundant Largemouth Bass are more likely to be sampled, but the potential for food competition between adult Largemouth Bass and other adult sport fish is low.

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INTRODUCTION

Minnesota is currently undergoing long-term shifts in climate resulting in warmer and more variable weather patterns (Pryor et al. 2014). Johnson and Stefan (2006) found ice duration on lakes in Minnesota from 1979 to 2002 became shorter, indicating a longer growing season. Lottig et al. (2014) demonstrated that water clarity has increased in many northern Midwestern temperate lentic systems, including Minnesota's lakes. These changes are likely to influence fish populations and may be resulting in more Centrarchids where Percids previously dominated the sport fish community (e.g., Hansen et al. 2017).

Specifically, Largemouth Bass *Micropterus salmoides* may benefit from warmer temperatures and increased water clarity and aquatic vegetation. Largemouth Bass survival and growth are mediated by water temperature (Ludsin and DeVries 1997, Fullerton et al. 2000). The water temperature at which Largemouth Bass nest and hatch in the spring determines the size at which juveniles enter their first winter (Pine III et al. 2000) which in turn influences mortality and recruitment as larger individuals are more likely to survive (Miranda and Hubbard 1994, Garvey et al. 1998, Fullerton et al. 2000). Largemouth Bass abundance and condition are also influenced by littoral habitat, such as submerged aquatic vegetation (Annett et al. 1996, Hayse and Wissing 1996, Durocher et al. 1984, Pothoven et al. 1999, Valley and Bremigan 2002, Olson et al. 2003). For example, adult Largemouth Bass feed most efficiently at moderate vegetation densities which allow them to ambush prey (Bettoli et al. 1992) and higher vegetation abundance provides more cover and feeding habitat for juveniles, which can increase recruitment and abundance (Durocher et al. 1984, Miranda and Hubbard 1994, Miranda and Pugh 1997).

While Largemouth Bass are sought after by some Minnesotans, most anglers have traditionally preferred other species such as Walleye *Sander vitreus* and Northern Pike *Esox lucius* (Cook and Younk 1998, Schroeder 2012). Schroeder (2012) reported that 22% of anglers living in northeast Minnesota fished at least once for Largemouth Bass in local lakes compared to 44% fishing at least once for Northern Pike and 75% fishing at least once for Walleye. However, the number of lakes where Largemouth Bass are sampled in Minnesota has increased over the past century (Minnesota Department of Natural Resources (MN DNR), unpublished data), generating more opportunities to target the species. Some fish communities appear to be transitioning from coolwater fisheries dominated by Walleye or Northern Pike and Yellow Perch *Perca flavescens* to warmwater fisheries dominated by Largemouth Bass and *Lepomis* species such as Bluegill *Lepomis macrochirus* and Pumpkinseed *Lepomis gibbosus* (MN DNR, unpublished data). Constituent groups (lake associations and angler groups) are concerned by this transition and would like more information about how fish communities respond to increases in Largemouth Bass abundance.

A specific concern about Largemouth Bass relates to their trophic interactions with other fish, especially sport fish such as Walleye, whether in the form of competition or predation. Largemouth Bass are omnivorous and undergo dietary shifts throughout their ontogeny, consuming zooplankton and, invertebrates as juveniles, and invertebrates and fish as adults (Annett et al. 1996, Mittlebach and Persson 1998). Diet studies have sought to quantify the extent of resource competition or predation between Largemouth Bass and other sport fish but results have varied. Fayram et al. (2005) found Largemouth Bass did consume juvenile Walleyes in northern Wisconsin lakes, and stocked Walleye survival was negatively related to Largemouth Bass abundance. However, in an Iowa lake Liao et al. (2002) found diets of Largemouth Bass did not contain Walleye but Largemouth Bass, Northern Pike, and Walleye diets all contained high proportions of Yellow Perch. The availability of prey as well as in-lake habitat play a role in shaping sport fish diets and trophic interactions (e.g., Dibble and Harrel 1997).

While diet studies using stomach contents analysis are expensive, time consuming, and provide a description of organism only very recently consumed, stable isotope analysis (SIA) of carbon and nitrogen is useful for identifying and quantifying aquatic food web linkages and incorporates diet information over a longer time period (Vander Zanden et al. 1999, Fry 2006). The ratio of heavy to light carbon

($^{13}C/^{12}C$), or carbon stable isotope signature ($\delta^{13}C$), indicates the area of a lake (littoral vs. pelagic) from which an organism derives its prey (Vander Zanden and Rasmussen 1999, Post 2002). The ratio of heavy to light nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) relative to a system baseline (e.g., primary consumer) indicates the trophic level of an organism, or how high on the food chain it feeds (Vander Zanden and Rasmussen 1999, Post 2002). Thus, SIA provides a means of describing trophic position of fish in lakes with relatively small sample sizes compared to stomach contents analysis and integrates information over a longer time period.

The objectives of this study were to 1) link Largemouth Bass establishment to habitat metrics in northeastern Minnesota lakes and 2) compare trophic position of sport fish species across lakes with different Largemouth Bass populations to identify the ecological role of Largemouth Bass in different environments.

METHODS

Study lake selection

We contacted fisheries management staff in northeastern Minnesota during to develop a list of the best candidate lakes for the study (Figure 1, Table 1). We sought small lakes of similar size (less than 400 hectares) and lake class (Schupp 1992) within a practical driving distance range of the Duluth Area Fisheries Office with a public access developed enough to accommodate an electrofishing boat. We sought three lakes with historical Largemouth Bass populations (where Largemouth Bass were sampled regularly since before 1990), three lakes with recently established Largemouth Bass populations (sampled for the first time after 2004) and three lakes without Largemouth Bass. To isolate the effects of Largemouth Bass on the fish communities, only lakes without other *Micropterus* species (i.e., Smallmouth Bass *Micropterus dolomieu*) were considered.

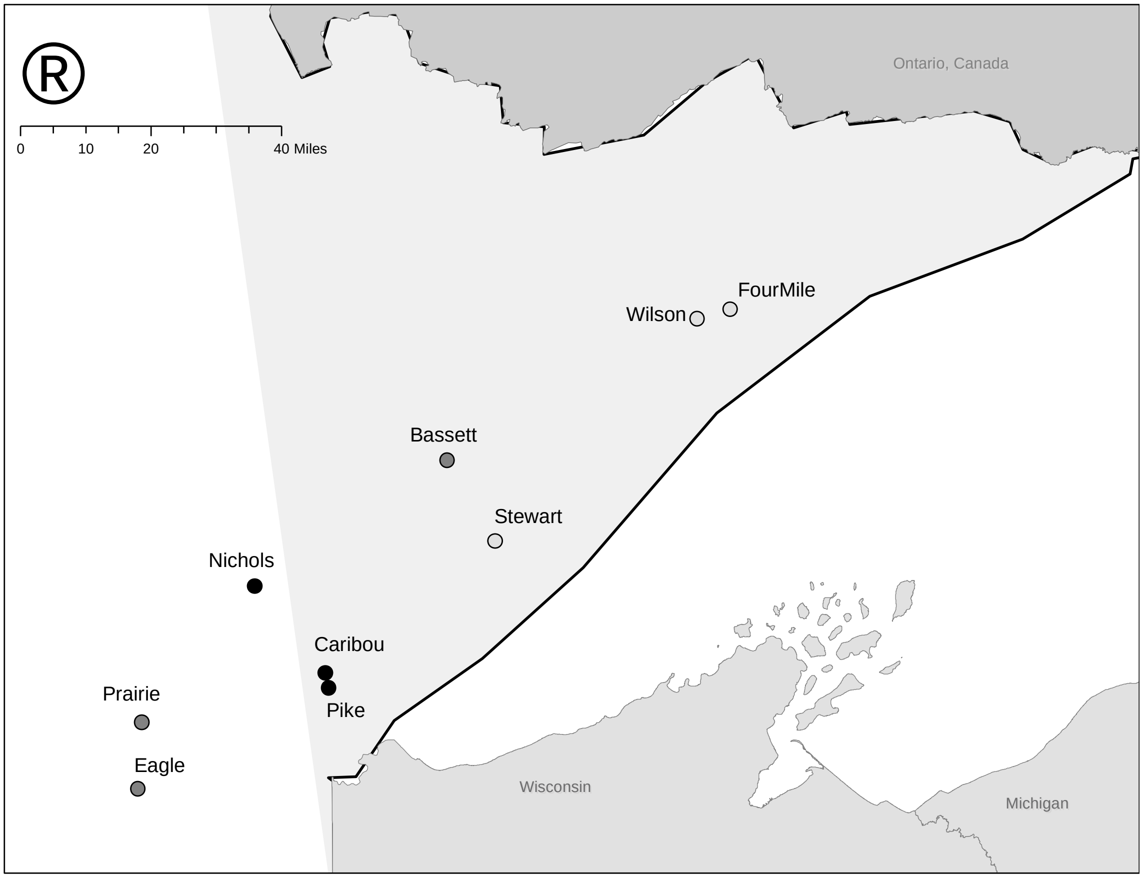

FIGURE 1. Locations of northeastern Minnesota study lakes with no (light gray), recently detected (dark gray), and historical (black) populations of Largemouth bass.

