# ABSTRACT

Bowfin (*Amia calva*) ecology in the Great Lakes is poorly understood, despite their role as a predator in one of the most economically important fisheries in the United States. The goal of this study was to investigate Bowfin ecology in Green Bay, Lake Michigan, described in the context of a stochastic ecosystem with latitudinal and longitudinal hydrogeomorphic and environmental gradients. Migratory activity was characterized over six years (2014-2019) in a seasonally flooded inland wetland connected to Green Bay. Moderate rates of interannual return were documented in tagged fish, and migration movements were positively associated with wetland water temperature. Bowfin were also collected from seven coastal wetland sites within Green Bay in 2014 and 2015 to assess age, growth, mortality, and trophic niche space. Bowfin age estimates ranged from 0 to 19 years and growth and mortality estimates are similar to reported rates in other Midwest systems. Between reader precision of age estimation from two hard structures was high (Lapilli mean coefficient of variation [CV] = 7.88%; Sagittae CV = 7.00%) and estimates from the two structures were similar (CV = 13.22%). Stable isotope analysis indicated a large and flexible trophic niche, with fish undergoing an ontogenetic diet shift in their first year to adults. Our description of Bowfin natural history in Green Bay supports the historic characterization as a flexible, generalist predator capable of using diverse wetland habitats and resources.

# INTRODUCTION

Bowfin (*Amia calva*) are a primitive fish native to the Great Lakes that range throughout the Eastern United States and Canada, south to the Gulf of Mexico (Page et al., 1991). Mature Bowfin are sexually dimorphic, as males reach a shorter maximum length and have a defined ocellus (eyespot) at the base of their caudal peduncle. The Bowfin is a species of many common names (e.g., dogfish and mudfish) that reflect their reputed aggression and preference for turbid, wetland-like habitats (Koch et al., 2009a). Bowfin have also been described as “a severe competitor to more esteemed fishes” (Coker, 1930); however, recent findings indicate that their competitive impacts on sportfish are less than previously thought (Scarnecchia, 1992).

The reproductive ecology of Bowfin is not unlike many potamodromous fishes of the Great Lakes. Bowfin migrate into and spawn in shallow wetlands in the early spring when water temperatures reach between 16°C and 19°C (Scott and Crossman, 1973). Males clear vegetation or debris to create a nest in which females deposit eggs, and females partition their eggs across multiple males. It is the male Bowfin, however, that uniquely defends a territory around their nest until the eggs hatch and will continue to guard their school of fry for an additional month (Reighard, 1903). Young-of-year develop and grow quickly in wetlands, exceeding 100 mm in their first summer, at which time they become solitary (Reighard, 1903; Holland, 1964). Juvenile Bowfin swim poorly in current (Hoover and Killgore, 2002) and likely utilize vegetated habitat near the hatch site, but little is known about their ecology, partially due to poor detectability (Koch et al., 2009a). Inter-annual variation in the timing of reproductive activity, individual spawning behavior over repeated reproductive cycles and the potential for external cues to migration and spawning have not been evaluated.

Increases in Bowfin harvest for caviar production has increased the need for accurate quantification of population dynamics. Several hard structures have been used to estimate ages when modeling Bowfin growth, including scales (Mundahl et al., 1998), gular plates (Holland, 1964; Davis, 2006; Koch et al., 2009b), sectioned pectoral fin rays (Koch et al., 2009a; Koch et al., 2009b; and Porter et al., 2014), and otoliths (Koch et al., 2009b) with mixed success and inconsistency across regions. Reports of adult Bowfin age, growth, and mortality show a latitudinal gradient across the species’ natural range. Bowfin in southern populations showed faster growth early in life, matured earlier, and had higher mortality rates than Bowfin captured in the Upper Mississippi River (Davis, 2006; Koch et al., 2009a; Porter et al., 2014).

