UNDERSTANDING SLIMY SCULPIN POPULATION DECLINES IN THE GREAT LAKES

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ABSTRACT

The Laurentian Great Lakes benthos has undergone a variety of changes related to invasive species, changing predator abundance, and shifting food webs that reduced the availability of native prey resources. Slimy sculpin (*Cottus cognatus*) were one of the most abundant benthic prey fish that were preyed upon by lake trout and burbot and slimy sculpin selectively fed upon on the historically abundant Diporeia (*Diporeia spp.*). Today, round goby (*Neogobius melanostomus*) and dreissenid mussels (zebra: *Dreissena polymorpha*; quagga: *D. bugensis*) are the dominant benthic prey fish and benthic macroinvertebrates and are hypothesized to be responsible for the decline of many native prey fish and macroinvertebrates. Within this dissertation we investigate hypotheses related to round goby competition, lake trout predation, or dreissenid mussel increases that could explain the recent declines in slimy sculpin abundance in many of the Great Lakes. In chapter 1, we observe competition over space and artificial spawning shelters between round goby and slimy sculpin in a laboratory setting at temperatures reflective of their offshore overlap. In chapter 2, we model slimy sculpin population dynamics at various regional ports trawl surveyed by the USGS dating from the 1970's to 2019 and test covariates related to changing lake trout predation pressure, round goby competition, and dreissenid mussel abundance. In chapter 3, we attempt to observe slimy sculpin and round goby presence/absence on rocky reef habitat in northern Lake Michigan with eDNA and camera drone surveys and report on fish community composition and laboratory experiments observing round goby and slimy sculpin eDNA shedding and decay rates. Overall, our goal was to better understand the potential drivers and mechanisms of slimy sculpin declines in the Great Lakes and provide information that could inform fisheries management about the potential to preserve native benthic prey fish diversity and the associated adaptive capacity of the Great Lakes.

PREFACE

The materials in chapter one are currently under review for publication in peer-reviewed journals and the language and formatting reflects the requirements of the submission process. The materials in chapters two and three were developed with the intention of refinement towards publication in peer-reviewed journals. First-person plural pronouns are used within the body of the chapters because multiple co-authors will be credited in publication.

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INTRODUCTION

The Laurentian Great Lakes have been subject to numerous anthropogenic perturbations, oftentimes related to aquatic invasive species. Most notably, the Great Lakes benthos have been reshaped by co-evolved Ponto-Caspian species (Lower et al. 2024) that have wide environmental tolerances and high phenotypic variability (Reid and Orlova 2002). Specifically, dreissenid mussels (hereafter zebra: *Dreissena polymorpha*; quagga: *D. bugensis*) and round goby (*Neogobius melanostomus*) have contributed to a nearshore shunt in nutrient cycling (Hecky et al. 2014, Stadig et al. 2020) and declines in native benthic invertebrates and prey fish (Janssen et al. 2001, Dermott et al. 2005, Barbiero et al. 2011, Kornis et al. 2012, Burlakova et al. 2018, Robinson et al. 2021). These species invasions vary across the lakes, but for most lakes, they were introduced in ports (Charlebois et al. 1997), proceeded to preferred nearshore hard substrates (Clapp et al. 2001, Kornis et al. 2012, Karatayev and Burlakova 2025), and have progressed offshore across all substrate types (Madenjian et al. 2015, Volkel et al. 2021, Jude et al. 2022, Karatayev and Burlakova 2025). Concurrently, the native deepwater fish community of the Great Lakes has declined in abundance in recent years (Riley et al. 2008, Gorman 2019, Robinson et al. 2021), and fisheries managers are concerned about the effects on ecologically linked predatory fishes such as lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) (Van Oosten & Deason 1938, Elrod and O'Gorman 1991, Madenjian et al. 1998, Weidel et al. 2019, Bunnell et al. 2020).

Slimy sculpin (*Cottus cognatus*), historically one of the most abundant native deepwater prey fish (Robinson et al. 2021), was an important forage fish for lake trout, particularly juvenile life stages (Madenjian et al. 1998). Slimy sculpin historically occupied habitat from nearshore rocky reefs to colder offshore habitat and are believed to rely on hard structure for shelter and spawning (Owens & Noguchi 1998). The shallower dwelling mottled sculpin (*Cottus bairdii*) has already been extirpated from parts of Lake Michigan, possibly due to round goby competition (Janssen et al. 2001), and there are concerns that slimy sculpin could be similarly affected because of pressure from round goby and native deepwater sculpin (*Myoxocephalus thompsonii*) (Volkel et al. 2021). Additionally, recent slimy sculpin declines could be related to increased lake trout predation from stocking and increased natural recruitment considering that historic declines in slimy sculpin abundance in Lakes Michigan and Ontario have been correlated with nearshore lake trout stocking and abundance (Owens and Bergstedt 1994, Madenjian et al.

2005). However, this hypothesis has not been revisited since stocking practices have shifted to offshore habitats (Holey et al. 1995), lake trout natural recruitment has surged in the lakes, and ecosystem dynamics have changed because of invasive species (Bunnell et al. 2014).

Within this dissertation, we aim to clarify some of the uncertainty related to the potential drivers of slimy sculpin abundance declines through a combination of controlled laboratory experiments, population dynamics modeling, and field observations of lesser studied and critical rocky reef habitats. In chapter 1, we observed slimy sculpin growth, shelter occupancy, chasing rates, spawning rates, and mortality rates with and without the presence of a round goby in a laboratory setting at cold water temperatures (5 °C) reflective of their potential offshore overlap. In chapter 2, we filled missing data from long-term benthic trawl survey data collected by the United States Geological Survey (USGS) with imputed values to develop observational time series of slimy sculpin density at ports spread throughout Lake Michigan and southern Lake Ontario and tested covariates reflecting lake trout, dreissenid mussels, and round goby as primary drivers of slimy sculpin population dynamics using Kalman filtering and smoothing. This approach allowed us to observe changes in slimy sculpin population depth structure and density through time and align them with observed invasion pathways and regionally varying lake trout stocking and recruitment. In chapter 3, we use metabarcoding and qPCR of eDNA and camera drone surveys of rocky reef habitat to estimate relative abundance and fish community composition. We used water samples from laboratory studies and experimental testing to evaluate the sensitivity and specificity of our qPCR assays and estimate rates of eDNA shedding and decay for slimy sculpin and round goby. The qPCR results for samples collected from Lake Michigan rocky reefs were analyzed using Bayesian hierarchical occupancy models (Dorazio and Erikson 2018) to understand the relationship between physical density of species and environmental covariates to determine the probability of detecting the species with eDNA.

Across all chapters, our goal was to better understand the potential drivers of slimy sculpin population declines and the potential mechanisms of round goby competition in offshore habitats where the species are expected to overlap, which could clarify uncertainty about the future function of the Great Lakes deepwater prey fish community and the potential for native species conservation.

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CHAPTER 1. COMPETITION BETWEEN ROUND GOBY AND SLIMY SCULPIN IN A LABORATORY SETTING

Abstract

Slimy sculpin (*Cottus cognatus*) abundance has substantially declined in many regions of the Laurentian Great Lakes coincident with the arrival and range expansions of invasive round goby (*Neogobius melanostomus*). Previous studies have found that round goby can outcompete native mottled sculpin (*C. bairdii*) for shelter and negatively affect native spoonhead sculpin (*Cottus ricei*) growth, but direct behavioral interactions of slimy sculpin and round goby have not been observed. In the Great Lakes, round goby overlap with slimy sculpin during spring spawning and offshore overwinter, which raises concerns that round goby competition could contribute to slimy sculpin decline. We conducted a laboratory experiment to observe slimy sculpin spawning success, aggressive interactions, artificial shelter occupancy, growth, and survival in the presence/absence of round goby at temperatures reflective of their potential offshore overlap and spawning activity in the Great Lakes. Slimy sculpin were less likely to occupy shelters and experienced increased intraspecific competition when round goby were present. However, round goby rarely chased slimy sculpin and slimy sculpin growth, spawning, and survival were not significantly different between groups. Cold temperatures reflective of spring deepwater conditions or slimy sculpin aggression may have mitigated round goby impacts, but the size advantages of round goby may still allow them to outcompete slimy sculpin for shelter. Our results indicate that round goby overlap could affect slimy sculpin through increased competition for shelter and space, but in the Great Lakes, food scarcity, round goby size advantages, and round goby densities may create greater stress for slimy sculpin than we observed.

Introduction

In the Laurentian Great Lakes, the establishment and spread of round goby (*Neogobius melanostomus*) has coincided with declining abundance of many native prey fish populations, including historically abundant sculpin species (Robinson et al. 2021). Round goby have been linked to declines in mottled sculpin (*Cottus bairdii*) abundance in Lake Michigan, potentially due to observed antagonistic interactions (chasing), competition for shelter, and egg predation

(Dubs and Corkum 1996, Janssen & Jude 2001, Lauer et al. 2004). Similarly, slimy sculpin (*Cottus cognatus*) abundance declinied coincident to round goby invasion and depth range expansion, raising concern that slimy sculpin abundance could continue to decrease if round goby populations continue to expand their depth range (Charlebois et al. 2001, Kornis et al. 2012, Robinson et al. 2021, Volkel et al. 2021). If so, we could expect the invasive round goby to further dominate the Great Lakes benthos, alter food web pathways, and reduce ecosystem resilience (Poos et al. 2010, Kornis et al. 2012, Rush et al. 2012, Lower et al. 2024).

Round goby and slimy sculpin likely overlap spatially throughout the winter in the Great Lakes because of round goby seasonal offshore migration (Carlson et al. 2021, Jude et al. 2022). However, round goby migrate from offshore to nearshore waters in spring slimy sculpin spawning is at its peak, and spawn multiple times from spring to fall (Kornis et al. 2012), and likely overlap significantly during their spawning periods (Selgeby 1988, Owens and Noguchi 1998, Kornis et al. 2012, Volkel 2019, Carlson et al. 2021). Round goby and slimy sculpin both prefer to shelter and spawn under rocks or other hard structures to avoid predation and guard their eggs, which increases the potential for spawning interference and habitat competition (Kornis et al. 2012, Robinson et al. 2021). Spatial overlap is somewhat mitigated once round goby fully migrate inshore for the summer to their preferred water temperatures between 15–28°C and their average depth of capture in the fall is ≈30m (Kornis et al. 2012, Andres et al. 2020, Christensen et al. 2021, Volkel et al. 2021, Behrens et al. 2022, Madenjian et al. 2022). Meanwhile, most slimy sculpin remain in relatively deeper waters (≥75m & ≈4–5°C; Beletsky & Schwab 2001, Volkel et al. 2021, Madenjian et al. 2022), but immature slimy sculpin are more likely to be nearshore closer to preferred temperatures that potentially maximize their growth rate (≈35m & 9–12°C; Otto and Rice 1977, Brandt 1986, Beletsky & Schwab 2001, Pennock et al. 2021).

In addition to possible competition for habitat, food resource competition between the species could also affect slimy sculpin populations. Round goby in the Great Lakes feed predominantly on dreissenid mussels (*Dreissena* spp.) and other bivalves in inshore waters, but they feed more heavily on *Mysis* in offshore waters (Walsh et al. 2008, Mychek-Londer et al. 2013, Bunnell et al. 2015, Pothoven 2018). Historically, slimy sculpin fed selectively on *Diporeia* (Kraft and Kitchell 1986, Hondorp et al. 2005, 2011, Davis et al. 2007), whereas deepwater sculpin (*Myoxocephalus thompsonii*) selected *Mysis*. More recently, slimy sculpin are increasingly reliant on *Mysis* as a prey resource (Owens and Dittman 2003, Walsh et al. 2008, Bunnell et al. 2015) ostensibly related to the substantial declines in *Diporeia* following dreissenid mussel invasion and subsequent nearshore shift in energy pathways (Wells 1980, Hecky et al. 2004, Nalepa et al. 2005, French III et al. 2010, Bunnell et al. 2015, Karatayev & Burlakova 2022). A previous study in Lake Ontario found that slimy sculpin fecundity was inversely related to slimy sculpin density, which may imply slimy sculpin populations were resource limited (Owens & Noguchi 1998). Today, slimy sculpin populations may be even more resource limited by nearshore shifts in energy pathways (Hecky et al. 2004, Dove & Chapra 2015). Therefore, when abundant round goby migrate offshore to overwinter, they may increase the energetic demand on slimy sculpin or outcompete them for *Mysis* and limit slimy sculpin growth and fecundity.

There is some evidence that slimy sculpin may be able to tolerate round goby and compete for space (Bergstrom and Mensigner 2009, Volkel et al. 2021). In artificial streams, slimy sculpin were able to maintain their weight in the presence of round goby during 21-day feeding trials, while spoonhead sculpin (*Cottus ricei*) and logperch (*Percina caprodes*) could not (Bergstrom and Mensinger 2009). Additionally, Volkel et al. (2021) hypothesized that slimy sculpin would shift into deeper waters to avoid round goby in Lakes Ontario, Michigan, and Huron, but they only found evidence of this in Lake Huron. Volkel et al. (2021) emphasized the importance of understanding behavioral interactions between these species to understand the risk of competition, but to date, there have been no direct observations of slimy sculpin and round goby interactions.

Overall, there is concern that round goby competition could be a driving factor in slimy sculpin declines in the Great Lakes, but uncertainty remains around the exact mechanisms, and the potential effects of other ecological factors (Volkel et al. 2021). Therefore, our study sought to observe round goby and slimy sculpin interactions and their consequences at temperatures reflective of their offshore overlap and preferred spawning temperatures. To do this, we conducted a laboratory experiment in which we evaluated the behavior of slimy sculpin pairs in the presence or absence of round goby at 5°C and documented shelter occupancy, aggressive interactions, spawning, growth, and mortality. We hypothesized that round goby are more aggressive competitors for space and predicted that round goby would chase slimy sculpin and decrease slimy sculpin shelter occupancy, growth, spawning activity, and survival.

Methodology

Specimen collection and husbandry

Round goby and slimy sculpin were collected during standardized spring benthic trawl surveys in Lakes Michigan and Superior (For additional details see; Tingley et al. 2020, Vinson et al. 2023). Fish were transported from the field to the laboratory in coolers maintained near a target temperature of 5 ±1ºC. All experiments were conducted at the U.S.G.S. Great Lakes Science Center (Ann Arbor, MI) and fish were housed beginning in May with experiments ending in August. Holding tanks in the laboratory were recirculating systems with mechanical, chemical, biological, and ultraviolet (UV) filtration. Holding tanks contained PVC pipes for shelter, had sand-covered bottoms, were mostly covered to limit light exposure, and room light and dark cycles mimicked the natural light cycles outside the building (Ann Arbor, MI). Fish in holding tanks were fed frozen *Mysis* (Brine Shrimp Direct; Ogden, Utah, USA) to satiation every weekday, and tanks were checked for mortalities and temperature daily. All fish were acclimated to the laboratory setting in the holding tanks for at least 30 days prior to trials in experimental tanks. All captures, transport, holding, and experiments conformed to Michigan State University IACUC animal standards and protocols (#PROTO202000106).

Competition trials

Twenty 75-liter tanks (53 cm L x 28 cm W x 40 cm H) were set up asynchronously with 10 treatment tanks (slimy sculpin pair and one round goby) and 10 control tanks (slimy sculpin pair absent round goby). Each tank contained one artificial spawning shelter with a plexiglass ceiling covered by plastic mesh to encourage fish to use the shelter for hiding while maintaining our ability to observe fish inside (Figures S1A & B). Tanks were maintained at 5 ±1ºC on the same recirculating systems with mechanical, chemical, biological, and ultraviolet (UV) filtration as holding tanks and had sand-covered bottoms. Tanks were mostly covered by a blockout board excluding the water intake area and had no cover during video recordings (≈1 h) approximately three times per week. The rooms' light and dark cycles mimicked the natural light cycles outside the building (Ann Arbor, MI).

Slimy sculpin were paired according to body size with the goal of matching a male and female together, but sexing sculpin proved difficult at smaller sizes when reproductive features were undeveloped. Round goby were introduced to treatment tanks simultaneous to slimy sculpin and were also selected to be of approximately similar size to their corresponding slimy sculpin pair. All mortalities throughout the experiment were replaced with fish of the closest size available. Upon fish introduction, we also noted 11 gravid female sculpins that were split between the control (n=6) and treatment tanks (n=5). All fish were weighed (0.01g) and measured for total length (mm) before being placed in tanks and again at the end of the experiments to determine growth. All weight measurements were wet weights to reduce handling time and stress on fish.

Each tank was fed frozen *Mysis* every weekday with daily serving sizes representing ≈3% of the total initial body mass of the fish in the tank, which is greater than observed daily consumption rates in Lake Michigan (0.2–0.8%; Mychek-Londer & Bunnell 2013) and comparable to the average stomach contents of slimy sculpin in Lake Superior (Selgeby 1988). Each tank was recorded for one hour approximately 3 times per week starting roughly 1–2 hours after feeding using a GoPro (San Mateo, CA) positioned above the tanks. All tanks were checked daily for mortalities, spawning activity, and temperature, and excess food was removed from the tank every weekday. All trials ran for a minimum of one month but varied in length from 33–63 days because of asynchronous setups. At the end of the experiment, fish were euthanized and sexed via internal assessment (male, female, or immature).

Video analysis of aggressive interactions and space occupancy

All video footage was collected during the daytime shortly (1–2 h) after feeding. We processed ≈180 hours of usable footage across all tanks in which we confidently could track all fish in a tank throughout the recording. Not all tanks had equal amounts of usable footage, but all tanks had at least four days of footage (≈1 h each day) during a month-long period that began when all tanks were set up. Footage was reviewed and summarized for the time that each fish spent in, out, or on top of the shelter. Initial positional locations and species identifications were made in-person and pointed out on the recording for the footage observer. Time spent in a position was recorded per fish and movement between positions was recorded if the positional change lasted more than 30 seconds to avoid conflating continuous chasing movement with an ultimate positional change. Chases were counted for each fish and summarized by species. Chases were defined as one fish rapidly swimming towards another (Lachance et al. 2010) and were recorded for each fish as either being chased or chasing. Continuous stop and go chases were only counted once every 30 seconds that it lasted. An example of typically observed chasing behavior and space occupancy of control and treatment tanks is found in the Electronic Supplementary Material (ESM).

Statistical analysis

We used randomization testing to analyze the behavioral and performance metrics of slimy sculpin pairs across control and treatment groups. Randomization testing is relatively robust to small datasets and makes limited assumptions about response variable probability distributions, which is beneficial for behavioral datasets that often have limited sample sizes and response variable data do not conform to a normal probability distribution (Adams & Anthony 1996; Craig & Fisher 2019). In our study, the units of replication were slimy sculpin pairs in a tank, and we randomized our response data by resampling original group labels without replacement for 999 iterations. Approximate p-values were calculated by determining the proportion of values representing the difference in mean responses between randomized groups that were greater or less than the observed difference in mean response. The measured response for testing chasing behavior was the average number of times a slimy sculpin was chased per hour in a tank. The measured response for testing shelter occupancy was the average proportion of time a slimy sculpin spent in a shelter in a tank. The measured response for testing slimy sculpin growth was the average relative growth rate (length & weight; Hopkins 1992) per day of a slimy sculpin in a tank. The measured response for testing spawning activity was the probability of slimy sculpin eggs being laid in any tank with female slimy sculpin present. All analyses were conducted in R (R Core Team, 2025). The predictions tested and their corresponding measured responses, test statistic procedures, and approximate p-values are summarized in Table 1.

Table 1. Predictions of slimy sculpin responses to round goby presence in laboratory studies designed to observe competition for spawning shelters and aggressive behavior between these species. The associated measured responses, test statistic, and estimated p-value from randomization testing. Asterisk denotes a significant result.

Predictions	Measured Response	Test Statistic	Result
Slimy sculpin chased more with round goby present	Average slimy sculpin chased rate per hour	Difference in group means	p=0.003*
Slimy sculpin spend less time in shelter with round goby present	Average proportion of time slimy sculpin spent in a shelter	Difference in group means	p=0.017*
Slimy sculpin growth decreases with round goby present	Average slimy sculpin relative (%) total length and weight change per day	Difference in group means	Length p=0.132 Weight p=0.214
Slimy sculpin spawning decreases with round goby present	Probability of eggs laid when female slimy sculpin present	Difference in group means	p=0.278
Slimy sculpin survival decreases with round goby present	Slimy sculpin mortality	Not tested because no mortalities in treatment group	N/A

Results

Slimy sculpin were chased significantly more in the presence of round goby (p=0.003; Tables 1 & 2). However, almost all chases (97.5%) that slimy sculpin experienced in the presence of round goby were slimy sculpin chasing each other, and slimy sculpin were 50% more likely to chase round goby than vice versa. The degree to which slimy sculpin were chased varied widely, with no chases recorded in ≈69% of daily footage in control tanks and ≈50% of daily footage in treatment tanks, and a maximum of 13 (control) and 22 (treatment) chases of slimy sculpin were observed in any daily footage across all tanks (Fig. 1).

Table 2. Average size, size range (), chasing, and chased rates of slimy sculpin and round goby across all daily footage collected in our laboratory study designed to observe competition for spawning shelters and aggressive behavior between these species. The control group is a pair of slimy sculpins without a round goby present and the treatment group is a pair of slimy sculpins in the presence of a round goby.

Species	Group	Length (mm)	Weight (g)	Chasing (#/hr)	Chased (#/hr)
Slimy Sculpin	Control	70.9 (56–93)	4.2 (1.9–10.3)	1.1	1.1
Slimy Sculpin	Treatment	74.6 (57–90)	4.9 (2.4–10.3)	2.5	2.6
Round Goby	Treatment	82.4 (64–104)	8.35 (3.3–15.7)	0.1	0.2

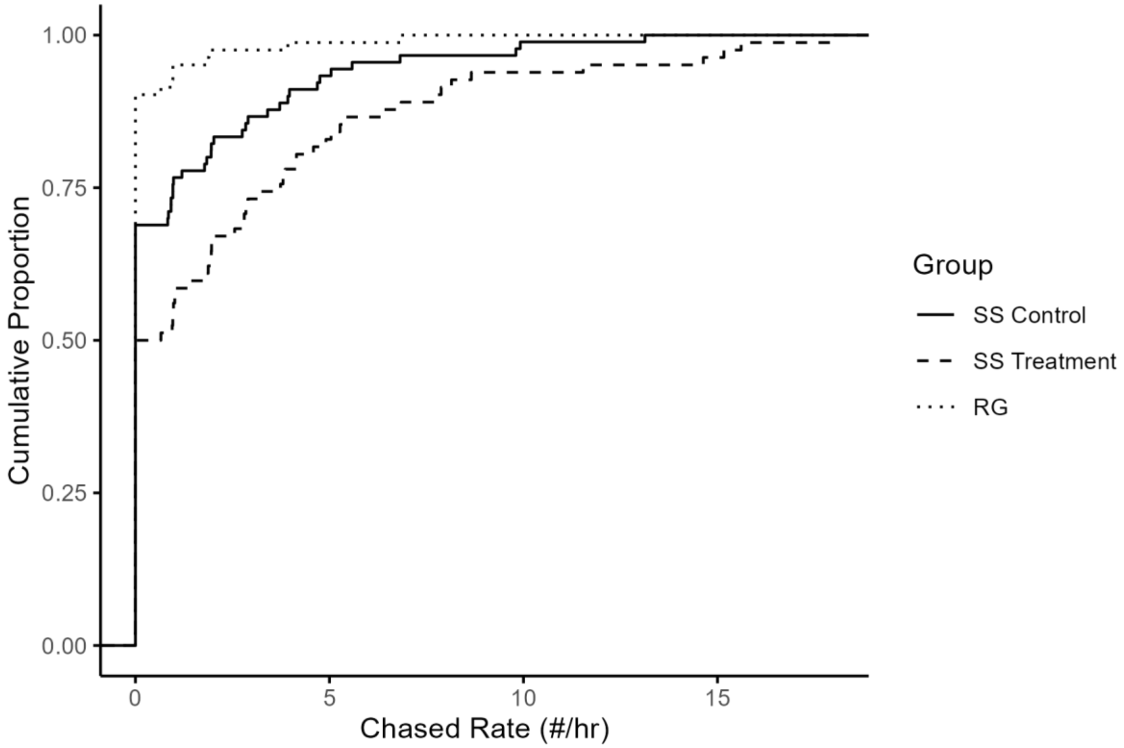

Figure 1. Cumulative distribution of all daily chased rates for the average fish in laboratory experiments conducted to understand spawning shelter competition and aggressive behavior between round goby (RG) and slimy sculpin (SS). The control group is a pair of slimy sculpins without a round goby present and the treatment group is a pair of slimy sculpins in the presence of a round goby.

Despite the limited chasing by round goby, slimy sculpin spent approximately 33% less time in shelters in the presence of round goby (Table 1, p=0.02), with round goby spending most of their time in shelters (Fig. 2A). The shelter was occupied by at least one slimy sculpin 87% of the time when round goby was absent and 54% of the time when round goby was present. Oftentimes when slimy sculpin were occupying the shelter in treatment tanks, they were sharing it with round goby (≈58%), usually it was the smaller sculpin, presumably trying to avoid being chased by the larger sculpin. The dominant slimy sculpin would occupy the shelter at similar rates between control and experimental tanks when the round goby was smaller than the dominant sculpin, but shelter occupancy was at least 70% less for the dominant sculpin in experimental tanks when the round goby was larger (Fig. 2B).

Although efforts were made to ensure all fish in a tank were of similar size, slimy sculpin average length was smaller than that of round goby (Tables 1 & 2), and round goby mortality often resulted in replacement with a larger round goby. Slimy sculpin average specific growth rate was roughly halved in the presence of round goby (Fig. 3), but this result was not statistically significant (Table 1, length p=0.13, weight p=0.21).

Slimy sculpin eggs were laid in 2 of 7 control tanks with female slimy sculpin and in 0 of 6 treatment tanks with female slimy sculpin, but this result was not statistically significant (Table 1, p = 0.28). The two largest female slimy sculpin in the control tanks spawned (87 and 93mm) and of the two largest female slimy sculpin in treatment tanks (85 and 89mm), one was gravid and the other was not gravid at the end of the experiment. We observed two slimy sculpin mortalities in control tanks, but no slimy sculpin died in the presence of round goby.

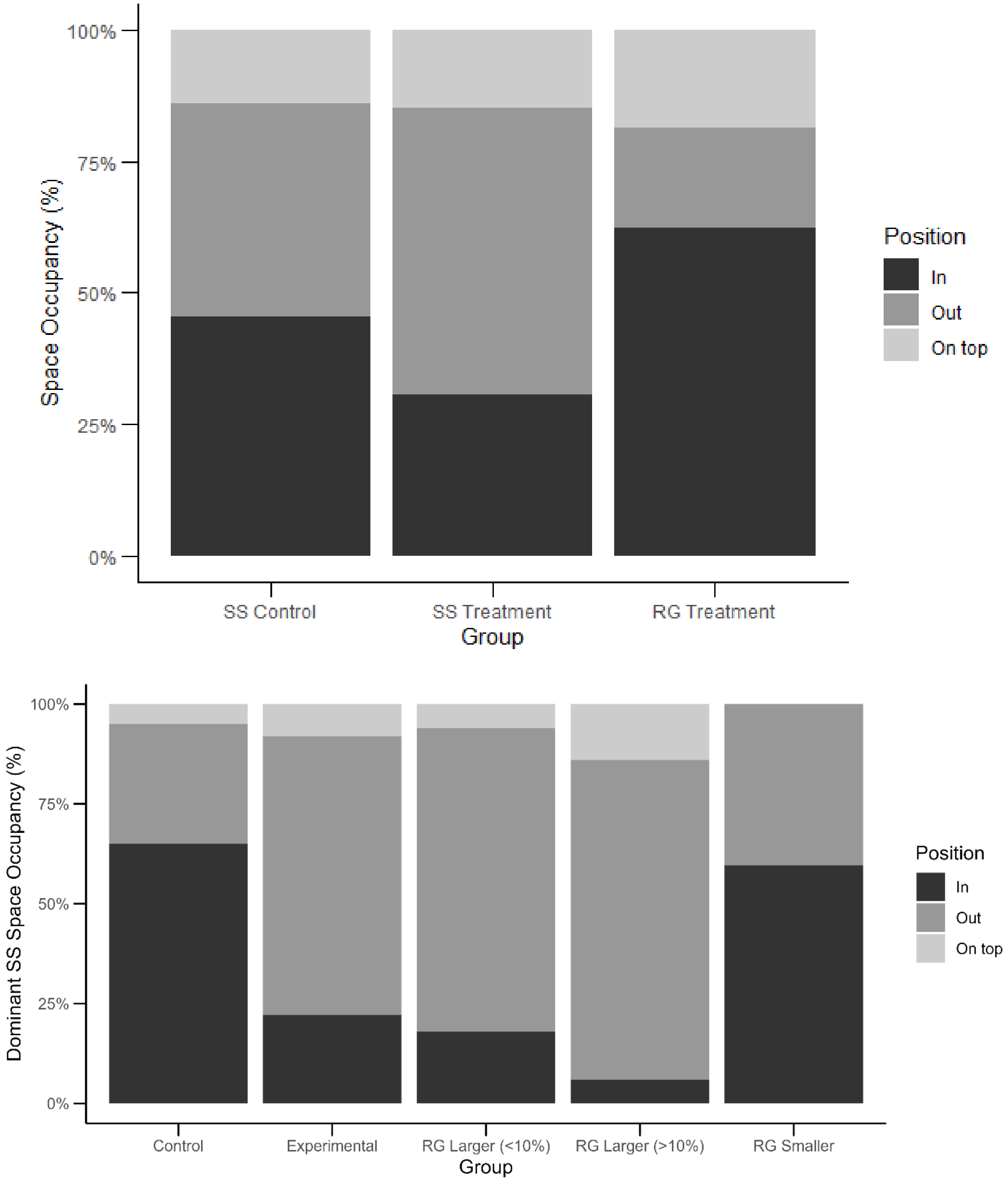

Figure 2. Average time (%) spent inside ("in") on top of ("on top"), or outside ("out") of an artificial spawning shelter (A: top) by the average slimy sculpin (SS) and round goby (RG) (B: bottom) and the dominant slimy sculpin depending on the experimental group and relative size difference (mm%) between the dominant slimy sculpin and round goby. Laboratory experiments were designed to observe the influence of round goby and slimy sculpin competition. The control group is a pair of slimy sculpins without a round goby present and the treatment group is a pair of slimy sculpins in the presence of a round goby. The dominant slimy sculpin was not identifiable in all daily footage, but a dominant slimy sculpin is classified as the one chasing other fish the most.

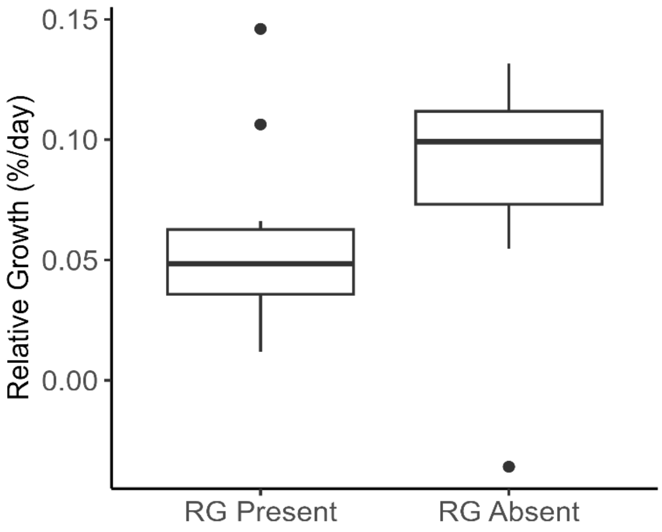

Figure 3. Distribution of average slimy sculpin relative growth rate (% length change per day) in laboratory experiments conducted to understand the impacts of spawning shelter competition and aggressive behavior between round goby (RG) and slimy sculpin. The control group is a pair of slimy sculpins without a round goby present (RG Absent) and the treatment group is a pair of slimy sculpins in the presence of a round goby (RG Present).

Discussion

Contrary to expectations, we observed limited evidence of round goby aggression toward slimy sculpin in this experiment. Although we did observe that round goby significantly affected slimy sculpin shelter occupancy, we did not detect significant effects of round goby on slimy sculpin growth, spawning activity, or survival. Furthermore, round goby rarely chased slimy sculpin; instead, slimy sculpin were more likely to chase round goby. It is important to note our experimental design cannot distinguish between the effect of increased fish density and round goby specific effects. However, abundant round goby populations seasonally migrating between nearshore and offshore is the most common sudden change in benthic prey fish community that slimy sculpin populations are likely to experience in the Great Lakes (Jude et al. 2022). Nevertheless, our study shows that an invading round goby could outcompete slimy sculpin for shelter if resources are limited, which has potential implications for spawning efficiency, even if aggressive interactions between round goby and slimy sculpin are muted by cold waters or slimy sculpin aggression.

Cold water temperatures in our study may have mitigated round goby aggression toward slimy sculpin. Round goby aerobic performance decreases notably at and below 10°C (Christensen et al. 2021) and round goby are cued to spawn between 9 and 26°C (Charlebois et al. 1997). Conversely, slimy sculpin prefer temperatures between 8 and 12°C and peak slimy sculpin spawning in Lake Ontario occurs around 4 – 5°C (Owens & Noguchi 1998). Previous laboratory studies observing round goby aggression were conducted between 8 and 18°C (Dubs and Corkum 1996, Janssen and Jude 2001). We chose 5°C because it likely reflects conditions during a critical period where round goby overlap with slimy sculpin as they overwinter and leading up to spring spawning. Our observations of slimy sculpin in spawning coloration and eggs being laid, and a lack of round goby in spawning coloration, indicates that our temperature selection was appropriate to mimic previous observations of natural responses.

The reduction in slimy sculpin shelter occupancy observed in our experiment could be exacerbated in the Great Lakes when round goby size advantage and abundance is greater than we observed. We hypothesize that the round goby size advantage allowed them to spend most of their time in shelters as slimy sculpin were often moving away from larger round goby without chases being initiated and fish are known to evaluate size differences prior to initiating aggression (Damsgård & Huntingford 2012). Some evidence suggests that aggressiveness can outcompete body size advantages up to 10% in fish with overlapping niches (Sanches et al. 2012), but in our experiment, body size appears to determine competitive advantages for space occupancy. In the offshore habitats of Lake Michigan, the average round goby was ≈30mm larger than the average slimy sculpin, and the largest round goby was ≈65mm larger than the largest slimy sculpin (Mychek-Londer 2011). Additionally, we would expect round goby to considerably outnumber slimy sculpin in many areas of the Great Lakes based on observations from remotely operated vehicles and density estimates from long-term trawl surveys (Jude et al. 2022, Warner et al. 2022). Altogether, if large and abundant round gobies overlap with slimy sculpin during spawning, increased competition for shelter could result in spawning interference for slimy sculpin or exclusion to sub-optimal habitat. To strengthen our hypothesis, further study with more realistic laboratory settings or in situ experiments in the Great Lakes would be beneficial.

Our observations of slimy sculpin aggressive behavior may help explain why slimy sculpin outperformed other native benthic fish species in the presence of round goby (Bergstrom and Mensinger 2009). We also did not observe a significant difference in slimy sculpin average growth rate in the presence of round goby (Bergstrom and Mensinger 2009), but average slimy sculpin growth rate was roughly halved. However, both studies fed fish to satiation, so differences in growth likely reflected changes in stress or energetic demand rather than competition for limited food. In our study, increased intraspecific aggression among slimy sculpins could have increased energetic demand, but not enough to significantly reduce growth when food was abundant. In the Great Lakes, there is evidence that slimy sculpin populations were resource limited prior to round goby arrival (Owens & Noguchi 1998, Owens & Dittman 2003). Therefore, when round goby occupies optimal habitat and compete for limited *Mysis*, the consequences for slimy sculpin growth may be greater than what we were able to observe.

We did not observe slimy sculpin spawning in the presence of round goby, and spawning occurred in ≈28% of control tanks with female slimy sculpin, but differences in spawning rates were not significant. Previous field observations noted that female slimy sculpin were consistently gravid or spent at ≥85mm (Owens & Noguchi 1998), and there were only two female slimy sculpin in each group above this threshold size, of which, the females in control tanks both spawned. It is possible that round goby interfered with slimy sculpin spawning, but a larger sample size and further study focused on larger (≥85mm) female slimy sculpin responses to round goby would be required to validate an effect. Additionally, a control group of three slimy sculpin compared to an experimental group of two slimy sculpin and a round goby would better identify the effects of density versus species composition. Of the two slimy sculpin egg masses we observed, one was laid early in the experiment and was guarded and fanned by the male slimy sculpin with the egg mass hatching successfully. The other was laid near the end of the experiment; the slimy sculpin was removed for measurement and eggs developed a fungus and did not hatch. These observations are comparable to what was observed with fourhorn sculpin (*Myoxocephalus quadricornis*) (Westin 1969) and highlight the risks of shelter displacement of slimy sculpin during spawning periods.

A concern with round goby establishment and expansion in the Great Lakes is increasing overlap with slimy sculpin, which may cause increased competition for food, space, and result in spawning interference (Poos et al. 2010, Kornis et al. 2012). We found evidence of round goby outcompeting slimy sculpin for shelter and causing increased intraspecific competition among slimy sculpin in sub-optimal habitat. Considering the observed role of hard structure being associated with slimy sculpin spawning (Owens & Noguchi 1998), we believe increased competition for shelter could result in reduced spawning efficiency, but our study did not have enough mature females to validate this prediction. Slimy sculpin aggression and colder temperatures could limit the severity of round goby effects, but in the Great Lakes, round goby advantages and competition for food may have a greater effect on slimy sculpin than we were able to explore in this study.