TABLE 1. Summary information for study lakes, including the Division of Waters id number (DOW), the type of Largemouth Bass population present in the lake, the surface area, maximum depth, Schupp lake class, year of last survey by the MN DNR. Also included are the mean relative abundance of key species from the last MN DNR survey, including Largemouth Bass catch per hour via boat electrofishing (LMB CPH), and the catch per unit effort (CPUE) via gill netting of Walleye (WAE), Northern Pike (NOP), and Yellow Perch (YEP), and CPUE via trap netting of Bluegill (BLG).

Lake Name	DOW	Population Type	Area (ha)	Max Depth (m)	Lake Class	Last Survey	LMB CPH	WAE CPUE	NOP CPUE	YEP CPUE	BLG CPUE
Prairie	69084800	Recent	322	14.3	5	2015	0.0	1.6	2.9	15.4	3.3
Bassett	69004100	Recent	163	6.4	5	2016	2.3	2.2	1.9	56.2	25.8
Eagle	09005700	Recent	157	10.7	5	2016	3.9	8.3	2.1	54.8	1.5
Caribou	69048900	Historical	218	6.4	16	2015	11.8	4.3	8.1	12.9	16.3
Nichols	69062700	Historical	169	9.5	16	2012	26.9	1.1	5.6	1.6	7.0
Pike	69049000	Historical	198	18.3	5	2014	35.2	15.8	0.6	2.0	1.7
Stewart	38074400	None	96	7.3	19	2014		2.0	8.2	32.0	10.3
Wilson	38004700	None	263	16.2	5	2011		27.3	1.3	20.0	0.0
Four Mile	16063900	None	240	6.1	16	2011		17.1	0.4	3.0	0.0

Fish sampling

We quantified Largemouth Bass relative abundance (catch per hour, CPH) using spring, night-time boat electrofishing following MN DNR (2017) standard sampling protocols in 2015 (Table 2) and 2016 (Table 3). Electrofishing began at sunset and we targeted surface water temperatures between 13 to 20 °C, beginning sampling with lakes at the southern edge of the region and moving northward throughout the period. We used DC current at an amperage ranging from 2-8 set to maximize fish response. In both years a boat with two spider anode arrays and the hull as the cathode were used for sampling. In 2015 a Smith Root boat equipped with a GPP 5.0 control box, in 2016 we used an Oquakwa boat equipped with an ETS Electrofishing Systems MBS-2DPH control box. A single netter was used to dip fish, the same netter was used for all lakes within each year but netters were different across years. Four randomly selected starting points were identified for every lake and the boat drove parallel to the shoreline for 20 minutes at each site with continuous application of current (e.g., the pedal was depressed the for the entire run), with the exception of the smallest lake, Stewart, where the entire shoreline was electrofished. In 2016 an anode array was lost while electrofishing Lake Nichols and a replacement could not be obtained in the timeframe we needed to sample so the remainder of electrofishing (all but Eagle Lake) was completed with a single anode. Conductivity and surface water temperature were measured at each lake before electrofishing.

TABLE 2. Sampling dates for the 2015 sampling season.

Lake	Temperature Loggers Set	Electrofishing	Short-term Gillnetting	Plant Hydroacoustics	Young of the Year Seining	Temperature Loggers Retrieved
Bassett	4/29	6/18	7/17	8/10	9/18	11/10
Caribou	4/30	6/10	7/02	8/04	9/02	11/10
Eagle	4/27	6/04	7/01	7/22	9/01	11/15
Four Mile	5/04	6/23	7/21	8/13	9/10	
Nichols	4/28	6/11	7/08	7/31	9/08	11/10
Pike	4/30	6/05	7/14	8/05	9/02	11/10
Prairie	4/27	6/15	7/09	7/28	8/31	11/15
Stewart	4/29	6/22	7/13	8/01	9/18	
Wilson	5/04	6/23	7/20	8/11	9/10	11/08

TABLE 3. Sampling timetable for the 2016 sampling season.

Lake	Temperature Loggers Set	Electrofishing	Short-term Gillnetting	Plant Hydroacoustics	Final Baseline Collection	Temperature Loggers Retrieved
Bassett	4/19	5/29	6/16	8/10	9/28	11/03
Caribou	4/18	5/24	6/14	8/04	9/29	
Eagle	4/15	5/26	6/11	7/29	10/3	11/08
Four Mile	4/26	6/02	6/27	8/16	10/01	
Nichols	4/18	5/24	6/09	8/01	9/29	11/07
Pike	4/21	6/01	6/02	8/11	9/30	11/09
Prairie	4/15	5/27	6/01	8/08	10/03	
Stewart	4/26	5/30	6/15	7/31	9/28	11/03
Wilson	5/04	6/02	6/28	8/12	10/01	11/04

We measured (total length (TL, mm) of all Largemouth Bass sampled and collected scales for SIA. In calcified structures, such as fish scales, the isotope signature is incorporated over the whole life of an organism (Hutchinson and Trueman 2006); thus fish scales represent an isotope signature that is integrated over several months or years and reflect a longer-term description of diet than muscle (Perga and Gerdeaux 2003). Up to ten fish from other sport fish species (Walleye, Yellow Perch, Northern Pike, and Bluegill) were also sampled for TL and scales for SIA (Table 4). Where sufficient samples were not obtained during electrofishing, follow-up sampling was conducted < 1 month later (Tables 2 and 3) using short-term (1-2 h) daytime gill net sets. Gill nets were 76-m long, 1.8-m high consisted of five 15.2-m panels with 19-, 25-, 32-, 38-, and 51-mm bar mesh (MN DNR 2017).

In the early fall of 2015 (Table 2), to sample young-of-the-year Largemouth Bass, sampling with a beach seine was conducted at 3 – 5 sites per lake, sites were randomly selected in areas with habitat most navigable with a seine. The seine was 15.2-m long and 1.5-m high with 6.3-mm mesh and was used with the fixed-pole, arc method. We seined the no bass lakes as well as the lakes known to have Largemouth Bass to help ensure the species was not present. Fish age was not estimated; Largemouth Bass < 100 mm TL were assumed to be age-0. All Largemouth Bass captured were counted and measured.

TABLE 4. Number (N) and mean total length (TL, mm (standard error)) of fish sampled from nine northeastern Minnesota lakes in 2015 and 2016 and analyzed for carbon and nitrogen stable isotopes.

	Largemouth Bass		Walleye		Northern Pike		Bluegill		Yellow Perch	
Lake	N	Mean TL	N	Mean TL	N	Mean TL	N	Mean TL	N	Mean TL
Stewart	0		12	344 (26)	17	504 (19)	18	190 (8)	19	199 (10)
Wilson	0		15	348 (22)	15	579 (23)	0		20	161 (4)
Four Mile	0		19	309 (18)	7	376 (50)	0		5	229 (26)
Bassett	14	335 (15)	19	346 (24)	18	485 (28)	20	159 (5)	19	155 (9)
Eagle	11	388 (19)	21	310 (18)	9	463 (21)	20	190 (6)	20	136 (7)
Prairie	0		24	289 (23)	14	523 (43)	14	181 (4)	20	115 (7)
Caribou	20	377 (15)	14	305 (25)	11	547 (27)	20	159 (4)	17	162 (6)
Nichols	19	335 (19)	14	281 (17)	8	499 (14)	20	180 (5)	17	139 (7)
Pike	13	344 (21)	17	292 (20)	6	703 (80)	1	212	20	144 (8)

Invertebrate sampling

We collected stable isotope baseline invertebrate samples approximately every six weeks during the open water period both years. These collections were paired with other sampling (temperature logger sets, short-term gillnetting, plant hydroacoustics, temperature logger retrieval; Tables 2 and 3). Four sites were randomly selected for invertebrate collection and then maintained throughout the study. In 2015, zooplankton were sampled vertically from the water column using a Wisconsin zooplankton net with 363 μ-mesh and benthic invertebrates were collected from nearshore sites in < 2-m of water using an Eckman dredge and sediment was washed through a 541 μ-mesh wash bucket. Methods used in 2015 did not yield enough invertebrates in most collections and so we analyzed only samples from late summer (July or August) which generally had the highest biomass. In 2016 effort to collect invertebrates was increased. Zooplankton were collected with a larger net with smaller mesh (0.3 m diameter, 243 μ-mesh) using a horizontal tow along the surface for approximately 5 minutes in the deepest part of the lake. Littoral invertebrates were collected with kick nets and a stovepipe sampler at nearshore sites < 2-m deep. In both years, bivalve mussels and gastropods were collected from the littoral zone by hand opportunistically during plant hydroacoustic sampling in late summer. All invertebrate sample material collected with sampling gears was placed on ice and then frozen until it could be sorted in the laboratory.

