POND FOOD WEBS INFLUENCED BY THE ADDITION OF PREDATORY FISH

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ABSTRACT

In freshwater ecosystems, top predators can induce trophic cascades to influence the entire food web. In this study, we tested whether adding top predators into ponds could affect the population of species at lower trophic levels and alter the feeding strategy of the secondary consumers. Specifically, we tested whether the addition of largemouth bass (*Micropterus salmoides*) to ponds affected the diet composition of sunfish ((pumpkinseed and bluegill; *Lepomis gibbosus* and *Lepomis macrochirus*), the dominant secondary consumer in the study ponds. I determined the diet composition of sunfish by analyzing the gastrointestinal tract contents. I found that in the ponds where bass were added, sunfish consumed more macroinvertebrates instead of zooplankton and phytoplankton. Therefore, we found that top predators are able to regulate the food web structure and energy flow through trophic cascades in these study ponds.

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TABLE OF CONTENTS

LIST OF FIGURES 1

LIST OF ABBREVIATIONS 2

PREFACE 3

INTRODUCTION 6

MATERIAL AND METHODS 7

RESULTS 8

GASTROINTESTINAL TRACT MASS 8
RELATIVE FULLNESS 9
VASCULARIZED PLANT 10
PHYTOPLANKTON 10
ZOOPLANKTON 11
MACROINVERTEBRATE 12

DISCUSSION 13

CONCLUSION 15

REFERENCE 16

LIST OF FIGURES

Figure1. Trophic cascade in a four-trophic-level food web

Figure 2. Total stomach and gut mass (gram) of fish in bass and control ponds.

Bass: the ponds had bass addition. Minnow: the ponds had sunfish and minnows only.

Figure 3. Relative GIT fullness of fish in bass and control ponds.

Figure 4. Percentage of vascular plants in fish stomach and gut in control and bass ponds.

△: mean percentage of vascular plants consumed by fish.

Figure 5. Percentage of phytoplankton in fish stomach and gut in bass and control ponds.

△: mean percentage of vascular plants consumed by fish.

Figure 6. Percentage of zooplankton in fish stomach and gut in bass and control ponds.

△: mean percentage of vascular plants consumed by fish.

Figure 7. Percentage of macroinvertebrate in fish stomach and gut in bass and control ponds.

△: mean percentage of vascular plants consumed by fish.

LIST OF ABBREVIATIONS

GIT	gastrointestinal tract

PREFACE

This capstone project was developed as part of a larger study aiming to assess the impact of freshwater pond food web structure on greenhouse gas emissions and conducted in the lab of Freshwater Ecology directed by Dr. Meredith Holgerson, in the Department of Ecology and Evolutionary Biology. The overarching goal of this thesis was to determine whether predators can induce a trophic cascade in macrophyte based ponds by altering the diet composition of secondary consumers. The study aimed to determine if the addition of top predators (largemouth bass) to freshwater ponds alters the diet composition of the dominant secondary consumers (sunfish).

Background information: Trophic cascade

Food web structures reveal the interaction between species in the community and indicate the flow of energy and nutrients among various consumers (Kwak and Park, 2020). Every organism in the community can be assigned to a trophic level, which refers to its vertical position in the food web. In a four-level food web (Figure 1), the base of the food web starts with primary producers, which are eaten by primary consumers, which in turn are eaten by secondary consumers, which are preyed upon by top predators. The consumption of prey is a direct interaction in the food web and can induce top-down control on the abundances of each level in the food web. When the predators exert top-down control that influences at least two levels of the food chain below it, we call this a trophic cascade. An important component of the cascade is that the predator indirectly affects the abundances of organisms (i.e., dashed arrows in Figure 1).

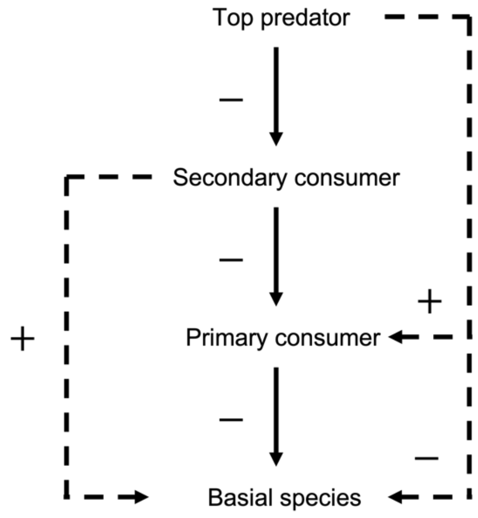

Figure 1. Trophic cascade in a four-trophic-level food web

The term trophic cascade was first introduced in 1980 by R. T. Paine (Paine, 1980) to describe the indirect interaction in the food web. However, early in 1859, Darwin first recorded this phenomenon in The Origin of Species (Darwin, 2004). He described that domestic cats could control the abundance of mice, which might consume honeycombs and reduce the bee populations, eventually leading to increased pollination and the spread of flowering plants. Another classic example of trophic cascades is that deer depleted the edible bush in America after their predator, the wolf, was extirpated (Leopold, 1949). Due to climate change and anthropogenic activity, many top predators are threatened. With fewer top predators, the population of secondary consumers can increase. Ultimately, the basal species, which often are plants, are depleted.