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CHAPTER 2. SLIMY SCULPIN POPULATION DYNAMICS IN LAKES MICHIGAN AND ONTARIO

Abstract

Slimy sculpin (*Cottus cognatus*) populations in many of the Great Lakes have been declining coincident to major ecological changes driven by species invasions and lake trout recovery. We tested three hypothesized primary drivers of recent slimy sculpin declines using time series data collected from long-term benthic trawl survey data from Lake Michigan and southern Lake Ontario: 1. increased lake trout (*Salvelinus namaycush*) predation, 2. increasing round goby (*Neogobius melanostomus*) competition, and 3. increasing dreissenid mussel (zebra [*Dreissena polymorpha*] and quagga [*D. bugensis*]) abundance. Specifically, we standardized observation data with delta generalized additive models and estimated slimy sculpin density at ports within lakes and through time using Kalman filtering and smoothing. Our best performing models found correlations between slimy sculpin declines and the spread of dreissenid mussels, specifically quagga mussels, and round goby that aligned with their known invasion pathways and observed high densities. We found some support ($\Delta AIC \leq 2$) for lake trout as a primary driver of modern slimy sculpin declines at one port in northern Lake Michigan that borders lake trout refuge habitat, and we observed historic trend changes in Lake Michigan slimy sculpin densities that aligned with regional stocking practices. Additionally, we observed changes in slimy sculpin spring depth distribution that aligned with dreissenid mussel and round goby invasion in southern Lake Ontario that suggest an initial shift away from nearshore (<40m) habitats followed by disappearance at depths <100m. These findings suggest slimy sculpin in southern Lake Ontario and most of Lake Michigan, except western mid-lake Michigan, may continue declining towards near zero densities or extirpation. The Great Lakes benthos could have lost some of the historical ecological functions performed by slimy sculpin. Given that observed declines align with species invasions, managers may need to increasingly focus on the dynamics of benthic food webs dominated by round goby and dreissenid mussels.

Introduction

Many recent perturbations in the Great Lakes ecosystems have altered the function and energetic pathways of pelagic and benthic ecosystems (Mills et al. 2003, Johnson et al 2005, Bunnell et al. 2014, Turschak et al. 2014, Madenjian et al. 2015, Ives et al. 2019). Within the

Great Lakes benthos, slimy sculpin (*Cottus cognatus*) abundance has decreased, potentially related to changes in food availability, competitive interactions, recruitment, and predation (Madenjian et al. 2005, Weidel et al. 2019, Robinson et al. 2021). These pressures are hypothesized to be linked to the invasion of round goby (*Neogobius melanostomus*) and dreissenid mussels (hereafter zebra: *Dreissena polymorpha*; quagga: *D. bugensis*), the collapse of *Diporeia spp*. (hereafter *Diporeia*), and variable lake trout (*Salvelinus namaycush*) stocking and recruitment (Madenjian et al. 2005, Nalepa et al. 2009, Barbiero et al. 2012, Bunnell et al. 2015, Robinson et al. 2021, Volkel et al. 2021). These perturbations have occurred during a period in which slimy sculpin populations have declined to historic lows (Robinson et al. 2021) and distinguishing among these potential primary drivers of slimy sculpin declines in the Great Lakes remains a challenge (Weidel et al. 2019, Bunnell et al. 2020, Volkel et al. 2021).

Prior to these perturbations, slimy sculpin were among the most abundant benthic prey fish in Lakes Michigan and Ontario, providing an energetic link between benthic invertebrates and native predators [e.g., juvenile lake trout and burbot (*Lota lota*)] (Van Oosten & Deason 1938, Elrod & O’Gorman 1991, Madenjian et al. 1998, Weidel et al. 2019, Bunnell et al. 2020). Today, slimy sculpin population abundances have declined to record lows, while invasive round goby are abundant in the diets of adult and juvenile lake trout (Happel et al. 2018, Luo et al. 2019, Nawrocki et al. 2022, Leonhardt et al. 2024). Some studies suggest that historic increases in lake trout biomass have led to declining slimy sculpin abundance in Lakes Michigan and Ontario (Christie et al. 1987, Owens & Bergstedt 1994, Madenjian et al. 2005), but it is unclear if this primary driver is as strong under current ecological conditions.

In Lake Michigan, lake trout abundance has increased due to a combination of stocking, changes in the targeted areas for lake trout recovery, and increased natural recruitment (Holey et al. 1995, Bronte et al. 2007, Patterson et al. 2016, Madenjian et al. 2023). The 1985 Lake Trout Restoration Plan (LMLTTC 1985) shifted stocking efforts from nearshore habitats, to offshore refuge and reef habitats, particularly in the Northern, Southern, and Clay Banks refuges, and imposed stricter harvest regulations (Holey et al. 1995). Other reefs in primary and secondary habitat received fewer stocked fish and less harvest restrictions (Holey et al. 1995), but these stocking efforts led to lake trout recruitment lake-wide (Hanson et al. 2013, Patterson et al. 2016, Madenjian et al. 2023). Given the nature of lake trout conservation action and recovery, it is possible slimy sculpin populations may face predation from both stocked and wild juvenile lake trout in areas with greater recruitment or stocking pressure. Therefore, this effect may be observable at a regional scale.

In addition to predation by native fishes, slimy sculpin in lakes Michigan and Ontario may be negatively affected by three prolific benthic invasive species from the Ponto-Caspian region: round goby and dreissenid mussels (zebra and quagga) (Vanderploeg et al. 2002). Round goby are known competitors for habitat with some native sculpin species (e.g., mottled sculpin: *Cottus bairdii*, spoonhead sculpin: *Cottus ricei*) and appear to be extending their spatial overlap with slimy sculpin (Janssen & Jude 2001, Bergstrom & Mensinger 2009, Robinson et al. 2021, Volkel et al. 2021). Round goby may threaten slimy sculpin at all life stages via competition for shelter, food, aggressive interactions, and egg predation (Janssen & Jude 2001, Bergstrom & Mensinger 2009, Kornis et al. 2012, Chapter 1). Historically, slimy sculpin spawned in habitats <75 m in depth during spring and summer and may rely on hard structures for spawning (Owens & Noguchi 1998). This same depth range is now occupied by round goby (Volkel et al. 2021), which also prefer hard substrate for spawning and shelter (Charlebois et al. 2001). It remains unclear whether slimy sculpin can adapt by spawning offshore (Volkel et al. 2021), and the degree of overlap and spawning interference may vary seasonally (Carson et al. 2021, Volkel et al 2021). However, round goby may also restrict spawning potential by competing with slimy sculpin for scarce food resources offshore over winter (Carlson et al. 2021, Pennuto et al. 2021, Jude et al. 2022).

Prior to the Great Lakes invasion by dreissenid mussels and round goby, slimy sculpin selectively preyed upon *Diporeia* whose availability was linked to slimy sculpin density (Wells 1980, Kraft & Kitchell 1986, Hondorp et al. 2005, Davis et al. 2007, Hondorp et al. 2011). Following the collapse of *Diporeia* in lakes Michigan and Ontario, slimy sculpin increasingly have relied on *Mysis diluviana* (hereafter *Mysis*), which are also consumed by other native and invasive fishes (Owens & Dittman 2003, Walsh et al. 2008, French et al. 2010, Bunnell et al. 2015). Round goby diets vary by depth, with individuals consuming primarily dreissenid mussels in nearshore habitats and greater proportions of *Mysis* in offshore habitats (Schaeffer et al. 2005, Walsh et al. 2007, Perello et al. 2015). Therefore, round goby may be significant competitors with slimy sculpin for winter forage. Previous evidence and our results suggest slimy sculpin are somewhat resilient to round goby invasion compared to other native benthic species (Bergstrom & Mensinger 2009, Chapter 1), but larger round goby can displace slimy sculpin from shelter

(Chapter 1). However, laboratory inferences should be treated cautiously without validation from field observations of negative interactions or negative relationships predicted from ecological time series analysis.

Round goby and quagga mussels invaded the Great Lakes during similar time periods, are ecologically linked, and co-evolved in the Ponto-Caspian region. As such, disentangling their individual effects may be challenging. Round goby populations were initially detected in southern Lake Michigan near Chicago, Illinois, in 1993 (Clapp et al. 2001) and in Lake Ontario by 1995 (Bunnell et al. 2020). However, they did not appear in prey fish trawl surveys until 2003 in Lake Michigan (Bunnell et al. 2020) and 2005 in Lake Ontario (Weidel et al. 2019). Zebra mussels established in Lakes Michigan and Ontario in 1989 (Hebert et al. 1991, Griffiths et al. 1991), but were largely replaced by the deeper-dwelling quagga mussels by the mid-2000s (Wilson et al. 2006, Nalepa et al. 2009). Quagga mussels have profoundly altered energy pathways in the Great Lakes and are hypothesized contributors to the collapse of *Diporei*a (Vanderploeg et al. 2002, Nalepa et al. 2009, Foley et al. 2014, Madenjian et al. 2015, Deroy et al. 2020), although the exact mechanism is unknown. They colonize almost any substrate, forming a benthos that can hinder slimy sculpin foraging efficiency (Beekey et al. 2004). If one or both of these invaders are affecting slimy sculpin populations, an analytic assessment may help parse through the spatial and temporal variability in population trends and allow us to consider the discrete timings and invasion pathways.

We hypothesize that three primary drivers could explain the current declines of slimy sculpin populations in Lakes Michigan and Ontario: 1. Lake trout, particularly juvenile lake trout abundance, has recently increased due to stocking and wild recruitment and may align with current slimy declines. 2. Round goby abundance has recently increased and may increase spawning interference, competition for habitat and food, and predation for slimy sculpin populations and cause slimy sculpin population declines. 3. Dreissenid mussels, particularly the dominant and deeper dwelling quagga mussel, have recently increased and could be causing slimy sculpin declines because of reduced foraging efficiency and declines in slimy sculpin's preferred prey, *Diporeia*. Our objective was to evaluate the relative support for each primary driver by analyzing long-term benthic trawl survey data for slimy sculpin density at regionally distributed ports, alongside long-term monitoring data for lake trout, round goby, and dreissenid mussel populations. Specifically, we first used delta generalized additive models to fill missing observation data across the survey extent (via spatial and temporal interpolation) and computed average slimy sculpin density across their depth distribution over time. Next, we modeled slimy sculpin population dynamics and tested covariates using Kalman filtering and smoothing. Ultimately, this approach aimed to identify correlations between slimy sculpin populations and proposed ecosystem drivers that may have affected slimy sculpin populations variably within and across lakes because of variable invasion pathways and targeted lake trout recovery efforts.

Methods

Description of Data Sources

We used data from annual bottom trawl surveys of Lakes Michigan and Ontario conducted by the United States Geological Survey (USGS; for additional details see Gorman et al. 2019) for measures of slimy sculpin, round goby, lake trout, and dreissenid mussel density (Table 2.1). Trawl survey depth ranges differed between lakes and varied in timing and gear used, but tows were conducted along depth contours ranging from the nearshore to the offshore (O'Malley et al. 2021; Tingley et al. 2021). Lake Michigan trawl surveys were conducted at seven ports (Fig. 2.1) in the fall from 1973 to 2019 and consistently covered depth ranges from 18m to 91m depth in ≈9m increments, as well as at 110m and 128m depth. Lake Ontario trawl surveys were conducted at six ports (Fig. 2.1) in the spring from 1979 to 2020 and covered depth ranges from 15m to 95m in 10m increments, as well as 110m to 150m in 15m increments. Covariate values were averaged across all tows from a port in a year. Dreissenid mussel captures in trawl surveys were incidental and are likely biased low because nets that became too heavy with dreissenid mussels were discarded in the field (R. Tingley, USGS, oral comm., 2025). In addition to overall density, dreissenid mussel densities at 60m of depth or greater were summarized to potentially better reflect offshore overlap of slimy sculpin and quagga mussels, as mussels collected in trawl surveys were not identified to species.

Estimates of juvenile lake trout (age 0–3) abundance between 1985 and 2019 in Lake Michigan were supplied by the Modeling Subcommittee and Lake Michigan Technical Committee (Janssen 2022) from statistical catch at age models at regional levels; estimates included both wild and stocked individuals. Lake trout abundances per statistical district (Gordon et al. 2021) were standardized to density (#/ha) using surface areas of the districts and ports along the western shore of Lake Michigan (Waukegan, Port Washington, Sturgeon Bay) were within the same district, but Ludington, Frankfort, and Manistique had unique districts.

Table 2.1. Summary of the data sources used for population dynamics modeling of slimy sculpin density in Lake Michigan and Lake Ontario.

Data Source	Lake	Measure	Years	Method
Slimy Sculpin	Michigan & Ontario	#/ha	1973–2019 1978–2019	USGS trawl survey
Dreissenid Mussel	Michigan & Ontario	g/ha	1999–2019 1993–2019	USGS trawl survey
Dreissenid Mussel at 60m+	Michigan & Ontario	g/ha	1999–2019 1995–2019	USGS trawl survey
Round Goby	Michigan & Ontario	#/ha	2003–2019 2002–2019	USGS trawl survey
Lake Trout	Michigan & Ontario	#/ha	1973–2019 1978–2019	USGS trawl survey
Juvenile Lake Trout (Age 0-3)	Michigan	#/ha	1985–2019	Statistical catch at age model outputs
Immature Lake Trout CPUE	Ontario	#/net	1980–2019	Gillnet surveys
All Lake Trout CPUE	Ontario	#/net	1980–2019	Gillnet surveys
Stocked Lake Trout	Ontario	#	1978–2019	US waters compiled

Note. Acronyms used represent United States Geological Survey (USGS) and catch per unit effort (CPUE).

Total stocked lake trout in the United States waters of Lake Ontario from 1979 to 2020 was calculated from the Great Lakes Fish Stocking database (*http://fsis.glfc.org/ [Accessed: March, 2021]*). Estimates of immature and all lake trout catch per unit effort (CPUE) in Lake Ontario were provided by the Great Lakes Fishery Commission Lake Ontario Lake Trout Committee (O'Malley et al. 2023). All covariates were normalized by dividing values by two times the standard deviation of covariate values over time at a port to place covariate values on a similar numerical scale to the slimy sculpin time series and each other covariate (Gelman 2008).

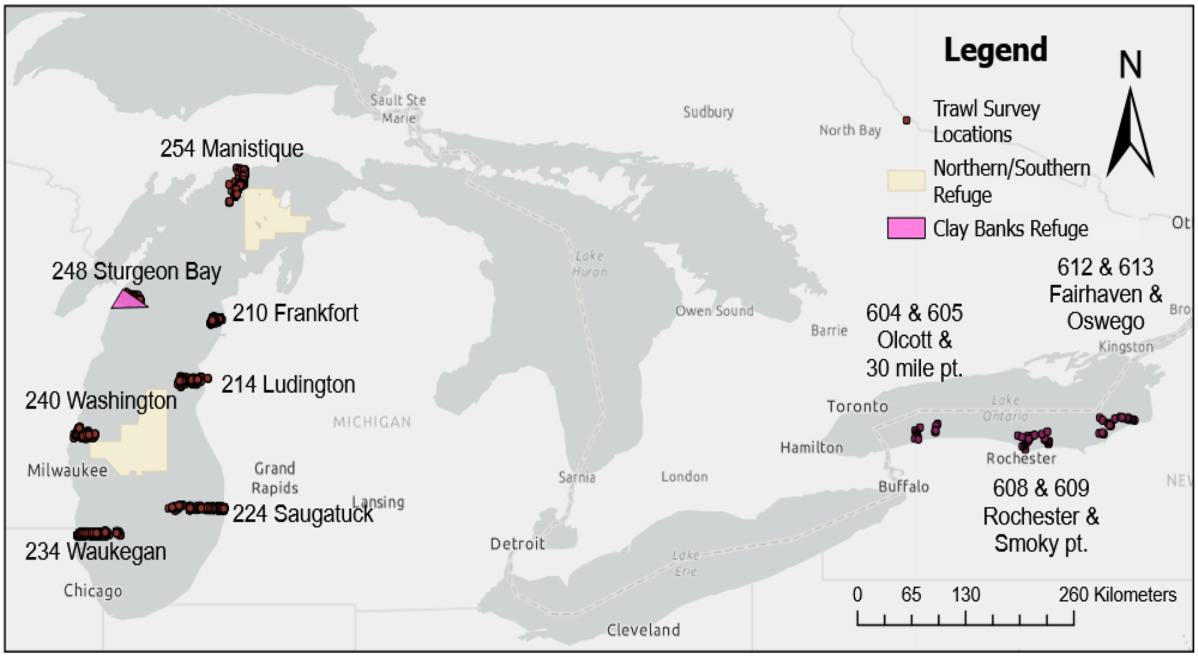

Figure 2.1. Overview of United States Geological Survey (USGS) trawl survey locations used in our delta generalized additive models and population dynamics models in Lake Ontario and Lake Michigan and the lake trout refugee habitat designated in 1985.

Standardizing Slimy Sculpin Observations

We standardized the slimy sculpin observation data across the survey extent of each port by creating a dataset of real and predicted observations, when necessary, to create equal depth increment representation of the survey extent. Predicted observations into unobserved space and time were made with delta generalized additive models (GAMs) and tensor product splines for each port that predicted density across depth by year informed by nearby real observations in space and time. We then calculated the arithmetic average of slimy sculpin density across all depth increments (both observed and predicted) for each year and port and used these values for our population dynamics modeling (Fig. 2.2).

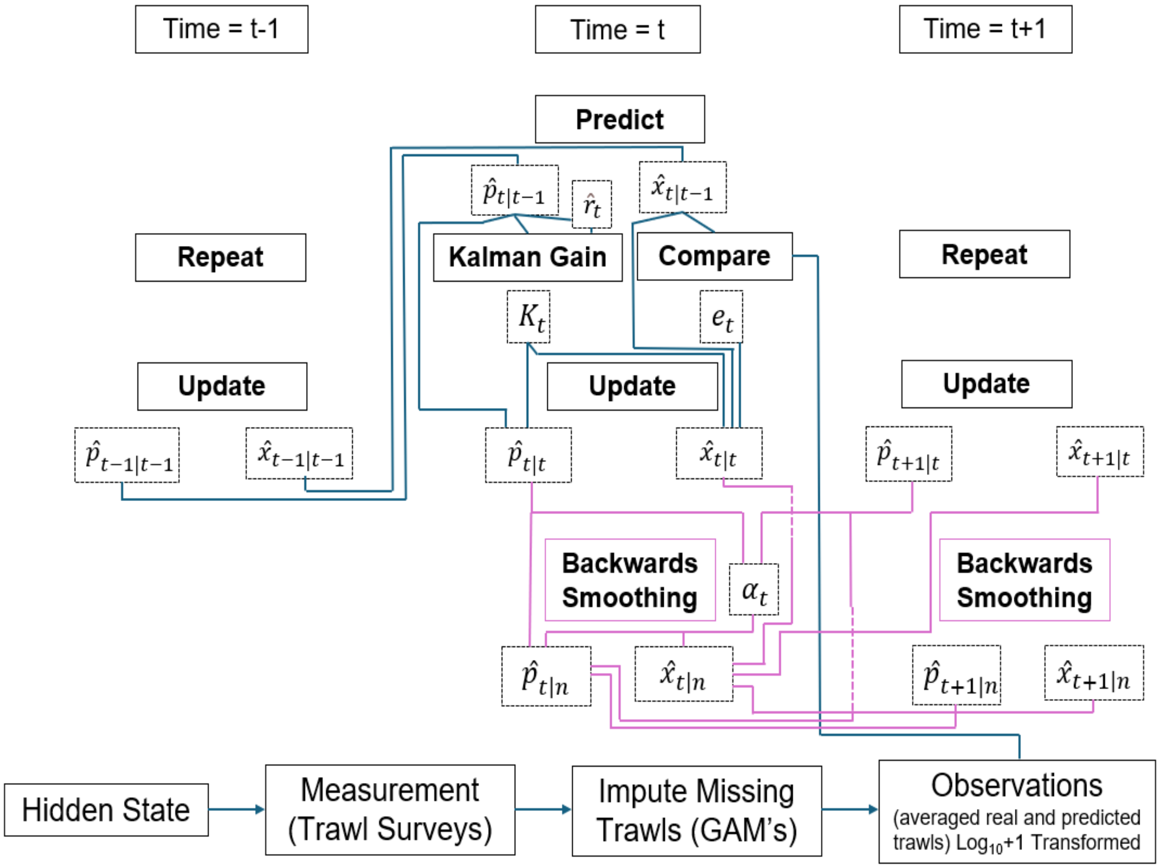

Figure 2.2. Overview of the Kalman filtering and smoothing procedure at time = t that was used to estimate slimy sculpin abundance (Log$_{10}$((#/ha)+1)) through time at each port. The Kalman filter assumes the states are evolving through a Markovian linear process with Gaussian process and measurement noise (Newman et al. 2014). The state transition process involves the updated estimated variance of the state for the previous year $\hat{p}_{t-1|t-1}$ and the updated estimate of the state in the previous year $\hat{x}_{t-1|t-1}$ to predict the estimate of the state $\hat{x}_{t|t-1}$ and the estimate of the variance of the state $\hat{p}_{t|t-1}$. The predicted estimate of the variance of the state is used to predict the variance of the observation $\hat{r}_t$ and both are used to estimate the Kalman gain K_t. The predicted estimate of the state is compared to the observations to calculate the residuals e_t, and the residuals, the Kalman gain, and the predicted estimate of the state are used to get the updated estimate of the state $\hat{x}_{t|t}$, while the predicted estimate of the variance of the state and the Kalman gain are used to get the updated estimate of the variance of the state $\hat{p}_{t|t}$. This process is repeated till the end of the time series and then the updated estimates of the state and estimates of the variance are backwards smoothed by first calculating the smoothing gain α_t with the next year's updated estimate of the variance of the state $\hat{p}_{t+1|t}$ and the current years updated estimate of the variance of the state. Then the smoothed estimate of the state $\hat{x}_{t|n}$ is calculated by the smoothing gain, the next year smoothed estimate of the state $\hat{x}_{t+1|n}$, and the current and next year updated estimate of the state. The smoothed estimate of the variance of the state $\hat{p}_{t|n}$ is similarly calculated by the smoothing gain, the next year smoothed estimate of the variance of the state $\hat{p}_{t+1|n}$, and the current and next year updated estimate of the variance of the state.

Trawl surveys were generally conducted at ≈10m increments across the depth distribution of the sampling extent, but standard sampling intervals were not equal increments across the depth range and not all intervals were conducted each year. The slimy sculpin population densities were variable across their depth distribution, and sampling variability would sometimes result in years with over or under sampling in the peak or tails of the depth and density distribution. Additionally, the observation data were collected when populations were at both high and low abundances; the low abundance period produced a dataset that was zero inflated with zero abundance being structured by depth and time.

Because inconsistent sampling data across ports and depth intervals could bias conclusions, we predicted trawl catches at ≈10m increments across the survey extent using observed catch data. We predicted trawl catches using two sub-models that predict the presence/absence of a species separately from positive catches and is referred to as a delta or hurdle model structure (Stefansson 1996, Thorson et al. 2015). Each sub-model used GAMs to smoothly model surfaces of slimy sculpin density across depth and time (Swartzman et al. 1992, Hinton & Maunder 2004, Gasper & Kruse 2013, Potts & Rose 2018) that were informed by nearby real observations in space and time. The delta GAMs were fit with tensor product splines using the mgcv package (Wood & Wood 2015, R Core Team 2025). We optimized the maximum basis dimensions of the tensor product splines to lower the Akaike Information Criterion (AIC) (Akaike 1973) while retaining smooth predictions across the full survey extent. For the first sub model, we predicted the probability value between 0 and 1 that a species was present in the trawl π by (Eq. 2.1):

$$logit(\pi(d, y)) = f_{bin}(d, y)$$

where d is depth, y is year, and $f_{bin}(d, y)$ is the smooth function of the interaction of depth and year fit using a binomial distribution with a logit link. We predicted nonzero density μ by (Eq. 2.2):

$$log(\mu(d, y)) = f_{pos}(d, y)$$

where d is depth, y is year, and $f_{pos}(d, y)$ is the smooth function of the interaction of depth and year fit using a gamma distribution with a log link (Eq. 2.2). The overall prediction of density at depth in a year $\hat{z}(d, y)$ was calculated by (Eq. 2.3):

$$\hat{z}(d,y) = \pi(d,y) \times \mu(d,y)$$

where $\pi(d,y)$ is the probability of a species' presence at depth in a year and $\mu(d,y)$ is the predicted nonzero density at depth in a year.

Overview of Time Series Used for Population Estimation

The arithmetic average density of all the standardized depth increments using real and predicted observations across the survey extent was used for population dynamics modeling. For Lake Michigan, standardized depths were 18 to 128m in ≈9m increments. For Lake Ontario, standardized depths were 15 to 95m and 110 to 150m in 10m increments, and real observations that were within ≈5m were rounded to the nearest interval. The standardized slimy sculpin density was $\log_{10}$ transformed with a +1 offset to normalize the observation data for population dynamics modeling.

Population Dynamics Model

We estimated slimy sculpin density at each port as a latent state evolving through time via Kalman filtering and smoothing (Newman et al. 2014). Kalman filtering is a recursive analytic procedure for estimating states (i.e., densities) and variances of the states over time using observations and estimates up until time t and assumes the states are evolving through a Markovian linear process with Gaussian process and measurement noise (Newman et al. 2014). After the Kalman filtering, Kalman smoothing (i.e., Rauch–Tung–Striebel smoothing) (Rauch et al. 1965) refined the estimates in a backwards pass that incorporated information into the state estimate from observations after time t. Overall, this process estimated the trajectory of the state across the time series and reflects a combination of short-term and long-term dynamics with a simplified estimate of uncertainty comprised of the estimated process and observation variance.

The first year's prediction of the latent state $\hat{x}_0$ (i.e., initial density of the time series) was an optimized free parameter, and the variance of that prediction for the first year $\hat{p}_0$ was the optimized initial process variance p_0, but the rest of the initialization followed the procedure described below. The transition between time steps began with the state transition equation which predicted slimy sculpin density $\hat{x}_{t|t-1}$ in the current year *t* by (Eq. 2.4):

$$\hat{x}_{t|t-1} = \hat{x}_{t-1|t-1} + \beta_i U_{i,t-1}$$

where the predicted state estimate $\hat{x}_{t|t-1}$ was a function of the updated latent state estimate of slimy sculpin density in the previous year $\hat{x}_{t-1}$ plus any covariates (Table 2.1) in the previous year $U_{i,t-1}$ multiplied by their respective coefficients β_i that are optimized from the data. The predicted estimate of variance of the predicted state estimate $\hat{p}_{t|t-1}$ of slimy sculpin density was estimated by (Eq. 2.5):

$$\hat{p}_{t|t-1} = \hat{p}_{t-1|t-1} + p_0$$

where $\hat{p}_{t-1|t-1}$ was the updated variance estimate of the previous year's state estimate of slimy sculpin density and p_0 is the optimized initial process variance. The prediction of variance of the observation $\hat{r}_t$ of slimy sculpin density was calculated by (Eq. 2.6):

$$\hat{r}_t = \hat{p}_{t|t-1} + r_0$$

where r_0 was the optimized initial observation variance. We then calculated the Kalman Gain K_t by (Eq. 2.7):

$$K_t = \hat{p}_{t|t-1} / \hat{r}_t$$

which was used to balance trust in the predicted state estimates compared to the new observation and produce an updated state estimate, and its derivation achieves minimal mean-squared error. The Kalman Gain was first used to update the prediction of the state estimate $\hat{x}_{t|t-1}$ to the updated state estimate $\hat{x}_{t|t}$ of slimy sculpin density in the current year by (Eq. 2.8):

$$\hat{x}_{t|t} = \hat{x}_{t|t-1} + K_t e_t$$

where e_t was the residual between the predicted state estimate of slimy sculpin density and the observed slimy sculpin density a time *t*. We then updated the predicted estimate of variance of slimy sculpin density $\hat{p}_{t|t-1}$ to the updated estimate of variance of slimy sculpin density in the current year $\hat{p}_{t|t}$ by (Eq. 2.9):

$$\hat{p}_{t|t} = \hat{p}_{t|t-1} - \hat{p}_t K_t$$

Lastly, once updated estimates of states (slimy sculpin density) and their variances were produced for all years, we smoothed these state estimates and their variances through time with a back calculation beginning in the second to last year. This backwards smoothing began by calculating the smoothing gain α_t by (Eq. 2.10):

$$\alpha_t = \hat{p}_t / \hat{p}_{t+1}$$

then we calculated the smoothed state estimates $x_{t|n}$ of slimy sculpin density by (Eq. 2.11):

$$\hat{x}_{t|n} = \hat{x}_t + \alpha_t \left(\hat{x}_{t+1|n} - \hat{x}_{t+1|t}\right)$$

and smoothed variance of the state estimates $p_{t|n}$ of slimy sculpin density by (Eq. 2.12):

$$\hat{p}_{t|n} = \hat{p}_{t|t} + \alpha_t^2 \left(\hat{p}_{t+1|n} - \hat{p}_{t+1|t}\right)$$

to produce the final estimates of the states (slimy sculpin density) and their variance that we report.

Model parameters were estimated and optimized with the *nlminb* function in R (R Core Team 2025), which is a gradient based algorithm that minimizes the negative log likelihood. The free parameters were the initial latent state $\hat{x}_0$, initial process p_0 and observation r_0 variance, and the covariates' (Table 2.1) coefficients β_i. We were unable to estimate an observation variance for port 210 in Lake Michigan with the imputed predictions and real observations of trawl catches from the GAM's. Instead, we estimated the observation variance using only the real trawl catches and fixed the observation variance to this value when we analyzed the combined observation dataset. Otherwise, the Kalman filter and smoother were run and estimated the remaining parameters for port 210 following the same procedure and included both real and predicted trawl catches in the observations. Once the free parameters were optimized and the final smoothed estimates were produced for each candidate model, the best performing models with stable beta estimates were determined via Akaike Information Criterion (AIC) and candidate models within 2 AIC of the best performing models were considered to have substantial support and discussed as well (Burnham & Anderson 2002). Models were tested with all possible combinations of up to two representative covariates (e.g., round goby & lake trout) at a time and covariates were treated as additive effects with a one-year time lag, and the candidate and best performing models were reported (Sup. File 1, Table 2.2).

Results

Observation Models Overview

The binomial GAMs on average described 47% (Lake Ontario) and 56% (Lake Michigan) of the adjusted proportion of variance in the presence/absence data, whereas the gamma GAMs on average described 67% (Lake Ontario) and 52% (Lake Michigan) of the adjusted proportion of variance in the nonzero density data (Table 2.2). The predicted observations at depth (Eq. 2.3) were used for 35% (Lake Ontario) and 28% (Lake Michigan) of the density estimates at standardized depth increments across the survey extent that were averaged by year and used for the population dynamics modeling (Sup Fig. 2.1 & 2.2). The observation models generally captured the trends through longer time scales but did not consistently capture large interannual changes in density that diverged from trends (Sup Fig. 2.3 & 2.4). Therefore, the state-space models using more predicted data may result in variance estimates being biased lower than if they had more real observations.

Table 2.2. Performance and parameterization of best observation models for each port in Lake Ontario (LO) and Lake Michigan (LM). Knots designate the maximum basis dimensions for depth (d) and year (y).

Lake and Port	GAM Presence/Absence			GAM Non-Zero Density		
	Knots (d_y)	AIC	R^2 adj.	Knots (d_y)	AIC	R^2 adj.
LO-604	5_20	339.85	0.53	7_25	2917.31	0.55
LO-605	5_20	329.74	0.51	8_25	2394.2	0.73
LO-608	5_20	415.93	0.45	7_30	3106.62	0.60
LO-609	3_20	352.52	0.46	7_25	2063.21	0.53
LO-612	2_20	242.37	0.38	7_25	2002.18	0.86
LO-613	5_20	432.83	0.49	7_35	2869.23	0.77
LM-210	3_20	283.06	0.61	8_25	4308.17	0.58
LM-214	6_20	405.14	0.58	8_30	2502.98	0.48
LM-234	4_20	409.62	0.53	7_30	1740.04	0.21
LM-240	6_20	400.71	0.42	7_30	2660.47	0.44
LM-248	4_20	386.21	0.55	8_30	2666.64	0.70
LM-254	4_20	349.42	0.65	8_25	2532.36	0.73

Predicted Depth Structure

Across Lake Ontario, the predictions of slimy sculpin population depth structure in spring were stable pre-1990s with the distribution peaking around 80m and slimy sculpin observed across the full survey extent. In the early 1990s, nearshore densities began declining, and the peak of the distribution shifted further offshore (Sup Fig. 2.5). In the eastern ports (604 & 605: Sup Fig. 2.5 A & B), slimy sculpin was predicted to be nearly absent in the nearshore (<40m) waters by the mid-2000s, and by the early 2010s, slimy sculpin were rarely predicted to be in waters less than 100m. In the central and western ports (608, 609, 612, & 613: Sup Fig. 2.5 C–F), the nearshore (<40m) slimy sculpin populations were predicted to be nearly absent by the early 2010s and by the mid-2010s, this extended to depths less than 90m.

In Lake Michigan, there were no consistent lake wide patterns of slimy sculpin population depth structure through time (Sup Fig. 2.6). At the northern and northwestern ports (254 & 248: Sup Fig. 2.6 E & F), slimy sculpin population depth structure was relatively stable until the 2000s when populations rapidly increased before beginning to decline heading into the 2010s. In the 2010s slimy sculpin progressively disappeared from the nearshore (<30m) at both ports (254 & 248) and from the offshore (>100m) at the northern port (254: Sup Fig. 2.6 F). At the western port (240: Sup Fig. 2.6 D), slimy sculpin population depth structure was relatively stable until the 1990s when densities increased across all depths until the 2010s, at which point densities began declining. At the southwestern port (234: Sup Fig. 2.6 C), the shape of the depth distribution of slimy sculpin does not change dramatically through time, but the peak of the distribution does shift from 65m to 40m heading into the 2000s. At the eastern port (214: Sup Fig. 2.6 B), slimy sculpin population densities were consistently low at depths less than ≈35m until the population recovered across the survey extent in the 1990s. The population (214) declined through the 2010s, and by the mid-2010s, slimy sculpin were nearly absent at depths less than ≈30m. At the northeastern port (210: Sup Fig. 2.6 A), slimy sculpin density varied through time and most notably at depths less than 40m and greater than 80m, but around 2010, the population began declining dramatically across all depths and slimy sculpin were absent at depths less than 40m by the end of the time series.

Population Dynamics and Covariates

Models were developed with all possible combinations of up to two representative covariates (i.e., round goby & lake trout) at a time and treated as additive effects with no interactions, and timing of current slimy sculpin population declines aligned with invasion pathways (Fig. 2.3). Dreissenid mussels or round goby were the covariates most commonly selected in the best performing models for Lake Michigan and Lake Ontario ports, and stable beta estimates always had a negative relationship with slimy sculpin density (Table 2.3 & 4). Lake trout covariates were rarely selected among the best performing models, and if they were, they were selected along with another covariate and had a beta estimate near zero with standard errors overlapping zero that suggested it was minimally contributing to the model (Arnold 2010).

Table 2.3 Results of candidate models using Kalman filter and smoother to predict slimy sculpin density for Lake Michigan ports. Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F). Covariates represent dreissenid mussels (DM), round goby (RG), and lake trout (LT). Dreissenid mussels were averaged across all depths (DM) and averaged across depths greater than 60 m (DM_60+) sampled by benthic trawls. Lake trout values represent the average value across all benthic trawl depths (LT_Trawl) and the age 0-3 estimates from a statistical catch at age model (LT_Juv). Green indicates the best performing model and models with substantial support that are within 2 AIC score of the best performing model. Grey indicates the model could not estimate a process error.

