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ASSESSING THE DISPERSAL AND RECRUITMENT OF STOCKED WALLEYE FRY IN A NORTHERN MINNESOTA CHAIN OF LAKES

by

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Joseph W. Amundson

As part of Minnesota's Walleye *Sander vitreus* egg take practice, 10% of eggs taken for hatchery purposes are stocked back into the donor lake. For Andrusia, part of the Cass Lake Chain of Lakes (Chain), this practice can result in elevated fry densities (mean: 17,000 fry/littoral hectare). However, if fry are able to disperse and use all available littoral hectares throughout the Chain, fry densities would be intermediate to typical stocking densities for Minnesota lakes (1,200 – 2,400 fry/littoral hectare). In 2016-2018, 3-3.5 million fry were mass-marked by immersion in oxytetracycline (OTC) prior to stocking into Andrusia to allow differentiation between these fish and those originating from natural reproduction or stocking of unmarked fry in other connected waters. Age-0 Walleyes were sampled throughout the Chain each fall (2016-2018) primarily by boat electrofishing. Each year, age-0 fish were widely distributed by late August. Combined mean marking rates (2016-2018) for each lake of the Chain ranged from 16 to 97% and cohort marking rates ranged from 71 to 78%. The ability of stocked fish to disperse throughout the Chain helped suppress density dependent effects, although, total length (mm) increased as catch-per-unit-effort (CPUE) decreased with distance from stocking site. In 2019, a chain-wide gill net assessment resulted in cohort (2016-2018) marking rates at ages 1 to 3 similar to their age-0 marking rates. The Chain was previously thought to be largely self-sustaining with put-back stocking considered a social aspect of management by compensating for the removal of eggs (i.e. potential recruits) rather than contributory to the Walleye population, but our results suggest that this stocking is substantially contributing to the population.

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ABSTRACT

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INTRODUCTION

Stocking Walleye *Sander vitreus* has been a management tool extensively used in North America, and a standard practice in Minnesota for over a half century (Li et al.

1996a, 1996b). Stocking rates are often based on the projected carrying capacity of the system being stocked and past evaluations of stocking success or failure of individual waters (MDNR 1996). Stocking lakes that have some degree of natural production may not add to the population and in some cases reduce the naturally produced population by intensifying intraspecific interactions (Li et al. 1996a; MDNR 1996). Fayram et al. (2005) reviewed past studies and showed stocking rates that are either too low or too high may negatively affect the cohort of stocked fish or the fish community in the receiving water. Stocking rates that are too low may result in low recruitment if the fish being stocked become prey for other species in the system. Conversely, stocking rates that are too high may lead to density-dependent reductions in growth and development rates, which may lead to increased predation, cannibalism and size-dependent mortality.

The Minnesota Department of Natural Resources (MNDNR) operates 13 Walleye egg take stations throughout the State to fulfill its annual hatchery needs of over 600 million eggs (Logsdon and Anderson 2018). Approximately 10% of eggs taken for hatchery purposes are supplementary stocked back into the egg-source lakes that support these spawning runs to compensate for the removal of eggs (i.e., potential recruits) from the system. The 10% put-back practice may result in elevated fry densities several times greater than recommended fry stocking density of 1,200 – 2,400 fry/littoral hectare (MDNR 1996). These densities associated with put-back stocking can reduce growth and survival affecting recruitment and subsequent year-class strength (Logsdon and Anderson 2018).

In the past, differentiating between naturally produced and hatchery reared fish was difficult because conventional fish marking methods are inadequate due to the small size (6-9 mm) of Walleye fry at the time of stocking (Fielder 2002; Lucchesi 2002). Chemical marking of potential food fish using oxytetracycline (OTC) was approved in 2004 (FDA 2017) following years of successful marking trials in Walleye fry (Brooks et al 1994; Fielder 2002; Lucchesi 2002). Subsequently, Logsdon et al. (2004) demonstrated newly hatched Walleye fry can be marked in large numbers with little adverse effects on growth and survival of the fish or disruption of day-to-day hatchery operations.

Stocking of OTC-marked Walleye fry in Minnesota was first done during the recovery of the Red Lakes at densities of 988-1,288 fry/ha, resulting in age-0 marking rates up to 97% and marks remaining detectable up to age-8 (Logsdon et al. 2016). Later studies were done on Leech Lake and Woman Lakes resulting in marking rates ranging 39% to 86% (Logsdon and Anderson 2018). These studies have shown that stocked fry can compose a large proportion of individual year classes and survive alongside wild fry.

While these studies outline what a powerful tool OTC marking can be to improve our understanding of Walleye fry dynamics, none of them were designed to document or quantify dispersal from the stocking location. The ability to disperse is essential, when stocking densities are high, because density dependent growth can result in decreased survival via increased predation risk and size-dependent mortality (Myrvold and Kennedy 2015), potentially reducing year class strength (Logsdon and Anderson 2018). Walleye fry have weak swimming abilities until ~21 mm in total length (Humphrey et al 2012). Prior to reaching this length (~20-30 days), the pelagic fry are subjected to wind-driven water circulation, upwellings and hydrodynamic characteristics, and river currents. These abiotic factors influence initial dispersal (Fraker et al. 2015) until the fish ultimately become demersal (Pratt and Fox 2001). Managers may choose to spread the fry using multiple stocking locations to alleviate potential density-dependent effects. However, in systems with extremely high stocking densities, this practice maybe futile. The objective of this study was to determine if elevated Walleye put-back stocking rates in a northern Minnesota chain of lakes creates density-dependent effects by, 1) assessing if Walleyes stocked into one lake in the chain disperse to other connected lakes, 2) comparing growth, condition, and relative abundance within and among lakes to detect any negative density-dependent effects and, 3) quantifying stocked Walleye recruitment to the chain of lakes and other connected waters.

METHODS

Study area – The Cass Lake Chain (Chain), located in north-central Minnesota bordering Beltrami and Cass Counties, contains four main lakes: Andrusia, Cass, Kitchi, Wolf and two connected lakes: Pike Bay and Big (Figure 1). The Chain is connected by two substantial river systems, the Mississippi River flowing west to east, through Wolf, Andrusia and Cass, and the Turtle River flowing north to south, through Kitchi and Cass. The only outflow is the Mississippi River, exiting the northeast corner of Cass. Andrusia is the egg-source lake in the Chain and thus receives all the put-back fry. The egg take station is situated on Big Lake Creek which exits Big's shallow west bay flowing south into the north end of Andrusia. Beaver *Castor canadensis* dam building activity on the creek creates impoundments that are breached each spring to provide adequate water flow to attract spawning Walleye. Pike Bay and Cass are connected via a shallow canal that can flow in both directions depending on water level fluctuations and wind/wave action. The natural flow is south to north from Pike Bay to Cass (Figure 1). Due to management agreements, fry stocking occurs (unmarked) every other year in Kitchi (odd years) and every year in Pike Bay and Big at densities of 2,400 fry/littoral hectare.