Habitat assessments

We quantified the percent of the water column that is occupied by plants where plants occur (plant biovolume; BVp) and percent of the lake bottom covered with vegetation (percent area covered; PAC) using hydroacoustic data collected with a commercial depth finder (Lowrance HDS 8) according to the BioBase standard operating procedure (Navico, Inc. 2014) by driving transects across the entire lake spread 50 m apart perpendicular to the longest shoreline at a speed less than 10 kph. We analyzed data using the cloud-based BioBase software's estimates of BVp and PAC derived using a geostatistical interpolated grid method. Grid estimates are interpolated and evenly spaced values representing kriged output of aggregated data points as opposed to using sonar pings from individual points.

Additionally, we measured the dissolved oxygen profiles, water temperature, and water clarity (Secchi depth [m]) throughout the growing season. Although we attempted to quantify water temperature in the study lakes using temperature logger chains, we unable to locate some of the chains for retrieval in both years of the study. Therefore, we used modeled lake temperature data obtained using methods described in Hansen et al. (2017) to estimate and compare growing degree days (base temperature 5 °C; GDD) for each lake. These temperature data were available from 1980 to 2015, but not for 2016.

Laboratory preparations

Fish scales were stored in manila envelopes then rinsed with de-ionized water and air dried for 3 days. We used scissors to cut a 1 mm wide segment from the center of the scale and dissected it into 1 mm pieces. For scales less than 5 mm wide, the entire scale was cut into 1 mm pieces. These scale fragments were stored in plastic assimilation vials and mailed to a laboratory for weighing, encapsulation, and SIA.

Frozen invertebrate samples were thawed and sorted in the laboratory with a dissecting microscope. We picked up to 5 mL volume of *Daphnia* species from zooplankton samples and stored them in 95% ethanol. When 5 mL of Daphnia could not be obtained from a sample, *Bosmina* were also included. Littoral and benthic macroinvertebrate samples were thawed and all species observed were removed from the plant and sediment material and stored in 95% ethanol. For SIA, we analyzed only chironomid species as that was the most abundant and ubiquitous group. In many cases, the amount of chironomid mass available from one site within a lake was not sufficient for SIA. Therefore, we combined chironomids from all sites for each sampling date within a lake into one composite sample. We thawed bivalves and gastropods and dissected foot muscle tissue which was then refrozen. Up to five individual bivalves or gastropods were combined to form one composite sample for each lake each year. We dried invertebrate tissue in an oven for 3 days at 50 °C, ground samples with a mortar and pestle, placed samples material in plastic assimilation vials, and then mailed samples to a laboratory for weighing, encapsulation, and SIA.

The University of Minnesota Department of Earth Sciences laboratory in St. Paul, MN completed weighing and $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis of the fish and invertebrate samples in 2015 but was unavailable the following year. Stable Isotope Tracer Technologies, Inc., in Waterloo, Ontario completed weighing and analysis in 2016. Because muscle tissue contains lipids which can be enriched relative to other tissues, we corrected gastropod and bivalve $\delta^{13}C$ using the equation developed by Post et al. (2007).

Data analysis

We used paired t-tests to test for differences in measured variables (e.g., CPH, habitat metrics) within lakes between years. We used a t-test to test for differences in seine catch per effort and mean length of seined fish in lakes with recent and historical populations. We used analysis of variance (ANOVA) to test for differences in measured variables among groups of lakes. When we found differences among groups ($p \leq 0.05$), we performed Tukey honestly significant different tests to determine which groups were different from each other. We developed linear regression models to explain variation in Largemouth Bass CPH with habitat metrics and compared models using the Akaike information criterion (AIC). We completed all analysis in R, version 3.4.3 (R Core Team (2017)). Herein, we report means (μ) ± standard error (SE) and test statistics with degrees of freedom (df) in subscript.

We had intended to estimate trophic position of all fish collected using a two-end member mixing model as described by Post (2002) which allows for differentiation between energy sources from the pelagic and benthic areas of a lake. However, difficulties in establishing an accurate baseline encompassing the gradient of littoral to pelagic $\delta^{13}C$ for each lake precluded this analysis. Instead we used biplots of the data to make relative comparisons of trophic position of different species within lakes. To inform sample collection in future studies, we also presented a summary of our invertebrate data and discuss its implications.

RESULTS

Study lakes comparison

Although catches in some lakes were higher in 2015 than 2016 (Figure 2), overall Largemouth Bass CPH was not different within lakes across years ($t_{df=8} = 1.7$, $p = 0.12$). Largemouth Bass CPH was different among groups (Figure 3, $F_{df=2} = 6.7$, $p = 0.03$), specifically CPH was higher in lakes with historical populations (μ = 9.8 ± 2.9) compared to lakes with no Largemouth Bass (μ = 0.0; $p = 0.03$). Between lakes with recent (μ = 3.1 ± 1.8) and historical populations, CPH was not different ($p = 0.11$) nor was it different between lakes with recent populations and lakes with no Largemouth Bass ($p = 0.55$).

We sampled age-0 Largemouth Bass with seining in all of the recent and historical lakes, but we did not find them in the no bass lakes. Catch rates of age-0 Largemouth Bass were generally low where the species was present (mean catch per effort ranged from 0.8 to 5.5 fish/haul) and but mean catch per effort was higher in historical lakes (μ = 3.5 ± 1.3) than recent lakes (μ = 1.7 ± 0.6; $t_{df=2} = -1.3$, Figure 4). Mean length of age-0 Largemouth Bass was higher in recent lakes (μ = 71 ± 4) than in historical lakes (μ = 54 ± 2; $t_{df=2} = 1.9$, Figure 4).

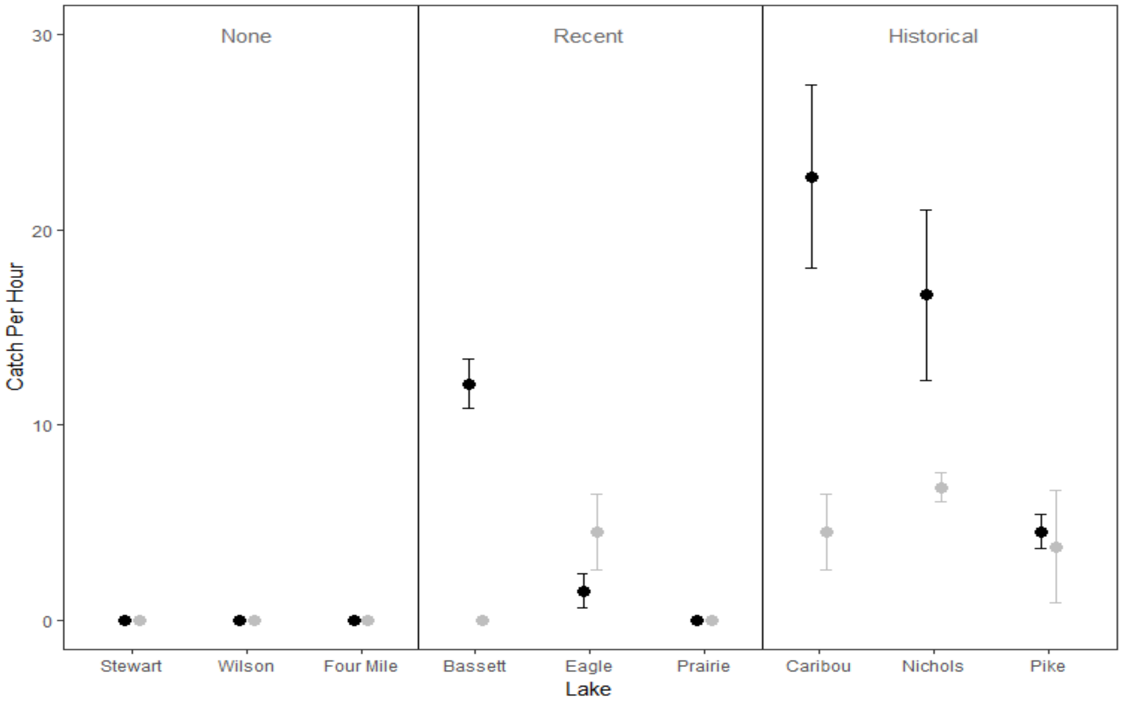

FIGURE 2. Mean catch per hour of Largemouth Bass (± standard error) sampled with spring, night-time boat electrofishing from nine lakes in northeastern Minnesota in 2015 (black circles) and 2016 (gray circles). Lakes had either no Largemouth Bass, historical populations of Largemouth Bass, or recently detected Largemouth Bass (three lakes per category). Largemouth Bass were known to be present in "historical" lakes for more than 30 years and less than 15 years in "recent" but have not been sampled in "none" lakes.