A

Covariates	AIC	Beta's
DM_60+	74.68	-0.44
DM	75.71	-0.31
DM_60+, LT_Trawl	76.5	-0.46, 0.03
DM_60+, LT_Juv	76.64	-0.46, 0.01
DM, LT_Trawl	77.3	-0.34, 0.05
DM, LT_Juv	77.63	-0.34, 0.01
LT_Juv	78.85	-0.02
None	78.39	
LT_Trawl	80.34	-0.02
RG		
DM_60+, RG		
DM, RG		
RG, LT_Juv		
RG, LT_Trawl		

B

Covariates	AIC	Beta's
DM, LT_Juv	38.7	-0.32, 0.03
DM	39.09	-0.22
DM_60+	40.04	-0.22
DM, RG	40.28	-0.15, -0.18
RG	40.5	-0.24
None	40.55	
DM_60+, LT_Juv	40.59	-0.29, 0.03
DM_60+, RG	40.97	-0.17, -0.17
DM, LT_Trawl	41.07	-0.22, -0.01
RG, LT_Juv	41.43	-0.31, 0.02
DM_60+, LT_Trawl	41.95	-0.22, -0.02
LT_Trawl	42.31	-0.04
LT_Juv	42.33	0.01
RG, LT_Trawl	42.4	-0.23, -0.02

Table 2.2 (cont'd)

C

Covariates	AIC	Beta's
RG	49.49	-0.27
DM_60+, LT_Juv	50.21	-0.26, 0.02
RG, LT_Juv	50.23	-0.35, 0.01
DM_60+	50.39	-0.17
DM, RG	51.00	-0.05, -0.23
RG, LT_Trawl	51.49	-0.27, -0.01
DM	51.58	-0.11
None	51.87	
DM, LT_Juv	51.88	-0.19, 0.02
DM_60+, RG	52.11	-0.14, -0.04
DM_60+, LT_Trawl	52.38	-0.18, 0.01
LT_Trawl	52.64	-0.05
DM, LT_Trawl	53.42	-0.09, -0.02
LT_Juv	53.85	-0.002

D

Covariates	AIC	Beta's
RG, LT_Trawl	50.27	-0.66, 0.03
RG	52.55	-0.45
DM_60+, LT_Juv	52.70	-0.41, 0.04
DM, RG	53.55	0.08, -0.55
RG, LT_Juv	54.35	-0.47, 0.03
DM_60+, LT_Trawl	54.35	-0.47, 0.03
DM_60+, RG	54.4	-0.54, 0.07
DM_60+	55.59	-0.21
None	56.28	
LT_Juv	58.11	0.01
DM	58.16	-0.03
LT_Trawl	58.25	-0.01
DM, LT_Juv	59.46	-0.1, 0.02
DM, LT_Trawl	60.15	-0.03, -0.01

E

Covariates	AIC	Beta's
RG	35.95	-0.40
RG, LT_Juv	40.98	-0.46, 0.02
DM_60+, RG	41.82	-0.06, -0.39
DM, RG	41.88	-0.03, -0.39
RG, LT_Trawl	41.9	-0.40, -0.02
None	43.76	
DM	45.24	-0.10
DM_60+	45.27	-0.12
LT_Trawl	45.68	-0.02
LT_Juv	45.76	0.01
DM, LT_Juv	47.11	-0.13, 0.01
DM_60+, LT_Juv	47.17	-0.14, 0.01
DM, LT_Trawl	47.18	-0.10, -0.03
DM_60+, LT_Trawl	47.21	-0.11, -0.02

F

Covariates	AIC	Beta's
DM_60+	70.37	-0.32
DM	71.1	-0.28
None	71.79	
DM_60+, LT_Trawl	71.79	-0.28, -0.10
DM_60+, RG	71.9	-0.29, -0.15
LT_Trawl	72.15	-0.14
LT_Juv	72.27	-0.04
DM_60+, LT_Juv	72.36	-0.3, -0.01
DM, LT_Trawl	72.41	-0.24, -0.10
RG	72.47	-0.21
DM, RG	72.6	-0.25, -0.16
DM, LT_Juv	73.03	-0.25, -0.01
RG, LT_Trawl	73.34	-0.20, -0.13
RG, LT_Juv	73.88	-0.15, -0.03

Table 2.4 Results of candidate models using Kalman filter and smoother to predict slimy sculpin density for Lake Ontario ports. Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F). Covariates represent dreissenid mussels (DM), round goby (RG), and lake trout (LT). Dreissenid mussels were averaged across all depths (DM) and averaged across depths greater than 60 m (DM_60+) sampled by benthic trawls. Lake trout values represent the average value across all benthic trawl depths (LT_Trawl), the lake trout stocked in the US waters of Lake Ontario (LT_stocked), and lake trout catch per unit of effort from gill net surveys in the US waters of Lake Ontario across all ages (All_LT_CPUE) and only immature lake trout (Juv_LT_CPUE). Green indicates the best performing model and models with substantial support that are within 2 AIC score of the best performing model. Grey indicates the model could not estimate a process error and red indicates a model with unstable beta estimates that was not considered as a best performing model.

A

Covariates	AIC	Beta's
RG	38	-0.16
RG, DM	38.27	-0.14, -0.13
None	38.5	
DM	38.7	-0.14
All_LT_CPUE	39.5	-0.02
LT_Stocked	39.5	-0.03
All_LT_CPUE, RG	39.6	-0.01, -0.14
LT_Stocked, RG	39.6	-0.02, -0.14
LT_Trawl, RG	39.95	0.01, -0.16
Juv_LT_CPUE, RG	39.97	-0.01, -0.16
LT_Stocked, DM	40.22	-0.02, -0.11
Juv_LT_CPUE	40.4	-0.02
All_LT_CPUE, DM	40.4	-0.01, -0.11
LT_Trawl	40.5	-0.01
LT_Trawl, DM	40.6	0.01, -0.14
Juv_LT_CPUE, DM	40.67	-0.01, -0.13
DM_60+		
LT_Stocked, DM_60+		
Juv_LT_CPUE, DM_60+		
All_LT_CPUE, DM_60+		
LT_Trawl, DM_60+		
RG, DM_60+		

B

Covariates	AIC	Beta's
RG	13.55	-0.18
RG, DM_60+	13.91	-0.17, -0.12
LT_Trawl, RG	14.55	0.05, -0.19
All_LT_CPUE, RG	15.45	-0.01, -0.17
Juv_LT_CPUE, RG	15.47	0.01, -0.19
RG, DM	15.51	-0.18, 0.02
LT_Stocked, RG	15.52	-0.01, -0.18
None	15.67	
DM_60+	15.68	-0.14
LT_Trawl, DM_60+	16.8	0.05, -0.15
All_LT_CPUE	17.01	-0.02
LT_Trawl	17.04	0.05
LT_Stocked	17.19	-0.02
All_LT_CPUE, DM_60+	17.42	-0.01, -0.13
LT_Stocked, DM_60+	17.5	-0.01, -0.13
Juv_LT_CPUE	17.67	-0.01
DM	17.67	0.01
Juv_LT_CPUE, DM_60+	17.68	0.01, -0.14
All_LT_CPUE, DM	18.93	-0.02, 0.03
LT_Trawl, DM	19.02	0.05, -0.02
LT_Stocked, DM	19.16	-0.02, 0.02
Juv_LT_CPUE, DM	19.67	-0.01, 0.01

Table 2.4 (cont'd)

C

Covariates	AIC	Beta's
DM	24.8	-0.17
None	25.2	
RG	25.26	-0.15
RG, DM	26.2	-0.09, -0.13
DM_60+	26.23	-0.10
LT_Stocked	26.34	-0.03
LT_Stocked, DM	26.46	-0.02, -0.15
All_LT_CPUE	26.73	-0.02
LT_Trawl, DM	26.73	0.01, -0.18
All_LT_CPUE, DM	26.74	-0.01, -0.16
Juv_LT_CPUE, DM	26.78	-0.01, -0.17
RG, DM_60+	26.87	-0.29, 0.14
LT_Stocked, RG	26.97	-0.02, -0.13
Juv_LT_CPUE	27.06	-0.01
LT_Trawl, RG	27.18	0.02, -0.16
LT_Trawl	27.19	-0.01
All_LT_CPUE, RG	27.2	-0.01, -0.14
Juv_LT_CPUE, RG	27.24	-0.01, -0.15
LT_Stocked, DM_60+	27.68	-0.02, -0.08
All_LT_CPUE, DM_60+	28.02	-0.01, -0.08
Juv_LT_CPUE, DM_60+	28.17	-0.01, -0.09
LT_Trawl, DM_60+	28.21	0.01, -0.10

D

Covariates	AIC	Beta's
None	18.12	
LT_Trawl	19.74	-0.04
All_LT_CPUE	19.87	-0.01
RG	19.92	-0.05
Juv_LT_CPUE	20.003	0.02
DM 60+	20.02	-0.05
DM	20.07	-0.04
LT_Stocked	20.12	-0.01
LT_Trawl, RG	21.6	-0.03, -0.04
LT_Trawl, DM_60+	21.68	-0.03, -0.04
LT_Trawl, DM	21.72	-0.04, -0.03
Juv_LT_CPUE, RG	21.76	0.02, -0.06
All_LT_CPUE, RG	21.77	-0.01, -0.04
All_LT_CPUE, DM_60+	21.83	-0.01, -0.04
All_LT_CPUE, DM	21.85	-0.01, -0.02
RG, DM	21.85	-0.10, 0.09
Juv_LT_CPUE, DM_60+	21.89	0.02, -0.06
RG, DM_60+	21.91	-0.07, 0.03
LT_Stocked, RG	21.92	0.01, -0.05
Juv_LT_CPUE, DM	21.94	0.02, -0.04
LT_Stocked, DM_60+	22.02	0.01, -0.05
LT_Stocked, DM	22.07	0.01, -0.04

Table 2.4 (cont’d)

E

Covariates	AIC	Beta's
RG, DM	14.52	0.68, -0.94
DM	15.52	-0.24
RG, DM_60+	15.58	0.60, -0.87
DM_60+	15.8	-0.24
LT_Trawl, DM	16.83	-0.05, -0.25
LT_Trawl, DM_60+	17.13	-0.05, -0.25
All_LT_CPUE, DM	17.2	-0.02, -0.22
RG	17.44	-0.18
All_LT_CPUE, DM_60+	17.48	-0.02, -0.22
Juv_LT_CPUE, DM	17.49	-0.01, -0.24
LT_Stocked, DM	17.51	-0.01, -0.24
Juv_LT_CPUE, DM_60+	17.78	-0.01, -0.24
LT_Stocked, DM 60+	17.8	-0.01, -0.24
LT_Trawl, RG	18.91	-0.04, -0.18
All_LT_CPUE, RG	18.92	-0.02, -0.16
Juv_LT_CPUE, RG	19.4	-0.01, -0.18
LT_Stocked, RG	19.41	-0.01, -0.18
None	20.42	
All_LT_CPUE	20.69	-0.03
LT_Trawl	21.88	-0.04
LT_Stocked	22.05	-0.02
Juv_LT_CPUE	22.14	-0.02

F

Covariates	AIC	Beta's
DM	29.44	-0.16
All_LT_CPUE, DM	29.89	-0.04, -0.14
All_LT_CPUE	29.98	-0.05
None	30.09	
DM_60+	30.19	-0.14
All_LT_CPUE, DM_60+	30.46	-0.04, -0.12
LT_Trawl, DM	30.75	-0.07, -0.16
LT_Stocked, DM	31.07	-0.03, -0.15
Juv_LT_CPUE, DM	31.09	-0.03, -0.16
RG, DM	31.35	0.07, -0.17
LT_Stocked	31.41	-0.04
LT_Trawl	31.47	-0.07
LT_Trawl, DM_60+	31.50	-0.07, -0.14
Juv_LT_CPUE	31.68	-0.04
LT_Stocked, DM_60+	31.73	-0.03, -0.13
Juv_LT_CPUE, DM_60+	31.82	-0.04, -0.14
All_LT_CPUE, RG	31.95	-0.05, 0.04
RG	32.02	-0.06
RG, DM_60+	32.14	0.05, -0.15
LT_Stocked, RG	33.41	-0.04, -0.02
LT_Trawl, RG	33.41	-0.07, -0.06
Juv_LT_CPUE, RG	33.66	-0.04, -0.04

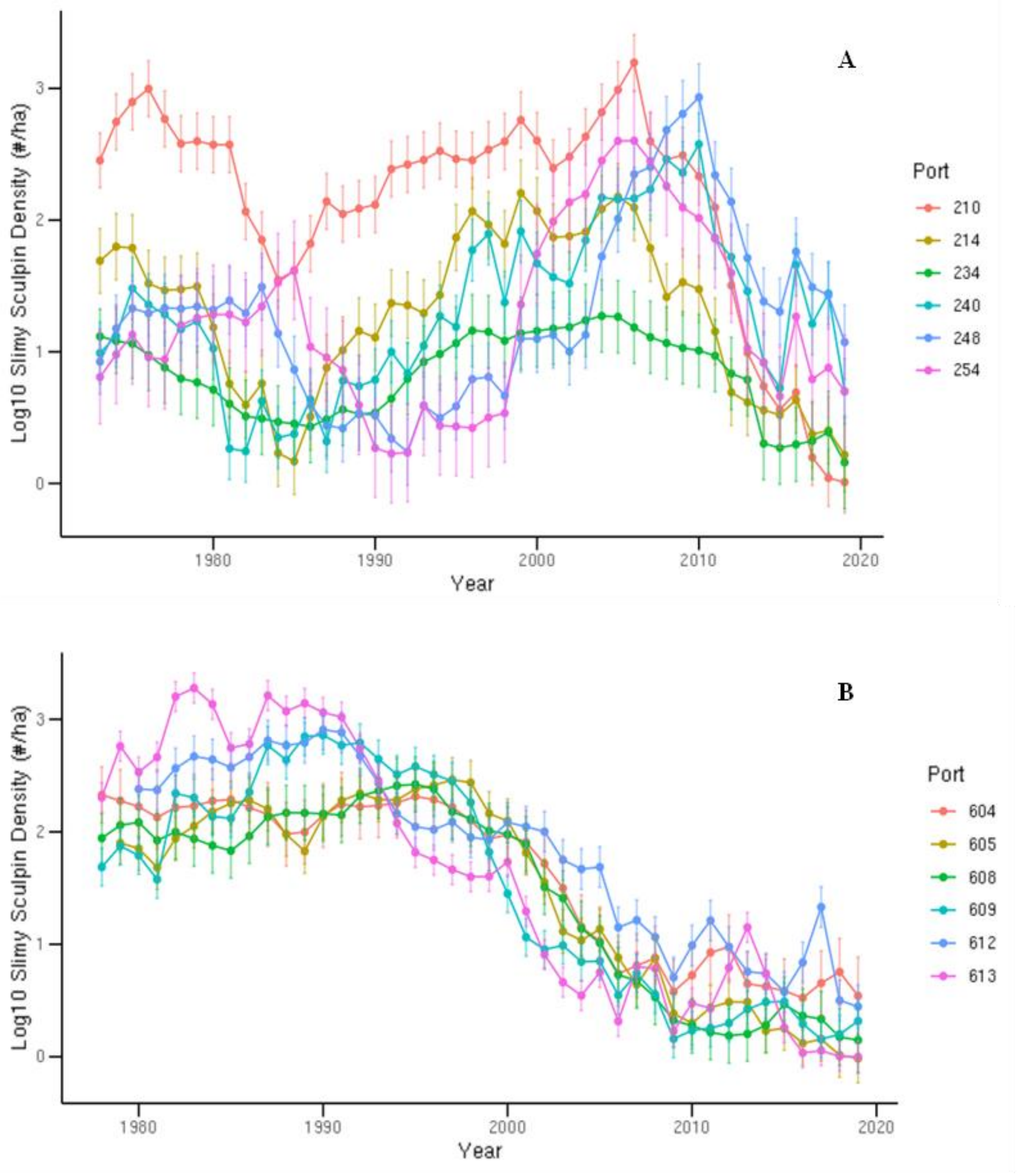

Figure 2.3. Kalman filter and smoother estimates and 95% confidence interval bars of slimy sculpin density ($\log_{10}$+1 (#/ha)) across the survey extent for Lake Michigan ports from 1973 to 2019 (A) and Lake Ontario ports from 1978 to 2019 (B).

In the Lake Ontario models, slimy sculpin density at all ports was generally stable or trending upward through the 1980s. At the three easternmost ports (609, 612, & 613), slimy sculpin density began to trend downward in 1990–1991, stabilized briefly at the two far east ports (612 & 613) from 1995 to 2000 before they began declining again in 2001 (Fig. 2.3 B, Sup Fig. 2.8 D-F). At the westernmost ports (604, 605, & 608), slimy sculpin density was relatively stable until 1996–1998, and declines progressed rapidly until 2010. At three of the four east and mid lake ports (608, 612, & 613), the best performing models only included dreissenid mussels (Table 2.4). In the two westernmost ports, where average round goby densities were the highest, the best performing models only included round goby (604 & 605: Table 2.4, Sup. Fig. 2.7 A). Across all ports, the list of candidate models that received substantial support (ΔAIC ≤ 2) included round goby and dreissenid mussel covariates (Table 2.4). However, at three ports there were candidate models that received substantial support (ΔAIC ≤ 2) that did not include any covariates (604, 608, 613 Table 2.4) and one port had a best performing model including no covariates (609, Table 2.4). Dreissenid mussels were collected more consistently in the western ports and the first surge in dreissenid mussel density was observed in 1995–1996 followed by another surge observed in 2001 (Fig. 2.4 B & C). Round goby were first detected in ports in the west in 2002 and spread eastward with the most eastern port (613) first detecting round goby in 2008 (Fig. 2.4 A).

In the Lake Michigan models, there were regional patterns in slimy sculpin population trend changes around 1985, and covariate selection often aligned with modern declines. At the north and northwestern ports (254 & 248: Fig. 2.3 A, Sup Fig. 2.10 E & F), slimy sculpin density trended upward from 1973 until 1984–1986, followed by declines until 1993–1994 when densities reached their lowest levels of the time series. Slimy sculpin densities then gradually increased until 1999 (254) and 2004 (248) when densities rapidly increased from ≈10 fish/ha or less to 100s of fish/ha at their peak 6 or 7 years later. At the southwestern and eastern ports (210, 214, 234, & 240: Fig. 2.3 A, Sup Fig. 2.10 A–D), slimy sculpin densities were generally trending downward from 1973 until 1984–1986 when densities began to generally trend upward until the beginning of the modern declines (post-2000).

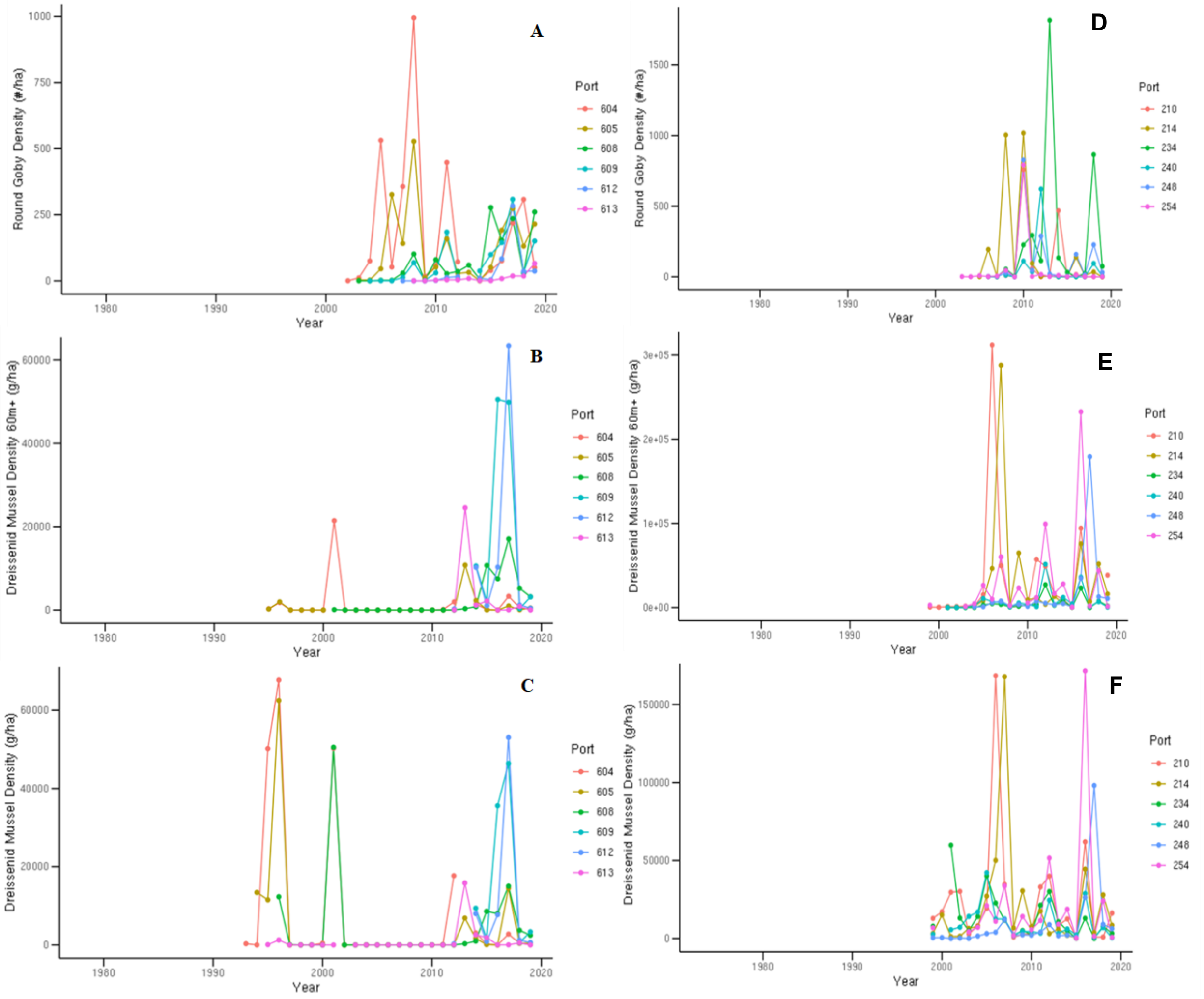

Figure 2.4. Lake Ontario density of round goby (#/ha) (A), dreissenid mussels (g/ha) at 60m of depth of more (B), and dreissenid mussels (g/ha) (C) from USGS trawl survey data collected from 1978 to 2019. Lake Michigan density of round goby (#/ha) (D), dreissenid mussels (g/ha) at 60m of depth of more (E), and dreissenid mussels (g/ha) (F) from USGS trawl survey data collected from 1973 to 2019.

The modern declines in slimy sculpin density in Lake Michigan were best explained by dreissenid mussels in the northern and eastern ports (210, 214, & 254: Table 2.3) which also had the highest average dreissenid mussel density (Sup Fig. 2.11), while the western ports were best explained by round goby (234, 240, & 248: Table 2.3). Most best performing and candidate models with substantial support (ΔAIC ≤ 2) that included lake trout covariates estimated the beta near zero with standard errors overlapping zero that suggested it was minimally contributing to the model (Table 2.3) (Arnold 2010). The exception was the northern port (254: Table 2.3) candidate models with substantial support (ΔAIC ≤ 2) including lake trout capture in trawls that estimated a negative relationship and had standard errors not overlapping zero. The northern, eastern, and southwestern ports' (254, 210, 214, & 234) modern declines in slimy sculpin density (post-2000's) began between 2006 and 2007 (Fig. 2.3 A), which closely corresponds with the first observed lake wide surge in dreissenid mussels and offshore (60m+) dreissenid mussel densities between 2005 and 2007 (Fig. 2.4 E & F). Notably, the surge in offshore dreissenid mussel density was greatest in all ports in which dreissenid mussels best explained slimy sculpin density in the models (210, 214, & 254: Fig. 2.4 E, Sup Fig. 2.12). The modern decline in slimy sculpin density in the two mid lake western ports began in 2011 (240 & 248: Fig. 2.3 A), which closely follows the first lake level surge in round goby density in 2010 (Fig. 2.4 D). The northern and eastern ports recorded the highest densities of round goby in 2010 (254, 210, & 214: Fig. 2.4 D), and 2011 marked another phase in the rapid decline of these slimy sculpin densities (Fig. 2.3 A). There were two ports that had candidate models that included no covariates and received substantial support ($\Delta AIC \leq 2$) (214 & 254: Table 2.3) and three ports that had candidate models that included both round goby and dreissenid mussels and received substantial support ($\Delta AIC \leq 2$) (214 & 234 & 254: Table 2.3). One set of models for a southeastern port (Saugatuck: 224) was unable to estimate a process or observation error because of a highly variable time-series and the results of the modeling process are reported separately (Sup Fig. 13).

Discussion

In Lakes Michigan and Ontario, declines in slimy sculpin populations have occurred with some ports that historically averaged more than 1,000 fish/ha now averaging less than 1 fish/ha. The invasion of round goby and dreissenid mussels, as well as the recovery of lake trout populations, have been hypothesized as drivers of these modern declines in slimy sculpin populations, but we primarily found evidence supporting effects of dreissenid mussels and round goby. Patterns in the regional selection of covariates within our population dynamics models generally matched the ecosystem patterns of invasion pathways and high densities. At most ports, the beginning of modern declines closely aligned with the first observed lake-wide surge in dreissenid mussel densities from trawl surveys. In western Lake Michigan ports, modern declines also coincided with the first lake-wide surge in round goby in trawl surveys. Lake Trout population trends were generally only included in our models with effect estimates that were near and overlapping with zero. However, our juvenile lake trout estimates in Lake Michigan began in 1985 and we observed historically distinct trend changes in Lake Michigan's slimy sculpin populations around 1985 that corresponded regionally with changes in stocking practices (Holey et al. 1995).

Across both lakes, slimy sculpin populations appeared resistant to the initial expansion of zebra mussels, but quagga mussel expansion coincided with the onset of modern declines at most ports. Dreissenid mussels have been hypothesized to indirectly affect slimy sculpin through their effects on preferred forage (*Diporeia*) and directly by reducing foraging efficiency (Beekey et al. 2004, Nalepa et al. 2009, Deroy et al. 2020). Between 1995 and 2000 in Lake Michigan, zebra mussel densities were increasing at depths <50 m, while *Diporeia* declined by 75% at depths <30m and by 40% at depths >90m (Nalepa et al. 2009). Meanwhile, *Mysis* declined by about 50% both offshore and nearshore in southeastern Lake Michigan (Pothoven et al. 2010). Despite these changes, slimy sculpin populations at most ports were generally trending upward between 1995 and 2000. By 2003, *Mysis* were 82% of slimy sculpin fall diets in eastern Lake Michigan, but *Diporeia* were 54-69% of slimy sculpin fall diets in western Lake Michigan (French et al. 2010). It was not until quagga mussels began rapidly increasing in 2005–2006 in southern Lake Michigan (Nalepa et al. 2010) and dramatically increased across Lake Michigan's offshore (>90m) between 2005 and 2010 (Nalepa et al. 2020) that the northern and eastern slimy sculpin densities began to decline. These regions had the highest quagga mussel densities in 2005 and the greatest *Diporeia* declines between 2000 and 2005 (Nalepa et al. 2020). Meanwhile, western Lake Michigan maintained the highest *Diporeia* densities between 2005 and 2015 (Nalepa et al. 2020) and our best performing models did not select for dreissenid mussels.

Since 2010, quagga mussel densities have stabilized or declined in Lake Michigan, and *Diporeia* have been nearly absent at depths <50m (Nalepa et al. 2020). However, quagga mussels' range has continued to expand offshore, and by 2015, *Diporeia* were nearly absent at depths <125m (Nalepa et al. 2020, Zalusky et al. 2023). This decline in *Diporeia* resulted in a significant increase in slimy sculpin reliance on *Mysis* between 1995 and 2010 at eastern and western ports (Bunnell et al. 2015). Additionally, increased reliance on *Mysis* resulted in offshore slimy sculpin having high dietary overlap with deepwater sculpin (*Myoxocephalus thompsonii*) across the lake, high dietary overlap with large bloater (*Coregonus hoyi*) in the east, but low dietary overlap with large round goby (Bunnell et al. 2015). Meanwhile, *Mysis* populations have remained relatively stable offshore in southeastern Lake Michigan between 1999 and 2008

(Pothoven et al. 2010) and lake-wide between 2006 and 2016 (Jude et al. 2018). It appears that even with stable *Mysis* populations, the continued offshore expansion of quagga mussels or the loss of offshore *Diporeia* could be contributing to the ongoing decline of slimy sculpin populations in Lake Michigan.

In Lake Ontario, *Diporeia* populations practically disappeared between 1994 and 2008, progressing east to west along the track of quagga mussel expansion (Birkett et al. 2015), and since 2008, quagga mussel densities have continued to increase offshore (Karatayev et al. 2022). Zebra mussels were first observed in 1990 in western and central Lake Ontario, and quagga mussels were first detected in 1995 (Watkins et al. 2007). Slimy sculpin populations in those regions were generally stable or continued to increase until 1996–1998, at which point they began declining towards the low abundances observed today. In eastern Lake Ontario, slimy sculpin populations began declining in 1990–1991, and quagga mussels were the first dreissenid observed at around 100 m depth in 1990; by 1995, *Diporeia* at that depth had declined by more than 99% (Watkins et al. 2007). Since 2009, slimy sculpin population declines in Lake Ontario have slowed or stabilized, and *Diporeia* have been mostly absent (Burlakova et al. 2022).

Our results indicate that high densities of round goby could contribute to slimy sculpin population declines and may displace slimy sculpin from nearshore habitats. Round goby were detected in southern Lake Michigan and western Lake Ontario harbors by 1996 (Charlebois et al. 1997), but round goby did not appear in trawl surveys in western Lake Ontario until 2002–2003 and western Lake Michigan until 2006–2008. While round goby was expanding from harbors onto soft sediments sampled in USGS trawl surveys, slimy sculpin populations were still increasing in mid-lake western Lake Michigan but had begun declining in western Lake Ontario. However, once a surge in round goby density was recorded in Lake Michigan trawls, it marked either the beginning of declines in mid-lake western Lake Michigan or an acceleration of declines at other ports. In Lake Ontario, the most notable increases in round goby were observed after slimy sculpin populations had largely collapsed, yet nearshore disappearance of slimy sculpin was consistently observed once round goby expanded.

Historically, lake-wide slimy sculpin population trends in Lake Michigan were linked to juvenile lake trout predation via regression analyses (Madenjian et al. 2005) with assumed linear decreases and then increases pre/post 1985 as stocking practices changed to target offshore refuge habitat (Holey et al. 1995). We found supporting evidence for ≈1985 marking a shift in slimy sculpin population trends, but in the two northern ports bordering or overlapping the refuge habitat (Holey et al. 1995), slimy sculpin populations went from increasing to decreasing, whereas other ports showed the reverse. It is important to note that our estimates from catch at age models began when stocking practices had changed, and slimy sculpin populations had begun rising. This could have influenced the potential for the model to reliably detect a negative effect from juvenile lake trout estimates because the change in lake trout stocking overlap with slimy sculpin cannot be captured by our covariate that reflected broader regional changes in juvenile lake trout densities. The northern port (254) in Lake Michigan borders the northern refuge habitat designated for lake trout recovery and was closest to selecting a lake trout covariate with a meaningful negative covariate beta in a candidate model that received substantial support ($\Delta AIC \leq 2$) (Table 2.3) and was also the only port showing slimy sculpin disappearing from the deepest and shallowest extent of the survey. In the northern refuge, stocking is focused on offshore habitat since 1985, and stocking rates were stable and generally lower from 1995 to 2005 while sculpin populations were increasing. Between 2005 and 2010, stocking rates rose while slimy sculpin began declining, yet stocking stabilized in 2010 while slimy sculpin continued to decline (Madenjian et al. 2023). The southwestern port (234) in Lake Michigan is near well-documented primary stocking habitat (Patterson et al. 2016) and had lower stocking from 1995 to 2006 while slimy sculpin populations were generally trending upward. No stocking occurred in 2007, then stocking rates doubled in 2008, but slimy sculpin populations had already started declining in 2006, and there was no notable acceleration in the decline until 2014, which followed a surge in round goby density. Wild juvenile lake trout started to be captured more frequently in trawl surveys across Lake Michigan between 2005 and 2008 (Tingley et al. 2025), but the individual catch by port only received substantial support with meaningful beta estimates in the northern port (Table 2.3). Ports Ludington (214) and Washington (240) are in secondary stocking habitat with minimal or no nearby reefs, yet the timing of modern slimy sculpin declines still mirrored regional trends. Overall, lake trout stocking patterns or population trends are not as strongly tied to modern slimy sculpin declines as other co-occurring environmental stressors in Lake Michigan, but we saw some evidence for a negative relationship in the port bordering the northern refuge habitat that is more intensively stocked.

In both lakes, the most prominent change in slimy sculpin depth distribution during modern declines typically began with them disappearing from depths <40m, followed by outward expansions of this disappearance, and some populations completely collapsing. Our trawl survey data only extends to 150m depth and is more intensively collected at depths less than 110m. It is possible that declines in abundance could just be shifts beyond our survey extent, but examination of slimy sculpin distributions at the deepest transects do not currently support this hypothesis (Madenjian et al. 2022). Both dreissenid mussels and round goby followed similar nearshore-to-offshore invasion pathways, but we never observed nearshore displacement of slimy sculpin when only one invasion occurred. Dreissenid mussels usually expanded before round goby density surged in both lakes, but in Lake Ontario, we observed a consistent pattern of nearshore displacement of slimy sculpin following round goby appearance in trawl surveys. This nearshore displacement in spring is concerning because slimy sculpin historically spawned at depths <75m (Owens & Noguchi 1998), and by 2019 we almost never observed slimy sculpin at those depths. This displacement might suggest spawning interference; and the proportion of juvenile slimy sculpin in Lake Ontario trawl surveys has declined by more than 95% following round goby increases (Weidel et al. 2019). Further analysis of slimy sculpin demographic data coupled with our results could clarify whether reduced spawning began with dreissenid expansion and accelerated once round goby surged or only followed round goby population trends.

Our modeling procedures appear to generally capture slimy sculpin population trends through time and identify covariates that align with invasion pathways, but observation errors are likely underestimated because of generalized model assumptions to fit variable time series and data limitations. We treated each port as an independent set of observations, and within each year, the population was represented by a depth stratified average value from real and predicted observations which resulted in a single datapoint for each year at each port. This procedure allowed us to identify discrete regional differences and develop temporally distinct depth profiles of slimy sculpin density but did not include replicate samples or covariance among ports that may help inform observation uncertainty. Populations within a lake may be linked demographically through larval dispersal considering Lake Ontario slimy sculpin populations were found to be panmictic (Euclide et al. 2018) and inclusion of a covariance matrix may help explain more uncertainty in regional population trends. The Kalman filter assumes Gaussian noise as it transitions through states and we attempted to normalize our data with $\log_{10}$ transformation, but some datasets were skewed with heavy tails, and this could result in the model potentially chasing noisy observations and systematically underestimating uncertainty. If observations consistently had high year to year variance in trend direction, then final state estimates became less responsive to potential noisy observations because of the backwards smoothing procedure.

We did not use a full observation modeling process to generate all our observation data prior to our population dynamics modeling, and we cannot account for varying catchability or selectivity through time that could be associated with a change in gear type in Lake Ontario or changing benthos structure (quagga mussels) in both lakes. We tested gear type as a categorical additive effect during preliminary analysis of Lake Ontario data for standardization of observation data, but it did not emerge as significant and was dropped from subsequent analysis. Dreissenid mussel density can affect the observation process at very high densities when trawls become too heavy and need to be abandoned (Ralph Tingley, USGS, oral. comm., 2025), but we are uncertain how it affects the observation process of prey fish below those levels. Failed or missed trawl tows in the nearshore or offshore could significantly influence dreissenid mussel and round goby values included in our models because of their nearshore relative abundance. In Lake Ontario, observations were more consistently missed nearshore after dreissenid mussels arrived and likely resulted in biased low estimates of round goby and dreissenid mussel density. The change in gear type in Lake Ontario in 1997 was specifically motivated to avoid capturing dreissenid mussels (Weidel et al. 2019), and dreissenid mussels were not consistently recorded across Lake Ontario until 2014. Additionally, four of the six ports in Lake Ontario models with no covariates included were either the best performing model or received substantial support ($\Delta\text{AIC} \leq 2$), therefore, covariate selection in Lake Ontario should be treated more cautiously. In Lake Michigan, observations were consistently sparser offshore and therefore our estimates of round goby and dreissenid mussels are likely biased relatively higher. However, our estimates of all species captured in the trawl surveys are likely biased low because they do not sample rocky substrate that is preferred by these species

Dreissenid mussel density estimates from trawl surveys may be biased low from a selectivity bias from the mesh size relative to the body size of the organisms. The trawl cod end liner mesh size is 13mm stretch and average quagga mussel length was generally around 12mm in Lake Ontario in 1992 (Mills et al. 1993). Considering this potential mismatch, the estimates of dreissenid mussel density will be biased low, but captures may be dependent on the presence of large individuals or the formation of structural clusters or mats that make them collectively large enough to be captured by the net. Our estimates of dreissenid mussel density may only reflect larger individuals and high densities that are large enough to make clusters.

Covariate testing could be influenced by covariate normalization or the way we tested covariate effects as time lagged and additive. We normalized covariates by port using the observed variability of the port's time series of observations, rather than the lake-wide range of variability. This approach makes direct comparison of covariate and beta values between ports difficult because of the varying scales and could result in stronger covariate effects being predicted at ports with lower local densities, but this approach may better reflect the observable local variability and habitat conditions relative to the environmental covariate. Covariates selected often aligned with the highest densities observed across the lake despite this obscured relative magnitude from our covariate normalization process. Covariate inclusion in state estimates relied on an assumption of a one-year time lag effect of covariate measures as an additive effect on the measure of slimy sculpin populations. This time lag was selected over a current year measure of covariates as preliminary model testing showed that models generally performed better with one year lagged effects in Lake Michigan. The effects of dreissenid mussels and round goby may be better captured with a time lag effect because of the delayed effect of resource availability, competition for food, and spawning interference. Meanwhile, predation effects from lake trout may be more immediate and better represented with no time lag considering we see potential immediate responses to lake trout stocking practice in Lake Michigan in 1985. However, one year lagged lake trout population measures and stocking rates often tracked closely with the current year and most juvenile lake trout (Age 0–3) in the previous year will still be preying on slimy sculpin in the following year. We did not test interactive effects of covariates because it was outside the scope of the hypothesized relationships being discussed in the literature. Hypothetically, there could be interactive effects such as round goby competition with slimy sculpin changing in habitats where dreissenid mussels are an abundant food source for round goby (Griffin & Silliman 2011) or lake trout predation pressure on slimy sculpin changing in habitats where round goby are an abundant food source (Barraquand et al.