Egg collection, incubation and fry marking – The Minnesota Department of Natural Resources (MNDNR) obtains Walleye eggs from wild stock during the spawning migration from Big Lake Creek, a tributary to Andrusia. Eggs were stripped from female fish, fertilized and water hardened on site before transport to the MNDNR's Bemidji State Fish Hatchery for incubation. The Big Lake Creek egg station produces fry for numerous lakes within the northern reaches of the Mississippi River watershed.

After incubation (~ 3 weeks), the newly hatched Walleye fry (< 24 h post hatch, ~ 8 mm total length) were treated by immersion in an OTC solution using procedures first outlined by Brooks et al. (1994) and later modified to allow for the maximum Food and Drug Administration allowable OTC concertation, 700 mg/L, and immersion period, 6 hours, of active OTC (Fielder 2002; Lucchesi 2002; Logsdon et al. 2004; Logsdon et al. 2009). The OTC solution was prepared using Terramycin-343 (Pfizer, New York, NY), then buffered to pH 6.8 with sodium phosphate dibasic (Sigma, St. Louis, Missouri). Logsdon et al. (2009) further describes the procedures that are still currently used by the Bemidji hatchery and for this study. A silicon-based surfactant (No-Foam, Argent Chemical, Redmond, Washington) was added to the solution at a concentration of 0.04 mL/L to reduce foaming of the OTC solution. To reduce handling stress, the fry were treated directly in transport containers. These containers, which are commonly used by the MNDNR to transport walleye fry, consisted of collapsible, 19-L, clear plastic water jugs with the caps modified by the addition of automotive tire valve stems to facilitate inflation with oxygen. The fry were enumerated by weight and combined with the OTC solution in each container at a density of approximately 4,400 fry/L. To allow room for oxygen inflation, a maximum of 50,000 fry and 11.4 L of OTC solution were combined in each container. The fry remained immersed in the OTC solution for at least 6 h to ensure adequate time for the OTC to be metabolized and be incorporated into the fish's bony structures. Care was taken to reduce fry exposure to sunlight (OTC photodegrades) and changes in temperature during the entire process.

Stocking – Approximately 3 million fry were treated with OTC over a 2-d period at the Bemidji, MN hatchery in May 2016 and stocked into the pelagic zone of the north basin of Andrusia (Figure 1) to avoid obvious transport downstream via the Mississippi River. A sub-sample of OTC-treated fry was transferred to area rearing ponds to grow to fingerling size, at which time a mark efficacy evaluation was conducted. Approximately 3.5 million fry were treated and stocked over a 2- or 3-d period in 2017 and 2018, in the same manner as 2016. The increase in 2017 was due to observed fry mortality in the jugs due to a precipitate that sometimes forms during the immersion period.

Juvenile sampling – Age-0 and age-1 Walleyes were sampled using multiple methods each year. In 2016, 2017, 2018 and 2019 all lakes in the system were sampled via nighttime boat electrofishing during a two-week period from the end of August through the beginning of September at standardized locations (Figure 1). Target minimum sample size was n=50 for Andrusia, Wolf, Kitchi, Pike Bay, and Big, while a minimum target sample size of n=200 for Cass was set as this lake is much larger and more complex than the other lakes in the Chain. In cases where the minimum sample size was not obtained in the initial sampling event, additional effort was expended until the sample size was achieved or it was deemed too inefficient to continue attempting to collect fish from that lake. Additional boat electrofishing in October, as well as bottom trawling in August, were conducted on Cass (Figure 1) as part of standard sampling associated with Minnesota's Large Lake Monitoring Program. Age-0 (2016-2018) and age-1 (2017-2019) fish collected during electrofishing and trawling were placed on ice upon collection and then frozen until examination for an OTC mark. In the lab, fish were thawed, individually measured for total length (mm), weight, (g) and both sagittal otoliths were removed. All fish < 175 mm were assumed to be age-0 based on historical age length data, and fish ≥ 175 mm had their sagittal otoliths inspected using whole view methods described by Isermann et al. (2003) to determine age (Table 1).

Sub-adult sampling – In 2019, Walleyes were sampled from each lake in the Chain and connected waters during a chain-wide assessment with experimental gill nets during an 8-week period in August and September (Figure 1). The gill nets consisted of five 15.3-m panels of mesh measuring 19, 25, 32, 38, and 51 mm (measurements are bar length). The nets were fished for approximately 24 hours prior to retrieval, and all collected Walleyes were individually measured for total length, weighed, and both sagittal otoliths were removed. Age-3 (2016 cohort), age-2 (2017 cohort) and age-1 (2018 cohort) fish were identified by inspection of a broken sagittal otolith as described by Heidinger and Clodfelter (1987) or inspected using whole view methods described by Isermann et al. (2003). As part of the Minnesota Large Lake Program, experimental gill netting was conducted annually (2016-2018) on Cass (20 nets; Figure 1), fish aged to cohorts that included OTC-marked fish were collected and processed as described above.

OTC mark detection – Walleye otolith inspection for the presence of an OTC mark was conducted following the methods of Secor et al. (1991), Brooks et al. (1994) and Logsdon et al. (2009). Regardless of aging technique used, Isermann et al. (2003) or Heidinger and Clodfelter (1987), Walleyes sagittal otolith(s) were first placed in a black dish and submerged in water to clean remaining sacculus, if present, under a dissection microscope. Once cleaned, the otolith(s) were secured to a microscope slide, convex side down, with cyanoacrylate glue (i.e. Superglue) and left to dry for ≥ 45 minutes in the dark. Then otolith(s) were wet sanded with either 600- or 1000-grit waterproof sandpaper until the inner daily growth rings become visible, using immersion oil to help clarity, with transmitted light under 100x magnification. Inspection for mark was conducted under an epifluorescent microscope with ultraviolet (UV) light and filter blocks designed to optimize oxytetracycline fluorescence (Bumguardner 1991; Brooks et al. 1994; Logsdon et al. 2004). Each otolith was inspected by a single reader, a total of two different readers (2016-2017 and 2018-2019 field seasons) inspected otoliths over the 4-year study. Before starting on the project, readers were subjected to a blind 100 fish test to evaluate readers accuracy of distinguishing between known marked and unmarked fish. Both readers scored 100% (identifying 100/100 fish correctly), letting us presume that marking rates presented in this study reflect the actual proportions of marked and unmarked fish in the population.