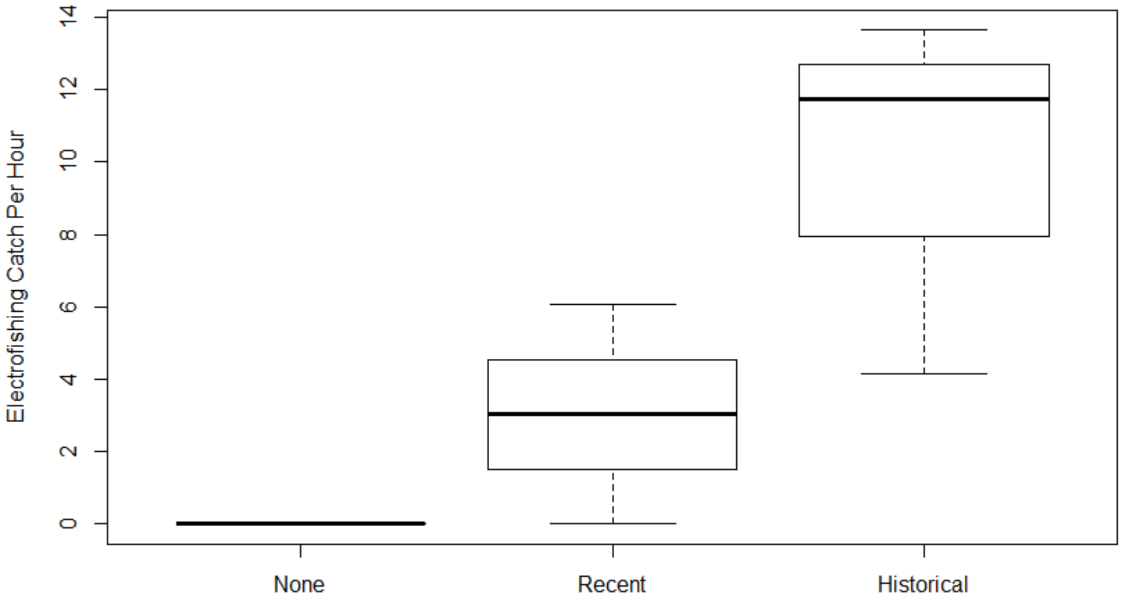

FIGURE 3. Mean catch per hour of Largemouth Bass (± standard error) sampled with spring, night-time boat electrofishing from nine lakes in northeastern Minnesota averaged by type of Largemouth Bass population (three lakes per category, catch averaged by lake over the 2015 and 2016 sampling). Largemouth Bass were known to be present in "historical" lakes for greater than 30 years and less than 15 years in "recent" lakes but have not been sampled in "none" lakes.

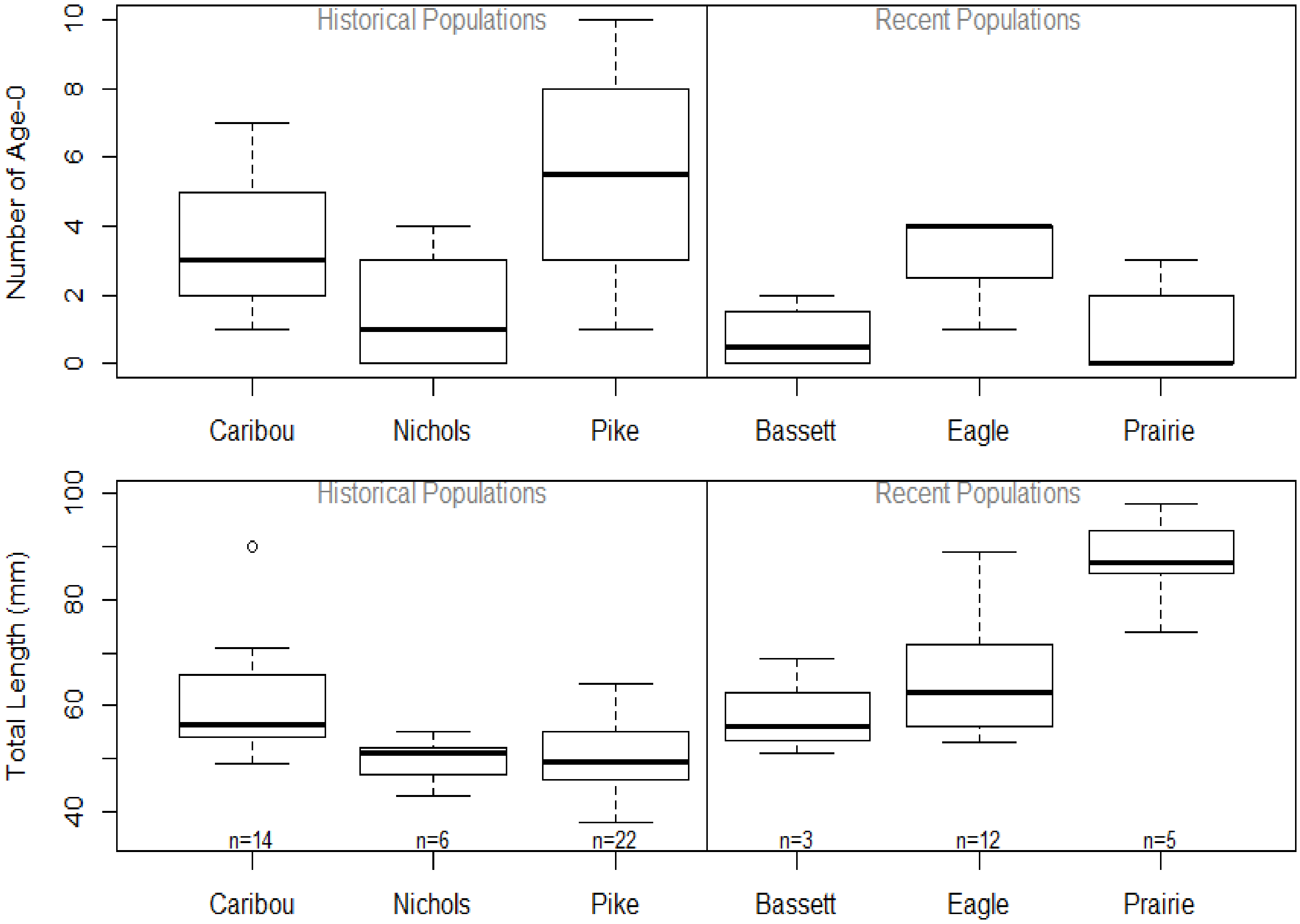

FIGURE 4. Box-and-whisker plots of age-0 Largemouth Bass per seine haul (top) and their associated length (bottom) in six lakes in northeastern Minnesota from August 31 – September 18, 2015. Largemouth Bass were known to be present in “historical” lakes for greater than 30 years and less than 15 years in “recent” lakes. The number (n) of Largemouth Bass sampled from each lake is along the x-axis of the bottom panel.

Habitat Comparisons

Vegetation

Percent Area Covered did not differ within lakes among years ($t_{df=8} = 1.5$, $p = 0.16$; Figure 5) nor was it different among lakes with different population types ($F_{df=2} = 0.8$, $p = 0.46$, Figure 6). Biovolume percent did not differ within lakes among years ($t_{df=8} = 2.0$, $p = 0.08$; Figure 5) but was significantly different among groups of lakes ($F_{df=2} = 7.3$, $p = 0.03$; Figure 6), where it was lower in lakes with no Largemouth Bass ($\mu = 24.3 \pm 6.3$) compared to lakes with historical Largemouth Bass ($\mu = 54.7 \pm 5.8$, $p = 0.02$). Biovolume percent did not differ between lakes with no Largemouth Bass and recently detected Largemouth Bass ($\mu = 46.0 \pm 5.4$; $P = 0.08$).

Temperature and Water Clarity

The average number of growing degree days measured annually from 1980 to 2015 (Figure 6) was significantly different among populations types ($p = 0.04$) and specifically was higher in the lakes with recent Largemouth Bass populations ($\mu = 2364 \pm 17.7$) than lakes without Largemouth Bass ($\mu = 2134 \pm 15.3$; $p = 0.03$). There was no significant difference among the other groups (historical $\mu = 2293.4 \pm 14.5$; $p > 0.1$; Figure 6). Mean Secchi depth was lower in 2016 than in 2015 ($p = 0.01$; Figure 5) but when averaged across years Secchi depth did not differ across population type ($p = 0.36$; Figure 6).

