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OPTIMIZING UNDERWATER CAMERA SAMPLING TO ASSESS OVERWINTERING BACKWATER FISH HABITAT ON THE UPPER MISSISSIPPI RIVER

A Chapter Style Thesis Paper Submitted in Partial Fulfillment of the Requirements
for the Degree of Biology – MS: Aquatic Science Concentration

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College of Science and Health

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OPTIMIZING UNDERWATER CAMERA SAMPLING TO ASSESS OVERWINTERING BACKWATER FISH HABITAT ON THE UPPER MISSISSIPPI RIVER

By Benjamin Patschull

We recommend acceptance of this thesis paper in partial fulfillment of the candidate's requirements for the degree of Biology – MS: Aquatic Science Concentration

The candidate has completed the oral defense of the thesis paper

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ABSTRACT

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Gear limitations imposed by ice conditions in temperate regions have created a significant knowledge gap regarding the winter habitat use of the backwater fish assemblage. We developed and optimized an underwater camera method for under – ice sampling in four freshwater backwaters on the Upper Mississippi River. We found that site depth ($\Sigma w_i = 1.0$), water clarity ($\Sigma w_i = 0.99$), snow depth ($\Sigma w_i = 0.89$), and ice depth ($\Sigma w_i = 0.86$) were the main factors influencing camera viewing distance. Rarefaction analysis showed 21 sampling sites per backwater and 15 – minute recordings sufficiently captured species richness and relative abundance. Using this optimized underwater camera method, we evaluated the effect of environmental factors on fish assemblage metrics (i.e., species richness and combined MaxN) and species – specific data for Bluegill, Largemouth Bass, and Yellow Perch. Random forest models ranked water temperature (°C) and dissolved oxygen (mg/L) as the primary factors influencing the fish assemblage metrics and the centrarchid presence and relative abundance. Yellow Perch presence and relative abundance were driven by site depth (m) and conductivity (µS), respectively. The results of this study can be used by managers to guide restoration strategies incorporating environmental factors that promote quality overwintering habitat for backwater fishes.

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CHAPTER I

OPTIMIZATION OF AN UNDERWATER CAMERA SAMPLING METHOD FOR FRESHWATER FISHERIES

M.S. Research Thesis

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ABSTRACT

Ice cover restricts our understanding of the habitat use and assemblage structure of fishes in temperate ecosystems by preventing the use of conventional sampling methods. Although underwater camera surveys have been used extensively in marine ecosystems, standardized methods are not available for freshwater systems due to decreased water clarity and lack of institutional knowledge. We developed a novel camera method for sampling fish assemblages in four backwaters on the Upper Mississippi River. To assess the effectiveness of cameras in diverse aquatic environments, water clarity (cm), aquatic vegetation cover, snow depth (cm), water depth (m), sky cover, and ice depth (cm) were measured at randomly selected sampling points throughout each backwater (*n* = 21 each). We found that site depth ($\Sigma w_i = 1.0$), water clarity ($\Sigma w_i = 0.99$), snow depth ($\Sigma w_i = 0.89$), and ice depth ($\Sigma w_i = 0.86$) strongly influenced camera viewing distance. We optimized the number of sampling sites and recording duration using rarefaction, finding that 21 sampling sites per backwater and 15 – minute recordings sufficiently capture species richness and relative abundance. This standardization of underwater camera sampling methods will allow fisheries managers and to sample freshwater systems once inaccessible.

Introduction

Most fisheries sampling gears and methods are not suitable during the ice-covered periods of the year in temperate climates, so relatively little is known about fish assemblage structure during winter (Bartels et al. 2008). Past research efforts have used costly and labor-intensive techniques to study fishes on large river ecosystems in temperate climates during winter. For example, telemetry studies have been used to monitor fish movements and identify overwintering habitat use of sportfishes like Bluegill *Lepomis macrochirus*, Largemouth Bass *Micropterus nigricans*, and Black Crappie *Pomoxis nigromaculatus* (Sheehan et al. 1990; Gent et al. 1995; Knights et al. 1995; Johnson et al. 1998). However, the high cost and effort necessary for telemetry efforts have restricted studies to a small number of species of direct economic or conservation value (Thorstad et al. 2013). New sampling methods are needed to effectively survey more species and describe fish assemblage structure during winter.

Underwater cameras have been used to describe habitat and fish assemblage relationships (Cappo et al. 2004; Easton et al. 2015), estimate species richness (Jessop et al. 2022), and assess fish behavior in marine environments where conventional sampling methods are not feasible (Watson et al. 2005). In comparative studies, underwater camera surveys detect more fish species than conventional fisheries gears in marine systems (Bacheler et al. 2013). Despite demonstrated success in marine environments, underwater cameras have rarely been used in freshwater fisheries assessments, partially due to the generally poor water clarity and lack of established camera sampling methods (Chidami et al. 2007; Wilson et al. 2014). Recent technological advancements like infrared lighting have improved performance of underwater cameras in systems with poor water clarity (Chidami et al. 2007) and, when used in freshwater pools, underwater camera surveys have successfully indexed fish abundance (Wilson et al. 2014; Hitt et al. 2020). To our knowledge, standardized methods for underwater camera surveys have not yet been developed for estimating fish species richness and relative abundance in large, freshwater ecosystems.

To address knowledge gaps about under – ice fish assemblages and habitat use, we developed a standardized method for using underwater cameras to survey fish during ice – covered periods on the Upper Mississippi River (UMR). To evaluate our method, we (1) estimated camera viewing distance in different environmental conditions (e.g., water depth, ice thickness, water clarity, vegetative cover) and determined whether estimated viewing distance influenced species richness and relative abundance metrics; and (2) tested the effects of sampling time and the number of replicate sampling sites on estimates of species richness and species-specific relative abundances in backwater lakes. This underwater camera sampling method for freshwater systems will facilitate future descriptions of overwintering habitat and the community dynamics of diverse fishes during winter.

Methods

Study Area

The UMR encompasses the commercially navigable reach of the Mississippi River north of Cairo, Illinois to Minneapolis, Minnesota, including the navigable tributaries, and has a total basin area of 500,000 km^2 (Houser et al. 2022). In the 1930's, the UMR was divided into pools as the result of the construction of 27 lock and dams (Ickes et al. 2022). A diverse assemblage of ~140 fish species occur within the basin (Houser et al. 2022). Four backwater lakes were selected based on having water quality characteristics favorable for overwintering fishes within Pools 7 and 8 of the UMR in an area with an array of habitat types (Wilcox 1993; Table 1.1).

Study Design

Video samples were collected during the winter of 2025, at Stoddard Island Complex (*n* = 21), Lawrence Lake (*n* = 21), Airport Bay (*n* = 21) and Pettibone Lagoon (*n* = 21). To ensure adequate spatial distribution of sites, each backwater lake was divided into three longitudinal sections of equal area: the upper section, middle section, and lower section. Video samples were distributed equally among each section. Areas within the backwaters with depths < 0.4 m were not sampled to allow adequate depth for the camera apparatus to be deployed.

Underwater Camera Design

Videos were collected using an Aqua Vu Quad HD unit (MFR #200-5132, Aqua-Vu), recorded in 720p HD color using four lenses to yield a 360-degree view and saved to a 512 GB SD card using an Avermedia capture card (MFR #GC513, Avermedia) (Figure 1.1). Infrared lights on the camera body provided light for video in turbid environments (Figure 1.1). An aluminum camera housing (12 cm diameter, 40 cm height) was fabricated to protect the camera housing and lenses while deployed (Figure 1.1). The housing was attached to a pole for sites with < 2 m of water, and to a cable for sites > 2 m. Both the camera and the capture card were powered by a 12-volt portable battery (Figure 1.1).

Video Capture and Viewing Distance

At each site, a single hole (20 cm diameter) was drilled in the ice for the underwater camera, and the water depth (cm) was measured using a Vexilar digital depth sounder (MFR #LPS-1, Vexilar). The recorded video sample was collected 0.4 m from the benthic surface at all sites. We began recording 150 seconds after the camera was deployed to allow fish to acclimate to the camera apparatus (Watson et al. 2005) before a 20 – minute recording was collected from each sampling site. After video recordings, the Secchi depth was measured (cm) and vegetation density was visually indexed as: 0 (0% coverage), 1 (1-19% coverage), 2 (20-49% coverage), 3 (50-90% coverage), or 4 (>90% coverage), at each site. Sky cover (sun covered by clouds or no cloud cover), ice thickness (cm), and snow depth (cm) were also measured at each site. Water quality measurements of dissolved oxygen (mg/L) and water temperature (°C) were recorded using a YSI Pro2030.

Holes were drilled in the ice at 1 m increments from each fish sampling site. A vertically facing Secchi disk facing the camera direction was lowered to the recording depth in and moved away in 1 m increments until no longer visible (i.e., the viewing distance, cm).

Data Analysis

Multiple regression analysis with AICc model selection was used to analyze the effect of the environmental variables collected at each site (i.e., standard Secchi, site depth, sky cover, snow depth, sampling backwater, and ice thickness) on the field calculated viewing distance (Table 1.2). Vegetation score was removed from analysis as it was negatively correlated with site depth (Spearman's $\rho = -0.82$). The relative importance for each covariate was described by using the cumulative AICc weights (Σw_i). Plots were created to demonstrate the effect size of each environmental variable included in the top performing model under typical conditions and under extreme conditions. Using the top model generated from the viewing distance AICc analysis, viewing area was estimated for each site. Effect size was described by the change in estimated viewing distance between the observed quartile 1 and quartile 3 values (typical conditions) and the maximum and minimum values (extreme conditions). When calculating the maximum and minimum viewing distance value for a given environmental variable, the additional environmental variables were included in the calculation at their mean observed value.

Each detected fish was identified to the lowest possible taxonomic rank using visible characteristics, and the maximum number of each species in any single frame of the video was recorded as the MaxN (Cappo et al. 2004; Wilson et al. 2014; Campbell et al. 2015). Using MaxN as the measure of relative abundance eliminated the chance of double counting fish in the recorded samples (Wilson et al. 2014).

The influence of estimated viewing distance on response variables (i.e., Bluegill MaxN and species richness) was explored by applying zero inflated negative binomial modelling (ZINB) using the R package glmmTMB (Zuur et al. 2009; Brooks et al. 2017; McGillycuddy et al. 2025). The ZINB model was selected due to the high number of sites where Bluegill MaxN and species richness was zero.

Rarefaction was used to assess the required sampling duration to accurately estimate Bluegill MaxN. To evaluate sampling completeness in terms of species diversity, the iNext package in R was used (Chao et al. 2014; Chao et al. 2016) to develop a rarefaction curve describing the number of sampling sites necessary to accurately represent species richness.

