

Field and Laboratory Studies Suggest that Recruitment of the Invasive Common Carp is
Controlled by Native Fish in Stable Lakes of the Upper Mississippi Basin

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Abstract

Recent studies have shown that recruitment of the common carp in many lakes of the Upper Mississippi Basin is limited to areas that experience an ecological disturbance that alters the fish community. It has been hypothesized that recruitment of carp is limited by native predatory fish and that carp are only able to recruit in habitats where these native species have been excluded due to winter hypoxia. We tested this hypothesis by comparing the survival of carp eggs and larvae in the presence and absence of native predatory fishes in three experiments. First, we sampled fish diets and carp egg abundance on a daily basis in lakes where wild carp had spawned to identify fish predators and track carp egg abundance in the environment. We simultaneously estimated the date of carp egg hatching using eggs raised in the lab at water temperatures that represented lake temperatures. We found that the bluegill sunfish was the main predator of carp eggs (94% of egg predators were bluegill sunfish), and that egg abundance declined before the estimated date of hatching in areas where bluegill sunfish were present. In our second experiment, carp egg survival was tested in the presence and absence of bluegill sunfish in the laboratory. Carp eggs were fertilized in the laboratory, placed on artificial vegetation in 70 liter aquaria that contained a bluegill sunfish or no fish, and counted twice daily until hatching. We found that the survival to hatching of carp eggs in aquaria decreased from 74% to 15% in the presence of bluegill sunfish (p -value < 0.001). In our third experiment, carp larval survival was tested in the presence of bluegill and green sunfish in the laboratory. Larval carp were raised from eggs fertilized in the laboratory until they reached their free swimming stage and introduced into 1,600 liter tanks with five individuals of a single species of predatory fish or no fish. Larvae

were then sampled twice daily for two days. Both bluegill and green sunfish reduced larval carp survival to zero percent after 34 hours (p-value < 0.001). These results suggest that the bluegill sunfish is a voracious predator of the early life stages of common carp and is likely responsible for reducing the recruitment of common carp through predation on its eggs and larvae.

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Chapter 1

An Introduction

General Characteristics of the Common Carp

The common carp (*Cyprinus carpio*, hereafter carp) is a large, benthivorous minnow native to the drainages of the Caspian, Black, and Aral Seas (Balon 1995). After centuries of human introductions around the globe, the carp is currently found on every continent except Antarctica (Balon 1995). These introductions have allowed the carp to invade a variety of ecosystems around the world, and its presence in many of these systems is unwanted.

The carp is a long lived fish capable of living over 25 years and reaching a size of over 80 cm in total length (Brown et al. 2005; Swee and McCrimmon 1966). Male carp typically mature at ages two to four (25-35 cm) and female carp at ages four to five (30-45 cm) in temperate climates (Becker 1983; Billard et al. 1995; Swee and McCrimmon 1966). Once mature, female fecundity is positively correlated with caudal fork length and total weight, and a single female can produce over 2.2 million oocytes in a single spawning season (Sivakumaran et al. 2003; Swee and McCrimmon 1966). It is interesting to note that the fecundity of the carp is an order of magnitude greater than almost all native North American fishes (Winemiller and Rose 1992).

The diet of carp is very broad and includes various benthic invertebrates, plant debris, and detritus (Becker 1983; Garcia-Berthou 2001). Its foraging behavior is characterized by rooting in the substratum and has been shown to affect the chemistry and macrophyte community of lakes it inhabits (Crivelli 1983). The presence of carp is

correlated with increases in dissolved phosphorus and nitrogen concentrations, declines in the abundance of aquatic macrophytes, and increases in turbidity (Crivelli 1983; Moss et al. 2002; Schrage and Downing 2004). While these effects are not directly harmful to most aquatic species, they have many negative indirect effects, especially on the aquatic communities of North America (Lougheed et al. 2004; Parkos et al. 2003). These negative effects have made the carp a target of eradication programs for over a century (Lubinski et al. 1986). Many of these programs have attempted to decrease populations through the removal of adults using a variety of methods including poisoning (rotenone), water draw-down, and large scale capture, but few past programs have had long term success (Bajer et al. 2009; Lubinski et al. 1986).

Life History of the Common Carp

The carp is classified as a non-guarding, open substratum, egg scattering, obligatory plant spawner (Balon 1995). It typically spawns from mid-spring to early-summer in northern temperate climates (Balon 1995), but has been observed spawning through July and into August in Lake St. Lawrence, Ontario (Swee and McCrimmon 1966). Most spawning activity takes place in the late morning and early afternoon (Billard 1999; Swee and McCrimmon 1966). Spawning has been observed at water temperatures ranging from 16 to 28°C, although most spawning takes place at water temperatures between 19 and 23°C (Balon 1995; Billard 1999; Swee and McCrimmon 1966). Spawning areas are characterized by beds of submerged and emergent vegetation, and are typically in less than 1m of water (Balon 1995; Swee and McCrimmon 1966).

Females release their eggs in multiple spawning bouts that occur over a 10 to 14 day period (Balon 1995). Spawning behavior is characterized by groups of several males following a single female and repeatedly pushing against the body of the female with their heads (Balon 1995; Swee and McCrimmon 1966). When a female is ready to spawn, she thrashes her caudal fin violently and expel a mass of eggs over a bed of vegetation (Swee and McCrimmon 1966). The males follow alongside the female and discharge milt as the female releases her eggs to fertilize them (Swee and McCrimmon 1966). This act is carried out in two to five seconds, and is repeated multiple times throughout the day (Balon 1995; Swee and McCrimmon 1966).

Carp eggs swell to 1.5 to 1.8 mm in diameter once they are exposed to water (Balon 1995). The outer membrane is adhesive, allowing them to stick to submerged vegetation and keeping them from sinking to the bottom (Balon 1995; Swee and McCrimmon 1966). Information on hatching is limited, but suggests that hatching occurs within three to six days of fertilization and is dependent on water temperature (Balon 1995; Smallwood and Smallwood 1931; Swee and McCrimmon 1966).

Newly hatched larvae remain attached to vegetation and possess a yolk sack that is absorbed over three days (Smallwood and Smallwood 1931). After the yolk has been absorbed, larvae begin to feed on plankton (Smallwood and Smallwood 1931). Larval carp can grow up to 0.5 mm per day if food is not limiting (Carvalho et al. 1997). Nothing has been published about the habits and behavior of larvae other than the fact that they become extremely abundant in Australian rivers as flood waters recede (Stuart and Jones 2006).

The transition from the larval to juvenile stage happens 20 to 30 days after hatching at a standard length of 20 to 25 mm (Vilizzi and Walker 1999). This transition is characterized by a change in morphology, feeding habits, and locomotion (Vilizzi and Walker 1999). At the juvenile stage, carp resemble adults in both morphology and behavior (Vilizzi and Walker 1999).

Very little information on the survival of carp eggs and larvae in the wild has been documented, and no one has tested what factors affect survival of eggs and larvae in the wild. This information is important in understanding the ecology of carp as the survival of early life stages affects not only the size and growth of a population, but also the age structure within the population.

Effects of Predation on the Recruitment of Fish

Recruitment can be defined as when an individual becomes a member of a population, and is one of the most important factors that determines population size (Bailey and Houde 1989). Recruitment is impacted by a number of factors that affect the survival of an organism, including abiotic factors such as temperature, dissolved oxygen, and pH, and biotic factors such as predation and competition (Jackson et al. 2001). The survival of individuals to the age or size where they can recruit to the population is essential for the persistence of a population in the wild (Jackson et al. 2001).

In both marine and freshwater ecosystems, predation has been shown to be a major factor controlling recruitment in fish (Bailey and Houde 1989; Jackson et al. 2001). Koster and Mollmann (2000) showed that consumption of cod (*Gadus morhua*) eggs by herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in the Baltic Sea is correlated

with low recruitment of cod. Further modeling of cod recruitment in the Baltic Sea by Andersen et al. (2008) showed that changes in salinity and dissolved oxygen could not explain trends in cod recruitment alone, and that predation by sprat was an important indicator of cod recruitment. Fauchald (2010) suggested that decreases in the population of cod due to overfishing have allowed a superabundance of their prey (sprat and herring), which now restrict the recovery of the cod population by preying upon the early life stages of cod. He argues that this predator-prey reversal is acting to control cod recruitment and maintain small populations of cod in the Baltic.

Recruitment on coral reefs has also been shown to be affected by predation. Carr and Hixon (1995) showed that by experimentally removing resident predators from certain reefs in the Bahamas, the recruitment of rainbow wrasse (*Halichoeres pictus*) and blue chromis (*Chromis cyanea*) increased by 37.6% and 31.9%, respectively. The invasion of Caribbean reefs by the Indo-Pacific lionfish (*Pterois volitans*) has also been shown to decrease recruitment of native fish species. Albins and Hixon (2008) showed that the presence of lionfish on a reef decreased native fish recruitment by an average of 79%.

Predation is important to the recruitment of fish in freshwater systems as well. Tonn et al. (1992) showed that predation by Eurasian perch (*Perca fluviatilis*) was able to reduce recruitment of crucian carp (*Carassius carassius*) in small shallow lakes in eastern Finland. Not only was the survival of young-of-the-year crucian carp reduced in the presence of predatory perch, but their growth rate was also reduced. Kim and DeVries (2001) showed that mature bluegill sunfish (*Lepomis macrochirus*) and white crappie (*Pomoxis annularis*) decreased survival of larval bluegill sunfish in 5,300 liter

mesocosms by 55% and 40%, respectively. This reduction took place within a relatively short four hour test period, and likely would have been much higher if the experiment had lasted longer due to the fact that wild bluegill sunfish forage continuously during the day while white crappie follow a crepuscular foraging strategy (Becker 1983). Regardless of the length of the experiment, it still suggests that predators have a large impact on the recruitment of young bluegill.

The recruitment of carp may also be regulated by predators during its early life stages. Carp eggs are similar to those of cod in that they are broadcast during spawning rather than laid in nests, are not guarded by either parent, and possess no defense mechanisms such as toxins to protect themselves from predators (Becker 1983). It is therefore possible that carp would experience the same negative effects on recruitment that cod experience if egg predators are prevalent in the ecosystem when they spawn.

Larval carp also likely face a severe risk from predators. Kim and DeVries (2001) showed that two of the most common centrarchids found in North American lakes, bluegill sunfish and white crappie, are voracious predators of larval fish. Margulies (1990) also showed that fish larvae are vulnerable to bluegill sunfish when he tested the vulnerability of different sizes of larval white perch (*Morone americana*) and showed a decrease in mortality as larval size and age increased. Litvak and Leggett (1992) showed that young larvae are more vulnerable to predators when they tested the vulnerability of larval capelin (*Mallotus villosus*) to predation by three-spined stickleback (*Gasterosteus aculeatus*) at different ages. However, they also showed that the risk of an attack increases as the size of the larvae increases, suggesting predation may be heavier on older larvae compared to younger larvae.

Potential Predators of Carp in North America

As in other areas of the world, freshwater fish native to North America feed on a wide variety of items including vegetation, plankton, molluscs, crustaceans, arthropods, other fish, detritus, or a combination of these (Becker 1983). Wainwright and Richard (1995) examined the diet and morphology of four centrarchid species commonly found in North America and suggested that diet can be predicted based on the body size, mouth gape, and jaw-lever mechanism of a species. They showed that prey type correlates with the morphology of the predator, and that as the predator's morphology changes during growth, its prey changes as well.

As carp eggs are found adhered to aquatic vegetation, potential egg predators must be able to maneuver in vegetative habitats and handle small (2mm) prey. Wainwright et al. (2007) showed that fish with small mouths have greater accuracy when using suction feeding than fish with larger mouths. Larger mouthed fish would therefore be unlikely predators of carp eggs due to their inability to target and manipulate small prey. However, fish that normally feed on invertebrates found on aquatic vegetation, or species that consume plant material directly, could prey on carp eggs. These species include bluegill sunfish, green sunfish (*Lepomis cyanellus*), pumpkinseed sunfish (*Lepomis gibbosus*), and juvenile largemouth bass (*Micropterus salmoides*) (Becker 1983).

Predators of carp larvae face different challenges. After carp larvae absorb their yolk sack, they begin to forage on plankton in the water column (Carvalho et al. 1997). This suggests that zooplankton predators would be best at consuming carp larvae. Such

potential predators include bluegill sunfish, pumpkinseed sunfish, and black and white crappie (Becker 1983).

Recent Research on Carp Recruitment in the Upper Mississippi Basin

Recent research on the population ecology of carp in the Upper Mississippi Basin has yielded new information suggesting the recruitment of carp in North America may be controlled by predatory fish. Bajer and Sorensen (2010) found that adult carp in several interconnected chain of lakes in the Upper Mississippi Basin were represented by only a few age classes, and that these age classes were highly correlated with low winter dissolved oxygen concentrations in the lake system. These hypoxic events often lead to winterkills where a substantial proportion of the fish community experiences mortality (Cooper and Washburn 1949; Petrosky and Magnuson 1973). Farwell et al. (2007) and Tonn and Magnuson (1982) have shown that two very different fish communities develop in lakes that regularly experience winter hypoxia. A hypoxic-tolerant fish community dominated by cyprinids normally develops in lakes that experience low winter dissolved oxygen levels while a hypoxic-intolerant fish community dominated by centrarchids normally is found in lakes that do not experience low winter dissolved oxygen levels. Bajer and Sorensen (2010) suggested that carp may be taking advantage of these different fish communities by moving into areas that have experienced a winterkill when they spawn. They hypothesized that the early life stages of carp are able to survive better in areas that have experienced winter hypoxia due to a release from fish predators that would otherwise consume their eggs and larvae.