In contrast to a trophic cascade where predators exert top-down control, food webs are also influenced by bottom-up processes. Bottom-up processes are where nutrient inputs and primary production exert upwards control on the food web. The concept is that more food resources will increase abundances of organisms at all levels of the food web. While top-down and bottom-up controls have been debated (Ripple et al., 2016), we now acknowledge that both top-down control and bottom-up control regulate the food web at the same time. For example, sea otters (*Enhydra* *lutris*) protect aquatic microalgae, such as kelp, by regulating the sea urchins (*Strongylocentrotus polyacanthus*) through trophic cascade. At the same time, the density of kelp can affect the abundance and diet of the kelp forest fish (rock greenling, *Hexagrammos lagocephalus*), which is regarded as bottom-up control (Reisewitz et al., 2006).

In this study, we tested whether an addition of a top predator (largemouth bass, *Micropterus salmoides*) to freshwater pond ecosystems would induce a trophic cascade and fundamentally alter food web properties.

The approach used to assess whether largemouth bass addition induced a trophic cascade was by measuring their impact on the diet composition and abundance of their dominant prey item, the sunfish (pumpkinseed and bluegill; *Lepomis gibbosus* and *Lepomis macrochirus*). Prior to this study, the sunfish population was stunted due to high population levels and presumed competition for prey. It was hypothesized that addition of largemouth bass would alter the diet composition of the secondary consumers by reducing the sunfish numbers, thereby easing competition. While the presence of highly digested feeds in the hindgut renders the identification of prey type difficult, presence of undigested feeds in the foregut allows easy identification and classification of prey type. Stomach and gut content analyses can be divided into three categories. The primary analysis is the occurrence frequency. The simplest method is to calculate the proportion of the stomach and gut with or without food. Another aspect is to analyze the occurrence of each prey type in the fish population, which indicates the number of individuals that consumed each specific prey type. Lastly, the abundance of each prey type can be estimated through numerical, volumetric, and gravimetric approaches. The numerical approach is to count all individuals of specific prey taxa represented in the stomach and gut. The presence-absence of prey can be a good indicator of prey importance. The volumetric and gravimetric methods can better quantify the prey composition by either measuring the volume of prey or the mass of prey, respectively.

Introduction

Food webs represent the pathways in which species consume one another and are thereby linked together in terms of energy flow (Mittelbach and McGill, 2019). The organic matter and energy flow is transmitted from primary producers to higher trophic levels through the food web. Understanding the source of nutrition and the flux of energy contributes to study the productivity of primary producers, trophic niche of species, and interspecies interaction. Trophic cascade means the interspecies indirect interactions between predators and preys, enabling the regulation of abundance and distribution of population through the food web (Paine, 1980). With the discovery of trophic cascades, there is strong evidence showing that the length of the food web (and presence of predators) can significantly influence the food web structure and abundance of organisms at each level of the food web. In turn, these changes can modify energy flow and ecosystem functions (Schindler et al., 1997). For example, sea otters (*Enhydra lutris*) protect aquatic microalgae, such as kelp, by regulating the abundance of sea urchins (*Strongylocentrotus sp.*) (Estes and Palmisano, 1974). The presence of a predator can therefore serve to promote biodiversity and regulate the densities of organisms below it on the food chain.

Theories of freshwater trophic cascades have stemmed from research in lakes, where phytoplankton are the dominant basal resource (piscivorous - zooplanktivorous - herbivores - phytoplankton). If piscivorous fish are introduced into lakes as top predators to form a four-level food web, the number of zooplanktivorous fish will decrease, and the number of herbivores will increase or the growth rate of herbivores will be higher (Schindler et al., 1997). As a result, the number of primary producers, which is phytoplankton, will increase. Yet, in shallow systems, the dominant basal resource can be either phytoplankton or macrophytes. It is currently unknown whether the trophic cascade theory will hold in macrophyte dominated systems, such as ponds.

