Movements and Spawning Habitat of Muskellunge *Esox masquinongy* in Green Bay, Lake Michigan

By

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Executive Summary

Muskellunge *(Esox masquinongy*) have been extirpated or have declined significantly in many areas of the Great Lakes, including Green Bay. Restoring a self-sustaining population of Muskellunge to Green Bay is a goal of several management efforts. Currently, annual stocking supports a world-renowned trophy fishery for anglers seeking to catch large Muskellunge. However, there has been limited evidence of natural reproduction as few juvenile Muskellunge of wild origin have been collected. Lack of juvenile Muskellunge in Green Bay suggests natural recruitment is limited, possibly due to habitat limitations.

Previous work has identified potential Muskellunge spawning locations in Green Bay and provided initial descriptions of Muskellunge spawning habitat, but it is not known whether Muskellunge successfully hatch at these locations or what habitat characteristics result in successful hatching. Additionally, it is not known if Muskellunge in Green Bay exhibit reproductive homing and return to the same locations to spawn in consecutive years, or if Muskellunge return to their stocking location to spawn.

Due to the uncertainties surrounding Muskellunge reproduction in Green Bay, my objectives were to determine if: 1) Muskellunge spawning locations and occurrence of successful hatching were related to habitat variables, 2) proportions of Muskellunge spawning in or outside of tributaries to Lower Green Bay were different, 3) most Muskellunge (>75%) returned to stocking locations to spawn, and 4) adult Muskellunge display high site fidelity (>75%), returning to the same locations to spawn in consecutive years. Results from my research allowed me to quantify the availability of suitable spawning habitat in the Fox and Menominee rivers, and to qualitatively characterize general movement patterns of Muskellunge in Green Bay and its tributaries.

My study was the culmination of a 4-year effort focusing on Muskellunge spawning behavior and success in southern Green Bay and its tributaries. During 2017-2019, adult Muskellunge (N = 60) were captured and surgically implanted with radio and acoustic transmitters. Muskellunge were actively tracked during the open water season to identify putative spawning sites using a combination of aerial and boat-based telemetry. Muskellunge were also monitored passively using acoustic receivers at fixed locations. Egg sampling was conducted at putative spawning site using an airlift pump, followed by larval sampling using conical ichthyoplankton nets, D-frame nets, and quatrefoil light traps. Habitat variables were recorded at each putative spawning site and at a large number of random sites that were also sampled for eggs. Multiple logistic regression was used to model the habitat variables significantly influencing the probability of Muskellunge egg presence at a given location. Data from acoustic receivers and active tracking efforts were used to determine seasonal movement patterns and tributary use of Muskellunge. Chi-square tests were used to compare proportions of Muskellunge spawning in tributary vs. bay habitats, as well as analyze reproductive homing to stocking locations and spawning site fidelity. Side-scan sonar in combination with my logistic regression model was used to quantify the amount of suitable Muskellunge spawning habitat in the Fox and Menominee rivers.

In total, 278 sites were sampled for eggs, and as a result 58 egg sites were identified. Additionally, 436 individual sampling events resulted in the capture of two larval Muskellunge. Because only two larval Muskellunge were captured, I was not able to quantify habitat characteristics related to successful hatching. Therefore, my final logistic model only includes habitat variables related to egg deposition. My final logistic regression model indicates bottom slope, depth, dissolved oxygen, distance from shore, percent of gravel substrate, and percent of organic matter as a substrate best predict Muskellunge egg presence at a given location. Of these habitat variables, bottom slope had the greatest influence on presence of Muskellunge eggs. The likelihood Muskellunge eggs were present at a given site decreased by 50% when slope steepness increased by 0.5 m/m. A 1-m increase in depth resulted in a 49% decrease in probability Muskellunge eggs were present. A one unit increase in the dissolved oxygen concentration (1 mg/L) at a given site increased the probability Muskellunge eggs were present 1.4 times. Additionally, for every 1 m increase in distance to shore the probability of Muskellunge egg presence decreased by 14%. Finally, a 10% increase of gravel or organic matter in the substrate increased the probability of Muskellunge egg presence by 27% and 19%, respectively. Currently, based on surface area, 1.3% of the Fox River and 8.3% of the Menominee River are suitable Muskellunge spawning habitat (suitable for egg deposition).

Proportions of Muskellunge spawning in or outside of tributaries were not significantly different in 2018, 2019, or 2020 based on chi-square analysis. Of the Muskellunge spawning in tributaries to Green Bay during my study, the majority spawned in the Fox River. Telemetry data suggest Muskellunge are spawning in the Fox, Menominee, Suamico, and Peshtigo rivers, and potentially in Duck Creek. Eggs were collected to confirm spawning in the Fox, Menominee, Suamico, and Peshtigo rivers and in multiple locations throughout Green Bay proper. In all but one case, Muskellunge with known stocking locations (N = 6) returned to their stocking location to spawn. Finally, Muskellunge in Green Bay displayed moderate levels of spawning site fidelity (45-70%), and the upper proportion of this range was not significantly different than 75%.

Muskellunge in Green Bay generally moved south towards the mouth of the Fox River as water temperatures cooled in fall. A handful of Muskellunge overwintered in the Fox River in 2018, 2019, and 2020. Peak spring spawning activity was observed at water temperatures

comparatively higher to reported Muskellunge spawning activity in most locations. Entry into tributaries for spawning each spring was staggered over a long period of time prior to peak spawn, whereas exit out of tributaries after presumed spawning was comparatively more truncated. In the Fox River, average residency times for each year were 27, 27, and 21 days respectively, with males generally residing longer than females.

My results demonstrate that habitat suitable for Muskellunge egg deposition may be lacking in the Fox and Menominee rivers. Additionally, the lack of Muskellunge larvae captured during my study suggests that successful hatching may not be occurring in Lower Green Bay and the Fox River or is occurring at relatively low levels. A lack of naturally produced recruits indicates the Green Bay Muskellunge population is not self-sustaining, and stocking is still required to support the current fishery. My results also demonstrate that choice of stocking locations may play an import role as a management tool, as Muskellunge are likely to return to stocking locations to spawn and generally display moderate site fidelity. Stocking Muskellunge in tributaries at shallow areas with minimal human alteration of the shoreline may result in increased egg deposition and subsequent natural reproduction. Furthermore, stocking Muskellunge in Green Bay proper at sites closely resembling wetland or coastal marshes, such as in Deadhorse Bay, may increase Muskellunge spawning in these locations and lead to successful hatching. Due to the proportion of Muskellunge in Green Bay not spawning in tributary habitats, known spawning areas in Green Bay proper should be considered for enhancement or protection efforts in addition to those in tributaries. Management objectives and goals related to the Green Bay Muskellunge population should emphasize actions that result in increased knowledge of factors regulating successful hatching, enhancement and protection of suitable spawning habitat, and identifying natural reproduction.

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Introduction

Habitat is fundamental to the sustainability of fish populations and is broadly defined as those physical and chemical features of aquatic systems that affect survival, growth, reproduction, and recruitment (Bozek et al. 2011). While habitat is critical at all life stages, self-sustaining fish populations require spawning habitat where eggs can successfully develop and hatch. Inadequate recruitment resulting from limited spawning habitat can create a population bottleneck (Rosenfeld and Hatfield 2006; Weller et al. 2016). While certain characteristics of spawning habitat vary between fish species at the microhabitat scale, similar factors generally define what constitutes viable spawning habitat. Factors such as temperature and dissolved oxygen levels (Ringler et al. 1975), structural complexity of habitat (i.e. vegetation/woody cover; Robillard et al. 2001), water flow (Weyers et al. 2003), and composition of substrate (Snickars et al. 2010) all contribute to the suitability of a spawning habitat. Additionally, abiotic factors such as water level, turbidity, and wave action (Raabe et al. 2015), anthropogenic factors such as shoreline development and runoff (Dombeck et al. 1986; Rust et al. 2002) and biotic factors such as spawning site fidelity (Bronte et al. 2007), competition with other species with similar feeding and habitat needs (Smith 1976), and predation on eggs and young (Crowder 1980) can also affect reproductive success. Lack of all necessary habitat components specific to a given species can be detrimental to egg development (Kondolf 2001; Palm et al. 2007).

To effectively create, enhance, protect, or restore spawning habitat, it is important to identify habitat that is conducive to successful production of young fish given the species and environment (Taylor et al. 2017). Previous studies have attempted to distinguish what comprises spawning habitat through a variety of methods. GIS based spatial models using the distribution of eggs, and thus habitat suitability, to model spawning areas (Eastwood et al. 2001), along with statistical models such as the Physical Habitat Simulation Model (PHABSIM; Geist and Dauble 1998), have primarily been used to identify spawning habitat. Additionally, methods such as photographing substrate (Chiotti et al. 2007), hydrographic measurements (Fitzsimons 1995), visually identifying spawning fish (Gosch et al. 2006), and both active (Ickes et al. 1999) and passive (Walters et al. 2009) telemetry have also been used. Because of the varied habitats used by fishes for spawning, it is important that a variety of methods exist to identify locations in which spawning is hypothesized to be most successful.

Lack of suitable spawning habitat where eggs can develop and eventually hatch can be more detrimental to certain fish populations or species than others. Fish species with delayed maturation, intermittent spawning, or generally low density are especially susceptible to a lack of spawning habitat, as these life history traits can lead to inherently low reproductive success regardless of spawning habitat availability (Auer 1996; Reynolds et al. 2005; Rowell et al. 2008). Anthropogenic effects on spawning habitat also can have a more detrimental effect on certain fish species. Dams inhibiting access to spawning areas may affect migratory species such as Chinook Salmon *Oncorhynchus tshawytscha* (Mertz and Setka 2004), Lake Sturgeon *Acipenser fulvescens* (Auer 1996), and Striped Bass *Morone saxatilis* (Beasley and Hightower 1999), while channelization may threaten riverine species that require stable flows and the presence of gravel or cobble substrate to successfully spawn (Carline and Klosiewski 1985; Lau et al. 2006). At the smallest spatial scale, habitat type and quality can affect the reproductive success of individual fish (Rosenfeld and Hatfield 2006).

Muskellunge *Esox masquinongy* are a large predatory gamefish that support popular fisheries in many states and provinces within North America (Bozek et al. 1999; Gilbert and Sass

2016). Although Muskellunge are native to a variety of habitats, they are seldom abundant in any of the systems in which they occur (Cook and Solomon 1987). Additionally, natural recruitment of Muskellunge occurs at low levels in many systems (Zorn et al. 1998; Crane et al. 2015) and stocking is routinely used to supplement or maintain Muskellunge fisheries (Crossman 1986; Simonson and Hewett 1999; Gilbert and Sass 2016). Moreover, natural reproduction of Muskellunge has declined in some waters that previously supported self-sustaining populations (Dombeck 1984; Zorn et al. 1998; Rust et al. 2002).

In the Great Lakes, several Muskellunge populations have shown declines in abundance (Mackay and Werner 1934; Buss 1960). Muskellunge populations in Green Bay, Lake Michigan (Kapuscinski et al. 2007), Spanish River, Lake Huron (Liskauskas 2017), the St. Lawrence River (Farrell et al. 2007), Toronto Bay, Lake Ontario (Whillans 1979) and Sandusky Bay, Lake Erie (Crossman 1986) have all exhibited declines in overall Muskellunge abundance in the last century or more. While the decline of Muskellunge populations within the Great Lakes is complex and can be attributed to a number of different factors, recruitment failure demonstrated by a lack of naturally produced recruits has negatively impacted efforts to restore both the Green Bay and St. Lawrence population (Rowe and Lange 2009; Farrell et al. 2017). In some regions, propagation and stocking have been implemented to address declines and supplement these degraded populations (Crossman 1986; Farrell et al. 2007; Crane et al. 2015).

The Muskellunge population in Green Bay has been the focus of many rehabilitation efforts (WDNR 1986; Kerr 2011; Battige 2011). Muskellunge in southern Green Bay were decimated during the early to mid-1900s by habitat destruction, pollution, and over-exploitation (Kapuscinski 2007; Lake Michigan Fisheries Team 2010) and the decline in Muskellunge contributed to an unbalanced fish community in Green Bay. A combination of an unbalanced fish community, water quality issues, contaminated sediment concerns, as well as habitat loss and degradation resulted in the creation of the Lower Green Bay Remedial Action Plan (LGBRAP) by the Wisconsin Department of Natural Resources (WDNR) in 1988. The LGBRAP contained management objectives that focused on the rehabilitation of the Fox River Area of Concern (AOC), which includes the Fox River along with an area denoted by an arbitrary line from Long Tail Point to Point Au Sable and south (Figure 1). One of the many needs identified in this action plan was to increase the populations of predatory fishes within southern Green Bay. At the same time, the WDNR in cooperation with several local Muskellunge angling clubs and the Musky Clubs Alliance of Wisconsin initiated a Great Lakes strain Muskellunge reintroduction program for the Green Bay waters of Lake Michigan (Lake Michigan Fisheries Team 2017). In 1989, the WDNR stocked 2,461 fingerling Muskellunge in the Fox River, 1,350 fingerling Muskellunge in the Menominee River, and 1,450 fingerling Muskellunge into Lower Green Bay. From 1989 through 2019, a total of 24,906 yearling and 170,588 fingerling Muskellunge have been stocked in the Fox, Menominee, Peshtigo, Oconto, Suamico and Pensaukee rivers, Little Sturgeon Bay and Sturgeon Bay, along with Lower Green Bay (Kapuscinski et al. 2007; Lake Michigan Fisheries Team 2020).

Stocking of Muskellunge supports a world-renowned fishery and has made Green Bay a top destination for anglers seeking to catch large Muskellunge. Given the popularity of this fishery, the WDNR conducts annual assessments of the Muskellunge population in Green Bay that includes creel surveys, electrofishing, and fyke (trap) netting. Muskellunge harvest in Lake Michigan waters including Green Bay is regulated by a 54” minimum length limit (WDNR 2019) and very few harvested Muskellunge have been observed in WDNR creel surveys (Lake

Michigan Fisheries Team 2017). Low harvest reflects the aforementioned minimum length limit and the fact that most Muskellunge anglers practice catch and release (Simonson and Hewett 1999; Fayram 2003; Simonson 2012). Overall catch of Muskellunge on Green Bay has increased since 2012, and the average length of both male and female Muskellunge has increased from 2003 (males: 952 mm; females: 1183 mm) to 2019 (males: 1120 mm; females: 1289 mm; Hogler and Surendonk 2019).

A lack of naturally produced juvenile Muskellunge in Green Bay suggests there are factors limiting natural reproduction to the point that the Green Bay population is not considered self-sustaining, an important goal of rehabilitation efforts (Lake Michigan Fisheries Team 2017). Eggs have been collected from Muskellunge previously stocked into Green Bay and these eggs have been fertilized and hatched in an artificial setting (Rowe and Hogler 2012), demonstrating that viable eggs are produced. However, no confirmed naturally reproduced juvenile Muskellunge have been observed in the southern portion of Green Bay, and very few have been collected elsewhere in Green Bay (Kapuscinski et al. 2007).

Inherently low density, generally low recruitment, and evaluation of propagation efforts across their range has resulted in prior research surrounding Muskellunge spawning. In general, once water temperatures are between 8°C – 16°C in spring (Lane et al. 1996), female Muskellunge broadcast spawn intermittently over substrates where the non-adhesive eggs may settle and potentially develop (Dombeck 1984). General spawning locations include shallow backwaters, vegetated marshes, and shoals within rivers (Lane et al. 1996; Rust et al. 2002; Crane et al. 2015). Characteristics of Muskellunge spawning habitat that may positively influence reproductive success include vegetation (Farrell et al. 2007), presence of woody debris

(Rust et al. 2002), and composition of substrate (Crane et al. 2014). Specific conditions within a habitat such as water flow (Crane et al. 2014), dissolved oxygen concentration (Dombeck et al. 1984; Zorn et al. 1998), depth, and slope (Nohner and Diana 2015) also contribute to Muskellunge reproductive success. Additionally, competition with other species with similar feeding and habitat needs such as Northern Pike *Esox lucius* (Dombeck et al. 1986; Cooper et al. 2008) and predation on eggs and larvae (Stein et al. 1981) can negatively affect success, as well as human alterations, such as shoreline development of littoral zones (Rust et al. 2002), and both nutrient loading and sedimentation (Battige 2011).

The spawning behavior of Muskellunge specifically in the Great Lakes has been examined in a variety of studies (Lane et al. 1996; Farrel 2001; Cooper et al. 2008), although the majority of the documentation surrounding the habitat preferences of these fish is highly variable (Cook and Solomon 1987). For example, Muskellunge in the St. Lawrence River and Lake St. Clair have been documented spawning in both shallow (< 1 m) and deep (> 3 m) water and in areas with high, moderate, or no vegetation (Haas 1978; Farrell et al. 1996). Spawning locations ranged from open water to vegetated marshes, shoals in main river channels, and shallow backwaters along river margins (Farrell et al. 1996; Battige 2011; Crane et al. 2015); preferred substrates consisted of rock, gravel, sand, and silt (Haas 1978; Farrell et al. 1996; Younk et al. 1996). While these previous studies provided some information on Muskellunge spawning in the Great Lakes, a more recent study (Battige 2011) provided initial understanding of the potential habitat characteristics of Muskellunge spawning behavior in Green Bay specifically. Battige (2011) implanted small radio transmitters into the oviducts of mature female Muskellunge prior to spawning. Transmitters were located after expulsion, presumably at egg deposition sites. Microhabitat characteristics were collected in the areas in which the transmitters were deposited, and then compared to surrounding areas. Muskellunge were observed spawning in the Fox and Menominee Rivers, along with Little Sturgeon Bay and Lower Green Bay. Battige (2011) used habitat characteristics in the vicinity of expelled transmitters in the Menominee River to create a predictive model describing selection of spawning habitat. Battige (2011) concluded that Muskellunge preferred areas with a presence of woody debris, high levels of vegetation (34-100%), and bottoms containing fine substrate (< 2 mm) with little to no slope (0-3%).