Bowfin were historically characterized as foraging on more “desirable” recreational species, but recent research highlights a generalist diet. Lagler and Hubbs (1940) described Bowfin as preying extensively upon game and pan fish, especially centrarchids, but also found that crayfish were an important component in some individuals’ diets. Likewise, Ashley and Rachels (1998) found that crayfish are a preferred prey item, followed by numerous centrarchid species. In laboratory-based experiments, Mundahl et al. (1998) reported that adult Bowfin preferentially preyed upon crayfish and cyprinids over centrarchids, potentially reflecting why Bowfin have historically not been an effective management tool for controlling stunted bluegill populations. A contemporary diet study combining stomach contents with stable isotope analysis of fish collected within the Lake Huron – Lake Erie corridor found that Bowfin act as a generalist and occupy a relatively large trophic niche at the population level; however, individual Bowfin specialize on specific prey types (Nawrocki et al., 2016). Patterns of Bowfin resource use in wetlands are variable, and likely affected by ontogeny and ecological setting.

Green Bay, Lake Michigan (Green Bay) stretches 190 km from the mouth of the Fox River of Wisconsin to the Bays de Noc in Michigan, transitioning from hypereutrophic in the lower bay to meso-oligotrophic in the deeper upper bay (Sager and Richman, 1991). Green Bay historically contained abundant wetlands that have been degraded and reduced, but still form a diverse system containing tributaries with backwater wetlands and fringing coastal wetlands that Bowfin may utilize (Scott and Crossman, 1973; Davis, 2006; Frieswyk and Zedler, 2007; Flood, 2015). Studies of Green Bay and Lake Michigan fish communities continue to note the presence of Bowfin (Wagner, 1972; Jude and Pappas, 1992; Brazner and Beals, 1997); however, no dedicated investigations of Bowfin ecology or dynamic rates (i.e. growth, mortality, and recruitment) have been conducted in Lake Michigan. Assessing the ecological role Bowfin play in Green Bay wetlands may provide valuable insight into coastal wetland ecology (Jude and Pappas, 1992; Brazner, 1997; Brazner and Beals, 1997; Lamberti et al., 2010).

This study seeks to provide a comprehensive description of Bowfin ecology in waters of Green Bay, Lake Michigan. Our first objective was to determine if patterns exist with respect to intra- and inter-annual migratory and spawning behavior by quantifying the association between migratory activity and environmental cues. We expected a relationship between water temperature and fish movement, and consistency in migration timing for individuals that returned to the wetland over multiple years. Second, we used various calcified structures to estimate age, assessed interpretability and precision of age estimates, and modeled growth and mortality. Pectoral fin rays were anticipated to provide more interpretable structures than the three pairs of otoliths, given their successful use in other systems (Koch et al., 2009b; Porter, 2014), but otoliths were expected to have greater precision. We expected growth and mortality to differ between sexes, with females reaching greater lengths and ages. Our third objective was to determine Bowfin diet composition and establish relative intrapopulation trophic characteristics using a combination of stomach contents and stable isotope analysis. We expected trophic ecology to differ based on Bowfin length and among wetlands due to the north-south trophic gradient of Green Bay and variability in wetland morphology and degradation.

# MATERIALS AND METHODS

**Study site.—**Seven coastal wetlands in Green Bay (Fig. 1) were sampled during the summer and early fall in 2014 and 2015, consisting, from south to north, of Point Sable, Dead Horse Bay, Pensaukee River, Little Sturgeon Bay, Peshtigo River, Cedar River, and Rapid River. Dead Horse Bay lacks a permanent tributary and has fringing wetland habitat with little submergent vegetation. Point Sable and Little Sturgeon Bay have small tributaries with drowned mouths. Pensaukee River and Cedar River have channelized mouths with riverine wetlands and fringing wetlands along the bay shore. Peshtigo River and Rapid River are less impacted than the other wetlands, and contain braided channels, riverine wetlands, multiple channels at their mouths, and fringing wetlands along the bay shore. Reproductive movements were assessed at a restored wetland that seasonally connects to Dead Horse Bay through a roadside ditch (Fig. 2).  
**Migratory movements.—**Migratory movements of Bowfin were investigated in the restored wetland during spring from 2014 to 2019 as incidental catch in a Northern Pike (*Esox lucius*) study. Adult Bowfin were captured entering and exiting the focal wetland using two fyke nets (0.9m x 1.8m frame) that were mounted to lateral fencing to create a complete impasse within the channel for the entire spawning season, beginning prior to Northern Pike movements that occurred weeks before Bowfin began moving.. Nets were checked daily and fish were released in the direction of travel; nets were checked before leaving to ensure that no fish immediately re-entered. Field processing consisted of determining sex by gamete expression or dimorphic coloration, measuring total length (TL; mm), and tagging Bowfin at the base of the dorsal fin using numbered FD-94 Floy T-bar tags. Water temperature was recorded hourly using a data logger placed in the middle of the wetland channel before the onset of migration. Data from 2018 was removed from analysis due to an unexpected snowfall in April that blocked fish movement.