The goal of this study was to determine if the addition of a top predator would induce a trophic cascade in artificial ponds dominated by macrophytes. Specifically, we assessed if the addition of a top predator caused secondary consumers to change their diet. We hypothesized that the abundance of secondary consumers will decrease following the addition of top predators in ponds. Therefore, secondary consumers may have less intraspecies competition with the addition of top predators and under trophic cascade control, the increased population of zooplankton provides more prey for secondary consumers. Furthermore, with the presence of top predators, secondary consumers may spend more energy on avoiding predation instead of feeding, leading to changes in diets of secondary consumers (Preisser et al., 2005). The objective of this study was to determine the prey types and abundances in the gastrointestinal tract (GIT) of zooplanktivorous fish.

Material and methods

Eight artificial ponds at the Cornell University Experimental Ponds Facility in Freeville, New York were used. The ponds were each 30 × 30 m in surface area and 2.1 m in maximum depth. All 8 ponds had high densities of zooplanktivorous fishes, which were primarily two types of sunfish, i.e., pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*), with smaller numbers of fathead minnows (*Pimephales promelas*). Largemouth bass (*Micropterus salmoides*) were added to 4 of the 8 ponds as top predators in May 2022. The 4 ponds without added largemouth bass were used as control ponds. For the remainder of this manuscript, ponds with added largemouth bass are referred to as bass ponds.

At the end of the experiment in September 2022, 30 fish were collected from each pond, with 240 fish in total. All fish were humanely euthanized in MS222 (TMS, tricaine methanesulfonate and frozen. The gastrointestinal tract (GIT) comprising the stomach and intestine, was excised from each fish, and preserved in 70% ethanol until analysis.

Twenty fish per pond were selected for GIT content analysis, for a total of 160 fish. The GIT was weighed on weighing paper after blotting dry. The fullness of the GIT was determined based on a combination of previously published methods (Lang, 2004; Garrido et al., 2008). The GIT contents of each fish were examined under microscope (LEICA M165 C, Leica Biosystems). Gastrointestinal tract fullness was visually assessed and divided into 5 levels as follows: level 1, empty; level 2, less than 25% full; level 3, 25 to 50% full; level 4, 50 to 75% full; level 5, 75 to

100% full. The prey types were identified and assigned to one of four categories: vascularized plant, phytoplankton, zooplankton, and macroinvertebrate, and expressed in percentage.

The GIT fullness and proportion of prey type categories were compared between the bass and control ponds using analysis of variance (ANOVA). Differences between treatments were determined at a $P < 0.05$ and trends at $P < 0.1$.

Results

Gastrointestinal tract mass

The GIT weight averaged 0.0765 g, and ranged from 0.0089 to 0.5251 g. The GIT weight did not differ between the bass and control ponds ($P = 0.944$) (Figure 2).