While it is not known if Green Bay Muskellunge exhibit reproductive homing to a large extent, initial results of previous research suggest a majority of Muskellunge in Green Bay (75%) demonstrate spawning site fidelity to some degree (Sheffer 2019). Moreover, evidence of spawning site fidelity has been observed during studies conducted in other Great Lakes (Farrell et al. 2007) and also in small inland systems (Crossman 1990; Jennings et al. 2011). Identifying the preferred spawning habitat of Muskellunge in the Great Lakes, specifically in the southern end of Green Bay, may allow managers to improve these habitats, thus improving natural reproduction. If Muskellunge in Green Bay do exhibit reproductive homing to a large extent, selection of stocking locations of fingerlings and yearlings may be important to reproductive success, as stocking in areas with suitable spawning habitat may increase natural reproduction (Farrell et al. 2007). Because predictive models of potential spawning habitat created by Battige (2011) only included habitat data gathered from the Menominee River, their spatial application may be limited. Identifying the characteristics representing suitable Muskellunge spawning habitat in a variety of locations within southern Green Bay will help guide future habitat improvement projects and stocking strategies.

Habitat improvement projects, stocking strategies, and assessments focused on restoring the Lower Green Bay and Fox River Muskellunge population to self-sustaining levels is of utmost importance in the Green Bay watershed. My research aligns with portions of a variety of management plans surrounding these goals. The 2017-2026 Lake Michigan Integrated Fisheries Management Plan: Vision 1, Goal A, Objective 1 aims to inventory, evaluate, restore, and/or enhance spawning and nursery habitat of Muskellunge in Green Bay; Vision 1, Goal B, Objective 4 aims to restore self-sustaining populations of Great Lakes Spotted Muskellunge to Green Bay; Vision 1, Goal A, Objective 6 aims to restore habitat that has been previously degraded due to waterway alteration. My research also aligns with objective 4.2.3.6B in the WDNR Integrated Management Plan for the Lower Fox River, which calls for the continued re-establishment and assessment of Muskellunge. Finally, my research relates to two goals of the 2012 Green Bay Great Lakes Spotted Musky Management Plan: Goal 2, Objective 1, which calls for the re-establishment of a naturally reproducing Muskellunge population, specifically in the Lower Bay/Fox River management area, and Goal 5, Objective 1, which is to restore/rehabilitate Muskellunge habitat within Green Bay.

My study was a continuation of Muskellunge research in Green Bay that began in 2017 (Sheffer 2019). Due to the uncertainties surrounding Muskellunge reproduction in Green Bay, my objectives were to determine if: 1) Muskellunge spawning locations and occurrence of successful hatching were related to habitat variables, 2) proportions of Muskellunge spawning in or outside of tributaries to Lower Green Bay were different, 3) most Muskellunge (>75%) returned to stocking locations to spawn, and 4) adult Muskellunge display high site fidelity (>75%), returning to the same locations to spawn in consecutive years. Results from my research allowed me to quantify the availability of suitable spawning habitat in the Fox and Menominee rivers, and to qualitatively characterize general movement patterns of Muskellunge in Green Bay and its tributaries.

Methods

Study Site

My portion of this study was conducted during 2019-2020 in the southern portion of Green Bay and tributaries to Lower Green Bay in Marinette, Brown, Door, and Oconto counties in northeastern Wisconsin. Initial research (Sheffer 2019) occurred during 2017-2018. My research focused on the lower portion of Green Bay, defined by an arbitrary line from Long Tail Point to Point au Sable, along with the Fox, Menominee, and Peshtigo rivers and Duck Creek (Figure 1). I focused on the Fox River from the De Pere Dam downstream to the mouth (approximately 11 km); the Menominee River from safe and navigable water nearest the Menominee Dam downstream to the mouth (approximately 4 km); and the Peshtigo River from the mouth to a distance of approximately 11 km upstream, or until water is no longer navigable. Additional tributaries (e.g., Duck Creek, Suamico. Little Suamico, Oconto, and Pensaukee rivers) became focal areas as my data collection progressed.

Adult Sampling and Transmitter Implantation

A total of 62 Muskellunge (> 965 mm total length) were captured between fall 2017 and spring 2019 for transmitter implantation. Muskellunge were captured using a variety of sampling gears including hook-and-line angling, boat electrofishing, and fyke nets. Hook-and-line collected Muskellunge were captured during fall by professional angling guides. Muskellunge collected by boat electrofishing were captured during fall using standard Wisconsin-style boom shockers and pulsed direct current. Muskellunge collected using fyke nets (22.86 cm mesh, 1.45 m diameter hoop, 1.45 m x 22.86 m lead) were captured within tributaries during spring.

After capture, Muskellunge were held in an aerated, 545-L holding tank and monitored for normal activity (e.g. able to maintain equilibrium, regular opercular activity). All fish were measured to the nearest centimeter (total length; TL), and scanned with a handheld reader (Biomark, Boise, ID; Biomark® 601 Reader) for passive integrated transponders (PIT; 12 mm; 125 kHz). If no PIT tag was detected, one was implanted in the dorsal musculature via syringe. An external Floy tag (Floy Manufacturing, Seattle, Washington) with a unique four-digit number and contact information for the Wisconsin Cooperative Fishery Research Unit was inserted between the dorsal pterygiophores in each Muskellunge allowing for individual external identification. Floy tags were used to provide supplemental information on locations of individual fish captured by anglers given that the Floy tag was present when captured. Any previous fin clips were noted, and sex was determined visually (Lebeau and Pageau 1989).

Both radio (Advanced Telemetry Systems [ATS], Isanti, Minnesota; ATS® Model F1845B: 26g, 857-day battery life) and acoustic transmitters (Vemco, Bedford, Nova Scotia; Vemco® Model V16-6H: 34g, 3,541-day battery life) were surgically implanted in the body cavity of each Muskellunge greater than 965 mm total length (Figure 2). The battery life of acoustic transmitters allowed for Muskellunge to be located throughout this study and beyond. Radio transmitters implanted in Muskellunge tagged in 2017 (N = 20) began to expire in spring 2020 due to battery longevity. Muskellunge were placed ventral side up in a mesh sling with water continuously pumped over the gills. Fish were immobilized using transcutaneous electrical nerve stimulation (TENS) administered by a hand-held unit (Bio Protech, Chino, CA; MAXTENS® 1000). A 4-cm incision was made halfway between the pectoral and pelvic girdles along the ventral midline (Figure 3). Acoustic transmitters were inserted through the incision and into the body cavity (Figure 4). A hole, approximately 3-cm posterior to the incision was made by inserting a curved, hollow needle through the incision and using the needle to puncture the body wall just posterior to the incision (Figure 5). The radio antenna was fed through the needle so that the antenna was outside the fish while the transmitter was inserted into the body cavity (Figure 6). Incisions were closed using three interrupted sutures (Ethicon, Somerville, NJ; PDS® II, 2-0; Figure 7). Following surgery, fish were returned to the holding tank to fully recover before being released. The capture and release locations of Muskellunge were recorded with a handheld GPS (Garmin International, Plathe, KS; GPSMAP ® 765; Figure 9).

Telemetry

Muskellunge were located actively during the open water season using a combination of aerial and boat-based telemetry. A Cessna 185 fitted with two 4-element Yagi antennas and an ATS® Model R4000 receiver with a 4-second scan rate was used to determine general locations of Muskellunge using radio transmitters. When a radio frequency was detected during an aerial survey, the receiver was tuned to that frequency until a location was determined. Antennas were used individually to achieve an approximate bearing toward the fish, and GPS coordinates were taken when the receiver signal was loudest, indicating that the airplane was directly above the fish. Fish locations delineated by the plane were transferred to handheld GPS units, which guided on-the-water telemetry.

I attempted to locate Muskellunge multiple times per week leading up to and during the spawn. Following presumed spawning, aerial-based telemetry was used once per month to locate Muskellunge during the remainder of the open water season. Boat-based telemetry was used to determine locations of Muskellunge using both acoustic and radio telemetry. A Vemco® VR100 receiver and Vemco® VH165 omni-directional hydrophone was used to determine locations of tagged Muskellunge. For radio telemetry, a 3-element Yagi antenna and ATS® Model R2000 receiver was used to locate fish. After detecting a Muskellunge with either method, radio telemetry was used to determine the fine-scale location of a tagged fish based on highest signal strength.

Movements of Muskellunge were also monitored passively throughout the year using Vemco® VR2W acoustic receivers placed at fixed locations. Receivers in tributaries were attached to a steel pipe encased in a concrete block, submerged within 20 m of shore, and secured with chain to the shoreline (Figure 8). There were at least two VR2Ws placed in each tributary within my study site. This included the Fox, Menominee, Peshtigo, Oconto, Pensaukee, Little Suamico, and Suamico rivers, along with Duck Creek (Figure 9). These receivers allowed me to determine directional movement of Muskellunge and residency within tributaries. Additional receivers associated with other studies were located throughout Green Bay (Figure 10). Data was downloaded from the receivers at least once per year.

Defining Spawning Periods and Locations

To define Muskellunge spawning sites during my study, I established a spawning period each year. Once water temperatures surpassed 8°C in spring (United States Geological Survey (USGS) temperature gage; Figure 14), I determined a peak spawn date using a number of observations: movement of Muskellunge into tributaries, locations of these tracked fish in reference to depth and distance from shore, and visual observation of spawning pairs. I then defined the Muskellunge spawning period as beginning ten days prior to the peak spawn date and ending ten days after the peak spawn date, resulting in a 21-day spawning period each year.

Spawning sites for individual Muskellunge were determined based on locations during the spawning period. An individual Muskellunge's location within the spawning period nearest the peak spawn date was considered that fish's spawning site. Acoustic receivers deployed at entrances to tributaries were placed slightly upstream from the mouth (Figure 9). Therefore, Muskellunge were considered to have entered and spawned in a tributary if receiver detections indicated they were in the river or near the mouth. If Muskellunge were located at multiple tributary entrances during the spawning period without entry, a spawning site was not determined. Muskellunge that overwintered in tributaries were considered spawning in that tributary if exit the following spring occurred after the peak spawn date. Finally, Muskellunge that did not cross the Chambers Island receiver line (Figure 10) and were not located in bay or tributary habitats were considered to have spawned at unknown locations in southern Green Bay.

Spawning Habitat

Putative spawning sites were delineated as locations where individual or pairs of Muskellunge were repeatedly located via telemetry, physically observed during daylight, or via nighttime spotlight surveys during the spawning period (Crane et al. 2014; Diana et al. 2015). During 2018, 2019, and 2020, habitat data were collected at each putative spawning site, 120 randomly selected sites in the Fox River, 35 random sites in the Menominee River, 15 random sites in Duck Creek, and 15 random sites in Lower Green Bay. Random sites were selected prior to sampling using shoreline measurements and a random number generator in Program R (Version 4.0.2). At each putative and random site, dissolved oxygen (mg/L) and temperature (°C) were measured at the substrate-water interface with an YSI^{TM} 556 Multi-Probe System mounted to a pole. A Marsh-McBirney^{TM} flow meter was used to measure water velocity. Substrate was collected with a substrate grab and suction pump, and percent composition was determined visually to the nearest 5% according to a modified Wentworth (1922) scale, including fine (diameter < 2 mm), gravel (diameter > 2 mm and < 16 mm diameter), pebble (diameter > 16 mm and < 64 mm diameter), cobble (diameter > 64 mm and < 256 mm), and boulder (diameter > 256 mm), as well as organic matter (leaf matter, detached and decaying vegetation, decaying woody debris).

Relative abundance of coarse woody structure (CWS) and bottom slope was also determined at each site. Coarse woody structure was defined as any piece of wood 2 cm or greater in diameter. The number of individual CWS was determined within a 10-m radius of putative spawning sites either visually or using a side-scan sonar unit. Relative abundance of CWS was also ranked on a relative scale to the maximum number of CWS observed in the study: 1) 0 – 33.33% of the maximum; 2) 33.34 – 66.66% of the maximum; and 3) 66.67 – 100% of the maximum. To determine bottom slope, a depth measurement was taken on each side of the boat as the boat was positioned parallel to the shoreline. This produced a slope measurement (m/m) along a 2.3 m transect (sampling boat width) perpendicular to shore. The resulting slope was calculated as the difference in depth (m) divided by the 2.3 m transect.

Relative abundance of aquatic macrophytes was determined using a double-headed vegetation rake attached to a telescoping pole. The rake was lowered to the substrate at a single location within the putative spawning site and twisted until two complete circles were made and then pulled to the surface. Relative abundance was determined was determined based on a rake fullness scale: 1) few plants, single layer across tines; 2) plants covered rake in single layer but tines were visible; and 3) rake is completely covered and tines were not visible. The number of rake tines that were covered in vegetation was noted. Distance to shore of the sample site was also measured. Additionally, the 50 m of shoreline adjacent to spawning sites was ranked in terms of anthropogenic alteration following Rust et al. (2002). The three categories include: 1) totally altered: vegetation completely cleared, sea walls, riprap, imported beach sand or structure was present on the shoreline; 2) partially altered: houses or buildings were built near the shoreline but riparian vegetation remained and minimal alteration of the shoreline itself; and 3) unaltered shoreline, where no human impact was observed.

Egg Sampling

Putative spawning sites where Muskellunge were located several times during the spawning period, or areas in which spawning pairs of Muskellunge were observed were searched for eggs using an airlift pump (Figure 11). The pump was operated at these sites until five minutes of sampling effort had been expended while the boat was anchored over the putative spawning site. The pump was moved around underneath the boat, and the size of the area searched by the pump varied based on movement of Muskellunge during tracking or observation. The airlift pump consisted of three parts: 1) a generator; 2) an air compressor and air hose; and 3) an adjustable PVC pipe. The pipe length was adjusted to reach the substrate while approximately 80% of the pipe was submerged. Compressed air was injected near the bottom end of the PVC pipe to dispel eggs and substrate upwards. Contents were discharged into a 1.2 mm stretch mesh net and searched for eggs (Figure 12). All eggs were preserved in 95% ethanol and sent to the Molecular Conservation Genetics Laboratory at the University of Wisconsin-Stevens Point for genetic identification.

Randomly selected sites in the Fox and Menominee rivers, Duck Creek, and Lower Green Bay where habitat characteristics were measured were also sampled for eggs. At each random site, a specific random point was selected between the shoreline and a maximum depth of 2.5 meters to sample for eggs. Muskellunge typically spawn from less than 1 m to 2.5 m deep (Lane et al. 1996; Farrell 2001; Crane et al. 2014), which is why I only selected sampling locations at depths 2.5 m and shallower. Additionally, our ability to egg sample was limited at depths > 2.5 m. Each sample point was recorded with a handheld GPS unit (Garmin

International, Olathe, KS; GPSMAP® 765). The airlift pump was operated for five minutes at each sampling point within a 2 m by 1 m square of the recorded GPS position.

Larval Sampling

Larval Muskellunge were sampled using conical ichthyoplankton nets (SEA-GEAR, Melbourne, Florida; Model 9000), D-frame nets, and quatrefoil light traps in 2018 and 2019. When using D-frame nets, boats were positioned 1 – 2 m from shore and the net was swept from the shoreline towards the boat working the net in and around woody structure when present. Ichthyoplankton nets were towed at the surface within each defined spawning site for five minutes. Light traps were set at dusk and collected at dawn the following morning.

Previous research in 2018 (Sheffer 2019) resulted in capture of only two larval Muskellunge within the Menominee River. Consequently, in spring 2019, staff with the Wisconsin Cooperative Fishery Research Unit tested my larval collection methods on Snipe Lake, an 87-hectare mesotrophic lake in northern Wisconsin supporting a high density Muskellunge population that is solely supported by natural reproduction (Eslinger 2015). Testing my collection methods in Snipe Lake helped confirm their effectiveness, as larval Muskellunge were captured using all sampling gears. The assessment on Snipe Lake determined that the use of D-frame nets while wading was the most efficient method of capturing larval Muskellunge.

Based on results of the Snipe Lake assessment, I exclusively used D-frame nets to sample for larval Muskellunge in southern Green Bay during spring 2020. Moving parallel to the shoreline from a central sampling point, D-frame nets were swept from the shoreline out, as well as in and around woody structure until 50 m on each side of the site had been sampled. All larvae were preserved in 95% ethanol and visually identified using a larval fish identification key (Auer

1982). Larvae not identified visually were sent to the Molecular Conservation Genetics Laboratory at the University of Wisconsin-Stevens Point for genetic identification.

Habitat Assessment Using Side-Scan Sonar

Side imaging sonar (Lowrance StructureScan® HD LSS- 2/Lowrance HDS-9 Carbon/StructureScan 3D Module) was used to map habitat within the Fox and Menominee Rivers used by Muskellunge for spawning. Individual scans 36.5 m wide were recorded between the shoreline and a depth of 2.5 m (Lane et al. 1996; Battige 2011; Crane et al. 2014). I recorded multiple overlapping scans in areas where the distance from the shoreline to a depth of 2.5 m was greater than a single 36.5 m wide scan. Muskellunge typically spawn from less than 1 m to 2.5 m deep (Haas 1978; Farrell 2001; Crane et al. 2014), which is why I chose to scan depths 2.5 m and shallower. Scans were recorded only in a downstream direction at speeds approximately 5.6-6.8 km/h (Kaeser and Litts 2011; Richter 2015). Bottom slope was determined using the distance from the shoreline (0 m) to a depth of 2.5 m. Shoreline alteration was recorded for each scan location using the aforementioned guidelines (Rust et al. 2002). Additionally, habitat data collected during egg and larval sampling, aerial imagery, and additional random substrate samples were used to ensure my interpretation of substrate type, presence/absence of coarse woody structure, and vegetation being displayed on the side-scan images was accurate.