DATA ANALYSIS

Stocked Walleye dispersal and marking rates – Yearly dispersal of stocked Walleyes was determined using relative abundance (catch-per-unit-effort; CPUE) of OTC marked fish caught during boat electrofishing conducted during late summer (end of August through beginning of September) at standardized sites in every study lake. CPUE calculations were made using the total number of marked fish caught divided by sampling time for each lake and year. Fish collected from all sampling gears and sites each year (Figure 1; Figure 2) were used in marking rate analysis. A generalized linear model (glm) was developed in program R (R Core Team 2014), glm(Mark ~ Lake, family=binomial), where Mark, categorized as yes or no, is a function of Lake captured in, to estimate the marking rate and standard error. This process was used for age-0 in 2016, 2017, and 2018. Based on preliminary results of fry dispersal, migration into lakes Pike Bay and Big was very limited. For the remainder of this manuscript the Chain will refer to Andrusia, Cass, Kitchi and Wolf. Pike Bay and Big will be referred to as, other connected waters.

Growth and condition – To evaluate possible variability in total length between marked and unmarked Walleyes within each lake during the first summer of life each year, fish collected during the late-August, chain-wide nighttime electrofishing assessment were tested 1) for normality using Shapiro-Wilk test, 2) if both were considered normal ($P > 0.05$) a *t*-test was performed or, 3) if one or both were considered non-normal ($P < 0.05$) a Wilcoxon test was performed (Table 2). To evaluate marked age-0 Walleye growth among lakes, multiple linear regression-based candidate models were developed. A set of candidate models was developed to assess whether lake and year were meaningful predictors of fish total length. All candidate models included a capture date, day of year (DOY), predictor variable plus year and/or lake. The candidate models were compared using Akaike's information criterion (AIC) to determine which was the best supported model (Akaike 1973; Table 3). Using capture dates from all fish collected in the Chain each year, the median sampling DOY was established. The best supported model was then used to predict the mean total length and standard error on the median day of year.

A set of candidate models was developed to assess whether distance from stocking site was a meaningful predictor of fish total length. All candidate models included a capture DOY predictor variable and year of capture with either shoreline distance (ShoreDist) from stocking site or direct distance (DirectDist) from the stocking site in Andrusia to each electrofishing and trawling sites in the Chain (Figure 1). The candidate models were compared using Akaike's information criterion (AIC) to determine which was the best supported model (Akaike 1973; Table 3). Using the median DOY, the best AIC supported model was used to predict the mean total length and standard error across the range of distances sampling sites were from the stocking location.

The relative condition (Kn) of age-0 Walleye was determined by the equation:

$$Kn = W/W',$$

where W is the weight (g) of an individual fish and W' is the length-specific expected weight for a fish in the population under study as predicted by a length weight-length regression equation calculated for that population (Le Cren 1951). A set of candidate models was developed to assess whether lake, ShoreDist or catch-per-unit-effort (CPUE) were a meaningful predictors of fish relative condition each year. The candidate models were compared using Akaike's information criterion (AIC) to determine which was the best supported model (Akaike 1973; Table 5). The best supported model was used to predict the mean Kn and standard error.

Marked Population – The percent of the population of Walleyes ages 1-3 (2018-2016 cohorts) in each lake was estimated from the gill-net catches of the 2019 chain-wide assessment using Anderson's (1998) gill-net catchability model (q_{abg} model). Catchability ($q[l]$) was first calculated for each 10-mm length-group using the formula

$$q(l) = 1.32 \cdot \alpha(l)\ \Sigma_{meshes}\, \beta_m \gamma(x),$$

where l is the midpoint of the length-group, $\alpha(l)$ is the encounter probability per length group for the entire gill net, βm is the contact coefficient per mesh size, and $\gamma(x)$ is the retention function for each mesh size where x is the fish/mesh perimeter ratio. The abundance estimates of marked and unmarked (N) fish were then calculated for each lake with the formula

$$\widehat{N} = \Sigma_{lengths} CPUE_l\ /\ q(l),$$

where CPUE is the catch per unit effort of age 1-3 Walleyes per 10 mm length interval, and q(l) is the catchability coefficient. Lakes were analyzed separately to account for lake size and number of nets used. Abundance estimates of marked and unmarked fish were run separately to allow for any sized differences to influence predicted total population and marking rates. The total population of marked fish in the Chain, ages 1 to 3, was divided by the total Chain population of fish from these cohorts to determine the proportion of fish that originated from the Andrusia stockings.

RESULTS

Stocked Walleye dispersal and marking rates – In 2016, age-0 fish dispersed into every lake in the Chain and into Pike Bay, with no marked fish caught in Big (Table 4; Figure 3). Within the Chain, the highest marked CPUE was in Andrusia (199.4 fish/hr) followed by Wolf (50.0 fish/hr), Cass (36.5 fish/hr), and Kitchi (6.2 fish/hr). Other connected waters age-0 marked CPUE were, Pike Bay (1.0 fish/hr) and Big (0.0 fish/hr). The overall age-0 marking rate for the Chain was 72%. The highest rates were in Andrusia (96%; SE = 0.02), followed by Cass (84%; SE = 0.02), Wolf (39%; SE = 0.05) and Kitchi (24%; SE = 0.06; Figure 2). The other connected waters age-0 marking rates were, Pike Bay (2%; SE = 0.02) and Big (0%; SE < 0.01).

In 2017, Age-0 fish dispersed into every lake in the Chain except Kitchi and no marked fish were caught in Pike Bay or Big (Table 4; Figure 3). Within the Chain, the highest marked CPUE was in Andrusia (73.6 fish/hr) followed by Wolf (22.7 fish/hr), and Cass (14.0 fish/hr). The overall age-0 marking rate for the Chain was 78%. The highest marking rates were in Andrusia (99%; SE = 0.01), followed by Cass (97%; SE = 0.01) and Wolf (25%; SE = 0.05; Figure 2). The other connected waters of Pike Bay (0%; SE < 0.01) and Big (0%; SE < 0.01) did not have any marked age-0 Walleye detected.