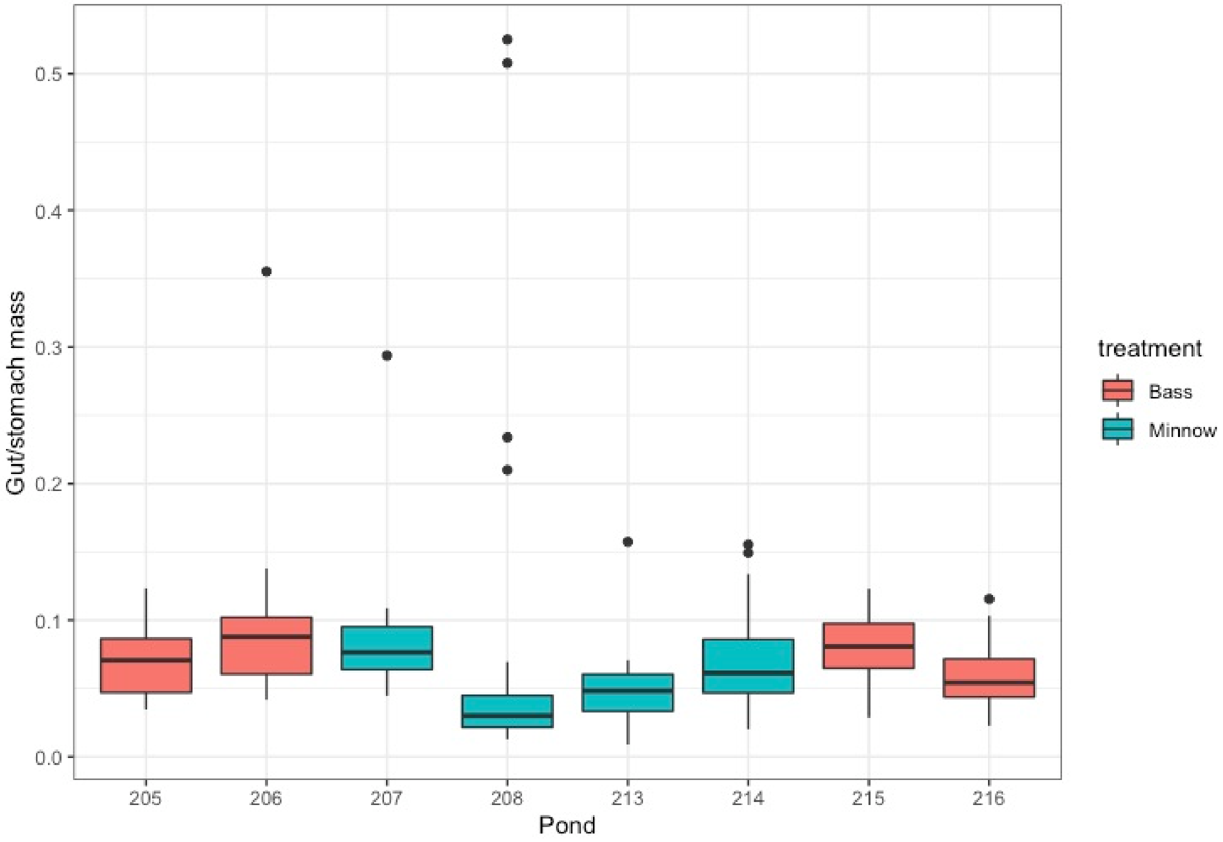

Figure 2. Total stomach and gut mass (gram) of fish in bass and control ponds. Bass: the ponds had bass addition. Minnow: the ponds had sunfish and minnows only.

Relative fullness

Sunfish in bass ponds had greater GIT fullness than those in control ponds ($P < 0.001$) (Figure 3). Sunfish in bass ponds had an average stomach fullness of 3.4, and sunfish in control ponds had an average stomach fullness of 2.7.

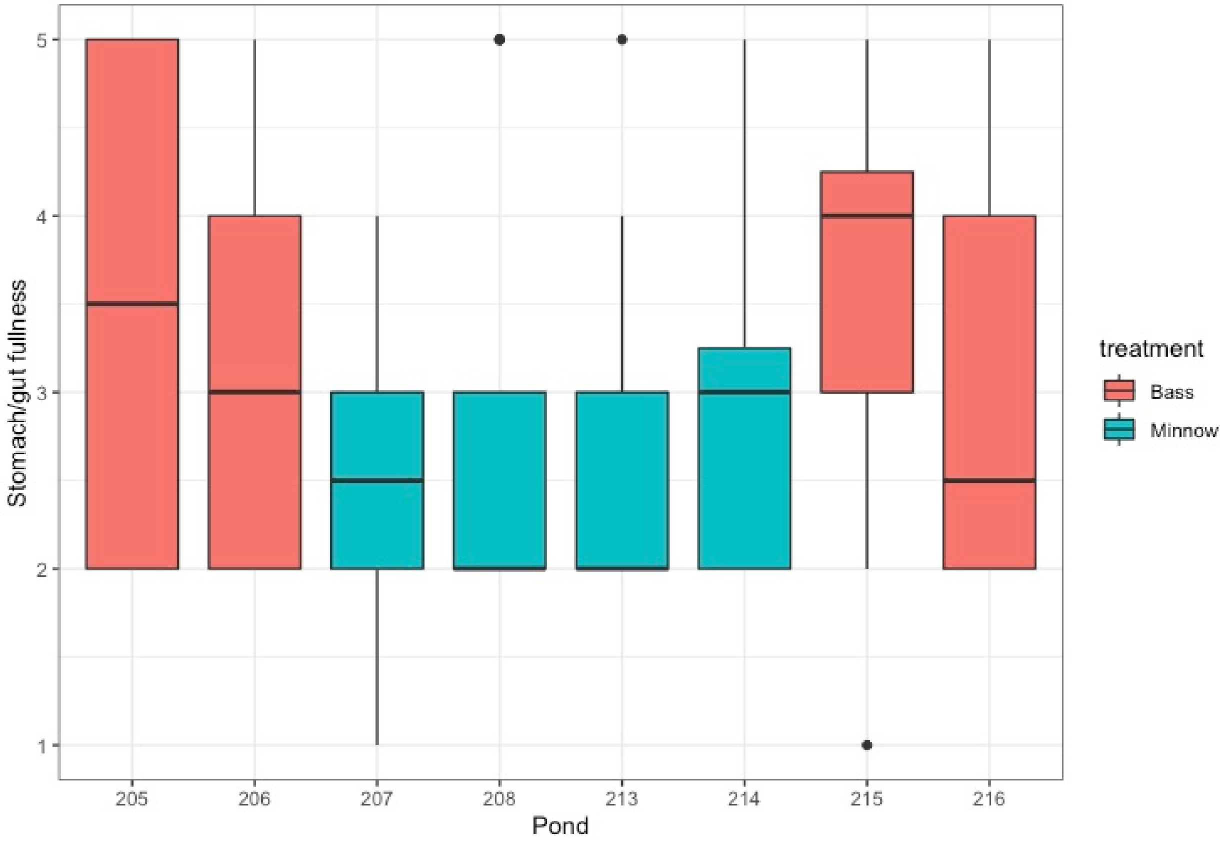

Figure 3. Relative GIT fullness of fish in bass and control ponds.