Data Analysis

For objective 1, I used multiple logistic regression to determine which habitat variables were significantly related to the probability that Muskellunge eggs were deposited at a location, where presence of eggs was considered “success” and subsequently denoted a 1, and absence of eggs was considered “failure” and denoted a 0. The presence of eggs was modeled in relation to depth, bottom slope, temperature, distance from shore, dissolved oxygen, flow, vegetation rake fullness, number of tines covered in vegetation, presence or absence of CWS, relative abundance of CWS, presence or absence of algae, shoreline alteration rank, percent of modified shoreline, presence or absence of organic matter, the percentage of organic matter as substrate, and the percentage of each substrate type.

I quantified the extent of correlation among all predictor variables in the full model by calculating the variance inflation factor (VIF) of each predictor using the vif() function from the package "car" in R (Version 4.0.2). A VIF value greater than 5 indicated individual predictors were highly correlated and warranted potential removal from the full model (Quinn and Keough 2002; Rahel and Jackson 2007). After determining the extent of multicollinearity among individual predictors and removing correlated variables, remaining predictor variables were analyzed in the following logistic regression model:

$$\ln\left[\frac{\pi(x)}{1-\pi(x)}\right] = \beta_0 + \beta_1(\text{predictor 1})_i + \beta_2(\text{predictor 2})_i + \beta_3(\text{predictor 3})_i + \ldots +\beta_p(\text{predictor p})_i$$

where $\pi(x)$ is the probability that eggs are present for a given variable value, β_0 is the intercept (the natural log of odds of eggs or larvae being present relative to being absent), and β_1, β_2, β_3, … β_p are the partial regression coefficients for each predictor variable, holding the remaining predictors constant. The null hypothesis was that there was no relationship between the presence of eggs and the value of each predictor variable, while all other predictor variables were held constant. The alternate hypothesis was that at least one partial regression coefficient was not equal to zero. To test the null hypothesis I compared the fit of the null model (β_1, β_2, β_3… β_p terms set to 0) to the fit of the model with remaining predictor variables using the pchisq() function (Peng et al. 2002). A *P* value less than 0.05 suggested the model containing remaining predictor variables provides a significantly better fit to the data than the null model. If the null hypothesis was rejected ($P < 0.05$), I tested individual remaining predictors for their significance to the model using the glm() function.

Only significant predictor variables ($P < 0.05$) were retained for the final model. I removed variables from the final model I considered biologically insignificant (Hosmer and Lemeshow 2000; Burnham and Anderson 2002) and determined which combination of remaining variables resulted in the most parsimonious model using the lowest AIC score (Burnham and Anderson 2002; Rahel and Jackson 2007). Odds ratios were used to evaluate the relative importance of each predictor variable in the most parsimonious model (Peng et al. 2002; Ditton and Sutton 2004). The odds ratio of a predictor variable is a measure of how the odds (e.g., probability that eggs are present relative to being absent) of Muskellunge eggs occurring change for a one-unit increase of that predictor variable. The odds ratio is calculated as e^{β_i}, where β_i is the regression coefficient if the ith predictor variable.

To quantify the availability of suitable Muskellunge spawning habitat (habitat suitable for egg deposition) in the Fox and Menominee rivers, I imported side scan images into ReefMaster software (2017 ReefMaster Software Limited Version 2.0) where images were fit to the geographic image space in the location they were collected. Images were exported to QGIS (Version 3.14.0) where a mosaic of all scans was created for each individual tributary. The resulting mosaics were further exported to ArcMap (Version 10.8) where I manually digitized benthic features based on visual interpretation (Andrews 2003; Figures 17, 18, 19).

After digitizing the benthic features within the scanned images, I used significant predictor variables from my logistic regression model to identify areas of suitable Muskellunge spawning habitat. For an area to be considered suitable spawning habitat, it needed to meet the following requirements: 1) Significant predictor variables quantified by presence/absence that positively influenced the presence of Muskellunge eggs had to be present, and significant predictor variables negatively influencing the presence of eggs had to be absent, 2) for significant predictors quantified numerically, the upper quartile of the range of each predictor variable at all locations where eggs were collected was considered the upper threshold for suitable spawning habitat, and anything greater than the upper quartile was not considered suitable, and 3) any substrate type that was considered a significant predictor variable positively influencing egg presence needed to be the dominant substrate type ($\geq$ 50%) at a location. I quantified the amount of suitable Muskellunge spawning habitat within the Fox and Menominee Rivers as a percentage of each tributary's total surface area from the tributary mouth to the nearest upstream dam.

For objectives 2-4, locations of tagged Muskellunge during spawning were used to determine if proportions of Muskellunge spawning in or outside of tributaries to Lower Green Bay were different, if adult Muskellunge in Green Bay returned to stocking locations to spawn, and to determine spawning site fidelity. For objective 2, spring-tagged Muskellunge captured in the Fox River (2018: N = 10; 2019: N = 6; Table 1) were not included in analyses in each respective year to reduce bias towards tributary spawning Muskellunge. For objective 2, I used a chi-square test ($\alpha = 0.05$) to determine if proportions of Muskellunge spawning in or outside of tributaries were significantly different.

Spring-tagged Muskellunge were included in analyses for objectives 3 and 4. For objective 3, stocking locations for individual Muskellunge were determined via PIT tags previously implanted by WDNR staff. Muskellunge stocked in Green Bay proper were classified as returning to stocking location to spawn if active or passive telemetry indicated they were spawning within 1 km of their stocking location. Muskellunge stocked in tributaries to Lower

Green Bay were classified as returning to stocking location to spawn if telemetry indicated they spawned somewhere in the tributary, and Muskellunge stocked at the mouth of a tributary were classified as returning to stocking location to spawn if telemetry indicated they spawned within 1 km of the stocking location, either in Green Bay proper or within the tributary.

For objective 4, I considered individual Muskellunge as displaying spawning site fidelity if located in either year (2019 or 2020) at the same spawning location as a previous year's (2018 or 2019) observation during the spawning period. I considered the display of spawning site fidelity in Green Bay proper as an individual Muskellunge spawning within 1 km of a previous spawning location. In tributaries to Lower Green Bay, I considered the display of spawning site fidelity as Muskellunge returning to the same tributary in its entirety.

For objectives 3 and 4, I performed a one-proportion Z-test using the prop.test() function in R. For objective 3, setting the expected frequency to 0.75 allows the prop.test() function to return a p-value (α = 0.05) testing the null hypothesis that the true probability of Muskellunge returning to stocking locations to spawn is greater than 75%. For objective 4, setting the expected frequency to 0.75 allows the prop.test() function to return a p-value (α = 0.05) testing the null hypothesis that the true probability of Muskellunge displaying spawning site fidelity is greater than 75%.

Because the fate (dead or alive) of Muskellunge presumed to have spawned at unknown locations in southern Green Bay was undetermined, I calculated a range of spawning site fidelity rates. I calculated the lower rate of site fidelity by including all fish with at least one known spawning location or those confirmed to be alive via at least one successful tracking event during the study. Muskellunge that were presumed to have spawned at unknown locations in southern Green Bay were included in this analysis. Conversely, I calculated the upper rate of site fidelity by including only Muskellunge with at least two known spawning locations or those confirmed to be alive via a successful tracking event in 2020. The prop.test() function was used to test the null hypothesis that the probability of Muskellunge displaying spawning site fidelity was greater than 75% for both the upper and lower rates of probability.

Results

Adult Sampling and Tagging

Two of the 62 Muskellunge we tagged (mean = 1168 mm, SD = 123 mm; Table 1; Figures 9 and 13) died immediately after tagging and 60 fish provided data used in analyses. Forty-one Muskellunge were captured via hook-and-line angling, five were collected by boat electrofishing, and 16 were collected using fyke nets. In fall 2017, a total of 20 Muskellunge were captured: 18 via hook-and-line angling and two via boat electrofishing. All hook-and-line captured Muskellunge were implanted with transmitters and released in southern Green Bay. The two Muskellunge captured via boat electrofishing were implanted with transmitters and released in the Fox River.

In spring 2018, 10 Muskellunge were captured using fyke nets. All 10 Muskellunge captured in spring 2018 were implanted with transmitters and released near Fox Point in the Fox River. One Muskellunge tagged in spring 2018 died soon after tagging, likely due to the stress of capture and subsequent surgery. In fall 2018, a total of 26 Muskellunge were captured: 23 via hook-and-line angling and three via boat electrofishing. All hook-and-line captured Muskellunge were implanted with transmitters and released in southern Green Bay. The three Muskellunge captured via boat electrofishing were implanted with transmitters and released at the mouth of the Fox River. One Muskellunge captured via hook-and-line angling died soon after tagging likely due to the stress of capture, subsequent surgery, and warm water temperatures.

Finally, in spring 2019, six Muskellunge were captured using fyke nets. All six Muskellunge captured in spring 2019 were implanted with transmitters and released near Fox Point in the Fox River. Of the 60 Muskellunge that provided data for my study, 38 were female, 15 were male, and sex was not determined for seven fish.

Spawning Periods

In spring 2018, 2019, and 2020, spawning pairs of Muskellunge were observed at a wide range of water temperatures. Spawning behavior was observed at temperatures as low as 11°C and as high as 20°C, with peak spawning activity observed between 13.6 – 15.5°C. I determined the peak spawn date each spring as May 12, May 24, and May 22 respectively. Therefore, the 2018 spawning period ranged from May 2 – May 22, the 2019 spawning period ranged from May 14 – June 3, and the 2020 spawning period ranged from May 12 – June 1.

Objective 1: Suitable Spawning Habitat Characteristics and Quantification

In spring 2018, Muskellunge eggs were collected at 15 of 48 sites (31%). All 15 were confirmed to be Muskellunge eggs via genetic analysis. Muskellunge eggs collected in 2018 were found in a variety of locations including Point au Sable, and in the Fox, Suamico, Menominee, and Peshtigo rivers (Figures 14 and 15). Of the 15 sites where Muskellunge eggs were collected, five (33%) had been identified through telemetry, six (40%) were identified via observation of spawning pairs, and four (27%) were chosen based on the presence of potentially suitable spawning habitat.

A total of 111 individual larval fish sampling events were completed in 2018. Fifty-seven (51%) of these occurred in areas of potentially suitable spawning habitat, and the remaining 54 (49%) occurred at previously identified Muskellunge egg sites. Ichthyoplankton trawls were used for 29 (26%) sampling events, D-frame nets were used for 70 (63%) sampling events, and quatrefoil light traps were used for 12 (11%) sampling events. Sampling occurred at 32 (29%) sites in the Menominee River, eight (7%) sites in the Suamico River, seven (6%) sites in the Peshtigo River, 44 (40%) sites in the Fox River, and 20 (18%) sites in southern Green Bay. Only two larval Muskellunge, with total lengths of 36 and 37 mm, were captured in the Menominee River using D-frame nets.

In spring 2019, Muskellunge eggs were collected at 25 of 112 sites (22%). Genetic analysis confirmed that eggs collected at 22 of these sites were from Muskellunge. Genetic sequencing failed for eggs collected at four sites. Based on visual interpretation and comparison to known Muskellunge eggs, I determined that eggs from three of the four sites were from Muskellunge. Muskellunge eggs collected in 2019 were found in a variety of locations including the Fox River, Menominee River, and Deadhorse Bay (Figures 14 and 15). Of the 25 sites where Muskellunge eggs were sampled, seven (28%) were identified via telemetry, three (12%) were identified via observation of spawning pairs, and 15 (60%) were randomly selected.

A total of 247 individual larval fish sampling events were completed in 2019. Thirty-three (13%) occurred in areas of potentially suitable spawning habitat, four (2%) occurred at the spring 2018 larval capture site, and 210 (85%) occurred at previously identified Muskellunge egg sites. Ichthyoplankton trawls were used for 74 (30%) sampling events, D-frame nets were used for 127 (51%) sampling events, and quatrefoil light traps were used for 46 (19%) sampling events. Sampling occurred at 38 (15%) sites in the Menominee River, 18 (7%) sites in the Suamico River, eight (3%) sites in the Peshtigo River, 161 (65%) sites in the Fox River, and 22 (9%) sites in southern Green Bay. No larval Muskellunge were captured.

In spring 2020, Muskellunge eggs were collected at 18 of 118 sites (15%). Eggs collected at 17 sites were confirmed to be from Muskellunge via genetic analysis. Genetic sequencing failed for eggs collected at two sites. Based on visual interpretation and comparison to known Muskellunge eggs, I determined eggs from one of the two sites were from Muskellunge. Muskellunge eggs collected in 2020 were found in a variety of locations including Point au Sable, the Fox River, and the Menominee River (Figures 14 and 15). Of the 18 sites where Muskellunge eggs were collected, four (22%) were identified via telemetry, one (6%) was identified via observation of spawning pairs, and 13 (72%) were randomly selected.

A total of 88 individual larval fish sampling events were completed in 2020. Two (2%) occurred at the spring 2018 larval capture site, the remaining 86 (98%) occurred at previously identified Muskellunge egg sites. D-frame dip nets were used for all sampling events. Sampling occurred at 21 (24%) sites in the Menominee River, 54 (61%) sites in the Fox River, and 13 (15%) sites in southern Green Bay. No larval Muskellunge were captured.

Due to the lack of Muskellunge larvae, the logistic regression model I constructed included only habitat variables influencing the probability that Muskellunge eggs were present at a site, rather than hatching success. Percent fine substrate, percent modified shoreline, and number of vegetation tines covered were initially removed as predictor variables due to collinearity (VIF > 5; Table 2). Percent fine substrate was collinear with all other substrate types, percent of modified shoreline was collinear with shoreline alteration rank, and number of vegetation tines covered was collinear with vegetation rank. I chose to remove percent fine substrate, percent modified shoreline, and number of vegetation tines as I felt variables that remained were more easily quantified and explanatory.

Null hypothesis testing suggested the model with remaining predictor variables (Table 2) provided a significantly better fit to the data than the model fit with only the intercept term ($P < 0.001$). Significant predictor variables retained for the final model included depth, dissolved oxygen concentration, bottom slope, distance to shore, percent gravel substrate, and percent of organic matter as substrate (Table 3). The most parsimonious model predicting the presence of Muskellunge eggs included slope, depth, dissolved oxygen concentration, distance to shore, percent gravel substrate, and percent organic matter as a substrate. AIC scores appear in Appendix A. Thus, the final logistic model was:

$$\ln\left[\frac{\pi(x)}{1-\pi(x)}\right] = -2.786 - 5.269S - 0.682D + 0.304DO - 0.157DS + 0.027GS + 0.019OM$$

where S represents bottom slope (m/m), D represents depth (m), DO represents dissolved oxygen concentration (mg/L), DS represents distance to shore (m), GS represents the percent of gravel in the substrate, and OM represents the percent of organic matter in the substrate. Ranges of predictor variables included in the final logistic model for sites where Muskellunge eggs were collected are shown in Table 4.

The final logistic regression model suggested bottom slope had the greatest influence on Muskellunge egg presence (Table 3). Probability of Muskellunge egg presence in relation to each significant predictor variable is shown in Figure 16. The likelihood that Muskellunge eggs were present at a specific site decreased by 50% when slope increased by 0.5 m/m. A 1-m increase in depth resulted in a 49% decrease in probability Muskellunge eggs were present. A one unit increase in the dissolved oxygen concentration (1 mg/L) at a given site increased the probability of Muskellunge eggs being present by 1.4 times. Additionally, for every 1-m increase in distance to shore the probability of Muskellunge egg presence decreased by 14%. Finally, a 10% increase of gravel or organic matter in the substrate increased the probability of Muskellunge egg presence by 27% and 19%, respectively.

Based on results from logistic regression and the distribution of habitat measurements at sites where eggs were found, I considered habitat in the Fox and Menominee rivers that was near shore (≤ 15 m), shallow (≤ 1.3 m), with a gradually sloping bottom (≤ 0.17 m), and organic matter or gravel as the dominant substrate (≥ 50%) as suitable for Muskellunge egg deposition. Maximum slope (0.17 m) and depth (1.3 m) represented the upper quartiles of the distributions of these variables for sites where eggs were found (Table 4). Additionally, only one site where Muskellunge eggs were collected was > 15 m from shore. Finally, I chose to classify suitable spawning habitat using only the dominant substrate (≥ 50%) in a given location, due to the difficulty of identifying multiple substrate types at a single location when using visual interpretation of side scan imagery.

Only 13.2 ha of the Fox River was considered suitable Muskellunge spawning habitat (suitable for egg deposition), representing 1.3% of the river's surface area from the mouth upstream to the De Pere Dam is approximately (1,002 ha). If only the area of the Fox River under 2.5 m deep is considered potential Muskellunge spawning habitat, approximately 6.0% was considered suitable spawning habitat in terms of egg deposition. The surface area of the Menominee River from the mouth upstream to the Menominee Dam is approximately 221 ha, of which 18.4 ha (8.3%) was considered suitable Muskellunge spawning habitat. If only the area of the Menominee River under 2.5 m deep is considered potential Muskellunge spawning habitat, approximately 23.8% was considered suitable spawning habitat (Table 5; Figures 17, 18, 19).