A generalized linear mixed model using the GLIMMIX procedure in SAS (SAS Institute, 2013) was used to evaluate the association between the total number of Bowfin captured each day and water temperature at the time of capture (Forsythe et al., 2012). Migratory activity was modeled as a Poisson random process due to a strong positive skew attributed to many days with zero captures. Temporal autocorrelation in daily counts of migratory fish was accounted for using a low-rank time-based smoothing algorithm. Water temperature and Julian Day were the fixed explanatory variables of interest and year was treated as a random effect. Changes in water temperature (i.e., “lagged” effects) can also be a predictor of migratory activity for some Great Lakes primitive fish (lake sturgeon; Forsythe et al., 2012); thus, we also explored water temperature lags of 1 and 2 days prior to the observed catch. The best fitting model was determined using the lowest calculated AIC (Akaike, 1974).

**Coastal wetland sampling.—**Seven coastal wetland complexes were sampled in late spring and summer 2014 and 2015 using fyke nets and boat electrofishing (Schoen et al., 2016). Two short and two tall fyke nets (0.5m x 1.0m and 1.0m x 1.0m frame) were fished at depths where the frame was fully submerged but approximately even with the water surface. Fyke nets were set with a 20m lead extending perpendicular from shore approximately one hour before sunset and checked the following morning. Boat electrofishing was conducted in areas within the wetlands less than 2m deep and power was standardized by water conductivity (Miranda, 2009). Bowfin were put on ice at capture and processed at the laboratory; total length (TL, mm), weight (W, g), and sex were recorded for each fish, and otoliths, pectoral fin rays, stomachs, and tissue samples were collected.

Sagittal otoliths were collected in 2014 and 2015 for aging, growth estimation and microchemical analysis. To compare aging structures, all three pairs of otoliths (sagittae, lapilli, and asteriscii) and pectoral fin rays were collected in 2015 (see Supporting Information). Otoliths were mounted in EpoFix epoxy and sectioned (0.750 mm in width) using a low-speed Isometric Saw (PACE Technologies PICO155). Otolith cross sections were sanded, polished, and photographed for analysis. Fin rays were removed from interradial membranes and encapsulated in epoxy following the guidelines in Koch and Quist (2007). Fin rays were sectioned from the proximal end (0.750mm thick) and photographed.

***Estimating age, growth, and mortality.—***A weight-length relationship was derived using a least squares regression for pooled fish of both sexes captured from all gear types in all wetlands: log10*W* = log10α + βlog10*TL*

Parameter values and associated confidence intervals for *α* and *β* were estimated along with a coefficient of determination (*r2*).

Otoliths were mounted in EpoFix epoxy and a 0.750 mm section was cut along the transverse plane using a PACE Technologies PICO155 Precision cutter. Otolith cross-sections were sanded to expose the primordium, polished, and photographed using an AmScope MU300 Microscope Digital Camera. Two readers independently evaluated photographs and marked annuli using FishBC. Consensus annuli (annuli that both readers marked) from lapilli were used to back-calculate length-at-age to estimate growth.

Since fish were sampled over the entirety of a summer while growth could continuously occur, back-calculated lengths at age were used to determine length at age relationships. Length at age was estimated using the direct proportion method, which assumes otolith growth is directly proportional to growth in total length throughout the life of the fish (Schramm et al., 1992; DeVries and Frie, 1996; Koch et al., 2009b), and is calculated as:

where is the total length of the fish at a previous age *a*, is the total length of the fish at capture, is the annular radius (distance from the otolith core to the annulus) for previous age *a*, is the capture radius (distance from the otolith core to the outer edge of the otolith, along the same transect as ). Estimated length-at-age tables were created for all consensus individuals and mean length-at-age was calculated separately for each sex. Due to a limited sample size, growth will be presented as the mean length at age 2 for males and age 3 for females, which is the age at which each sex would reach maturity (Koch et al. 2009).