Vascularized plant

The percentage of vascular plants consumed by sunfish did not differ between control and bass ponds (P = 0.443) (Figure 4).

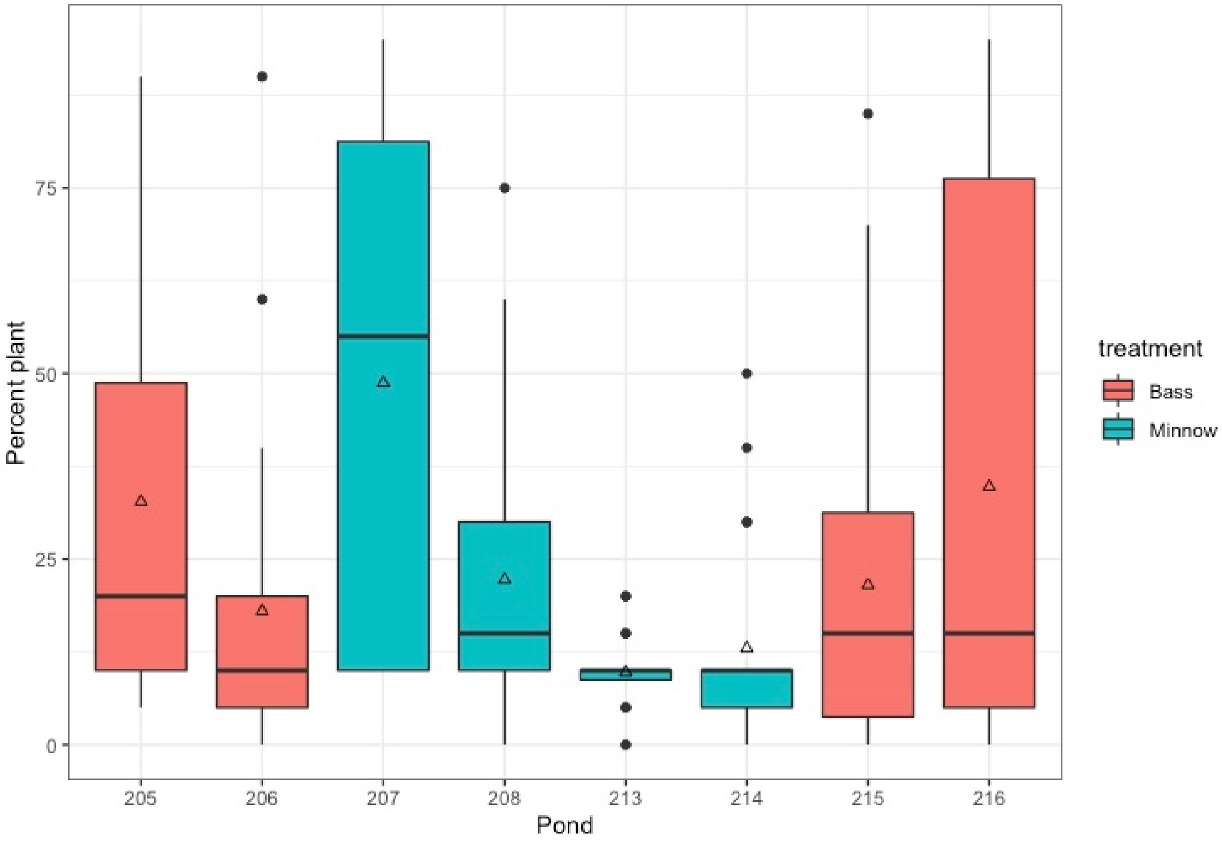

Figure 4. Percentage of vascular plants in fish stomach and gut in control and bass ponds. △: mean percentage of vascular plants consumed by fish.

Phytoplankton

The percentage of phytoplankton consumed by sunfish in bass ponds was lower compared to that of sunfish in control ponds (P < 0.001) (Figure 5). The average percentage of phytoplankton consumed by sunfish in bass and control ponds were 7.06% and 18.81%, respectively.