Objective 2: Spawning Locations

Of the 20 Muskellunge available for tracking in spring 2018, 19 were considered for analysis of spawning locations (Table 1). Nine of the 19 fish (47%) spawned in Green Bay proper, seven (37%) spawned in the Fox River, and one fish each (5%) spawned in the Menominee, Peshtigo, and Suamico rivers, respectively. The single Muskellunge not included in analysis was located in bay and tributary habitats during the spawning period, therefore a spawning location could not be determined. Overall, nine of 19 (47%) Muskellunge spawned in Green Bay proper and ten of 19 (53%) spawned in tributaries. Chi-square analysis for the 2018 spawning period suggested that the proportions of Muskellunge spawning in or outside of tributaries were not significantly different ($\chi^2 = 0.05$, df = 1, $P = 0.819$).

Of the 54 Muskellunge available for tracking in 2019, 47 were considered for analysis of spawning locations (Table 1). Twenty five of the 47 fish (53%) spawned in Green Bay proper, 15 (32%) spawned in the Fox River, four (9%) met criteria for spawning in Duck Creek, and one fish each (2%) spawned in the Menominee, Peshtigo, and Suamico rivers. Two of the Muskellunge not included in analysis were considered dead via telemetry. The remaining seven fish that were not included in analysis were located in both bay and tributary habitats during the spawning period, therefore a spawning location could not be determined. Overall, 25 of 47 (53%) Muskellunge spawned in Green Bay proper and 22 of 47 (47%) spawned in tributaries. Chi-square analysis for the 2019 spawning period suggested that the proportions of Muskellunge spawning in or outside of tributaries were not significantly different ($\chi^2 = 0.191$, df = 1, $P = 0.662$).

Of the 58 Muskellunge available for tracking in 2020, 50 were considered for analysis of spawning locations in 2020 (Table 1). Twenty-six of the 50 fish (52%) spawned in Green Bay proper, 19 (38%) spawned in the Fox River, two (4%) met criteria for spawning in Duck Creek, and one fish each (2%) spawned in the Menominee, Peshtigo, and Suamico rivers. Two Muskellunge were not included due to inconclusive telemetry data, and the remaining six were located in both bay and tributary habitats during the spawning period, therefore a spawning location could not be determined. Overall, 26 of 50 (52%) Muskellunge spawned in Green Bay proper and 24 of 50 (48%) spawned in tributaries. Chi-square analysis for the 2020 spawning period suggested that the proportions of Muskellunge spawning in or outside of tributaries were not significantly different ($\chi^2 = 0.08$, df = 1, $P = 0.777$).

Objective 3: Reproductive Homing to Stocking Locations

Six Muskellunge in my study had known stocking locations (Table 1). Three were stocked at Fox Point in the Fox River, two were stocked at Fox Metro Boat Launch at the mouth of the Fox River, and one was stocked at Boom Landing in the Menominee River (Figure 20). Three of these Muskellunge had transmitters implanted during the 2018 spawning period, and all returned to their stocking location that year. One fish returned to its stocking location in the Menominee River, one returned to its stocking location in the Fox River, and one was tagged during the spawning period at its stocking location in the Fox River. The remaining three Muskellunge had transmitters implanted during the spawning period in 2019.

In 2019, four of six Muskellunge returned to stocking locations to spawn. The Muskellunge stocked in the Menominee River returned, two Muskellunge stocked at Fox Metro Boat Launch returned to within 1 km of Fox Metro Boat Launch during the spawning period, and one Muskellunge was tagged in the Fox River at its Fox Point stocking location. One Muskellunge stocked at Fox Point did not return to its stocking location to spawn and was located in Green Bay proper near the Peshtigo River. Finally, one Muskellunge stocked at Fox Point was not located.

The four Muskellunge that returned to stocking locations to spawn in 2019 also returned to stocking locations to spawn in 2020. The Muskellunge that did not return to its stocking location to spawn in 2019 was not located during the 2020 spawning period. Additionally, in 2020, I did not locate the same Muskellunge originally stocked in the Fox River that I was unable to locate in 2019. In all cases where Muskellunge with known stocking sites were located during the spawning period (2018: N = 3; 2019: N = 5; 2020: N = 4), Muskellunge returned to stocking locations to spawn in all but one instance (Table 1). While these observations indicate the majority (> 75%) of Muskellunge in Green Bay return to stocking locations to spawn, I was unable to test this statistically due to small sample size.

Objective 4: Spawning Site Fidelity

Twenty-three Muskellunge in my study were located at the same spawning site in at least two of three spawning periods (2018, 2019, 2020), and therefore displayed evidence of spawning site fidelity (Table 1). I included 51 Muskellunge to estimate the lower end of the range for Muskellunge spawning site fidelity, and 33 Muskellunge to estimate the upper end of the range. Twenty-three of 51 Muskellunge displaying spawning site fidelity resulted in a proportion of 45% (lower rate), and this proportion was not significantly higher than 75% ($\chi^2 = 22.752$, df = 1, $P = .0001$). Conversely, 23 of 33 Muskellunge displaying spawning site fidelity resulted in a proportion of 70% (upper rate). Analysis of the upper rate did not significantly differ from 75% ($\chi^2 = 0.253$, df = 1, $P = 0.308$). Two of the Muskellunge not included in analyses were considered dead via telemetry, one was not included due to inconclusive telemetry data, and six were located at multiple tributaries during the spawning period, therefore a spawning site was not determined (Table 1).

General Movement Patterns

In general, as water temperatures cooled in fall, Muskellunge were observed making southerly movements from mid-summer ranges north of Long Tail Point towards southern Green Bay and the Fox River. From September through December 2017, seven Muskellunge were detected by receivers at the mouth of the Fox River. Only one of these seven Muskellunge was detected on an upstream receiver, and that Muskellunge also resided in the Fox River over the winter before exiting near the peak spawn date in spring 2018. From September through December of 2018, fourteen Muskellunge were detected by receivers at the mouth of the Fox River. Only three of these fourteen were detected by an upstream receiver, and two of the fourteen Muskellunge resided over the winter before exiting after the spawning period in spring 2019. From September through December 2019, 19 Muskellunge were detected by receivers at the mouth of the Fox River. Six of these 19 Muskellunge were detected on an upstream receiver, and five of the 19 resided over the winter before exiting after the spawning period in spring 2020.

During my study Muskellunge entered a variety of tributaries each spring including the Fox, Menominee, Peshtigo, and Suamico rivers along with Duck Creek (Table 1). Of the eight Muskellunge that spawned in the Fox River in 2018, seven entered in spring prior to presumed spawning. Entry dates ranged from 13 March 2018 to 14 May 2018, with the average date of entry 18 April 2018. Residency times ranged from five to 64 days, with an average residency time of 27 days for all fish. Exit dates of Muskellunge that spawned in the Fox River ranged from 2 May 2018 to 25 May 2018, with average exit date 18 May 2018. The one male Muskellunge that entered the Fox River to spawn in 2018 resided for 64 days, while the six females resided for an average of 21 days. One female Muskellunge entered the Menominee River to spawn on 10 April 2018, where it resided for 48 days before exiting the river on 28 May 2018. Additionally, one female Muskellunge entered the Suamico River on 27 April 2018, where it resided for 23 days before exiting the river on 20 May 2018. Finally, one female Muskellunge entered the Peshtigo River to spawn on 10 May 2018, where it resided for 15 days before exiting the river on 25 May 2018.

Of the 15 Muskellunge that spawned in the Fox River in 2019, 13 entered in spring prior to presumed spawning. Entry dates ranged from 5 April 2019 to 31 May 2019, with the average date of entry 6 May 2019. Residency times for all fish ranged from one to 58 days, with an average residency of 27 days. Exit dates of Muskellunge that spawned in the Fox River ranged from 19 May 2019 to 18 June 2019, with an average exit 1 June 2019. The four male Muskellunge that spawned in the Fox River in 2019 had an average residency time of 40 days, while the six females had an average residency time of 25 days. A sex was not identified during tagging for three Muskellunge that entered the Fox River to spawn in 2019. Additionally, one female Muskellunge entered the Menominee River to spawn on 9 April 2019, where it resided for 56 days before exiting the river on 4 June 2019. One female Muskellunge entered the Suamico River to spawn on 7 May 2019, where it resided for 25 days before exiting the river on 1 June 2019. One female Muskellunge entered the Peshtigo River to spawn on 6 May 2019, where it resided for 29 days before exiting the river on 4 June 2019. Finally, four Muskellunge entered Duck Creek in 2019, with an average entry date of 17 May 2019, and average exit date of 25 May 2019. One Muskellunge that entered Duck Creek resided for 25 days. However, the remaining three fish that entered all resided less than 2 days.

Of the 19 Muskellunge that spawned in the Fox River in 2020, 14 entered in spring prior to presumed spawning. Entry dates ranged from 27 March 2020 to 31 May 2020, with an average entry date of 8 May 2020. Residency times for all fish ranged from two to 61 days, with an average residency of 21 days. Exit dates of Muskellunge that spawned in the Fox River in 2020 ranged from 20 May 2020 to 7 June 2020, with an average exit of 29 May 2020. The two male Muskellunge that spawned in the Fox River in 2020 resided in the river for an average of 48 days, while the nine females resided for an average of 19 days. A sex was not identified during tagging for three Muskellunge that entered the Fox River to spawn in 2020. Additionally, one female Muskellunge entered the Menominee River to spawn on 8 April 2020, where it resided for 56 days before exiting the river on 3 June 2020. One female Muskellunge entered the Suamico River to spawn on 2 May 2020, where it resided for 21 days before exiting the river on 23 May 2020. One female Muskellunge entered the Peshtigo River to spawn on 4 May 2020, where it resided for 30 days before exiting the river on 3 June 2020. Finally, two Muskellunge entered Duck Creek in 2020, with an average entry date of 18 May 2020 and average exit date of 19 May 2020. Both fish that entered Duck Creek in 2020 resided for less than two days.

Discussion

Objective 1: Spawning Habitat Characteristics and Quantification

My results suggest Muskellunge in Green Bay and its tributaries are spawning in gradually sloping, shallow (< 1.3 m), near shore (< 5 m) habitats with organic matter or gravel substrates. Additionally, these habitats contain high levels of dissolved oxygen (> 8 mg/L) at the substrate interface. The habitat variables I determined to be significant predictors of Muskellunge egg presence differed slightly from preliminary results reported by Sheffer (2019). Sheffer (2019) reported dissolved oxygen levels, distance to shore, depth, and gravel substrate as significant predictor variables of Muskellunge egg presence. The inclusion of organic matter and bottom slope as additional significant predictors provides more conclusive evidence regarding what constitutes suitable Muskellunge spawning habitat (habitat suitable for egg deposition) in the Green Bay watershed. Ninety-five percent of the Muskellunge eggs I collected were in depths shallower than 1.5 m, suggesting shallow water habitats are preferred spawning locations of Green Bay Muskellunge. Shallow water spawning activity of Green Bay Muskellunge aligns with previous results from both Great Lakes and inland systems (Farrell 1996; Nohner and Diana 2015). Additionally, high levels of dissolved oxygen (> 8.0 mg/L) throughout the majority of Green Bay and its tributaries, with the exception of Duck Creek, indicate that dissolved oxygen levels are likely not a limiting factor for Muskellunge spawning habitat in Green Bay, based on minimum requirements described in previous literature (> 6.0 mg/L; Dombeck et al. 1984; Lebeau 1992). Furthermore, the significance of both organic matter and gravel substrate in my model indicate Green Bay Muskellunge are spawning over a variety of different substrates, as organic matter and gravel were generally not found in the same sampling locations.

The influence of bottom slope on Muskellunge egg presence in my study is similar to results of research previously conducted in the Green Bay watershed, in which slope had the greatest influence on the building of predictive models identifying Muskellunge spawning locations (Battige 2011). In addition, Muskellunge have been observed spawning in areas with minimal to moderate slopes both in the Great Lakes (Battige 2011) and within inland lakes (Nohner and Diana 2015). Likely, high levels of human development along Fox River shorelines is the mechanism behind the significance of slope in my model, as seawalls, breakwaters, and the construction of docking slips has made many areas of the Fox River have steeper slopes than what would naturally be present. To test the relationship between slope and human alteration of the shoreline, I performed a Welch two sample *t*-test. Results of that *t-test* indicated that slopes at altered shorelines (> 15% modified, mean slope = 0.32 m/m) were significantly steeper than at locations with minimal shoreline alteration unaltered shorelines (≤ 15% modified; mean slope = 0.11 m/m). Overall, my findings suggest bottom slope is a factor limiting Muskellunge spawning habitat in Lower Green Bay and the Fox River.

Near shore spawning activity of Muskellunge has been documented previously in the Great Lakes (Farrell 2001; Crane and Farrell 2015) and based on my visual and telemetry observations and egg collection, most Green Bay Muskellunge, particularly in tributaries, are spawning in near shore (< 5 m) habitats. While previous research has not defined bounds for what distance from shore is considered "near shore," in Green Bay, most Muskellunge spawning in tributaries deposit eggs within 5 m of shore. I observed many spawning pairs staying < 1 m from shore during the duration of their broadcast spawning activity. The range of distances from shore I collected eggs confirms Green Bay Muskellunge near shore spawning behavior (0.50 – 40 m; mean = 3.68 m). In bay habitats, some Muskellunge may be spawning comparatively further from shore than those in tributaries. While I still collected eggs in Green Bay proper in near shore habitats, each spawning period I located Muskellunge in the coastal marsh habitats of Deadhorse Bay a significant distance (> 100 m) from shore, suggesting limited offshore spawning may be occurring. The strong trend of tributary spawning Green Bay Muskellunge to deposit eggs extraordinarily close to shore is advantageous for fishery managers, as spawning habitat restoration efforts can focus solely on near shore, shallow areas.

Interestingly, both coarse woody structure (CWS) and aquatic vegetation have been identified as significant habitat variables in previous studies as characteristics of suitable Muskellunge spawning and nursery habitat (Dombeck 1979; Leblanc et al. 2014; Crane et al. 2015), although neither were significant in my study. Coarse woody structure is very common throughout Green Bay and its tributaries. Because of this, many sampling locations had CWS present regardless of egg presence. It is likely the sheer number of sites CWS was present within my study (75%) made it difficult for the model to differentiate CWS as a significant predictor variable. Similarly, it is likely the lack of aquatic macrophyte coverage in many areas of my study site, particularly during time of year Muskellunge are spawning, that led to aquatic vegetation being insignificant as a predictor of Muskellunge egg presence in Green Bay. For example, the Fox River, Point au Sable, and majority of Green Bay proper all lack significant aquatic vegetation coverage during the Muskellunge spawning period. Conversely, Deadhorse Bay and the Menominee River have comparatively higher levels of aquatic vegetation. Other studies have identified the importance of aquatic vegetation as it relates to Muskellunge spawning locations and habitat of newly hatched larvae (Pierce et al. 2007; Leblanc et al. 2014; Diana et al. 2015). Therefore, there is potential that the presence of aquatic vegetation in the Menominee River is more conducive to the survival of newly hatched Muskellunge larvae and might explain why larvae have been captured there. If Muskellunge eggs are successfully hatching, lack of aquatic vegetation in many Muskellunge spawning areas results in poor nursery habitat and this could be a limiting factor regarding natural reproduction in Green Bay and its tributaries.

No larval Muskellunge were collected in the Lower Green Bay/Fox River Area of Concern despite 43 confirmed egg sites, numerous observed spawning pairs of Muskellunge, and over 300 larval sampling events. During initial research (Sheffer 2019), the only two larval Muskellunge captured were collected in the Menominee River. Confirming the effectiveness of our larval sampling methods on Snipe Lake suggests that successful hatching may not be occurring or high initial mortality is occurring particularly in the Fox River and potentially elsewhere in the Lower Green Bay/Fox River Area of Concern.

In the Fox River specifically, spring-time operation of the De Pere Dam, as well as seiche events causes highly variable water levels during Muskellunge egg development (USGS gage; Figure 22). The De Pere Dam is operated under a Federal Energy Regulatory Commission license to provides stable flows from April through mid-June for spawning Lake Sturgeon. Consistent flows out of the Fox River combined with seiche events as a result of strong northerly winds, may cause short term water level changes of over 1 m. Possibly, these fluctuating water levels are transporting eggs onto shorelines or away from suitable habitat for development, as variable water levels have been shown to impact recruitment and successful hatching in other systems (Martin et al. 1981; Moore 1989; Bolle et al. 2009). Additionally, the Fox River is a major supplier of anthropogenically-induced suspended sediment into Green Bay (Khazaei et al. 2018). The negative impact of fine sediment deposits, particularly on the development of eggs in riverine systems, is well documented (Farmer and Chow-Fraser 2004; Jensen et al. 2009; Kemp et al. 2011). Because Muskellunge spawning in Lower Green Bay and the Fox River are spawning in shallow, near-shore areas with minimal flow, it is possible eggs are being covered and suffocated. Finally, the observation of Round Goby *Neogobius melanostomus* and Yellow Perch *Perca flavescens* at Muskellunge egg deposition sites during larval sampling suggests Muskellunge may be suffering from predation before or shortly after hatching. Round Goby and Yellow Perch both have been documented preying on fish eggs and larvae (Chotkowski and Marsden 1999; Fullhart et al. 2002) and it is possible the spawning behavior of Muskellunge (e.g. broadcast spawning, unguarded eggs) leaves developing eggs susceptible to predation. Stabilizing water levels during Muskellunge egg development, as well as watershed level management practices to reduce fine sediment loading may be required for Green Bay Muskellunge to successfully hatch. In addition, future research should consider the potential for predation on Muskellunge eggs and larvae by Yellow Perch and Round Goby.