Results

Viewing Distance

The observed viewing distance (mean ± standard error [SE]) across all sampling sites was 136.78 ± 14.9 cm, while the estimated viewing distance was 127.84 ± 13.94 cm. Site depth, water clarity, snow depth, and water clarity were the main predictors of observed viewing distance (Table 1.3). Support was also present for sampling backwater, as it was included in one of the two top performing models (Table 1.3). Site depth ($\Sigma w_i = 1.0$) and ice depth ($\Sigma w_i = 0.86$) had positive effect on viewing distance, while water clarity ($\Sigma w_i = 0.99$) and snow depth (0.89) had negative effect on viewing distance (Table 1.4). Additionally, there was relatively little difference between viewing distance across the sampling backwaters ($\Sigma w_i = 0.42$; Table 1.4). The results of the marginal effect size (ME) analysis under typical conditions (i.e., observed quartile 1 – quartile 3 values) demonstrated that site depth (ME = -29 cm) and ice depth (ME = -15 cm) generated negative changes in viewing distance while water clarity (ME = 18 cm) and snow depth (ME = 12 cm) created positive changes in viewing distance (Figure 1.2A). Under the extreme conditions (i.e., observed minimum – maximum values), the change in viewing distance decreased for the maximum observed site depth (ME = -160 cm) and ice depth (ME =

-50) and increased for water clarity (ME = 55 cm) and snow depth (ME = 56 cm; Figure 1.2B).

Relative Abundance and Species Richness Correction

The estimated viewing distance did not have a significant influence on observed Bluegill MaxN for the conditional (count) model ($p > 0.05$). The zero – inflation component of the ZINB model for excess zeros (i.e., zeros due to observer error) did not produce a significant result ($p > 0.05$). The results from the ZINB model for species richness and estimated viewing distance were not significant ($p > 0.05$) for the conditional or zero – inflation model.

Optimizing Sampling Duration and Number of Sites

After 5 minutes, roughly 50 % of sampling sites achieved their Bluegill MaxN (Figure 1.3). After 15 minutes, nearly 90% of sites had achieved their Bluegill MaxN value (Figure 1.3). The number of sampling sites necessary to attain the maximize site species richness increased rapidly to an inflection point at 15 samples in Airport Bay, Lawrence Lake, and Stoddard (Figure 1.4). Pettibone Lagoon did not display a clear inflection point (Figure 1.4).

Discussion

We developed and described the limitations of an underwater camera sampling method for lentic systems in temperate climates using four backwater lakes of the UMR. Our goal was to evaluate the effect of environmental drivers on camera viewing distance and its effect on relative abundance and species richness estimates; and determine the optimum sampling site number and sampling duration to develop standardized methods for the future use of this sampling technique. We found that ice depth and site water depth negatively affected viewing distance, while snow depth, and water clarity had a positive effect; and that relative abundance results can be standardized to account for variation in viewing distance. Our results show that by collecting 15 – minute recorded samples at 21 sampling sites, the underwater camera sampling method produces comparable results across various freshwater backwaters. This standardized underwater camera sampling method will allow fish assemblage sampling in seasons where conventional sampling methods are unsuitable.

Based on our observations, we found that at sites where visual secchi measurements were less than 70 cm (estimated viewing distance = 100 cm) fishes became difficult to identify, indicating that the underwater camera method mirrors electrofishing, where efficiency is increased in clearer water (Lyon et al. 2014). These results suggest that the underwater sampling methods proposed here should not be used when water clarity (secchi depth) is < 70 cm or when estimated viewing distance is < 100 cm. Simlar to electrofishing, which is most effective in depths from 2 – 4 m, the underwater camera sampling method has an effective depth range (Flotemersch and Blocksom 2005; Macnaughton et al. 2014; Pritchard et al. 2021). There was a 434% decrease in viewing distance between the maximum site depth under typical conditions (i.e., 2.2 m) and the maximum site depth under extreme conditions (i.e., 6.5 m). To maintain an estimated viewing distance of 100 cm, the effective depth for the underwater camera method is 3 m, a common maximum depth for shallow freshwater backwaters and slightly beyond our maximum site depth under typical conditions (i.e., mean ± [SE] observed site depth = 2.00 ± .02 m). We found that ice depth can reduce viewing distance by 20 cm in extreme conditions (i.e., max. ice depth = 61 cm), suggesting that when all other conditions are typical (i.e., site depth = 2.0 m, water clarity = 110 cm, snow depth = 2 cm), ice depth should not exceed 61 cm to maintain an estimated viewing distance of 100 cm. Our results also showed that snow depth had a positive effect on viewing distance, which we predict is due to decreased algal production under lower light conditions, providing clearer water (Garcia et al. 2019). Although backwater location was included in one of the two top performing models, it had a relatively small influence on viewing distance compared to direct measures of environmental features (i.e., 0.43). Thus, this underwater sampling method is transferrable to similar freshwater systems outside of the area of study. Under typical conditions for other covariates, we have provided water clarity measurements of 70 cm, site depths of 3 m, and ice depths of 61 cm as distinct limits for the application of the underwater camera sampling method.

We assessed the effect of viewing distance on the fisheries data collected by the underwater camera, finding that estimated viewing distance did not significantly influence Bluegill relative abundance (MaxN) or species richness. These results suggest that relative abundances collected from sites with varying viewing distances remain comparable, adding confidence to the method being transferrable across freshwater systems.

A standardized sampling duration facilitates efficient and effective fisheries surveys. 15 minutes is an optimal sampling time for backwater lakes of the Upper Mississippi River, aligning with the results found in estuary habitats, where species richness was accurately represented after 15 minutes of recording (Ebner and Morgan 2013). Sampling effort, in terms of the number of sampling sites, is also a major consideration for fisheries managers. We found a clear species richness inflection point prior to the observed sampling site value (*n* = 21), with minimal increase in expected species richness for Lawrence Lake, Stoddard, and Airport Bay. Pettibone Lagoon, the smallest of our four sampling backwaters, did not reach a clear species richness inflection point suggesting that sampling area alone may not accurately predict the necessary number of sampling sites. Our results indicate that at least 21 sampling sites are needed to capture representative species richness in 15 – 200 ha backwaters, compared to saltwater studies using drop cameras in larger areas (~1500 ha) that sample up to 272 sites (Easton et al. 2015). These results suggest the number of sampling sites be designated based on species richness rarefaction rather than relying on geographic area. The demonstrated effectiveness of 15 – minute recorded samples from 21 sampling sites per backwater supports their use in future freshwater underwater camera surveys.

Extrapolated rarefaction results showed little increase in species richness, suggesting that observed values approach the maximum detectable in the sampled backwaters with the underwater camera method. Additionally, we did not find evidence indicating viewing distance significantly influenced the species richness captured at each site. The underwater camera method provided a low observed species richness value in this winter study compared to species richness values captured from open-water sampling efforts using conventional fisheries sampling methods (UMRR - LTRM 2024). However, the underwater camera method did produce relatively high MaxN values at many sites, leading us to conclude that this method effectively captures the fishes present in backwater habitats in the highest abundances during the winter. We suspect the low species richness values collected by the underwater camera method may be due to a variety of factors. Some fish have been found to experience lethargy in cold water temperatures (Crawshaw et al. 1982) and may remain immobile in backwaters during the winter making them more difficult to locate using the underwater camera, which relies on capturing moving fish as they pass by the camera. Other fishes may move between habitats under the ice depending on environmental conditions (e.g., winter severity, oxygen depletion; Gent et al. 1995). To answer these questions, we recommend further underwater camera sampling efforts take place across river ecosystems in temperate climates.

Our underwater camera sampling method provides fisheries managers with an additional tool for sampling areas when conventional methods are not practical. For successful under – ice camera sampling, we were able to define limits for environmental factors influencing viewing distance. We also estimated the required sampling time and number of sampling sites to apply underwater camera sampling to other freshwater systems. The sampling guidelines developed in this study expand the spatial and temporal scope of freshwater fisheries surveys by allowing for the collection of fisheries data in historically underrepresented habitats.

Table 1.1. Water temperature (ºC), dissolved oxygen (mg/L), and turbidity (NTU) values collected from January Long Term Resource Monitoring program water quality sampling. Data reported for Airport Bay was collected from nearby fixed sampling site approximately 0.65 km east of Airport Bay from years 1992 – 2024. Data reported for Pettibone, Lawrence Lake, and Stoddard was collected from stratified random sampling sites located within each backwater from years 1994 – 2024.

				Water Temp. (ºC)			**Dissolved Oxy. (mg/L)**			**Turbidity (NTU)**		
Backwater	**n**	**Area (ha)**	**Mean Depth (m)**	**Mean**	**SE**	**Min-max**	**Mean**	**SE**	**Min-max**	**Mean**	**SE**	**Min-max**
Airport Bay	15	29.2	2.1	0.6	0.22	0.1 - 3.5	11.0	0.55	8.7 - 16.6	5.1	0.51	3 - 10
Pettibone	24	15.0	1.9	0.6	0.18	0 - 2.2	10.3	1.12	0 - 15.6	2.9	0.30	2 - 9
Lawrence Lake	184	105.6	.8	1.1	0.07	0 - 5.3	7.8	0.47	0 - 22.9	14.5	3.50	1 - 400
Stoddard	42	201.2	1.2	0.6	0.09	0 - 2.4	10.6	0.5	0 - 19.5	3.5	0.25	2 - 9

Table 1.2. Environmental covariates measured at underwater camera sampling sites (n=84) and included in AICc analysis. Sampling performed in Airport Bay on Pool 7, and Pettibone Lagoon, Lawrence Lake and Stoddard Island Complex on Pool 8 of the Upper Mississippi River near La Crosse, WI.

Covariate	**Variable type**	**Mean**	**Standard error**	**Min-max**
Ice depth (cm)	Continuous	37.0	4.0	12 – 61
Backwater	Nominal	NA	NA	NA
Sky cover	Nominal	NA	NA	0 (Cloud) - 1 (Sun)
Site depth (m)	Continuous	2.0	0.2	0.7 – 6.5
Snow depth (cm)	Continuous	1.7	0.2	0 – 18
Wtr. clarity (cm)	Continuous	110.4	12.1	55 - 215

Table 1.3. Results of AICc modeling analyzing the effect of water clarity, sky cover, vegetation score, and ice depth on viewing distance of underwater camera. Table includes only models with ΔAICc > 2.

Model covariates	**df**	**AICc**	**ΔAICc**	**Weight**
Ice depth + site depth + snow depth + water clarity	6	762.3	0	0.38
Ice depth + sampling backwater + site depth + snow depth + water clarity	7	763.5	1.17	0.21

Table 1.4. AICc sum of weights for covariates used to analyze the effect of environmental variables on viewing distance of underwater camera.