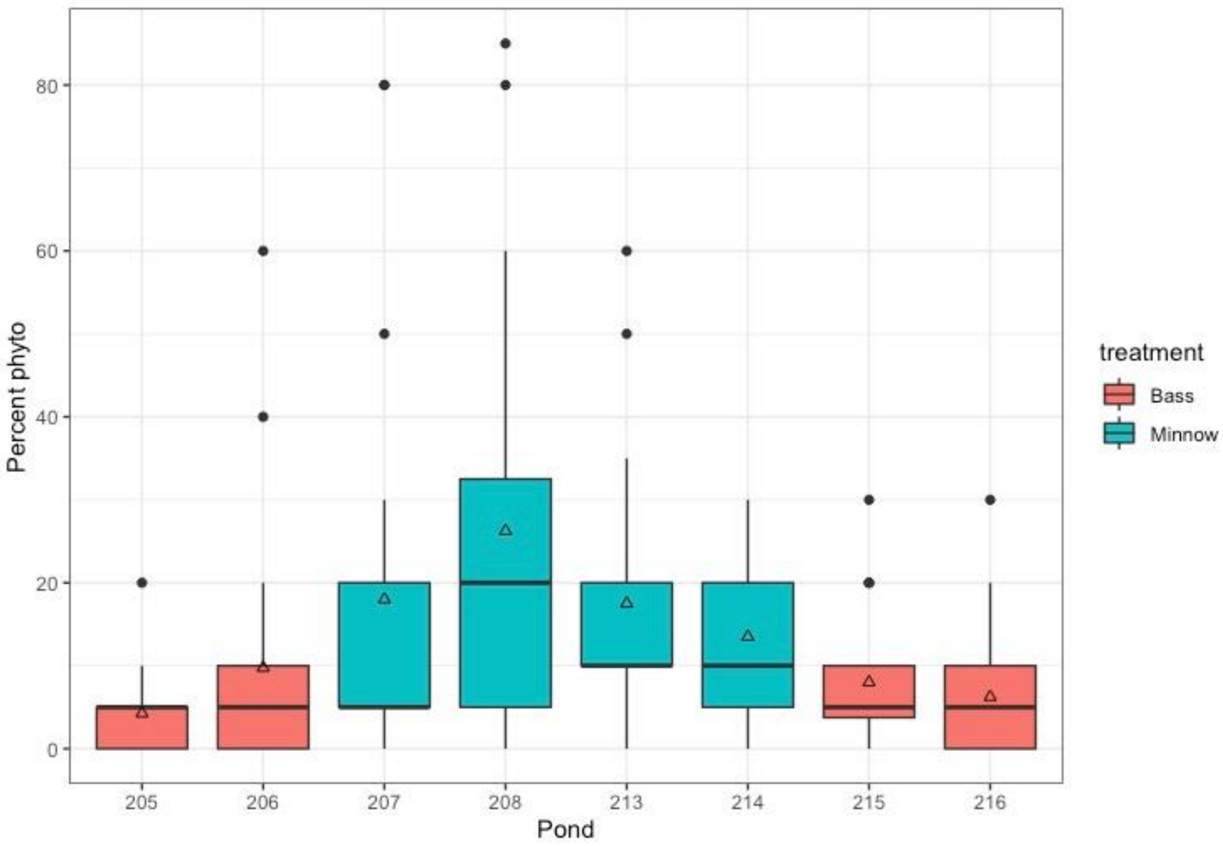

Figure 5. Percentage of phytoplankton in fish stomach and gut in bass and control ponds. △: mean percentage of vascular plants consumed by fish.

Zooplankton

Sunfish in bass ponds consumed less zooplankton ($P < 0.01$) compared to those in control ponds (Figure 6). Zooplankton comprised 24 and 41% of the matter in the GIT of sunfish in bass and control ponds, respectively.