While the amount of suitable spawning habitat (habitat suitable for egg deposition) in the Fox and Menominee rivers is an approximation, it is likely that the difference in the proportion of available suitable spawning habitat is a result of anthropogenic effects (Table 5). Each significant predictor variable negatively influencing the probability of Muskellunge egg deposition, and thus the areas of suitable spawning habitat, seemed to correspond with human alteration. In the Fox River, 73% of egg sampling sites had 15% or more of the shoreline altered by human effects, while in the Menominee River 58% of sites had 15% or more of the shoreline altered. This human impact was reflected in both the proportion and locations of suitable Muskellunge spawning habitat identified within these tributaries (Table 5; Figure 17, 18, 19).

During my quantification of suitable Muskellunge spawning habitat (habitat suitable for egg deposition), I chose not to include dissolved oxygen as a parameter due to diel fluctuations and relatively high levels (> 8.0 mg/L) throughout my study site. Furthermore, restricting the surface area of potential spawning habitat to locations under 2.5 m likely provides a better understanding of the proportion of suitable spawning habitat available to Muskellunge within these tributaries (Table 5). However, despite this depth restriction, areas of suitable Muskellunge spawning habitat are still relatively small in the Fox and Menominee Rivers. Therefore, it is likely that the amount of available suitable spawning habitat for Green Bay Muskellunge is limiting natural reproduction.

Objective 2: Spawning Locations

Similar to Sheffer (2019) and Battige (2011) my results suggest Muskellunge in Green Bay are spawning in both tributary and bay habitats and use multiple tributaries for spawning each year. Confirmed spawning in the Fox, Menominee, Peshtigo, and Suamico rivers indicates suitable spawning habitat (habitat suitable for egg deposition) is available in multiple Green Bay tributaries. Alternatively, some Muskellunge entered Duck Creek during the spawning period (Table 1), however I was unable to successfully collect eggs within Duck Creek or observe pairs of Muskellunge to confirm spawning. Only one Muskellunge that entered Duck Creek during the spawning window resided for more than two days, whereas Muskellunge spawning in other tributaries resided approximately 25 days. Furthermore, some Muskellunge were detected only on the downstream receiver at Duck Creek without entering the tributary, suggesting Muskellunge may have been spawning in marsh habitats outside of the Duck Creek mouth. Muskellunge have been observed spawning in Great Lakes marsh and coastal wetland habitats in previous studies, and there is a large wetland complex located immediately outside of Duck Creek that may provide better spawning habitat (Farrell 2001; Weller et al. 2016; Schaeffer et al. 2020). Low residency times, comparatively low dissolved oxygen levels (6.68 mg/L to 8.79 mg/L), absence of eggs and spawning pairs, and potentially better spawning habitat outside of the tributary suggest Muskellunge are not using Duck Creek as a primary spawning area.

While the fate (i.e. dead or alive) of Muskellunge not located via telemetry is undetermined, I chose to include Muskellunge with at least one successful tracking event during the study in analyses of bay vs. tributary spawning Muskellunge. This decision likely biased proportions slightly towards bay-spawning fish, however I do not believe that shift in proportion was high enough to make the proportions of bay spawning and tributary spawning Muskellunge in Green Bay significantly different. Furthermore, Muskellunge are highly mobile during the spawning period. This mobility made the determination of spawning locations difficult for a subset of Muskellunge each year. Multiple Muskellunge were detected during the 2019 and 2020 spawning periods on receivers at the mouth of the Fox River, the mouth of Duck Creek, and located actively in bay habitats between the two tributaries. Therefore, I was not able to determine a spawning location for fish displaying this behavior (Table 1). The exclusion of these fish from proportion analyses likely had little effect on my results, as I expect approximately equal proportions of the excluded Muskellunge spawned in bay vs. tributary habitats each respective year.

The tendency of Green Bay Muskellunge to spawn in Green Bay proper and multiple tributaries in approximately equal proportions indicates that current assessment efforts focused largely in the Fox River may need to be expanded. Sampling that encompasses multiple tributaries and bay habitats may be required to obtain a representative sample of the Green Bay Muskellunge population. While I did not create habitat maps of the Peshtigo and Suamico rivers due to time constraints, confirming spawning via egg collection, as well as the appearance of potentially suitable Muskellunge spawning habitat throughout these tributaries suggests that any previous habitat availability concerns are unwarranted, and the inclusion of these tributaries should be considered in future population assessments and stocking efforts. Conversely, further research may be required to confirm Muskellunge spawning activity in Duck Creek before habitat restoration efforts, expanded stocking locations, or populations assessments are conducted within the tributary.

Objective 3: Reproductive Homing to Stocking Locations

The trend of Green Bay Muskellunge returning to stocking locations to spawn reported by Sheffer (2019) continued throughout my study. Two Muskellunge stocked at Fox Metro Boat Launch had undetermined spawning locations in 2019 and 2020 (Table 1; Figure 20). However, their locations during the spawning period each year were within 1 km of Fox Metro boat launch and they likely spawned in that area, therefore I classified them as returning to stocking locations to spawn. The remaining Muskellunge in my study that returned to stocking locations did so consistently and in some cases were in the same locations within tributaries. For example, I located the Muskellunge stocked in the Menominee River (Table 1; Figure 21) in all three years within 10 m of the same location each spawning period.

Additionally, Sheffer (2019) confirmed that Muskellunge in previous studies and those previously tagged by WDNR staff often return to their stocking locations to spawn. Using PIT tag data, Sheffer (2019) confirmed six Muskellunge tagged on Green Bay by Battige (2011) returned to their stocking locations to spawn. Also, information gathered from the WDNR PIT tag database showed 152 out of 154 adult Muskellunge recaptured during annual spring surveys from 1994 to 2013 were captured in the tributary they were stocked (Sheffer 2019; Steven Hogler, WDNR, *unpublished data*).

The results of my study, preliminary research (Sheffer 2019), and WDNR PIT tag data suggest that most Muskellunge in Green Bay return to their stocking locations to spawn. The tendency of most Muskellunge in Green Bay to return to stocking locations to spawn indicates that WDNR staff may be able to increase natural reproduction by altering current stocking strategies to areas where suitable spawning habitat is present. Stocking Muskellunge in areas of suitable spawning habitat may increase egg deposition and subsequent natural reproduction, as fish are likely to return to their location of stocking origin.

Objective 4: Spawning Site Fidelity

My results suggest Muskellunge in Green Bay display moderate spawning site fidelity. These results differ slightly from preliminary results (Sheffer 2019), in which most (> 75%) Muskellunge displayed spawning site fidelity. A more robust sample size, as well as the inclusion of fish with an undetermined fate likely led to these differences. Rather than bias estimates either direction by including or excluding fish with an undetermined fate in a single analysis, it was necessary to calculate a range of fidelity for Muskellunge in Green Bay. Thus, analysis of the lower proportion included 28 Muskellunge with unknown fate that were assumed to have spawned at locations in Green Bay proper. Likely, a portion of these Muskellunge did display spawning site fidelity in Green Bay proper at a site I never located, or they died during the study. Therefore, this lower proportion is likely biased slightly low. Analysis of my upper proportion resulted in a much larger proportion of Green Bay Muskellunge displaying spawning site fidelity. Sheffer's (2019) analysis, in which 77% of Muskellunge displayed spawning site fidelity, also included only fish that two spawning locations were identified. Because fish included in my upper proportion were confirmed alive, it is a better representation of the true tendencies of Muskellunge in Green Bay to exhibit reproductive homing.

Muskellunge have previously demonstrated reproductive homing in inland systems (Crossman 1990; Margenau 1994; Younk et al. 1996) and even in previous Great Lakes research (Farrell et al. 2007; Weller et al. 2016). The display of reproductive homing by Muskellunge in large systems such as Green Bay is likely a better representation of the true tendencies of Muskellunge to display spawning site fidelity than in inland systems. In Green Bay and its tributaries, Muskellunge have many spawning locations to select from, whereas in inland lakes or smaller systems suitable spawning habitat may be limited. Information regarding the tendency of Green Bay Muskellunge to display spawning site fidelity allows managers to enhance habitat at known spawning locations to increase egg deposition and potentially natural reproduction, as fish are somewhat likely to return to these areas.

General Movement Patterns

Similar to Sheffer (2019), I hypothesized that southerly movement of Muskellunge in late summer and fall is likely in response to predatory foraging. The Fox River is a known spawning location for Lake Whitefish *Coregonus clupeaformis*, and the timing of Muskellunge detected on

Fox River receivers each fall suggests Muskellunge are using this source of prey. Future research analyzing Muskellunge diet items during late fall may lend further insight into mechanisms behind these southerly movements. Additionally, a handful of Muskellunge in my study overwintered in the Fox River each respective year. Slightly warmer water temperatures in comparison to Green Bay proper, increased dissolved oxygen levels, or concentrations of prey species in the Fox River during the winter months may have led to this behavior.

Water temperatures that I observed spawning Muskellunge in Green Bay were similar to Battige (2011), but slightly warmer than previously reported in other Great Lakes studies (13.6-15.5°C vs. 8-16°C; Lane et al. 1996; Farrell 2001; Farmer and Chow-Fraser 2004). Furthermore, tributary spawning Muskellunge appear to enter tributaries over a prolonged period well before presumed spawning but exit over a comparatively more truncated period after presumed spawning. In addition, male Muskellunge entered tributaries earlier, resided longer, and exited later than females in general. Thus, seasonal congregations of Muskellunge during late fall, variable spring tributary residency time, and differences in entry and exit dates during the spawning period indicates that the potential exists for alterations to standard population assessments. Possibly, electrofishing, fyke netting, or other standard sampling during late fall when Muskellunge are congregated in southern Green Bay may allow for a better overall representation of the Green Bay Muskellunge population.

During my study, quantifying locations of Muskellunge throughout mid-summer and mid-winter months was difficult. Movement of Muskellunge out of near shore bay and tributary habitats after presumed spawning made detection success much lower than when fish were concentrated during the spawn and pre-spawn periods. Movement of Muskellunge throughout Green Bay proper, residency in deeper water, and less tracking effort during mid-summer months likely led to a decrease in Muskellunge located throughout July and August. Additionally, active tracking was not possible in winter months, as radio detections via air-based telemetry became difficult due to ice cover. Similar to Sheffer (2019), only one Muskellunge in my study left southern Green Bay and was detected north of the Chambers Island receiver line (Figure 10), suggesting most Muskellunge in southern Green Bay remain south of Chambers Island annually. Interestingly, the single Muskellunge that left my study area was last detected by acoustic receivers southwest of Manistique, Michigan, over 320 km from Green Bay, Wisconsin. The tendency of Muskellunge in southern Green Bay to remain south of Chambers Island provides additional insight to fishery managers as decisions are made regarding population level management of Green Bay Muskellunge.

Management Recommendations

My results demonstrate the importance of the enhancement and protection of current suitable Muskellunge spawning and nursery habitat in Green Bay as a management strategy for reestablishing a self-sustaining population. Current availability of Muskellunge spawning habitat suitable for egg deposition in the Fox and Menominee rivers is minimal and may be contributing to the lack of naturally reproduced Muskellunge in Green Bay. Protection of known Muskellunge spawning areas, as well as the restoration of habitat negatively altered by anthropogenic effects particularly in the Fox River, may lead to increases in the amount of suitable spawning habitat available to Green Bay Muskellunge. Based on my results, habitat restoration and protection efforts should be focused on shallow, near shore, gradually sloping areas with minimal human alteration of the shoreline. Potential near shore habitat enhancements include the construction of riparian vegetation along areas of minimally altered shoreline to increase the amount of organic matter in the substrate, placement of gravel substrate in areas with minimal fine sediment deposits, and removal of human-placed structures contributing to steep bottom slopes along shorelines.

Potentially, the mitigation of fine sediment into the Fox River may help alleviate the possible suffocation of Muskellunge eggs during development and increase hatching success. Agriculture has been identified as the primary contributor to sediment loads in the Fox River Wisconsin Watershed (Robertson and Saad 2011). Additionally, a recent large-scale cleanup of the Fox River to remove polychlorinated biphenyls (PCBs) resulted in dredging activity that actively resuspends fine sediment. During my sampling, majority of this dredging activity was occurring on or near Muskellunge egg deposition sites. While the Fox River PCB cleanup and dredging activity is complete, the threat of sediment loading via agricultural practices still exists in the Fox River watershed. Management practices that mitigate fine sediment loading such as the creation of riparian buffers, erosion control construction along shorelines, and diversion of stormwater runoff may be necessary before successful hatching of Muskellunge is observed in the Fox River and Lower Green Bay (Krishnappan and Marsalek 2002; Chesapeake Bay Program 2006; Wilkes et al. 2018). Equally important to reducing fine sediment deposits as a method to increase hatching is the stabilization of spring water levels in the Fox River. While I acknowledge it may be difficult to accomplish, strategic operation of the De Pere Dam as a method to stabilize water levels during Muskellunge egg development in response to springtime seiche events may reduce egg movement out of suitable spawning habitat and increase hatching success. To maximize potential natural reproduction, watershed-level management practices related to reducing fine sediment deposits, as well as potentially stabilizing water levels during Muskellunge egg development, should be pursued in combination with aforementioned river-level habitat restoration efforts.

Additionally, the results of my objectives related to the movement and spawning locations of Muskellunge suggest that the choice of stocking locations may play an important role in future natural recruitment. Sheffer (2019) also acknowledged this aspect of potential Muskellunge management in Green Bay and noted that the strategic choice of stocking locations by fishery managers in areas of suitable spawning habitat may lead to a greater probability of egg deposition and subsequent hatching. The tendency of Lower Green Bay and Fox River Muskellunge to return to stocking locations, as well as display of spawning site fidelity, allows fishery managers to alter stocking sites to locations with the most suitable spawning habitat, as Muskellunge will likely return to those locations to spawn. While I was not able to determine strong spawning site fidelity for locations in Green Bay proper, the potential also exists for new stocking locations in bay habitats. Potentially, stocking Muskellunge in wetland or coastal marsh habitats similar to those found outside of Duck Creek and in Deadhorse Bay, described in other studies as Muskellunge spawning and nursery habitat, may increase the number of Muskellunge spawning in those locations and as a result increase natural reproduction (Farrell 2001; Weller et al. 2016; Schaeffer et al. 2020). Furthermore, confirming Muskellunge spawning in the Peshtigo and Suamico rivers indicates the potential for additional alternate stocking locations in other tributaries. Because my results suggest minimal suitable Muskellunge spawning habitat exists in the Fox and Menominee rivers, which encompasses four current stocking locations (Figure 19), pursuing stocking options in bay habitats and other tributaries to Green Bay may be advantageous.

Finally, the identification of naturally reproduced Muskellunge in Lower Green Bay and the Fox River is of utmost importance to fishery managers regarding the reestablishment of a self-sustaining population. Currently, all fingerling Muskellunge stocked into Green Bay receive a left-ventral fin clip prior to stocking, and all yearling Muskellunge receive a right-ventral fin clip prior to stocking. Fin-clipping Muskellunge allows for the identification of stocked vs. naturally reproduced fish at young ages, however fin regeneration and decreased confidence identifying clips can make this differentiation difficult at the adult stage (McNeil and Crossman 1979; Nielsen 1992). In addition to fin-clipping, 20% of the yearling Muskellunge stocked in Green Bay and tributaries receive PIT tags to identify individual fish and assess growth rates. PIT tags allow fisheries managers to identify individual fish, and due to their high retention rates are ideal for long-lived species like Muskellunge (Rude et al. 2011; Weber and Flammang 2017). Moreover, sampling juvenile Muskellunge is inherently difficult and catch rates are generally low (Dembkowski et al. 2020). Therefore, naturally reproduced Muskellunge may go undetected at the juvenile life stage when fin-clips are more readily identified. In Green Bay and its tributaries, PIT tagging all Muskellunge prior to stocking would allow for fisheries managers to index naturally reproduced fish at any age during annual population assessments. Additionally, increased information on age and growth rates as well as reproductive homing to stocking locations would be discernable from increases in PIT tag data. Through current PIT tagging efforts, the WDNR has established a long-term mark-recapture data set that is used to index population parameters and for other management objectives (Kapuscinski et al. 2007; Sheffer 2019). However, only having a limited number of Muskellunge currently implanted with PIT tags does not allow for Green Bay fishery managers to successfully identify all Muskellunge that were stocked vs. those naturally reproduced.

Similar to Sheffer (2019), my results suggest the Lower Green Bay and Fox River Muskellunge population has not reached self-sustaining levels. Stocking of Muskellunge in Green Bay and its tributaries is still required to support the trophy fishery currently available to recreational anglers. Future management objectives and goals should focus on habitat improvements related to protecting and enhancing areas of suitable Muskellunge spawning habitat, watershed level changes that potentially increase the occurrence of successful hatching, the development of alternate stocking locations, and increasing the ability of fisheries managers to identify any naturally reproduced recruits.

Further Research

The overall goal of my study was to provide information to fishery managers that helps restore the Lower Green Bay and Fox River Muskellunge population to self-sustaining levels. While I successfully identified Muskellunge spawning locations and related habitat conditions that result in egg deposition, I was unable to confirm successful hatching of Muskellunge larvae. Therefore, alternate research may need to be conducted to determine the factors regulating hatching success of larval Muskellunge in Lower Green Bay and its tributaries. To address my hypotheses as to why Muskellunge eggs are not hatching in Lower Green Bay and the Fox River specifically, I suggest considering the following research ideas.