Instantaneous natural mortality (*M*) was determined using the estimator published by Then et al. (2015) following the model recommendation for data-poor stocks that relies on maximum age (*t*max), calculated for this study using age estimates from sagittal otoliths, where *M* = 4.899*t*max-0.916. Exploitation estimates for Bowfin within Green Bay are not available so a conditional natural mortality rate (*n*; mortality that occurs in the absence of fishing)was calculated for males and females separately assuming a type 2 fishery (i.e. fishing and natural mortality act concurrently) where *n* = 1-*e*-*M* (Ricker, 1975).

## **Trophic analysis.—**

Wetland residency was determined using otolith microchemistry. Sagittal otoliths were mounted in epoxy, polished, and analyzed for trace elements using laser ablation inductively couple plasma mass spectrometry at the Center for Elemental and Isotopic Analysis at Central Michigan University following methods detailed by Schoen et al. (2016). Ablation transects spanned from the otolith core to edge, however due to the shape of a Bowfin’s sagittal otolith the transect did not run perpendicular to all annuli. Trace element concentrations of barium (137Ba), calcium (43Ca), copper (65Cu), lead (208Pb), magnesium (25Mg), manganese (55Mn), strontium (88Sr) and zinc (66Zn) isotopes were reported as a ratio against Ca.

A random forest model was used to classify Bowfin to their wetland of residence using trace element ratios of the outer 8 microns of the ablation transect, such that high classification success would reflect an identifiable microchemistry signature of the wetlands in which the fish were captured (Breiman, 2001; Hansen et al., 2015; Watson et al., 2018). Random forest modeling was selected because it can identify nonlinear relationships and is internally validated through out-of-bag error estimation (Breiman, 2001; Liaw and Weiner, 2002). Only sites with a total number of 10 replicate fish, Pensaukee and Peshtigo, were analyzed in the random forest procedure for effective bootstrapping (Chernick, 1999). Computations were performed in the R statistical environment (R Core Team, 2020) using the ‘randomForest’ package (Liaw and Wiener, 2002). All models consisted of 20,000 trees and an *m* value of 2 while initial models were trained with all *p* predictor parameters. Variable importance for each predictor (i.e., trace element) was described by the raw importance values, which is the mean decrease in classification accuracy when that predictor is permuted across all trees while holding other predictors constant (Liaw and Wiener, 2002). Trace element ratios found to negatively influence model accuracy were iteratively removed until a best performing model was reached. The predictive function within the random forest was used from the best-fit model to assign previous site use classifications for all Peshtigo and Pensaukee adults to the outer 10% of each ablation.

Isotopic signatures were used to determine trophic niche. Samples of muscle tissue for stable isotope analysis were collected dorsally of the lateral line and approximately 25mm posterior of the cleithrum. Isotopic signatures of YOY and YAO fish were used to identify ontogenetic shifts in trophic position, trophic niche size, and trophic niche overlap between life stages. Due to sample size limitations, Peshtigo was excluded from further analysis and we only examined Bowfin resident to Pensaukee wetland during the previous growing season, as classified using otolith microchemistry. Only fish with greater than 75% predicted site classification to Pensaukee in the outer 10% of their ablation path from the best fit random forest model were included in this analysis. Since isotopic turnover time for muscle tissue is approximately 1-3 months (Boecklen et al., 2011), retained individuals’ isotopic signatures should reflect prey consumed in the Pensaukee wetland.

Muscle tissue samples were placed in pre-ashed vials, dried at 60℃, then ground into a fine powder. Samples were analyzed for N and C isotope ratios using a Thermo-Finnegan isotope ratio mass spectrometer with atmospheric air and PeeDee belemnite as standards for nitrogen and carbon, respectively. Ratios of δ13C indicate basal energy source and can illustrate a gradient from wetland to nearshore production, as differences in biological processes and carbon availability cause wetland primary producers to be more depleted in δ13C than those in associated nearshore areas (Zieman et al., 1984; Keough et al., 1998; Turschak et al., 2014).