In 2018, Age-0 fish dispersed into every lake in the Chain, but no marked fish were caught in Pike Bay or Big during August or September sampling (Table 4; Figure 3). Within the Chain, the highest marked CPUE was in Andrusia (185.0 fish/hr) followed by Wolf (100.1 fish/hr), Cass (45.0 fish/hr) and Kitchi (10.6 fish/hr). The overall age-0 marking rate for the Chain was 78%. The highest marking rates were in Andrusia (97%; SE = 0.01), followed by Cass (80%; SE = 0.02), Wolf (33%: SE = 0.04) and Kitchi (16%;

SE = 0.05; Figure 2). The other connected waters age-0 marking rates were, Pike Bay (40%; SE = 0.2; two of five fish sampled during supplemental sampling in October) and Big (0%; SE < 0.01).

Marking rates of age-0 Walleyes were similar throughout the years in each of the study lakes. Within the Chain, relative abundance of OTC marked fish was highest in Andrusia each year, followed by Wolf, Cass and Kitchi (Table 4; Figure 2). Pike Bay had one marked fish captured during standard sampling (2016) and Big did not have a marked fish captured (Table 4; Figure 2). Marking rates within the Chain where highest in Andrusia each year, followed by Cass, Wolf and Kitchi (Figure 2).

Growth and condition – In 2016, lake-specific marked and unmarked fish mean total lengths ranged from 139 (SD = 2.38) to 151.3 mm (SD = 2.68) and 138.5 (SD = 1.82) to 147.9 mm (SD = 1.31), respectively (Table 2). There were no statistical differences between marked and unmarked total lengths within each lake ($P > 0.05$; Table 2). In 2017, lake-specific mean total lengths of marked and unmarked fish ranged from 134.9 (SD = 1.01) to 141.9 mm (SD = 1.00) and 128 (SD = 4.36) to 144 mm (SD = NA), respectively (Table 2). There were no statistical differences between marked and unmarked fish total length within each lake ($P > 0.05$; Table 2). In 2018, lake-specific mean total lengths of marked and unmarked fish ranged from 138.5 (SD = 0.72) to 148 mm (SD = 2.42) and 134.6 (SD = 2.93) to 148.5 mm (SD = 1.35), respectively (Table 2). There were statistical differences between marked and unmarked fish mean total length in lakes Cass ($P = 0.01$) and Wolf ($P = 0.00$; Table 2). Marked fish in Cass were larger (148 mm) than unmarked fish (141 mm) and the opposite occurred in Wolf where marked fish were shorter (139 mm) than unmarked fish (144 mm; Table 2).

For among lake analyses of marked Walleye growth using all age-0 fish captured during the field season, the best supported AIC score was associated with the model TL ~ Year · Lake + DOY (Table 3). A mean length projection of fish in each Chain lake on day 239 of each year (2016-218) was created (Figure 4). In 2016 and 2017, mean lengths were similar in Andrusia, Wolf and Cass (difference of mean length in 2016, 2.2 mm, and 2017, 2.7 mm; Table 2). However, in 2016 the model estimated mean length of fish in Kitchi was 5.7 mm longer than any other lake in the Chain (Table 2). In 2018, mean length increased as fish dispersed from the stocking site in Andrusia. The mean lengths were, Andrusia 138.5 mm (SE = 0.87), Wolf 141.9 mm (SE = 1.9), Cass 148.1 mm (SE = 0.75) and largest in Kitchi 152.8 mm (SE = 3.67; Table 2).

Dispersal distance from stocking site analysis of marked Walleye growth had two near identically supported models (Table 3). Both models support that distance fish traveled from the stocking site, year and DOY is related to total length. After further analysis, the model that best matched the data was determined to be, TL ~ Year · ShoreDist + DOY. As fish dispersed each year (2016-2018), mean total length increased as the distance traveled from the stocking site increased (Figure 6).

Among lake analysis of marked age-0 relative condition resulted in two equally supported models (Table 3). The model, Kn ~ Year · Lake, was chosen for ease of among lake comparisons (Figure 4). Relative condition of marked fish within the Chain was highest in 2016 and lowest in 2017 (Figure 4). Among these lakes, Andrusia relative condition was highest, and Cass was lowest, except in 2017. Kitchi was the only lake to have fish relative condition at or above 1.00 in the years marked fish were sampled. All Chain lakes yearly pooled relative condition were above 1.00 except for Cass (0.98).

Marked population – Age-1, -2 and -3 (2018, 2017 and 2016 cohorts) Chain Walleye estimated marked and unmarked cohort populations according to Anderson (1998) gill net selectivity model were 217,046 and 109,065 (2018), 38,610 and 7837 (2017), 28,254 and 9,248 (2016) respectively (Table 5). OTC marked populations of cohorts represented 66.7% (2018), 83.1% (2017), and 75.3% (2016) of the total estimated populations (Table 5). Cass represents 78.2% of the total surface area within the Chain but held an even higher cohort percentage of the total Walleye population, 94.1% (2018), 94.9% (2017) and 85.6% (2016). Pike Bay did not have a marked fish sampled in the 2016 and 2017 cohorts, although 65.3% of the 2018 cohorts total estimated population of 3,449 was from fish marked in Andrusia that year (Table 5). No OTC marked fish from any cohort (2016-2018) were detected in Big during the 2019 gill net sampling (Table 5).

DISCUSSION

Marked age-0 Walleyes showed the ability to disperse throughout the Chain within the first three months after stocking. Movement downstream into Cass via the Mississippi River from Andrusia was expected, although the extent of movement upstream into Wolf and Kitchi was higher than anticipated. Electrofishing catch rates of marked fish in Wolf, which lies 3.2 km upstream for Andrusia via the Mississippi River, ranged from 22.7 to 100.1 fish/hr and were greater than Cass (which lies downstream) each year. Marked fish were also detected in Kitchi in 2016 and 2018 at electrofishing catch rates of 6.2 and 10.6 fish/hr. Although these catch rates are modest, they represent the capture of dozens of individuals. These fish moved a considerable distance from the stocking site (20 km) and had to first move downstream within the Mississippi River, through Cass, then upstream through the Turtle River to reach the sampling sites on the north end of Kitchi. Due to sampling timeframes, it is not exactly clear when these upstream movements occurred. Humphrey et al. (2012) found that Walleye fry do not have the ability to swim against currents greater than 20 cm$\cdot$s^{-1} (water velocities of the Mississippi R. and Turtle R. were not measured) until they reach 25 mm in total length (approximately 3 weeks posthatch). This increased swimming ability closely coincides with the fry's shift from pelagic to demersal (Pratt and Fox 2001). Therefore, the absolute earliest attempts to move upstream by marked fry in this study would have been around early June (1-12; 3 weeks after yearly stocking dates).