Egg enclosures that allow researchers to observe hatching success in a natural environment have been used previously for a variety of fish species (Cowan et al. 1992; Viavant 1998). However, due to the non-adhesive properties of Muskellunge eggs, replication of natural habitat conditions during the development of eggs would be challenging. Natural movement of eggs, sediment deposits, and natural water flows would be difficult to replicate in an enclosure even when placed at known Muskellunge spawning locations in Green Bay. To determine the potential impact of fine sediment on Muskellunge eggs during development, placing sediment traps at known Muskellunge spawning locations would allow managers to quantify the amount of fine sediment deposited over the time period a Muskellunge egg is developing. Following this quantification, fine sediment deposits could be replicated in a laboratory setting with artificially fertilized Muskellunge eggs to measure hatching success in response to potential suffocation. In addition, diet analysis of fish species found in Lower Green Bay and the Fox River near known Muskellunge spawning locations may lend fishery managers insight into potential predation on Muskellunge eggs and larvae. The deployment of baited cloverleaf traps, mini fyke nets, and dip nets to collect fish species such as Round Goby and Yellow Perch in known Muskellunge spawning locations, would allow researchers to analyze diet contents and determine if predation on Muskellunge eggs or larvae is occurring.

Previous research has resulted in the collection of Muskellunge larvae in Sturgeon Bay during WDNR assessments, as well as in the Menominee River during initial phases of my study (Sheffer 2019). Potentially, habitat assessments in Sturgeon Bay and additional sampling effort in the Menominee River may result in the identification of habitat that is conducive to successful hatching of Muskellunge larvae rather than just egg deposition. Following this, the possibility exists to use this information as a guide to identify areas within Green Bay proper that are favorable for the enhancement or creation of Muskellunge spawning and nursery habitat.

Finally, similar to Sheffer (2019), the use of a variety of telemetry techniques allowed me to locate Muskellunge on both broad and fine scales throughout my study. The use of radio telemetry was preferred to identify spawning locations and spawning behavior, as fine scale movements were easily determined during boat-based tracking, especially in shallow water. In addition to boat-based radio tracking, aerial surveys allowed us to gain information on Muskellunge throughout the expanse of southern Green Bay, something that would not have been possible using only boat-based techniques. These aerial surveys provided information on many Muskellunge that did enter tributaries or had home ranges outside of the southern-most portion of Green Bay proper, and increased tracking efficiency immensely. Finally, all Muskellunge in my study were implanted with acoustic transmitters capable of operating for nearly 10 years (3,541 d), therefore passive receiver arrays in southern Green Bay will allow fisheries managers to continue to monitor movement and tributary use of Muskellunge in southern Green Bay and its tributaries for years to come. My study demonstrated the advantages of using multiple telemetry techniques to investigate different aspects of movement and reproductive behavior of Muskellunge, and future research projects should consider employing a variety of tracking methods when investigating these ecologically and economically important long-lived fish.

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Table 1. Total length (TL), sex, date of tagging, stocking location, delineation of bay vs. tributary spawning locations, and general spawning locations of Muskellunge implanted with acoustic and radio transmitters from fall 2017 through spring 2019 in Green Bay Lake Michigan. Sexes represent M = Male, F = female, and U = unknown. Bay vs. Tributary spawning locations represent B = Bay, T = Tributary, U = undetermined.

						Bay vs. Tributary Spawn Location			Spawning Location		
Acoustic ID	**Radio Freq.**	**Date Tagged**	**Sex**	**TL (mm)**	**Stocking**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
13276	165.123	8/22/2017	F	1029		B	B	B	N. Point Au Sable	Bay - Unknown	Bay - Unknown
13278	165.252	8/22/2017	F	1283		T	T	T	Fox River	Fox River	Fox River
13268	164.893	8/23/2017	F	1334		B	B	B	Deadhorse Bay	Bay - Unknown	Bay - Unknown
13270	165.164	8/23/2017	F	1143		T	B	B	Fox River	Bay - Unknown	Bay - Unknown
13272	164.646	8/23/2017	F	1143	Menominee	T	T	T	Menominee River	Menominee River	Menominee River
13274	165.214	8/23/2017	F	1257		T	B	T	Fox River	Bay - Unknown	Fox River
13279	164.765	8/29/2017	F	1245		T	T	T	Peshtigo River	Peshtigo River	Peshtigo River
13287	165.044	10/5/2017	F	1219		T	T	T	Fox River	Fox River	Fox River
13289	165.143	10/5/2017	F	1308		B	T	T	Kidney Island	Duck Creek	Fox River
13291	165.085	10/5/2017	F	1295		B	T	T	Deadhorse Bay	Duck Creek	Fox River
13283	164.804	10/6/2017	F	1359		T	U	U	Suamico River	Not determined	Not determined
13285	165.022	10/6/2017	F	1162		B	B	B	N. Point Au Sable	Bay - Unknown	Bay - Unknown
13281	165.107	10/10/2017	F	1130		B	B	B	N. Point Au Sable	Bay - Unknown	Bay - Unknown
13292	164.094	10/13/2017	F	1016		B	T	T	Point Au Sable	Fox River	Fox River
13277	165.065	10/19/2017	M	1232	Fox River	T	B	B	Fox River	Bay - Near Peshtigo R.	Bay - Unknown
13302	164.188	10/23/2017	F	1003		U	U	U	Not determined	Not determined	Not determined
13269	164.705	10/26/2017	M	997		B	T	T	Bay- Unknown	Duck Creek	Duck Creek
13294	164.824	10/26/2017	F	1175		T	T	T	Fox River	Duck Creek	Suamico River
13284	164.745	11/2/2017	F	1359		B	B	B	S. Point Au Sable	N. Long Tail Point	Bay - Unknown
13280	164.606	11/7/2017	F	1219		T	B	B	Fox River	Bay - Unknown	Bay - Unknown
13271	164.684	5/9/2018	M	1207			U	U	Fox River	Dead	Dead
13273	165.235	5/9/2018	F	1213			T	U	Fox River	Fox River	Unknown
13282	164.225	5/9/2018	M	1219			B	B	Fox River	Bay - Unknown	Bay - Unknown

Table 1 continued

						Bay vs. Tributary Spawn Location			Spawning Location		
Acoustic ID	**Radio Freq.**	**Date Tagged**	**Sex**	**TL (mm)**	**Stocking**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
13286	164.845	5/9/2018	M	1041			B	B	Fox River	Bay - Unknown	Bay - Unknown
13290	164.245	5/9/2018	M	1099	Fox River		B	B	Fox River	Bay - Unknown	Bay - Unknown
13296	164.206	5/9/2018	M	1130			T	T	Fox River	Fox River	Fox River
13298	164.383	5/9/2018	F	1289			U	U	Fox River	Dead	Dead
13300	164.485	5/9/2018	F	1219			U	U	Fox River	Not determined	Not determined
13301	165.272	5/9/2018	F	1308			B	B	Fox River	Bay - Unknown	Bay - Unknown
13295	164.875	8/30/2018	F	940			B	U		N. Point Au Sable	Lake Michigan
13305	164.055	8/30/2018	M	1105			B	B		Little tail	Bay - Unknown
13275	164.785	9/7/2018	F	1353			B	B		Deadhorse Bay	Deadhorse Bay
13306	164.076	9/12/2018	M	1264			B	B		N. Point Au Sable	Bay - Unknown
13312	164.144	9/12/2018	F	1327			T	U		Suamico River	Not determined
13313	164.564	9/12/2018	F	1118			B	B		Deadhorse Bay	Deadhorse bay
13314	164.665	9/12/2018	U	1130			T	T		Fox River	Fox River
13293	164.445	9/13/2018	M	1016			T	T		Fox River	Fox River
13297	164.367	9/13/2018	F	1232			B	B		Bay - Unknown	Bay - Unknown
13288	164.526	9/14/2018	F	1378			B	B		Point Au Sable	Bay - Unknown
13303	164.914	9/14/2018	U	940			T	T		Fox River	Fox River
13318	164.126	9/29/2018	F	1181			T	T		Fox River	Duck Creek
13319	164.404	9/29/2018	F	1207			B	B		W. Long Tail Point	Bay - Unknown
13320	164.426	10/14/2018	M	1029			T	T		Fox River	Fox River
13317	164.547	10/18/2018	U	953	Fox River - Metro		U	T		Not determined	Fox River
13323	164.285	10/19/2018	F	1010			B	B		Kidney Island	Kidney Island
13325	165.305	10/19/2018	F	959			B	B		N. Point Au Sable	Bay - Unknown
13316	164.626	10/20/2018	F	1048			T	T		Fox River	Fox River
13307	164.586	10/22/2018	F	1080			B	B		Little Sturgeon Bay	Bay - Unknown
13327	164.466	10/25/2018	F	1213			T	T		Fox River	Fox River

Table 1 continued

						Bay vs. Tributary Spawn Location			Spawning Location		
Acoustic ID	**Radio Freq.**	**Date Tagged**	**Sex**	**TL (mm)**	**Stocking**	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
13324	164.347	10/26/2018	F	1340			B	B		N. Point Au Sable	Bay - Unknown
13326	164.935	10/26/2018	U	1080			T	T		Fox River	Fox River
13309	164.165	10/29/2018	M	1156			T	T		Fox River	Fox River
13321	164.035	10/29/2018	M	1149	Fox River - Metro		U	U		Not determined	Not determined
13322	165.184	10/29/2018	M	1003			T	T		Fox River	Fox River
13299	164.728	5/16/2019	F	1327				B		Fox River	Point Au Sable
13304	164.307	5/16/2019	M	1067				U		Fox River	Not determined
13308	164.017	5/16/2019	F	1168				T		Fox River	Fox River
13310	164.506	5/16/2019	M	1029				B		Fox River	Bay - Unknown
13311	164.324	5/16/2019	F	1130				B		Fox River	Bay - Unknown
13315	164.264	5/16/2019	F	1295	Fox River			T		Fox River	Fox River

Table 2. Summary of the remaining variables included in the multiple logistic regression model after collinear predictors were removed. The fit of the full model (all β terms set to 0) was initially compared to the fit of the model with remaining predictor variables using the pchisq() function. Once the null hypothesis was rejected ($P < 0.05$), I tested the predictors included in this table for their significance using the glm() function. Variables with significant P-values ($P < 0.05$; indicated with an asterisk) were retained for determination of the most parsimonious model using AIC scores.

Habitat Variable	Coefficient	SE	P-value
Intercept	-0.928	2.340	0.692
Depth (m)	-1.411	0.596	0.018*
Slope (m/m)	-5.508	1.821	0.002*
Flow (m3/s)	6.860	4.692	0.144
Dissolved Oxygen (mg/L)	0.322	0.095	0.001*
Temperature	-0.070	0.097	0.471
Vegetation Rank 1	-0.397	0.519	0.444
Vegetation Rank 2	-0.913	0.938	0.330
Percent Gravel	0.031	0.012	0.013*
Percent Pebble	-0.027	0.025	0.282
Percent Cobble	-0.071	0.067	0.287
Percent Boulder	0.000	0.012	0.973
Percent Organic Matter	0.149	0.008	0.049*
Organic Matter - Presence	0.539	0.561	0.337
Algae - Presence	-1.005	0.689	0.145
CWS - Medium	-0.309	0.610	0.613
CWS - High	0.412	0.506	0.416
CWS - Presence	0.592	0.794	0.456
Alteration Rank - 2	-0.938	0.736	0.203
Alteration Rank - 3	-0.933	0.734	0.204
Distance from shore (m)	-0.155	0.060	0.010*

Table 3. Summary of significant habitat variables in the most parsimonious model used to predict the probability of Muskellunge egg presence in Green Bay, Lake Michigan. The odds ratio of a predictor variable is a measure of how the odds (e.g. probability that eggs are present relative to being absent) of Muskellunge eggs occurring change for a one-unit increase in that predictor variable. The odds ratio is calculated as e^{β_i}, where β_i is the regression coefficient if the ith predictor variable.

Habitat Variable	Regression Coefficient	SE	Odds ratio (95% confidence interval)
Intercept	-2.786	1.104	
Depth	-0.682	0.459	0.505 (0.201 – 1.229)
DO concentration	0.304	0.087	1.355 (1.149 – 1.615)
Bottom slope	-5.269	1.643	0.005 (0 – 0.094)
Percent gravel	0.027	0.010	1.027 (1.006 – 1.049)
Percent organic matter	0.019	0.005	1.019 (1.008-1.030)
Distance to shore	-0.157	0.055	0.855 (0.761-0.943)

Table 4. Range of predictor variables retained in the final logistic regression model at locations where eggs were collected and locations where eggs were not collected.

Habitat Variable	Locations with Eggs (mean)	Locations without Eggs (mean)
Depth (m)	0.20 - 1.80 (0.98)	0.37-2.90 (1.19)
DO concentration (mg/L)	6.09 -16.46 (9.42)	2.51 -16.20 (8.51)
Bottom slope (m/m)	0 - 0.37 (0.11)	0 - 1.04 (0.22)
Percent gravel	0 - 75 (15.12)	0 - 85 (11.00)
Percent organic matter	0 - 100 (42.51)	0 - 100 (27.07)
Distance to shore (m)	0.50 - 40 (3.68)	0.15 - 1,500 (19.94)

Table 5. Summary of the availability of suitable Muskellunge spawning habitat in the Fox and Menominee River, from the mouth of each river to the nearest upstream dam.

	Fox River	Menominee River
Total surface area (ha)	1001.60	220.58
Total surface area under 2.5 m (ha)	218.38	77.21
Total surface area of suitable spawning habitat (ha)	13.20	18.41
Percent suitable spawning habitat	1.32%	8.35%
Percent suitable spawning habitat under 2.5 m	6.04%	23.84%

Figure 1: Map of Lower Green Bay including my study area denoted in yellow. The LGBFR - Area of Concern is encompassed by the hashed yellow markings.

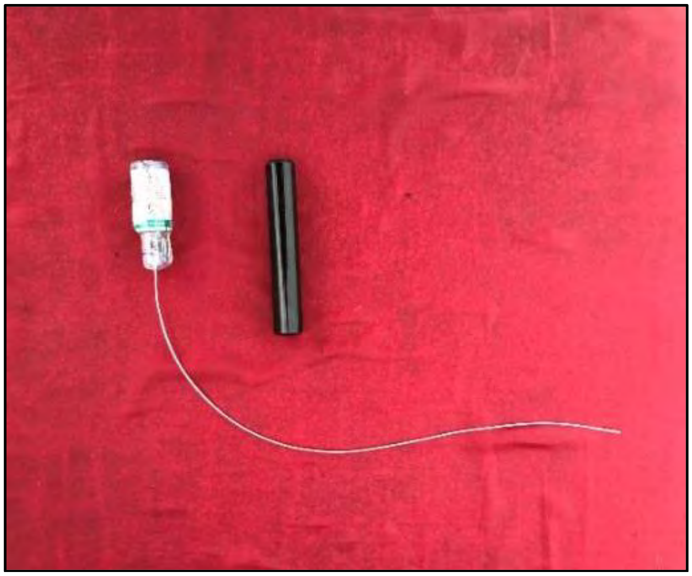


Figure 2: Radio Transmitters (left) and acoustic transmitters (right) were surgically implanted into Muskellunge to track movements and determine spawning locations.

Figure 3: A 4-cm incision was made along the ventral midline between the pectoral and pelvic fins of the Muskellunge.

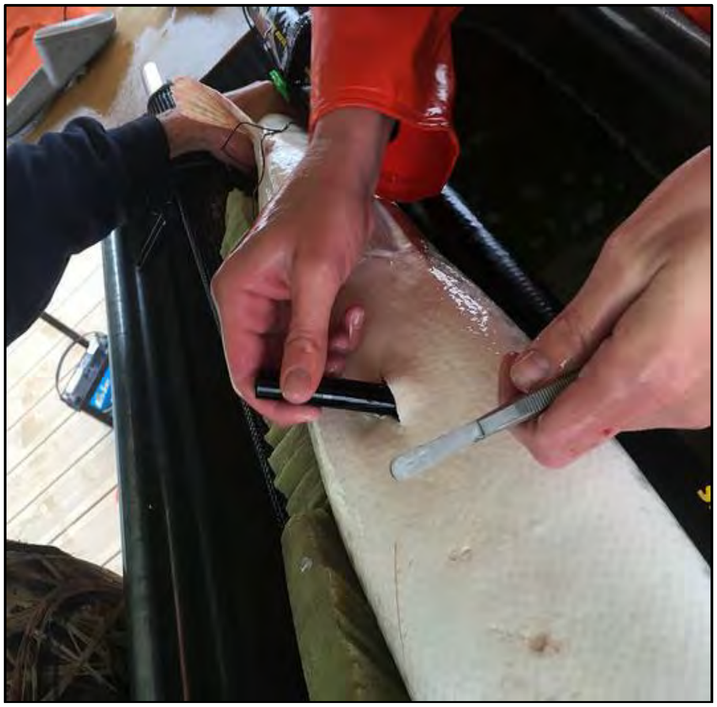


Figure 4: Acoustic transmitters were inserted through the incision in the Muskellunge body cavity.

Figure 5: A curved hollow needle was inserted through the incision on the Muskellunge and used to puncture a hole just posterior/lateral to the incision. The radio antenna was run through the needle.