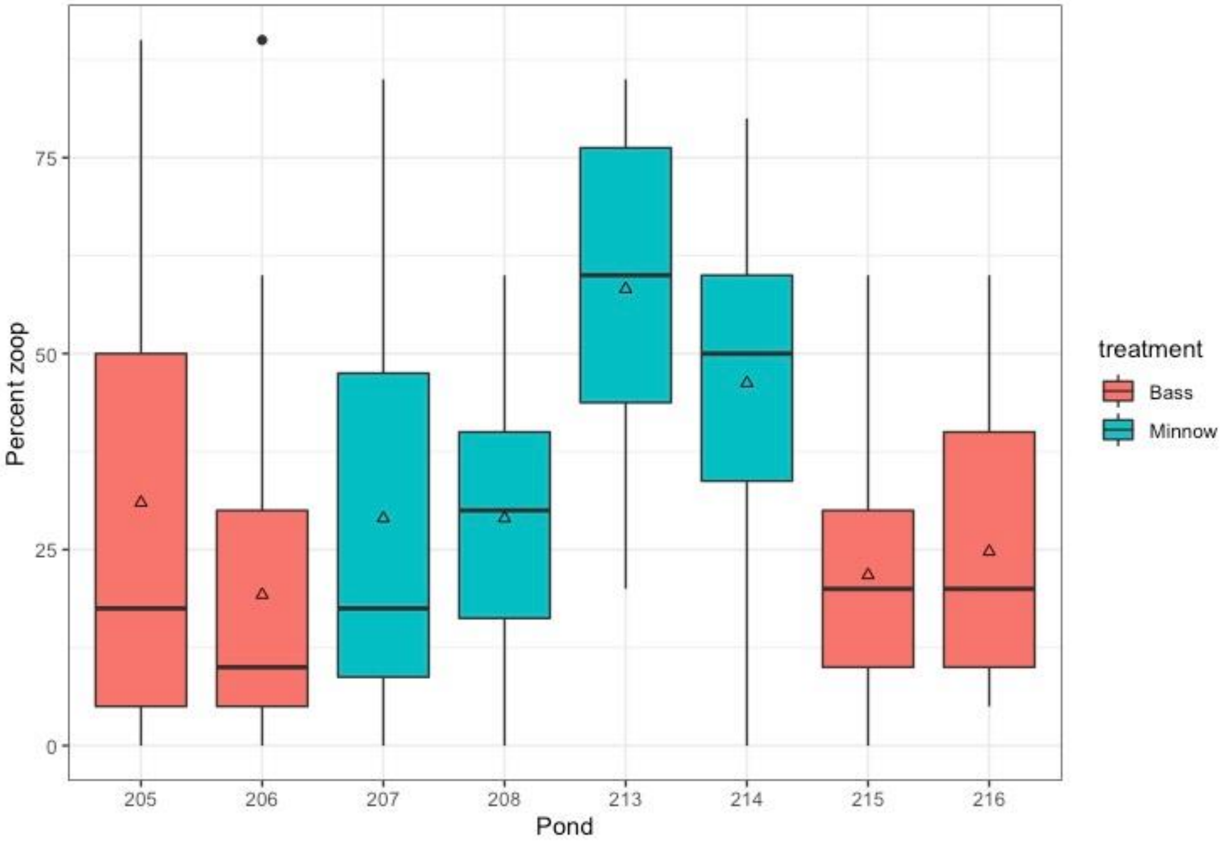

Figure 6. Percentage of zooplankton in fish stomach and gut in bass and control ponds. △: mean percentage of vascular plants consumed by fish.

Macroinvertebrate

The percentage of macroinvertebrate consumed by sunfish in bass ponds was greater ($P < 0.0001$) compared to that of sunfish in control ponds (Figure 7). The percentage of macroinvertebrate consumption was 40.69 and 16.81% in bass and control ponds, respectively.