Marked fish were detected in every lake in the Chain each year, except Kitchi in 2017. Only three marked age-0 fish were sampled in Pike Bay throughout the study (2016-2018) further indicating the interaction between Cass and Pike Bay is minimal (Strand 1980; Kennedy 2011) and zero marked fish were sampled in Big. OTC marked fish made up the majority, 71 to 78%, of the age-0 Walleyes sampled within the Chain each year. Although this percentage is combined data from four lakes, the results are similar to Logsdon and Anderson (2018) where they found other Minnesota egg-source lakes Walleye year-classes can be compromised of a substantial proportion of stocked fish (range, 4 to 97%). Marking rates in Andrusia and Cass were least 80% and each lake in the Chain had a marking rate of 16% or greater each year with one exception in Kitchi (2017: 0%). Even though no marked fish were sampled in Kitchi in 2017 (n = 33; the only year the minimum target sample size, n = 50, was not achieved), it is unrealistic that OTC marked fish did not disperse into Kitchi that year with marking rates from 2016 (24%) and 2018 (16%) showing it is well connected to the other Chain lakes. Kitchi and the two lakes directly upstream (Big Rice and Little Rice) were stocked in 2017 with unmarked fry, at a rate of 2,400 fry/littoral hectare, totaling around 2.1 million total fry between the three lakes. This stocking event may have diluted the possibility of capturing a marked fish in the sample size that was achieved.

Marked and unmarked fish growth (TL) within each Chain lake at age-0 were similar. There were only two instances in which mean total length of marked and unmarked fry were significantly different. Both instances occurred in 2018 with opposite outcomes, marked fish were larger in Cass and unmarked were larger in Wolf. Further, investigation into possible differences in relative condition between marked and unmarked fish within lakes at age-0 revealed no instances where condition was significantly different, even in 2018 when total lengths of marked and unmarked fish were in Cass and Wolf. These results are similar to Logsdon (2006) pond experiments where no advantages in growth of survival between of OTC-treated fry and non-treated fry were found when they were stocked together. If one group of fish (marked or unmarked) experienced growth depression within or among lakes (within the first three months), there was time for compensatory growth to occur when favorable conditions were restored prior to capture (Ali et al. 2003). The similar growth and condition within each lake of marked or unmarked fry allowed for similar overwintering success as later capture at age-1 to age-3 resulted in marking rates close to each cohort's age-0 marking rates (± 10%). These results mirror Logsdon (2006) where the 1999 Walleye cohort of the Red Lakes age-1 to age-5 were within 10% of the cohorts age-0 rates.

Relative condition of marked fish varied by year in each lake in the Chain. Total densities Chain wide were highest in 2018, followed by 2016 and lowest in 2017. A trend did emerge within each Chain lake as relative condition of marked fish was highest in 2016 and lowest in 2017, even though densities (total CPUE) were lowest in 2017 (expect Kitchi, condition was highest in 2018). Despite high stocking densities, Andrusia yearly average relative condition (Kn) of age-0 fish was highest in 2016 (1.05) and second highest in 2017 and 2018 (0.97 and 1.00, respectively). These results indicate that relative condition of the marked fish in the Chain is not solely dependent on stocking density.

Alternatively, growth of marked Walleyes increased with distance from stocking site in 2016 and 2018 and remained steady in 2017 (lowest total CPUE in each lake during the study). The increase in growth coincides with the decrease in total CPUE creating an inverse relationship and suggesting there is density-dependent growth occurring in the Chain. This relationship could also be attributed to the increasing swimming capabilities of juvenile Walleyes as they grow (Humphrey et al. 2012) and bigger fish were able to swim farther by the time of sampling. Unfortunately, the way the data was collected does not allow for clear interpretation of which mechanism is driving the relationship between growth and distance, but unmarked and marked fry had similar lengths in each lake and our evidence points towards the former (density-dependent). Furthermore, the Walleyes each year (combined marked and unmarked) in Kitchi had longer total lengths than any other lake in the Chain coupled with the lowest total CPUE each year (expect for 2018) suggesting there is some degree of density-dependent growth occurring from one end of the Chain to the other.

The results of this study have shown that fry stocked into Andrusia have the capabilities to disperse throughout the Chain, downstream and upstream, during the first three months of life. Subsequently, OTC marked cohorts (2016 -2018) were captured via gill nets at nearly the same marking rate as they were captured at age-0, Although there seems to be no advantages between marked and unmarked fish in growth or condition in each lake, marked fish made up the majority of each cohort. Growth modeling across distance from stocking site showed total lengths of marked fish increased as total CPUE declined, suggesting some degree of density-dependent growth Chain wide but it is currently unknown if those differences are biologically significant.

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TABLE 1. Total number of Walleyes analyzed for OTC marks each field season, 2016-2019, by age.

Year	Age-0	Age-1	Age-2	Age-3	Total
2016	610	-	-	-	610
2017	538	223	-	-	761
2018	952	39	42	-	1,033
2019	-	245	97	137	479
Total	2,100	507	139	137	2,883

TABLE 2. Growth comparison between marked (Y) and unmarked (N) fry captured during the chain-wide electrofishing at the end of August through the beginning of September 2016-2018. Standard errors are presented in parentheses.

Year	n		Mean TL mm		Shapiro-Wilk		*t*-test	Wilcox
	Y	N	Y	N	Y	N		
				Andrusia				
2016	115	5	141 (1.46)	138.8 (7.9)	0.0006	0.7928	-	0.7376
2017	110	1	134.9 (1.01)	144 (NA)	0.0000	NA	-	0.2743
2018	193	5	138.5 (0.72)	134.6 (2.93)	0.0002	0.3866	-	0.2465
				Cass				
2016	143	24	142.7 (0.87)	142.3 (3.3)	0.0052	0.8120	-	0.7013
2017	138	3	141.9 (1)	129 (4.36)	0.2418	0.7804	0.0912	-
2018	102	6	147.9 (0.89)	141.2 (1.92)	0.8650	0.4445	0.0147	-
				Wolf				
2016	32	50	139 (2.38)	144.9 (1.32)	0.0349	0.0553	-	0.0518
2017	17	52	139.2 (3.98)	138.5 (1.82)	0.0480	0.0338	-	0.6705
2018	41	78	138.9 (1.35)	144.2 (1.02)	0.0539	0.3041	0.0023	-
				Kitchi				
2016	14	45	151.3 (2.68)	147.9 (1.31)	0.3011	0.8085	0.2712	-
2018	11	58	148 (2.42)	148.5 (1.35)	0.8287	0.0035	-	0.7181

TABLE 3. Model rankings to explain variation in marked age-0 Walleye growth and relative condition in the Cass Lake Chain, 2016-2018. Total length (TL) and relative condition (Kn) of marked Walleye at the time of capture where predicted using Akaike information criterion with the one or more predictor variables: Year of sampling, Lake sampled from, Day of Year (DOY; capture date), density (CPUE), distance in km from stocking site in Andrusia Lake; Direct Distance (DirectDist) by water and Shoreline Distance (ShoreDist) by water (ΔAIC; the difference between each model with the smallest value, highest ranked, model representing the best fitted model best fitted AIC model). The four highest ranking models and the null model are represented.