Covariate	**Sum of weights**	**Direction of effect**
Site depth	1.00	-
Water clarity	0.99	+
Ice depth	0.89	-
Snow depth	0.86	+
Sampling backwater	0.42	NA
Sky cover	0.24	NA

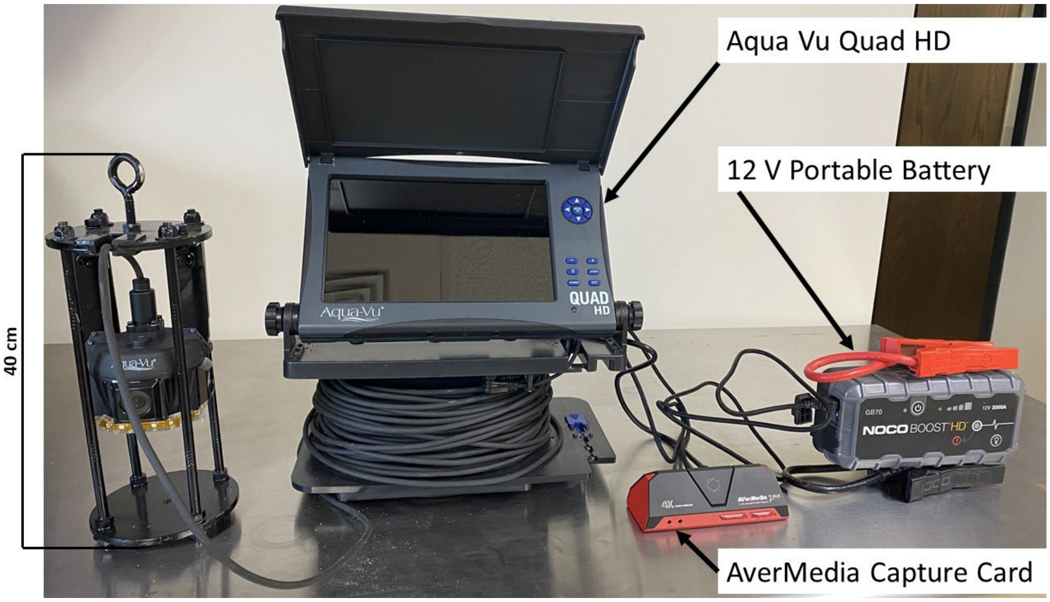

Figure 1.1. Underwater camera sampling instruments used in this study.

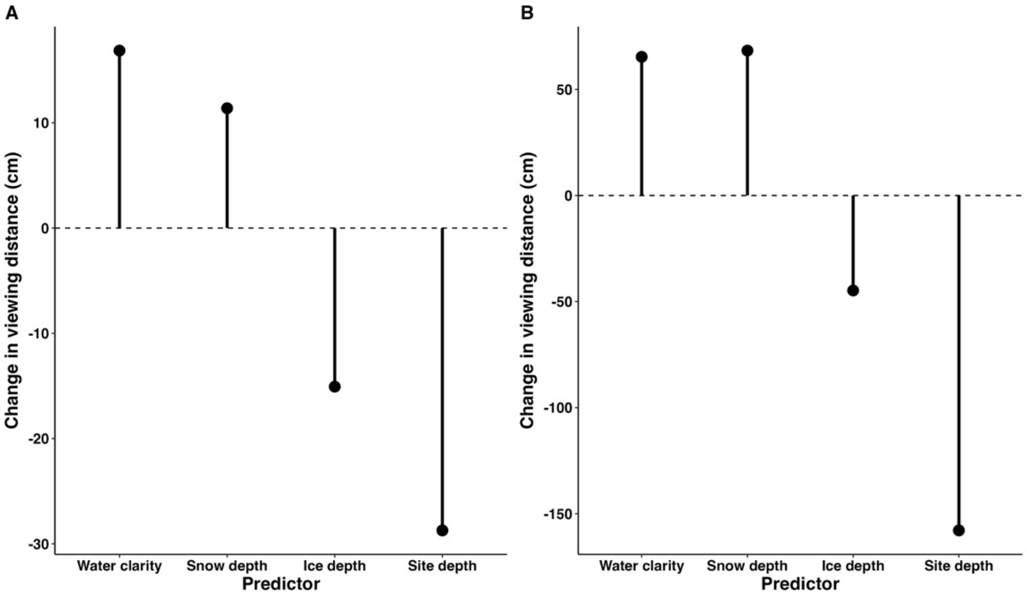

Figure 1.2. (A) Change in underwater camera viewing distance (cm) for each predictor included in top AICc model under typical conditions. (B) Change in underwater camera viewing distance (cm) for each predictor included in top AICc model under extreme conditions. All other predictors were held at their mean value when calculating change in viewing distance.

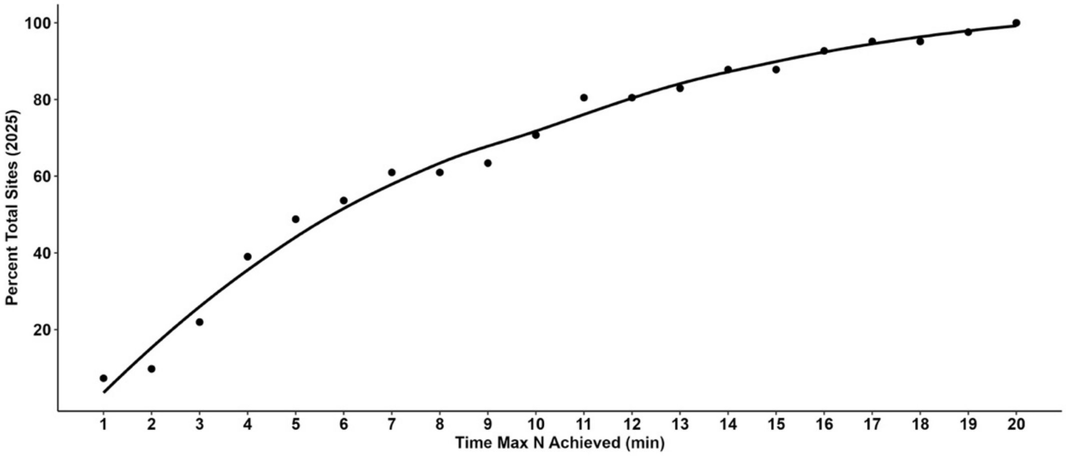

Figure 1.3. Percent of sites that reached maximum bluegill count at each minute of the 20 – minute sampling period during 2025 sampling. Bluegill MaxN values from underwater camera samples collected from Airport Bay on Pool 7; and Pettibone Lagoon, Lawrence Lake and Stoddard Island Complex on Pool 8 of the Upper Mississippi River near La Crosse, WI.

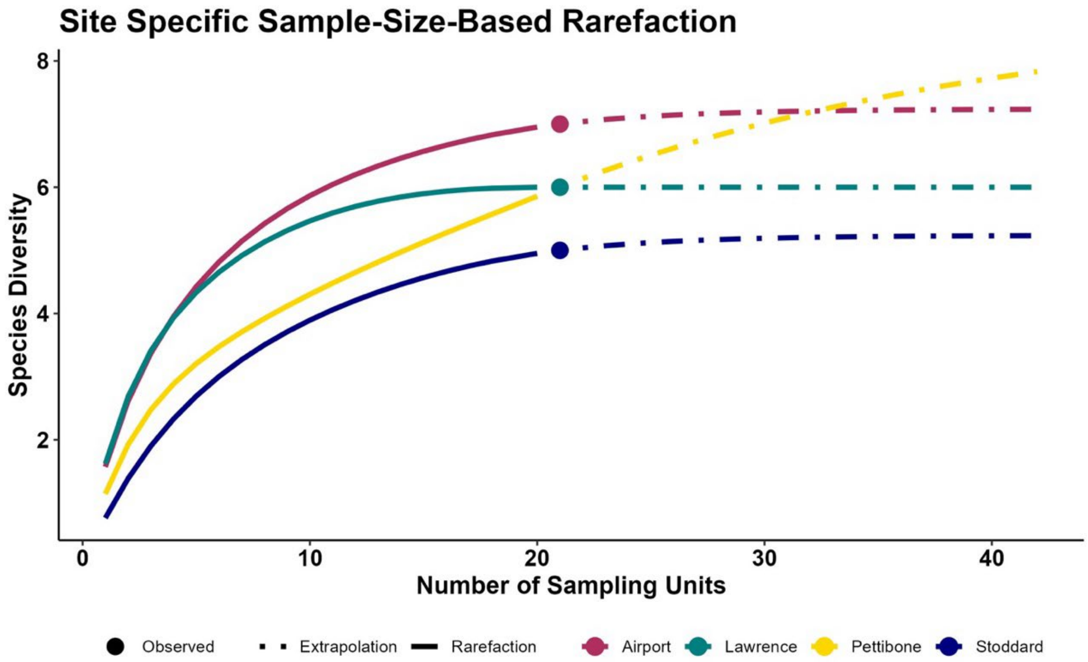

Figure 1.4. Number of sampling sites to achieve desired species diversity based on data collected from under-ice underwater camera sampling performed in Lawrence Lake, Stoddard, Pettibone Lagoon, and Airport Bay backwaters within Pool 7 and Pool 8 near La Crosse, WI during the 2025 sampling winter.

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CHAPTER II

INFLUENCE OF ENVIRONMENTAL FACTORS ON OVERWINTERING HABITAT USE BY MISSISSIPPI RIVER BACKWATER FISHES

M.S. Research Thesis

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ABSTRACT

Backwater habitats throughout large river ecosystems in temperate climates can provide suitable environmental factors during winter for several economically important backwater fishes. The limitations of conventional fisheries sampling gears imposed by ice cover has left the habitat use of many backwater fishes beyond several centrarchid species poorly understood. We used underwater cameras to survey nine backwater species in two reaches of the Upper Mississippi River. We used random forest analysis to rank the relative importance of environmental covariates on fish assemblage metrics (i.e., species richness and combined MaxN) and species – specific data for Bluegill, Largemouth Bass, and Yellow Perch. We found that water temperature (°C) and dissolved oxygen (mg/L) were the most important factors influencing fish assemblage metrics and the species – specific data for Bluegill and Largemouth Bass presence and relative abundance. We also found that filamentous algae presence had a negative relationship with Bluegill relative abundance and that site depth (m) and conductivity (µS) were the most important factors influencing Yellow Perch presence and relative abundance. This study supports the development of backwater habitat that contains a mosaic of both physical and chemical features used by diverse limnophilic fishes.

Introduction

Habitat types such as backwaters, side channels, and main channels allow large river ecosystems to support a diversity of fishes. For example, the Upper Mississippi River (UMR) supports over 150 species of fish (Houser et al. 2022). Backwaters, side channels, and main channels differ in physical and chemical characteristics such as velocity, substrate, and depth (Wilcox 1993). The diversity and abundance of many endemic species is dynamic, as movement between deeper channels with higher velocities (i.e., main and side channels) and shallow backwaters with low velocities is required for survival or reproduction throughout the year (Dettmers et al. 2001). While habitat use during open water periods is well documented for many fishes, much less is known about the habitat use of most species in large temperate rivers during winter.