Phelps et al. (2008) noted a similar phenomenon in the age structure of carp populations in isolated lakes in eastern South Dakota. They noted that carp age classes correlated with increased temperatures, more precipitation, and less wind during the spring, summer, and fall. However, their analysis concluded that recruitment did not correlate with winter severity. While both studies suggested different mechanisms for the variation in age class recruitment, it is possible carp recruitment is determined by different mechanisms in the two different systems. The isolated lakes of eastern South Dakota had no history of winterkill when the study was conducted, which could explain why the analysis by Phelps et al. (2008) did not identify winter severity as significantly affecting carp recruitment.

My Thesis

The goal of this thesis was to test the hypothesis suggested by Bajer and Sorensen (2010) that predatory fish control the recruitment of carp in the Upper Mississippi Basin through predation on the early life stages of carp. It has two chapters. The first chapter includes background information on carp and explains how predation affects recruitment in selected aquatic ecosystems. The second chapter describes a study that tested whether fish predators are responsible for controlling the survival of carp to the juvenile stage in lakes of the Upper Mississippi Basin. This thesis was intended to expand on a recent study spearheaded by Dr. Przemyslaw Bajer (see Appendix 1). I assisted Dr. Bajer in carrying out the study and am a coauthor of the manuscript presented in Appendix 1 that is being prepared for submission to *Biological Invasions*. The study presented in Appendix 1 found that carp eggs experienced higher mortality in lakes that had not

experienced an ecological disturbance (i.e. winterkill) unless they were protected from fish predators using mesh netting. It was also found that the presence of bluegill sunfish is strongly correlated with low carp recruitment. However, the study in Appendix 1 was unable to state whether naturally occurring carp eggs face a high risk of predation because all eggs used in the study were artificially fertilized and placed on pieces of yarn.

The objective of the study presented in Chapter 2 was to test whether fish predators are responsible for controlling the survival of carp to the juvenile stage by 1) tracking carp egg abundance and egg predation by fish in natural carp spawning areas, 2) measuring the effect fish predators have on carp egg survival in the lab, and 3) measuring the effect fish predators have on carp larval survival in a simulated carp spawning habitat. If the hypothesis suggested by Bajer and Sorensen (2010) can be supported by a more natural field study and rigorous laboratory experiments, it may allow fisheries managers to manage the recruitment of invasive carp populations through the careful stocking of native predators or through the prevention of winter hypoxia in designated lakes using winter aeration. This technique could be a great help in controlling invasive common carp in the North American Midwest.

The manuscript presented in Chapter 2 is written in the style of Transactions of the American Fisheries Society. We anticipate submitting the manuscript for publication with coauthors Christopher J. Chizinski and Peter W. Sorensen following publication of the manuscript found in Appendix 1.

Chapter 2

Field and Laboratory Studies Suggest that Recruitment of the Invasive Common Carp is Controlled by Native Fish in Stable Lakes of the Upper Mississippi Basin

Chapter Summary

Recent studies have shown that recruitment of the common carp in many lakes of the Upper Mississippi Basin is limited to areas that experience an ecological disturbance that alters the fish community. It has been hypothesized that recruitment of carp is limited by native predatory fish and that carp are only able to recruit in habitats where these native species have been excluded due to winter hypoxia. We tested this hypothesis by comparing the survival of carp eggs and larvae in the presence and absence of native predatory fishes in three experiments. First, we sampled fish diets and carp egg abundance on a daily basis in lakes where wild carp had spawned to identify fish predators and track carp egg abundance in the environment. We simultaneously estimated the date of carp egg hatching using eggs raised in the lab at water temperatures that represented lake temperatures. We found that the bluegill sunfish was the main predator of carp eggs (94% of egg predators were bluegill sunfish), and that egg abundance declined before the estimated date of hatching in areas where bluegill sunfish were present. In our second experiment, carp egg survival was tested in the presence and absence of bluegill sunfish in the laboratory. Carp eggs were fertilized in the laboratory, placed on artificial vegetation in 70 liter aquaria that contained a bluegill sunfish or no fish, and counted twice daily until hatching. We found that the survival to hatching of

carp eggs in aquaria decreased from 74% to 15% in the presence of bluegill sunfish (p-value < 0.001). In our third experiment, carp larval survival was tested in the presence of bluegill and green sunfish in the laboratory. Larval carp were raised from eggs fertilized in the laboratory until they reached their free swimming stage and introduced into 1,600 liter tanks with five individuals of a single species of predatory fish or no fish. Larvae were then sampled twice daily for two days. Both bluegill and green sunfish reduced larval carp survival to zero percent after 34 hours (p-value < 0.001). These results suggest that the bluegill sunfish is a voracious predator of the early life stages of common carp and is likely responsible for reducing the recruitment of common carp through predation on its eggs and larvae.

Introduction

The common carp (*Cyprinus carpio*, hereafter carp) is a large, benthivorous minnow which is native to the drainages of the Caspian, Black, and Aral Seas (Balon 1995). After centuries of human introductions around the globe, the carp is currently found on every continent except Antarctica (Balon 1995). The carp is considered a pest in both North America and Australia (Clark et al. 1991; Koehn 2004), and has many negative effects on its environment when it reaches high densities (Bajer et al. 2009). Its presence is correlated with increases in dissolved nutrient concentrations, declines in the abundance of aquatic macrophytes, and increases in turbidity (Crivelli 1983; Moss et al. 2002; Schrage and Downing 2004). It has become particularly damaging in North American and Australian freshwater ecosystems due to its high abundance in those ecosystems (Bajer et al. 2009; Pinto et al. 2005). This has made the carp a target of

eradication programs in North America for over a century (Lubinski et al. 1986). A variety of methods including poisoning, water draw-downs, and large scale netting operations have been developed to decrease population sizes of carp, but few past programs have had long term success (Bajer et al. 2009; Lubinski et al. 1986).

Recent research has suggested that young-of-the-year carp are only able to recruit (i.e. survive to adulthood) under certain circumstances in interconnected lakes of the Upper Mississippi Basin (Bajer and Sorensen 2010). Recruitment is one of the most important factors which determines the size of populations over time (Bailey and Houde 1989), and is affected by both abiotic and biotic factors (Jackson et al. 2001). In many species of fish, predation on immature individuals is a major determining factor in the recruitment of a species, typically because young fish are more vulnerable to predators than mature individuals (Tonn et al. 1992). Koster and Mollmann (2000) suggested that the recruitment of cod in the Baltic Sea is likely controlled by egg predators, and Andersen et al. (2008) later supported this hypothesis by showing that the abundance of egg predators correlated with recruitment better than stock structure or maternal age or size. Litvak and Leggett (1992) showed that larval capelin (*Mallotus villosus*) experience higher mortality in the presence of three-spined stickleback (*Gasterosteus aculeatus*) when they are younger, but that the risk of an attack increases as the size of the larvae increases, suggesting that young larvae are more vulnerable to predators but that larvae may be preyed upon more heavily as they grow and mature.

Predation may also be an important factor in determining the recruitment of carp. Bajer and Sorensen (2010) showed that the size of carp age classes in an interconnected chain of lakes in the Upper Mississippi Basin was negatively correlated with winter

dissolved oxygen levels in the lakes, and suggested that carp are only able to recruit in areas that have experienced an ecological disturbance (i.e. winterkill). Carp were also shown to overwinter in deep lakes that did not experience winter hypoxia and migrate through streams during the spring prior to spawning to nearby shallow lakes that had experienced winter hypoxia (Bajer and Sorensen 2010). This movement to lakes that experience winter hypoxia is relevant because lakes of the Upper Mississippi Basin can be characterized by two distinct fish assemblages that are correlated with winter dissolved oxygen levels (Tonn and Magnuson 1982). The first assemblage is dominated by centrarchids and characterized by high winter dissolved oxygen levels, while the second is dominated of cyprinids and characterized by low winter dissolved oxygen levels. The ability of carp to move between these two types of lakes may allow its young to develop in areas that are not accessible to certain species of predatory fish (e.g. sunfishes), therefore allowing young carp to escape the negative effects of these predators.

Unfortunately, previous studies did not directly test whether predation was the cause of the stochastic recruitment of carp. In the present study, we tested the hypothesis that predatory fish restrict the recruitment of carp in interconnected lakes of the Upper Mississippi Basin by measuring the ability of carp eggs and larvae to survive in the presence of native fish. The objectives of this study were 1) to track carp egg abundance and fish predation on carp eggs in natural carp spawning areas, 2) to test the effect of fish predators on carp egg survival under controlled laboratory conditions, and 3) to test the effect of fish predators on larval carp survival in a simulated carp spawning habitat. We hypothesized that a number of native fish species would prey on carp eggs in the wild,

and that these predators would significantly decrease the survival of carp egg and larval life stages in the lab.

Methods

Study Area

This study took place in a chain of interconnected lakes near St. Paul, Minnesota (Figure 1). Two lakes were selected from within this chain to represent a lake which experienced winter oxygen levels less than 2 mg/L (termed ‘hypoxic’) and a lake which did not experience winter oxygen levels less than 2 mg/L (termed ‘normoxic’). This distinction was made because Bajer and Sorensen (2010) suggested that carp only recruit in lakes that have experienced a disturbance (i.e. winter hypoxia), and 2 mg/L is considered the threshold for winter hypoxia in lakes of the Upper Mississippi Basin (Knights et al. 1995).

Lake Casey (45°01' N, 93°01' W) is a 4.8 ha lake with a maximum depth of 1.5 m and an average depth of 1.0 m. It has a history of winter hypoxia, and had a minimum dissolved oxygen concentration of 0.6 mg/L the winter after this study was conducted (Bajer, unpublished data). Dense stands of cattails (*Typha sp.*) covered 95 % of the shore line of Lake Casey, but no vegetation was present in the center of the lake. The fish community in the lake was composed of black bullhead (*Ameiurus melas*), green sunfish (*Lepomis cyanellus*), fathead minnow (*Pimephales promelas*), and common carp.

Lake Keller (45°00' N, 93°04' W) is a 27.3 ha lake with a maximum depth of 2.5 m and an average depth of 2.1 m. It does not have a history of winter hypoxia, and reached a minimum dissolved oxygen concentration of 3.2 mg/L the winter after this

study was conducted (Bajer, unpublished data). At the time of this study, approximately 53 % of the littoral zone was covered by beds of curly leaf pondweed (*Potamogeton crispus*), Eurasian water milfoil (*Myriophyllum spicatum*), and coontail (*Ceratophyllum demersum*). The fish community in Lake Keller was composed of black bullhead, bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*Lepomis gibbosus*), largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*), walleye (*Sander vitreus*), yellow perch (*Perca flavescens*), and common carp.

Experiment 1: Does predation by native fish on wild carp eggs affect carp recruitment?

Bajer and Sorensen (2010) suggested that predation by fish on carp eggs and larvae is restricting carp recruitment in lakes of the Upper Mississippi Basin. To test this hypothesis, we carried out a three part experiment. First, the hatching rate of carp eggs was estimated using eggs raised in the lab to allow us to estimate the time carp eggs were vulnerable to predators in the wild. Second, a survey of natural carp spawning areas in our two study lakes identified predators of carp eggs and measured carp egg survival through time during late spring and early summer. Third, our study lakes were sampled again in late summer to see if juvenile carp were present.

Experiment 1a: What is the hatching rate of carp eggs at temperatures between 15 and 30°C?

The literature on carp reproductive biology does not include a precise estimate of the time between fertilization and hatching of carp eggs at various water temperatures. To predict how long carp eggs would be present in wild carp spawning areas, the lower

developmental threshold for carp egg development and the degree days necessary for carp eggs to fully develop was calculated using eggs raised at a range of temperatures representative of lake water temperatures during the carp spawning season. Eggs were artificially fertilized and raised in the lab so water temperature and time to hatching could be measured more precisely.

Male and female carp were captured in Lake Keller during May 2010, separated by sex, and transported to 1,000 L holding tanks in the laboratory. The evening of capture, all carp were injected with a 0.5 mg/kg injection of Ovaprim (Western Chemical Inc.) to induce ovulation and spermatation (Brzuska and Adamek 1999). Injections were made into the ventral body cavity slightly posterior to the pelvic fins at 1900 hours and carp were returned to their holding tanks overnight. Twelve to sixteen hours after injection, eggs and milt were stripped into a 5.7 L bowl using abdominal pressure and mixed for 10 minutes using a spatula. Fifty mL of water was added to the fertilized eggs to begin the water hardening process, and the eggs were mixed for an additional 10 minutes. Eggs were then placed on 30 cm long pieces of artificial vegetation (green yarn; 20% wool, 80% acrylic) at a mean density of 209 eggs per strand (SD = 28). Each piece of yarn was placed into a separate 10 L flow through tank and maintained at a constant temperature using a submersible aquarium heater (Hydor THEO; 50 W, 100 W, or 150 W depending on desired temperature). Temperatures were chosen to represent a range of temperatures that lakes experience during the carp spawning season (i.e. late spring and early summer). Eggs were examined at 12 hour intervals by removing the strands of yarn from the tanks and counting the number of clear and opaque eggs on each strand. The distinction between clear and opaque eggs was made because clear eggs appeared to be

viable and eventually hatch, while opaque eggs turned to mush and eventually disintegrated.