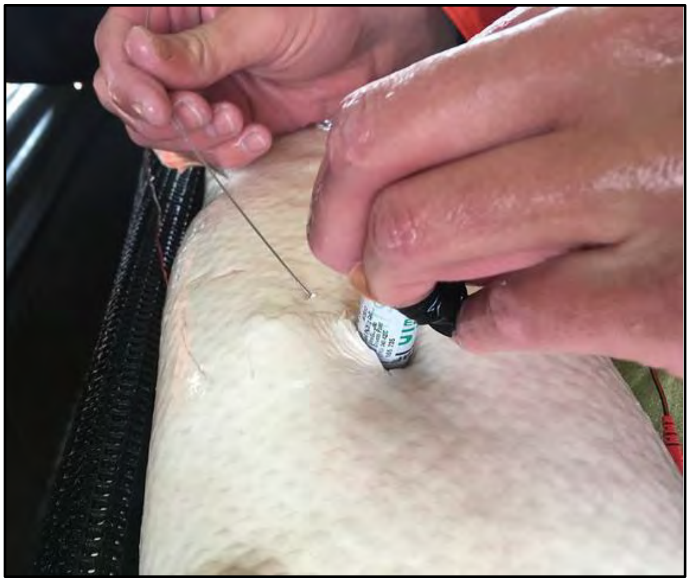


Figure 6: The needle was pulled out, and the radio transmitter inserted into the Muskellunge body cavity, allowing the antenna to trail externally.

Figure 7: Three sutures were used to close the incision. Throughout the surgery, Muskellunge were placed on a sling and water was pumped through the gills.

Figure 8: In tributaries, each VR2W acoustic receiver was attached to a steel pipe encased in a concrete block, submerged 20 m offshore, and secured to onshore trees with a chain.

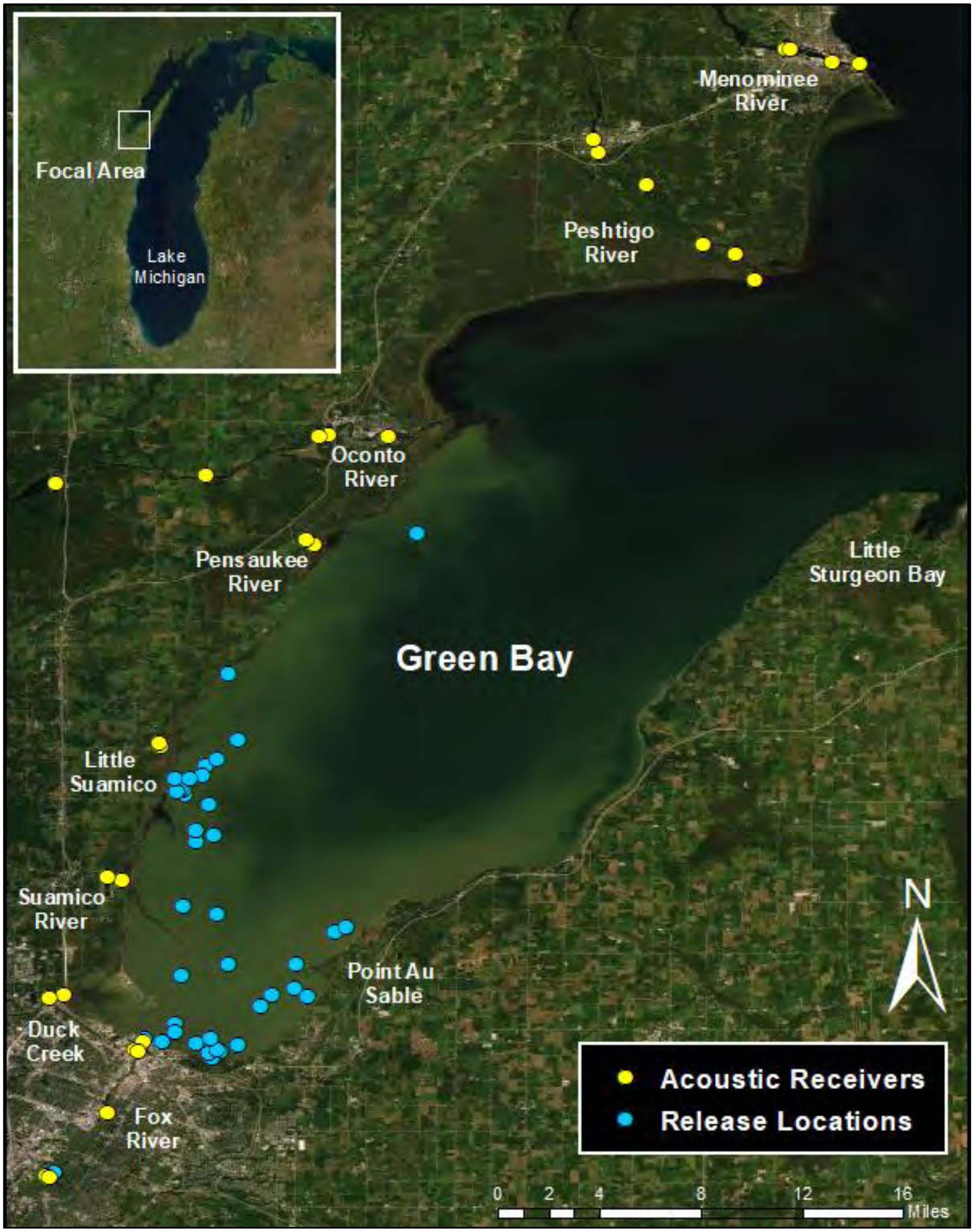


Figure 9: Array of acoustic receivers placed in tributaries to Lower Green Bay (yellow circles), and release locations of Muskellunge (N = 60; blue circles) tagged from fall 2017 – spring 2019.

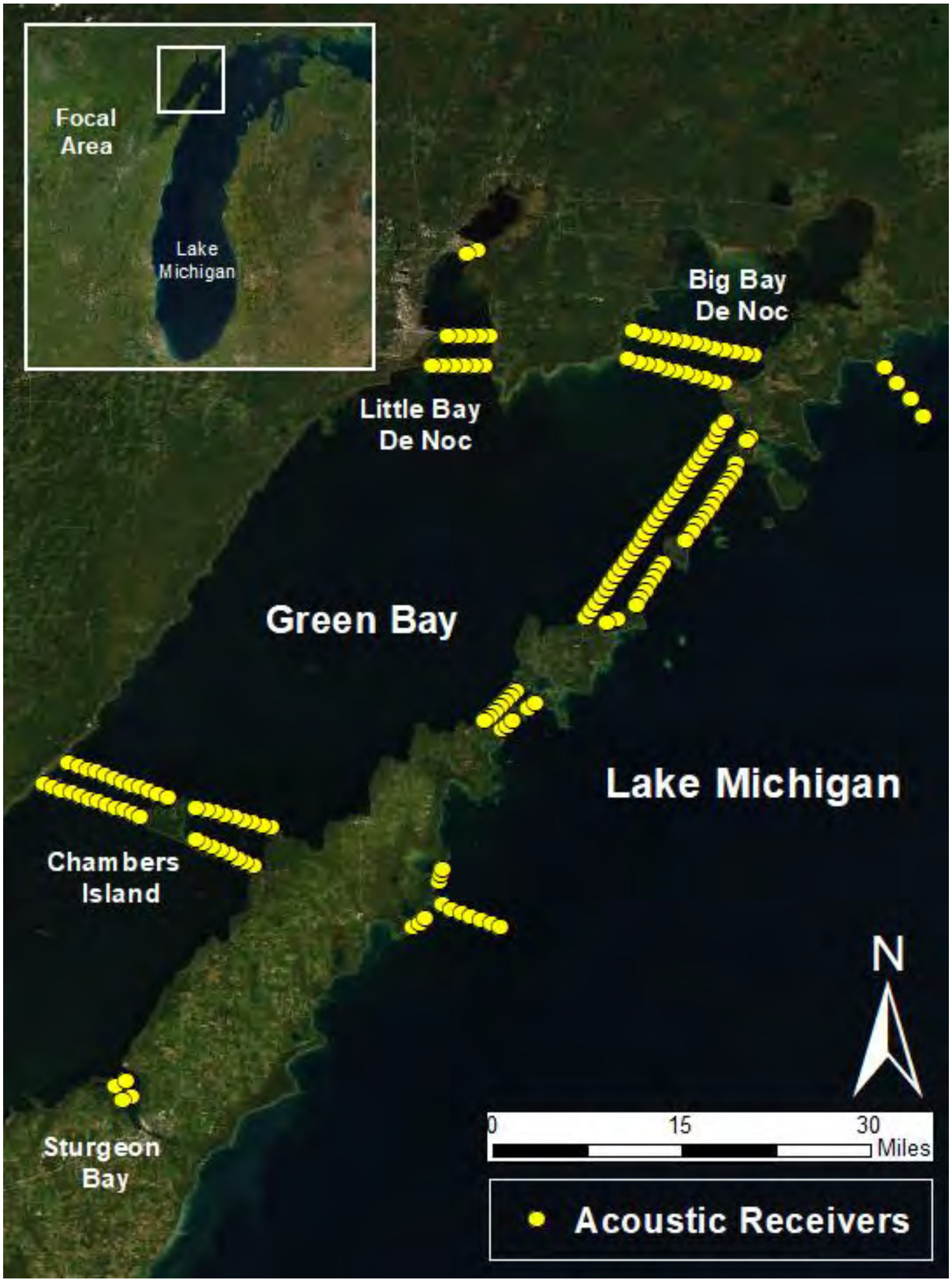


Figure 10: Array of acoustic receivers (yellow circles) in northern Green Bay and Lake Michigan.

Figure 11: Our airlift pump, which consisted of a generator, air compressor, air hose, and PVC pipe.

Figure 12: Contents were dispelled into 1.2 mm stretch mesh, in which fine sediment could be sieved. Contents were searched for eggs.

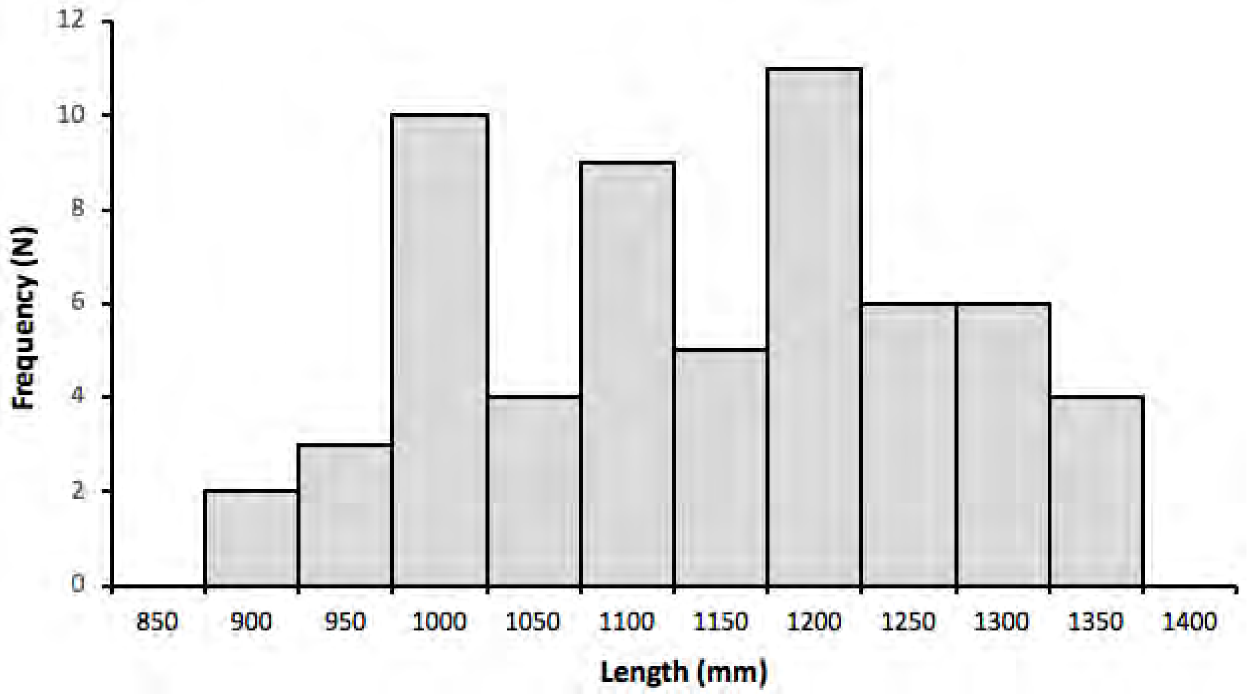


Figure 13: Length frequency of Muskellunge implanted with transmitters (N = 60) in southern Green Bay and its tributaries.

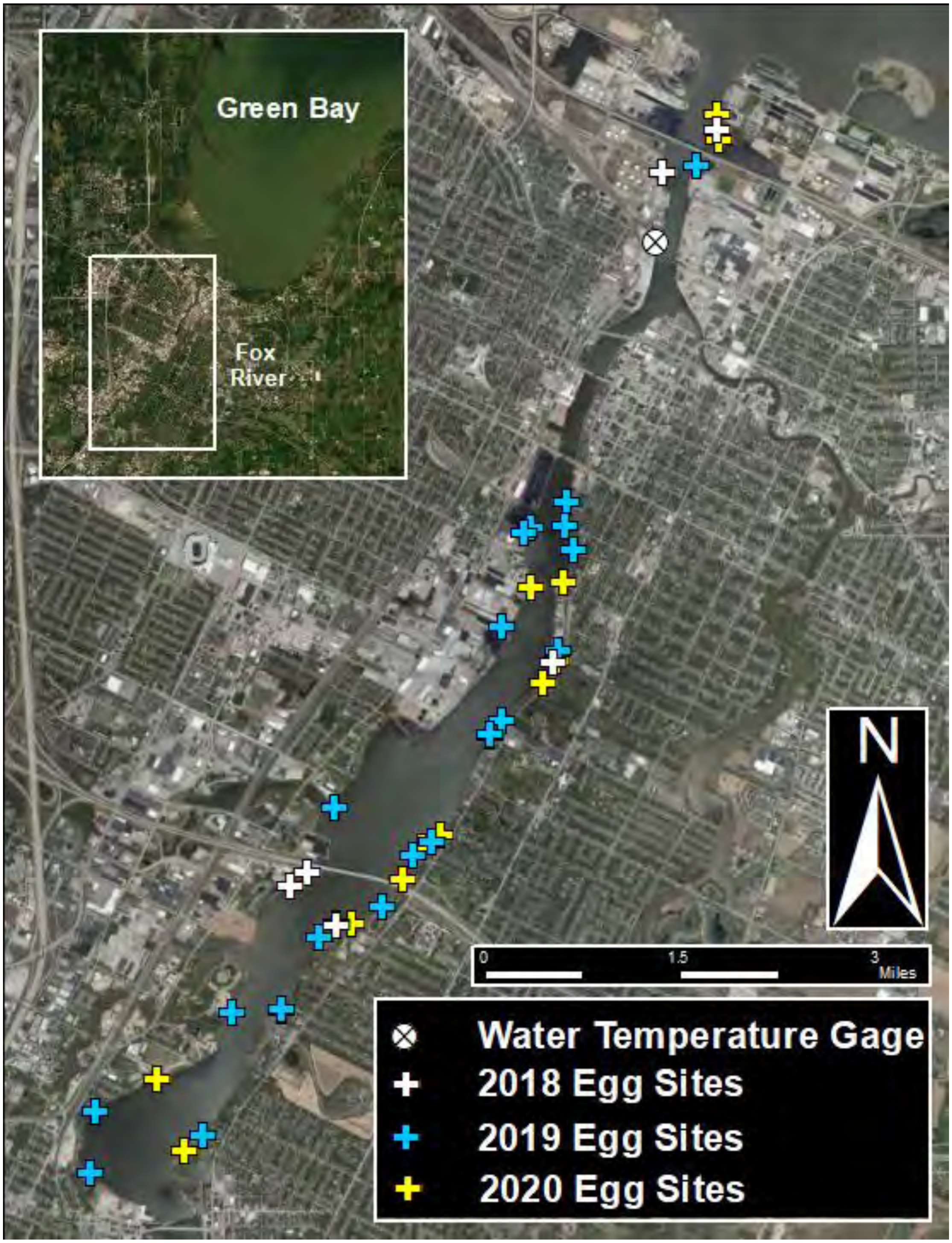


Figure 14: Map of all sites in the Fox River where Muskellunge eggs were collected between spring 2018 and spring 2020. The approximate location of the USGS water temperature gage is denoted by the white circle with the black "X."