Equation	Δ AIC
Growth by Lake	
TL ~ Year · Lake + DOY	0
TL ~ Year + Lake + DOY	42.2
TL ~ Year · DOY	61.7
TL ~ Year + DOY	67.8
TL ~ 1 + DOY	96.3
Growth by Distance	
TL ~ Year + ShoreDist + DOY	0
TL ~ Year · ShoreDist + DOY	0.05
TL ~ Year · DirectDist + DOY	2.7
TL ~ Year + DirectDist + DOY	3.0
TL ~ 1 + DOY	72.7
Condition	
Kn ~ Year · Lake	0
Kn ~ Year · SD	0.5
Kn ~ Year + Lake	6.6
Kn ~ Year · CPUE	8.2
Kn ~ 1	88.8

TABLE 4. Total boat electrofishing catch-per-unit-effort (CPUE) of age-0 walleye in the Cass Lake Chain and other connected waters late August through early September, 2016-2018.

CPUE	Cass Lake Chain				Other Connected Waters	
(fish/hr)	Andrusia	Cass	Wolf	Kitchi	Pike Bay	Big
			2016			
Marked	199.4	36.5	50.0	6.2	1.0	0
Unmarked	8.7	6.5	78.0	16.1	53.7	95.5
Total	208.1	43.0	128.0	22.3	54.7	95.5
Effort (hrs)	0.58	2.63	0.64	0.81	0.97	0.59
			2017			
Marked	73.6	14.0	22.7	0	0	0
Unmarked	1.4	0.6	69.7	11.9	4.9	23.9
Total	75.0	14.6	92.4	11.9	4.9	23.9
Effort (hrs)	0.73	3.00	0.7	1.09	0.68	0.5
			2018			
Marked	185.0	45.0	100.1	10.6	0	0
Unmarked	5.7	4.6	193.2	55.6	0	13.8
Total	190.7	49.6	293.3	66.2	0	13.8
Effort (hrs)	0.53	1.31	0.56	1.04	1.01	0.72

TABLE 5: Abundance estimates generated by Anderson (1998) gill net selectivity model using 2019 gill net catches for each cohort for each lake in the Cass Lake Chain and connected waters.

		Mark		
Lake	Age	Yes	No	Rate
Chain				
Andrusia	1	4,230 (1,071 - 7,649)	2,791 (0 - 6,878)	60%
	2	837 (0 - 2,093)	306 (0 - 919)	73%
	3	2,567 (1,521 - 3,817)	711 (194 - 1,143)	78%
Cass	1	210,233 (116,912 - 326,623)	96,642 (46,246 - 445,550)	69%
	2	37,773 (23,104 - 52,380)	6,283 (2,578 - 10,523)	86%
	3	24,394 (13,136 - 38,529)	7,722 (3,601 - 12,665)	76%
Wolf	1	2,134 (463 - 4,730)	4,387 (1,953 - 6,903)	33%
	2	0	878 (0 - 1,907)	0%
	3	1,201 (508 - 2,000)	630 (206 - 1,122)	66%
Kitchi	1	5,244 (2,169 - 8,681)	450 (0 - 1,350)	8%
	2	370 (0 - 905)	0	0%
	3	92 (0 - 276)	184 (0 - 498)	33%
Total	1	217,046 (118,446 - 340,352)	109,065 (63,673 - 160,917)	67%
	2	38,610 (23,104 - 54,472)	7.837 (3,240 - 12,444)	83%
	3	28,254 (14,895 - 44,622)	9,248 (5,890 - 12,683)	75%
Other connected				
Pike Bay	1	2,251 (0 - 5,556)	1,198 (0 - 3,598)	65%
	2	0	25,126 (10,675 - 42,533)	0%
	3	0	9,371 (3,295 - 16,546)	0%
Big	1	0	4,936 (0 - 12,341)	0%
	2	0	2,885 (0 - 6,872)	0%
	3	0	6,623 (3,783 - 9,531)	0%