The rate of hatching was calculated using three steps. First, the disappearance of clear eggs from the pieces of yarn was used as an approximation of carp egg hatching. The disappearance of clear eggs was measured by plotting the number of clear and opaque eggs against time and looking for a decrease in the number of clear eggs without a corresponding increase in the number of opaque eggs. This would represent that clear eggs were actually disappearing from the yarn and not simply turning opaque. The time from fertilization to when the clear eggs began to disappear was calculated at each temperature, and the inverse of this time to disappearance was plotted against temperature to represent the hatching rate of carp eggs at different temperatures.

Second, a weighted general linear model (R 2.13.0) was fitted to the hatching rate data to estimate the change in hatching rate across various temperatures (Kocourek et al. 1994). A weighted general linear model was used because all clear eggs did not disappear within a single 12 hour time period between sampling events, and a weighted linear model allowed us to account for this difference in hatching rates. The proportion of clear eggs that disappeared within a given time period (and therefore at a given rate) was calculated by dividing the number of clear eggs that disappeared during that time period by the number of clear eggs present directly before the clear eggs began to disappear and plotted as an individual rate against temperature.

Third, the weighted general linear model was used to estimate the lower developmental threshold of carp egg development (i.e. the x intercept of the model) and the degree days necessary for an egg to fully develop and hatch (i.e. the inverse of the

slope) (Kocourek et al. 1994). These two values were used in Experiment 1b to estimate how long carp eggs would be present in wild carp spawning areas.

The survivorship of carp eggs to the assumed date of hatching was also analyzed. Survivorship was calculated by dividing the number of clear eggs at the sampling period directly before the clear eggs began to disappear by the total number of eggs at the beginning of the experiment. A linear regression (R 2.13.0) was used to test whether survivorship varied with temperature.

Experiment 1b: Are carp eggs consumed by fish predators in naturally occurring carp spawning areas?

Bajer and Sorensen (2010) suggested that predation by fish on carp eggs and larvae is restricting carp recruitment in lakes of the Upper Mississippi Basin. We carried out a survey of natural carp spawning areas in two model lakes to identify predators of carp eggs and measure carp egg survival through time. Both study lakes were surveyed for spawning activity (groups of carp splashing at the surface) between 0800 and 1000 hours every day between April 27th and June 26th, 2009. Each lake was first scanned visually by two observers to identify areas where carp were spawning in the lake (carp splashing at the surface). If spawning activity was observed in an area, both observers counted the number of spawning events (a group of carp splashing at the surface) during a five minute interval. The two counts were averaged to quantify the magnitude of spawning in the area. The edges of the spawning area were then mapped by boat using a handheld GPS unit (Garmin Legend H). Once a spawning site was mapped, the area was sampled daily to quantify predation on carp eggs by fishes within the spawning area

and the persistence of carp eggs in the area through time. Sampling began on the day of spawning (Day 0) and continued for five days after spawning (Day 5).

Due to differences in the depth of the two lakes studied, fish were collected for diet analysis in two ways. In the shallower Lake Casey, fish were collected by making a single pass through the spawning area with a beach seine (15 m wide, 10 mm mesh). In the deeper Lake Keller, fish were collected by making a single pass through the spawning area with a pulsed direct current electrofishing boat (50-150 V, 5-15 A, Midwest Lake Management Inc.). The first pass through the spawning area each day was used to capture fish for diet analysis to sample the fish community before it was disturbed by additional passes through the spawning area. All captured fish were placed in a live well immediately after capture and held until the transect of the spawning area was completed. Each individual was identified to the species level and its total length was recorded. Fish diets were sampled using the gastric lavage technique described by Hartleb and Moring (1995). Briefly, a thin pipe was inserted through the fish's esophagus and into its stomach. The diameter of the pipe varied by fish size and species, and ranged from 4mm to 20mm. Water was then pumped down the pipe into the stomach using a hand held pressure sprayer and allowed to flow out around the pipe and wash the contents of the stomach into a collecting sieve (600 μm). Fish stomach contents were identified as one of four categories (vegetation, invertebrates, fish, or carp eggs) and the percent volume of the stomach contents in each category was recorded for each individual. The total number of carp eggs found in the stomach was also recorded. All fish were immediately released back into the spawning area after sampling.

The abundance of carp eggs in each spawning area was quantified by collecting seven samples of vegetation from different locations within each spawning area each day through the fifth day after spawning. This was carried out on a second pass through the spawning area after fish in the area had been sampled and released. A garden rake was used to collect vegetation samples by lowering it approximately 0.5m into the water and spinning it two complete rotations. Samples were not taken from deeper vegetation because carp eggs are found predominately at the surface (Balon 1995). Submerged aquatic vegetation became tangled in the forks of the rake and could then be raised out of the water. The mass of each vegetation sample was recorded (Ohaus CS5000) and the total number of carp eggs in the sample was counted by separating the vegetation into individual stems and visually checking each stem for carp eggs. No distinction was made between clear or opaque eggs in the wild to ensure that all spawning areas could be sampled in a timely manner.

The number of carp eggs observed in wild carp spawning areas was compared to an expected number of eggs in order to see whether carp eggs disappeared before they were expected to hatch. We calculated the expected number of carp eggs using two steps. First, the date of hatching was estimated by calculating the number of degree days each egg accumulated over time. Twenty-four hour temperature loggers were deployed in Lakes Keller and Casey in early May to track lake temperatures through the spawning season, but we were unable to retrieve either temperature logger at the end of the spawning season. Instead, temperature data collected in two nearby lakes (Goose Lake, Carver County, and Lake Cynthia, Scott County, MN; Bajer, unpublished) was used to estimate the temperatures of our study lakes. Lakes Goose and Cynthia were selected

because they had similar water temperatures compared to our study lakes (t-test, $p = 0.785$, $df = 15$, Appendix 3). The temperature data from Lakes Goose and Cynthia was converted to degree days by multiplying the mean temperature of the two lakes by the number of hours the lake was at that temperature and dividing this by 24 hours. The degree days above the lower developmental threshold (12.7°C ; Experiment 1a) were then added together for consecutive days starting on the day of spawning. The date of hatching was estimated to be the day when the accumulated degree days above the lower developmental threshold exceeded the degree days necessary for hatching (28.0; Experiment 1a).

Next, the expected number of eggs was plotted as either present or absent. Eggs were expected to be present on all days before the estimated date of hatching and absent on all days after the date of hatching. The number of expected eggs was equal to the maximum number of eggs observed in each spawning area.

Experiment 1c: Did young-of-the-year carp survive to the juvenile stage in our study lakes?

To confirm the survival of young-of-the-year carp to the juvenile stage, we sampled the fish communities in our study lakes during August 2009 using trap nets, a standard fish sampling technique (Cross et al. 1995). A lack of juvenile carp would suggest that low recruitment is set before the juvenile stage, while high abundance of juvenile carp would suggest that egg predators have little effect on carp recruitment. Trap nets were constructed of 13 mm mesh wrapped around two rectangular steel frames (1.0 x 1.8 m) and five hoops (0.8 m diameter) with a 1.0 x 12.2 m lead (Cross et al.

1995). Throats with openings of 18 and 13 cm in diameter were attached to the first and third hoop, respectively. Five nets were set perpendicular to shore at regular intervals around each lake with the lead extending to the shore line. All nets were set between 1200 and 1600 hours and sampled between 0800 and 1200 hours the next morning. All captured fish were identified to the species level and their total lengths were recorded. Fish were released immediately after sampling.

Experiment 2: Do bluegill sunfish significantly affect the survival of carp eggs to hatching?

Field observations showed that the bluegill sunfish was the main predator of carp eggs in our study lakes and that eggs seemed to disappear from wild carp spawning areas before they were able to hatch when bluegill were present in a lake. To test the extent of the effect that bluegill predation has on carp survival, bluegill sunfish were collected from Lake Keller on June 27th, 2009, and brought to the lab. Bluegill were held in a 500 L flow through tank with a photoperiod of 16 hours light to eight hours dark and allowed to acclimate to a diet of flake food (Aquatic Eco-Systems, Apopka, FL). Within one week, all bluegill were feeding on flake food.

Six bluegill of a similar size (150 mm TL, SD = 20 mm) were selected from the holding tank and placed into individual 75 L aquaria. They were held for an additional week under the same conditions as the holding tank to allow them to acclimate to the new tank. Carp eggs were fertilized and allowed to attach to artificial vegetation as in Experiment 1a. Three hundred carp eggs (SD = 117) were introduced to each tank containing bluegill as well as six identical control tanks that lacked fish. Eggs on the

artificial vegetation were examined and counted at 12 hour intervals until all eggs had hatched. A generalized linear model (R 2.13.0) was used to analyze the interaction between time and fish presence on carp egg abundance within tanks.

Experiment 3: Do the most abundant native fishes in normoxic and hypoxic lakes (bluegill sunfish and green sunfish) consume carp larvae under controlled laboratory conditions?

Laboratory tests showed that bluegill sunfish may not be able to consume all carp eggs in an area before the eggs are able to hatch. However, even if some carp eggs are able to survive to hatching, larval carp may experience high mortality due to predation just as carp eggs do. We selected the bluegill sunfish and green sunfish for this experiment because they were the most abundant species present in carp spawning areas. Bluegill sunfish represented 46.4% of fish caught in carp spawning areas in our normoxic lake while green sunfish represented 100% of fish caught in our hypoxic lake.

Bluegill and green sunfish were collected from Lakes Keller and Casey using beach seines (50 m long, 15 m wide, 10 mm mesh) during May 2010. Collected fish were placed into a 500 L flow through tank with a photoperiod of 16 hours light to eight hours dark and allowed to acclimate to a diet of flake food. After 10 days, all fish had switched to a diet of flake food.

After all fish had acclimated to lab conditions, four 1,600 L tanks (2.5 m x 2.5 m x 0.25 m) were each stocked with five individuals of a single species (two tanks with bluegill sunfish, two tanks with green sunfish). To replicate wild carp spawning habitat, natural vegetation (curly leaf pondweed and coontail) was collected from Lake Keller and

placed in all tanks until it covered 25% of the surface. Fish were allowed to acclimate to the new tanks over the course of one week. An additional two tanks were treated the same way, except no fish were introduced (i.e. no fish control). This test group of six tanks was repeated twice for a total of four replicates for each treatment (bluegill sunfish, green sunfish, or no fish control).

Carp eggs were collected and fertilized as in Experiments 1 and 2, but fertilized eggs were treated differently to maximize hatching success. Instead of attaching fertilized eggs to artificial vegetation, eggs were immediately treated with a solution of 3 g urea and 4 g NaCl per L of water to remove the adhesiveness of the eggs (Schoonbee and Brandt 1982). Treated eggs were then placed in six L Zoug jars (Hiner 1961) that kept the eggs suspended in the water column through the use of a bubbler at the bottom of the jar (Billard 1999). After hatching, larvae were collected in 75 L tanks and fed a diet of brine shrimp until they were introduced to the test tanks and the experiment began.

One thousand larvae ($SD = 74$) were distributed evenly throughout each of the six test tanks to begin the experiment. This density was used to ensure that larval carp could be sampled during the experiment. Larvae were introduced at 2400 hours because sunfish are visual predators and we wanted to limit predation until the larvae were able to acclimate to the test tank (artificial sunrise was at 0600 hours). Larvae were sampled using a dip net (35 cm x 25 cm; 350 μ m mesh) at 16 evenly spaced locations throughout the tank. To sample, the dip net was placed parallel to the surface of the water and swept through the water column in a “U” shape that touched the bottom of the tank and ended back at the surface next to where the net started. Larvae caught in each net sweep were immediately counted and released back into the tank at the same place they were

captured. Initial larval sampling took place two hours after larvae were introduced to the tank, and regular sampling was conducted at 1000 and 2100 hours for two days after the introduction of larvae. A generalized linear model (R 2.13.0) was used to analyze the interaction between time and treatment type (bluegill sunfish, green sunfish, or no fish) on carp larval densities within tanks.

Results

Experiment 1a

Clear eggs disappeared at different times depending on the water temperature they were reared in (Figure 2). A pattern consisting of three phases emerged when the number of clear and opaque eggs was plotted against time. The first phase occurred directly after fertilization and was characterized by a decrease in the number of clear eggs and an increase in the number of opaque eggs, but no net loss in the number of eggs on each piece of yarn. The second phase was characterized by little change in the number of either clear or opaque eggs. The third phase was characterized by a decrease in the number of clear eggs but no change in the number of opaque eggs. This third phase was assumed to be when eggs were hatching. Larvae were observed at all temperatures during and after the third phase.

The rate at which clear eggs disappeared showed a positive linear relationship with temperature ($a = 0.0357$, $b = -0.4528$, $p < 0.001$, $df = 34$, $r^2 = 0.84$; Figure 3a). The lower developmental threshold for development was estimated to be 12.7°C ($SD = 2.5^{\circ}\text{C}$), and degree days was estimated to be 28.0 ($SD = 2.0$). We estimate that it would

have taken four days for carp eggs to hatch in Lake Keller, and three days for eggs to hatch in Lake Casey.

Carp egg survival to hatching showed no significant differences when all temperatures were included ($p = 0.89$, $df = 18$; Figure 3b). However, a slight positive trend was found for temperatures between 14.5 and 26.9°C ($p = 0.04$, $df = 16$).

Experiment 1b

Spawning activity varied widely within and between lakes (Appendix 4). All spawning areas were active for only one day. Twelve spawning areas were observed in Lake Keller during May and June of 2009. Seven of these areas were not analyzed because we were unable to find carp eggs within them. A single spawning area was observed in Lake Casey on May 21st. However, we were unable to sample the spawning area on the day of spawning and the day after spawning due to the presence of multiple spawning areas in Lake Keller at the same time.