Overwintering refugia is important to native fishes by providing suitable water temperature and dissolve oxygen concentrations during winter (Johnson et al. 1998; Bodensteiner and Lewis 1992; Crawshaw 1984). During the winter, flowing main and side channels on the UMR maintain steady temperatures of 0 °C (Johnson et al. 1998; Bodensteiner and Lewis 1992). Several centrachid species are known to move to backwater habitats, where slow moving water allows temperatures to rise to avoid the unfavorable environmental conditions during the ice-covered season (Sheehan et al. 1990). Channel Catfish (*Ictalurus punctatus*) and Northern Pike (*Esox lucius*) have also been documented avoiding main channel water temperatures during the coldest part of winter (Sheehan et al. 1990). Past studies have shown that Largemouth Bass (*Micropterus nigricans*), Bluegill (*Lepomis macrochirus*) and Black Crappie (*Pomoxis nigromaculatus*) prefer habitats that maintain low velocities (<1 cm/s), suitable dissolved oxygen levels (>3 mg/l), and water temperatures greater than 1°C for overwintering (Raibley et al. 2011; Johnson et al. 1998).

Most available knowledge involving overwintering fishes has been collected from conventional fisheries surveys (e.g., electrofishing) during late fall (i.e., before ice cover), or from under-ice acoustic telemetry focused mostly on select centrarchid species (Bartels et al. 2008; Knights et al. 1995; Sheehan et al. 1990). Although it is generally thought that backwater fishes move to overwintering locations when water temperatures fall below 10 °C (Bartels et al. 2008), the under-ice movements and habitat use of many fishes are entirely unknown. Magnifying this knowledge gap, water temperature and dissolved oxygen levels can fluctuate during the ice-covered period on large river ecosystems (Obertegger et al. 2017; Gent et al. 1995), potentially leading to changes in habitat that cannot be observed without under-ice sampling. For example, a study on Pool 13 of the Upper Mississippi River tracked large-scale, 4-mile movements of radio – tagged Largemouth Bass after dissolved oxygen concentrations decreased to 3 ppm (Gent et al. 1995). Although the overwintering movements of several centrarchids (i.e., Bluegill and Largemouth Bass) has been documented using telemetry (Knights et al. 1995; Sheehan et al. 1990), the high financial and labor demands of the method have resulted in little information involving the backwater fish assemblage (Thorstad et al. 2013). These past studies show how the details describing overwintering habitat preferences of backwater fishes can be muddled when observations are restricted to pre-ice surveys and telemetry efforts focused on limited centrarchid species.

To-date, few studies have described fish diversity in backwater lakes during winter and how abundance is influenced by environmental factors during periods of ice cover. This study is among the first to describe the overwintering habitat use of several limnophilic fishes common to the backwater habitats of the Upper Mississippi River using camera sampling technology. Specifically, we: 1) ranked the relative importance of environmental factors on two measures of fish assemblage (e.g., species richness and combined fish abundance) and 2) ranked the relative importance of environmental factors on the habitat use of the three most abundant fishes found in our sampling (e.g., Bluegill, Largemouth Bass, and Yellow Perch (*Perca flavescens*)). Based on relationships between environmental factors and the fish assemblage, we will compare the habitat characteristics that may promote overwinter survival for centrarchid species and the wider backwater fish assemblage.

Methods

Study Design

The UMR is a large river ecosystem with complex habitats defined by diverse physical features sustaining a wide fish assemblage (Junk et al. 2007). Since the construction of 29 locks and dams in the 1930s, the river has been divided into pools containing multiple habitat types which are typically delineated by depth, velocity, and distance from the main channel (Houser et al. 2022; Wilcox et al. 1993); with backwaters identified as preferred overwintering habitat (Johnson et al. 1998; Knights et al. 1995). Within Pools 7 and 8, four large backwater lakes were selected (i.e., Airport Bay, Pettibone Lagoon, Stoddard, and Lawrence Lake) to evaluate the habitat use of limnophilic fishes during winter (Figure 2.1). These backwaters were selected for study due to their water quality characteristics which may be hospitable for backwater fishes during winter (Table 2.1). Using a stratified random sampling design, we sampled 21 sites at each backwater lake between December 2024 and February 2025, dividing each lake into three equal strata (*n* = 9) to ensure spatial distribution of samples.

Camera Sampling

At each site, a single hole was drilled in the ice for an Aqua Vu Quad underwater camera (MFR #200-5132, Aqua-Vu) which was used to collect recordings 20 cm from the benthic surface (Figure 2.2). A 180-second acclimation period was used before recordings started to allow fish to become adjusted to the camera presence (Watson et al. 2005). At each site, we recorded a 20-minute video sample to an SD card using an Avermedia capture card (MFR #GC513, Avermedia; Figure 2.2), and later identified all observed fishes to the lowest taxonomic level using visible features. For each sample, the maximum number of a species present in any single frame was documented as the species MaxN and will be used to evaluate relative abundance (Campbell et al. 2015; Wilson et al. 2014; Cappo et al. 2004). MaxN allowed analysis to be conducted without double counting individual fish and overestimating abundance.

Environmental Covariate Field Methods

Dissolved oxygen (mg/L), conductivity (μS), and water temperature (°C) were collected at each site using a YSI Pro2030 unit at mid-depth (Table 2.2). A Vexilar

LPS-1 digital depth sounder (MFR #LPS-1, Vexilar) was used to measure water depth and vegetation and filamentous algae coverage were visually estimated (Table 2.2).

Statistical Analysis

Using the *ranger* package in R (Wright and Zeigler 2017), random forest models were used to rank the relative effect of the explanatory covariates on the fish assemblage measures (e.g., species richness class and combined MaxN) (Čandek et al. 2020) and the presence and relative abundances of Bluegill, Largemouth Bass, and Yellow Perch (Ngor et al. 2023; Arora et al. 2022). Random forest models are robust to zero inflated data (Siders et al. 2020; Garcia-Marti et al. 2019), can handle collinear covariates, and can identify non – linear relationships (DeLuca et al. 2023), which are each common for ecological count data (Ngor et al. 2023; Arora et al. 2022). Species richness values ranged from 0 to 4, with values of 3 and 4 being relatively rare in our sampling effort. To minimize model issues associated with sparse class representation, species richness was grouped into three categories (e.g., low: 0 species present, medium: 1 species present, and high: 2-4 species present). Combined MaxN represented the sum of all species – specific MaxN values at each site. Using the *pdp* package in R (Greenwell 2017), partial dependency plots were developed for all significant predictor variables to visualize the direction and magnitude of effect (Deluca et al. 2023).

Results

Species Richness

Water temperature was the most important covariate influencing species richness class, with a relative importance value approximately double that of the second ranked covariate in random forest models (Figure 2.3). The probability of observing zero species was higher at cold water temperatures (~0 – 1 °C), with a sharp decline after 2 °C (Figure 2.4). The probability of observing 1 species gradually increased with water temperature, while the probability of observing 2-4 species began low but increased sharply after 2 °C (Figure 2.4).

Combined MaxN

Combined MaxN ranged from 0 – 62 (mean ± standard error [SE] = 3.93 ± 0.4). Water temperature and dissolved oxygen were the most important covariates influencing combined MaxN (Figure 2.5). The predicted combined MaxN increased gradually with water temperature with a sharp increase at 2 °C (Figure 2.6A). Dissolved oxygen showed a gradual increase in predicted combined MaxN at lower values until a sharp decrease at ~10 mg/L (Figure 2.6B).

Presence/Absence

Bluegill were found at 38% of the sampling sites (mean MaxN ± [SE] = 1.55 ± 0.2; Table 2.3). Water temperature ranked as the covariate with the highest relative importance for Bluegill presence (Figure 2.7A). The probability that Bluegill occurred at water temperatures < 2 °C was relatively low, but the predicted probability of presence increased sharply at 2 °C and continued to rise with warmer temperatures (Figure 2.8A).

Largemouth Bass were found at 32% of sampled sites (mean MaxN ± [SE] = 0.46 ± 0.1; Table 2.3). Largemouth Bass presence was most strongly influenced by water temperature (Figure 2.9). Largemouth Bass presence at water temperatures < 2

°C was low with a general increase in predicted presence as water temperatures increased (Figure 2.10).

Yellow Perch were found at 17% of sampled sites (mean MaxN ± [SE] = 0.49 ± 0.1; Table 2.3). Site depth was the most important covariate for predicting Yellow Perch presence (Figure 2.11A). The probability of Yellow Perch presence was highest at site depths < 2 m, with a general decrease with site depth (Figure 2.12A).

Relative Abundance

Bluegill relative abundance was most strongly influenced by filamentous algae and dissolved oxygen (Figure 2.7B). Predicted Bluegill MaxN values were higher in areas without filamentous algae (Figure 2.8B). Predicted Bluegill MaxN values increased with dissolved oxygen levels until 10 mg/L, followed by a sharp decline as dissolved oxygen continued to increase beyond 10 mg/L (Figure 2.8C).

The effect of the environmental covariates on the abundance of Largemouth Bass was not examined, as the Largemouth Bass MaxN data lacked sufficient range and variability to test the covariate effects (Table 2.3).

Yellow Perch abundance was most greatly influenced by conductivity compared to other covariates (Figure 2.11B). Predicted Yellow Perch MaxN was low at 300 – 600 µS and increased at conductivities above 600 µS (Figure 2.12B).

Discussion

This study provides among the first insights into the habitat use of the backwater fish assemblage during ice covered periods in the UMR. To – date, backwater fish community dynamics during winter remain understudied, stressing the importance of this study for guiding overwintering management strategies for the wider backwater fish community. Water temperature and dissolved oxygen were the biggest determinants of assemblage metrics (i.e., species richness class and combined MaxN) and centrarchid species presence and relative abundance (i.e., Bluegill and Largemouth Bass). Our species – specific analysis also showed that filamentous algae presence was an important covariate influencing Bluegill abundance, while site depth and conductivity were the most important covariates influencing Yellow Perch presence and relative abundance. These results indicate that the environmental factors influencing overwintering habitat use for the wider backwater fish community (i.e., water temperature and dissolved oxygen) mirror those influencing the habitat use for Bluegill and Largemouth Bass, while Yellow Perch were driven by depth and conductivity.

Water temperature and dissolved oxygen best explained overwintering habitat use of the wider backwater fish community and the species – specific data for Bluegill and Largemouth Bass. Past studies have found a positive relationship between dissolved oxygen and centrarchid presence and relative abundance (Johnson et al. 1998; Knights et al. 1995), however we encountered many sites with high dissolved oxygen saturation during our study. Our partial dependence plots showed a sharp decrease in predicted combined MaxN and predicted Bluegill abundance at oxygen levels (> 10 mg/L) suggesting areas with high dissolved oxygen levels provide poor overwintering habitat for the backwater fish assemblage. The large number of sites with high dissolved oxygen levels may be a result of elevated filamentous algae presence under the ice and decreased snow cover allowing for increased levels of production during winter (Vadeboncoeur et al. 2021; Garcia et al. 2019; Kunkel et al. 2009).

Our results showed that Bluegill relative abundance was influenced negatively by presence of filamentous algae. We found that filamentous algae was primarily found at depths < 2 m (Figure 2.13). As backwaters on the UMR have been subject to increased sedimentation over time (Rogala et al. 2020), more backwater habitat is at risk of increased filamentous algae production during winter. Along with decreased snow accumulations stimulating winter production, backwater sedimentation poses a significant risk to overwintering habitat (Vadeboncoeur et al. 2021; Rogala et al. 2020; Kunkel et al. 2009). We recommend management strategies focused on mitigating backwater sedimentation and rehabilitation projects focused on creating overwintering areas with both deep (> 2 m) and shallow water (< 2 m) area to combat increased production and support backwater fish overwintering habitat.