2015), but the scale at which these interactions occur does not match the scale of our analysis and may be difficult to capture with our limited sample size and high covariate variability.

Despite these limitations, we were able to capture overall population trends and evaluate hypothesized primary drivers (lake trout, round goby, and dreissenid mussels) of slimy sculpin populations to find correlations with slimy sculpin population dynamics. Covariates selected in best-performing models aligned with invasion pathways and regionally higher densities despite the data transformations disguising their real-world scale. Slimy sculpin densities are nearing record observed lows in most regions of Lake Michigan and all of Lake Ontario. If slimy sculpin are now mostly limited to offshore habitats, then their density and persistence likely depends on offshore resource availability and competition. Quagga mussels now completely overlap with slimy sculpin, comprise the majority (>90%) of benthic animal biomass in both lakes, and are not a food resource to slimy sculpin (Bunnell et al. 2015, Vanni 2021). Alternatively, round goby can sustain dense nearshore populations in the summer, feed on quagga mussels, then migrate offshore overwinter and potentially compete with slimy sculpin for resources (Chapter 1). Historically, we have observed regional slimy sculpin populations decline in Lake Michigan and recover from near-zero densities when they appeared to be notably reduced nearshore, but we have never observed such low densities lake-wide, nor such a combination of threats with no immediate management actions available to effectively control round goby or quagga mussel populations. Within Lake Michigan, mid-lake western slimy sculpin populations maintain the highest densities and may be more resilient because of lower dreissenid mussel densities, greater availability of *Diporeia* (French et al. 2010), and average round goby densities. Within Lake Ontario, no region seems to be doing notably better and *Diporeia* are absent. If slimy sculpin declines continue towards near zero densities lake-wide or the species is extirpated, understanding the change in benthic ecological function from slimy sculpin absence and round goby dominance may be important to fisheries managers. The greatest concern is that slimy sculpin populations could risk extirpation within the lakes, resulting in the loss of the natural adaptive capacity of the lakes to function effectively under historic ecosystem conditions.

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CHAPTER 3. SURVEYING SLIMY SCULPIN AND ROUND GOBY IN ROCKY REEF HABITAT WITH EDNA

Abstract

Slimy sculpin (*Cottus cognatus*) populations have declined in Lake Michigan, as invasive round goby (*Neogobius melanostomus*) have become well established, which raises concerns about the ecological function of critical rocky reef habitat that both these species prefer. To better understand the status of these species in these habitats, we combined underwater camera drone surveys and eDNA processed with qPCR and metabarcoding to sample reefs in Grand Traverse Bay and Little Traverse Bay, Lake Michigan, at depths of 5–60m. We expected to see a transition from round goby to slimy sculpin across this depth range, but we never detected slimy sculpin eDNA in our field samples. Laboratory mesocosm experiments and assay testing for specificity and selectivity confirmed that slimy sculpin qPCR assays could detect slimy sculpin DNA, but densities were either too low or slimy sculpin were entirely absent from sampled habitat. We used hierarchical Bayesian occupancy models to assess the relationship between round goby density estimates from camera drone surveys and eDNA detections from qPCR, and evaluated the potential influence of depth, temperature, and reef on sample occupancy; only round goby density estimates were selected in the best fitting model. Metabarcoding results suggest that round goby were the most abundant fish species on these reefs (44% of reads), but in general, the vast majority (≈93%) of fish reads were invasive or introduced species. Overall, our results suggest that round goby and other invasive species dominate nearshore (<60m) rocky habitats in Little Traverse and Grand Traverse Bay in Lake Michigan which slimy sculpin historically occupied, but further observations of rocky reef habitats throughout the lake would be required to extrapolate these results to a lake wide pattern. If so, managers may need to consider how the function of rocky reef habitat that is often used for spawning and foraging has changed for other native Great Lakes species and explore if slimy sculpin life history is flexible enough to be restricted from nearshore (<60m) rocky substrates.

Introduction

The recent proliferation of invasive benthic species like the round goby (*Neogobius melanostomus*) and dreissenid mussels (zebra: *Dreissena polymorpha*; quagga: *D. bugensis*) in the Laurentian Great Lakes benthic ecosystems has raised questions about the current and future function of the benthic food web and role of our native species (Madenjian et al. 2012, Robinson et al. 2021, Tingley et al. 2021). Much of our understanding about Great Lakes benthic fish communities is limited to observations of soft sediments, but rocky reef habitat is often preferred, bioenergetically richer, and critical for the spawning and rearing of native fish species (Marsden et al. 1995, Janssen & Luebke 2004, Mclean et al. 2015, Robinson et al. 2021). In particular, slimy sculpin were historically an abundant benthic prey fish species in Lake Michigan that relied on rocky reef habitat for spawning and served as prey for lake trout (*Salvelinus namaycush*) during their juvenile life stage, which was spent on/near rocky reefs (Hudson et al. 1995, Madenjian et al. 1998, Houghton et al. 2010). Today, round goby are the dominant benthic prey fish in lake trout diets in Lake Michigan and slimy sculpin are less prevalent (Happel et al. 2018, Luo et al. 2019, Leonhardt et al. 2020). As indexed by bottom trawl surveys of Lake Michigan since 1973, slimy sculpin were at the lowest densities on record in 2020 and have remained low since (Ch. 2, Warner et al. 2024). However, it is uncertain whether these trends are similar in preferred rocky reef habitats where prey fish densities could be an order of magnitude greater than soft sediments (LMC 8 Whole-lake indexing of round goby abundances in Lake Michigan with GobyBot [Video] 2024).

Rocky reef habitat within the Great Lakes serves as both an energetic hotspot and spawning and rearing habitat for many native fish species (Marsden et al. 1995, Janssen & Luebke 2004, Kornis & Janssen 2011). However, invasion of rocky reefs by round goby threatens native sculpin species and reef spawning fish populations through resource competition, egg predation, and young fish predation (Janssen & Jude 2001, Fitzsimons et al. 2006, Bergstrom & Mensinger 2009). Historically, slimy sculpin spawning appeared to be dependent on hard structure and mostly restricted to nearshore habitats (<75m) in the summer in Lake Ontario (Owens & Noguchi 1998). However, with recent declines in slimy sculpin abundance and an apparent shift towards the offshore in some Great Lakes (i.e., Lakes Huron and Ontario, Ch. 2, Volkel et al. 2021), it is unknown if this continues today. Overall, researchers are increasingly focusing on understanding, restoring, and preserving rocky reef habitat, and effective methods for surveying this habitat will be critical (Marsden et al. 1995, Mclean et al. 2015, Roseman et al. 2017).

Underwater video surveys have been identified as the most useful technique for surveying round goby populations in the Great Lakes across a variety of substrates (Johnson et al. 2005). Additionally, recent advancements in autonomous underwater vehicle (AUV) technology have enabled camera drone surveys to cover large distances and create detailed maps of physical habitat while counting and measuring observable benthic fish using computer vision for object detection (USGS 2018, Goudah et al. 2023, LMC 8 Whole-lake indexing of round goby abundances in Lake Michigan with GobyBot [Video] 2024, Esselman et al. 2025, P. Esselman, USGS, oral comm., 2025). Computer vision generally involves the use of artificial intelligence to interpret images and videos, and object detection involves identifying specific classes (e.g., fish) within an image and placing a bounding box around them (Goudah et al. 2023). Recent advancements in deep learning based methods, such as YOLO [You Only Look Once], have improved the computational efficiency and accuracy of object detection and are being implemented in Great Lakes surveys for estimating round goby abundance and biomass (Goudah et al. 2023, LMC 8 Whole-lake indexing of round goby abundances in Lake Michigan with GobyBot [Video] 2024, P. Esselman, USGS, oral comm., 2025). However, these methods cannot currently distinguish between sculpin species and round goby, and biomass estimates are limited by the observability of naturally complex habitat in which benthic species can hide (P. Esselman, USGS, oral comm., 2025).

Another recent surveillance technique that has proved useful for surveying round goby in the Great Lakes is environmental DNA (eDNA) (Nevers et al. 2018, Przybyla-Kelly et al. 2023). An advantage of eDNA surveys is that they do not require direct capture or observation of fish to provide information on fish community composition and relative abundance (Nevers et al. 2018, Yates et al. 2019, Fediajevaite et al. 2021, Pukk et al. 2021). However, eDNA surveys can be influenced by a wide variety of physiological and environmental variables that affect DNA shedding, degradation, and movement in the environment (Barnes et al. 2014, Goldberg et al. 2015, Barnes & Turner 2016, Rourke et al. 2022).

Recent literature reviews of eDNA advancements and its application in ecological monitoring have identified limitations and concerns of interpreting eDNA data in detail, but there is an increasing consensus that eDNA is practical for biodiversity monitoring and a growing interest in the potential of eDNA for biomass estimation (Rees et al. 2014, Goldberg et al. 2016, Cristescu & Herbert 2018, Coble et al. 2019, Ruppert et al. 2019, Wang et al. 2021, Rourke et al.

2022). To continue advancing the applications of eDNA surveillance, studies need to validate the relationships between eDNA and fish biomass with physical surveys or laboratory experiments (Goldberg et al. 2016, Nevers et al. 2018, Yates et al. 2019, Pukk et al. 2021). Within the Great Lakes, estimating and detecting round goby and slimy sculpin eDNA in rocky habitat may be useful for improving our understanding of eDNA as a surveillance tool and could fill a knowledge gap concerning sampling within their preferred habitat.

Within the study, our overall goal was to better understand slimy sculpin and round goby distribution within rocky reef habitat and assess the viability of eDNA as tool to survey these fish in this preferred habitat. To achieve this, we developed three objectives: 1) Determine similarities or differences in round goby and slimy sculpin eDNA shedding and decay rates. 2) Estimate the fish community composition, occupancy, and relative abundance of slimy sculpin and round goby in Lake Michigan rocky reef habitat using eDNA. 3) Assess the relationship between round goby and slimy sculpin eDNA detection and biomass density estimates and environmental covariates. To accomplish these objectives, we examined eDNA shedding and decay rates of round goby and slimy sculpin in mesocosms to improve the interpretation of our field results. For our field study, we surveyed on and off rocky reef habitats in northeastern Lake Michigan by collecting water samples for eDNA analysis and conducting camera drone surveys to assess fish community compositions and estimate round goby and slimy sculpin occupancy. Our survey efforts were conducted across a range of depths (5–60m), and field eDNA samples were processed via metabarcoding of the 12S locus to assess community composition and via quantitative polymerase chain reaction (qPCR) for round goby and slimy sculpin to estimate detection probabilities. Lastly, we used the detections of round goby via qPCR from our field study and the estimates of round goby abundance from the camera drone surveys to develop Bayesian hierarchical occupancy models to understand the relationship between local fish biomass and qPCR detection probabilities.

Methodology

Fish Collections and Maintenance for Shedding and Decay Experiments

Mesocosm experiments were performed at the United States Geological Survey, Great Lakes Science Center, in Ann Arbor, Michigan, in the summer of 2022 using round goby and slimy sculpin obtained from Lake Michigan and the Detroit River. Round goby and slimy sculpin from Lake Michigan were captured via trawl surveys (Tingley et al. 2021) and transported to the lab between May 3rd and May 12th. Additional round goby were collected from the Detroit River via baited minnow traps on June 30th and stored with the other gobies. All gobies and sculpins were maintained in 151-liter holding tanks separated by species and fed frozen *Mysis* for at least 30 days prior to experimental trials. Holding tanks ranged in temperature from 4–8 ºC but were consistently held at 8 º C the week prior to starting the mesocosm experiments. Animal welfare in this study was approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC) and followed outlined protocols (PROTO202000106).

Experimental Design and Sample Collections

Mesocosm experiments began on August 15th and ended August 31st and included four periods that ran back-to-back (2 shedding and 2 decay periods). These experiments were conducted within three, 321-liter, unsterilized perforated acrylic aquaria, within a single 4277-liter recirculating aquaculture system (Fig. 3.1) sourced with treated city water. The three tanks were filled with sand roughly 5 cm deep, and two of the tanks held round gobies three weeks prior to the beginning of the experiments. Perforations in the aquaria of ≈1mm diameter (Fig. 3.1) allowed the water temperature to be maintained at the target temperature of 8 º C (± 1 º C) without allowing fish to leave their mesocosm, but DNA was also expected to flow through these perforations. Water samples were collected from the larger tank (between the three smaller tanks) to detect potential DNA outflow and mixing throughout systems (Fig. 3.1). The 4277-liter flow through system was maintained at the target temperature for a week prior and throughout the mesocosm experiments. All tanks were exposed to 12 h light and 12 h dark photoperiods.

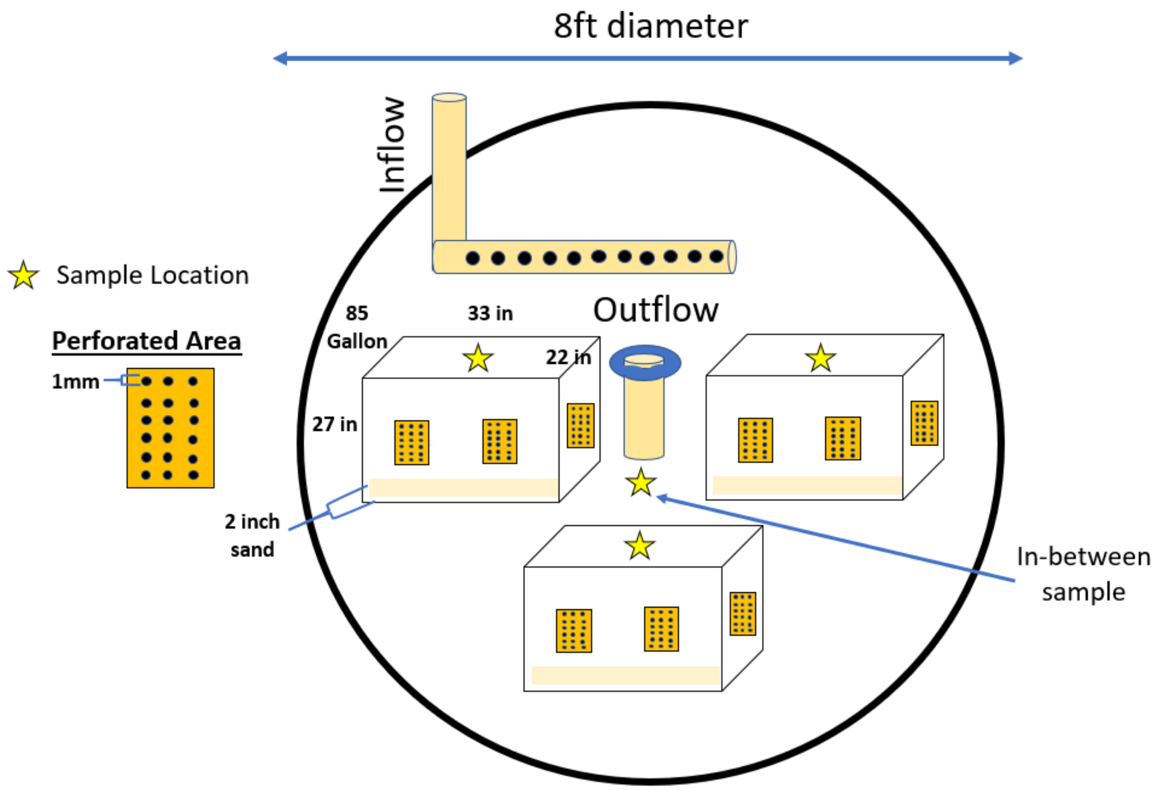

Figure 3.1 Layout of mesocosm experiments used to estimate slimy sculpin and round goby eDNA shedding and decay rates. Outflow pipe of the larger tank was an overflow outflow and water inflow pipe was submerged and pumped water near the bottom of the larger recirculating tank which contained the three experimental tanks that were submerged to the top ≈6 inches of the experimental tanks. Experimental tank 1 (left), tank 2 (bottom), and tank 3 (right).

The round goby mesocosm shedding and decay experiments used three gobies of similar size (116, 118, 130mm) which were weighed and measured prior to being placed in individual 321-liter tanks on August 15th. On August 18th, all gobies were removed, and tanks were left empty until August 22nd. On August 22nd, three sculpins of similar size (66, 67, 70mm) were selected, weighed, and measured, prior to being placed individually into the same three 321-liter tanks. On August 25th, all sculpins were removed, and water samples were collected until August 31st. Water collection efforts were restricted to daytime and weekday hours, which resulted in large gaps between sampling efforts.

One-liter surface water samples for eDNA analysis were collected using a sterile bottle and sterile gloves one hour prior to the beginning of the experiments, roughly 1, 3, 22, 26, 46, 50, and 68 hours after introduction, and roughly 3, 6, 25, 29, 72 or 97, 121, and 146 hours after removal, but exact timing and coverage varied by species (Supplemental Tables 3.1). Negative control samples (distilled water) were collected each day and poured directly into sterile bottles to test sterile handling techniques. All samples were filtered on site within 1hr of collection using sterile 0.45-µm MCE membrane filters, a sterilized reusable sample cup, and benchtop peristaltic pump. All equipment was sterilized in the same way as described for field samples. Filters were then placed in 90% ethanol and stored at room temperature until genomic DNA extraction.

Overview of DNA Processing

Methods for DNA processing are described in full detail below. Briefly, all lab samples were analyzed via qPCR in triplicate per species per sample. We used previously developed qPCR primer sets for both round goby (Nevers et al. 2018) and slimy sculpin (Rodgers et al. 2018) that are described in the field study methods. All testing of primer specificity and limits of detection and quantification were conducted as described in the field study. All PCR amplification and sequencing procedures were conducted as described in the field study.

Shedding and Decay Rates

To calculate shedding rates, eDNA concentrations must first reach steady state where eDNA shedding is in equilibrium with eDNA decay. If steady state was achieved, shedding rates were calculated following the methods of Nevers et al. (2018). Measures of copy number per liter at any time were standardized by subtracting the initial measures of background copy numbers per liter for a species from samples collected prior to the experiment beginning. Decay rates were visualized as the natural logarithm of copy number per liter at any time divided by the natural logarithm of the initial background levels of copy number per liter (Nevers et al. 2018). The limits of detection/quantification (Klymus et al. 2020) were not used for determining which values were included in this study, instead, any samples that exceeded background levels of fluorescence were included in occupancy models.

Field Study Area

Field eDNA collection efforts and camera drone surveys occurred in August 2022, in Grand Traverse Bay and Little Traverse Bay in northeastern Lake Michigan (Fig. 3.2). Surveys were conducted across a range of depths (5–60m) that were thought to represent previous slimy sculpin spawning habitat (Owens & Noguchi 1998) and overlap between round goby and slimy sculpin (Volkel et al. 2021). Water sampling for eDNA and camera drone surveys did not equally overlap in space (Fig. 3.2, Table 3.1) and time because of the opportunistic nature of our sampling; therefore, comparisons between eDNA and drone surveys assumed that biomass estimates from nearby drone surveys were representative of the density of round goby in the locations where eDNA collections occurred.

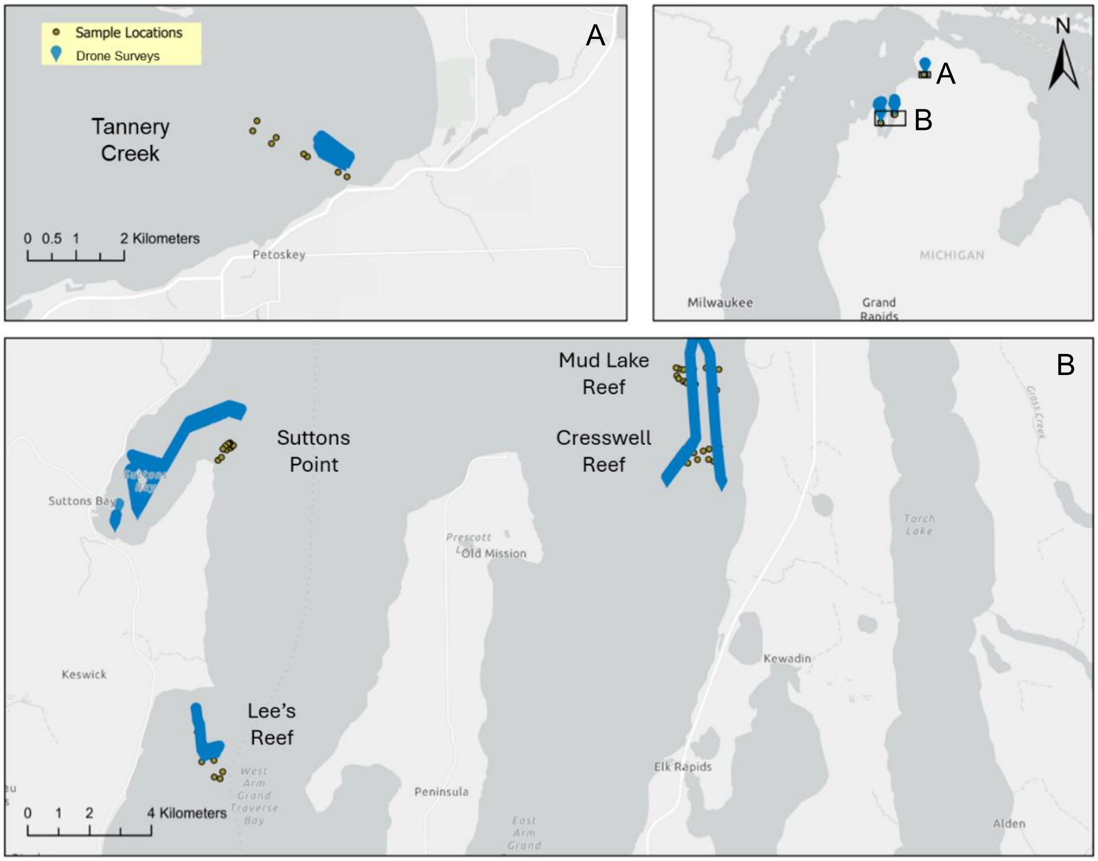

Figure. 3.2 eDNA water sample locations and camera drone survey paths on and off rocky reef habitat in Little (A) and Grand (B) Traverse Bay in Lake Michigan conducted in August 2022 to survey round goby and slimy sculpin.

Camera Drone Surveys, Image Processing, and Habitat Classification

Camera drone surveys were carried out using an L3Harris-Ocean Server Iver3 autonomous underwater vehicle to collect nadir images of the lakebed (for additional details refer to Geisz et al. 2024). Images were collected at five per second at a forward velocity of 1.5 knots (0.77 m/s) (Geisz et al. 2024) and were filtered to remove overlapping images. In total, we collected 5,179 unique usable geotagged images, and identification of round goby in images was conducted using the YOLO8 architecture which uses computer vision for object detection (for additional details refer to Goudah et al. 2023, LMC 8 Whole-lake indexing of round goby abundances in Lake Michigan with GobyBot [Video] 2024, Esselman et al. 2025). The training process for object detection involved developing a training dataset of manually labeled images with bounding boxes of round goby and a QA/QC process assessing the accuracy of computer vision labeled round goby. Computer vision labeled round goby that had an 80% probability of being correctly identified were included in the dataset and biomass estimates were produced using a length-weight relationship associated with the diagonal distance of the object bounding box with the open-source software "FishScale" (for additional details refer to LMC 8 Whole-lake indexing of round goby abundances in Lake Michigan with GobyBot [Video] 2024, Esselman et al. 2025). The object identifier is currently unable to distinguish between round goby and slimy sculpin (P. Esselman, USGS, oral comm., 2025), therefore, all fish were assumed to be round gobies and images were not used to detect slimy sculpin. Once fish were identified and biomass density (g/m^2) estimates were made using "FishScale" (for additional details refer to Esselman et al. 2025), these values were used for subsequent analysis. Substrate in each image was classified as either fine or coarse using a machine learning process (for additional details refer to Geisz et al. 2024) and proportional coarseness (%) of habitat was estimated across depth intervals.

eDNA Sample Collections

Across the five reefs, 55 water samples were collected for eDNA analysis (Table 3.1) and spatially positioned using GPS locations. We collected water samples in transects across a depth gradient in 5m to 15m increments using a Van Dorn sampler that collected water ≈4 m above the lake bottom (Table 3.1). Water samples were transferred into a sterile 1-L bottle while wearing nitrile gloves that were changed between each sample. Samples were probed with a YSI Pro ODO (YSI, Yellow Springs, OH, USA) for water temperature, conductivity, pH, and dissolved oxygen measurements before storing on ice to be processed at the end of each day (<12hrs). All bottles were sterilized before use in 20% bleach with a 10-minute soak (Prince and Andrus 1992). The Van Dorn was not sterilized between sites or samples, but two Van Dorn negative control samples (distilled water poured into the Van Dorn) were collected across the study during sampling events to assess contamination from the sampling device and probes, and four negative control samples (distilled water poured into a sterile bottle) were filtered during sampling events to assess sample handling and filtration techniques.

Table 3.1 Summary of field water samples collected for eDNA by reef and depth range to survey round goby and slimy sculpin in Little and Grand Traverse Bay in Lake Michigan, August of 2022.

Site	Total Samples (#)	0-5m	5-10m	10-20m	20-30m	30-40m	40-50m	50-60m
Lee's Reef	10	2	2	2	4	0	0	0
Suttons Point	11	1	2	2	6	0	0	0
Mud Lake Reef	13	2	1	2	2	2	2	2
Cresswell Reef	13	2	1	2	2	2	2	2
Tannery Creek	8	1	1	1	1	2	2	0

Water Sample Processing and DNA Extractions

All samples were filtered through a 0.45-µm mixed cellulose ester (MCE) membrane filter using a sterilized reusable sample cup and benchtop peristaltic pump, and we changed nitrile gloves between each sample. To sterilize the reusable sample cup between samples, we soaked the cup in a 20% bleach solution for 10 minutes and then rinsed it thoroughly in distilled water and dried with paper towels. Processed sample water was measured in a graduated beaker after each filtration to later adjust DNA concentration measurements. Filters were then placed in 90% ethanol and stored at room temperature until genomic DNA extraction.

DNA extractions were carried out in a dedicated laboratory space for eDNA handling that was routinely sanitized. All filters were removed from ethanol and air dried for 24 hours in a sterile designated eDNA hood prior to DNA extractions. DNA extractions used a combination of a QIAshredder homogenization kit (QIAGEN, Germantown, MD), DNeasy Blood and Tissue extraction kit (QIAGEN, Germantown, MD), and a OneStep PCR inhibitor removal kit (Zymo Research, Orange, CA) following a previously developed protocol (Laramie et al. 2015, Sard et al. 2019). All necessary materials and bench spaces were cleaned prior to use with 25% bleach and DNA Away (Thermo Fisher Scientific, Inc., Waltham, MA). One extraction negative control was included in each extraction event (≈20 samples) to test for contamination during the DNA extraction procedure.

qPCR Primer/Probes and Sequencing

Samples analyzed using qPCR were run in triplicate. Each plate included three replicates of a standard curve produced by 5-fold serial dilution of either a round goby cytochrome c oxidase subunit I (COI) or slimy sculpin cytochrome b (cytb) gBLOCK gene fragment (Integrated DNA Technologies, Coralville, IA) in 100 ng/µL yeast tRNA (Sigma Life Science, St. Louis, MO), with copy numbers ranging from 10 to 31,250. In addition, each plate contained three no template control reactions containing UV treated sterile nuclease free water and three positive reactions containing round goby or slimy sculpin genomic DNA at a copy number between 600 and 2000 at 1 ng/µL within 1 µL.

For round goby, we used mitochondrial COI TaqMan reagents developed by Nathan et al. (2015) and Nevers et al. (2018): GobyCOI-F2d: 5′- CTTCTGGCCTCCTCTGGTGTTG -3′, GobyCOIR2d: 5′- CCCTAGAATTGAGGAAATGCCGG -3′, and GobyCOI-Pr: 5′- 6-FAM-CAGGCAACTTGGCACATGCAG -BHQ-1 -3′. qPCR analysis for slimy sculpin used mitochondrial cytb TaqMan reagents based on those published by Rogers et al. (2018) but with the primers redesigned to better reflect sequences deposited in GenBank for this species from Lake Michigan: Cc-cytb-F1: 5'- CAACAAACTTGGGGGCGTT -3′, Cc-cytb-R1: 5'-GAGTTCAAAATAGGAATTGGGTTACG -3v, Cc-cytb-Pr: 5'- 6FAM-CATCCATCCTGGTGCTCAT -MGB-NFQ -3'. This resulted in an assay for round goby that amplifies 149 base pairs of COI (Nevers et al. 2018) and an assay for slimy sculpin that amplifies 128 base pairs of cytb.

qPCR reactions for both species contained 10 µL of 2X TaqMan Environmental Master Mix 2.0 (LifeTechnologies, Carlsbad, CA), 3 µL of eDNA template or UV-treated sterile nuclease-free water (for no template control reactions), and sufficient UV-treated sterile nuclease-free water to bring the reaction volume up to 20 µL. For round goby, reactions also contained 1.8 µL of each primer at 10 µM and 2.5 µL of probe at 2.5 µM, and for slimy sculpin, reactions also contained 0.6 µL of forward primer at 10 µM, 1.8 µL of reverse primer at 10 µM and 2 µL of probe at 2.5 µM. Instead of eDNA, standard curve reactions contained 1 µL of the corresponding gBLOCK DNA, and positive control reactions contained 1 µL of round goby or slimy sculpin genomic DNA at 0.1 ng/µL.

Reactions were carried out on a QuantStudio™ 6 Flex Real-Time PCR System (Thermo-Fisher, Waltham, MA) using the Fast 96-well block with cycling conditions set to 95° C for 10 minutes followed by 40 cycles of 95° C for 15 seconds and 60° C for one minute. Data were analyzed using QuantStudio™ Real-Time PCR Software v 1.2 (Thermo-Fisher, Waltham, MA), using a manual threshold for fluorescence that exceeds background levels of fluorescence and reaches the exponential part of the curve. A positive detection was classified when an amplification curve crossed this fluorescence threshold (above background fluorescence).

Assay Sensitivity and Specificity

Slimy sculpin qPCR assays were tested for species specificity using genomic DNA isolated from mottled sculpin (*Cottus bairdii*), deepwater sculpin (*Myoxocephalus thompsonii*), and slimy sculpin. The round goby qPCR assay was also tested using genomic DNA isolated from round goby. Template DNA isolations were tested in 10-fold serial dilutions of concentrations ranging from 1 ng/µL to 0.000001 ng/µL to produce 7 concentrations with two replicates, using 1 µL of DNA as template in each qPCR reaction. Each plate included a standard curve using a gBLOCK in triplicate, as described previously, and no-template controls. Both sets of reagents were tested for limits of detection (LOD) and quantification (LOQ) using the standard curves run with the gBLOCK following the guidance and scripts of Klymus et al. (2020). The limit of detection is defined as the lowest standard concentration at which 95% of the replicates produce positive amplification of the target DNA, and the limit of quantification is defined as the lowest standard concentration that could be quantified with a coefficient of variation below 35% (Klymus et al. 2020). It is important to note that amplifications of target

DNA at concentrations lower than the LOD/LOQ should not be dismissed, but LOD/LOQ are meant to serve as standardized way of reporting the accuracy of qPCR assays (Klymus et al. 2020).

Occupancy Modelling

We used Bayesian multi-scale hierarchical occupancy models (Dorazio and Erickson 2018) to estimate detection probabilities for round goby and slimy sculpin from qPCR across all samples using depth, temperature, and drone survey biomass as covariates influencing sample occupancy. Within our study, any replicate that exceeded the background threshold of fluorescence was treated as a positive detection. These multiscale occupancy models estimate a hierarchy of parameters that specify:

1. The probability of a species occurrence at a reef "ψ",
2. the conditional probability of a species' DNA being collected in a sample from a reef, given that the species is present "Θ" and
3. the conditional probability of a species detection in a qPCR replicate, given that the species is present in the sample "p".

All models were fit with the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm, with 2 chains of 20,000 iterations and assessed with a burn-in of 1,000 using the *eDNAoccupancy* R package (Dorazio and Erickson 2018, R Core Team 2025). Convergence was assessed with trace plots and Gelman-Rubin statistics using the *coda* R package (Plummer et al. 2015, R Core Team 2025). To assess model performance, we used Widely Applicable Information Criterion (WAIC) and Posterior Predictive Loss Criterion (PPLC) to quantify goodness of fit, predictive variance, and predictive power relative to the complexity of the models. We tested models with sample level covariates representing water temperature (^{o}C), depth (m), reef, and depth-specific round goby biomass density (g/m^2) from nearby camera drone surveys that may influence the probability of round goby eDNA being collected in water samples. Nearby camera drone survey biomass densities were averaged across images at five-meter depth intervals from the nearest continuous survey mission (Fig. 1).

Metabarcoding Taxonomic Database and Sequencing

All field samples were analyzed in duplicate via metabarcoding. We used a previously developed taxonomic database for metabarcoding (for additional details see Pukk et al. 2021) to detect up to 140 native and invasive Michigan fish species using the mitochondrial 12S rDNA locus with a region amplified by vertebrate-specific primers (Forward: 5′-ACTGGGATTAGATACCCC -3′, Reverse: 5′ TAGAACAGGCTCCTCTAG -3′) developed by Riaz et al. (2011). Additionally, the database was expanded to include other common vertebrate taxa in Michigan to reduce the number of unclassified operational taxonomic units (OTUs). All samples were amplified for metabarcoding using the PCR cycling conditions of 95° C for 10 minutes, followed by 35 cycles of 95° C for 30 seconds, 57° C for 30 seconds, and 72° C for 45 seconds and then one period of 72° C for 5 minutes. Libraries were prepared for sequencing by adding sample specific barcodes using the PCR cycling conditions of 95° C for 15 minutes, followed by 10 cycles of 95° C for 10 seconds, 65° C for 30 seconds, and 72° C for 30 seconds and then one period of 72° C for 5 minutes. Sequencing was carried out at the Michigan State University Research Technology Support Facility (RTSF) (https://rtsf.natsci. msu.edu/) using an Illumina MiSeq v2 flow cell lane to generate paired-end 150 base pair sequences.

Analysis of eDNA Community Composition Matrices

To develop our community composition matrices, we classified and counted sequences per unique operational taxonomic units, hereafter OTU or OTUs, which can be described as a group of closely related organisms (e.g., species or genus) (Sokal and Sneath 1963). All field sample sequencing data were initially processed using Mothur v1.46.1 (Schloss et al. 2009) following modified protocols described in Sard et al. (2019). The data were demultiplexed, and sequences were trimmed of primer regions, screened by size (size range 87–110 bp) and aligned to the 12S taxonomic database. Chimeric sequences were removed using Vsearch v2.16.0 (Rognes et al. 2016) and remaining reads were clustered into OTUs with a sequence similarity of 99% using default settings. Then, read counts per OTU were counted for each sample to create the community matrices. Nucleotide BLAST was used to query all OTUs that were not classified to species or genus to potentially classify other fish species missing or poorly represented in the taxonomic databases. Species that were classified but known to be extinct or unlikely to be in

Lake Michigan because they are outside of their distribution and are rare, were removed from the community matrix.

We needed to account for potential for contamination to falsely represent OTUs across samples because of the unsterilized Van-Dorn and from unintentional contamination. Therefore, we removed OTUs from each sample that had read counts beneath a threshold determined by calculating the median number of non-zero reads per classification from all negative control samples based on the procedure of Pukk et al. (2021). Additionally, human DNA contamination was evident within some of our samples, likely from the unsterilized Van Dorn, and may have unequally inhibited the potential to detect present fish species. Therefore, samples that did not have at least 1000 fish reads in one of the two replicates were removed from all subsequent analysis. All community matrix analyses were conducted in R v4.3.1 (R Core Team 2025) using the "tidyverse" package (Wickham & Wickham 2017).

Results

qPCR No-Template and Positive Controls

Across all objectives related to field samples, mesocosms, and assay sensitivity/specificity with genomic and synthetic DNA none of the no-template qPCR control reactions and extraction negatives showed amplification and all the qPCR positive controls with genomic DNA were positive (1 µL at 0.1 ng/µL).

qPCR Assay: Specificity and Sensitivity for Genomic DNA

During the serial dilutions testing the sensitivity of the round goby qPCR assay for genomic DNA, round goby amplified consistently at or above 0.01 ng/µL and in 1 of 2 replicates at 0.001 ng/µL but failed to amplify in replicates between 0.0001 ng/µL and 0.000001 ng/µL. During the serial dilutions testing the sensitivity of the slimy sculpin qPCR assay for genomic DNA, mottled sculpin never amplified, deepwater sculpin weakly amplified in 3 of the 4 replicates at the highest concentrations, and slimy sculpin amplified consistently at concentrations at or above 0.01 ng/µL and in 3 out of 4 replicates at 0.001 ng/µL, but failed to amplify in replicates between 0.0001 ng/µL and 0.000001 ng/µL.

Assay Sensitivity for Synthetic DNA

All round goby standard curves ranged between a slope of ≈ −3.3 to –3.5, $R^2 > 0.98$, and efficiency = ≈90% to 102%. Across the 10 plates with round goby standard curves, positive amplifications were consistently observed in samples with eDNA copy numbers at and above 250, while failure to amplify occurred in ≈6.6% of wells at 50 copies, and in 20% of wells at 10 copies. The LOD estimated for the round goby assay was 62.9 copies, while the LOQ was 324 copies (Sup Fig. 3.1 A).