Eight fish species were found within carp spawning areas in Lake Keller, while only one species of fish, the green sunfish, was found within the carp spawning area in Lake Casey (Figure 4, Table 1). Bluegill sunfish were the major predator of carp eggs, although two black bullhead and a single pumpkinseed sunfish were found with carp eggs in their stomachs (Figure 4b). Carp eggs were a major part of the diet of bluegill sunfish, and the number of carp eggs in fish stomach samples declined as the number of carp eggs in the environment declined (Appendix 5).

Egg abundance in the vegetation declined before the expected date of hatching in Lake Keller, suggesting carp eggs did not survive to hatching, while the abundance of carp eggs in Lake Casey did not decline before the expected date of hatching (Figure 5b).

Experiment 1c

Young-of-the-year carp were not found in Lake Keller, but a total of 23 young-of-the-year carp were caught in Lake Casey (Figure 6).

Experiment 2

Bluegill sunfish significantly decreased the survival of carp eggs before they began to hatch 79 hours after fertilization ($p = 0.008$, $df = 70$, Figure 7). Bluegill consumed 45% (SD = 34.7%) of carp eggs within the first 18 hours of the experiment, but their feeding rate decreased as eggs became scarcer. When bluegill sunfish were present, overall survival of carp eggs to hatching declined from 74 to 15% (SD = 18.2 and 6.8%, respectively). Eggs were observed falling off of the artificial vegetation in both treatments during counting, which might explain the decline in carp eggs in the absence of bluegill.

Experiment 3

The presence of bluegill sunfish or green sunfish significantly decreased the survival of carp larvae after 10 hours ($p < 0.001$, $df = 756$, Figure 8). By this time, bluegill sunfish had reduced carp larval density by 92.0% (SD = 10.1%) and green sunfish had reduced larval density by 89.7% (SD = 6.5%). No larvae were found in any

of the tanks where fish were present when they were drained through 350 μm mesh at the end of the experiment.

Discussion

Previous research has suggested that the sporadic recruitment of common carp in interconnected lakes of the Upper Mississippi Basin is due to abiotic disturbances that alter the fish community (Bajer and Sorensen 2010). Bajer et al. (Appendix 1) showed that the mortality of artificially fertilized carp eggs is higher in normoxic lakes unless eggs are protected from fish predators, and that carp recruitment is negatively correlated with the abundance of bluegill sunfish. The current study provides new evidence that supports the hypothesis that native predatory fish, and particularly bluegill sunfish, control the recruitment of invasive common carp in the Upper Mississippi Basin.

We found that the survival of naturally spawned carp eggs is extremely low in the presence of native predatory fish found in a normoxic lake. We also showed that carp survive to their juvenile stage in a hypoxic lake despite the ability of green sunfish, a fish commonly found in hypoxic lakes, to prey on carp larvae. Finally, we described how water temperature affects the hatching rate of carp eggs in greater detail than is currently available in the literature by calculating the lower developmental threshold of carp egg development and the degree days necessary for carp eggs to hatch. These results suggest that carp recruitment is controlled by predation on its early life history stages, and that egg predators play a larger role in controlling recruitment than larval predators in lakes of the Upper Mississippi Basin.

Our observations of wild carp spawning areas showed that fish did in fact consume naturally spawned carp eggs and that the vast majority of fish consuming carp eggs were bluegill sunfish. We also showed that carp eggs disappeared before their estimated date of hatching if bluegill were present in carp spawning areas. These results corroborate the findings of the study presented in Appendix 1 that carp egg survival is much lower when eggs are exposed to predatory fish. Although the study in Appendix 1 used artificially spawned eggs on pieces of yarn in their predation experiments, our analysis of naturally spawned carp eggs showed a similar decreasing trend in carp egg survival in the presence of predatory fish. Our laboratory experiments further demonstrated that individual bluegill sunfish are capable of significantly decreasing the survival of carp eggs to hatching. These results support the hypothesis that bluegill sunfish is a keystone species in the regulation of carp recruitment, and that the presence of bluegill in a lake may be necessary to keep carp from recruiting (Bajer et al. Appendix 1; Bajer and Sorensen 2010).

When considered in a broader perspective, it is not surprising that we found the bluegill sunfish to be a voracious predator of carp eggs. The ability of bluegill sunfish to prey on small prey items is well documented, and a great deal of literature has been published on their physiology and foraging behavior. In fact, much of the literature on the theory of optimal foraging behavior has used the bluegill sunfish as the model predator. Werner and Hall (1979) and Werner et al. (1983) showed the bluegill sunfish to be a habitat generalist that is able to switch between multiple habitat types as the profitability of foraging within these habitats changes over time. This ability to move in and out of different habitats as resources change would allow the bluegill to move

quickly into carp spawning areas to take advantage of the high abundance of carp eggs present in the area and then easily switch back to other habitats once the eggs had disappeared. O'Brien et al. (1976) and Werner and Hall (1974) showed that bluegill sunfish selectively prey on the more abundant or larger prey present in the environment. This would allow the bluegill to consume a greater amount of carp eggs in a given amount of time due to its ability to target carp eggs over other prey items that are not as abundant. Wainwright et al. (2007) showed that the bluegill sunfish has better accuracy when suction feeding than other centrarchid species due to the unique morphology of its mouth and jaw. This increased accuracy may give the bluegill sunfish an advantage in consuming small (2 mm) carp eggs that are stuck to pieces of vegetation. These characteristics suggest that the bluegill sunfish is well adapted to feeding in areas where carp spawn, and that its ability to consume carp eggs is greater than many other species present in lakes of the Upper Mississippi Basin.

Even though our field and laboratory results showed that the presence of bluegill sunfish drastically reduced the survival of carp eggs before they were able to hatch, it is possible that some carp eggs were able to escape predation and survive to the larval stage. However, our test of carp larval survival in the presence of the two most abundant species of fish in our normoxic and hypoxic lakes showed that both species were able to consume 90% of larval carp present within 4 hours of simulated daylight. We also showed that both species consumed all larval carp present within 34 hours of the larvae being introduced. It is interesting to note that young-of-the-year carp survived in our hypoxic study lake despite the ability of green sunfish to consume carp larvae at rates similar to bluegill sunfish. We hypothesize that the lack of predation by green sunfish on carp eggs

in wild carp spawning areas allowed enough carp eggs to hatch that larval predators were unable to consume enough larvae to cause recruitment to fail. This would suggest that egg predators determine the strength of carp recruitment in lakes of the Upper Mississippi Basin. In a related experiment, we tested the ability of black bullhead to prey on larval carp, but found no significant difference between the survival of larvae in the presence of black bullhead vs. control tanks (Appendix 6).

In our observation of fish diets in wild carp spawning areas, we found two additional species with carp eggs in their diets besides the bluegill sunfish. We sampled a single pumpkinseed sunfish and two black bullhead that had carp eggs in their stomachs. While these fish did consume carp eggs in the wild, they did not appear to represent a significant threat to egg survival due to the low abundances of both species in carp spawning areas. It is even possible that the two black bullhead found with carp eggs in their stomachs did not consume the eggs from the vegetation, but rather from the benthos where the eggs would have suffocated and died regardless of predation (Smallwood and Smallwood 1931).

While our observations of carp spawning areas were limited to two lakes, the fish communities in these lakes are representative of the two major types of communities found in lakes of the Upper Mississippi Basin. Tonn and Magnuson (1982) showed that lakes in Wisconsin had two distinct fish assemblages. The first was composed of centrarchids and pikes (*Esox sp.*) and was characterized by high winter oxygen and/or high lake connectedness. The second assemblage was composed of mudminnows (*Umbra sp.*) and cyprinids and characterized by low winter oxygen and low lake connectedness. Bajer et al. (Appendix 1) also showed a difference in the fish assemblage

of lakes that correlated with winter oxygen concentrations. Our study lakes followed this pattern. Lake Keller had high connectivity, high winter oxygen concentrations, and a fish community dominated by centrarchids. Lake Casey had low connectivity, low winter oxygen concentrations, and a fish community dominated by the hypoxic resistant green sunfish (Becker 1983).

This study also appears to be the first to show detailed information on how water temperature affects the hatching rate of carp eggs. Smallwood and Smallwood (1931) stated that eggs reared in the laboratory and exposed to direct sunlight would hatch between five and seven days, and that eggs that were not exposed to direct sunlight would take longer to hatch. Balon (1995) stated that eggs hatch within three days at temperatures between 20 and 23°C, and Billard (1999) he stated carp eggs would hatch in 60 to 70 degree days. However, none of these references gave a standardized procedure of how hatching time was measured or how changes in temperature affected hatching time. Our results show that hatching rate does increase at higher temperatures, and further suggests that the lower thermal limit of egg development is 12.7°C. We calculated degree days to hatching to be 28.0, which is much lower than the figure of 60 degree days stated by Billard (1999). This difference in estimating carp hatching rates could have a significant effect on the management of invasive carp populations as it would drastically alter the time that carp eggs are estimated to be available to predators, and would affect the density of predators necessary to control the recruitment of a given population of carp in a management situation.

The ability of the bluegill sunfish to consume both carp eggs and larvae suggests that it would be a good choice for fisheries managers to use in future carp control

programs. However, further research would benefit both the field of carp biology as well as the management of carp populations due to the lack of information on how efficient bluegill sunfish are at consuming both carp eggs and larvae under real life field conditions. It would be interesting to know whether abiotic factors such as turbidity, habitat complexity, and water temperature affect the ability of bluegill to prey on the early life history stages of carp, as well as whether bluegill will switch to other food sources present in the environment if carp eggs or larvae reach low densities.

It would also be interesting to test the abilities of a variety of other species of fish to prey on carp eggs and larvae in the lab. Our experiments were restricted by both time and space, and were thus limited to testing only a few species. We believe that other common North American fish species may also be excellent predators of the early life stages of carp. Becker (1983) states that minnows, catfish, and sunfish prey on carp eggs, but provides no detail on individual species known to eat their eggs. Pumpkinseed sunfish introduced to Spain have been shown to prey on fish eggs there (Garcia-Berthou and Moreno-Amich 2000), and Kim and DeVries (2001) have shown that white crappie are excellent predators of fish larvae. Testing the ability of these species and others would allow fisheries managers to manage for a more holistic fish community composed of multiple species that would work together to combat carp recruitment, rather than simply stocking a single species at an overwhelming density.

Finally, we suggest that the removal of adults as well as the restriction of movement of carp from one system to another should be implemented with the use of native predatory fish to restrict the recruitment of carp in lakes of the Upper Mississippi Basin. We believe that the use of multiple techniques to control populations of invasive

common carp will yield better results than simply focusing on one technique, and that an integrated pest management strategy that takes advantage of all the weaknesses inherent in the biology of carp is more likely to be successful in managing this invasive species.

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Table 1

Mean number of fish caught per transect, mean length of fish caught, mean number of fish with carp eggs in diet per transect, and mean number of carp eggs found in stomachs of fish consuming carp eggs. Green sunfish were only found in Lake Casey. All other species were caught in Lake Keller. Standard deviation is shown in parentheses. See Table A5-1 for diet of fish. (n_{Lake Keller} = 5; n_{Lake Casey} = 1)

Species	Fish / Transect	Length	Fish Consuming Eggs	Eggs / Fish
Black Bullhead	2.2 (2.7)	289.9 (19.9)	0.4 (0.9)	28.0 (na)
Black Crappie	0.6 (0.5)	94.0 (12.1)	0	
Bluegill Sunfish	23.2 (15.3)	134.4 (5.2)	9.2 (4.3)	25.9 (10.2)
Common Carp	16.0 (6.9)	633.6 (12.8)	0	
Largemouth Bass	6.4 (4.8)	227.1 (90.7)	0	
Pumpkinseed Sunfish	0.8 (0.4)	107.8 (14.2)	0.2 (0.4)	11.0 (na)
Walleye	0.2 (0.4)	472.0 (na)	0	
Yellow Perch	0.6 (0.9)	173.5 (34.6)	0	
Green Sunfish	15.0 (na)	94.1 (21.5)	0	