Trophic position can be described in relation to δ15N, with enrichment by 3-4‰ for each increase of one trophic position (Vander Zanden et al., 2001). Trophic position was calculated for YAO Bowfin relative to YOY Bowfin using a Bayesian framework in the ‘tRophicPosition’ package using the equation:

δ15NYAO = δ15NYOY + ΔN (TP-λ)

where δ15NAdults is adult Bowfin δ15N value, δ15NYOY is the YOY Bowfin δ15N value, ΔN is the trophic discrimination factor for nitrogen (3.4 ± 0.98; Post, 2002), TP is the trophic position of adult Bowfin, and λ is the trophic position of the baseline (Quezada-Romegialli et al., 2018). In this instance λ is set equal to 0, so that our adult trophic position is calculated relative to a YOY Bowfin baseline. Model chains were analyzed for convergence using Gelman and Rubin’s diagnostic approach that compares within-chain and between chain variance (1992). Models were created with 3 chains and 20,000 adaptive iterations with uninformed priors and the mode was used to describe central tendency of parameter estimates. Isospace overlap between YOY and YAO Bowfin was described using the probabilistic method outlined by Swanson et al. (2015) in the ‘nicheRover’ package (Lysy et al., 2014). Trophic niche region, NR,is defined in this study relative to the smallest bivariate elliptical space of the δ13C and δ15N ratios in which a randomly selected individual has a 95% probability of occurring. Ellipse size, overlap and uncertainty were determined using 10,000 iterations of a Bayesian framework with uninformed priors. Overlap was determined directionally where estimates represent the probability that an individual from life stage A will be found in the 95% niche region, NR, of life stage B (Swanson et al., 2015; Kornis et al., 2020).

# RESULTS

***Migratory movements.—***A total of 136 individuals were captured and tagged during spawning migrations from 2014 through 2019. Total annual sex ratios varied from 1.17 to 2.33 males per female (Table 1). On average, female Bowfin were larger than males with a mean total length of 630.4 mm (SD ± 51.8 mm) compared to male total mean length of 530.5 mm (SD ± 84.86 mm). A total of 83 adult Bowfin were only captured once during our study, 40 Bowfin were recaptured within the year they were tagged (moving in and out of the wetland during active migration) and 13 individuals were recaptured in multiple years during the study (Table 2). A female (tag 448) returned to the wetland in three consecutive years and its first arrival date fell within a range of 6 days (Table 2) across all years. Three individual nests, actively guarded by males and containing deposited eggs, were observed in 2019.

Migratory activity from 2014 through 2019 was initiated when water temperatures were between 7.1°C and 13.3°C. The earliest record of migratory activity occurred on March 30 in 2016 and spawning arrival dates varied interannually and related to warming water temperatures (Fig. 3). The onset of migration did not appear to be sex based, as males and females generally arrived at the wetland channel within a few days of each other. The best fitting GLIMMIX model (based on AIC) using the full suite of capture information collected (2014-2017; 2019) included Julian day (β = -0.020, SE = 0.045, F1,98 =0.20, P = 0.656) and water temperature 24 hours prior to the sampling date (β = 0.230, SE = 0.027, F1,136 = 70.06, P < 0.0001). The number of adult male and female Bowfin entering the wetland for spawning increased significantly during days or periods of warming water temperature. The dispersion parameter of this model, estimated by Pearson's chi-square statistic divided by its degrees of freedom, was 1.98 suggesting a small amount of overdispersion but overall strong fit to the observed data.

***Coastal wetland sampling.—***Coastal wetland sampling yielded a total of 58 Bowfin from the seven focal sites in 2014 (N = 21) and 2015 (N = 37). Bowfin were found in all focal coastal wetlands in 2014 but were only collected in the five lower and middle bay sites in 2015 (Table 3). No individuals were captured in the nearshore habitat in either year. Total length ranged from 129 mm to 716 mm, and females were consistently larger than males (Fig. 4).