Bluegill overwintering data is often used to guide overwintering backwater rehabilitation projects on the UMR (Mooney et al. 2025). Our results showed that the environmental factors influencing Bluegill presence and relative abundance mirrored the results for the backwater fish assemblage metrics, providing support for this management strategy. Developing backwaters with water temperatures > 2 °C, dissolved oxygen levels between 5 – 10 mg/L, and low filamentous algae levels are factors that are likely to promote overwintering habitat for the wider backwater community. However, we found that the environmental factors influencing Yellow Perch habitat use during winter (i.e., site depth and conductivity) differed from those influencing the backwater fish assemblage metrics and the species – specific data for

Bluegill and Largemouth Bass. We recommend future restoration project designs incorporate features that support the habitat preferences of other fishes that co – dominate the backwater assemblage, such as Yellow Perch.

This study has produced some of the first results that describe the overwintering habitat use of the backwater fish assemblage. We demonstrated that water quality characteristics (e.g., water temperature and dissolved oxygen) are important overwintering habitats for Bluegill and Largemouth Bass, as well as a broad suite of backwater fishes. We also found that site depth and conductivity influence overwintering habitat preference of Yellow Perch. We recommend future studies focus on identifying the overwintering habitat use of other limnophilic fishes that co – dominate the UMR backwater community during winter such as Black Crappie and Gizzard Shad. Habitat rehabilitation projects that incorporate features promoting overwintering survival for a wide range of backwater species ensure that large rivers like the UMR continue to support diverse fish communities. The results of this study can aid management in expanding the understanding of overwintering fish dynamics beyond the current centrarchid-centric models used for the construction of overwintering habitat structures in large river systems.

Table 2.1. Water temperature (ºC), dissolved oxygen (mg/L), and tubidity (NTU) values collected from January Long Term Resource Monitoring program water quality sampling (UMRR - LTRM 2024). Data reported for Airport Bay was collected from nearby fixed sampling site approximately 0.65 km east of Airport Bay from years 1992 – 2024. Data reported for Pettibone, Lawrence Lake, and Stoddard was collected from stratified random sampling sites located within each backwater from years 1994 – 2024.

				Water Temp. (ºC)			Dissolved Oxy. (mg/L)		
Backwater	**n**	**Area (ha)**	**Mean Depth (m)**	**Mean**	**SE**	**Min-max**	**Mean**	**SE**	**Min-max**
Airport Bay	15	29.2	2.1	0.6	0.22	0.1 - 3.5	11.0	0.55	8.7 - 16.6
Pettibone	24	15.0	1.9	0.6	0.18	0 - 2.2	10.3	1.12	0 - 15.6
Lawrence Lake	184	105.6	.8	1.1	0.07	0 - 5.3	7.8	0.47	0 - 22.9
Stoddard	42	201.2	1.2	0.6	0.09	0 - 2.4	10.6	0.5	0 - 19.5

Table 2.2. Summary statistics for covariates used in random forest modelling. Covariates were collected at each site sampled during this study. Measurements collected from samples in Pools 7 and 8 on the UMR.

Covariate	**Variable type**	**Mean**	**Standard error**	**Min-max**
Anglers present	Binary	NA	NA	0 (Absent) - 1 (Present)
Conductivity (µS)	Continuous	500.6	54.6	312 - 895
Dissolved oxygen (mg/L)	Continuous	13.7	1.5	2.4 - 25.5
Distance to anglers (m)	Continuous	159.7	19.8	5 - 500
Filamentous algae	Binary	NA	NA	0 (Absent) - 1 (Present)
Site depth (m)	Continuous	2.03	0.2	0.7 - 6.5
Vegetation score	Ordinal	NA	NA	0 (No vegetation) - 4 (Fully Vegetated)
Water temperature (°C)	Continuous	2.07	0.2	0.2 - 4.4

Table 2.3. Fishes collected from underwater camera sampling in backwaters in Pools 7 and 8 of the UMR. Number of sites found, mean and standard error (SE) MaxN values are reported. The maximum single site MaxN value and total summed MaxN value from all sites are also reported.

Species	**N sites present**	**µ MaxN**	**SE MaxN**	**Max MaxN**	**Tot MaxN**
Black Crappie	10	0.14	0.02	2	12
Bluegill	32	1.55	0.17	10	130
Largemouth Bass	27	0.46	0.05	4	39
Pumpkinseed	2	0.02	2.60E-03	1	2
Bowfin	4	0.05	5.00E-03	1	4
Common Carp	1	0.04	3.90E-03	3	3
Gizzard Shad	4	1.13	0.12	62	95
Northern Pike	4	0.05	5.20E-03	1	4
Yellow Perch	14	0.49	0.05	17	41

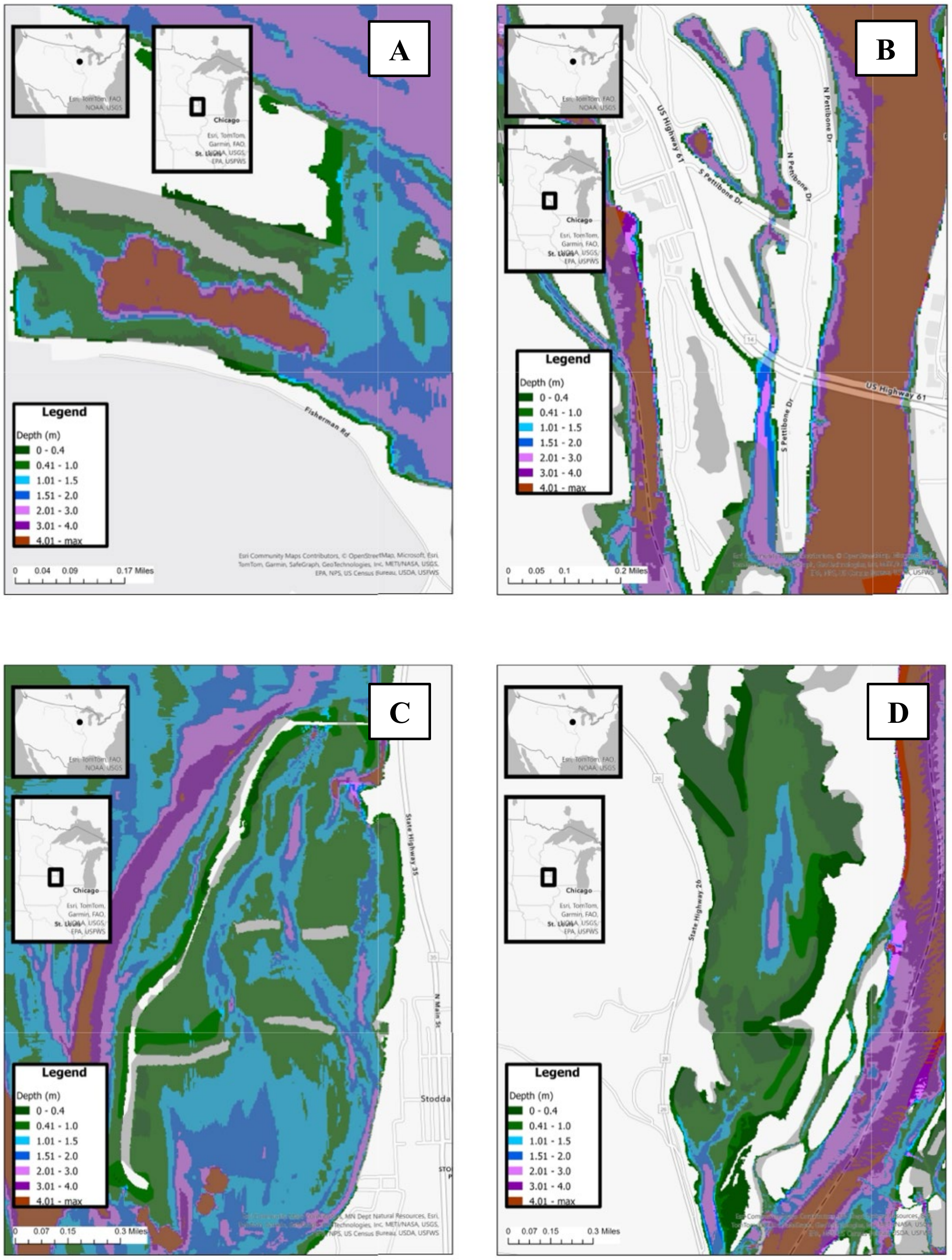

Figure 2.1. (A) Depth map of Airport Bay backwater in Pool 7 on the Upper Mississippi River. (B) Depth map of Pettibone Lagoon in Pool 8 on the Upper Mississippi River. (C) Depth map of Stoddard Bay backwater in Pool 8 on the Upper Mississippi River. (D) Depth map of Lawrence Lake Backwater in Pool 8 on the Upper Mississippi River. Depths based on 2010 bathymetry collected by the Long Term Resource Monitoring Program (Rogala 2019). Background map courtesy of ESRI Community Mapping Contributors.

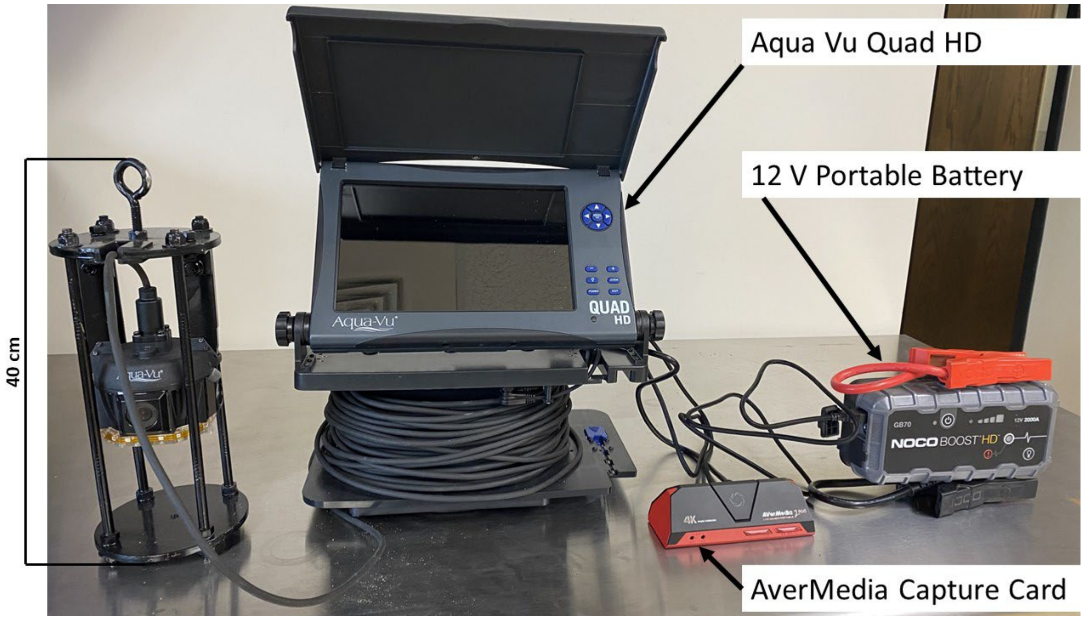

Figure 2.2. Aqua Vu Quad HD underwater camera used to collect samples used in this study. Avermedia capture card used to record samples onto SD card.