Figure 1

Map of the Phalen Chain of Lakes, St. Paul, Minnesota

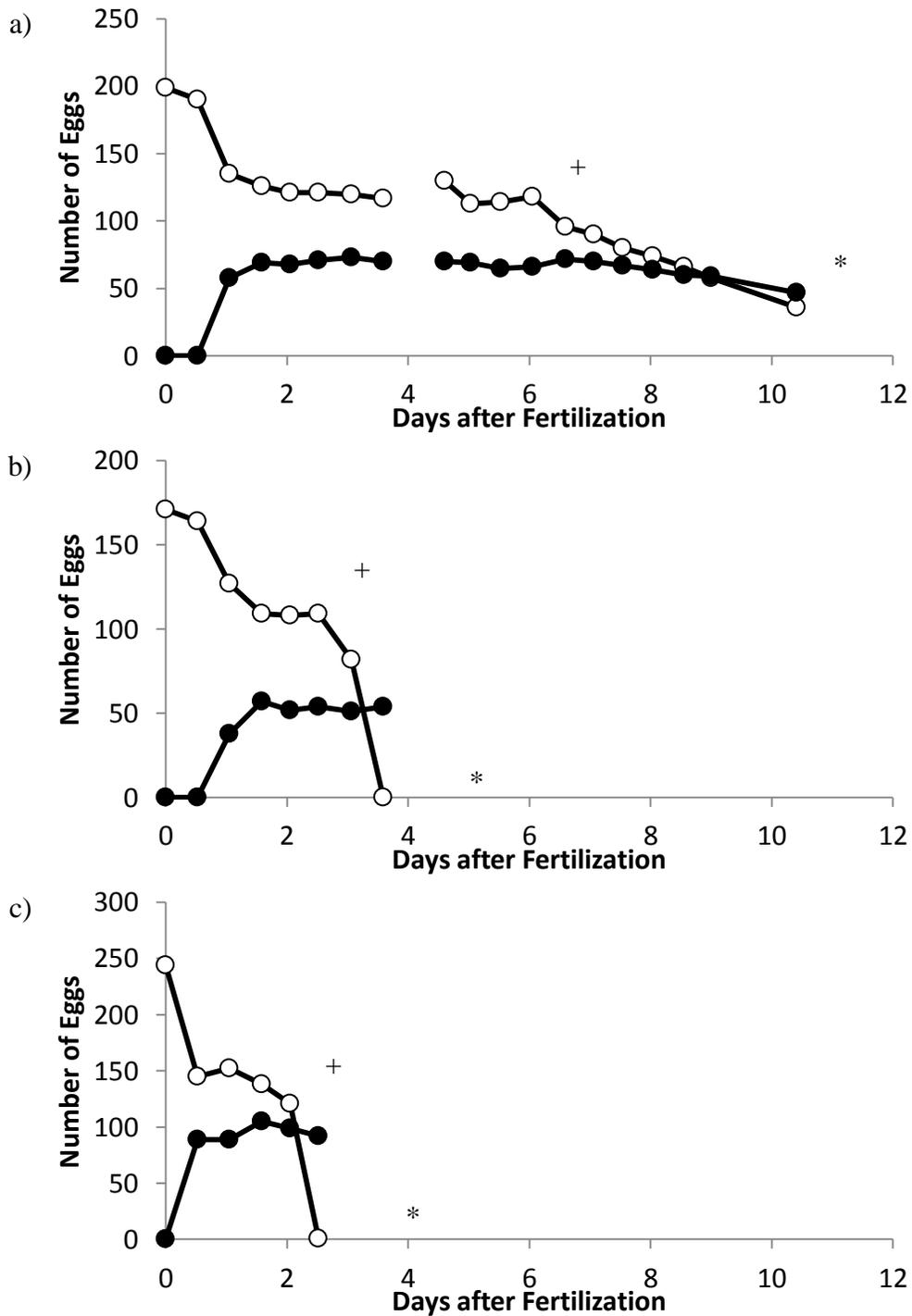


Figure 2

Number of clear (open circles) and opaque (closed circles) eggs observed at 14.5°C (a), 22.6°C (b), and 26.9°C (c). Beginning of egg hatching (+) and presence of larvae (*) is shown

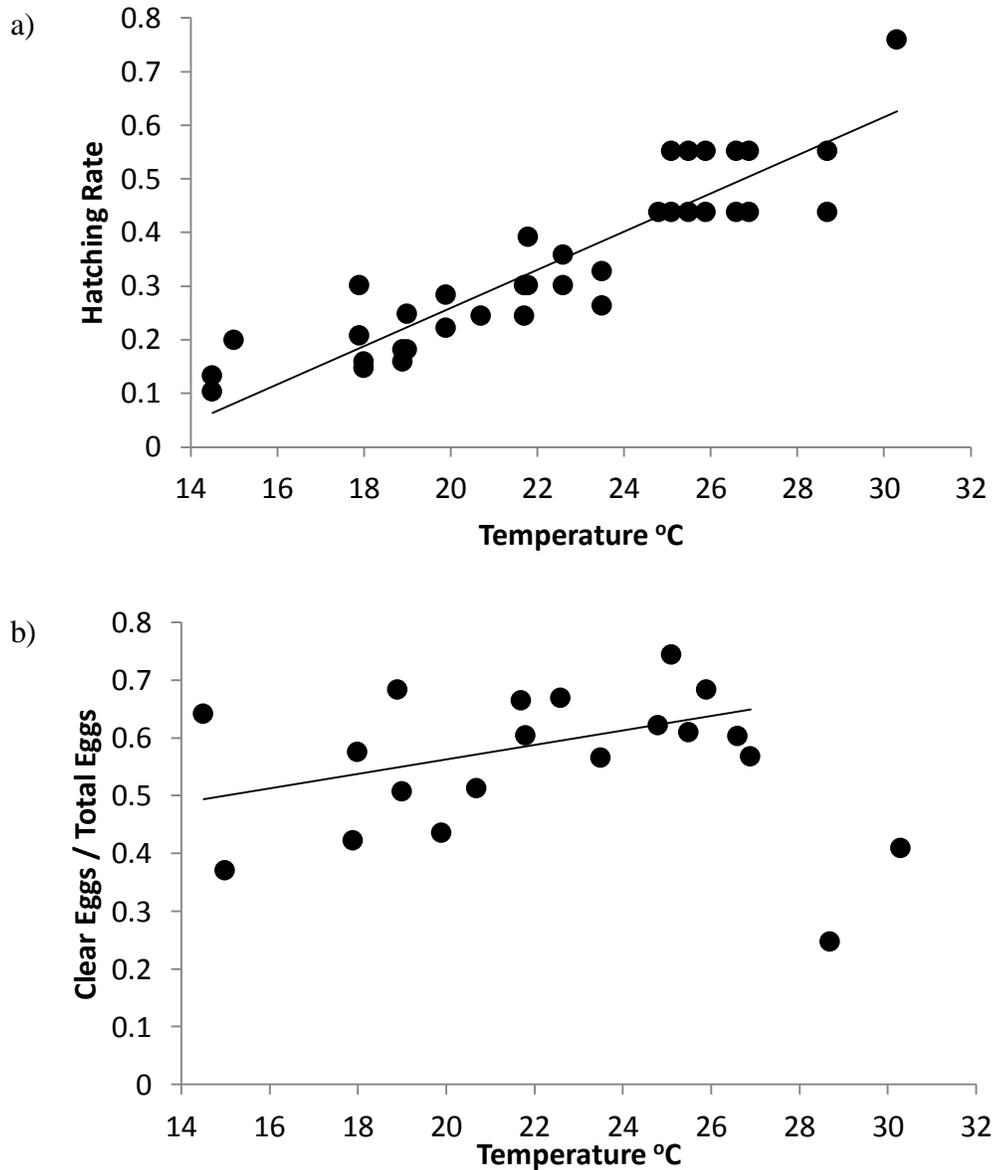


Figure 3

Hatching rate of carp eggs (a) and percentage of clear eggs before eggs disappeared (b) at various water temperatures. General linear model for hatching rate = $0.0357x - 0.4528$ ($r^2 = 0.84$, $p < 0.001$, $df = 34$). The lower developmental threshold for development was estimated to be 12.7°C ($SD = 2.5^\circ\text{C}$), and degree days was estimated to be 28.0 ($SD = 2.0$). A slight positive trend in survival to hatching was found between 14.5 and 26.9°C ($p = 0.04$, $df = 16$). ($n = 20$)

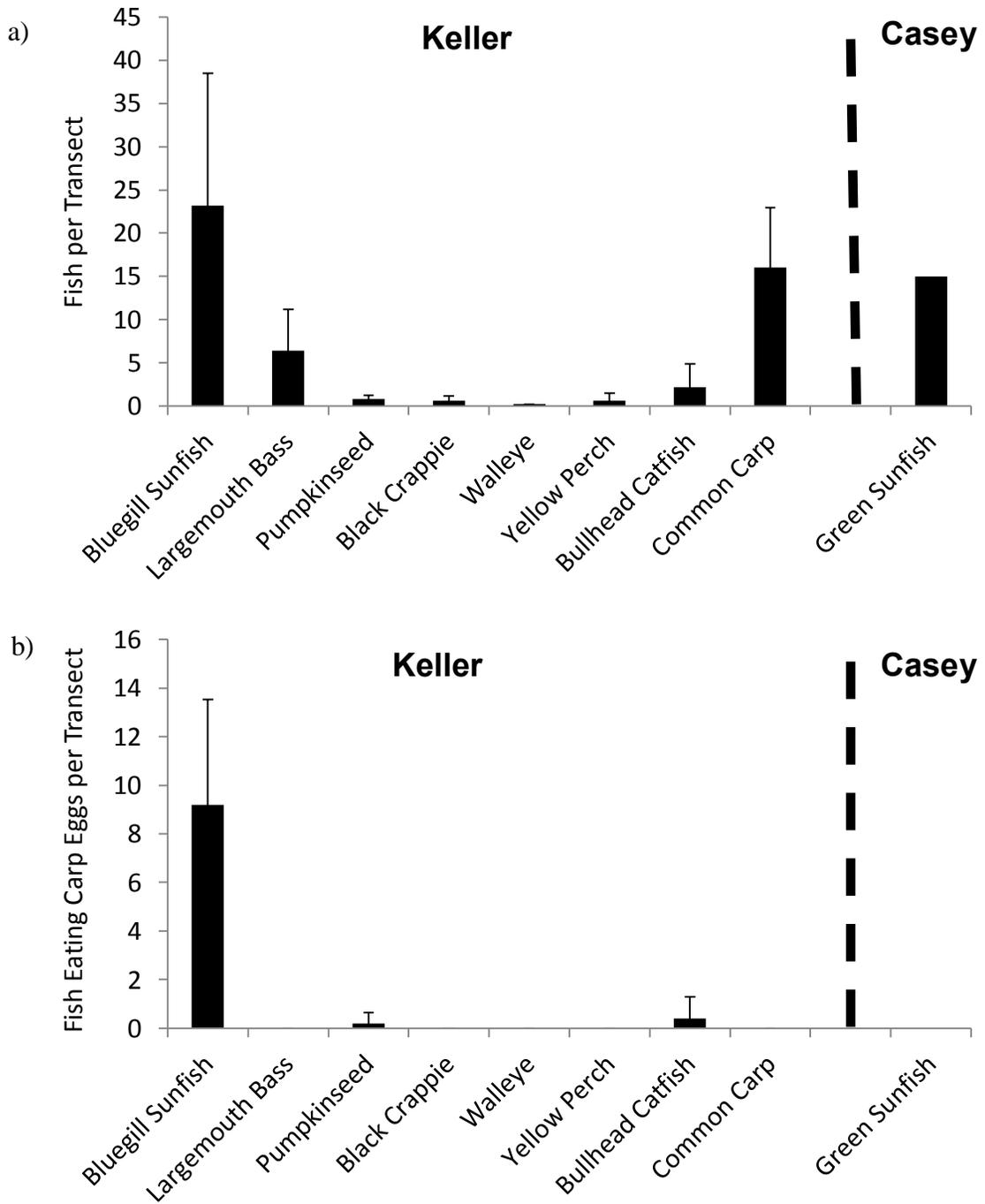


Figure 4

Mean number of fish caught in carp spawning areas (a) and mean number of fish with carp eggs in their stomach (b) in Lakes Keller and Casey. Bars represent standard deviation. ($n_{\text{Lake Keller}} = 5$; $n_{\text{Lake Casey}} = 1$)

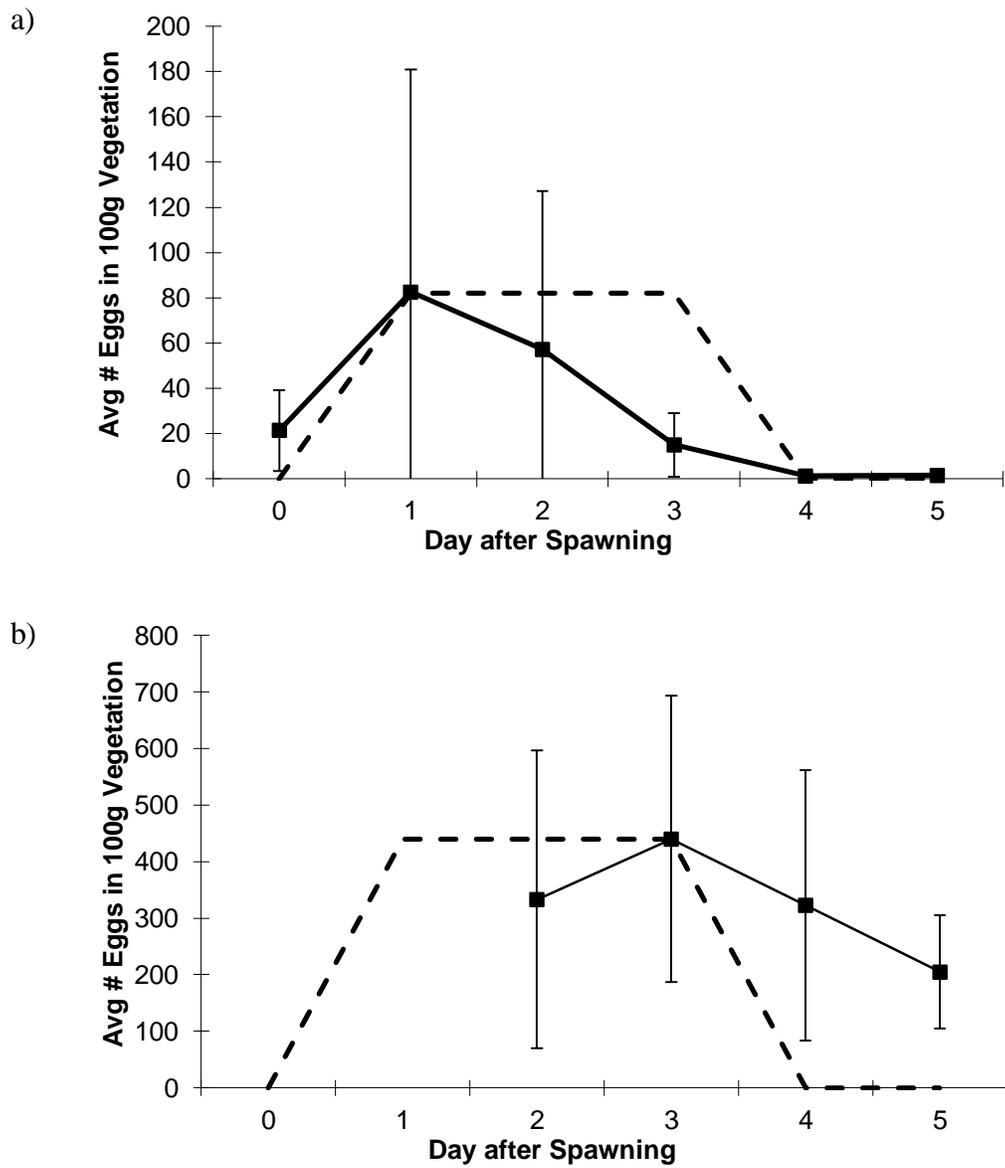


Figure 5

Mean abundance of carp eggs through time in wild carp spawning areas in Lake Keller