All slimy sculpin standard curves ranged between a slope of ≈ −3.3 to –3.5, $R^2 > 0.99$, and efficiency = ≈93% to 102%. Across the 8 plates with slimy sculpin standard curves, positive amplifications were consistently observed at and above 1,250 copies, and failure to amplify occurred in ≈4% of wells at 250 copies, ≈12.5% of wells at 50 copies, and ≈16.6% of wells at 10 copies. The LOD estimated for slimy sculpin copies per replicate was 79.4 and LOQ was 199 (Sup Fig. 3.1 B).

Mesocosm Experiments

We were unable to estimate shedding rates of round goby or slimy sculpin because eDNA concentrations had not reached a steady state within ≈70 hours (Fig. 3.3) in any of the tanks. Slimy sculpin DNA concentrations converged around ≈200–240 (CN/g/L) at the end of the experiment and tank 3 had been near that range for the last 24 hours, but a longer observation period would be needed to confirm a steady state given the variance we observed (Fig 3.4 B). Round goby DNA concentrations were ≈72–254 (CN/g/L) by the end of the experiment, but tanks 1 and 3 were declining and tank 2 concentrations were rising (Fig. 3.3 A). Decay rates could not be compared between species because slimy sculpin DNA detections and measurements were inconsistent (Fig. 3.4). Round goby DNA detections and measurements were consistent until roughly ≈200 hours after removal from the tanks (Fig. 3.4 A). Escapement of eDNA from the mesocosms was detected in the in-between samples in the larger recirculating tank, but eDNA concentrations of in-between samples were on average <1% and ≈2% of the round goby and slimy sculpin eDNA concentrations detected in mesocosms (Sup. Table 3.2 A & B).

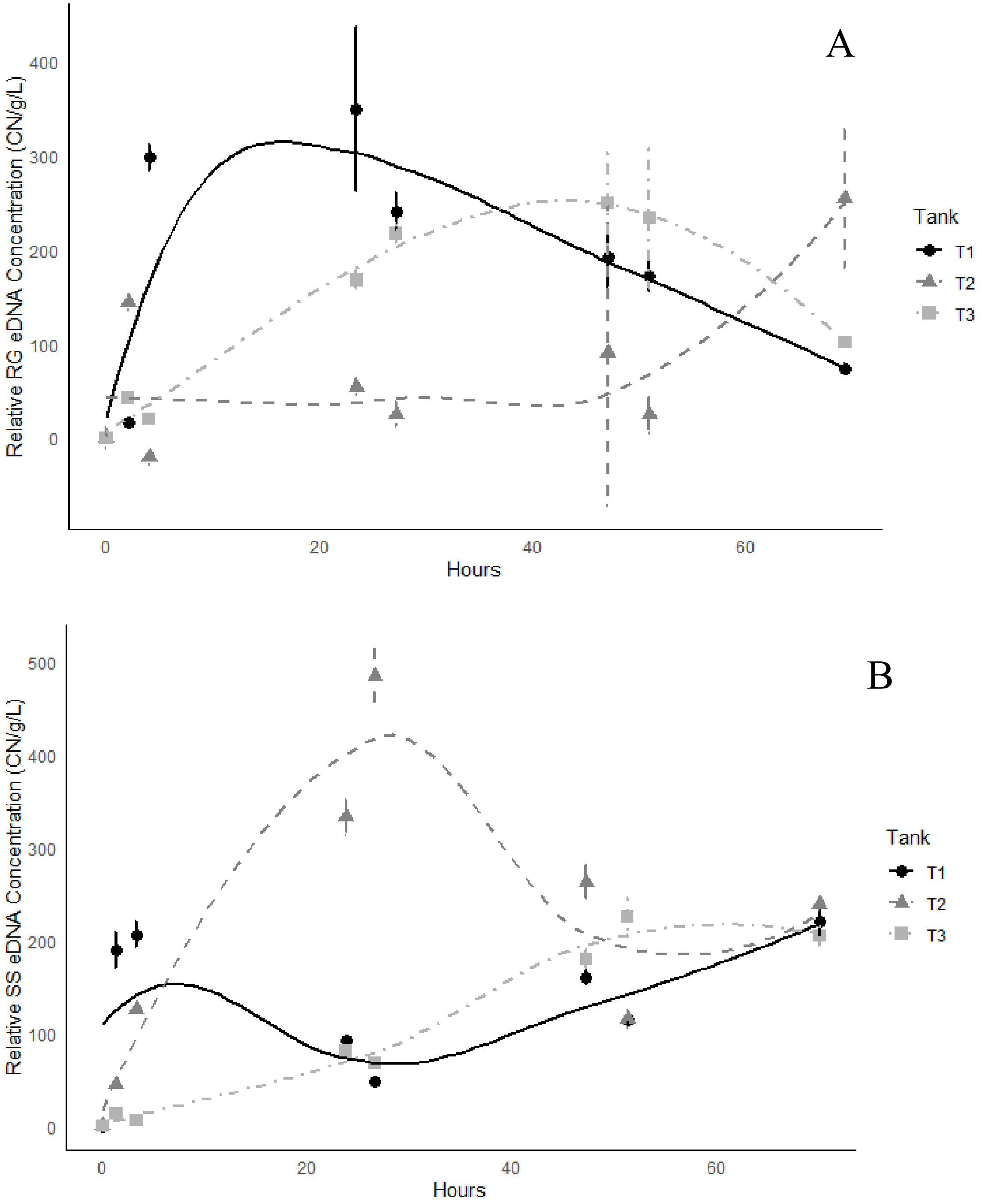

Figure 3.3 Observations of average eDNA concentrations over time and the smoothed relationship through time (Loess smoothing method) for mesocosm experiments with (A) one round goby and (B) one slimy sculpin per tank at 8 ° C (± 1 ° C) until they were removed. Relative eDNA values represent the concentration of target fish DNA per liter of water sampled (CN/L) at a time minus the initial concentration of target fish DNA before the experiment began and then standardized by the weight of the fish in the tank (g). Vertical lines through the points represent the standard deviation of the replicates of a sample.

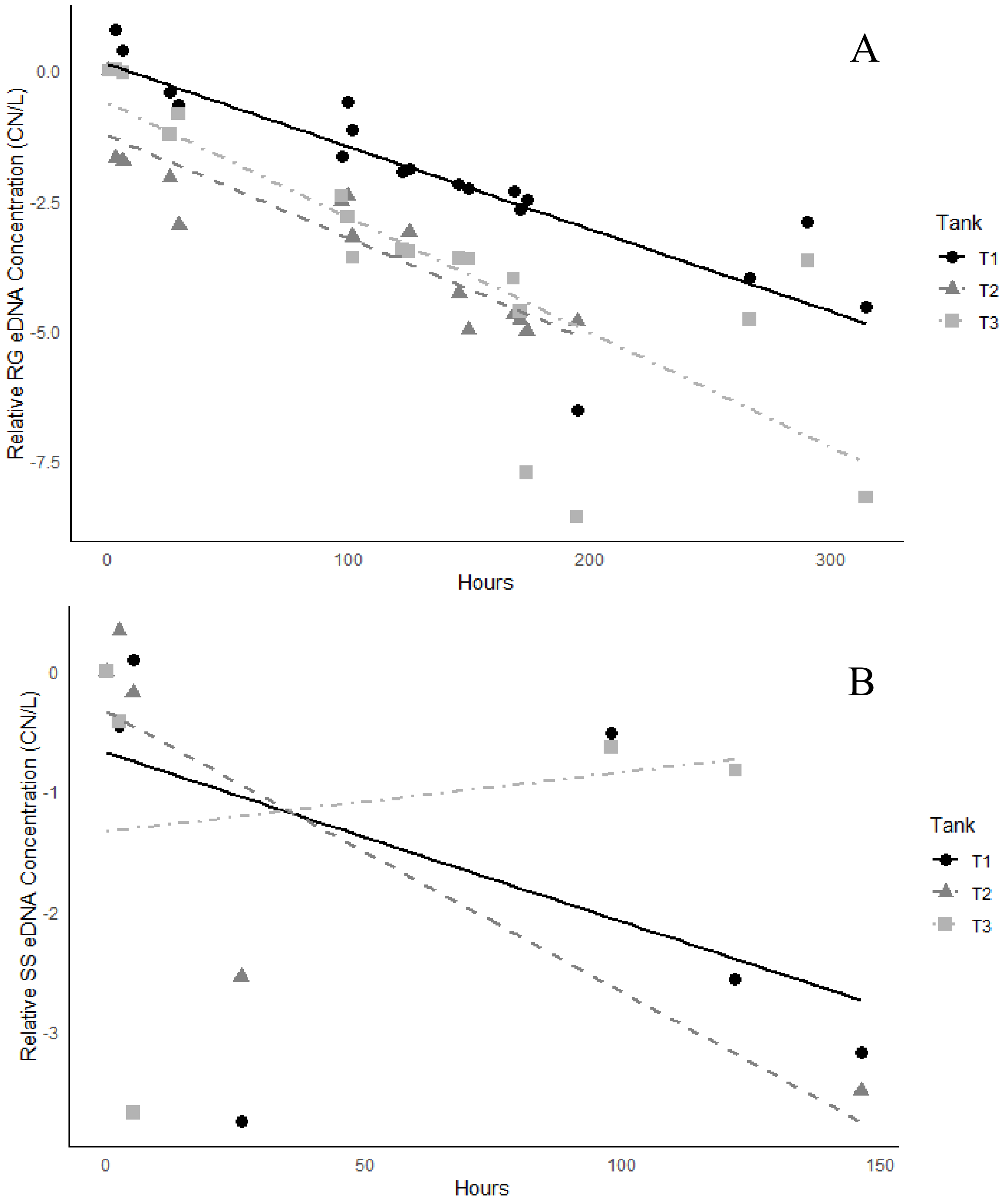

Figure 3.4 Observations of average eDNA concentrations over time and the linear relationship through time for mesocosm experiments with (A) one round goby and (B) one slimy sculpin per tank at 8 ° C (+ 1 ° C) after they were removed. Relative eDNA values represent the natural log of concentration of target fish DNA per liter of water sampled (CN/L) at a time divided by the initial concentration of target fish DNA right before the experiment began and then standardized by the weight of the fish in the tank (g).

Camera Drone Surveys and qPCR of Field Samples

Drone survey efforts and eDNA collections did not consistently overlap (Fig. 3.2, Table 3.2) and slimy sculpin were never detected in any of our field samples using qPCR or metabarcoding, which limited subsequent analyses accordingly. Cresswell Reef and Mud Lake Reef were surveyed with the camera drone at the same time and subsequent round goby density estimates were averaged from that survey, and each reef in occupancy models received the same values at depth intervals (Fig. 3.2, Table 3.2).

Across all eDNA samples, 75% (n=41) had drone surveys at that depth interval for the survey of that reef and were able to be used for occupancy modeling. Of these, 26% did not have any amplification of round goby DNA beyond the critical threshold of background fluorescence in any replicate. Round goby DNA was amplified at all sites, but only one eDNA sample had high enough round goby DNA density to meet LOD and none met the threshold for LOQ (Table 3.2, Sup Fig. 3.1). Nonetheless, we retained all detections of round goby for subsequent occupancy modeling.

Across all drone survey efforts, we estimated round goby density at 27 unique depth intervals (Table 3.2) that were surveyed by both camera drones and eDNA. Across these depth intervals, the average round goby density was 1.58 (g/m^2) and 18.5% of the intervals did not observe any round goby (Table 3.2), but these depth intervals were not equally surveyed (Fig. 3.2). The one eDNA sample that did meet reliable levels of detection was collected from a depth interval (≈10m) that also had the highest observed round goby density (12.91 g/m^2) from camera drone surveys (Table 3.2).

Table 3.2. Overview of average drone survey density estimates of round goby and average copy numbers of round goby (RG) DNA per replicate estimated from qPCR across depth intervals and by reef, collected on and off rocky reef habitat in Little and Grand Traverse Bay in Lake Michigan, August of 2022. Drone survey density estimates (g/m^2) averaged across all non-overlapping usable images from the nearest continuous drone survey to the eDNA collections. Round goby copy numbers were averaged across all replicates and samples that fell within that depth range for a reef surveyed.

	Suttons Point		**Lees Reef**		**Cresswell Reef**		**Mud Lake Reef**		**Tannery Creek**	
Depth (m)	Drone Density (g/m^2)	RG qPCR	Drone Density (g/m^2)	RG qPCR	Drone Density (g/m^2)	RG qPCR	Drone Density (g/m^2)	RG qPCR	Drone Density (g/m^2)	RG qPCR
4-5	2.59	0.00	2.19	15.03	1.71	3.87	1.71	12.31		10.32
>5-10	1.50	15.51	1.51	15.29	2.55	15.77	2.55	1.55	1.70	4.32
>10-15	1.58	2.00	1.16	5.06	6.99	0.52	6.99	5.80	0.40	
>15-20	1.40	0.00	0.07	0.48	12.91	16.40	12.91	7.89	0.00	0.00
>20-25	1.16	5.10	0.00	0.40	2.53	93.53	2.53	13.38		
>25-30	0.14	0.30		1.48	0.51	2.10	0.51	2.00		0.00
>30-35	0.23	0.00	0.00	0.00	0.37	0.75	0.37			
>35-40	0.24		0.00		0.26	0.00	0.26			0.00
>40-45	0.00		0.00		0.14	2.98	0.14	9.50		2.88
>45-50	0.58		0.00		0.00	0.00	0.00			0.00
>50-55	0.27					0.72		3.45		
>55-60	1.41				0.00	0.80	0.00	2.13		
>60-65	0.56				0.58	0.00	0.58	0.00		

Occupancy Models

We tested 11 candidate models to determine which sample level covariates would produce the lowest WAIC and PPLC score. Both criteria selected the model that only included round goby density estimates from camera drone surveys (RG) (Table 3.4). The next best fitting models within ≈2 WAIC included additive effects of sample depth and water temperature (Table 3.4, Sup Table 3.3 & 3.4). Among the top three models, we observed no difference in sample occupancy by round goby (Θ) across reefs based on 95% highest posterior density credible intervals (95% HPD) among estimates informed by covariates (Sup Table 3.3), but Suttons Point average (0.60–0.69) was notably lower than the other reefs (0.85–0.91) (Sup Table 3.3). The mean probability of round goby occupancy among reefs was 0.86 (95% HPD: 0.53–1.00), and the mean conditional probability of round goby DNA detection in a single replicate given that round goby DNA was present in the sample (p) was 0.68 (95% HPD: 0.58–0.77) (Sup Table 3.3). Using the complement of the individual probability of a replicate detection, the overall probability of detecting round goby DNA in at least one replicate of a sample with three replicates was 96.7%. The mean estimated covariate coefficient for round goby density estimates from camera drone surveys (RG) in the best performing model was 1.46 (95% HPD: 0.35–2.72) (Fig. 3.5) (Sup Table 3.4). In the second-best model, the mean estimated covariate coefficient for round goby density was 1.25 (95% HPD: 0.13–2.58), and depth (m) of the lake where the water sample was collected (depth) was -0.16 (95% HPD: -0.61–0.30) (Sup Table 3.4). In the third-best model, the mean estimated covariate coefficient for round goby density was 1.20 (95% HPD: 0.11–2.55), depth was 0.13 (95% HPD: -0.62–0.93), and temperature of the water sample (temp) was 0.36 (95% HPD: -0.43–1.22) (Sup Table 3.4). For the best fitting model, the sample level parameters exhibited high autocorrelation (Sup Fig. 3.2), but our effective sample sizes were above 1000 for all model parameters and Gelman-Rubin statistics from two chains were near 1.0, indicating adequate samples from parameter posterior distributions and convergence of the MCMC chain (Sup Fig. 3.3).

Table 3.3 Candidate set of hierarchical occupancy models used to estimate the probability of round goby eDNA occurrence among reefs (ψ) in Lake Michigan, 2022, the conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ), and the conditional probability of round goby eDNA detection in replicates given that round goby eDNA was present in the sample (p). Model comparisons were evaluated with Widely Applicable Information Criterion (WAIC) and Predictive Loss Criterion (PPLC) to find the model with the lowest scores. Covariates tested were round goby density estimates at a nearby depth interval during camera drone surveys (RG), lake depth at location of water sample for eDNA collections (depth), temperature of the water sample used for eDNA collections (temp), and the reef where samples were collected (reef).

Model	**WAIC**	**PPLC**
ψ(.), Θ(RG), p(.)	55.55	56.12
ψ(.), Θ(RG+depth), p(.)	56.37	56.61
ψ(.), Θ(RG+depth+temp), p(.)	57.05	57.22
ψ(.), Θ(RG+reef), p(.)	57.91	58.12
ψ(.), Θ(RG+temp), p(.)	59.95	57.07
ψ(.), Θ(depth), p(.)	60.74	59.42
ψ(.), Θ(temp), p(.)	60.91	59.85
ψ(.), Θ(.), p(.)	61.24	59.63
ψ(.), Θ(depth+temp), p(.)	61.92	60.56
ψ(.), Θ(RG*temp), p(.)	61.96	60.15
ψ(.), Θ(RG*depth), p(.)	62.51	60.46
ψ(.), Θ(depth*temp), p(.)	63.86	62.39
ψ(.), Θ(reef), p(.)	78.40	83.70

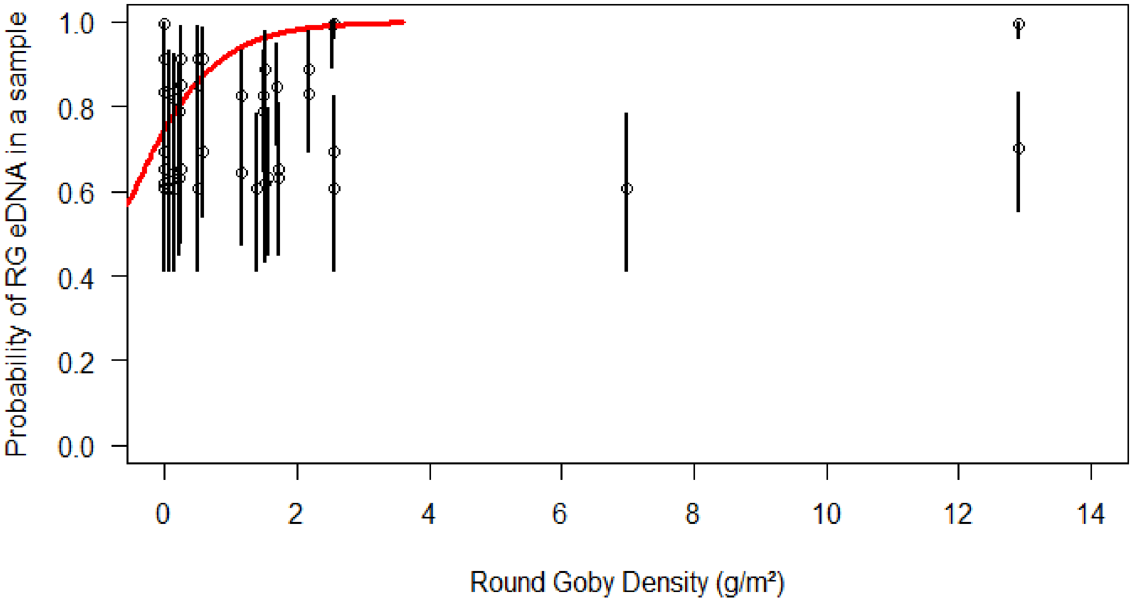

Figure 3.5 Mean posterior detection probability for round goby DNA occurrence (y-axis) in water samples collected on and off rocky reef habitat in Little and Grand Traverse Bay in Lake Michigan, August of 2022 (open circles) and 95 % credible intervals (black lines) from the best fitting hierarchical occupancy model. The x-axis is the average round goby biomass density (g/m^2) estimated from nearby camera drone surveys at the same depth interval as the eDNA sample. The red curved line is the logistic relationship between drone survey round goby density and round goby eDNA detection probability using the mean posterior parameter values. The best fitting model included average round goby biomass density (g/m^2) from nearby camera drone surveys as a sample level covariate for estimating the conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ).

Metabarcoding

Across all samples, round goby had the highest proportional representation of any detected fish species (44%: Table 3.4), and slimy sculpin were never classified via metabarcoding (Sup. File 1). A few reads were assigned to Cottidae unclassified, but those samples generally contained large read counts of deepwater sculpin and most likely represent sequencing errors (Sup. File 1). *Alosa* unclassified had the second highest proportional read counts overall (33%: Table 3.4). Due to a lack of interspecific sequence differences, the sequenced 12S fragment cannot distinguish among alewife (*Alosa pseudoharengus*), blueback herring (*Alosa aestivalus*), skipjack herring (*Alosa chrysochloris*), and American shad (*Alosa* *sapidissima*) (Sup. File 2), but only alewife is present in Lake Michigan, so it is most likely alewife. Atlantic salmon (*Salmo salar*) was the third most abundant OTU by fish read counts (11%: Table 3.4), it was highly unequally distributed across samples and reefs (Sup. File 1, Table 3.4), and we are uncertain about the reliability of these detections because of a lack of corresponding stocking of this species in Lake Michigan. After the top three most abundant OTU's by fish read count, the next top seven were each under 3% of the total fish read count beginning with white sucker (3%: *Catostomus commersonii*), rainbow smelt (2%: *Osmerus mordax*), common carp (2%: *Cyprinus carpio*), freshwater drum (1%: *Alpodinotus grunniens*), *Salmonidae* unclassified (1%), lake trout (1%: *Salvelinus namaycush*), and deepwater sculpin (1%: *Myoxocephalus thompsonii*) (Table 3.4). All other OTU's classified represented ≈1% of the total fish read counts and represented seven other species: blackchin shiner (*Notropis heterodon*), yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*), bluntnose minnow (*Pimephales notatus*), chinook salmon (*Oncorhynchus tshawytscha*), and lake whitefish (*Coregonus clupeaformis*). Fish species identified that are not expected to be found in Lake Michigan that were of low abundance were dismissed from our summary reporting but can be found within the supplementary file (Sup. File 2). This included: blue sucker (*Cycleptus elongatus*), amur grayling (*Thymallus tugarinae*), mountain whitefish (*Prosopium williamsoni*), beloribitsa (*Stenodus leucichthys*), razorback sucker (*Xyrauchen texanus*), dolly varden trout (*Salvelinus malma*), and pygmy whitefish (*Prosopium coulterii).* Additionally, ≈13% of our field samples were dismissed from our analysis because of too few fish reads (<1000 in 1 of 2 replicates). The median number of fish reads in our control samples was 4, and the copy numbers reported from our field samples were adjusted (-4) accordingly. Overall, invasive or introduced species represented ≈93% of the fish sequence reads from our surveys on or near rocky reef habitats (Sup. File 1).

Table 3.4 Overview of the proportional fish read abundance for the top ten fish operational taxonomic units (OTU) overall and by reef that were classified via metabarcoding of the 12S locus. Samples were collected on and off Lake Michigan rocky reef habitat in Little and Grand Traverse Bay in August of 2022.

OTU	All	Cresswell	Lees Reef	Mud Lake	Suttons Point	Tannery Creek
Neogobius melanostomus	44%	46%	25%	49%	58%	56%
Alosa unclassified	33%	27%	61%	18%	40%	27%
Salmo salar	11%	16%	0%	22%	0%	0%
Catostomus commersonii	3%	1%	4%	3%	1%	2%
Osmerus mordax	2%	2%	0%	1%	0%	8%
Cyprinus carpio	2%	0%	8%	0%	0%	8%
Aplodinotus grunniens	1%	5%	0%	0%	0%	0%
Salmonidae unclassified	1%	3%	0%	1%	0%	0%
Salvelinus namaycush	1%	1%	1%	3%	0%	0%
Myoxocephalus thompsonii	1%	0%	0%	3%	0%	0%
Other	≤1%	≤1%	≤1%	≤1%	≤1%	≤1%

Discussion

In Lake Michigan, we have observed dramatic declines of slimy sculpin in trawl surveys that occur on soft substrates (Ch. 2, Tingley et al. 2021), but researchers were uncertain if these observations were consistent with population changes in the species' preferred rocky reef habitat. Round goby have been hypothesized to be contributing to declines in slimy sculpin abundance through displacement and competition (Ch. 1, Robinson et al. 2021, Volkel et al. 2021). Our observations may suggest that slimy sculpin no longer consistently occupy nearshore rocky reef habitat in northeastern Lake Michigan and this habitat may be dominated by round goby.

We never detected slimy sculpin in our eDNA field samples, and round goby were the most abundant fish species by metabarcoding read counts. Our assays were able to successfully detect slimy sculpin and round goby DNA with similar sensitivities using genomic DNA, but slimy sculpin DNA detections in mesocosm experiments were inconsistent one day after the relatively small slimy sculpin were removed. The observable densities of round goby by camera drone surveys were the most important predictor when detecting round goby DNA in our samples and not all eDNA samples detected round goby despite their relative abundance. We planned to quantitatively compare eDNA shedding and decay rates between round goby and slimy sculpin to understand if these species are relatively equally detectable through time, but high variance in detections and estimates of eDNA made this unachievable. Our field observations suggest using eDNA collected from water samples representing natural densities of round goby and slimy sculpin on or near rocky reef habitat and reliably detecting low abundances or quantifying them via qPCR may present challenges.

Across all our samples, only one exceeded the estimated LOD for round goby and no samples reached the LOQ (Klymus et al. 2020). A previous study using the same round goby primers and probes for qPCR (Przybyla-Kelly et al. 2023) estimated much lower LOD and LOQ (7.46 CN/reaction) compared to our results (LOD: 62.9, LOQ: 324 CN/reaction), but our results are still within the range of values observed in other studies and labs using qPCR and reported in Klymus et al. (2020). This difference in standards could be a result of equipment, technique, and/or assay designs (Nevers et al. 2018) that decreased our amplification efficiency or consistency. Future studies using these qPCR assays may want to increase the number of replicate standard curves to potentially reduce variability in LOD/LOQ estimates. Additionally, if using these assays to observe natural densities of these species in the Great Lakes, future studies may want to increase the water volume filtered, the number of field and qPCR replicates, or the template volume used for qPCR to reduce variability in detections. Our field samples were collected in August when Przybyla-Kelly et al. (2023) also had the lowest detection rates of round goby eDNA (16%) in Lake Huron depth intervals. Round goby were only detected at 20m in their August surveys (Przybyla-Kelly et al. 2023) and concentrations were relatively lower than samples at this depth interval in April and October. It could be that round goby DNA shedding and decay in the Great Lakes in August biases round goby eDNA concentrations from qPCR low and impacted our field results.

Despite these limitations, we did frequently observe round goby DNA being amplified above background levels of fluorescence, which should not be excluded as detections (Klymus et al. 2020), and these detections were used in our occupancy modeling. We compared nearby camera drone surveys estimating round goby biomass density with our qPCR detections using Bayesian hierarchical occupancy models (Dorazio & Erikson 2018) and found that round goby biomass density estimated by camera drone surveys was the most important predictor of round goby DNA detections with greater biomass resulting in a greater detection probability in water samples. Round goby prefer warm nearshore waters in the summer (Carlson et al. 2021) and environmental covariates (depth & temperature) inclusion in the next best fitting models may have been a proxy for round goby density distribution. Disentangling the relationship between round goby's environmental preferences and the environmental effects on eDNA shedding and decay rates may be difficult within the confines of occupancy models or field observations. Laboratory observations are an effective way of estimating these environmental effects (Nevers et al. 2018), but translating them into field observations may require more complex models that try to estimate eDNA concentrations from detailed field observations of fish density and environmental parameters.

We were not able to model slimy sculpin detection probability because it was never detected in field samples via qPCR or metabarcoding. It is possible that slimy sculpin exist within or near reefs at such low densities that they are not able to be reliably detected. Notably, Jude et al. (2022) also surveyed the benthic fish community in Grand Traverse Bay, Lake Michigan, with remotely operated vehicles (ROV) taking images and observed very low slimy sculpin densities. Across ≈6 hours of footage at depths from 70–191m, they observed hundreds of round goby and over 100 deepwater sculpin, but only one slimy sculpin (Jude et al. 2022). Based on trawl surveys from soft substrates, slimy sculpin densities are low and round goby are the most abundant at depths <65m (Ch. 2, Volkel et al. 2021). Across multiple survey approaches for detecting benthic prey fish in northern Lake Michigan, there seems to be agreement that slimy sculpin are at low densities and round goby dominate the benthic prey fish community (Ch. 2).

Today, it appears that round goby and other invasive species are the most abundant fish in rocky habitat we surveyed at depths <65m where historically we may have observed slimy sculpin spawning in the summer (Owens & Noguchi 1998). However, extrapolating our observations from relatively small and narrow geographic areas may not be advisable as there may be geographic variation in slimy sculpin densities within and across lakes (Ch. 2). Our assays were able to detect slimy sculpin DNA from juvenile slimy sculpin in mesocosm studies, and after 48 hours of shedding, all tanks consistently measured concentrations above our limits of detection. Studies looking to revisit our hypothesis may want to target geographic regions where slimy sculpin are at higher densities and at different times of year, but could use or refine our methodology. If slimy sculpin occupancy is consistently restricted from nearshore rocky habitat year-round, it may be important to understand the flexibility of slimy sculpin life history in offshore (>65m) habitat and if nearshore displacement from rocky reef habitat causes reduced spawning and recruitment. Overall, our results indicate that the community and related function of rocky reef habitat has potentially changed in invaded Great Lakes systems and understanding how invasive species effect this critical habitat may be a priority for natural resource managers.

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CONCLUSION

The broad objective of my dissertation was to improve the understanding of slimy sculpin population declines in the Great Lakes and explore potential primary drivers of their population dynamics, including round goby competition, dreissenid mussel invasions, and lake trout predation.

In chapter 1, we observed slimy sculpin growth, shelter occupancy, chasing rates, spawning rates, and mortality rates with and without the presence of a round goby in a laboratory setting at cold water temperatures (5 °C) reflective of their potential offshore overlap. Slimy sculpin shelter occupancy significantly decreased in the presence of round goby, which appeared to be motivated by round goby size advantages. However, slimy sculpin were more likely to chase round goby, and we did not detect significant effects of round goby on slimy sculpin growth, spawning activity, or survival. On average, we observed a 50% decrease in slimy sculpin growth in the presence of round goby while feeding fish to satiation, but if offshore environments are food limited, then competition may be greater than we observed, and food competition could restrict spawning potential of slimy sculpin. Overall, the implications of these results would suggest that round goby could interfere with slimy sculpin populations in cold water (5 °C) offshore conditions. If researchers are seeking to extend our results to deeper dwelling deepwater sculpin, it is important to determine if the decreased aggression we observed from round goby was reflective of the cold water or a response to slimy sculpin aggression. Additionally, deepwater sculpin's ability to spawn in open sandy substrates (Jude et al. 2022) may help mitigate spawning habitat competition.

There are a few key uncertainties that could help researchers understand the ecological conditions for slimy sculpin persistence within the Great Lakes and their potential for recolonization if ecological conditions do vary in the future. Observing slimy sculpin spawning in the Great Lakes could be increasingly difficult given their low abundances (Ch. 2), but understanding the degree of overlap between slimy sculpin and round goby throughout their viable spawning season (spring to fall) may be informative of the potential for spawning interference. It is presumed that slimy sculpin spawn on hard structures (Owens and Noguchi 1998) with their adhesive eggs, but it may be worth testing if they can spawn in open sandy, gravelly, or dreissenid mussel substrates that may be more widely available and potentially decrease competition with round goby. We observed slimy sculpin spawning and eggs maturing in the lab without round goby or dreissenid mussels present. It may be worth examining if slimy sculpin spawning habitat quality could be reduced by dreissenid mussel presence because of their physical and chemical ecological engineering (Karatayev et al. 2002, Sousa et al. 2009). Additionally, examining how dominant male slimy sculpin guarding eggs or a shelter respond to larger round goby or multiple round gobies being introduced may reflect the potential for spawning interference within the Great Lakes.

In chapter 2, we modeled slimy sculpin population dynamics in Lake Michigan and southern Lake Ontario from the 1970's to 2020 and tested lake trout, round goby, and dreissenid mussel densities as primary drivers of slimy sculpin densities using Kalman filtering and smoothing. We modeled dramatic declines (>99.9%) in some slimy sculpin populations from their peaks in both lakes, but in the best case, declines were ≈90% or populations persisted around 10 fish per hectare rather than <1 fish per hectare. Most models supported the beginning of current slimy sculpin declines with initial dreissenid mussel expansions, specifically quagga mussels, and candidate models including dreissenid mussel abundance as a covariate were often selected as the best-fit models for slimy sculpin population dynamics. We also observed support for models that included round goby abundance in Lake Ontario western ports where the species first colonized and in western Lake Michigan ports where the initial increases in dreissenid mussel density during the quagga mussel expansion were relatively lower. In Lake Michigan, the first surge in round goby populations at a port aligned with the beginning of current slimy sculpin declines at the two mid-lake western ports but also aligned closely with accelerated declines across the lake. Lake trout were not selected in the best performing models with beta estimates that meaningfully contributed to the model, but we found substantial support for a candidate model that included a lake trout covariate with a significant negative relationship at the northern port in Lake Michigan that borders the lake trout refuge habitat. Additionally, we saw patterns in Lake Michigan slimy sculpin population trend changes around 1985 that aligned with the lake trout stocking changes (Holey et al. 1995). Lastly, both lakes showed changes in the depth distribution of slimy sculpin in the spring (Lake Ontario) and fall (Lake Michigan) during current slimy sculpin declines, that begin with nearshore (<40m) absence and proceed outward and in some cases result in complete population loss.

In Lake Michigan, the regions that were last to collapse or were doing relatively better than the rest in 2020 had some of the lowest average densities of dreissenid mussels and round goby and supported *Diporeia* populations until the end of our time series (Nalepa et al. 2020). In southern Lake Ontario, no region seems to be doing notably better than the rest, slimy sculpin were generally still gradually declining, and invertebrate surveys suggest *Diporeia* are absent (Birkett et al. 2015). Currently, there are no feasible management options to control dreissenid mussels and round goby populations lake wide and the persistence of slimy sculpin populations may be dependent on offshore conditions. Our chapter 3 results suggest that slimy sculpin populations are not fairing any better in lesser-surveyed nearshore rocky reef habitats. Therefore, fisheries managers may need to consider benthic ecosystem dynamics under a prey fish community dominated by round goby and retaining low density or absent slimy sculpin populations. This ultimately could represent a loss of adaptive capacity within the Great Lakes ecosystem, but we have seen regional populations of slimy sculpin recover from near zero densities in Lake Michigan. Additionally, genetic analysis of slimy sculpin within Lake Ontario suggests they are panmictic (Euclide et al. 2017) and it could be that localized populations may be adequate for preserving genetic diversity and long-term potential for recolonization. If slimy sculpin are extirpated within either lake, the next nearest population source may be within connected inland watersheds, but little is known about their genetic relatedness, overall abundance, and persistence.

In Chapter 3, our primary aim was to understand if slimy sculpin populations were potentially still abundant within nearshore (<60m) preferred rocky reef habitat, compared to the soft sediments surveyed for long term population estimates (Ch.2). We used metabarcoding and qPCR of eDNA alongside camera drone surveys in and near rocky reef habitat in northeastern Lake Michigan, and we never detected any slimy sculpin DNA. Overwhelmingly, we indexed invasive or introduced fish species reads (≈93%) and round goby were the most common fish species by read count (44%). We validated the specificity and sensitivity of our slimy sculpin qPCR assay within mesocosms and with experimental testing, but densities of slimy sculpin within our field surveys are either too low for us to detect or slimy sculpin are absent. Further surveying of rocky reef habitat where we expect slimy sculpin densities to be greater, such as mid-lake western Lake Michigan (Ch.2), may validate if slimy sculpin displacement from nearshore rocky reef habitat is likely lake wide.

Overall, there was consistency in our conclusions about the potential for competition from round goby to result in slimy sculpin displacement (Ch.1 & 3) and we observed a negative relationship between slimy sculpin population dynamics and round goby densities (Ch. 2). We found the strongest evidence for quagga mussels to be a primary driver of slimy sculpin declines in Lakes Michigan and Ontario (Ch. 2), potentially related to the loss of *Diporeia* or decreased foraging efficiency. Slimy sculpin populations within Lake Superior and inland lakes throughout North America have not been invaded to the same extent or invaded at all by round goby or quagga mussels, and detailed depth structured surveys of these populations and the associated benthic invertebrate communities may help us understand slimy sculpin dynamics in their absence. It is presumed that slimy sculpin populations within Lakes Michigan and Ontario were historically abundant, but our survey efforts began after widespread anthropogenic disturbances to the top predators occurred (Coble et al. 1990, Muir et al. 2012). We do not know what "normal" slimy sculpin densities would be under pre anthropogenic disturbance conditions and it is uncertain when these conditions may arise again. The resist-accept-direct (RAD) framework is increasingly being suggested for ecosystems and fisheries undergoing rapid ecological changes that result in new ecological states (Lynch et al. 2021, Lynch et al. 2022, Alofs et al. 2022, Kocik et al. 2022) and may help Great Lakes fisheries managers evaluate conservation efforts for slimy sculpin and other native benthic organisms under benthic ecosystem dynamics dominated by dreissenid mussels and round goby (Bunnell et al. 2014, Karatayev et al. 2014, Deroy and MacIsaac 2020, Burlakova et al. 2022, Eifert et al. 2023).