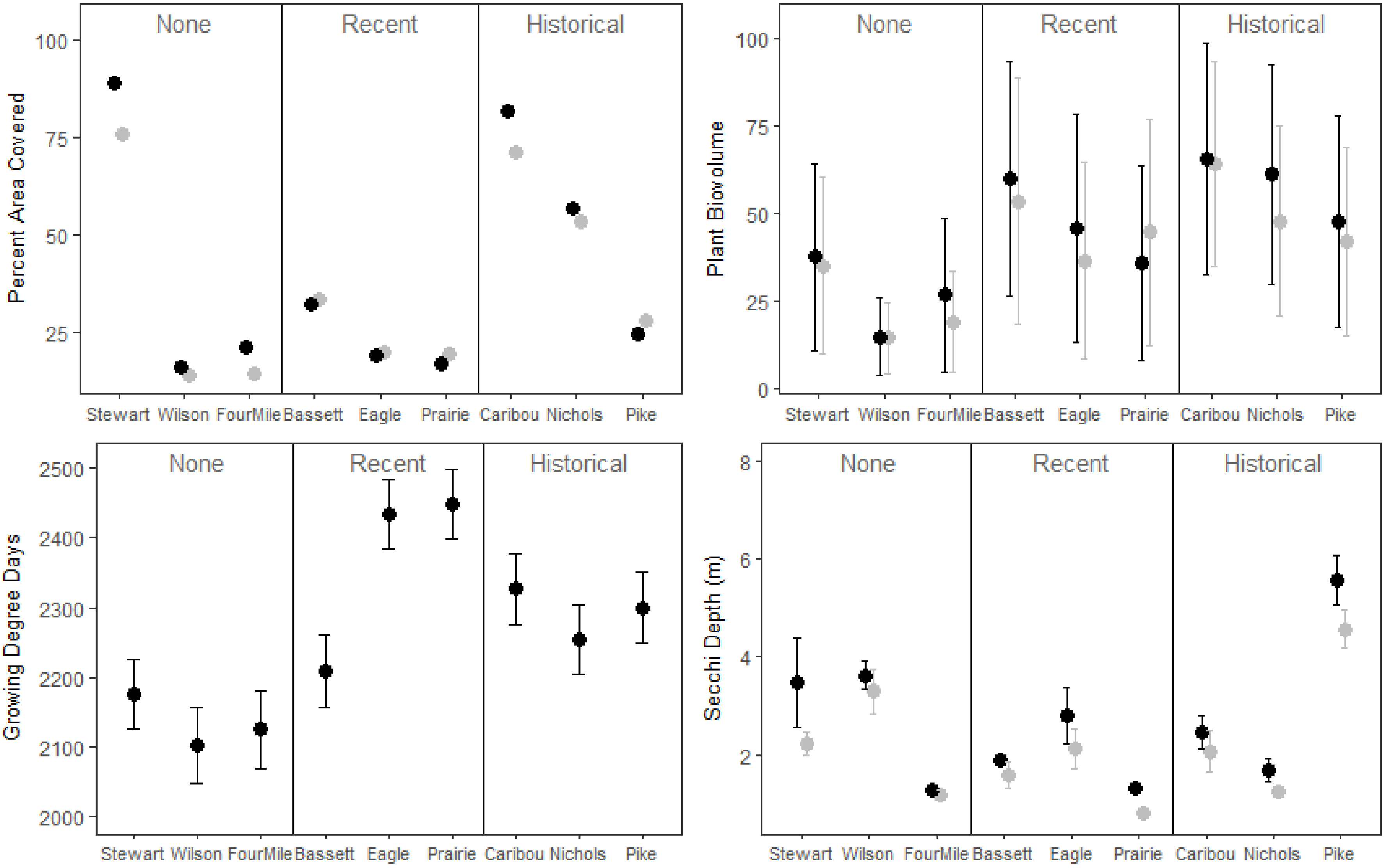

FIGURE 5. Habitat metrics of variables measured in nine northeastern Minnesota lakes in 2015 (black circles) and 2016 (gray circles). Largemouth Bass were known to be present in “historical” lakes for greater than 30 years and less than 15 years in “recent” lakes but have not been sampled in “none” lakes Metrics include percent of the lake bottom covered with vegetation (top left), mean percent of the water column occupied where plants are present (plant biovolume ± standard error; top right), number of growing degree days (base temperature 5°C averaged across estimates made from 1980 to 2015 ± 95% confidence interval; bottom left), and mean Secchi depth (± standard error; bottom right).

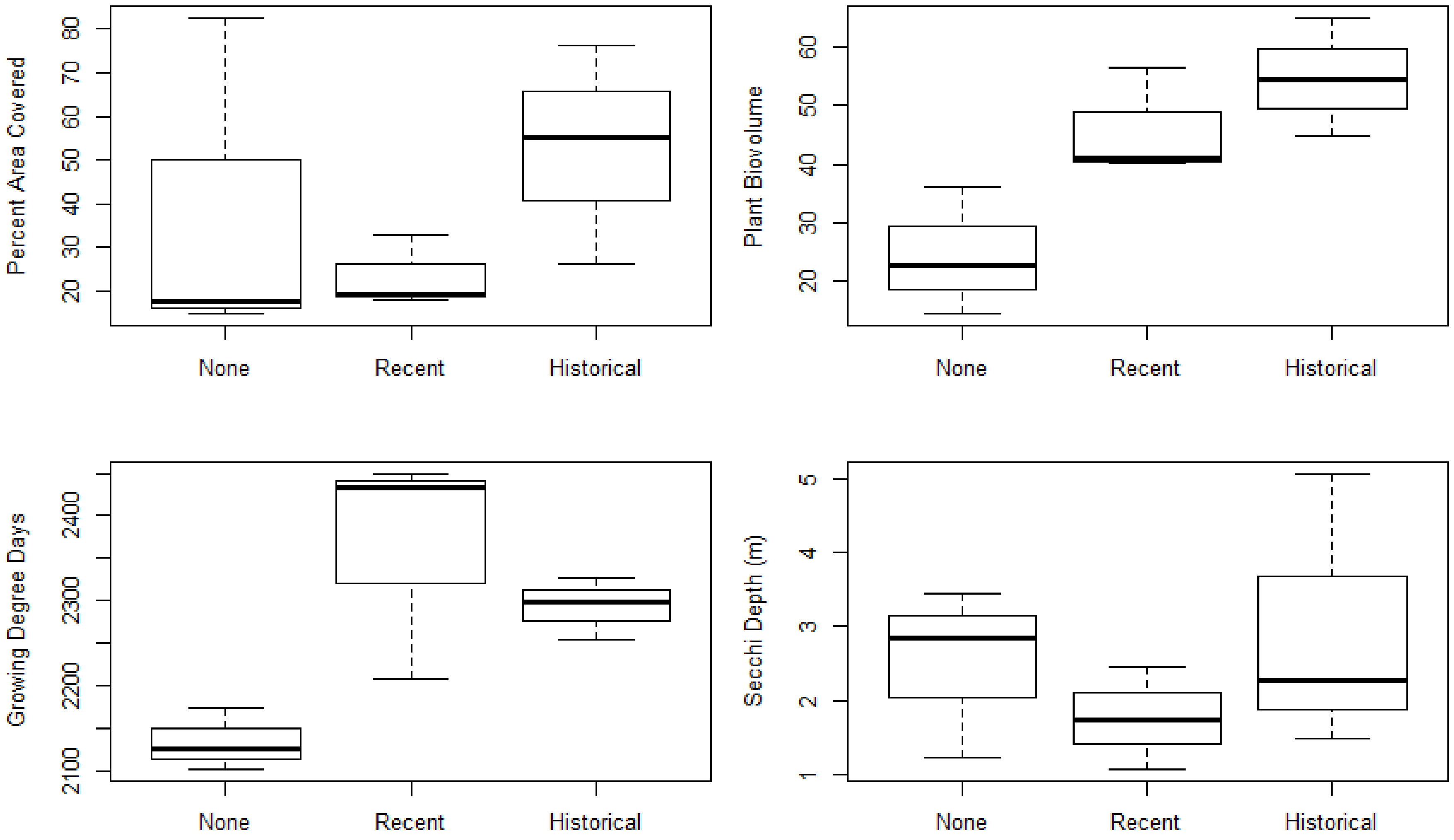

FIGURE 6. Box-and-whisker plots of habitat metrics of variables measured in nine northeastern Minnesota lakes averaged across groups with different Largemouth Bass population types. Largemouth Bass were known to be present in “historical” lakes for greater than 30 years and less than 15 years in “recent” lakes but have not been sampled in “none” lakes. Metrics were averaged across measurements taken 2015 and 2016 and include percent of the lake bottom covered with vegetation (top left), mean percent of the water column occupied where plants are present (plant biovolume; top right), number of growing degree days (base temperature 5 °C averaged across estimates made from 1980 to 2015; bottom left), and mean Secchi depth (bottom right).

Regression Analysis

We developed four linear regression models to explain variation in Largemouth Bass CPH. The first model included averaged values for all four habitat metrics (PAC, BVp, GDD, and Secchi depth; Table 5). Successive models eliminated habitat metrics one by one (with the metric that was least different among groups removed in each iteration) until only the metric (BVp) which was most significantly different among lakes with different lake types was included. Models including GDD and BVp and BVp alone were significant (Table 6). The AIC value for the model including only BVp was lowest indicating that model is most efficient at explaining variation in Largemouth Bass CPH (Figure 7), although it was only very marginally better than the model including GDD as well.

TABLE 5. Predictor variables used to explain Largemouth Bass relative abundance (catch per hour) in nine northeastern Minnesota lakes. Variables were averaged over 2015 and 2016, except growing degree days which were available only for 2015. Population type refers to Largemouth Bass where the species was known to be present in "historical" for more than 30 years and less than to 15 years in "recent" but have not been sampled in "none" lakes.