***Estimating age, growth, and mortality.—***The weight-length regression was significant for both the α and β parameters (F1,57 = 14745, p < 0.001, r2 = 0.996). The β estimate of 2.937 (2.888 – 2.985; 95% confidence intervals) showed a slightly negative allometric relationship and can be expressed as log10*W* = log10(-4.800) + (2.937) log10*TL*. Age at capture of Bowfin (based upon sagittal otolith estimates, which was the only structure for all individuals) ranged between YOY and 19 years; YOY and age-1 were the two most frequently sampled age classes. The three oldest fish collected during this study were all males.

Sagittae and lapilli otoliths were the most useful structures for age estimation. Astericii and pectoral fin rays did not display interpretable annuli, despite using analytical methods that were successful with fin rays in other systems. Lapilli provided a higher initial agreement percentage than sagittae (69.23% and 60.61% respectively), reader agreement within one year was high for both (96.15% and 96.97% respectively), and the mean coefficient of variation (CV) was low for both (7.88% and 7.00%, respectively). For cases of disagreement, the average age discrepancy was similar for both structures (1.13 years for lapilli, 1.08 years for sagittae). Consensus age estimates were consistent between lapilli and sagittae for individual fish. Exact agreement between consensus ages of lapilli and sagittae, of the 26 individuals for which both structures were collected, was 46.15%, agreement within one year was 84.62%, and mean CV was 13.22%. The greatest difference in age estimation was five years, where an individual was estimated to be 12 from lapilli and 7 from sagittae; all other discrepancies were 2 years or less. Lapilli were more precise than other calcified structures and provided a linear path along which back-calculated length at age could be estimated (Fig. 5), but they did underestimate more individuals over 10 years old relative to sagittae.

A total of 24 consensus individuals (male = 14; female = 10) with back calculated lengths at age (estimated using lapilli) were included in the hierarchical growth model estimates. The mean length of a female at age 3 was 474 mm (± 42 mm, SD) based on estimates from 8 individuals captured at three sites (Deadhorse Bay, Pensaukee, and Peshtigo), and the mean length of a male at age 2 was 350 mm (± 54 mm, SD) based on estimates from 5 individuals captured at three sites (Pensaukee, Peshtigo and Little Sturgeon Bay).

Instantaneous natural mortality (*M*) was estimated to be 0.3295 for males and 0.5447 for females, using the oldest estimated individuals (sagittae) for each sex (male =19 years old, female = 11 years old). These instantaneous rates are equal to conditional natural mortality (*n*) rates of 28.1% for males and 42.0% for females.

***Diet and Trophic analysis.—***

A total of 39 individuals (15 from Peshtigo and 24 from Pensaukee) were included in the random forest model to assess the degree of residency and probability of wetland reassignment. The final model, consisting of five trace element predictors, achieved a 79.5% success rate in assigning fish to the wetlands in which they were captured. Fish captured in the Pensaukee Wetland were successfully reclassified at a higher rate (83.3%) than fish captured in Peshtigo Wetland (73.3%). The most important trace element predictors were 88Sr and 137Ba (mean decrease in accuracy: 12.1% and 78.6%), followed by 55Mn, 25Mg, and 65Cu (mean decrease in accuracy: 35.1%, 18.9%, and 17.0%).

Due to poor classification and insufficient sample size of Bowfin assigned to their wetland of capture, Peshtigo was excluded from stable isotope analysis. One adult was removed from stable isotope analysis, as it only assigned to Pensaukee in 5% of predicted classifications, resulting in 12 YOY and 10 YAO individuals with isotope data for analysis. YAO Bowfin had a mode trophic position 0.945 (95% credible interval: 0.36-1.47) steps above YOY individuals. The niche region of YAO Bowfin was slightly larger (33.2± 10.5‰) than that of YOY Bowfin (25.8± 7.6‰). Niche overlap was moderate between life stages, YOY Bowfin had a 54% probability of occurring in the YAO niche (95% credible interval: 17% - 92%), while YAO Bowfin had a 33% probability of occurring in the YOY niche (95% credible interval: 10% – 69%; Fig. 9).