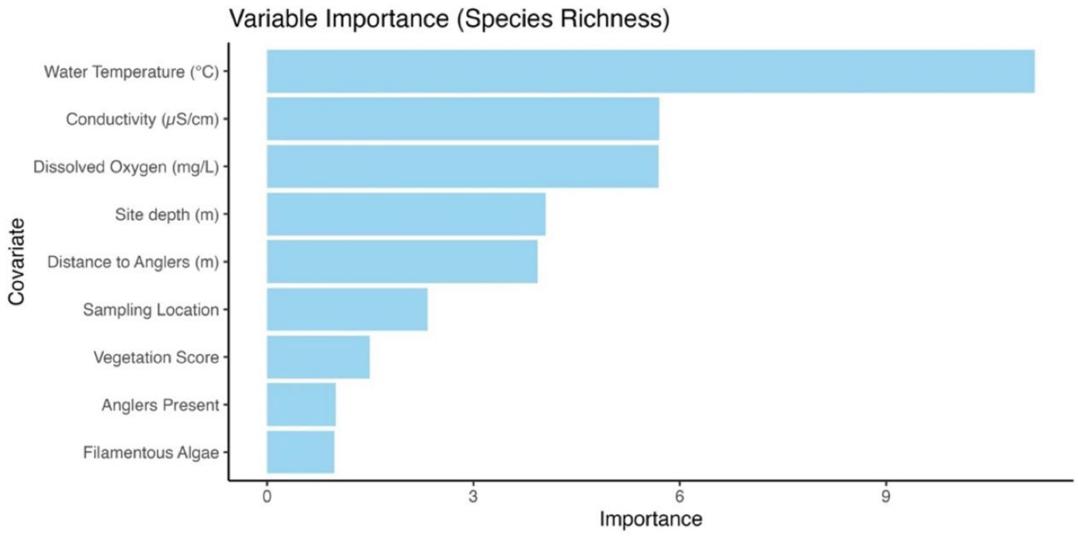

Figure 2.3. Variable importance values from random forest modelling for species richness classes (i.e., low = 0 species, medium = 1 species, high = 2 – 4 species). Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR. Variable importance values calculated using ranger package (Wright and Zeigler 2017) and plot created using ggplot2 package in R (Wickham 2016).

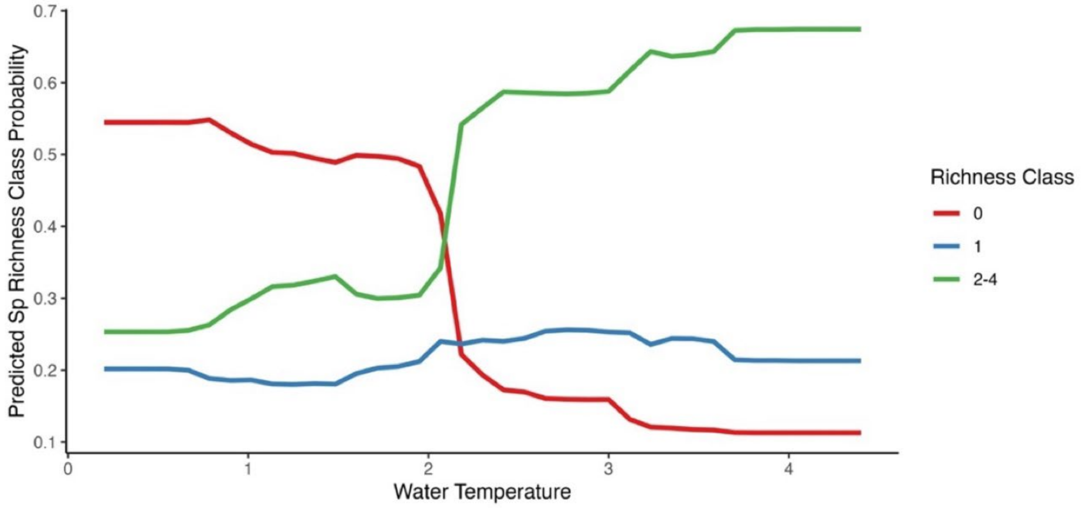

Figure 2.4. Partial dependence plot for predicted species richness classes (i.e., low = 0 species, medium = 1 species, high = 2 – 4 species) and water temperature (ºC). Partial dependence plots created using pdp (Greenwell 2017) and ggplot2 (Wickham 2016) packages in R. Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

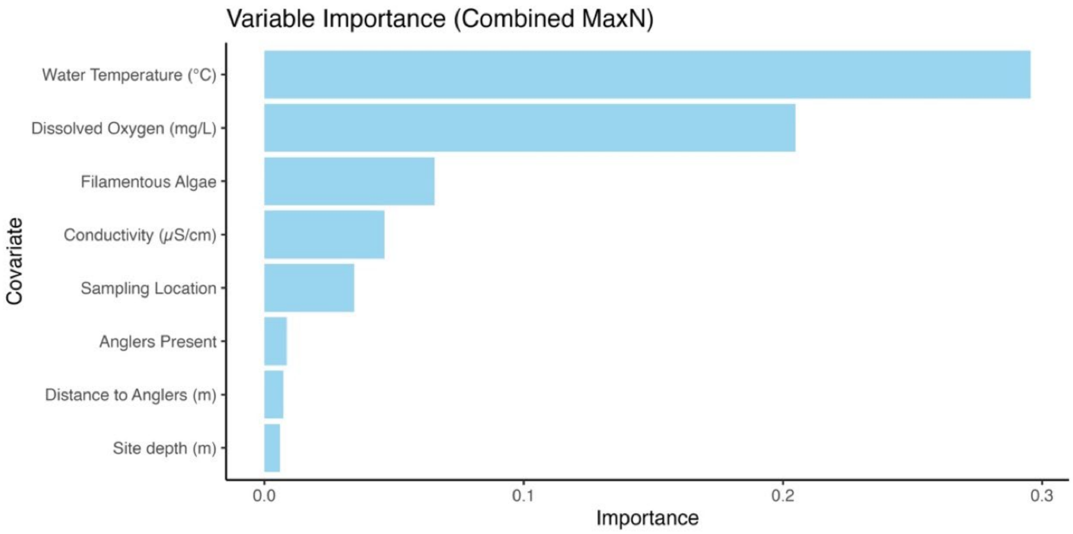

Figure 2.5. Variable importance values from random forest modelling for combined MaxN. Combined MaxN is sum of all species MaxN values from each site. Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

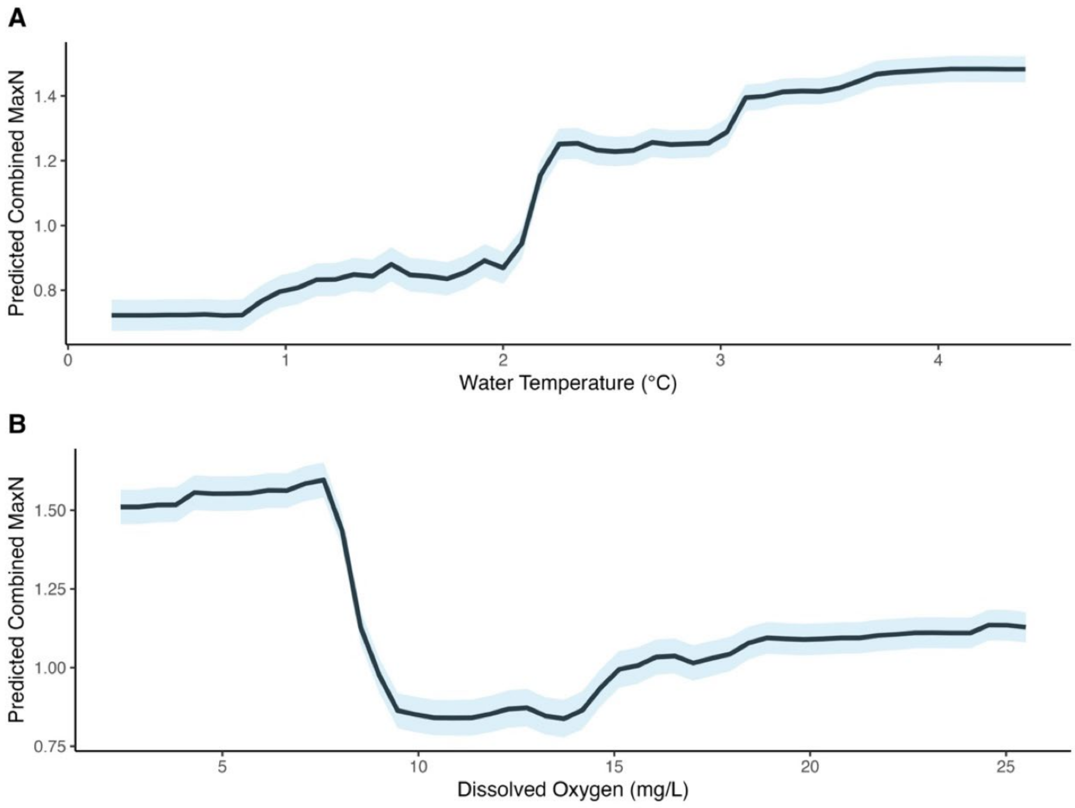

Figure 2.6. (A) Partial dependence plot for predicted combined MaxN and water temperature (ºC). (B) Partial dependence plot for predicted combine MaxN and dissolved oxygen (mg/L). Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