Lake	Population Type	Catch per Hour	Secchi Depth (m)	Plant Biovolume	Percent Area Covered	Growing Degree Days
Four Mile	None	0	1.2	22.7	17.7	2314
Stewart	None	0	2.9	36.1	82.4	2363
Wilson	None	0	11.4	14.5	14.7	2300
Caribou	Historical	13.6	7.5	64.9	76.5	2487
Nichols	Historical	11.8	4.8	54.4	55.1	2419
Pike	Historical	4.2	16.7	44.8	26.3	2474
Bassett	Recent	6.1	5.7	56.7	32.9	2381
Eagle	Recent	3	8.07	41.1	19.3	2589
Prairie	Recent	0	3.5	40.2	18.0	2368

TABLE 6. Summary statistics from linear models compiled to explain Largemouth Bass relative abundance in nine northeastern Minnesota lakes. Predictors included Secchi depth, percent of lake bottom covered by aquatic vegetation (PAC), growing degree days (GDD), and the percent of the water column occupied by plants where plants were present (BVp). Models were compared using the Akaike information criterion (AIC).

Predictors	Adjusted R^2	F	P	AIC
Secchi, PAC, GDD, BVp	0.42	2.46	0.20	55.16
Secchi, GDD, BVp	0.61	5.14	0.06	51.67
GDD, BVp	0.67	9.24	0.02	49.68
BVp	0.66	16.49	< 0.01	49.44

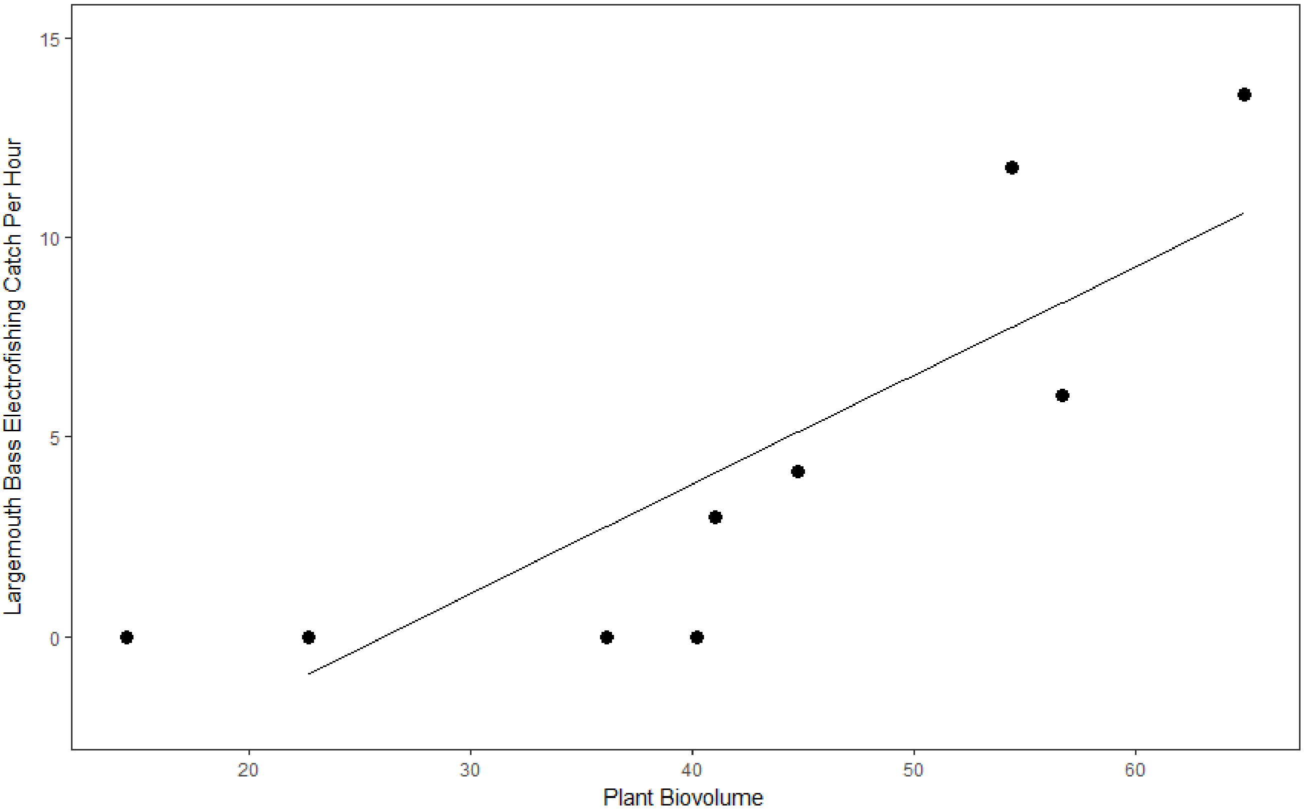

FIGURE 7. Mean Largemouth Bass electrofishing catch versus mean plant biovolume catch per hour from nine northeastern Minnesota lakes, averaged across samples taken in 2015 and 2016. Black line represents a linear regression (*p* = 0.01) and dashed gray line represents a potential threshold where all lakes with Largemouth Bass have greater than 40% plant biovolume.

Trophic Interactions

The relative trophic position of Largemouth Bass tended to be more littoral than any of the other fish species sampled and they occupied a trophic level generally above Bluegill and Yellow Perch but below Northern Pike and Walleye (Figure 8). Only in Eagle Lake, which has a recent population, was Largemouth Bass trophic level ($\delta^{15}N$) as high as Northern Pike and Walleye. Trophic position (mean $\delta^{13}C$ and $\delta^{15}N$ ± 95% confidence intervals) of Walleye and Largemouth Bass did not overlap in any of the lakes. However, in six out of the nine lakes trophic position of Walleye and Northern Pike did overlap, suggesting there is more similar prey consumed between those two species than between Walleye and Largemouth Bass. The trophic position of Yellow Perch and Bluegill overlapped in five of the seven lakes where they were both sampled but did not represent the range of littoral to pelagic habitats we expected.

The invertebrates we collected did not represent the full gradient of $\delta^{13}C$ consumed by fish in these lakes, with the sole exception of Pike Lake (Figure 9). Invertebrate baselines represented $\delta^{13}C$ as or more pelagic than the sport fish we studied, but neither of the sources we chose to encompass the littoral portion of the lake (gastropods and chironomids) were more littoral than our fish. While the $\delta^{15}N$ were generally below those measured in our fish, as expected, zooplankton sampled in spring (April) of 2016 in four of the nine lakes were unexpectedly high with values closer to fish compared to the other invertebrates (Figure 10). There was seasonal variability in baseline samples within lakes, but across lakes it did not appear to follow any specific patterns (Figures 9 and 10).