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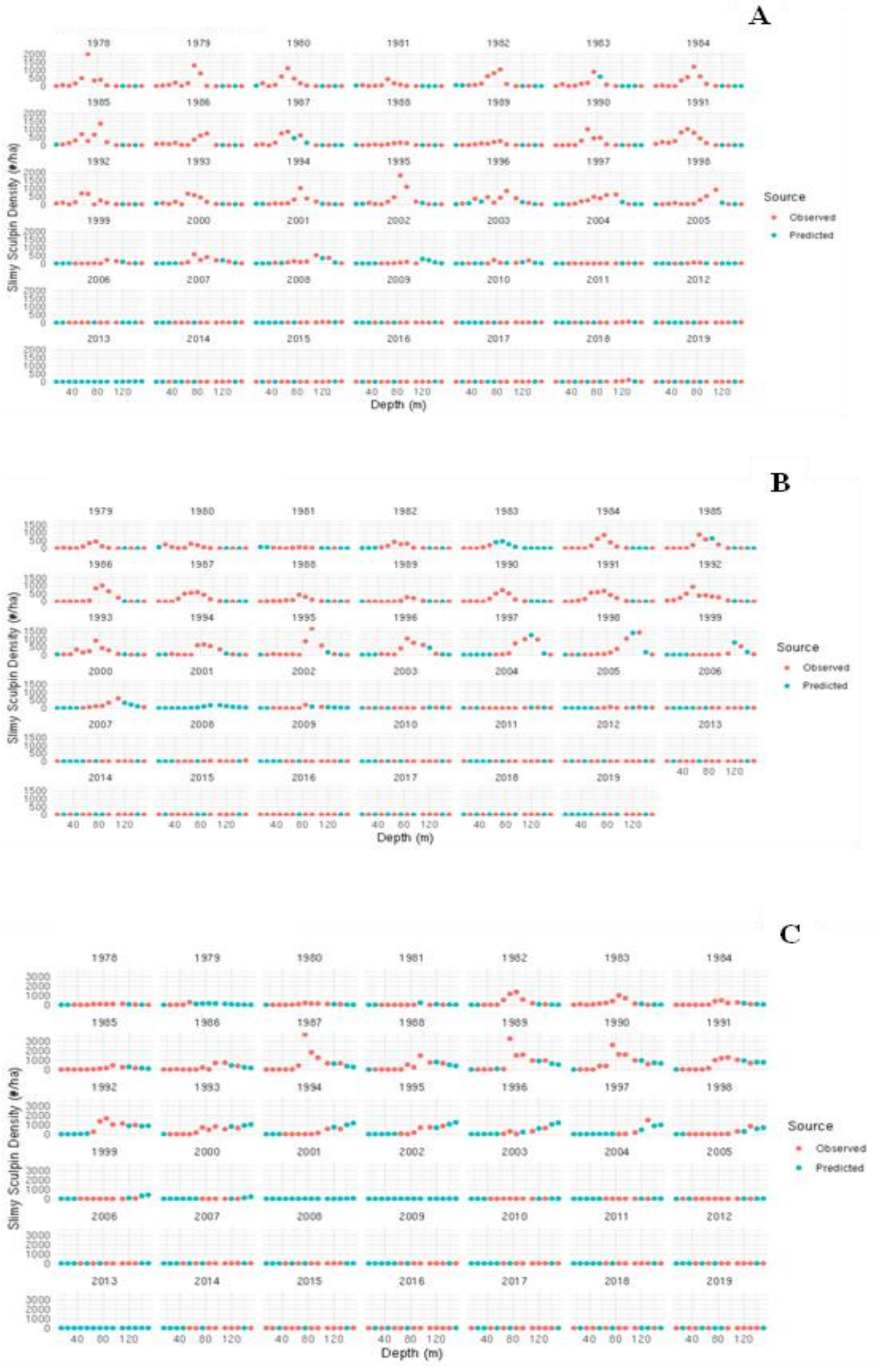
APPENDIX A: CH. 1 SUPPLEMENTAL

Supplemental file Video file of chases observed in laboratory experiments.

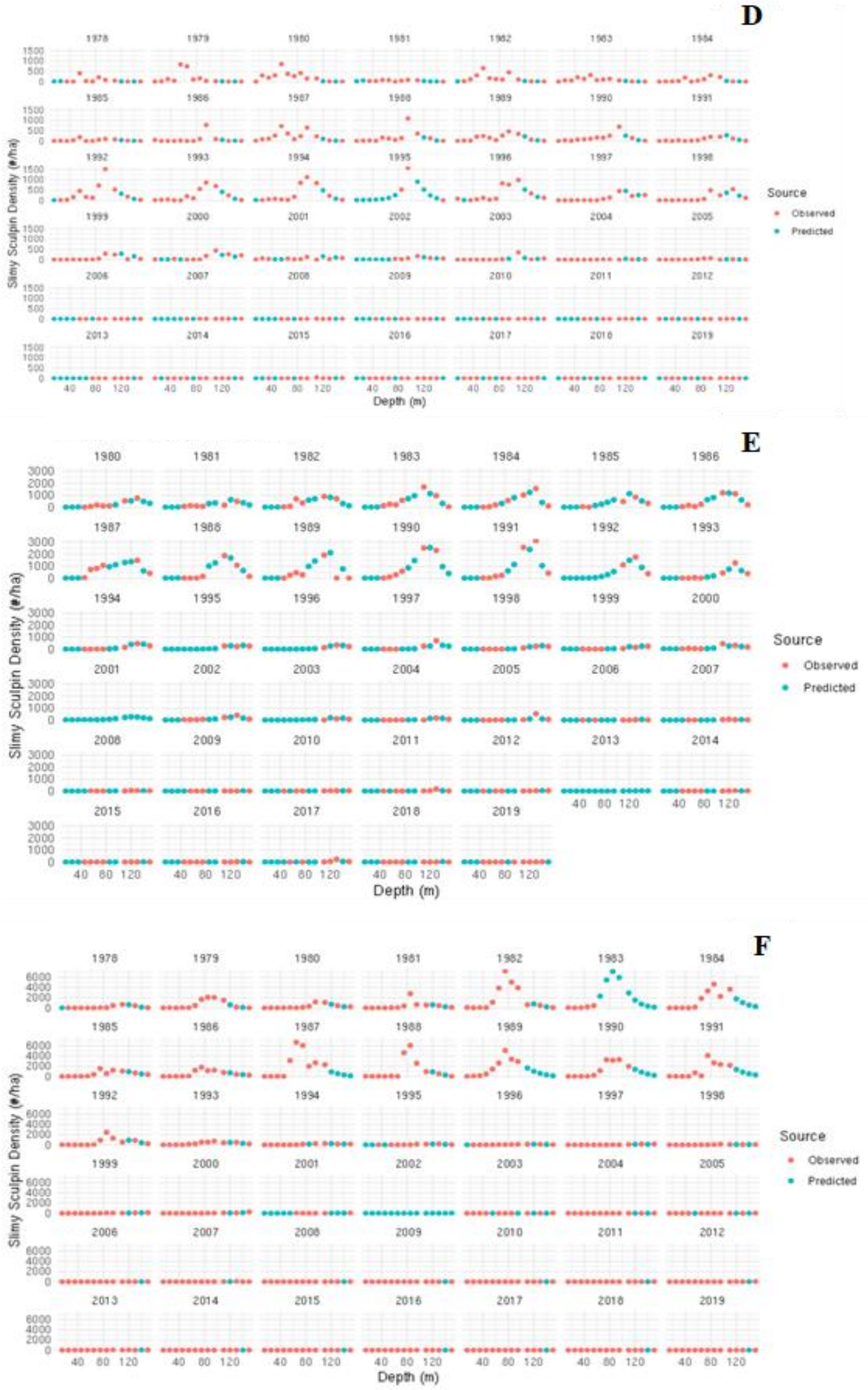
APPENDIX B: CH. 2 SUPPLEMENTAL

Supplemental File 1. Excel file containing the performance of candidate models for lakes Michigan and Ontario.



<u>Figure S2.1.</u> Combined data set of real trawl observations and predicted observations from delta generalized additive models (GAM's) of slimy sculpin density (#/ha) across the survey extent (15-150m) in ≈10m increments for each year from 1978-2019. Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F).

Figure S2.1. (cont'd).



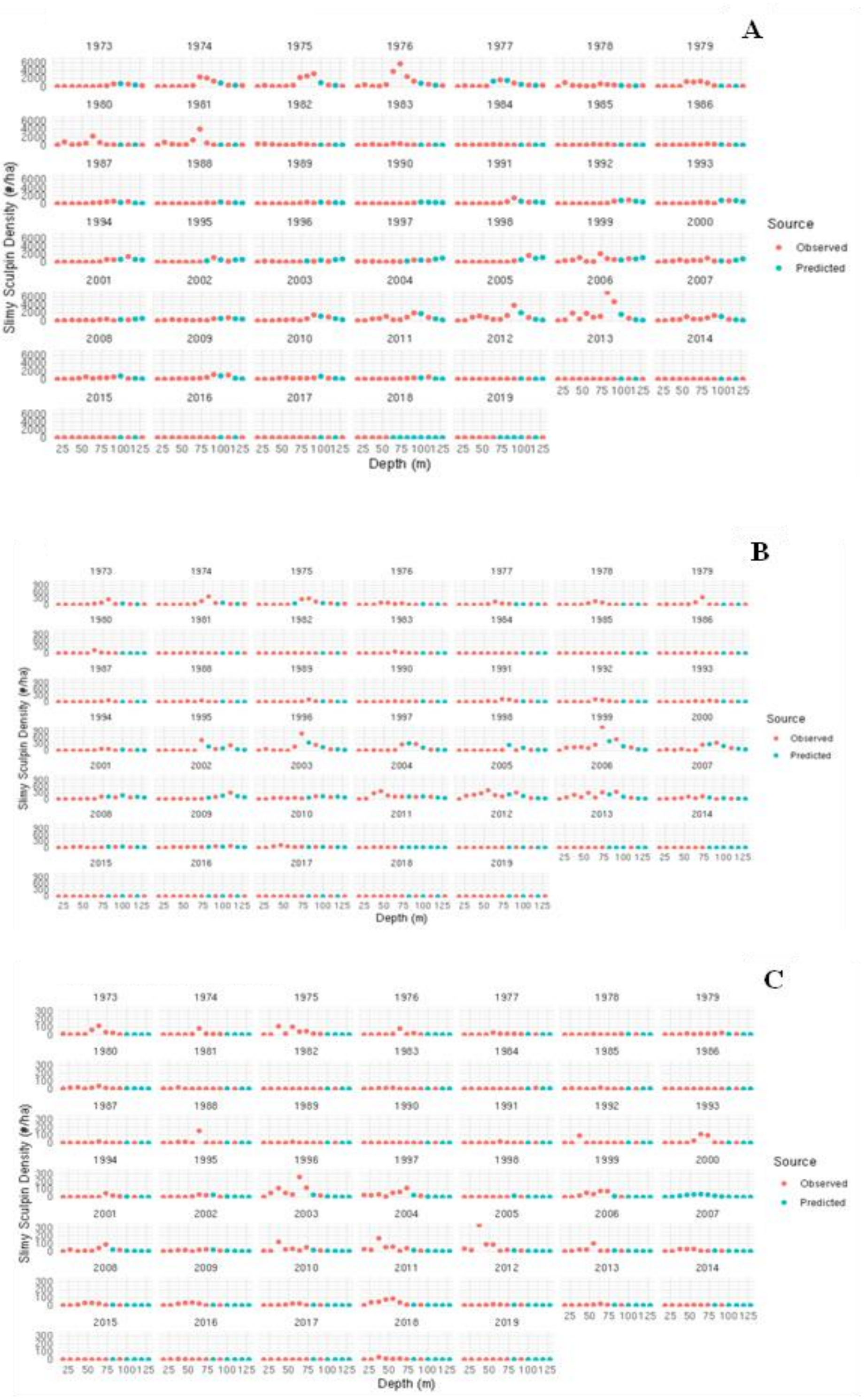
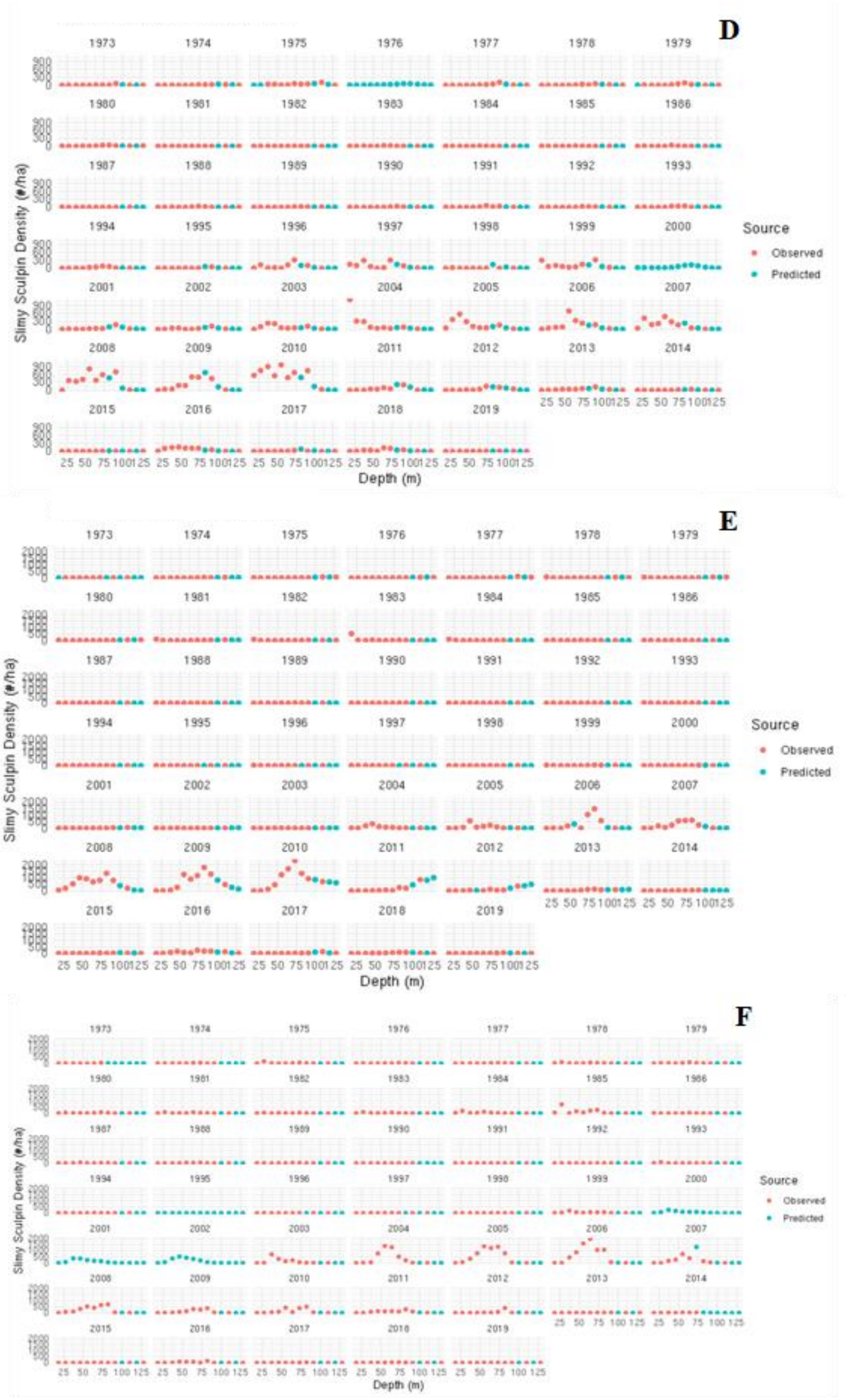


Figure S2.2. Combined data set of real trawl observations and predicted observations from delta generalized additive models (GAM's) of slimy sculpin density (#/ha) across the survey extent (18-128m) in ≈9m increments for each year from 1978-2019. Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F).

Figure S2.2. (cont'd)



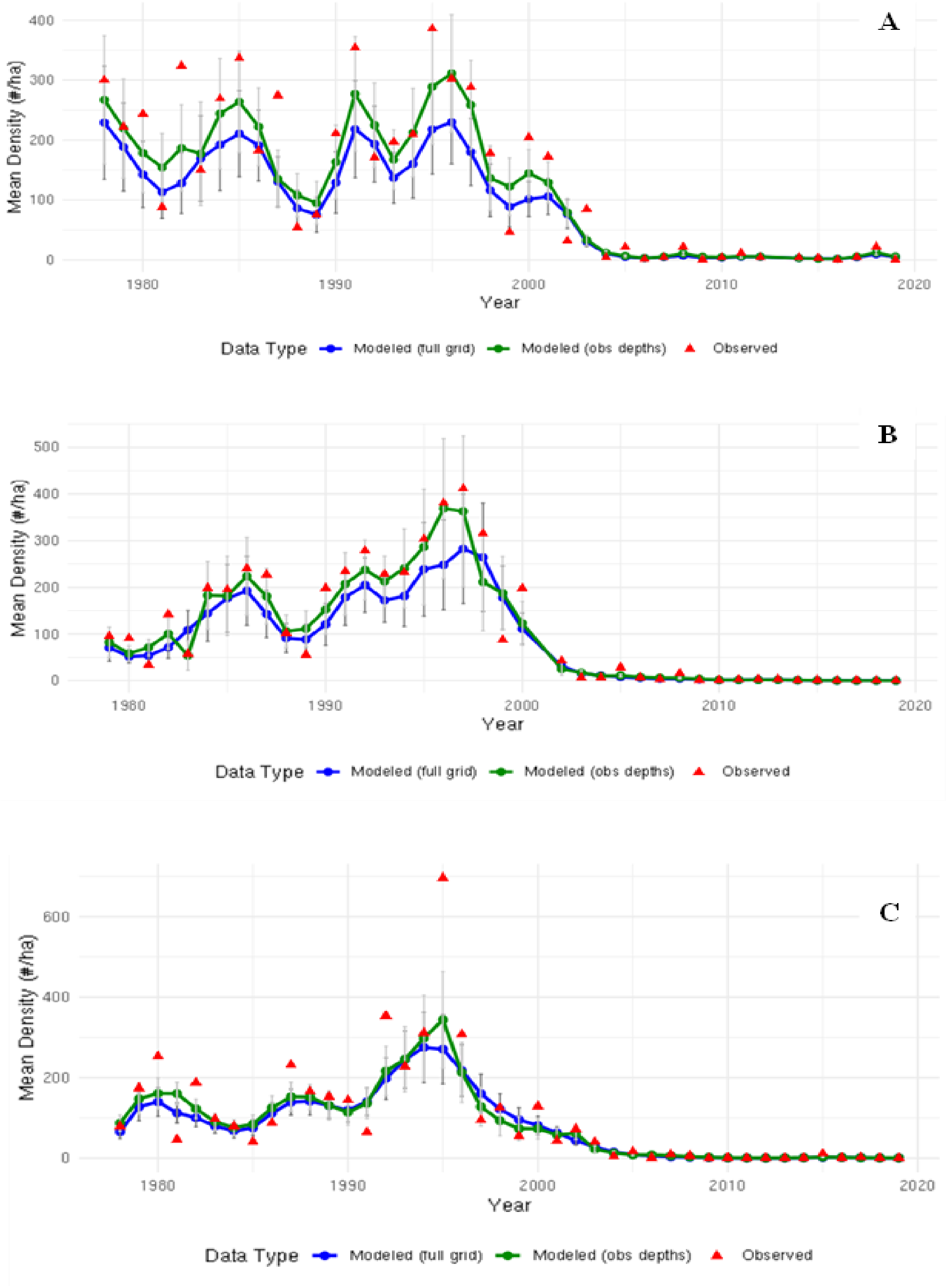
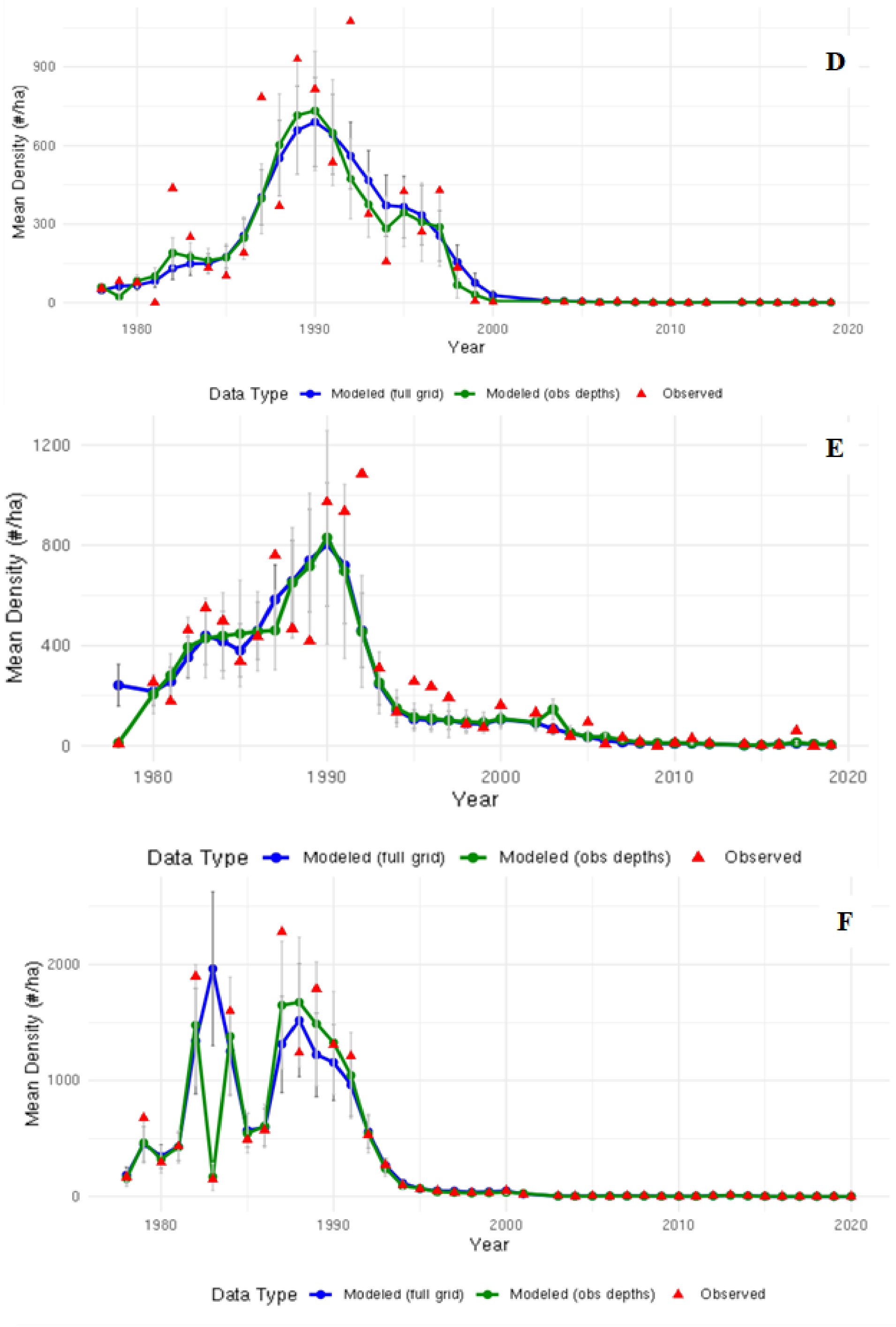


Figure S2.3. Average predicted Slimy Sculpin density (#/ha) and standard error bars from the observation models across the full standardized survey extent and increments (Modeled (full grid) & Blue). Average predicted Slimy Sculpin density (#/ha) and standard error bars from the delta generalized additive models (GAM's) at only the observed transects each year (Modeled (obs depth) & Green). Average observed density from the trawl surveys each year without standardization (Observed & Red). Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F).

Figure S2.3. (cont'd)



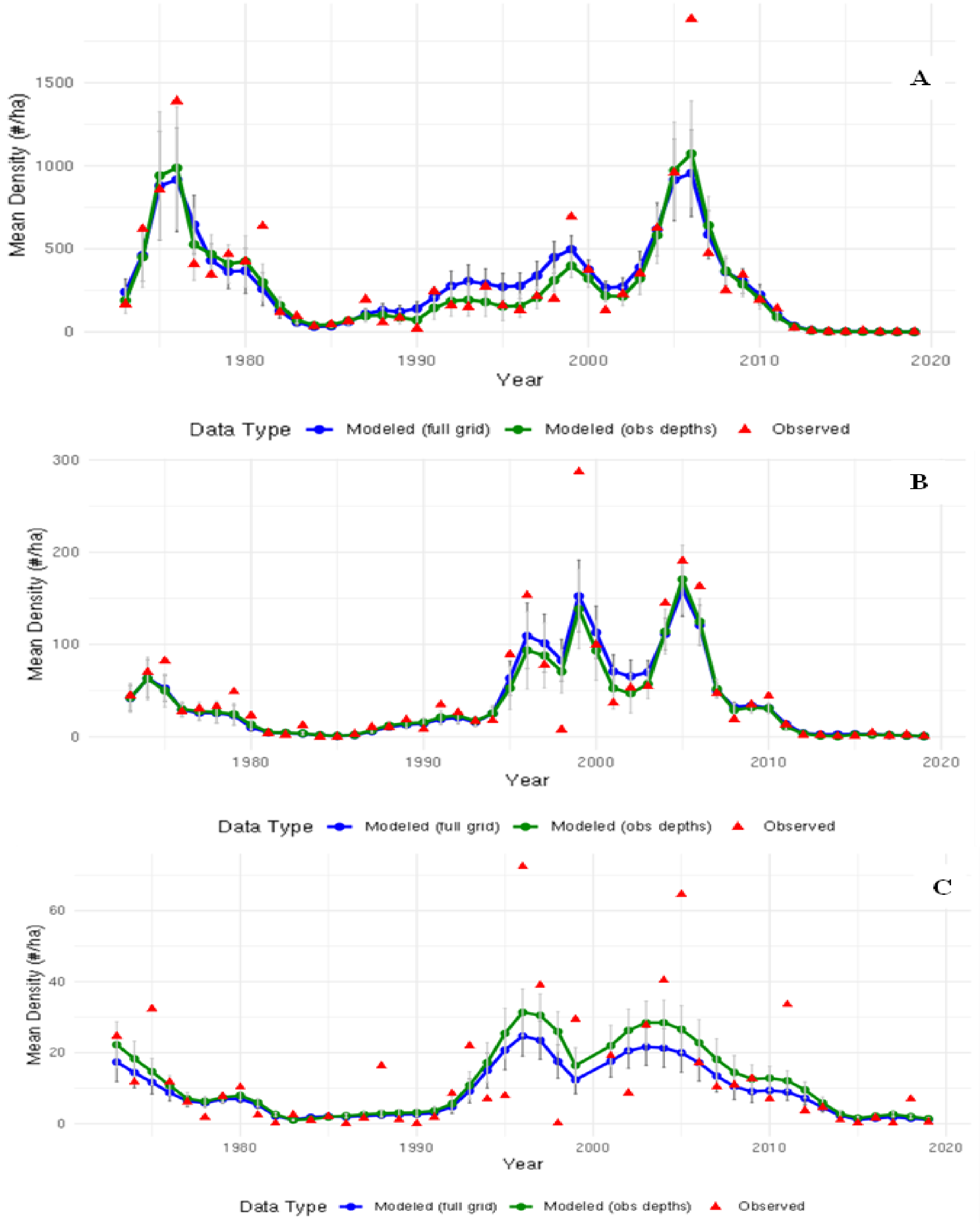
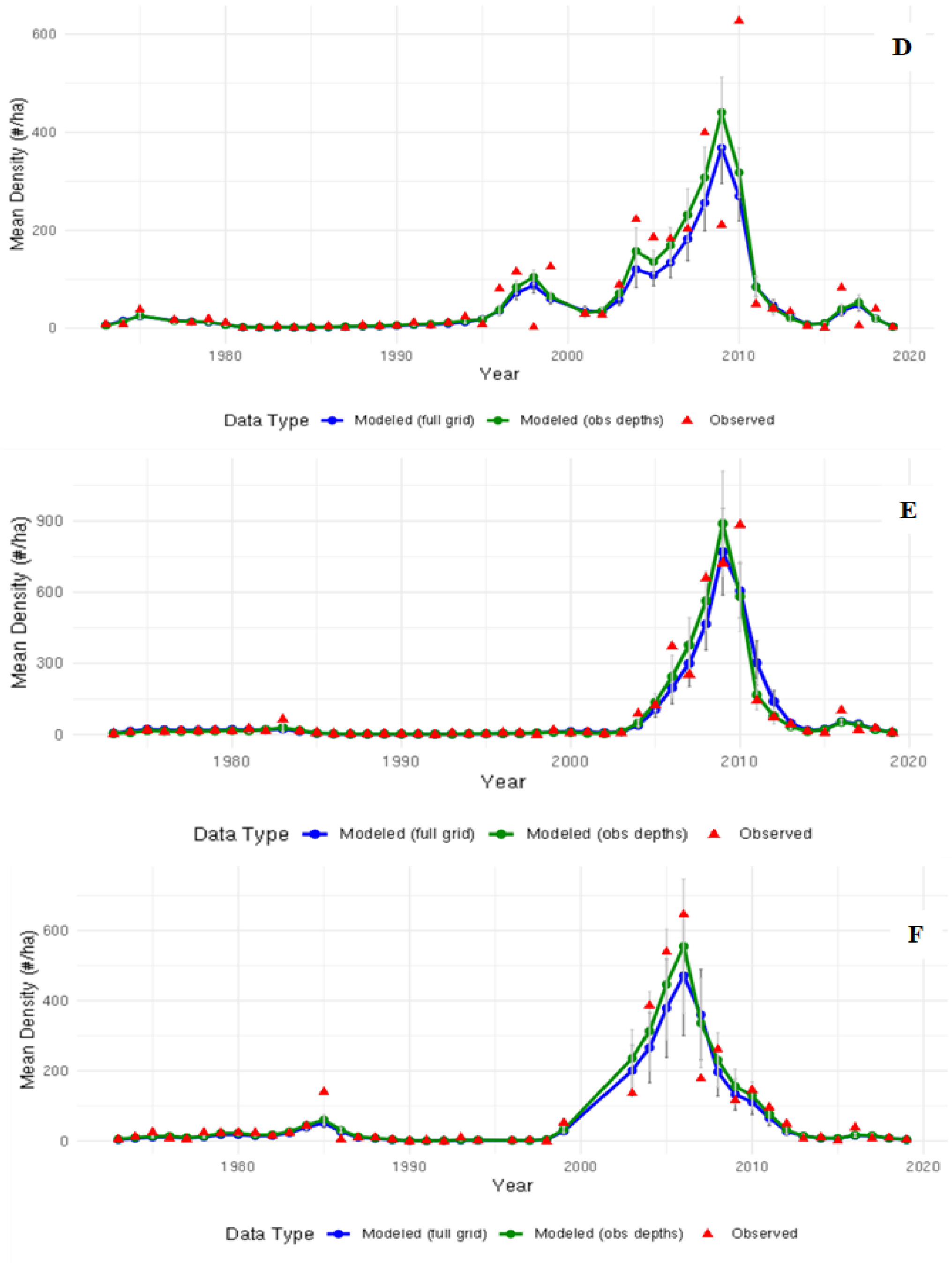


Figure S2.4. Average predicted Slimy Sculpin density (#/ha) and standard error bars from the observation models across the full standardized survey extent and increments (Modeled (full grid) & Blue). Average predicted Slimy Sculpin density (#/ha) and standard error bars from the delta generalized additive models (GAM’s) at only the observed transects each year (Modeled (obs depth) & Green). Average observed density from the trawl surveys each year without standardization (Observed & Red). Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F).

Figure S2.4. (cont'd)



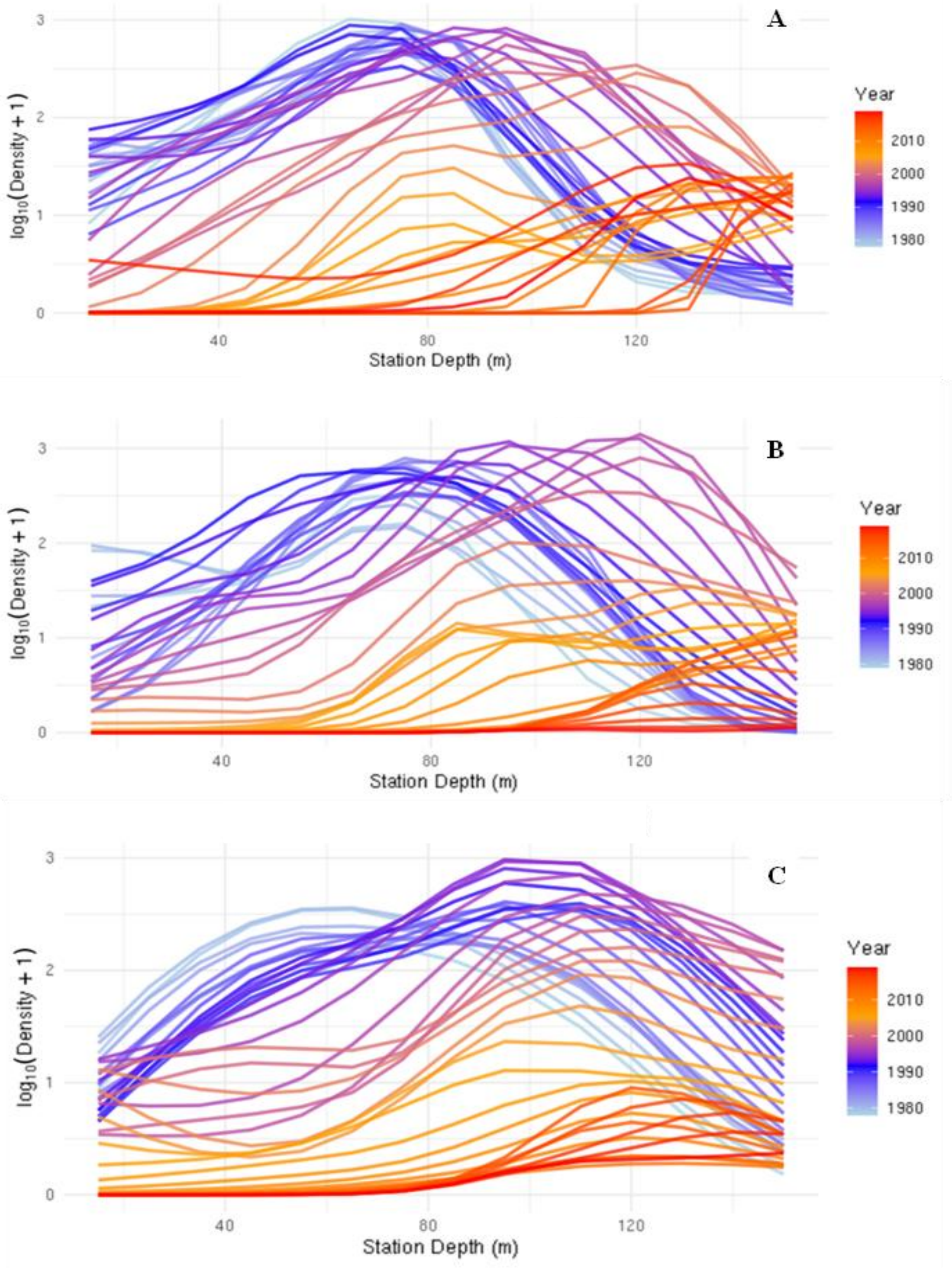
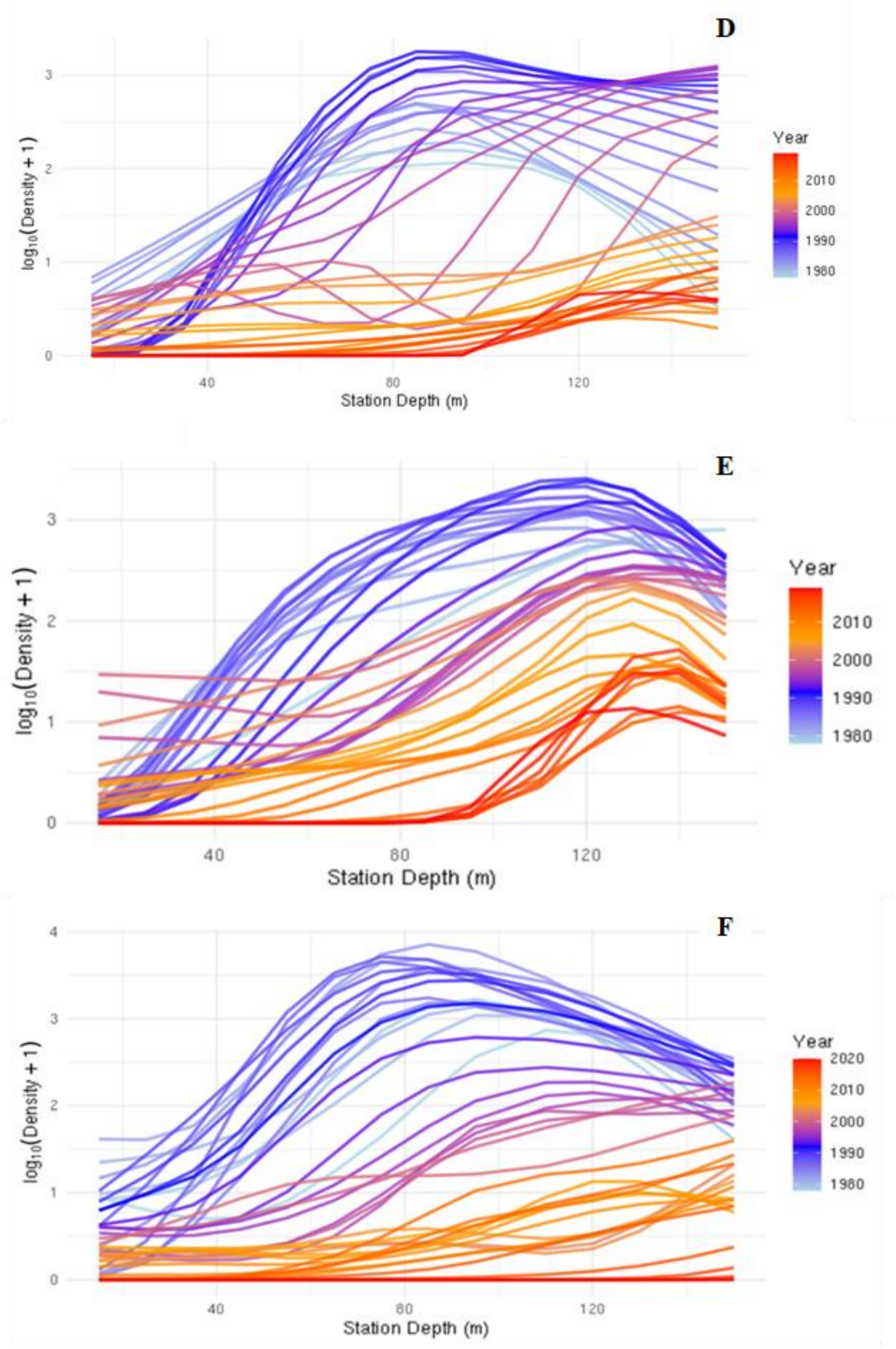


Figure S2.5. Predicted slimy sculpin density ($\log_{10}$+1 (#/ha)) at depth by year from delta generalized additive models (GAM's) for Lake Ontario ports. Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F).