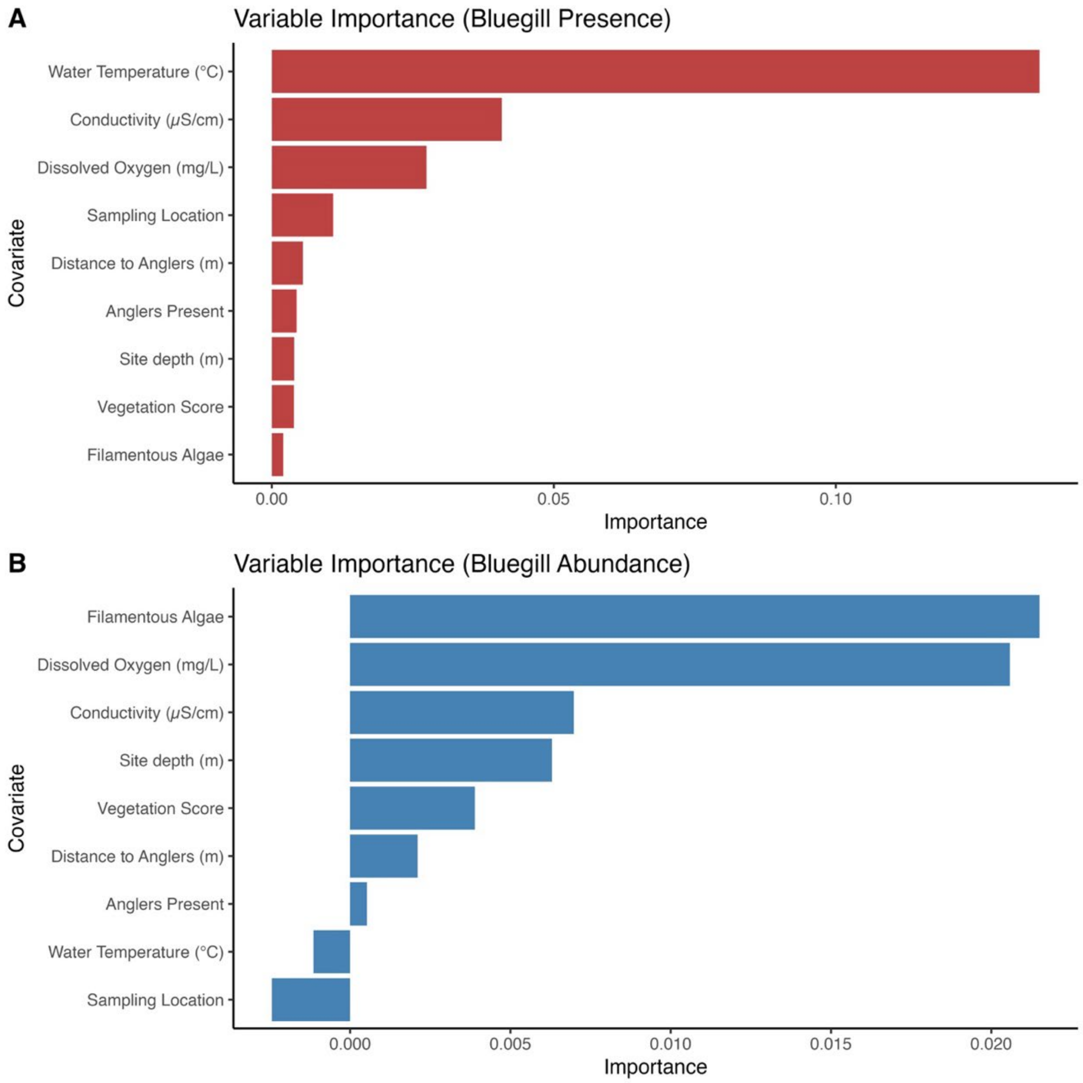

Figure 2.7. (A) Variable importance values from random forest modelling for Bluegill presence. (B) Variable importance values from random forest modelling for Bluegill abundance. Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

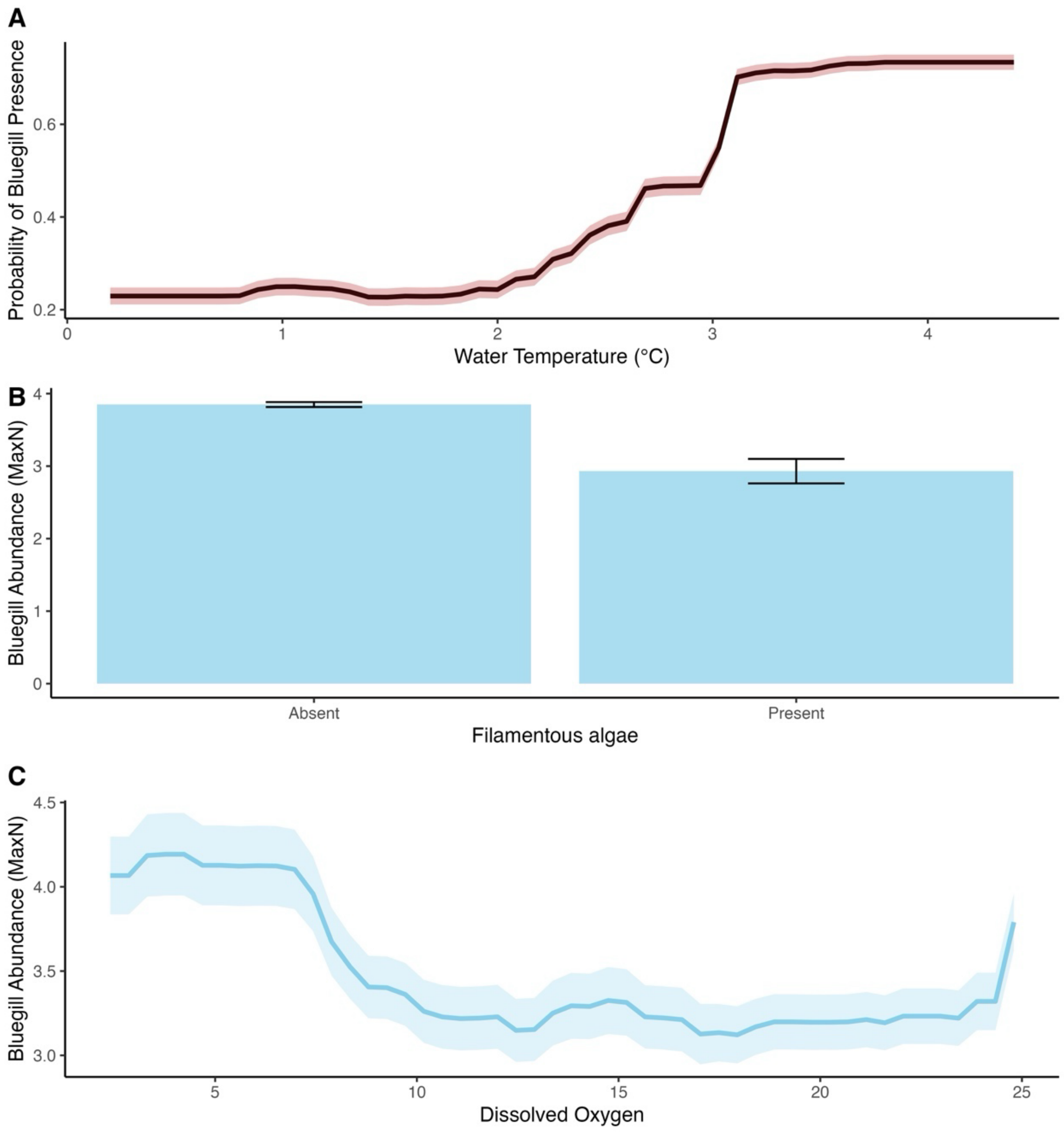

Figure 2.8. (A) Partial dependence plot for predicted Bluegill presence and water temperature (ºC). (B) Partial dependence plot for predicted Bluegill MaxN and filamentous algae. (C) Partial dependence plot for predicted Bluegill MaxN and dissolved oxygen (mg/L). Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

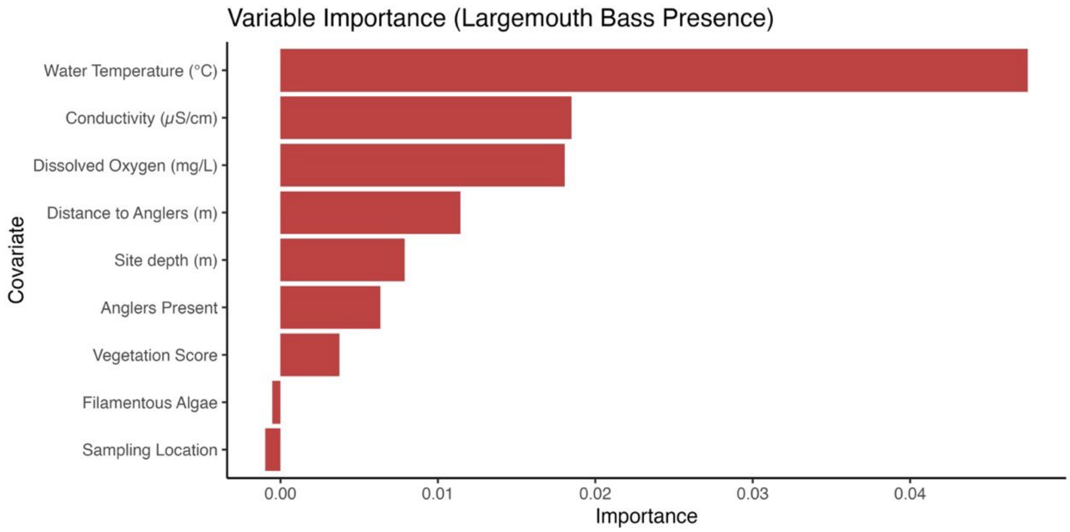

Figure 2.9. Variable importance values from random forest modelling for Largemouth Bass presence. Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

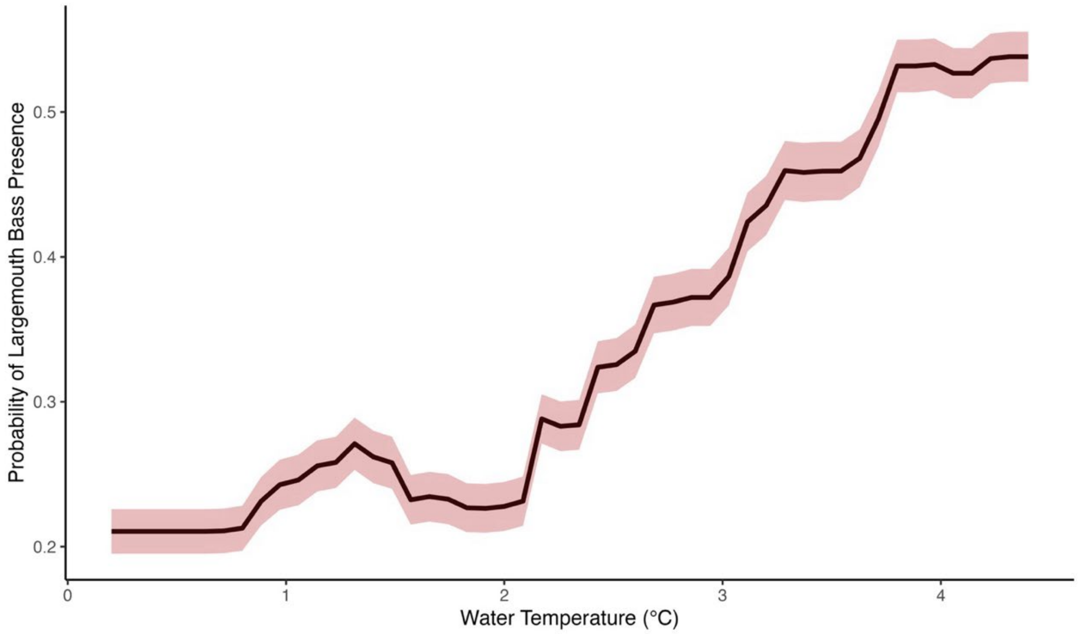

Figure 2.10. Partial dependence plot for predicted Yellow Perch presence and water temperature (ºC). Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