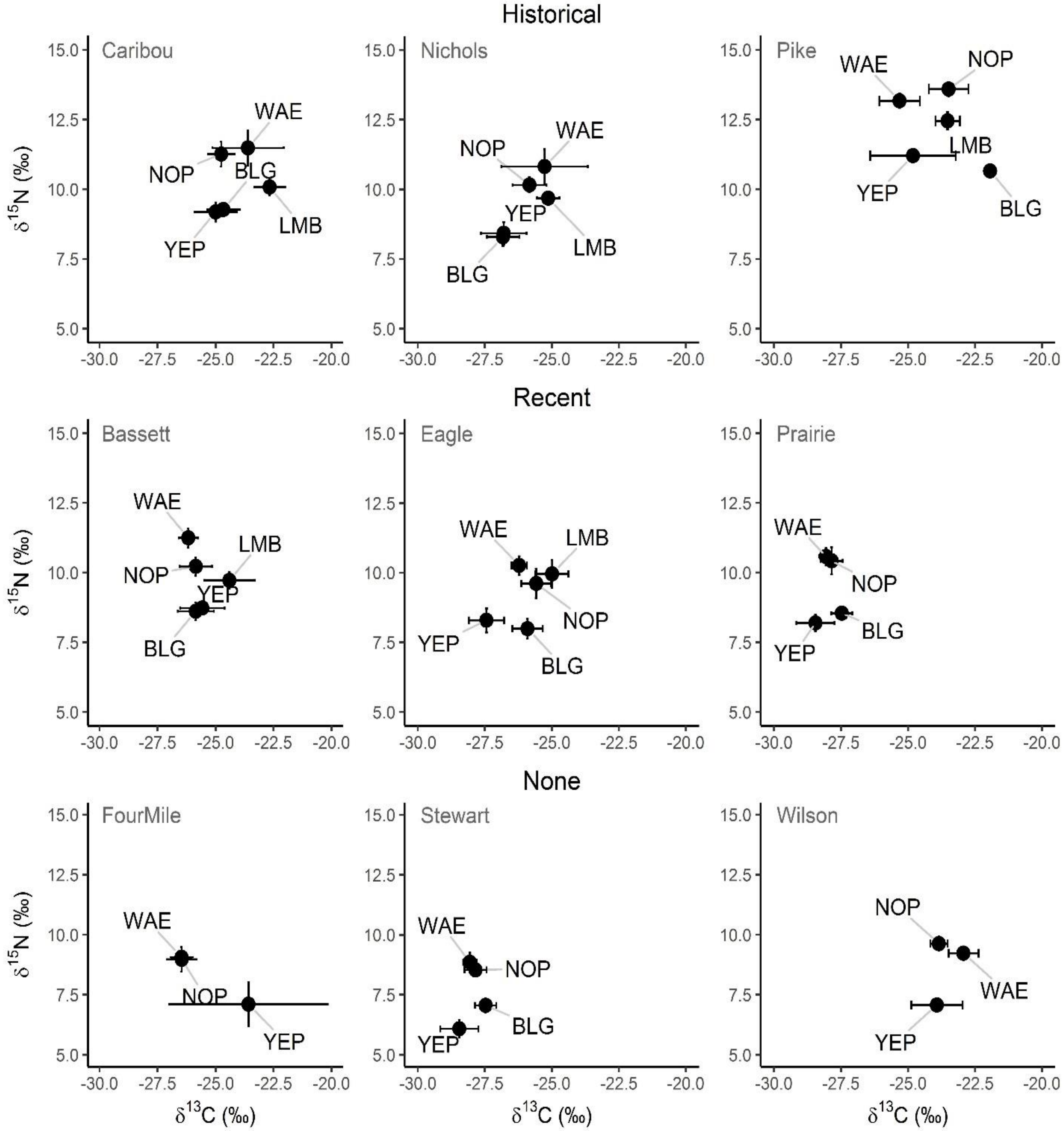

FIGURE 8. Mean Nitrogen (δ^{15}N) and Carbon (δ^{13}C) stable isotope signatures of Largemouth Bass (LMB), Walleye (WAE), Northern Pike (NOP), Yellow Perch (YEP), and Bluegill (BLG) scale tissue sampled from nine lakes in northeastern Minnesota in 2015 and 2016. Three lakes had historical populations of Largemouth Bass where the species had been present in the sampling history for more than 30 years (top row), three lakes had recently detected where the species had been present in the sampling history for less than 15 years (middle row), and three lakes had no Largemouth Bass present in the sampling history (bottom row). Lake names are presented in light gray at the top left of each graph. Error bars represent 95% confidence intervals.

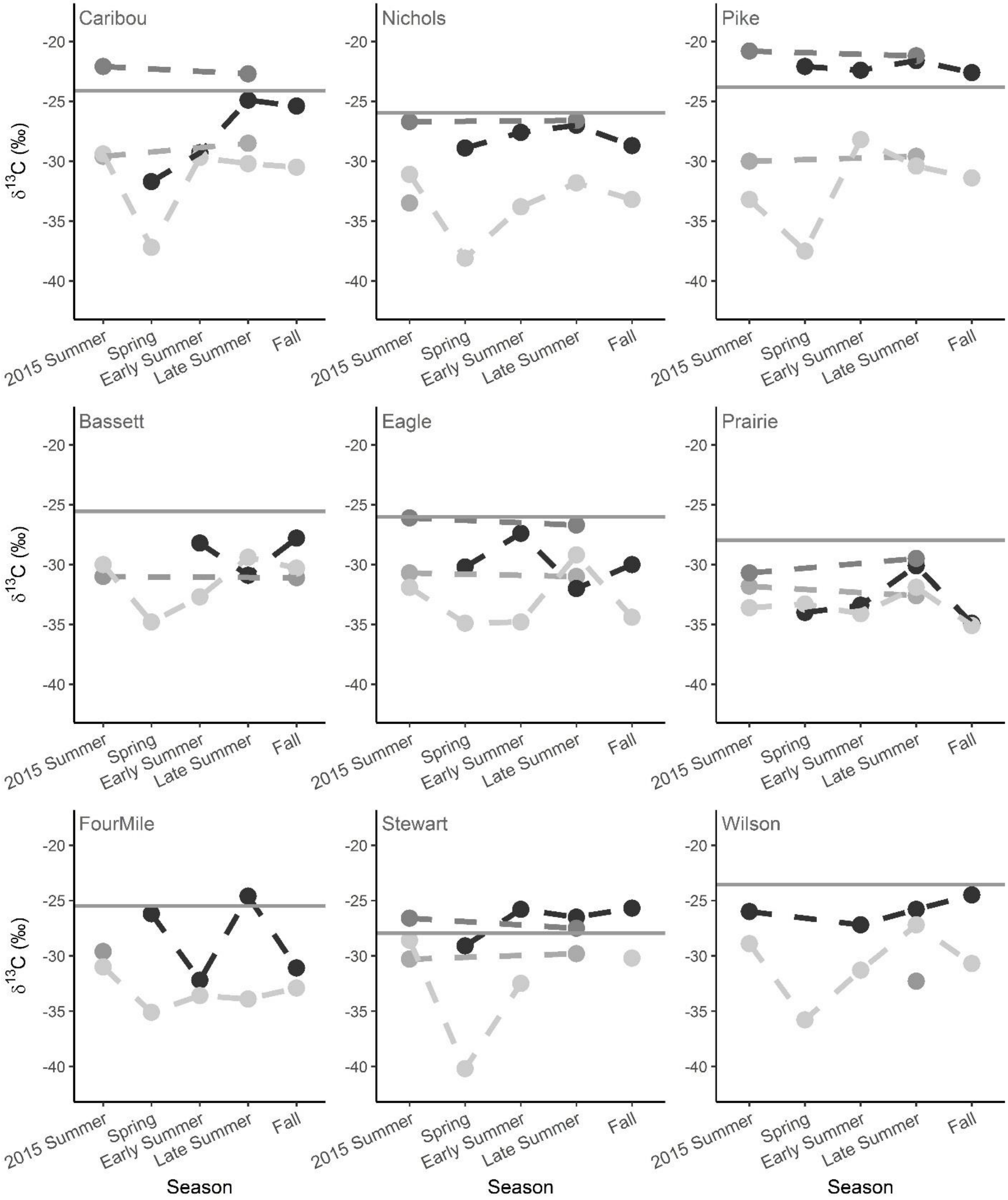

FIGURE 9. Seasonal variation in Carbon ($\delta^{13}C$) stable isotope ratios of baseline invertebrates sampled from nine lakes in northeastern Minnesota in 2015 and 2016, ordered chronologically. Nitrogen seasonal trends in baselines. Solid gray line represents the mean of all fish in each lake. Dashed lines and points represent baseline invertebrate signatures including cladaceron zooplankton (light gray), bivalves (medium gray), gastropods (dark gray), and chironomid larvae (black).