**DISCUSSION**

Our monitoring efforts over 6 years suggest that adult Bowfin use and occasionally return to the same inland wetland for nest construction and spawning activity. Bowfin migrated from Green Bay into this seasonal wetland in response to thermal cues, which varied interannually with environmental conditions. Bowfin, like other primitive fishes (e.g., sturgeon and gar), show seasonal trends in spawning movements and repeatability in spawning behavior as a function of environmental cues (Johnson and Noltie, 1996; Forsythe et al., 2012). This seasonal wetland, and its tributary network, fills from snowmelt and precipitation, causing discharge and water temperature to fluctuate in response to local climate conditions. Should future climate change affect temperature or precipitation patterns, adaptive plasticity will be necessary for opportunistic Bowfin spawning migrations.

Our sampling efforts collected Bowfin of a wide range of ages, and Bowfin were present in all seven wetlands around Green Bay, Lake Michigan; however, differences among wetlands impacted sampling effectiveness. Rapid River, Cedar River, and Little Sturgeon Bay had extensive vegetated areas that may have contained Bowfin but were too deep to electrofish and too far from shore to sample with fyke nets. Other sites may have been too small to support a large enough resident population for modeling (Point Sable) or had small, disjunct areas of quality habitat that may impact sampling efficiency or resident population size (Dead Horse Bay). We collected enough adults in only two of the wetlands (Peshtigo River and Pensaukee River) to analyze wetland residency relative to wetland of capture within the current growing season (approximately three months prior to capture) using otolith microchemistry. Bowfin from these wetlands showed distinct microchemistry signatures that indicated these individuals exhibited limited migration in the growing season prior to capture, similar to Oele et al (2015), who successfully delineated Northern Pike otolith microchemistry signatures at a watershed scale in Green Bay. The degree of site misclassification in this study could be a function of fish movement, but results may be confounded by wind-driven seiche events that circulate water from nearshore areas into coastal wetlands, temporarily mixing chemical signatures of the wetland and nearshore habitats.

Stable isotope analysis from Pensaukee River indicated an increase in trophic position with ontogeny. YAO Bowfin generally consumed prey one step higher in trophic position than YOY individuals, likely due to larger gape size and increased metabolic demand. YAO Bowfin also consumed a greater diversity of prey, so their niche space was larger than that of younger fish. Trophic niche regions for both YOY and YAO Bowfin were larger than those observed in Bowfin collected from Lake Huron-Erie Corridor wetlands in the spring and fall (Nawrocki et al., 2016), although this may be influenced by our smaller sample size. Overlap of almost half of the YOY niche region with the YAO niche region indicates that older Bowfin retained some ability to utilize the varied resources on which they relied in early life stages. Previous work in the Great Lakes has found larger trophic niches for Bowfin than other native species including Walleye, Northern Pike, Longnose Gar, and Muskellunge (Nawrocki, 2015). Diet plasticity at all life stages and generalist consumption at the population scale should allow Bowfin to persist in degraded habitats and possibly provide stability to local food webs.

Lackmann (2022) showed that using otoliths may result in estimates of much older populations

Bowfin resident to Green Bay grew at similar rates to Bowfin from other systems, despite likely differences in productivity, forage base, and growing season. For example, based on sex specific von Bertalanffy equations, male Bowfin from the Upper Mississippi River would be approximately 313 mm at age 2 compared with the estimated 350 mm age 2 bowfin, while females in the same system would be 400 mm at age 3 compared to the green bay estimate of 474 mm; however, Bowfin in the Upper Mississippi River did reach greater observed lengths (Koch et al., 2009a). Bowfin captured in the waters of Virginia were also observed with a greater maximum length, though both studies had small sample sizes of fish estimated to be older than age-6. First year growth of Bowfin in Louisiana (Davis 2006) was greater than that exhibited in Green Bay, but Green Bay fish grew faster in the ensuing three years. Bowfin collected in Lake Lindsay Grace, a small inland lake in Georgia, were shorter lived than other populations, but their growth to age-5 appears similar to Green Bay (Porter et al., 2014). Additionally, weight coefficient (β) for Green Bay Bowfin was similar to those from Michigan (β = 2.960; Schneider et al., 2000), indicating similar consumption efficiency across lengths and regions.