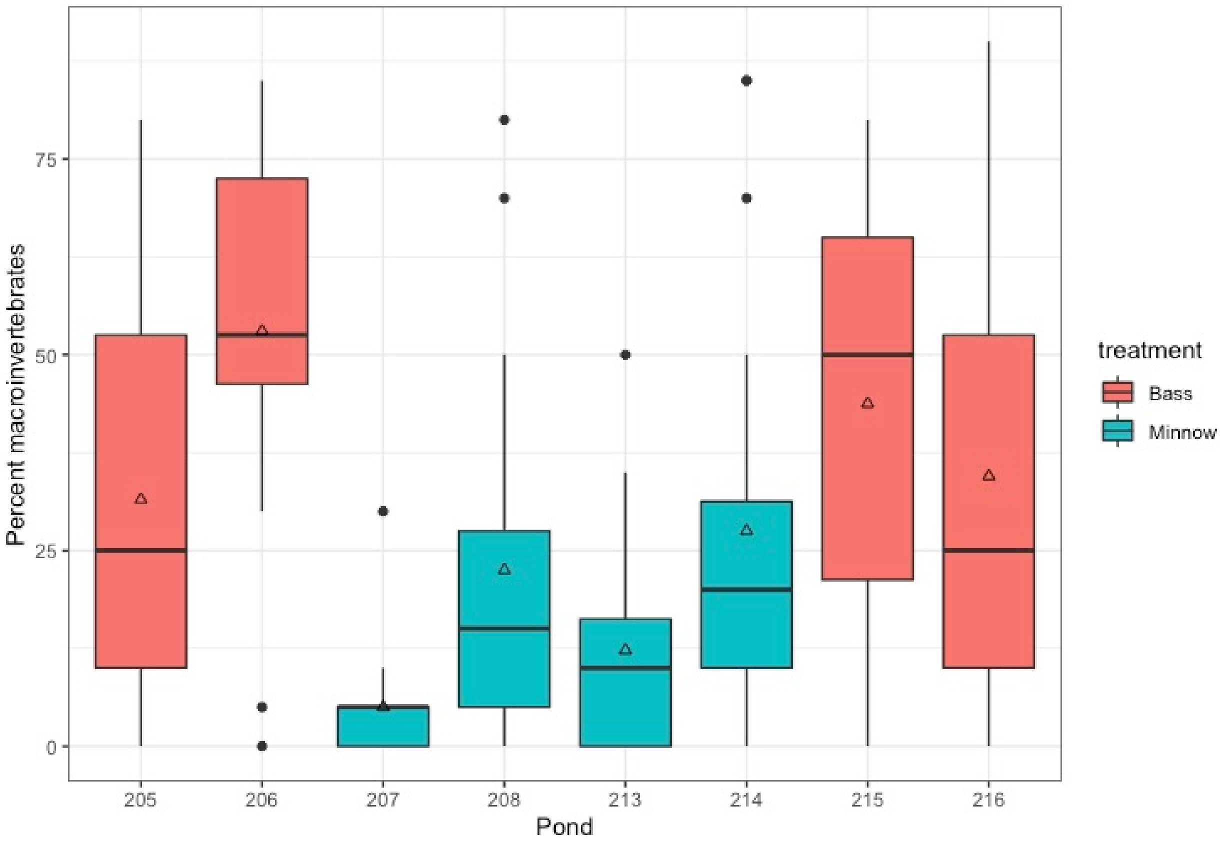

Figure 7. Percentage of macroinvertebrate in fish stomach and gut in bass and control ponds. △: mean percentage of vascular plants consumed by fish.

Discussion

Adding piscivorous fish to freshwater ecosystems can alter the food web through direct and indirect interactions across multiple trophic levels (Carpenter and Kitchell, 1996). We hypothesized that adding top predators to macrophyte-dominated ponds would induce a trophic cascade. We tested this by evaluating whether secondary consumers shifted their diet in response to addition of predators.

The relative GIT fullness was greater in sunfish from bass ponds, despite the total GIT mass not differing between the two treatments. The presence or absence of top predators may affect the feeding intake of secondary consumers. There are two possible explanations for why sunfish in the bass ponds had more prey in their GIT. First, largemouth bass directly reduced the sunfish population (data not shown), potentially releasing the sunfish from intraspecies competition. As a result, the sunfish may have had more food to eat, such as zooplankton or macroinvertebrates. This finding is consistent with that of Koel et al. (2019) who found that the growth of piscivorous fish population led to the reduction of prey fish and increased the biomass of large zooplankton. While differences in GIT fullness were observed among treatments, no differences in GIT mass across the treatments were found. We speculate that this is due to the gut and stomach tissues comprising a high biomass.

We hypothesized that by adding bass to the ponds, sunfish would change their diets and feeding strategy, which was supported by our results. , Sunfish in ponds with bass consumed less zooplankton and phytoplankton compared to sunfish in ponds without bass. Instead, sunfish in bass ponds consumed more macroinvertebrates than sunfish in ponds without bass. Fish may turn to high energy feedings when they are under severe intraspecies competition. Vesterinen et al. (2021) reported that zooplankton contain more docosahexaenoic acid (DHA) than do macroinvertebrates. Generalist fish were shown to modify their diets to zooplankton as a more efficient feeding strategy during a high-density period (Hayden et al., 2014). This finding also supports our results, whereby sunfish had a large population in ponds without bass and thus were prone to consume more zooplankton. Another possible reason is that the cascade interaction between predators and macroinvertebrates was strong. Large population of sunfish decrease the number of macroinvertebrates. In bass ponds, there were less sunfish, and thus more macroinvertebrates available for consumption. The result is support by Kipp and Ricciardi (2012), who found that the diversity and population of macroinvertebrates declined with the increasing density of predators. As observed in our study, sunfish in bass ponds consumed more macroinvertebrates than sunfish in control ponds. Sunfish may change their diet because of prey population, distribution, and availability (Blanco et al., 2003).

The sunfish diet composition showed significant differences in phytoplankton, zooplankton, and macroinvertebrate prey between ponds with and without bass. However, there was no difference in the proportion of vascularized plants. Neither pumpkinseed nor bluegill have shown to feed on plants throughout their life history (Mittelbach, 1984; Johnson and Dropkin, 1993; Werner et al., 1996), so this finding was unexpected. There is no evidence however suggesting that the consumption of plants is caused by the presence of top predators. It is possible that sunfish may accidentally feed on vascularized plants when predating on other prey, such as zooplankton or macroinvertebrates.

Conclusion

The addition of a top predator, the largemouth bass, to artificial ponds induced a trophic cascade by reducing sunfish populations and altering sunfish diets. Specifically, sunfish in ponds with bass consumed less phytoplankton and zooplankton and consumed more macroinvertebrates. The potential mechanism is that the bass reduced the sunfish populations, releasing them from competition, and indirectly increasing the biomass of their macroinvertebrate prey. Furthermore, top predators affected the food chain length and the food web structure in macrophyte based ponds and influenced the primary producers and energy flow from lower trophic level

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