(a) and Lake Casey (b). Solid lines represent eggs observed. Dashed lines represent eggs

expected based on calculated hatching rate. Bars represent standard deviation. (n_{Lake}

Keller = 5; $n_{\text{Lake Casey}} = 1$)

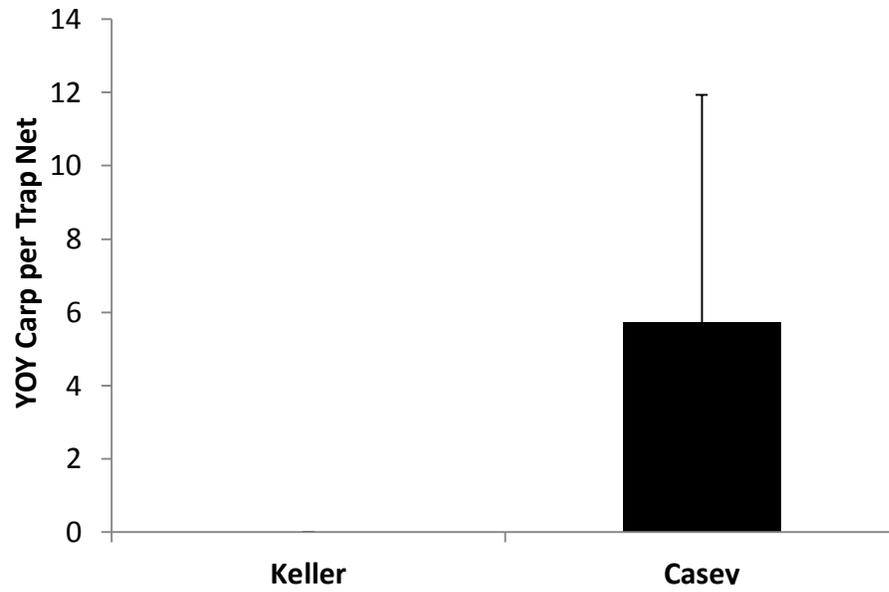


Figure 6

Mean number of young-of-the-year (YOY) carp caught per trap net in Lakes Keller and Casev in August 2009. Bars represent standard deviation. (n = 5)

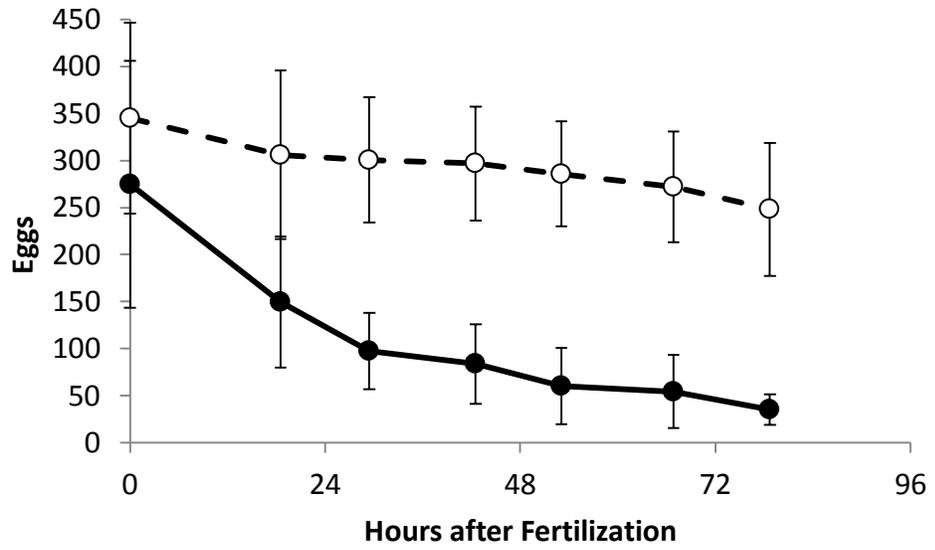


Figure 7

Mean number of carp eggs in the presence (solid line) and absence (dashed line) of bluegill sunfish. Bars represent standard deviation. (n = 6)

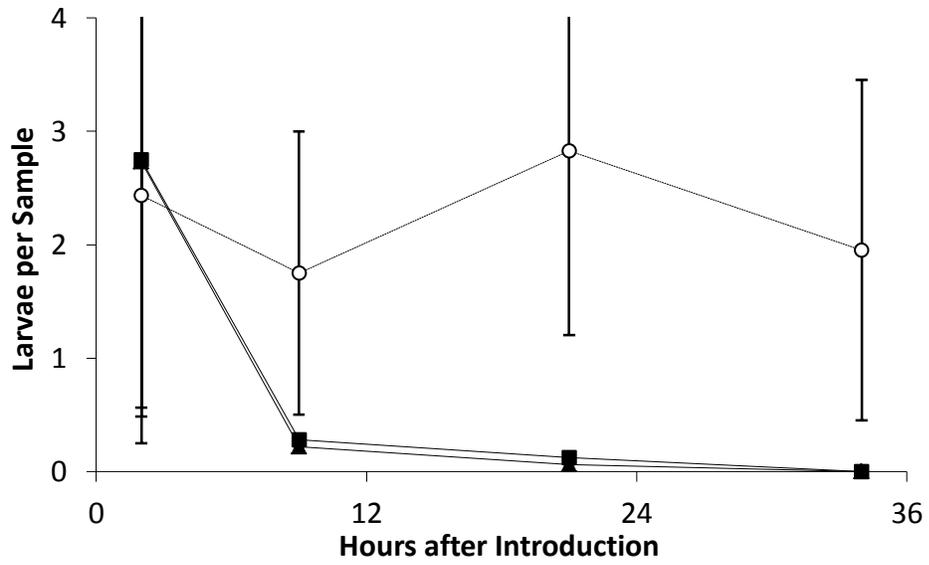


Figure 8

Mean number of carp larvae present sampled per dip net sweep in the presence and absence of fish predators. Open circles represent control (no fish), squares represent green sunfish, and triangles represent bluegill sunfish. Bars represent standard deviation.

(n = 4)

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Appendix 1

The following manuscript describes a recent study spearheaded by Dr. Przemyslaw Bajer exploring the correlation between the abundance of native predatory fish and common carp egg survival in lakes of the Upper Mississippi Basin. I assisted Dr. Bajer in carrying out the study and am a coauthor of the manuscript. We are currently preparing to submit this manuscript to Biological Invasions. We show that carp eggs experience higher mortality in lakes that have not experienced an ecological disturbance (i.e. winterkill) unless they are protected from fish predators using mesh netting. We also show that the abundance of bluegill sunfish has a strong negative correlation with carp recruitment.

Variation in micro-predator abundance controls the recruitment of an
invasive fish in a naturally unstable environment

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Abstract. Why certain species of fish become invasive is poorly understood and a key obstacle to restoring many of the world's ecosystems. In this study we tested whether spatial variation in biotic resistance exerted by native micro-predators might explain the reproductive success of the common carp, a large and fecund invasive species which typically spawns in outlying and unstable shallow habitat. An initial three-year study of the relative abundance of young-of-year (YOY) carp in 19 interconnected lakes in the Upper Mississippi River Basin discovered that YOY carp are only found in shallow waters that experience winter hypoxia (winterkill) and have low densities of native fish micro-predators which otherwise dominate these locales. A follow-up experiment tested if this distribution could be explained by native fish preying on carp eggs. It found that while carp eggs survived in winterkill lakes, they only survived in non-winterkill lakes if protected by mesh that excluded fish. Large numbers of carp eggs were also found in the stomachs of native fish inhabiting lakes that did not winterkill. We conclude that common carp, and likely many other invasive fish, have evolved life histories to avoid egg predators and can become invasive when they are absent.

INTRODUCTION

Dramatic increases in both the number and abundance of invasive fishes over the past few decades have caused precipitous declines in the biotic integrity of many aquatic ecosystems across the globe (Mack et al. 2000, Britton et al. 2010). While it is recognized that invasiveness in fish is often linked to complex combinations of local abiotic conditions and biotic resistance exerted by native competitors and predators, the precise nature of these relationships is poorly understood. Of special interest to aquatic ecologists

and managers is whether native predators might control the abundance of invasive fish in natural ecosystems (Moyle and Light 1996a, b, Marchetti et al. 2004, Moyle and Marchetti 2006). Although it has been demonstrated that native predators can control invasive aquatic invertebrates and amphibians, (Hill and Lodge 1999, DeRivera et al. 2005, Ward-Fear et al. 2010), no studies we know of provide direct (experimental) support for this possibility in fish. We speculate that one reason for this may simply be that the high mobility of many fishes coupled with the complexity of their life histories has made them difficult to study. This poor understanding has precluded development of meaningful biological control schemes.

The possibility that biotic resistance may often take the form of predatory pressure on the eggs and larvae of invasive fishes (and thus impose a recruitment bottleneck) is compelling. First, although most invasive fishes are extremely fecund (Kolar and Lodge 2002), their eggs and larvae tend to be small and defenseless, suggesting that they are susceptible to predation. Second, the vulnerability of early life stages is supported by the life histories of many invasive fishes which appear to employ predator-swamping strategies (Ims 1990). In particular, many highly fecund invasive fishes have adult phases that are mobile and spawn in a synchronous fashion in unstable and peripheral areas that presumably have fewer predators. The common carp (*Cyprinus carpio*), Asian carps (*Hypophthalmichthys* sp.), sea lamprey (*Petromyzon marinus*), and northern pike (*Esox lucius*) are all invasive fish species in which highly fecund adults migrate long distances to disperse their gametes in seasonally-unstable peripheral habitats (Potter 1980, Koed et al. 2006, Lohmeyer and Garvey 2009, Bajer and Sorensen 2010). Whether this suite of life history characteristics evolved to reduce exposure to egg and/or

larval predators does not appear to have been explicitly considered, nor does the possibility that differences in predator abundance might explain the success of invasive fishes in non-native habitats. Nevertheless, examples from the marine environment suggest that recruitment of fecund fishes (ex. cod [*Gadus morhua*]) can be controlled by egg and larval predators (Koster and Mollmann 2000) in at least some circumstances.

The common carp (hereafter ‘carp’) is one of world’s most invasive fish (Britton et al. 2010) and a good model to address the role of egg predation and its possible role in invasiveness. This species evolved in large and seasonally unstable rivers of Eastern Europe, is extremely fecund (up to 3 million eggs/ female), and performs annual spawning migrations into peripheral floodplains and marshes (Balon 1995, Barus 2001). Over the course of the past century it has been introduced worldwide and reaches extreme densities in temperate regions of North and South America and south-central Australia (Sorensen and Bajer 2011) where it is also extremely damaging to ecosystems (Weber and Brown 2009). We, and others, have noted that these regions are both large and characterized by seasonal environmental extremes that include winter and summer hypoxia and flooding (King et al. 2003, Stuart and Jones 2006, Bajer and Sorensen 2010). Population-ageing studies conducted in the Upper Mississippi River Basin demonstrate that invasiveness of carp in this region can be attributed to punctuated and sporadic recruitment events (Phelps et al. 2008) that precisely coincide with severe winter hypoxia in peripheral shallow areas (Bajer and Sorensen 2010). Although several factors could explain this relationship, we have hypothesized that temporary reductions in biotic resistance triggered by winter hypoxia (winterkill) are the cause (Bajer and Sorensen 2010). In support of this possibility, we have described regular spawning movements of

adult carp to and from seasonally-hypoxic areas (Bajer and Sorensen, 2010). Furthermore, and most importantly, these areas are normally dominated by numerous species of centrarchids (sunfishes), which are voracious micro-predators (Gross and MacMillan 1981), but which are also very sensitive to hypoxia and decline in abundance following winterkills (Rahel 1984).

In the present study we employed a combination of correlative observations and controlled experiments to test the hypothesis that recruitment and invasiveness of the common carp can be explained by localized reductions in biotic resistance associated with seasonal declines in native micro-predators in unstable spawning habitat (Bajer and Sorensen 2010, Sorensen and Bajer 2011). We found strong evidence that native egg predators and bluegill sunfish (*Lepomis macrochirus*) in particular, control carp recruitment by exerting strong biotic resistance that is reduced following instability events including winterkills. This discovery opens up the possibility of managing native fishes to control carp and suggests that other species of invasive fish should also be examined for this possibility.