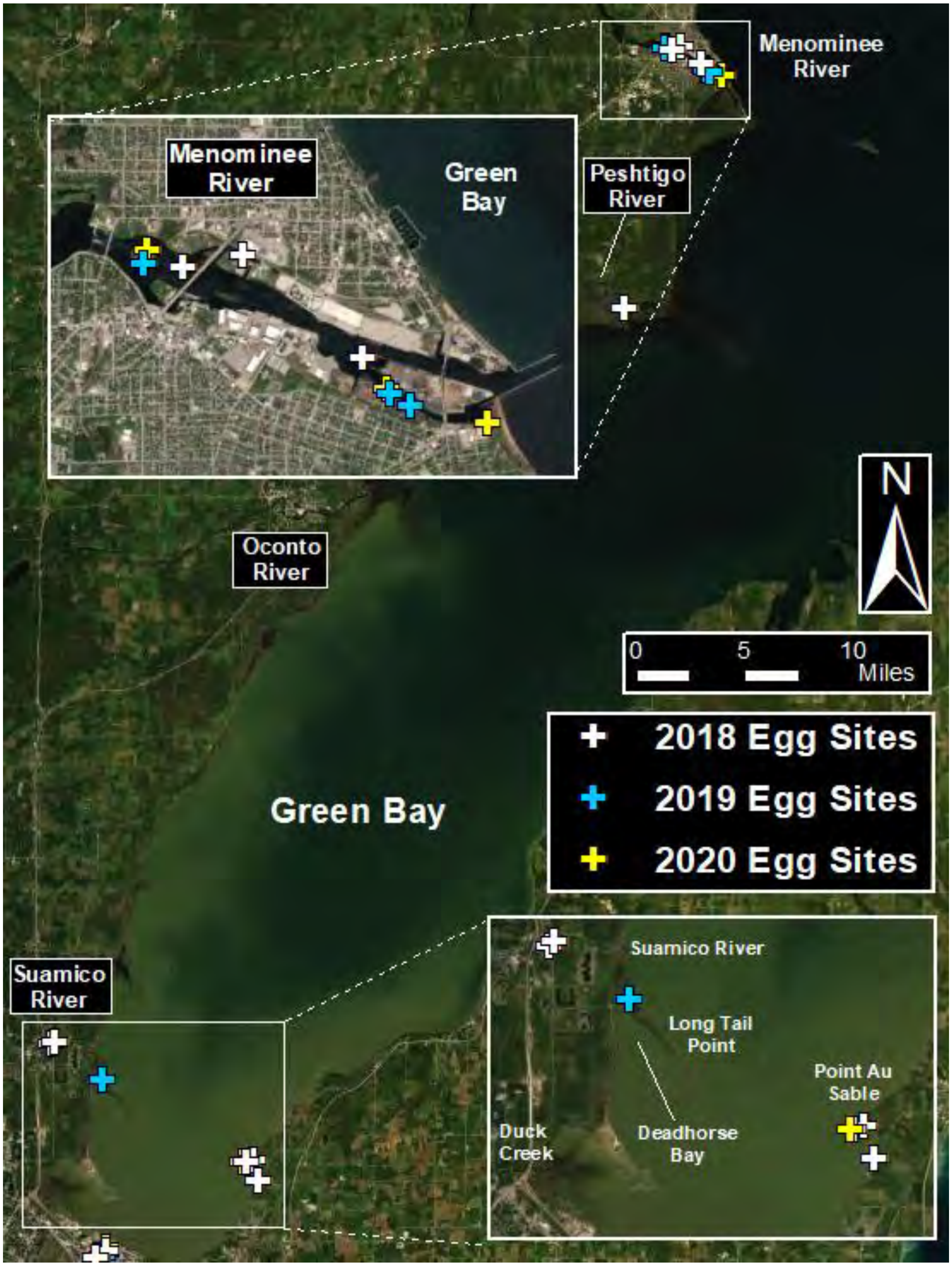


Figure 15: Map of sites outside of the Fox River where Muskellunge eggs were collected from spring 2018 through spring 2020.

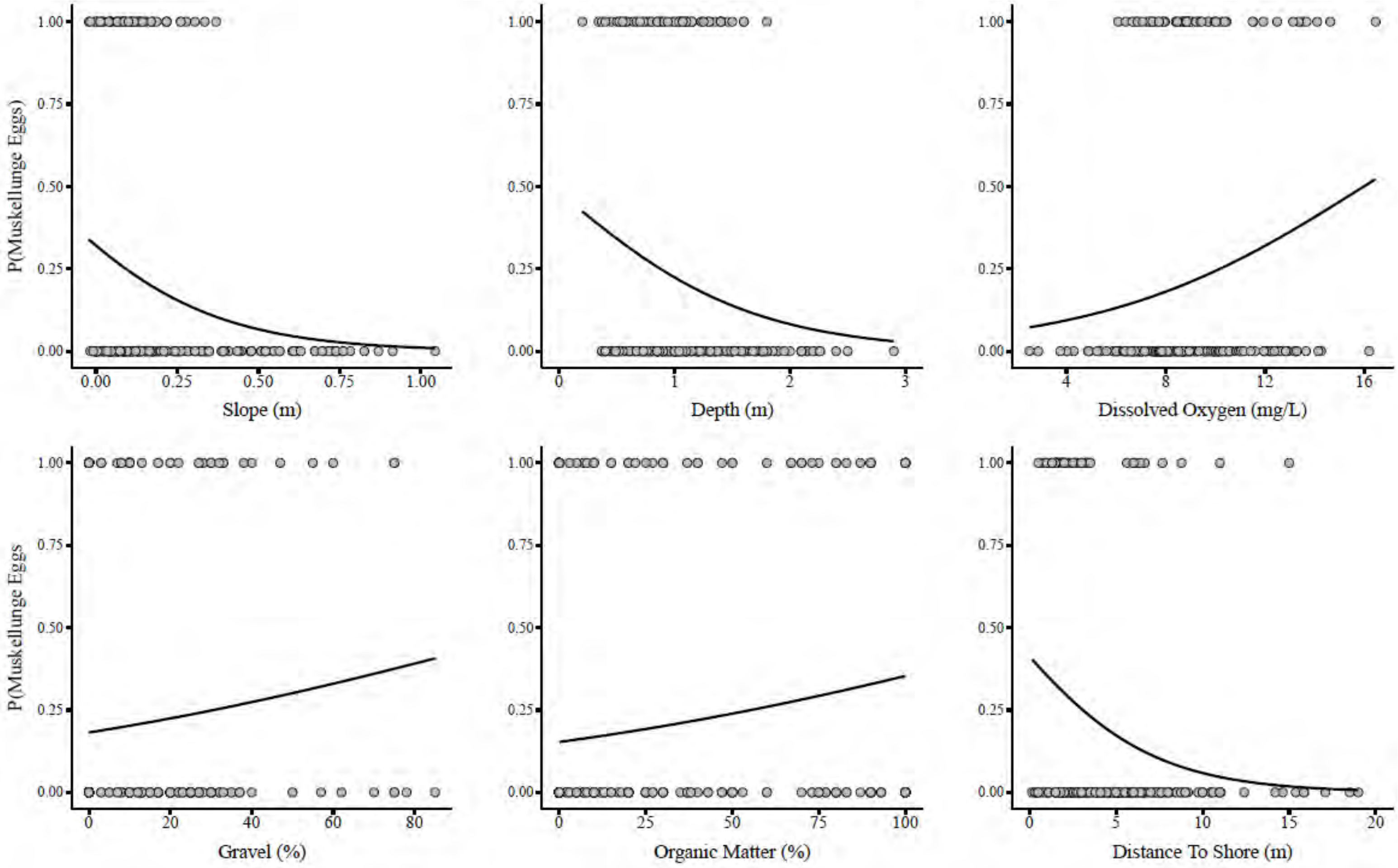


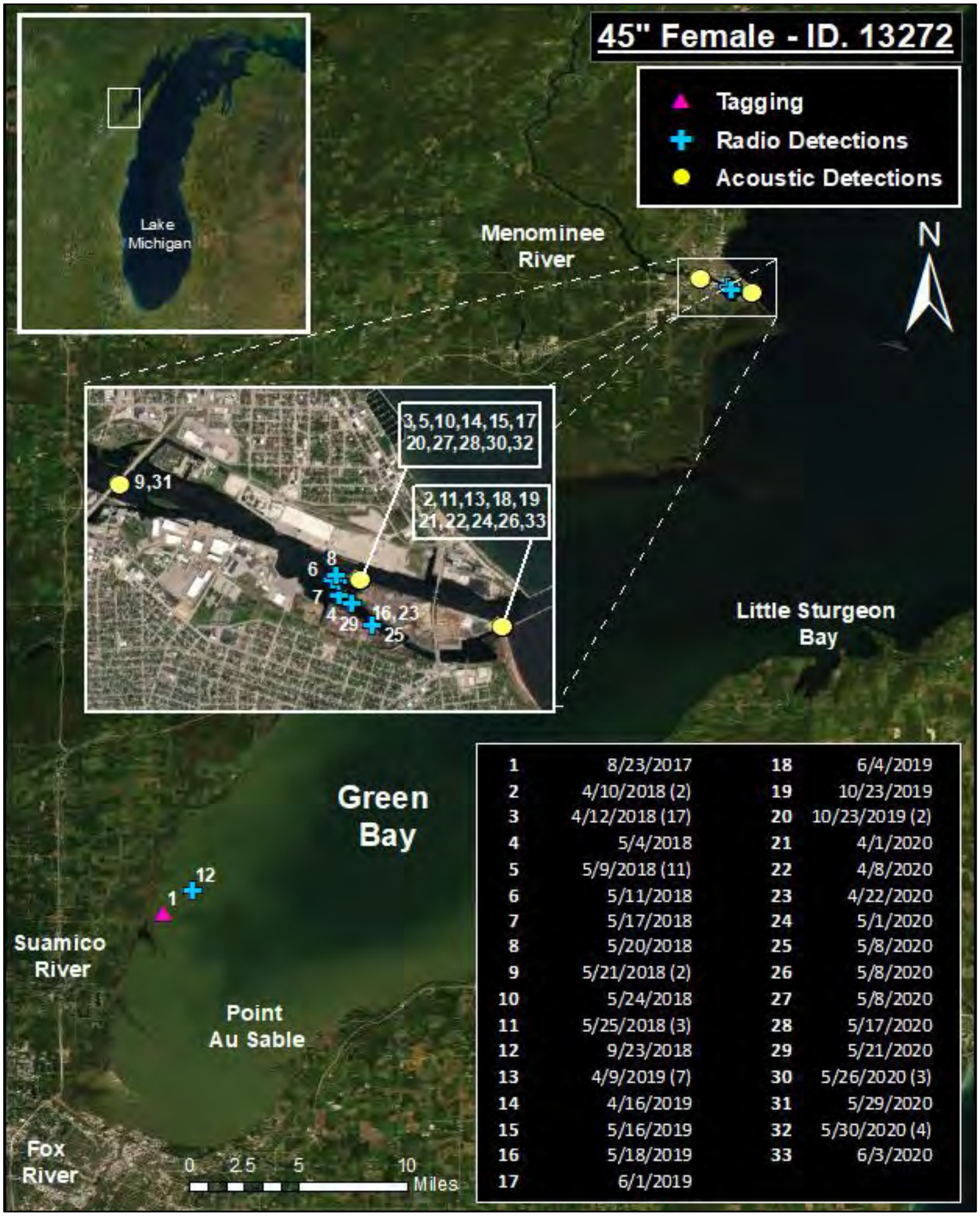
Figure 16: Univariate logistic regression plots of each predictor variable significantly influencing the probability Muskellunge eggs were present at a given location. No eggs were collected at sampling locations > 20 m from shore therefore those sampling sites (N = 17) were omitted on the distance to shore plot to improve clarity.

Figure 17: Map of suitable Muskellunge spawning habitat just downstream of Voyageur Park in the Fox River. Slope, depth, distance from shore, and substrate thresholds were met in these areas and thus they were considered suitable Muskellunge spawning habitat.

Figure 18: Map of suitable Muskellunge spawning habitat just downstream of the Highway 172 bridge in the Fox River. Slope, depth, distance from shore, and substrate thresholds were met in these areas and thus they were considered suitable Muskellunge spawning habitat.

Figure 19: Map of suitable Muskellunge spawning habitat in a back channel near the mouth of the Menominee River. Slope, depth, distance from shore, and substrate thresholds were met in these areas and thus they were considered suitable Muskellunge spawning habitat. This area was the presumed spawning site of Muskellunge with acoustic transmitter 13272 in the spring of 2018, 2019, and 2020.

Figure 20: Map of stocking locations of Muskellunge in Lower Green Bay (blue circles). Yellow crosses denote the locations where Muskellunge in my study with known stocking locations were originally stocked into Green Bay.



1	8/23/2017	18	6/4/2019
2	4/10/2018 (2)	19	10/23/2019
3	4/12/2018 (17)	20	10/23/2019 (2)
4	5/4/2018	21	4/1/2020
5	5/9/2018 (11)	22	4/8/2020
6	5/11/2018	23	4/22/2020
7	5/17/2018	24	5/1/2020
8	5/20/2018	25	5/8/2020
9	5/21/2018 (2)	26	5/8/2020
10	5/24/2018	27	5/8/2020
11	5/25/2018 (3)	28	5/17/2020
12	9/23/2018	29	5/21/2020
13	4/9/2019 (7)	30	5/26/2020 (3)
14	4/16/2019	31	5/29/2020
15	5/16/2019	32	5/30/2020 (4)
16	5/18/2019	33	6/3/2020
17	6/1/2019		

Figure 21: Map of all locations for Muskellunge with acoustic transmitter 13272 during my study. Acoustic detections are denoted by yellow circles, radio detections are denoted by blue crosses, and tagging is denoted by the pink triangle. Numbers in the legend correspond to the date of detection, and numbers in parentheses denote the number of continuous days detected by a given acoustic receiver.

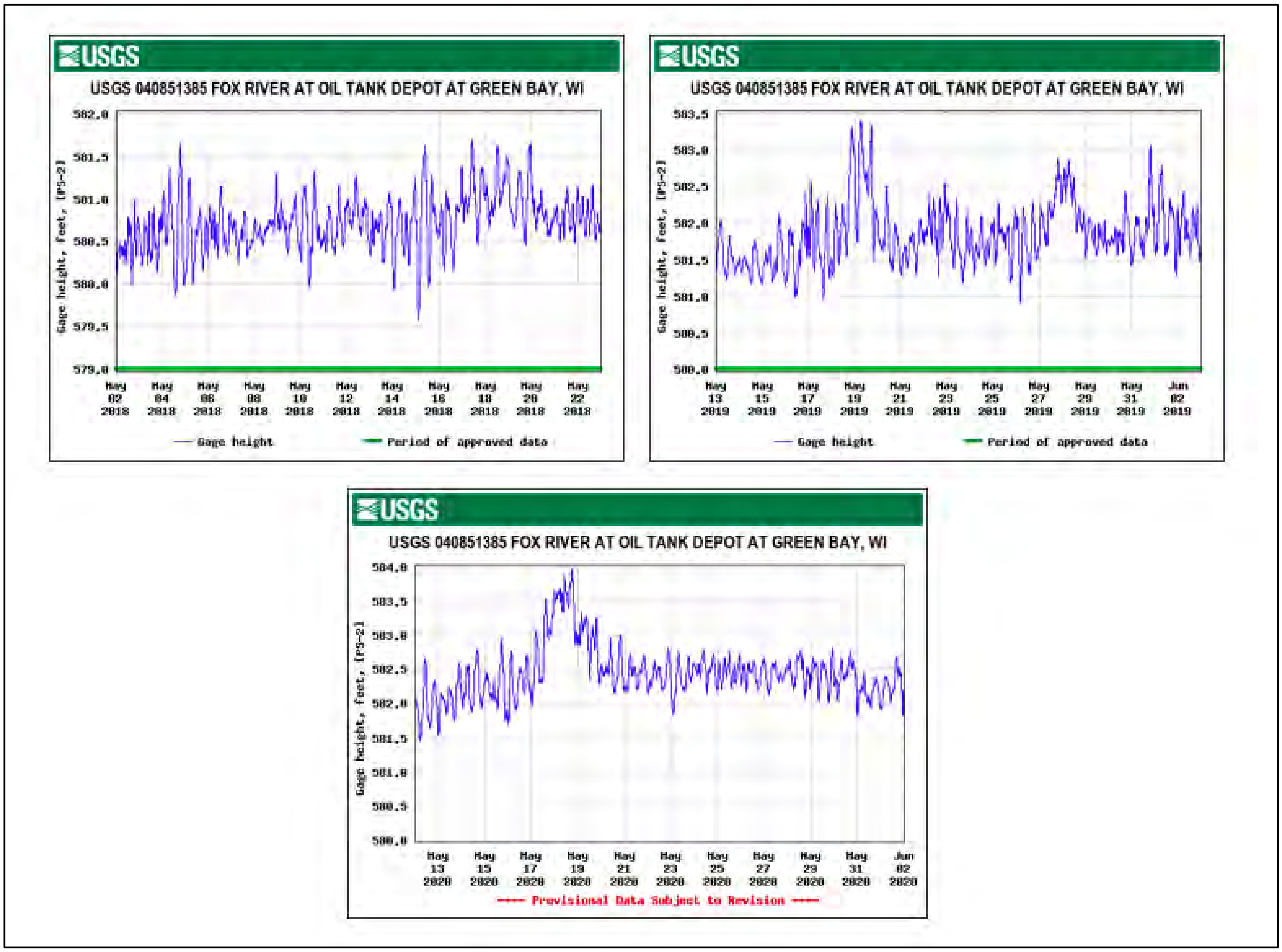


Figure 22: Fox River hydrograph during the 2018, 2019, and 2020 Muskellunge spawning periods (clockwise – from top left). USGS gage 040851385 is located approximately 1.5 miles upstream from the mouth of the Fox River and is denoted in figure 14.

Appendices

APPENDIX A.

Model	K	AIC	∆AIC
Depth + Slope + DS + DO + GS + OM	7	234.40	-
Slope + DS + DO + GS + OM	6	236.65	2.25
Depth + Slope + DS + DO + OM	6	238.98	2.33
Depth + Slope + DS + DO	5	244.48	5.5
Depth + Slope + DO + GS + DS	6	245.03	0.55
Slope + DO + DS	4	245.31	0.28
Depth + Slope + GS + DS + OM	6	245.91	0.6
Slope + DS + DO + GS	5	245.93	0.02
Depth + Slope + GS + DS	5	249.86	3.93
Depth + Slope + DS	4	250.92	1.06

Appendix A.1. Model combinations of variables significantly influencing the probability of Muskellunge egg presence and 10 lowest subsequent AIC scores. “DS” represents distance from shore (m), “DO” represents dissolved oxygen (mg/L), “GS” represents gravel substrate (%), and OM represents organic matter as substrate (%).