Our estimates of conditional natural mortality (*n*=28.1% – 42.0%) encompassed the estimated total annual mortality rate in the commercially-exploited Upper Mississippi River (*A*=35%; Koch et al., 2009) but were lower than estimates from populations in Louisiana (*A*=58%; Davis, 2006) and a South Georgia reservoir (*A*=68%; Porter et al., 2014). Exploitation rates of Bowfin in Green Bay are believed to be low, given limited encounters in creel surveys (Paoli pers. comm.), and if no exploitation exists our estimate of *n* would also represent *A*. Our research supports previously documented latitudinal gradients in fish population dynamics, in that northern populations display lower mortality rates than southern populations (Ebert et al., 1999; Braaten and Guy, 2002; Heibo et al., 2005). Sex specific differences in mortality were also identified by Koch et al. (2009); however, males experienced higher mortality in that system despite targeted harvest of gravid females. Two factors may affect the relatively greater female mortality rate found in this study: the aging structure used, which may improve interpretation of outer annuli, and statistical methodology, as the Then et al. (2015) estimator relies only on maximum age to estimate *M*. Hoenig (1983) noted that maximum age depends on the number of animals in the sample, and this study lacked the sample size of approximately 200 fish that Kritzer *et al.* (2001) demonstrated may be sufficient for precisely estimating population-level parameters; however, the Then et al. (2015) recommended method for data-poor stocks should provide a useable estimate for the ceiling of *M* in Green Bay Bowfin.

This study is the first to paint a broad picture of Bowfin natural history and ecology in the waters of Green Bay. Bowfin returned annually to spawn at a recently restored wetland, responding to warming water temperatures, however the recruitment dynamics of the population remain unknown. Growth, mortality, and niche were similar between Bowfin in Green Bay and stable populations elsewhere in North America, highlighting their adaptability to a variety of systems and habitat conditions. In Green Bay, wetland restoration targeting Northern Pike recruitment may also benefit Bowfin, as both native predators use similar spawning habitats. Across the Great Lakes region, however, climate change will likely impact timing of spawning migrations and success of egg incubation, and habitat degradation will impact wetland-resident predatory fish. The ecological role of Bowfin may lend some stability to wetland food webs (Scarnecchia, 1992; Nawrocki et al., 2015), and if these important habitats continue to decline in the future, Bowfin may still persist as a flexible, generalist predator.

# DATA ACCESSIBILITY

Supplemental material is available at and <https://www.ichthyologyandherpetology.org/XXX>. Data and code are available upon request and at <https://github.com/jshrov/Bowfin_Ecology_in_Green_Bay>

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# FIGURE CAPTIONS

**Fig. 1.** Map showing Malchow Wetland sampling location and the hydrologic connection to Green Bay, Lake Michigan fish use for migration.

**Fig. 2.** Map of Green Bay, Lake Michigan wetlands sampled during summers of 2014 and 2015.

**Fig. 3.** Migratory activity and environmental conditions in Malchow Wetland. Total counts of Bowfin (*Amia calva*) moving in and out of the wetland are shown as points overlaying model predictions and 95% confidence intervals (represented as a solid black line and gray band). Daily average water temperature of the outflowing channel is shown by a dashed line.

**Fig. 4.** Length frequency graph by sex of all Bowfin (*Amia calva*) captured from 2014-2019 in wetlands of Green Bay, Lake Michigan.

**Fig. 5.** Images of otolith cross-sections used to estimate age taken under a dissecting scope (panes A and B) and phase contrast microscope (C and D). Both readers had the same age estimate (6 years) for sagittae (A and C) and lapilli (B and D) from this Bowfin, which was collected from Dead Horse Bay in 2015.

**Fig. 6.** Gompertz growth curves for back-calculated length-at-age estimates of male and female Bowfin (*Amia calva*). Back-calculated length-at-age estimates are shown as a blue “X” for males and red circle for females. Dashed lines represent the mean of individual growth curves, stratified by sex. Bowfin were collected in summers of 2014 and 2015 from wetlands around Green Bay, Lake Michigan.

**Fig. 7.** Density plots illustrating the distribution of A) δ15N and D) δ13C stable isotope ratios for adult (yearling-and-older) and young-of-the-year Bowfin (*Amia calva*) collected in Pensaukee River. C) Raw data in two-dimensional isospace was used to inform B) elliptical projections of trophic niche regions. For each age class, the ten ellipses in panel B have a 95% probability of containing the isotopic signature of a randomly-selected individual from panel C.