METHODS

The study region and its fish

This study was conducted in lake systems of the Upper Mississippi River Basin. This region is characterized by a dense network of lakes, marshes, and interconnecting streams, which drain into larger rivers (Fig. 1). Adult carp inhabiting these systems typically overwinter in deeper lakes which have high oxygen concentrations but migrate in large numbers into shallow interconnected basins to spawn their sticky eggs on

floating vegetation in the spring (Bajer and Sorensen 2010; Fig. 1). Those carp that do not migrate spawn at the edges of deeper lakes and propagule pressure is extremely high across entire watersheds (up to 25 million carp eggs per ha) (Fig. 1). Severe winters characterize this region and winter hypoxia commonly occurs in outlying shallow basins where oxygen levels fall below 2 mg/L (Tonn et al. 1990). This level of oxygen is lethal to many native fish including the bluegill sunfish, which otherwise dominate these regions (Petrosky and Magnuson 1973, Rahel 1984). The bluegill is a relatively small (~15 cm TL) micro-predator that forages on invertebrates, zooplankton, fish eggs and larvae and typically comprises 50% - 70% of the native fish biomass (Gross and MacMillan 1981; Roth et al. 2007; Spotte 2007). Other abundant species include the black crappie (*Pomoxis nigromaculatus*), green sunfish (*Lepomis cyanellus*), pumpkinseed (*L. gibbosus*), yellow perch (*Perca flavescens*) and black bullhead (*Ictalurus melas*), all of which specialize in larger prey items and are relatively tolerant of hypoxia (Spotte 2007).

Experiment 1: Reproductive success of carp in stable vs. unstable habitats and how it correlates with the density of native predators

Winter dissolved-oxygen (DO) levels, the relative abundance of native fishes, and the relative abundance of YOY carp were measured in 19 lakes in five chains of interconnected lakes for three consecutive years (2008-2010). Chains of connected lakes were selected based on their accessibility, the presence of adult carp, and because they were deemed to be representative of the region. Each chain had 2-7 lakes, approximately half of which are shallower than 5 m and frequently experienced winter hypoxia while

the other half were deeper and did not (Fig. 1; S1). This study had three steps. First, we monitored oxygen levels in each lake each winter, then we confirmed that adult carp were present in each system each spring, and finally, we sampled each lake for YOY carp and native fish at summer's end. Dissolved oxygen (DO) was measured 1-2 times a month in all lakes between December and March. To accomplish this, holes were drilled through the ice at two locations of each lake and oxygen measured using an electronic meter (YSI 85, Yellow Springs, Ohio) from just below the surface to the bottom of the lake at 0.5m depth intervals. These values were then used to calculate winter DO minima following established protocols (Rahel 1984, Bajer and Sorensen 2010). The presence of adult (sexually-mature) carp was confirmed using either seining and/or boat electrofishing following established protocols each April-May (Bajer et al. 2010). Precise population estimates were available for several lakes because of ongoing mark-recapture studies being conducted for other reasons (Bajer and Sorensen 2010; Bajer et al. in press) (S1). Lastly, the fish communities of these lakes were sampled in late summer using standard fish survey traps (15 mm mesh trapnets with a 10 m lead; Rahel 1984) that we know to effectively catch young carp and other fish larger than 40 mm in total length, a size that carp typically surpass within two months in the region. In each case, five traps were set at evenly spaced intervals around the entire perimeter of each lake for one 24-h period.

To evaluate the relationship between winter DO minima and trapnet catch rates we used Poisson regressions analyses (procedure GENMOD in SAS 9.2; SAS Institute, Cary, North Carolina). This technique was used because trapnet catch rates are ordinal (zeros and discrete positive counts) and followed a Poisson distribution. Separate regressions were conducted for YOY carp and the four most common species of native

fish, as they comprised over 95% of all captured individuals. All native fish species less than a year old were excluded from this analysis because with the exception of northern pike, which we captured only occasionally, they hatch after carp and thus could not have been foraging on carp eggs or larvae.

Experiment 2: Testing predation by native fish on carp eggs in stable vs. unstable habitats

This experiment tested whether the presence or absence of YOY carp in lakes that winterkill or not can be explained by native fish predation on carp eggs. It was conducted in two hypoxic (winter DO < 1.5 mg/L) and two normoxic (winter DO > 1.5 mg/L) lakes that contained representative native fish communities (S2). These lakes were selected among the systems studied in Experiment 1. The experiment began in early May 2010 by monitoring daily carp spawning activity in each lake. When carp were observed spawning they were captured using an electrofishing boat and their eggs stripped into plastic containers and mixed with sperm (Billard 1999). Fertilized eggs were then placed onto 30-cm long pieces of artificial vegetation (green yarn) tied in loose clumps. Carp eggs were added to create densities similar to those on natural vegetation (one per ~ 1 cm) and clumps of yarn with 200 (± 20) eggs were then attached to an anchored line with a small float to mimic floating plants on which carp typically spawn. Eight clumps were placed (~ 10 m apart) into each of the two areas of each lake where spawning had recently been observed, while another set of 8 was individually inserted into either coarse- mesh bags to exclude fish (5 mm mesh, 4 per site) or fine- mesh bags to exclude macroinvertebrates (0.5 mm mesh, 4 per site) and also placed in the spawning sites. Each

clump was examined daily and the number of attached eggs counted before it was placed back into the water (eggs that fell off the yarn were also counted for the fine mesh controls). This procedure continued until larvae were seen in the fine-mesh controls. For data analysis, survival to hatch date was calculated as the proportion of eggs that were still found on the yarn on the last day before first larvae were observed. Egg survival was then analyzed using a nested ANOVA (SAS 9.2) to test for lake type (hypoxic versus normoxic), treatment type (open, coarse-mesh, fine-mesh), and interactions. In addition to monitoring egg mortality rates, native fish were collected from carp spawning sites in one of the normoxic lakes using an electrofishing boat and their stomach contents examined for carp eggs using gastric lavage. Finally, each lake was surveyed for YOY carp in late summer to confirm that the egg predation we observed could explain the presence or absence of YOY carp in these lakes.

RESULTS

Experiment 1

Five species comprised over 95% of the fish captured in our 19 study lakes: YOY carp, bluegill sunfish, black crappie, green sunfish, and black bullhead. Poisson regression analyses revealed that the catch rates of YOY carp were negatively influenced by winter DO ($P < 0.001$). With one exception, YOY carp were present only in lakes in which DO values fell below ~ 1.5 mg/L (Fig. 2). The opposite trend was determined for bluegill sunfish whose abundance was positively influenced by winter DO ($P = 0.01$); this species was only found in large numbers in lakes with winter DO > 1.5 mg/L (Fig. 2). We found no significant effect of winter DO on black crappies ($P = 0.25$), although these fish were also largely absent in lakes with DO < 1.5 mg/L (Fig. 2). Catch rates of green

sunfish were generally low but tended to increase in lakes with lower DO ($P < 0.01$) (Fig. 2). Catch rates of black bullheads were highly variable and not influenced by winter DO ($P = 0.96$) (Fig. 2). Overall, the median catch rate of native fish in lakes with winter DO > 1.5 mg/L was 2.5 times higher than that in lakes with lower DO levels and 43% of the catch was bluegill sunfish.

Experiment 2

While 47% of unprotected eggs survived to hatching in hypoxic lakes, only 0.8% of unprotected eggs survived in normoxic lakes and over 90% of these disappeared within the first 24-h (Fig. 3, $P < 0.05$). In contrast, nearly 70% of the eggs placed into either the fine-mesh or coarse-mesh bags survived to hatching (4 days) in both the hypoxic and normoxic lakes. These values did not differ from each other but were higher than the survival rates of unprotected eggs in both hypoxic and normoxic lakes (Fig. 3). These survival rates were explained by an interaction among lake and treatment types (both main effects and the interaction were significant; $P < 0.05$). Although we did not quantify larval survival, larvae were observed in the fine-mesh bags after 4 days and we estimated that no more than 5% of eggs fell off the yarn. Electrofishing surveys of carp spawning habitats in the normoxic lake resulted in the capture of 74 native fish including 45 bluegill sunfish. Thirty five of the bluegills were large enough for their diets to be examined and 29 of these had carp eggs in their stomachs (on average 71 eggs per bluegill). We also captured 8 yellow perch of which 6 consumed carp eggs (an average of 118 eggs per fish). Late summer trapnet surveys caught an average of 3.0 (SE = 1.58) and 4.75 (SE = 2.86) YOY carp per trapnet in both of the hypoxic lakes, but no YOY carp were sampled in the two normoxic lakes.

DISCUSSION

Although it has been previously demonstrated that native predators can control invasive invertebrates and amphibians (Hill and Lodge 1999, deRivera et al. 2005, Ward-Fear et al. 2010), this study provides the first direct evidence for a fish. Using both correlative analyses and controlled experiments in a complex and large but relatively easily studied system, we demonstrate that although carp exert high propagule pressure across wide range of habitats, native fish can control their recruitment by foraging on their eggs. However, when the densities of these native fishes are suppressed because of severe instability events (in this case winter hypoxia), the carp were able to overcome this recruitment bottleneck. This scenario appears to explain the invasiveness of carp in large portions of the Upper Mississippi River Basin and possibly other regions, such as the Murray Darling Basin that are also characterized by expansive and interconnected watersheds that experience seasonal extremes such as floods, droughts and summer hypoxia (King et al. 2003). We speculate that carp evolved to grow large and migrate into outlying shallow floodplains of the Ponto-Caspian region to escape predation by cyprinids native to this region whose proclivity for carp eggs has already been noted (Koblitskaya 1977, Barus et al. 2001).

As demonstrated by this study, explaining invasiveness of fish in specific locales may not be possible without considering specific life history requirements and behaviors exhibited by invaders in local habitats (Korsu et al. 2007). Key to the success of the carp is its propensity to move into shallow unstable areas to spawn (Bajer and Sorensen 2010, King et al. 2003). But this strategy may only be effective in ecosystems where unstable

nurseries that lack predators are at least periodically connected with stable overwintering / foraging habitats. Adaptations of native predators to instability events in shallow waters may also help explain the success of carp in different areas of the world. In our study region, the success of carp appears to be explained by high sensitivity of the dominant egg predators to hypoxia. On the other hand, the fact that carp are typically not invasive in large tropical rivers, in which summer hypoxia commonly occurs, may be explained by unique adaptations of native species in these systems to survive hypoxia (Welcomme 1995, Chippari-Gomes et al. 2005). Notably, although hypoxia has previously been shown to play a major role in structuring fish assemblages in North America (Tonn and Magnuson 1982, Rahel 1984, Tonn et al. 1990), it seemingly has not been considered as an explanation for the success of invasive species..

Many of the world's most invasive fish are mobile and complete their life cycles over large areas using specialized nursery habitats. Of special interest are the silver and bighead carp, which invaded the Mississippi River Basin and now threaten the Laurentian Great Lakes. These species employ long upstream migrations to spawn in open river habitat during spring floods, and their larvae drift into backwater flooded habitats located up to 200 km downstream (Lohmeyer and Garvey 2009). Flooded backwaters would presumably be expected to have fewer predators. Another example is the northern pike, which is highly invasive in western North America and has a life history similar to that of the common carp as it also migrates to shallow peripheral regions to spawn after winterkill (Koed et al. 2006). Similarly, invasive salmonids and landlocked sea lamprey migrate into shallow streams to spawn (Potter 1980, Henderson et al. 2000). Whether these behaviors function to protect their young from predators has not been explicitly

studied, although it has been suggested for the lamprey (Potter 1980). Our study shows that this possibility needs to be examined to explain invasiveness of these fish in different regions of the world.

Although we have not examined the effects of all native predators on all developmental stages of carp, our data strongly suggest that predation by native fish, and bluegill sunfish in particular, on carp eggs largely explains the invasiveness of carp in the Upper Mississippi River Basin. Bluegills are typically very abundant in this region, specialize in foraging on small prey in vegetated habitats (Keenlyside 1972, Werner and Hall 1974), and have been previously shown to be voracious egg predators (Gross and MacNeil 1981). We do not propose that bluegills necessarily consume all carp eggs, but rather that the extremely high predatory pressure they exert on eggs allows the other sympatric predators to control the few remaining larvae and fry. While sympatric predators may have important additive effects, the strong reciprocal relationship between bluegill sunfish and YOY carp in our study lakes suggest that the bluegill sunfish is the keystone element of biotic resistance in lakes of the Upper Mississippi region. Interestingly, bluegill sunfish have also been shown to play a role in controlling the early life stages of the invasive rusty crayfish (Roth et al. 2007). Alternative explanations for the lack of carp recruitment in normoxic systems (food, habitat, competitors, etc.) are unlikely as productivity and habitat changed little from year to year in our systems, but YOY carp were only present following hypoxic events. Lakes that had YOY carp typically also had high densities of larvae and juveniles of native species (data not shown) suggesting that competition for planktonic food plays a relatively minor role in the survival of YOY carp.

Finally, this study suggests new and more sustainable paths to control the common carp, which to date, have been controlled using nonspecific toxins, barriers and water draw-downs (Marking 1992, Wiley 2008). While poisons, barriers and drawdowns may on occasion provide short-term improvements in carp numbers and water quality, they also increase ecosystem fragmentation and may reduce biotic resistance and enhance the need for continuing intensive management. This study suggests that the common carp, and possibly other invaders that employ similar life histories, could instead be controlled using more sustainable approaches that strengthen native fish communities.