Figure S2.5. (cont'd)



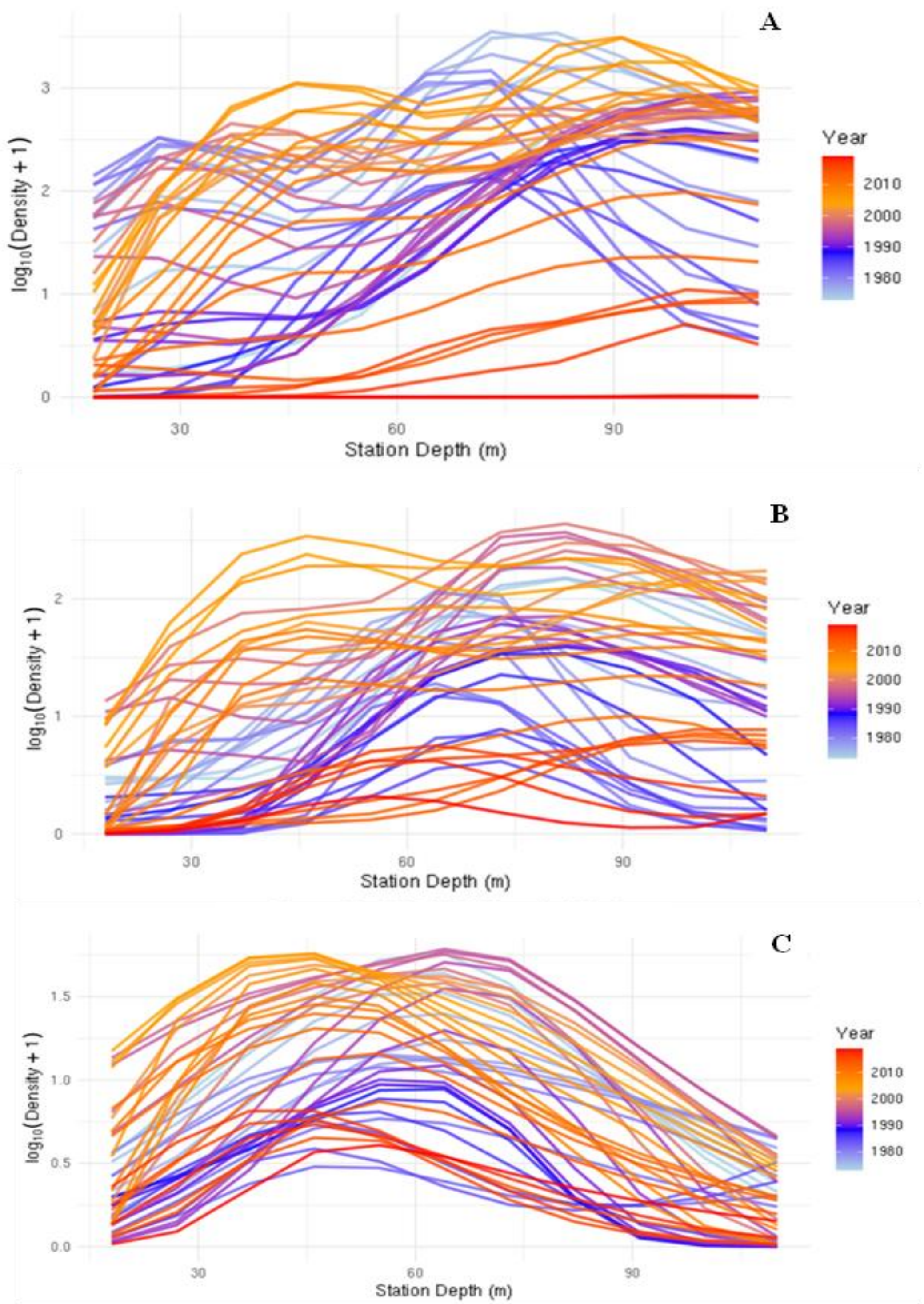
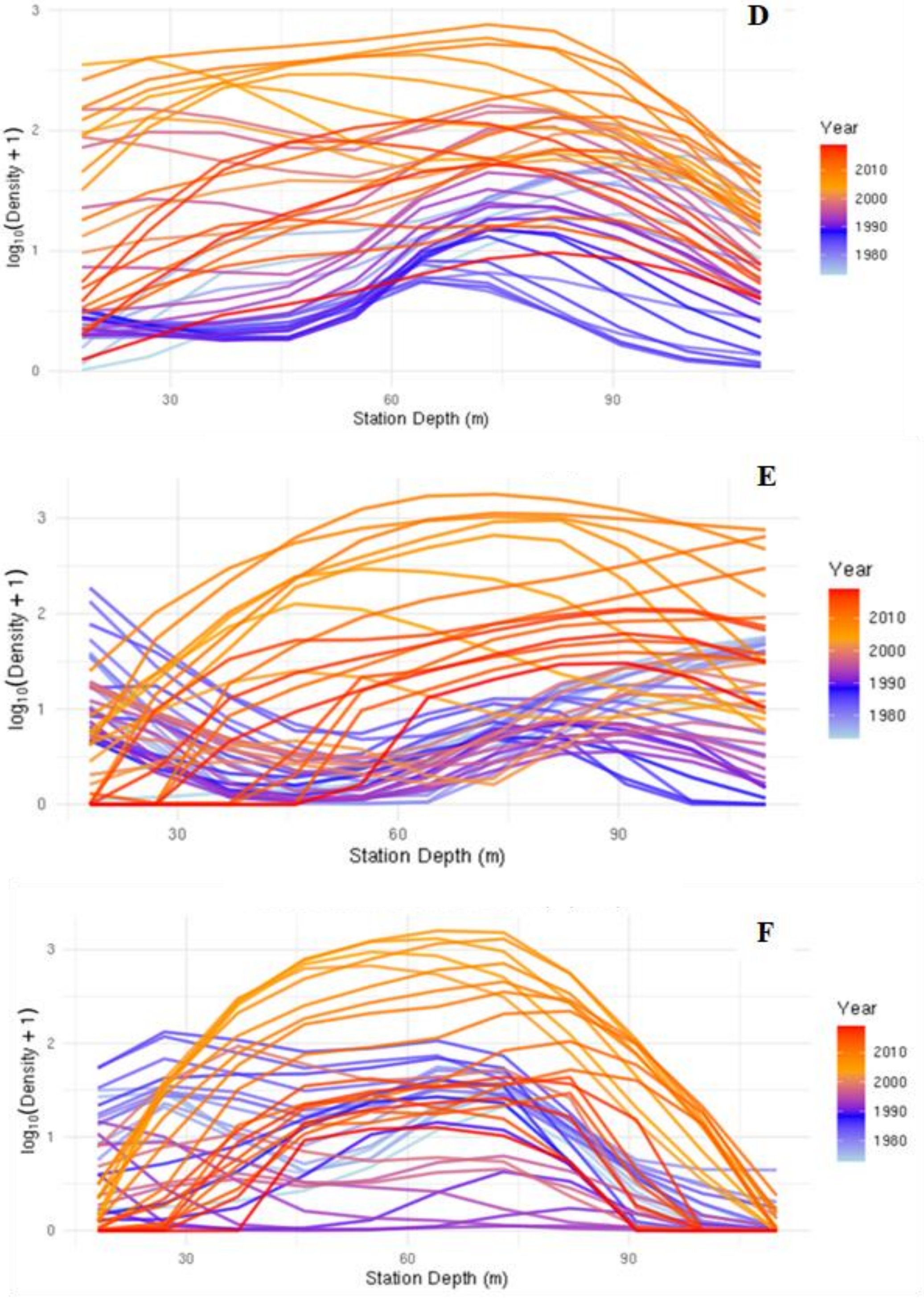
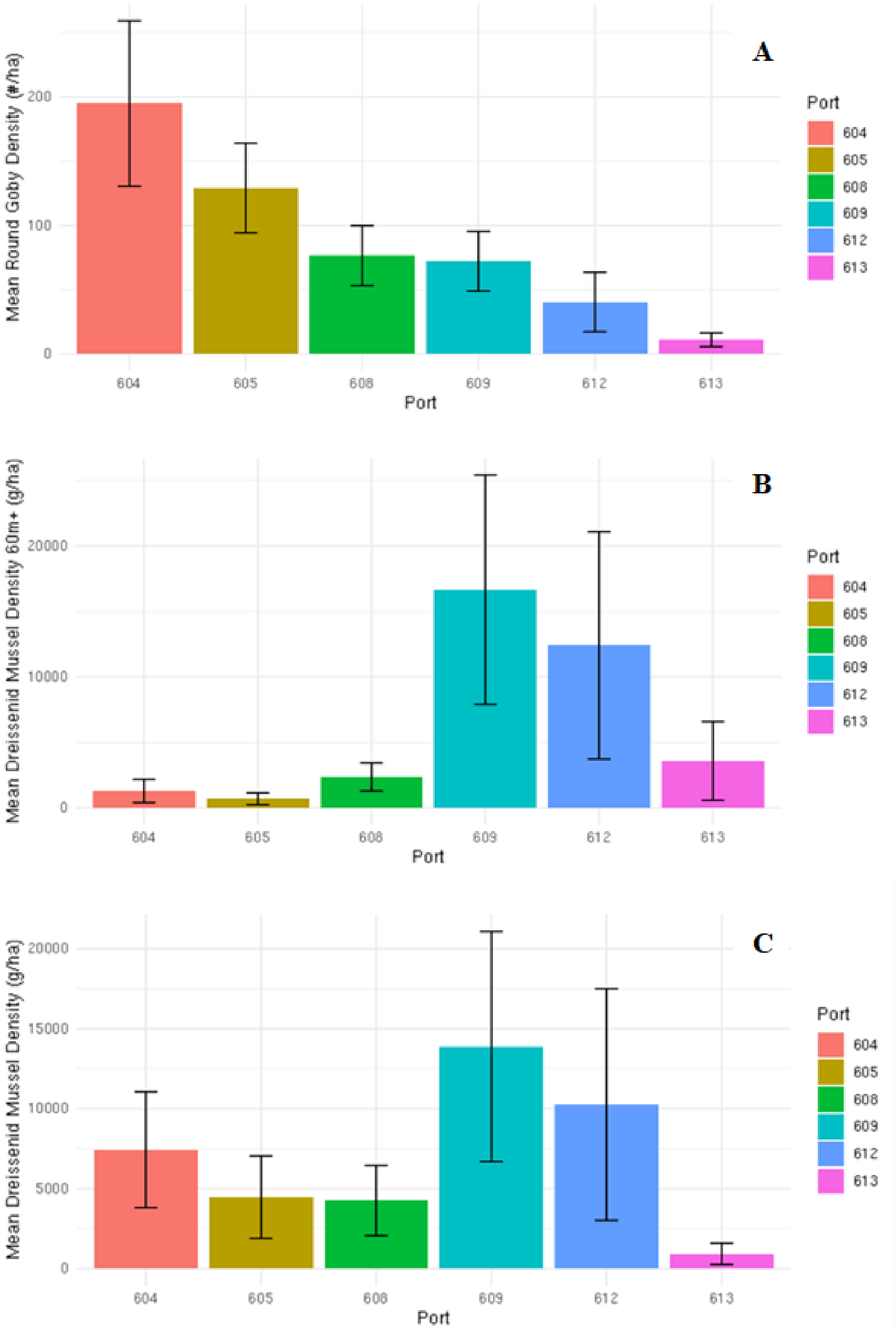


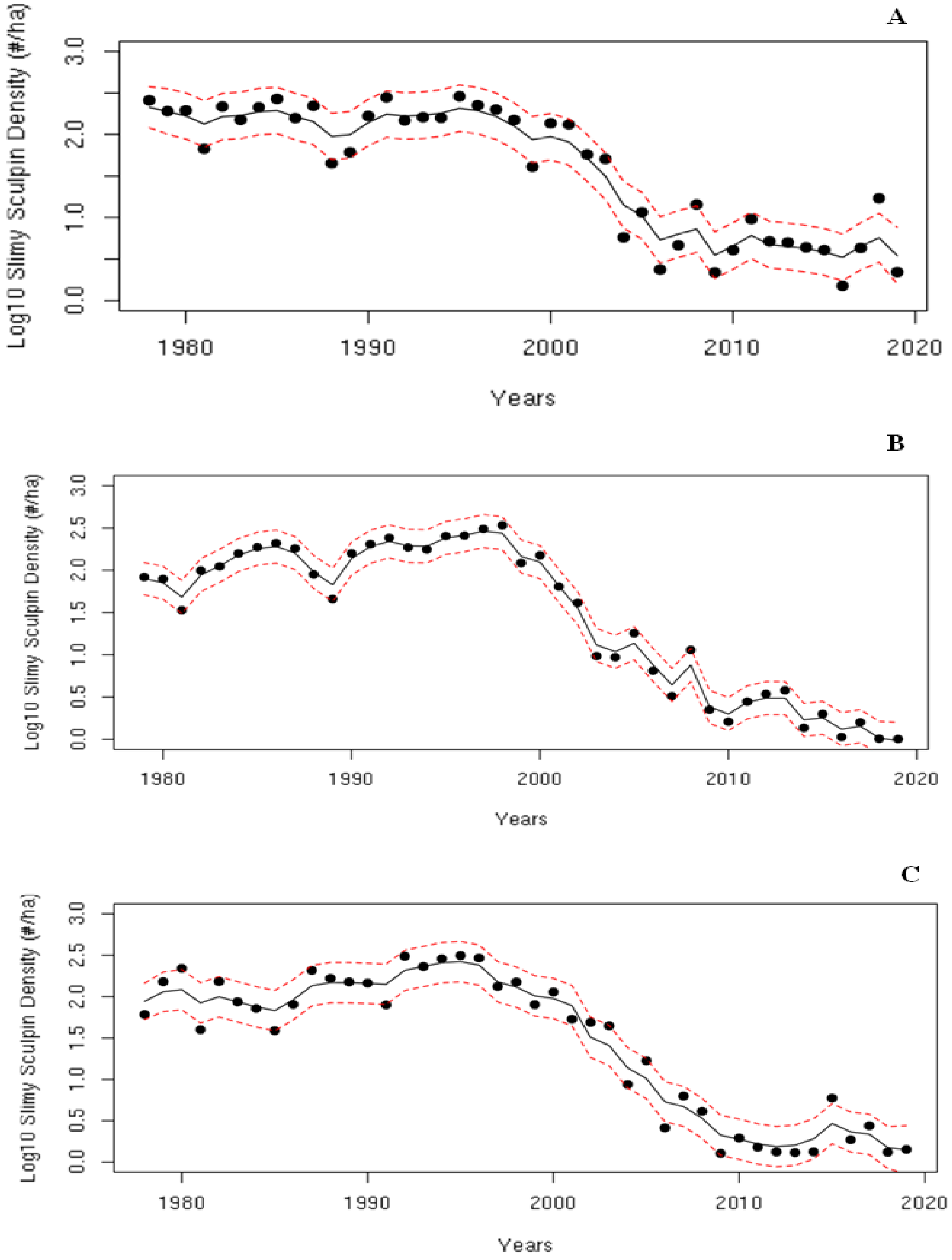
Figure S2.6. Predicted slimy sculpin density ($\log_{10}$+1 (#/ha)) at depth by year from delta generalized additive models (GAM's) for Lake Michigan ports. Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F).

Figure S2.6. (cont'd)



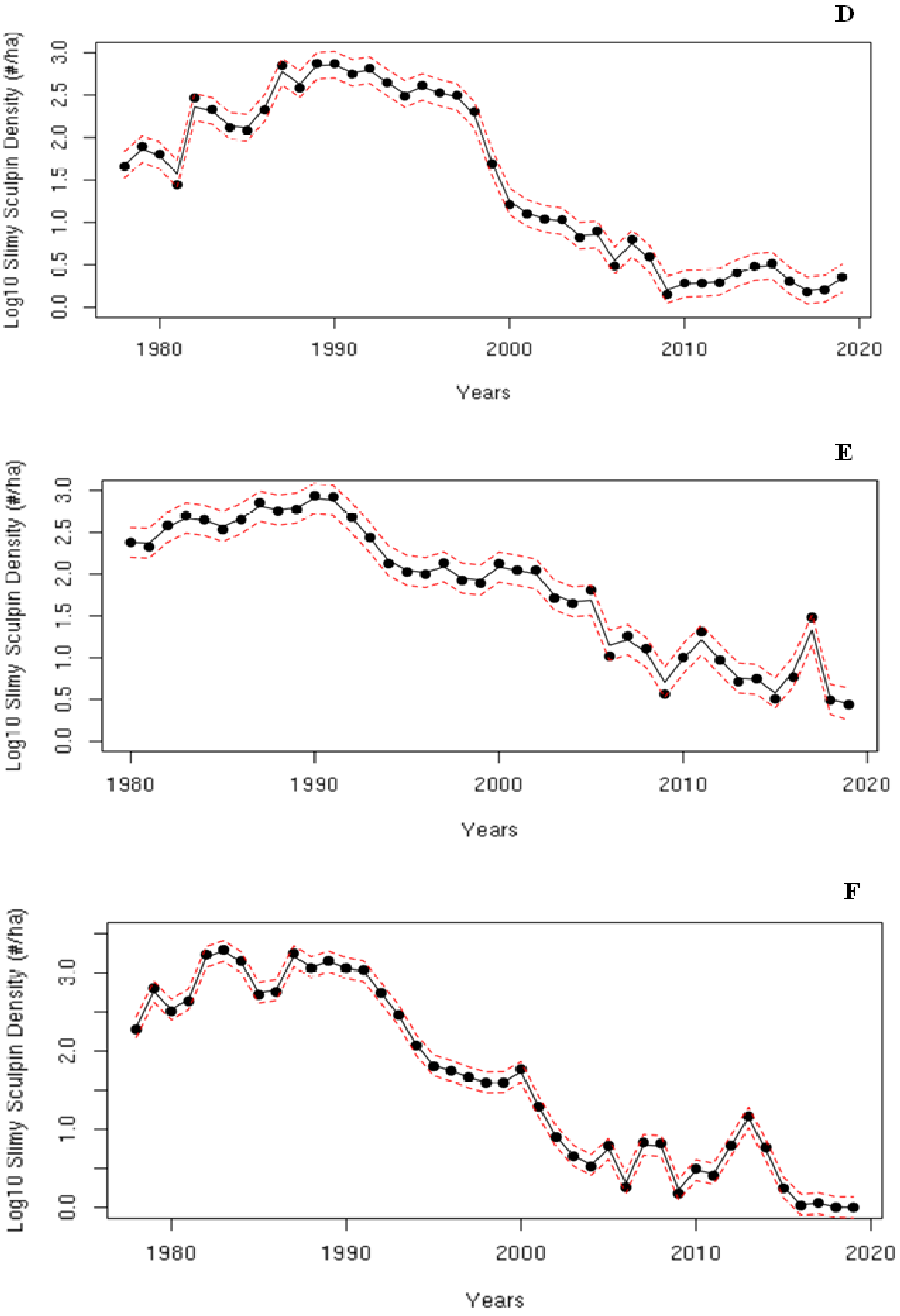


<u>Figure S2.7.</u> Lake Ontario mean density and standard deviation of round goby (A), dreissenid mussels at 60m of depth of more (B), and dreissenid mussels (C) across all years from the USGS trawl survey data from 1978-2019.



<u>Figure S2.8.</u> Estimated slimy sculpin density ($\log_{10}+1$ (#/ha)) (black line) and variance (red line) overtime using the Kalman filter and smoother with the observation data used (black dots). Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F).

Figure S2.8. (cont'd)



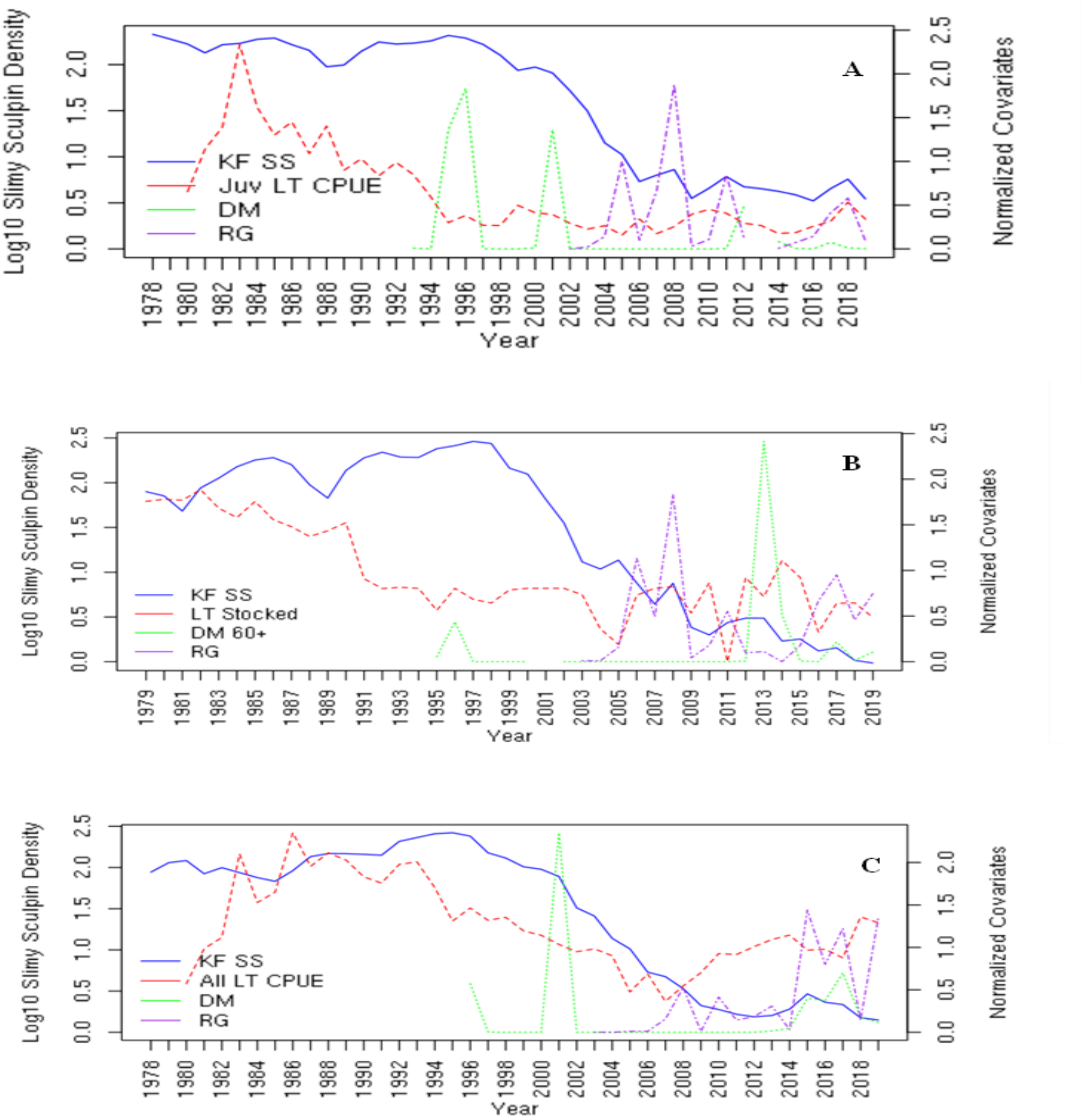
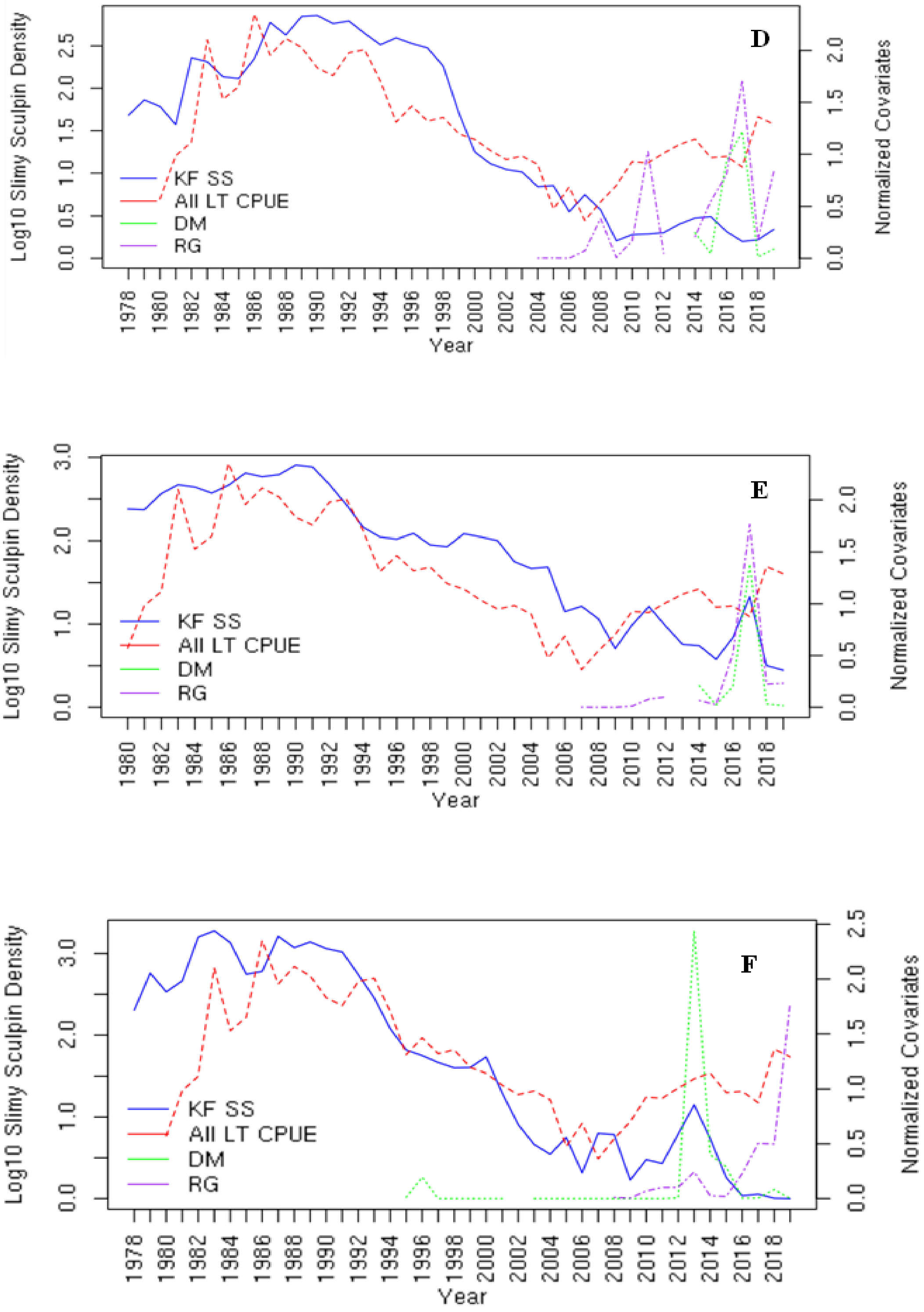


Figure S2.9. Estimated slimy sculpin density ($\log_{10}$+1(#/ha)) from the Kalman filter and smoother (KF SS) and normalized covariates that were closest to being selected for each category (dreissenid mussels = DM, round goby = RG, and lake trout = LT). Lake trout covariates represented all age groups of lake trout caught in gillnet surveys standardized by effort (All LT CPUE), just immature lake trout caught in gillnet surveys and standardized by effort (Juv LT CPUE), and the lake trout annual stocking numbers for United States waters (LT Stocked). Driessenid mussel covariates represented average density (g/ha) across all trawl surveys (DM) and average density (g/ha) at trawl surveys collected at 60m of depth or greater (DM 60m+). The round goby covariate (RG) represented the average density (#/ha) of round goby collected across all trawl surveys. Lake Ontario ports are Olcott 604 (A), 30-Mile pt 605 (B), Rochester 608 (C), Smoky pt 609 (D), Fairhaven 612 (E), and Oswego 613 (F).

Figure S2.9. (cont'd)



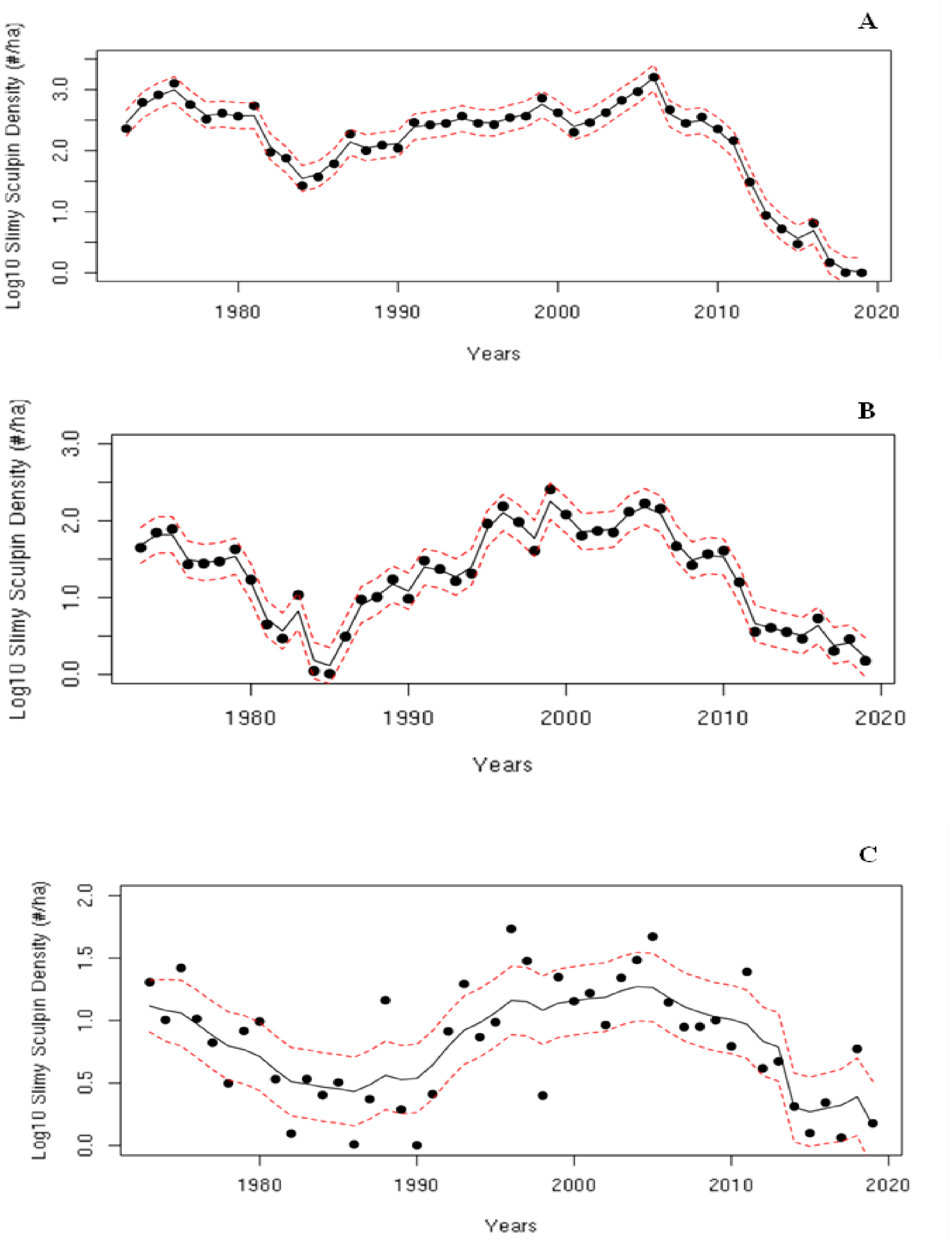
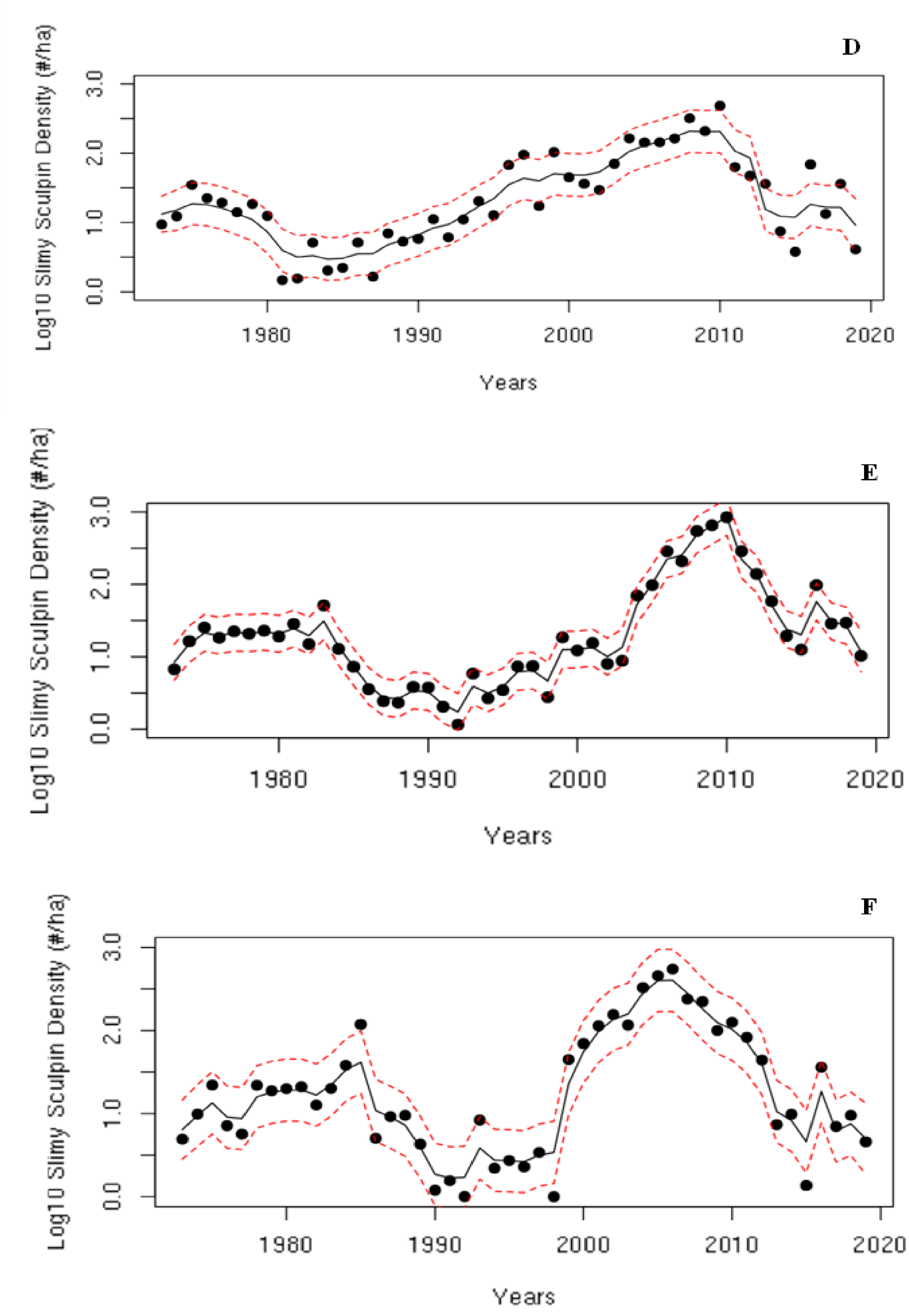


Figure S2.10. Estimated slimy sculpin density ($\log_{10}$+1 (#/ha)) (black line) and variance (red line) overtime using the Kalman filter and smoother with the observation data used (black dots). Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F).