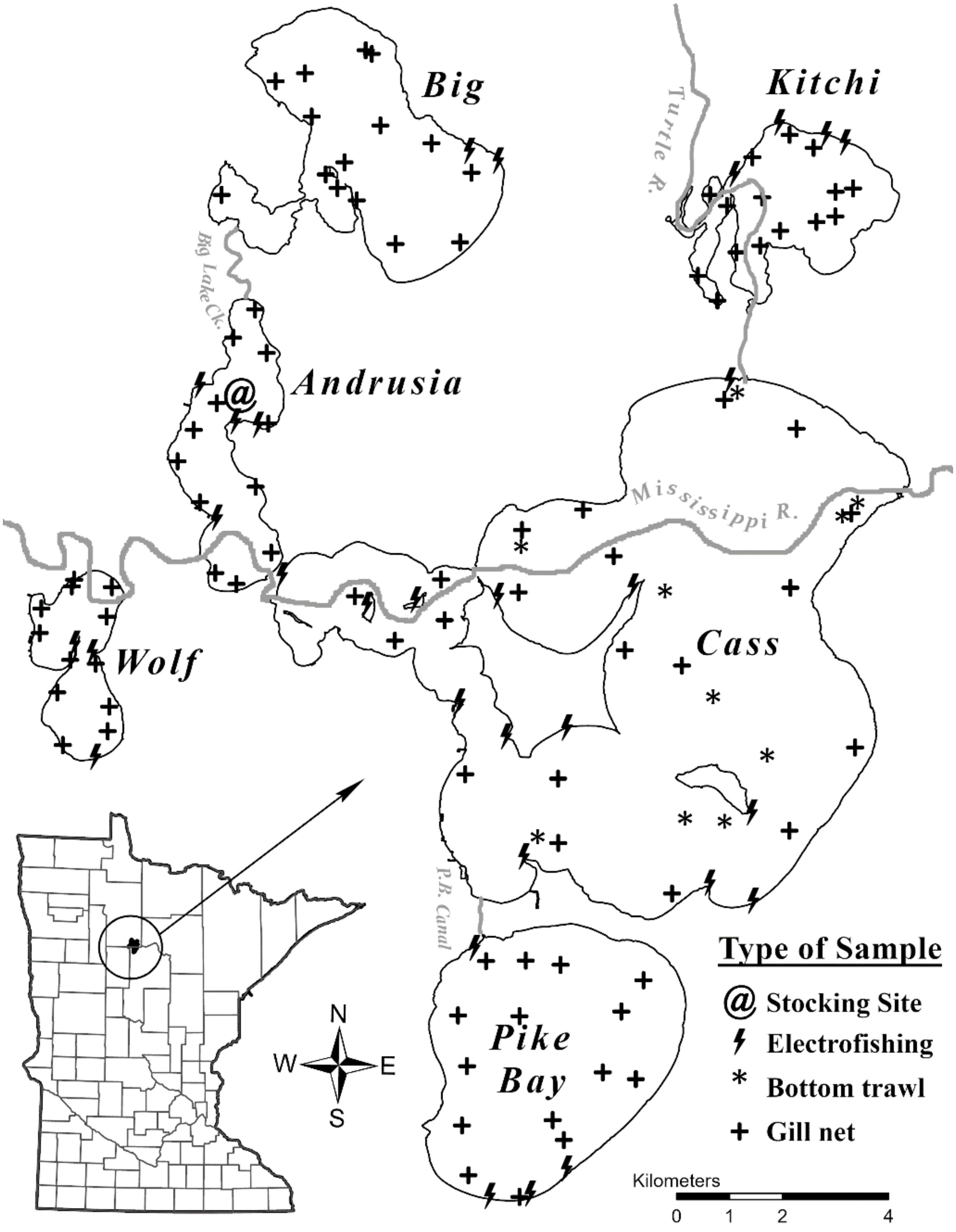

FIGURE 1. Cass Lake Chain stocking site, sampling sites and water ways.

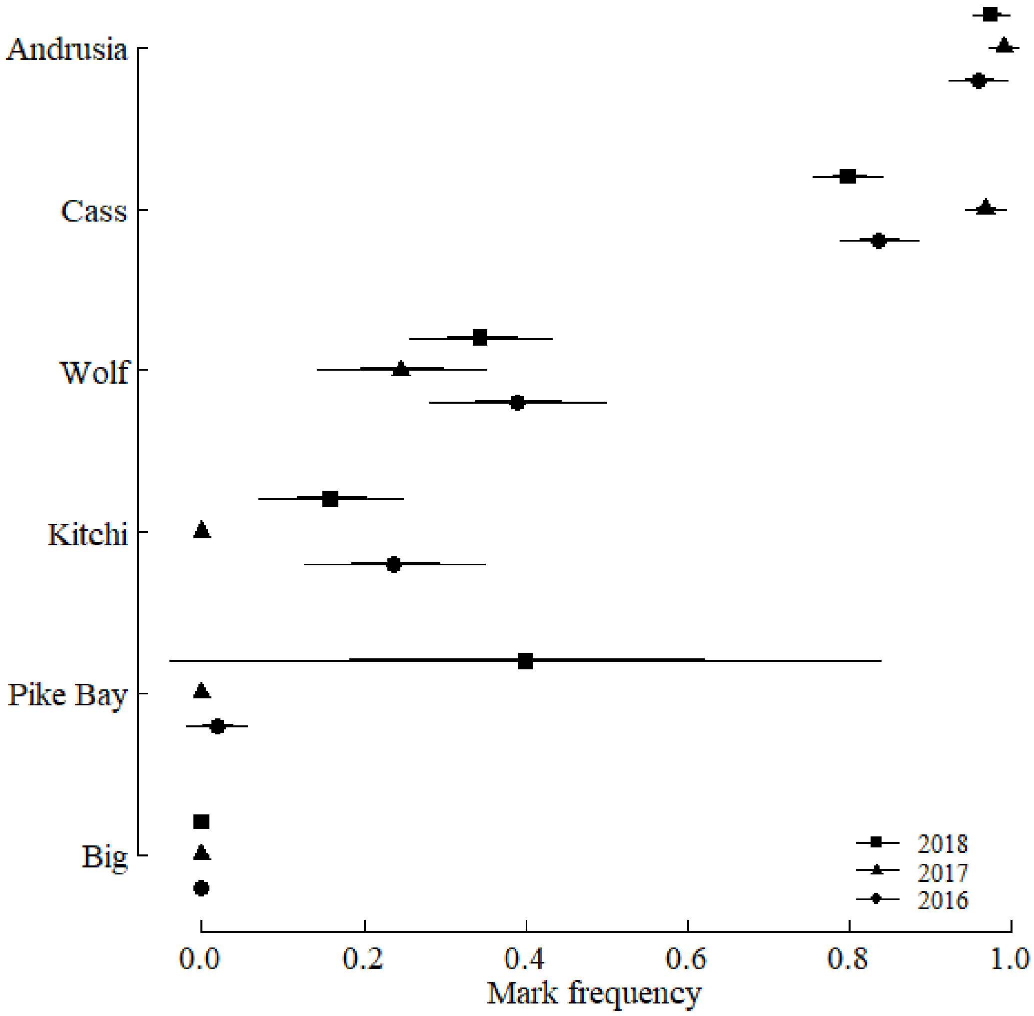

FIGURE 2. Age-0 Walleye OTC marking frequency of all fish analyzed for each lake by year. Dot represents marking frequency with lines representing one and two standard errors.