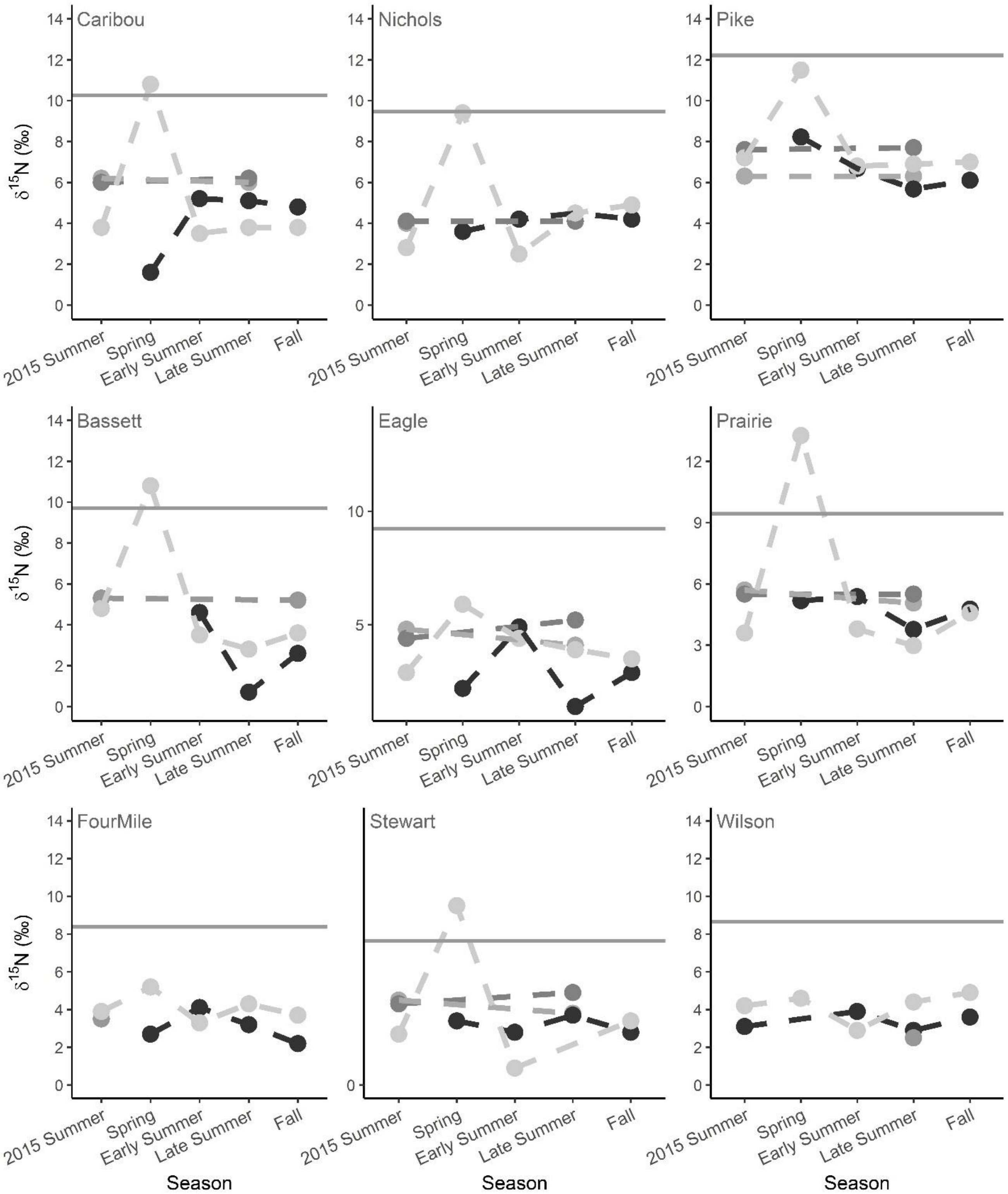

FIGURE 10. Seasonal variation in Nitrogen ($\delta^{15}N$) stable isotope ratios of baseline invertebrates sampled from nine lakes in northeastern Minnesota in 2015 and 2016, ordered chronologically. Nitrogen seasonal trends in baselines. Solid gray line represents the mean of all fish in each lake. Dashed lines and points represent baseline invertebrate signatures including cladaceron zooplankton (light gray), bivalves (medium gray), gastropods (dark gray), and chironomid larvae (black).

DISCUSSION

We found relative abundance of Largemouth Bass was higher in lakes with historical compared to recent populations; conversely mean size of age-0 Largemouth Bass was larger in recently detected populations. Bettoli et al. (1992) linked growth of age-0 Largemouth Bass to piscivory driven by the abundance of aquatic vegetation, however all lakes with Largemouth Bass had similar densities of aquatic vegetation in our study. Instead, the difference in age-0 length between the groups of lakes could be a density-dependent response where populations with fewer Largemouth Bass experience less competition for prey and have faster growth (e.g, Ludsin and DeVries 1997). However, if the age-0 fish in lower density lakes are able to have a higher probability of surviving the winter and thus better recruitment (Post et al. 1998), ultimately these lakes could see an overall increase in Largemouth Bass abundance.

Plant biovolume appears to be important in determining Largemouth Bass abundance and furthermore may provide a threshold for management given lakes with Largemouth Bass in this study had a BVp of at least 40%. Adding more estimates of Largemouth Bass relative abundance and BVp to this dataset would help refine the potential relationship between those metrics and it would be especially valuable to make the comparison in other types (e.g., larger, more mesotrophic) of lakes. Our measurements of BVp were consistent in the two years of this study, indicating that one sampling event of that metric may be adequate to describe it in a lake. Given the relatively low expense of measuring BVp with the BioBase method, it is possible that management staff may be able to sample BVp where they are concerned about Largemouth Bass expanding. Although other methods of measuring plant metrics with hydroacoustics are available, if more comparisons between BVp and Largemouth Bass abundance were to be compared to this study in the future it would be important to use consistent methods as estimates made using different equipment and data processing techniques can be significantly different and estimates may vary seasonally within a lake (Holbrook and Radomski 2014, Reed 2016).

Temperature, in the form of GDD, was higher in lakes with recently detected Largemouth Bass populations compared to lakes without Largemouth Bass. Although we would have expected GDD to also be higher in lakes with historical populations compared to lakes without Largemouth Bass we did not find that to be true. Two of the lakes in this study with recent populations were at the southern edge of the study area (Figure 1) and since water temperature can be highly driven by local weather patterns (e.g., Stefan et al. 1996) it is possible that the lower latitude of those lakes drove the differences we saw in GDD. A better understanding of the relationship between temperature and Largemouth Bass abundance could potentially be gained with continuous in-lake monitoring of using water temperature sensors deployed throughout the entire growing season. The protocol we used to collect temperature data with logger chains was not sufficient to gain these data even in lakes where we were able to retrieve the data as without year-round monitoring we did not capture the entire growing season.

Hansen et al. (2017) identified a threshold in Wisconsin lakes where the probability of Largemouth Bass occurring increased in lakes with more than 2,500 GDD. The estimated GDD for 2015 for all lakes in this study where electrofishing CPH was nonzero also was > 2,500. This suggests that the same approximate threshold may have applicability for better understanding Largemouth Bass presence in Minnesota as well. The number of GDD in lakes is likely to increase with climate change and it is possible that lakes currently without Largemouth Bass currently could potentially become more suitable for the species in the future (Fang et al. 2009).

Secchi depth and PAC were not significant in determining Largemouth Bass presence or abundance. When selecting lakes for this study we attempted to choose lakes with a similar range of productivity for each group of lakes. As Secchi depth is related to productivity in many Minnesota lakes (Cross and McInerny 1995), we may have limited our ability to make inferences about water clarity through our lake selection process. Durocher et al. (1984) identified a linear relationship between the percent of a lake covered with submerged vegetation and abundance of Largemouth Bass. In the case of our study, we found that the simple metric of PAC was not as useful in understanding Largemouth Bass abundance as BVp which actually takes into account the amount of plants present rather than just the presence or absence of plants.

The inference we were able to make about trophic resources used by sport fish was limited by our inability to properly quantify stable isotope baselines in these lakes. When source baselines are adequately described, isotope mixing models are a powerful tool for estimating the contribution of different prey sources to predators and comparing trophic position among organisms (Semmens et al. 2009, Parnell et al. 2010); without quantification of baselines, isotope mixing models cannot be applied. Collecting stable isotope baselines is a nontrivial component of stable isotope studies requiring a substantial amount of effort, especially in oligotrophic systems. Although the baseline organisms we choose to describe littoral and pelagic resources have been useful in other studies (Post 2002, Vander Zanden et al. 1998) they were did not capture the full gradient of $\delta^{13}C$ used by fish in our study lakes.

Additionally, the unusually high $\delta^{15}N$ signature of cladaceron zooplankton collected in April of 2016 confounded our ability to confidently establish a trophic level baseline. Yohannes et al. (2014) found particulate organic material collection from the pelagic water column had enriched $\delta^{15}N$ in April compared to other months at several depths. While enriched $\delta^{15}N$ from profundal baseline sources has been previously noted (Vander Zanden and Rasmussen 1999) the complexities of $\delta^{15}N$ from throughout the pelagic to profundal portion of lakes is still under investigation especially as it relates to nutrient cycling in aquatic systems (e.g., Sierszen et al. 2006). Because this zooplankton enrichment was observed only in April it is possible that a seasonal lake process (e.g., turnover) caused an upwelling of profundal resources. Research providing better insight into variation in stable isotope baselines in lakes, especially across related to the temporal and spatial variability within systems, continues to be published (e.g., Grey 2006, Cummings and Schindler 2013) and should be used to better inform future studies incorporating SIA.

Despite the limitations of the stable isotope data we collected, we were able to make relative comparisons about the trophic position occupied by Largemouth Bass and other sport fish. Because adult Largemouth Bass and Walleye trophic position did not overlap we can infer that the two species are not sharing (and thus unlikely competing for) prey resources in these lakes. This is in contrast to Fayram et al. (2005) which found Walleye diets to be more similar to Largemouth Bass diets compared to Northern Pike in northern Wisconsin lakes. In our study, regardless of presence or absence of Largemouth Bass, Walleye occupied a trophic position similar to that occupied by Northern Pike. In the majority of lakes we sampled, the $\delta^{13}C$ of Yellow Perch and Bluegill was similar to that of Walleye and Northern Pike, which would support the idea that those top-level predators are consuming fish such as Yellow Perch and Bluegill.

Other studies have found adult Largemouth Bass diets include large proportions of Yellow Perch (Liao et al. 2002, Paukert et al. 2003). In our study Largemouth Bass consumed more littoral prey resources and had a lower trophic level than we would expect if they were consuming adult Yellow Perch. However, it is common for fish to demonstrate ontogenetic shifts in diets and smaller Yellow Perch may have different trophic position than the size we sampled. We completed SIA on Yellow Perch with a mean size ranging from 115 to 229 mm, but Laio et al. (2002) found that Largemouth Bass primarily consumed fish < 100 mm. Therefore, we may not have captured the trophic position of fish most likely to be consumed by Largemouth Bass in this study. We also may have failed to encompass invertebrate prey important to Largemouth Bass, such as crayfish, and in general this study would have benefitted from a broader quantification of littoral prey resources given the dietary flexibility of Largemouth Bass (Hodgson and Kitchell 1987).

Climate change has the potential to increase the length of the growing season at the current edge of the Largemouth Bass range, potentially leading to more recruitment and higher abundances of the species (Hansen et al. 2017). Monitoring lake temperature and BVp can provide information about whether a lake is likely to support Largemouth Bass under these changing conditions. Although the trophic position of Largemouth Bass and other sport fish did not overlap at the adult stage, future research should focus on the trophic interactions among sport fish at pre-adult life stages.

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