Figure S2.10. (cont'd)



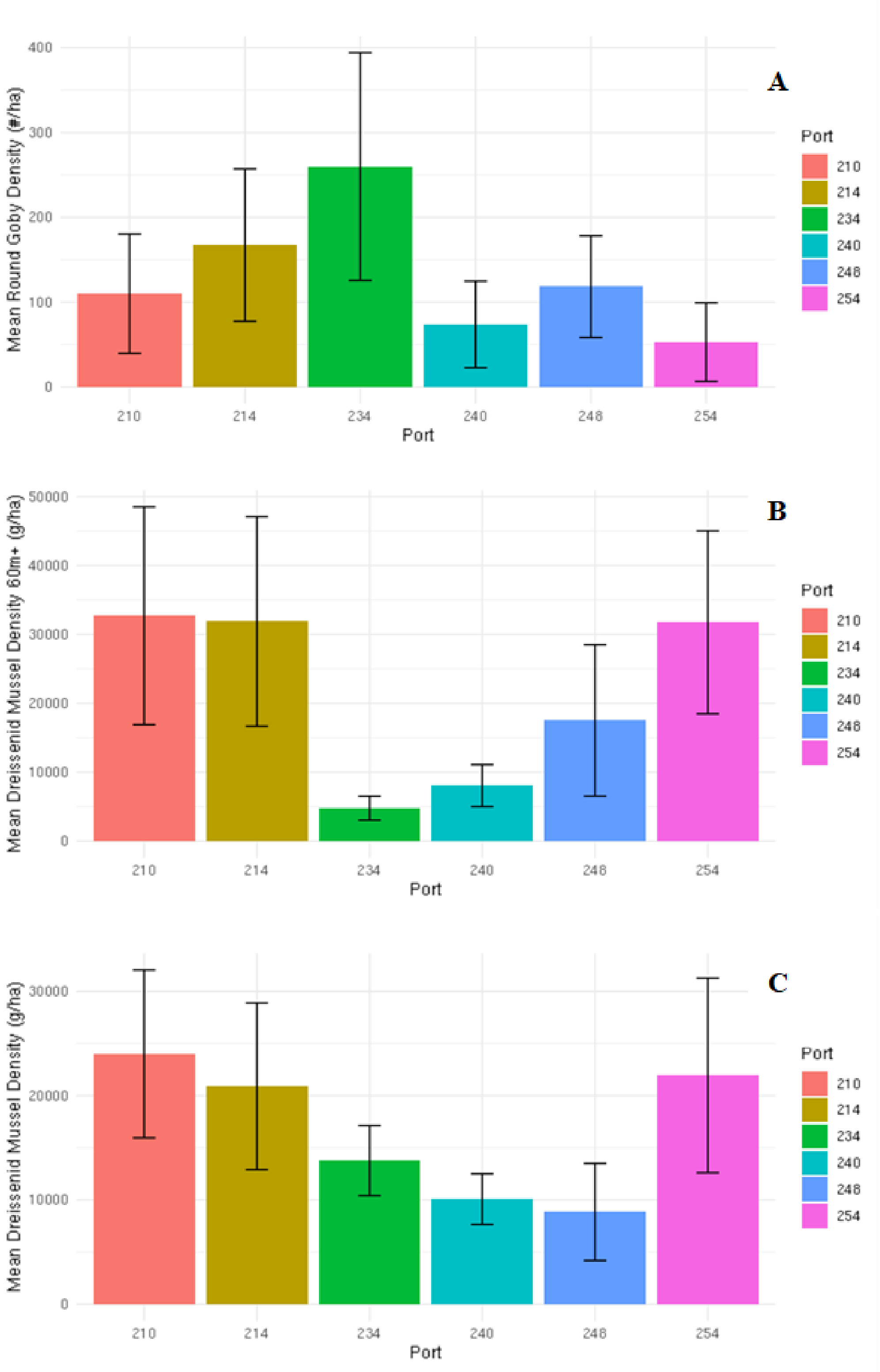


Figure S2.11. Lake Michigan mean density and standard deviation of round goby (A), dreissenid mussels at 60m of depth of more (B), and dreissenid mussels (C) across all years of the USGS trawl survey data from 1973-2019.

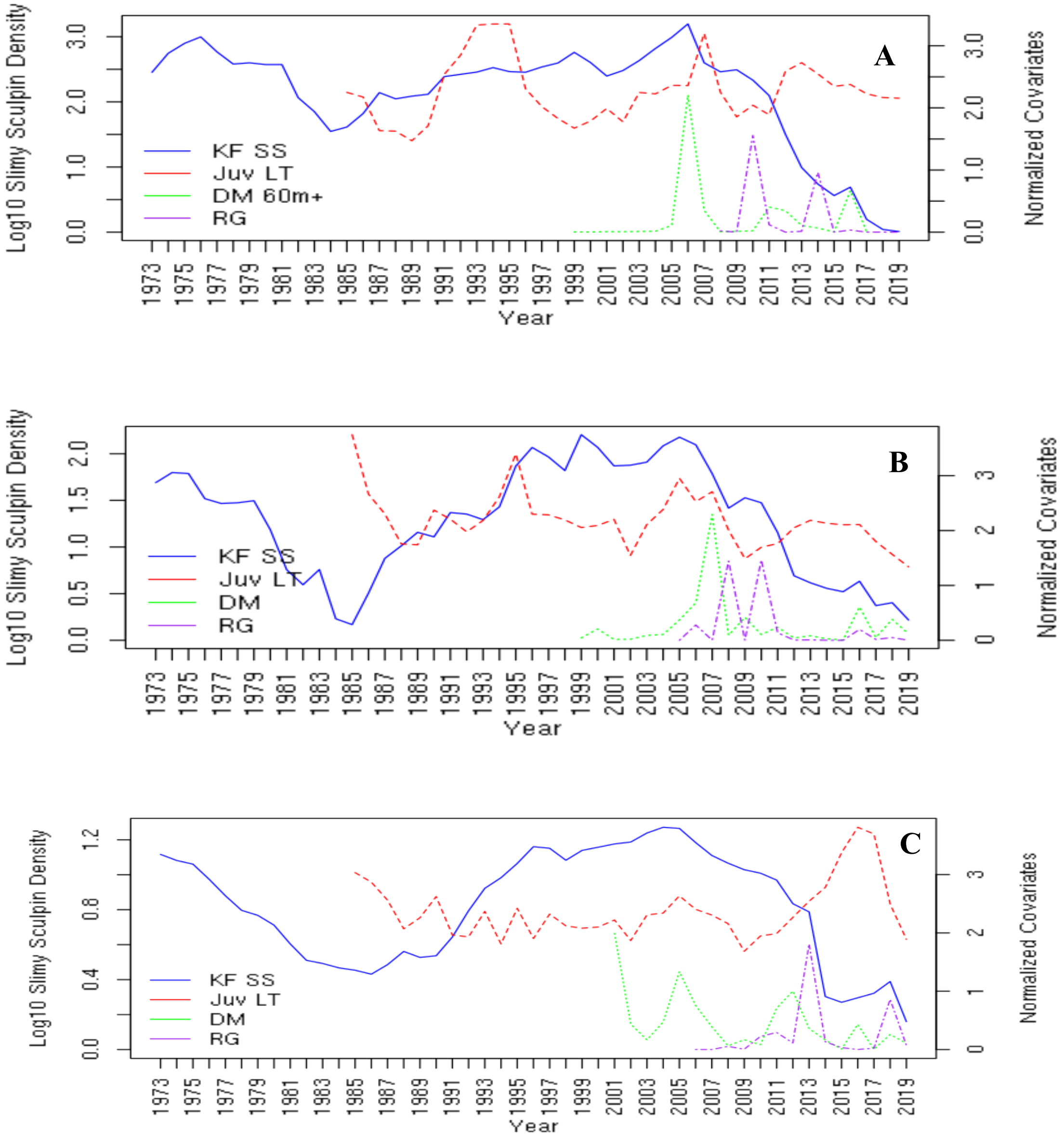
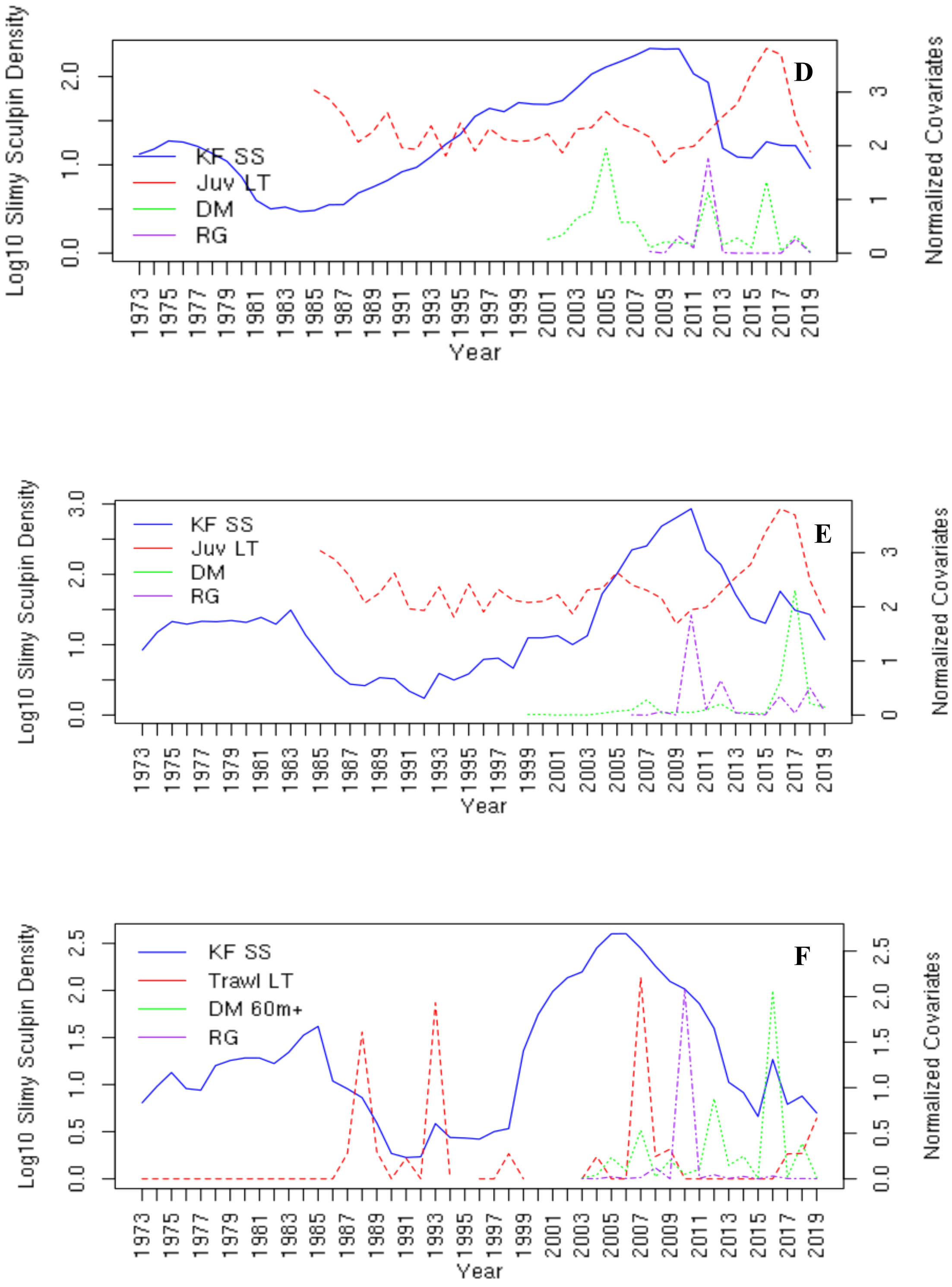


Figure S2.12. Estimated slimy sculpin density ($\log_{10}$+1(#/ha)) from the Kalman filter/smoother (KF SS) and normalized covariates that were closest to being selected for each category (dreissenid mussels = DM, round goby = RG, and lake trout = LT). Lake Trout covariates represented estimated juvenile (Age 0-3) Lake Trout density (#/ha) from statistical catch at age models at regional levels (Juv LT) and Lake Trout density (#/ha) in trawl surveys (Trawl LT). Dreissenid Mussel covariates represented average density (g/ha) across all trawl surveys (DM) and average density (g/ha) at trawl surveys collected at 60m of depth or greater (DM 60m+). The Round Goby covariate (RG) represented the average density (#/ha) of Round Goby collected across all trawl surveys. Lake Michigan ports are Frankfort 210 (A), Ludington 214 (B), Waukegan 234 (C), Port Washington 240 (D), Sturgeon Bay 248 (E), and Manistique 254 (F).

Figure S2.12. (cont'd)



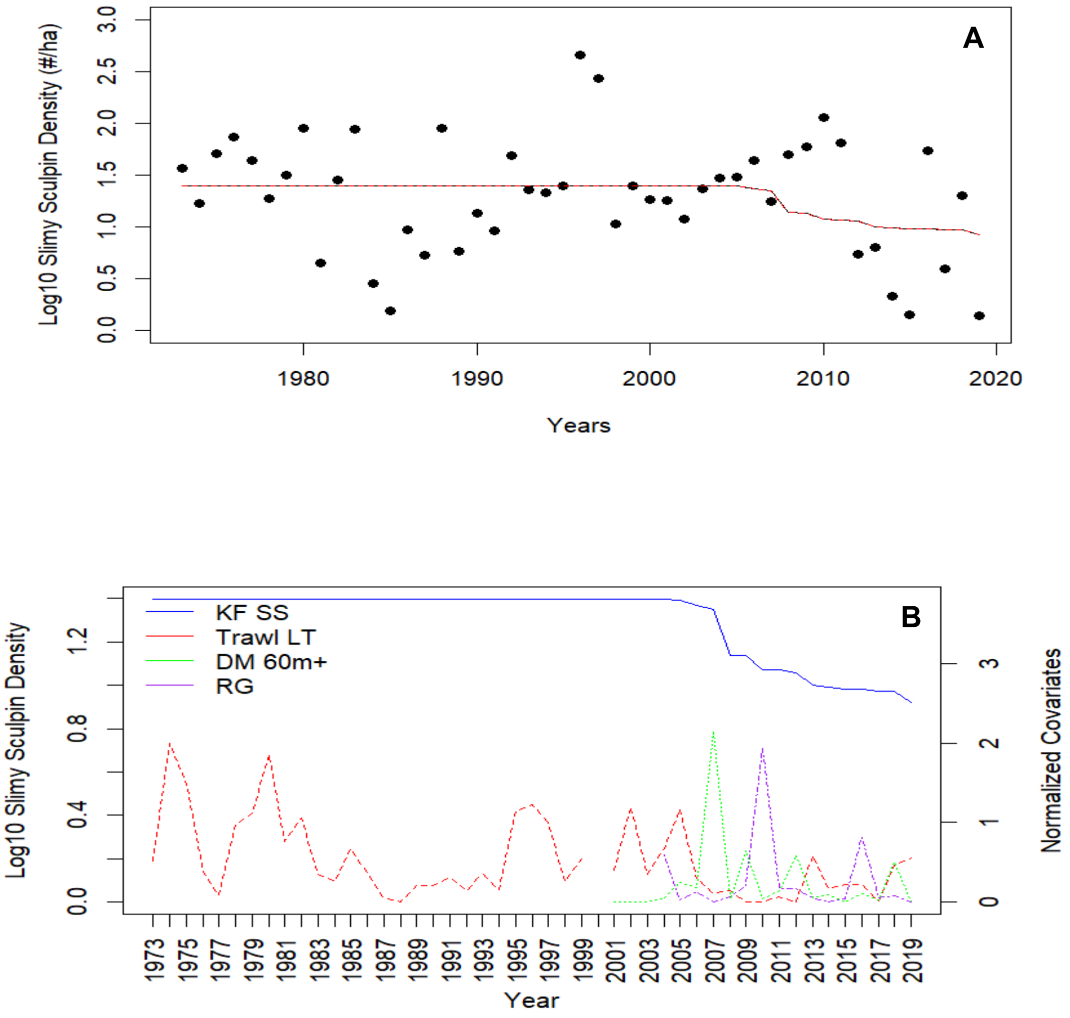
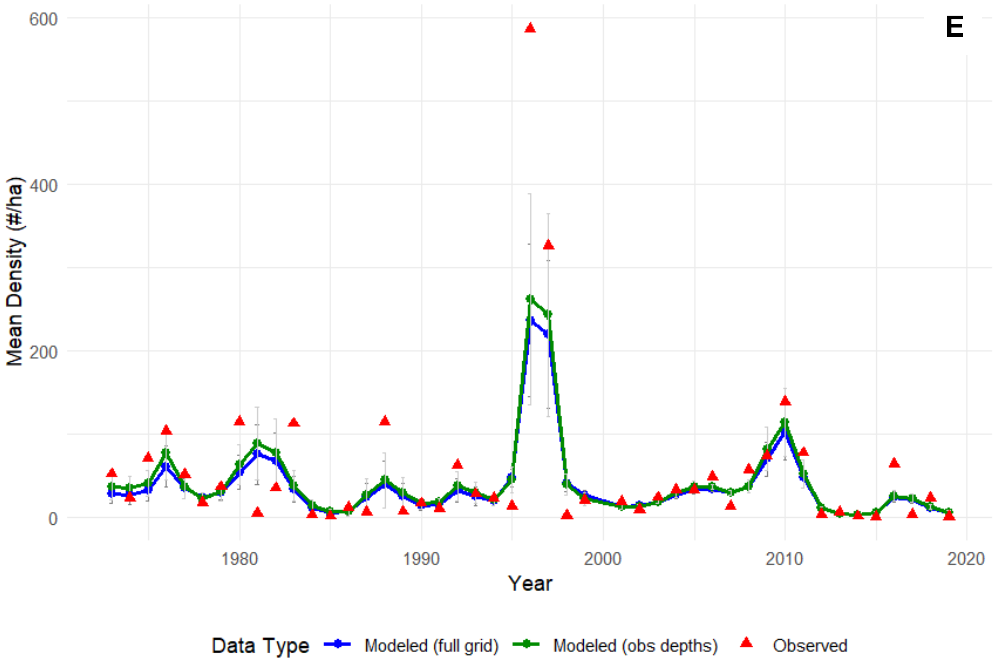


Figure S2.13. Results of the modeling procedure for Lake Michigan port 224 (Saugatuck) that was not able to estimate the process and observation errors via our modeling process. (A) Estimated slimy sculpin density ($\log_{10}$+1(#/ha)) from the Kalman filter/smoother (line) and the observations used. (B) Estimated slimy sculpin density ($\log_{10}$+1(#/ha)) from the Kalman filter/smoother (KF SS) and normalized covariates that were closest to being selected for each category (dreissenid mussels = DM, round goby = RG, and lake trout = LT). (C) The data sources and values before averaging and transforming that were used for the observation time series. (D) The $\log_{10}$+1 transformed predicted slimy sculpin densities across depth from the generalized additive models fit to the trawl survey data. (E) The average predicted Slimy Sculpin density (#/ha) and standard error bars from the observation models across the full standardized survey extent and increments (Modeled (full grid) & Blue). Average predicted Slimy Sculpin density (#/ha) and standard error bars from the delta generalized additive models (GAM's) at only the observed transects each year (Modeled (obs depth) & Green). Average observed density from the trawl surveys each year without standardization (Observed & Red)

Figure S2.13. (cont'd)

Figure S2.13. (cont'd)



APPENDIX C: CH. 3 SUPPLEMENTAL

Table S3.1. (A-D) Summary of laboratory water sample collections from mesocosms for round goby (A-B) and slimy sculpin (C-D) shedding and decay rates. In-between samples were added to the sampling procedure as the experiment was running to detect potential DNA mixing or loss from the perforations in mesocosms. In-between samples represent the water that surrounds all the partially immersed mesocosms within the larger tank (Fig. 1).

(A) Round Goby DNA Shedding

Date	Sample time	Elapsed Time (hrs)	In-between sample
8/15/2022	13:00	0	No
8/15/2022	15:10	1	No
8/15/2022	17:10	3	No
8/16/2022	12:30	22.5	No
8/16/2022	4:12	26.25	No
8/17/2022	12:05	46	No
8/17/2022	4:00	50	No
8/18/2022	10:20	68.33	No

Note: Goby added 8/15 (14:10) and goby removed 8/18 (11:05)

(B) Round Goby Only DNA Decay

Date	Sample time	Elapsed Time (hrs)	In-between sample
8/18/2022	14:05	3	No
8/18/2022	17:05	6	No
8/19/2022	12:25	25.33	No
8/19/2022	16:05	29	Yes
8/22/2022	11:40	72.42	Yes

Note: Sculpin are added 8/22 (13:00) but Goby DNA decay continues

Table S3.1. (cont'd)

(C) Slimy Sculpin DNA Shedding

Date	Sample time	Elapsed Time (hrs)	In-between sample
8/22/2022	14:40	1.66	Yes
8/22/2022	16:20	3.33	Yes
8/23/2022	12:50	23.83	Yes
8/23/2022	15:40	26.66	Yes
8/24/2022	12:20	47.33	Yes
8/24/2022	16:20	51.33	Yes
8/25/2022	11:10	70.17	Yes

Note: Sculpin are removed 8/25 (11:18)

(D) Slimy Sculpin DNA Decay

Date	Sample time	Elapsed Time (hrs)	In-between sample
8/25/2022	13:48	2.5	Yes
8/25/2022	16:28	5.17	Yes
8/26/2022	1:20	26	Yes
8/29/2022	13:00	97.7	Yes
8/30/2022	13:00	121.7	No
8/31/2022	13:25	146.12	Yes

Note: Sculpin were removed 8/25 (11:18)

Table S3.2. (A & B) Summary of round goby (A: Top) and slimy sculpin (B: Bottom) eDNA concentrations (copy number per liter) within smaller mesocosm tanks and from in-between the mesocosm tanks within the larger recirculating tank (Fig. 1) during experiments observing round goby and slimy sculpin eDNA shedding and decay rates.

Status	Hour	Average Tank (CN/L)	In-between (CN/L)	Proportion %
During	29.0	1162.7	14.2	1.2%
During	72.4	961.3	4.4	0.5%
After	74.0	477.0	2.6	0.5%
After with SS	75.6	686.0	2.1	0.3%
After with SS	77.3	220.3	2.5	1.1%
After with SS	97.8	270.0	0.0	0.0%
After with SS	100.7	142.7	0.0	0.0%
After with SS	121.3	116.0	0.0	0.0%
After with SS	125.3	109.7	0.0	0.0%
After with SS	144.2	79.5	1.9	2.4%
After	146.7	72.5	1.9	2.6%
After	149.3	26.7	0.0	0.0%
After	241.9	8.7	0.0	0.0%

Note: Status defines the stage of the experiments: during indicates that a round goby was present, after indicates that the round goby was removed, and after with SS indicates that the round goby was removed but there is a slimy sculpin present. Hour indicates the hours since the round goby were first introduced to the tanks.

Status	Hour	Average Tank (CN/L)	In-between (CN/L)	Proportion %
Before	0	0.0	0.0	0.0%
During	1.66	268.0	0.0	0.0%
During	3.33	379.0	0.9	0.2%
During	23.83	580.0	6.3	1.1%
During	26.66	685.0	3.9	0.6%
During	47.33	666.3	10.7	1.6%
During	51.33	497.7	10.1	2.0%
During	70.17	730.7	20.0	2.7%
During	72.67	676.7	12.0	1.8%
After	75.34	499.1	9.2	1.8%
After	96.17	27.5	2.7	9.8%
After	216.29	21.4	0.7	3.4%

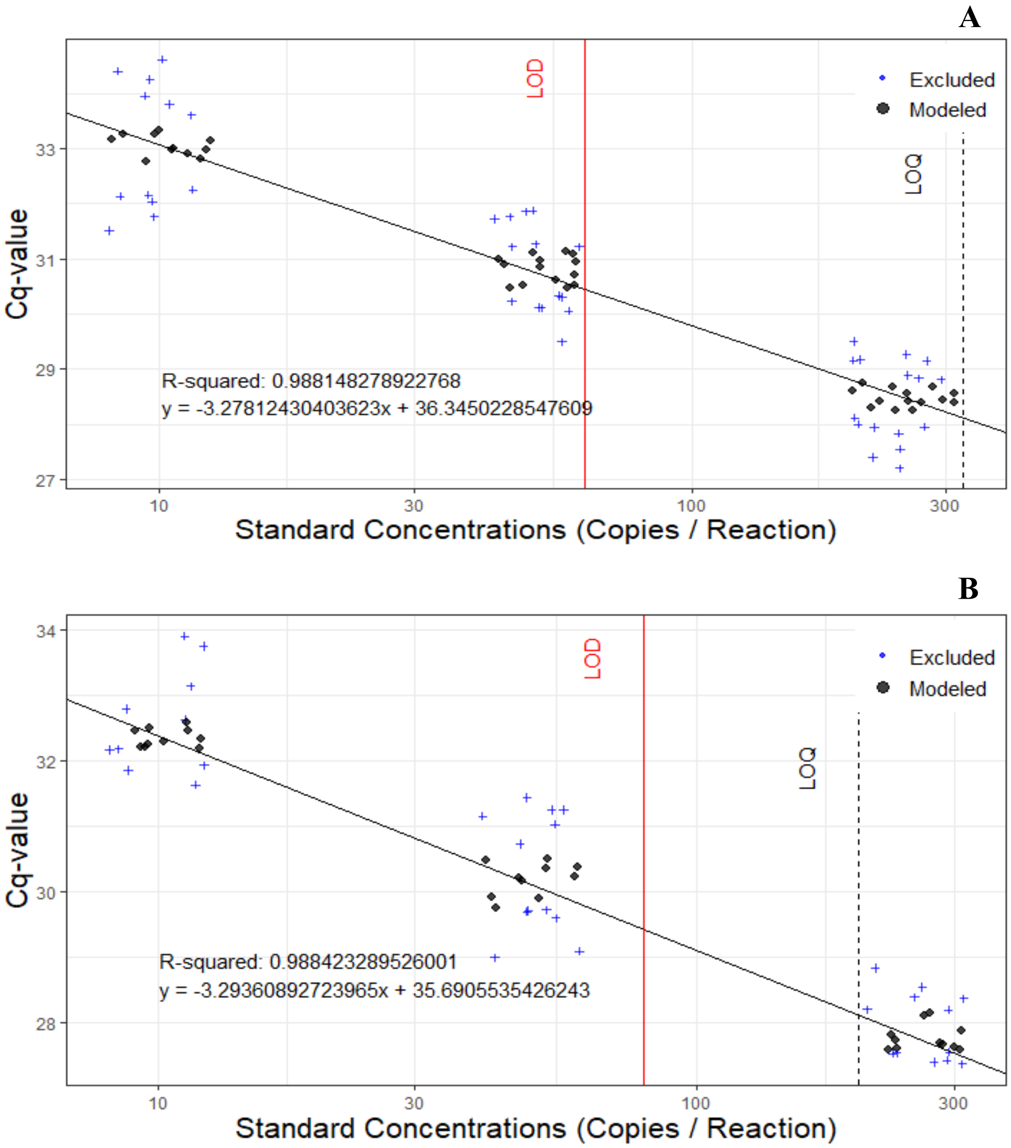
Note: Status defines the stage of the experiments: Before indicates before slimy sculpin was introduced, during indicates a slimy sculpin was present, and after indicates that the slimy sculpin was removed. Hour indicates the hours since the slimy sculpin were first introduced to the tanks.

Table S3.3. The top three hierarchical occupancy models and their mean estimated probability of round goby eDNA occurrence among reefs (ψ) from water samples collected in Lake Michigan in 2022, mean conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ), and mean conditional probability of round goby eDNA detection in replicates given that round goby eDNA was present in the sample (*p*), along with their upper and lower 95% highest posterior density credible intervals (95% HPD). Reefs sampled were Cresswell Reef (CR), Lee's Reef (LR), Mud Lake Reef (MLR), Sutton's Point (SP), and Tannery Creek (TC). Covariates tested were round goby density estimates at a nearby depth interval during camera drone surveys (RG), lake depth at location of water sample for eDNA collections (depth), and temperature of the water sample used for eDNA collections (temp).

	Models		
Parameters	ψ(.), Θ(RG), p(.)	ψ(.), Θ(RG+depth), p(.)	ψ(.), Θ(RG+depth+temp), p(.)
ψ (95% HPD)	0.86 (0.53-1.00)	0.86 (0.54-1.00)	0.86 (0.54-1.00)
Θ CR (95% HPD)	0.85 (0.70-0.96)	0.88 (0.71-0.98)	0.89 (0.59-0.99)
Θ LR (95% HPD)	0.89 (0.73-0.98)	0.91 (0.75-0.99)	0.87 (0.59-0.99)
Θ MLR (95% HPD)	0.85 (0.70-0.96)	0.88 (0.71-0.98)	0.90 (0.72-99)
Θ SP (95% HPD)	0.60 (0.40-0.78)	0.69 (0.36-0.93)	0.68 (0.33-0.93)
Θ TC (95% HPD)	0.85 (0.69-0.95)	0.88 (0.71-0.98)	0.89 (0.72-0.99)
p (95% HPD)	0.68 (0.59-0.77)	0.68 (0.59-0.77)	0.68 (0.58-0.77)

Table S3.4. The top three hierarchical occupancy models and their mean estimated parameters for the probability of round goby eDNA occurrence among reefs (ψ) from water samples collected in Lake Michigan, 2022, mean conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ), and mean conditional probability of round goby eDNA detection in replicates given that round goby eDNA was present in the sample (p), along with their upper and lower 95% highest posterior density credible intervals (95% HPD). Covariates tested were round goby density estimates at a nearby depth interval during camera drone surveys (RG), lake depth at location of water sample for eDNA collections (depth), and temperature of the water sample used for eDNA collections (temp).

		Models	
Parameters	ψ(.), Θ(RG), p(.)	ψ(.), Θ(RG+depth), p(.)	ψ(.), Θ(RG+depth+temp), p(.)
ψ intercept (95% HPD)	1.26 (0.10-2.64)	1.27 (0.10-2.65)	1.27 (0.10-2.62)
Θ intercept (95% HPD)	1.08 (0.55-1.68)	1.07 (0.54-1.68)	1.10 (0.55-1.75)
Θ RG (95% HPD)	1.45 (0.35-2.72)	1.29 (0.16-2.62)	1.21 (0.10-2.59)
Θ depth (95% HPD)		-0.15 (-0.61-0.33)	0.14 (-0.65-0.93)
Θ temp (95% HPD)			0.37 (-0.45-1.24)
p intercept (95% HPD)	0.48 (0.22-0.74)	0.48 (0.21-0.74)	0.47 (0.21-0.73)



<u>Figure S3.1.</u> Limits of detection (LOD) and quantification (LOQ) for round goby (A) and slimy sculpin (B) using the calibration curve from Klymus et al. (2020). The cycle threshold (Cq-value) was set to exceed background levels of fluorescence and reach the exponential phase of the amplification curve. Points drawn with black circles are the middle 2 quartiles of standards with ≥50% detection and are included in the linear regression calculations. Points drawn with blue pluses (+) are outside the middle 2 quartiles or for standards with <50% detection and are not included in the linear regression calculations.

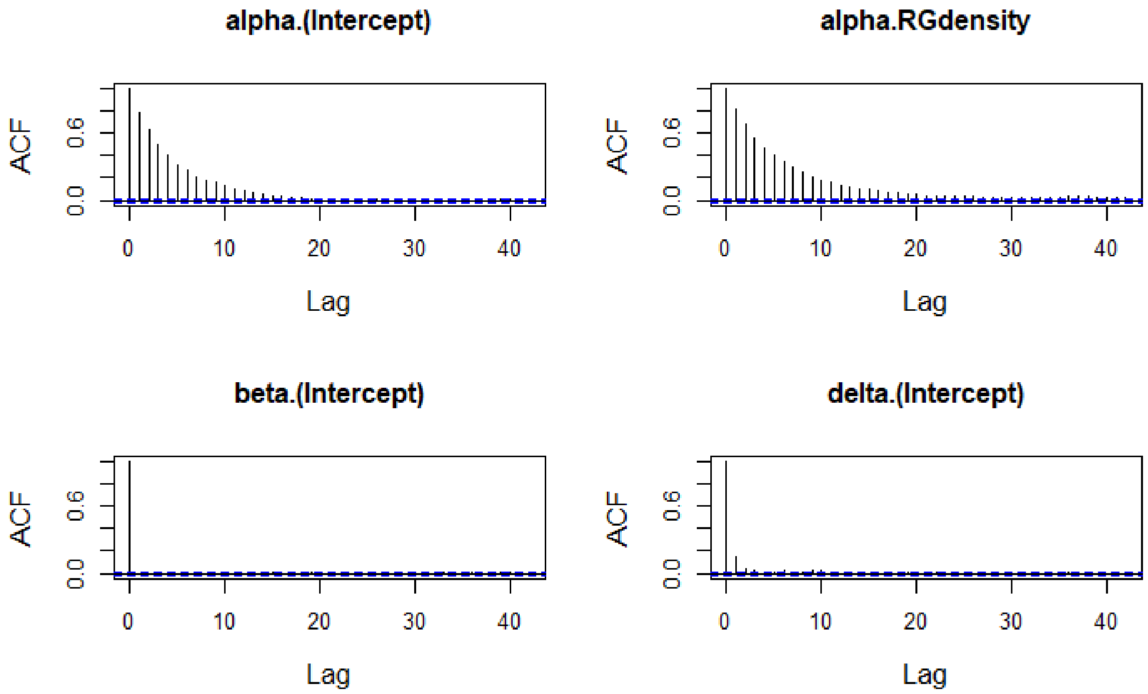


Figure S3.2. Autocorrelation plots of parameters in the best fitting hierarchical occupancy model. Parameter definitions: ψ = beta.(Intercept), (Θ) = alpha.(Intercept), p = (delta..Intercept), and $((\Theta)RG)$ = alpha.RGdensity . The best fitting model included average round goby biomass density (g/m^2) from nearby camera drone surveys as a sample level covariate for estimating the conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ). The model was fitted by running the MCMC algorithm for 20,000 iterations and retaining the last 19,000.

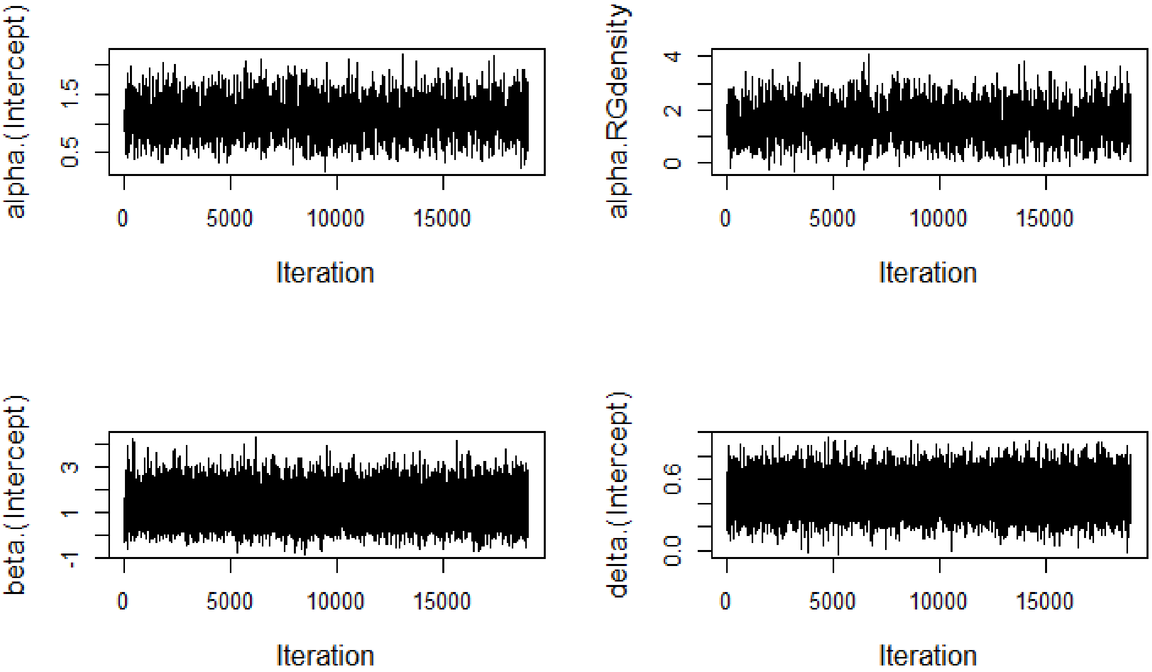


Figure S3.3. Trace plots of parameter estimates from the best fitting hierarchical occupancy model. Parameter definitions: ψ = beta.(Intercept), (Θ) = alpha.(Intercept), p = (delta..Intercept), and ((Θ)RG) = alpha.RGdensity . The best fitting model included average round goby biomass density (g/m^2) from nearby camera drone surveys as a sample level covariate for estimating the conditional probability of round goby eDNA occurrence in a sample within a reef given that round goby were present at a reef (Θ). The model was fitted by running the MCMC algorithm for 20,000 iterations and retaining the last 19,000.

Supplemental File 1. Excel file containing the read counts for each sample processed via metabarcoding and the primary authors notes for interpreting the file.

Supplemental File 2. Excel file of a distance matrix produced from our metabarcoding reference library.