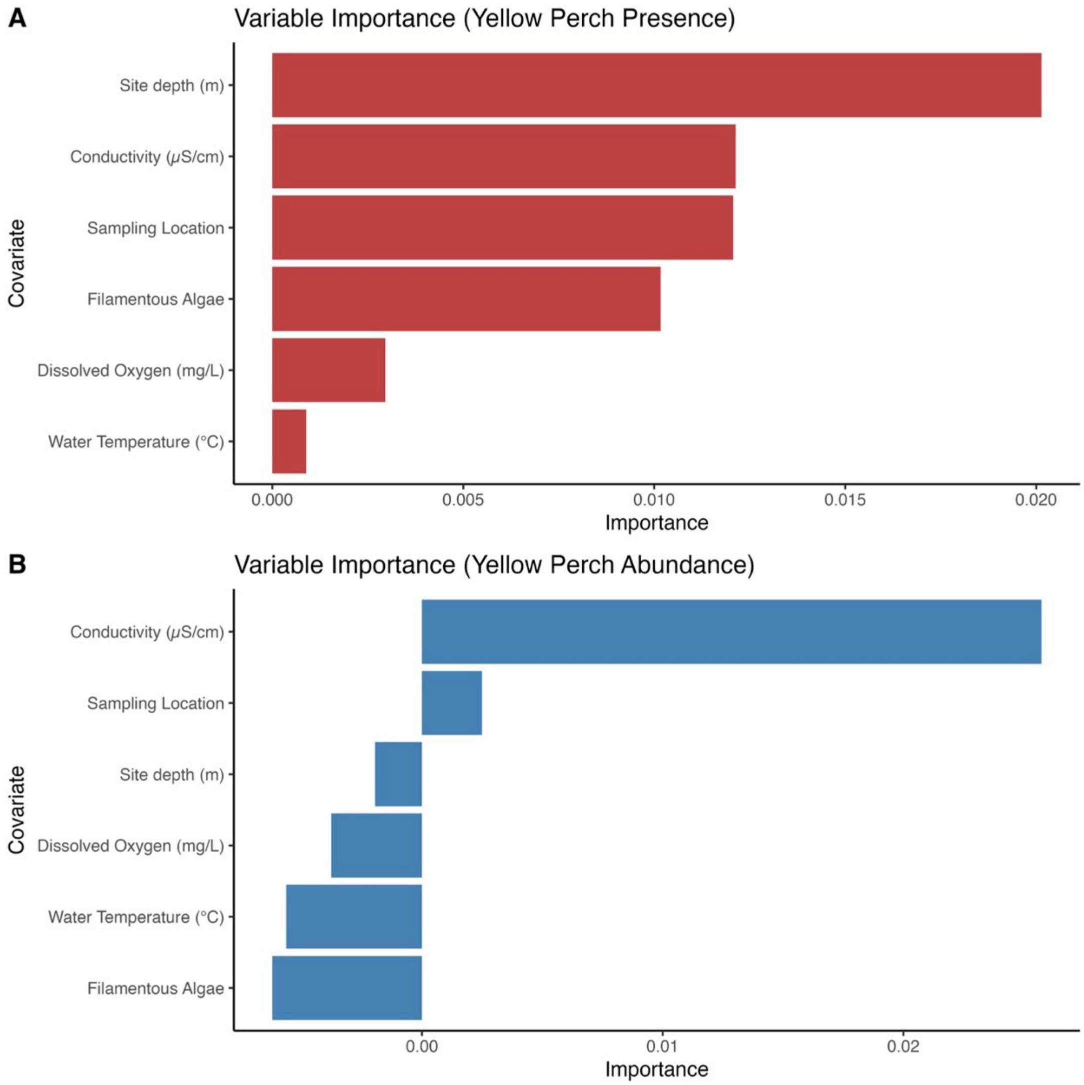

Figure 2.11. (A) Variable importance values from random forest modelling for Yellow Perch presence. (B) Variable importance values from random forest modelling for Yellow Perch abundance. Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR. Variable importance values calculated using ranger package (Wright and Zeigler 2017) and plot created using ggplot2 package in R (Wickham 2016).

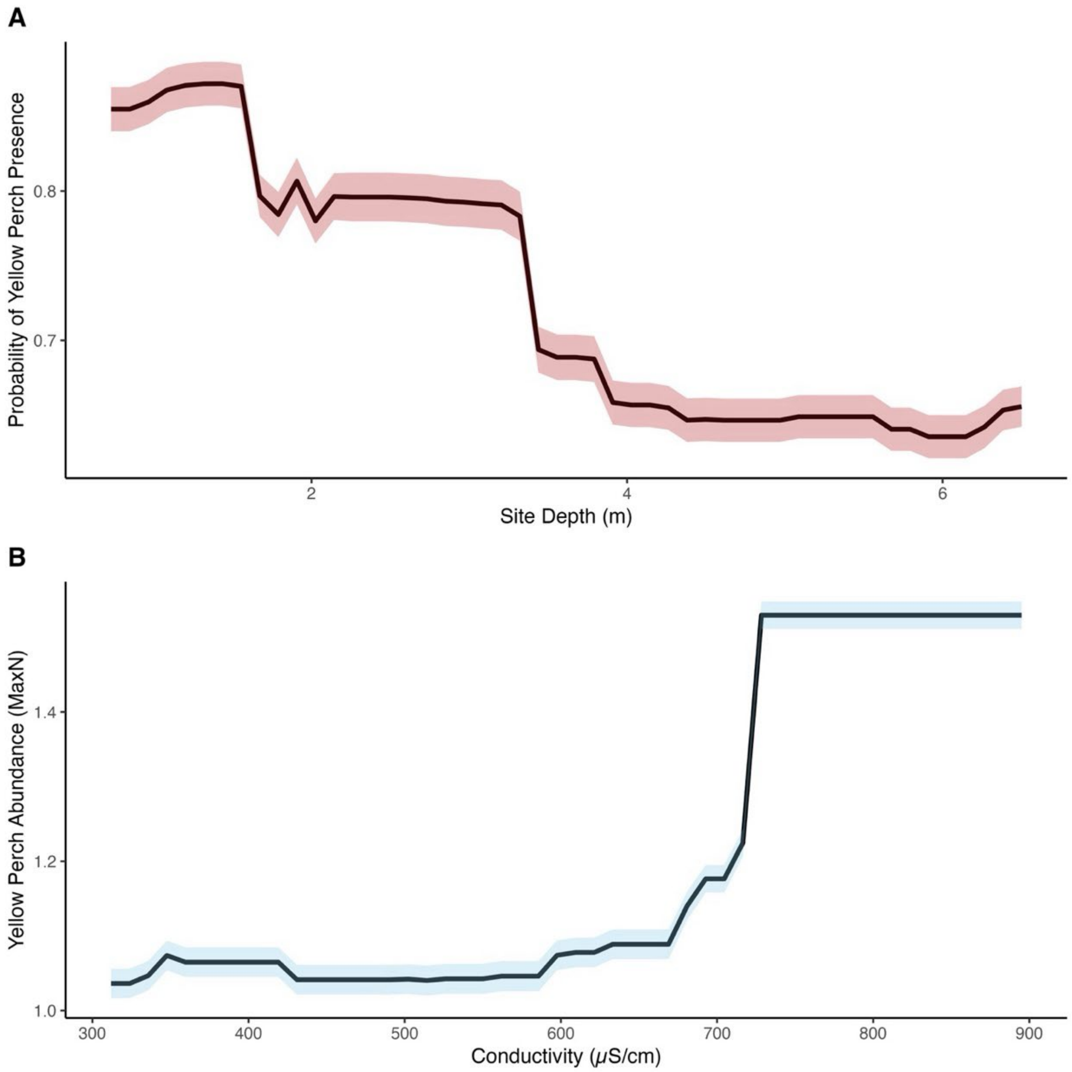

Figure 2.12. (A) Partial dependence plot for predicted Yellow Perch presence and site depth (m). (B) Partial dependence plot for predicted Yellow Perch MaxN and Conductivity (µS). Covariate measurements collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

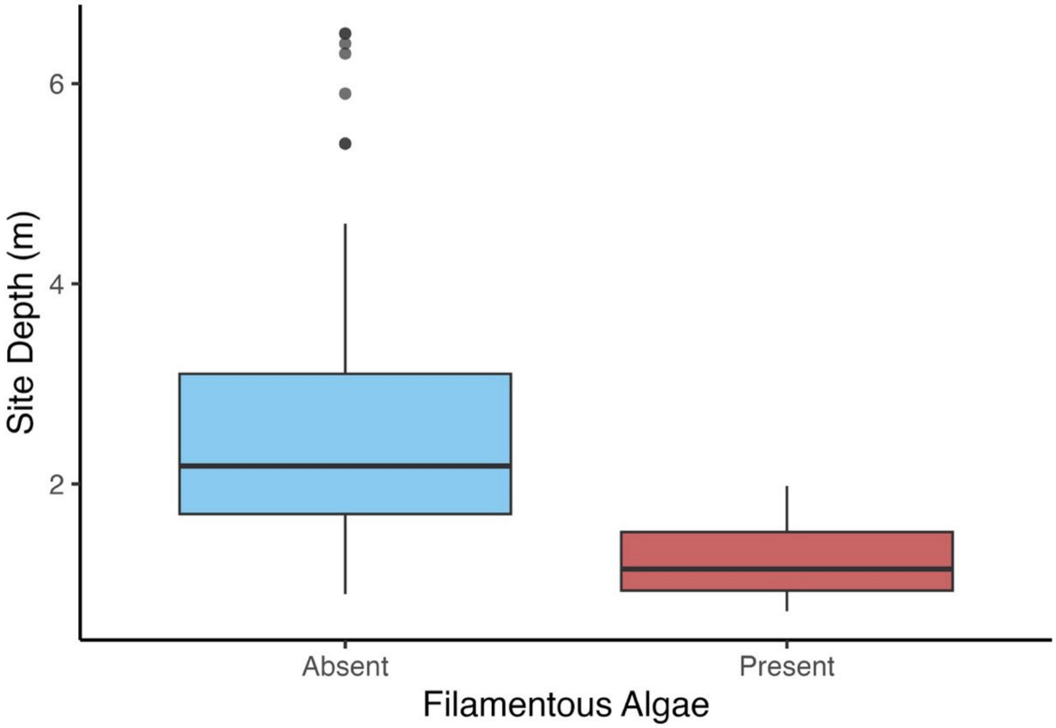

Figure 2.13. Filamentous presence and site depth. Boxes represent the 1st – 3rd quartile, with thick black line in representing median value. Box plot whiskers extend to the smallest and largest values that are not outliers. Outliers reported as dots beyond whiskers. Filamentous algae presence collected from each sampling site in four backwaters on Pools 7 and 8 of the UMR.

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