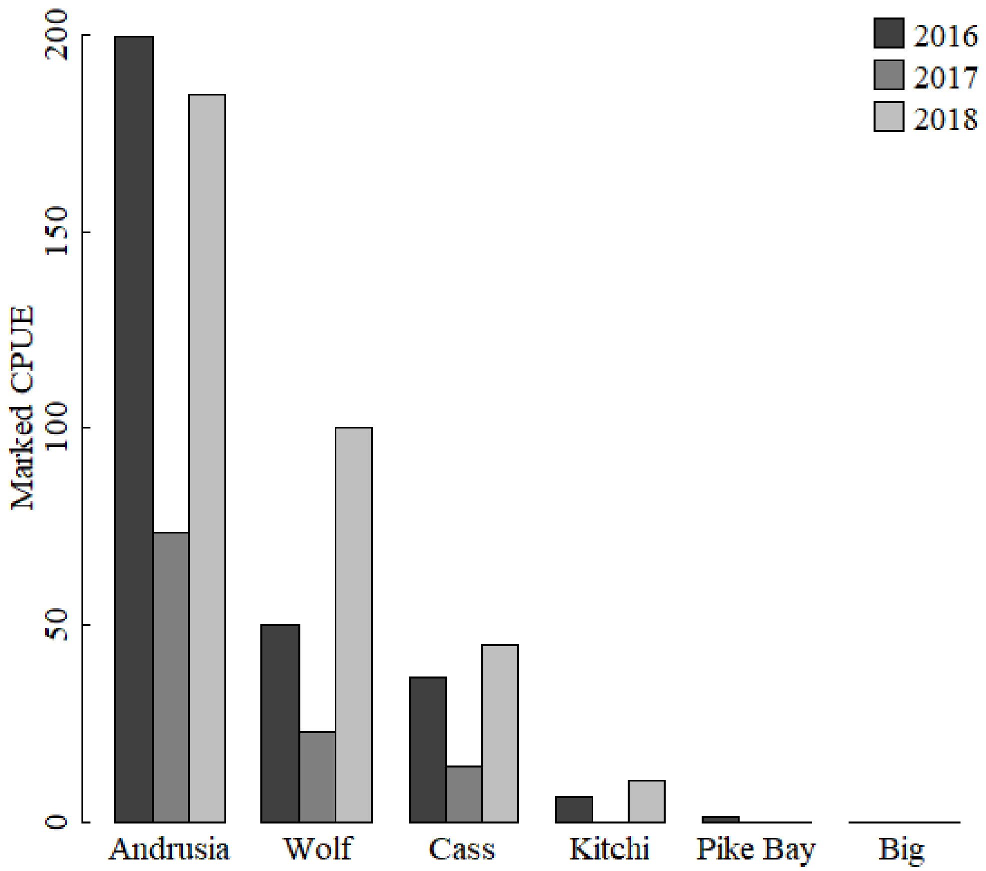

FIGURE 3. Total catch-per-unit-effort (fish/hour) of OTC marked age-0 Walleye of all study lakes, 2016-2018, during late August through early September assessment.

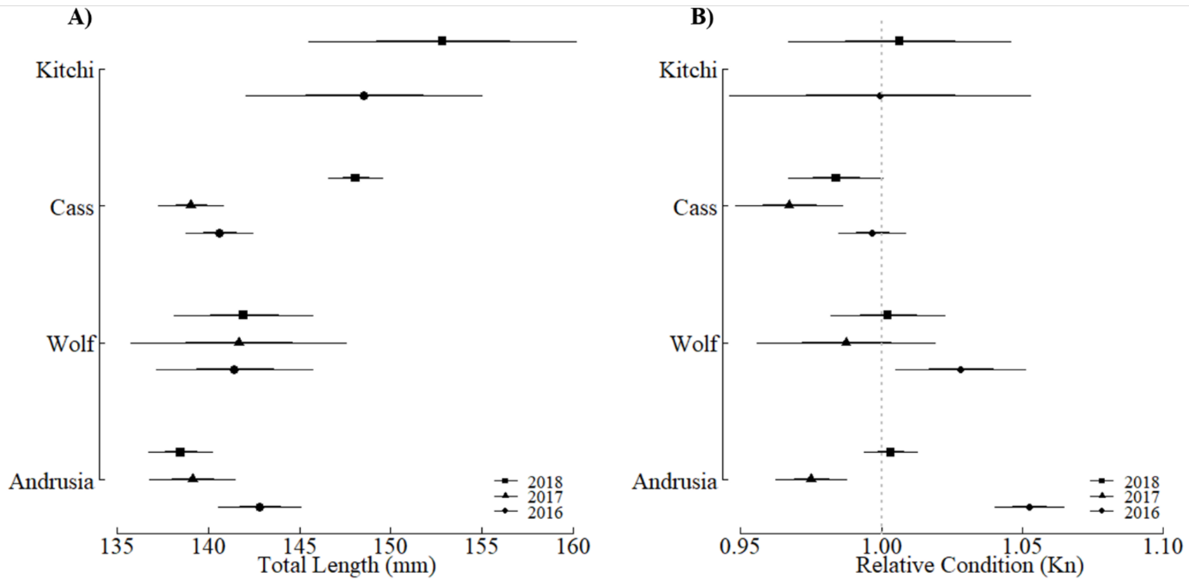

FIGURE 4. **A)** Marked age-0 Walleye mean total length (mm) for the Cass Lake Chain. Dot represents mean length with lines representing one and two standard errors. **B)** Annual mean relative condition (Kn) of OTC marked age-0 Walleye by lake with bars representing one and two standard errors.

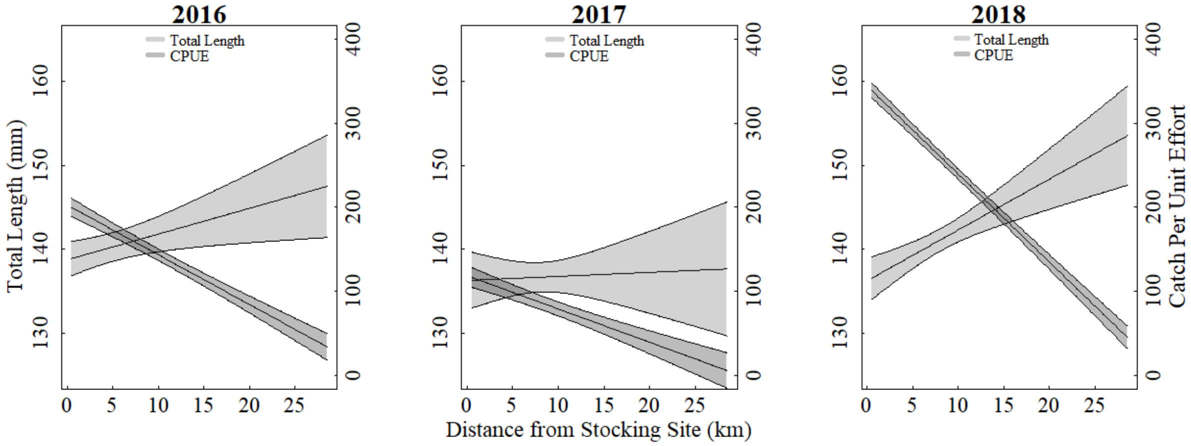

FIGURE 5. Predicted total length (TL) in millimeters (mm) and predicted total catch-per-unit-effort (CPUE) each year as a product of shoreline distance (km) from stocking site.