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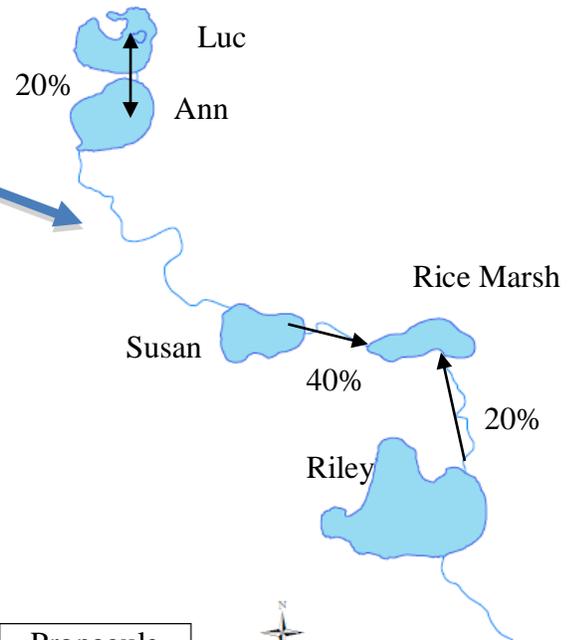
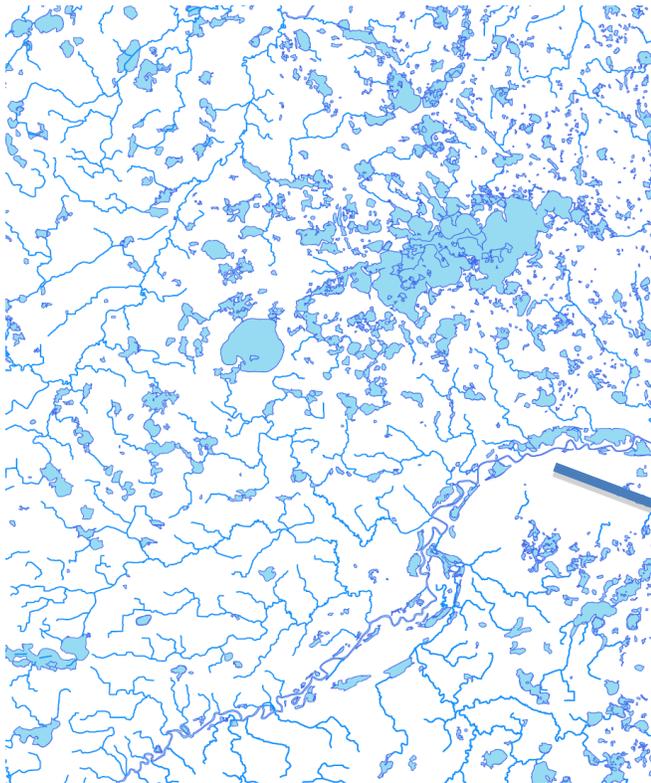
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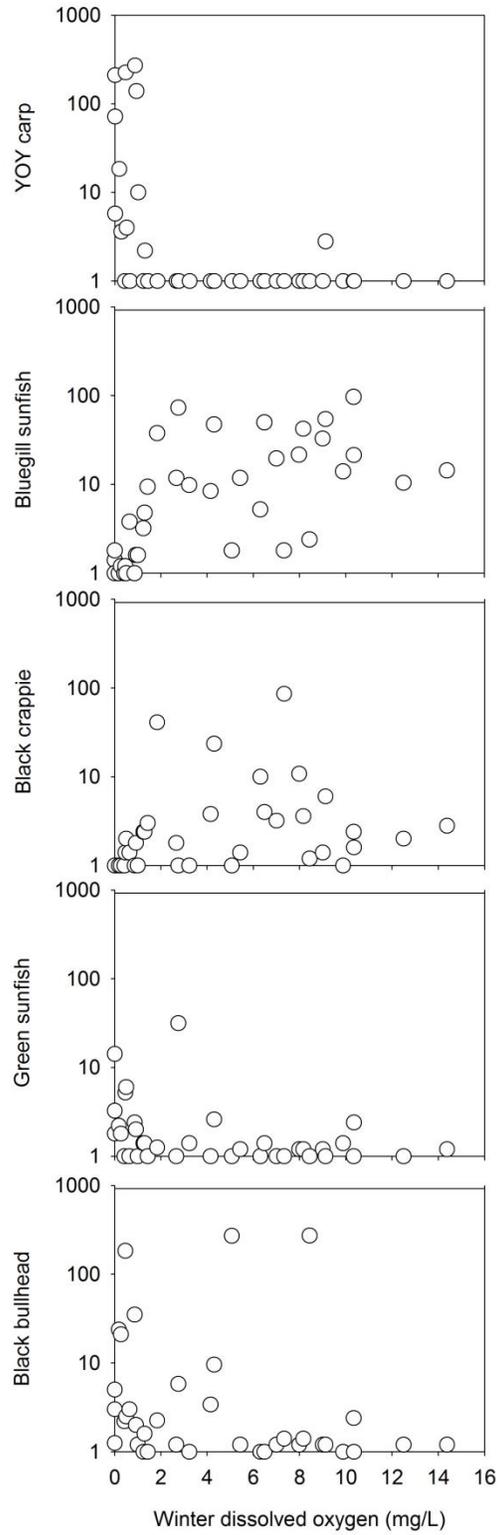
Figure 1. Hydrography of the study region. The insert to the right and table below illustrate the abundance, movement and propagule pressure (millions [mln] of carp eggs per hectare) of adult common carp in the Riley Creek chain of lakes, one of the five chains that we studied (for details see Bajer and Sorensen 2010). Each year, between 20% and 40% of adult carp move from lakes Susan and Riley, in which they overwinter, to spawn in the shallow, seasonally-hypoxic Rice Marsh Lake (arrows). The carp that do not move, spawn along the edges of lakes Susan and Riley. The two upper lakes are isolated and inhabited by a smaller number of carp some of which also move between the two basins during spawning season.

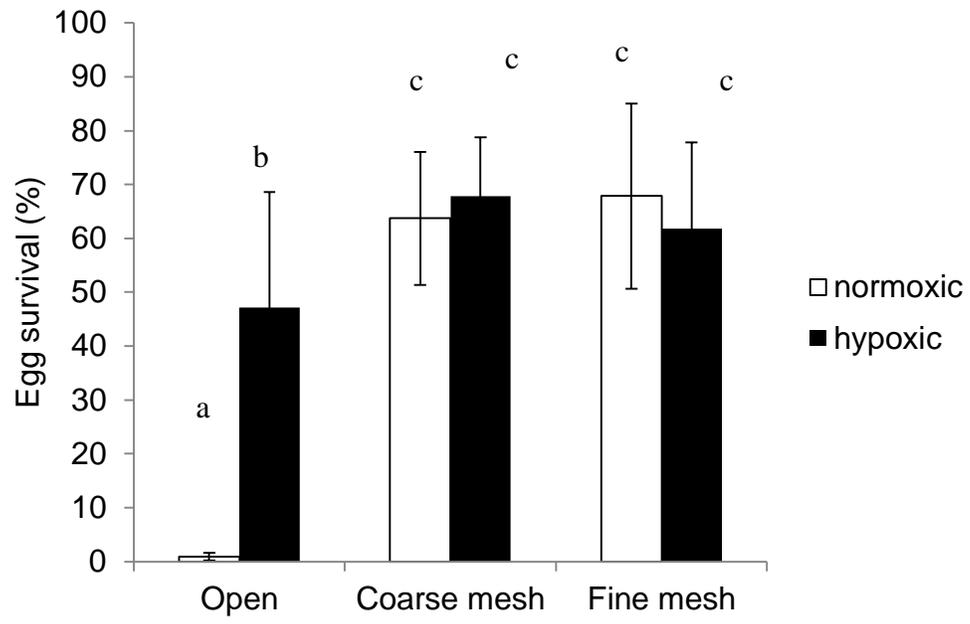
Figure 2. Catch rates of young-of-the-year (YOY) carp and native fish species versus winter dissolved oxygen minima in the study lakes.

Figure 3. Survival rates of carp eggs to hatching in normoxic and hypoxic lakes. Eggs were subjected to one of three treatments: 1) unprotected eggs were placed in open water within carp spawning areas, 2) eggs were placed into coarse-mesh bags (fish excluded but invertebrate predators allowed), 3) eggs were placed in fine-mesh bags (both fish and invertebrates excluded). Vertical bars represent standard deviation. Letters indicate statistical differences (nested ANOVA; $P = 0.05$).



Lake	Max. depth (m)	Adult carp population estimate	Propagule pressure (mln eggs/ha)
Lucy	6	685	5.8
Ann	15	200	2.2
Susan	5	4,054	25.8
Rice Marsh	3	1,000- 3,000	15.0
Riley	15	6,484	15.9





The size and depth of our study lakes. Lakes are listed from upstream to downstream in each chain and the plus sign indicates which lakes were sampled each year for YOY carp and native fish. The presence of adult carp was verified in each lake using seining and mark-recapture (MR) analyses and/ or boat electrofishing (EF).

Chain	Lake	Size (ha)	Max depth (m)	Adult carp	2008	2009	2010
Riley Creek	Lucy	35.0	6.1	MR		+	+
	Ann	44.4	13.7	EF		+	+
	Susan	37.5	5.1	MR	+	+	+
	Rice Marsh	40.0	3.0	MR	+	+	+
	Riley	120.0	14.9	MR	+	+	+
Carver Creek	Goose	159.2	3.05	EF	+	+	+
	Hydes	89.7	5.5	EF	+	+	+
	Reitz	31.9	11.0	EF	+	+	+
	Rice	50.0	2.5	EF	+	+	+
Sandy Creek	St. Catherine	38.0	3.0	EF	+	+	+
	Cynthia	80.0	3.1	EF	+	+	+
Purgatory Creek	Wetland	70.0	1.5	EF			+
	Staring	66.2	4.9	MR			+
Phalen Creek	Casey	4.7	1.1	MR			+
	Markham	7.0	2.0	MR			+
	Kohlman	29.9	2.7	MR			+
	Gervais	94.5	12.5	MR			+
	Keller	29.0	2.4	MR			+
	Phalen	79.6	27.8	EF			+

S2

The abundance (g/m^2) of native fish in the two hypoxic and two normoxic lakes used in Experiment 2. Fish abundance was determined by collecting five $\sim 100 \text{ m}^2$ littoral seine hauls in each lake. Lake characteristics are presented in S1.

	Hypoxic		Normoxic	
	Casey	Markham	Riley	Keller
Bluegill sunfish	--	--	9.81	23.66
Black crappie	--	--	--	0.18
Yellow perch	--	--	0.20	0.68
Largemouth bass	--	--	0.64	0.36
Northern pike	--	--	0.68	--
Pumpkinseed	0.06	--	0.05	0.18
Green sunfish	0.42	--	--	0.05
Black bullhead	3.18	--	--	--
Other native	--	0.09	--	--

Appendix 2

Carp Egg Hatching Data

Table A2-1

Number of clear eggs observed in Experiment 1a at 12 hour intervals at various temperatures (°C).

Temp	0h	12h	25h	38h	49h	60h	73h	86h	96h	110h	120h	132h	145h	158h	169h	181h	193h	205h	216h	250h
14.5	199	190	135	126	121	121	120	117		130	113	114	118	96	90	80	74	66	58	36
15.0	205		148	139		124	104	78	66		54		33							
17.9	229		124	121		86	78	47	31		25		7							
18.0	184	180	124	100	104	117	122	118		109	103	98	96	35	1					
18.9	253	251	191	172	170	166	157	157		160	153	122	47	9						
19.0	175		118	113		93	78	65	55		43		4							
19.9	220		135	130		78	74	63	33		0									
20.7	245	216	151	108	123	122	117	106		0										
21.7	225	205	169	128	127	134	127	45		1										
21.8	179		120	108	99	68	41	4												
22.5	171	164	127	109	108	109	82	0												
23.5	179		92	91	85	82	65	26	9											
24.8	246	238	169	145	141	0														
25.1	170		143	128	89	1														
25.4	197	199	144	117	87	0														
25.9	206		154	127	49	1														
26.5	215		148	120	54	0														
26.9	244	145	152	138	121	1														
28.6	241	240	105	57	14	2														
30.3	197		76	7																

Table A2-2

Number of opaque eggs observed in Experiment 1a at 12 hour intervals at various temperatures ($^{\circ}\text{C}$).

Temp	0h	12h	25h	38h	49h	60h	73h	86h	96h	110h	120h	132h	145h	158h	169h	181h	193h	205h	216h	250h
14.5	0	0	58	69	68	71	73	70		70	69	65	66	72	70	67	64	60	59	47
15.0	0		54	60		61	80	101	112		111		101							
17.9	0		88	87		100	107	120	129		118		93							
18.0	0	0	57	72	70	65	65	71		71	72	71	71	69	66					
18.9	0	0	59	74	71	79	77	69		68	71	70	68	68						
19.0	0		50	51		65	76	81	82		72		69							
19.9	0		83	81		99	96	78	71		49									
20.7	0	0	73	94	104	97	99	101		77										
21.7	0	0	51	83	80	74	64	59		61										
21.8	0		53	57	65	75	68	56												
22.5	0	0	38	57	52	54	51	54												
23.5	0		76	74	77	63	68	70	61											
24.8	0	0	61	80	86	78														
25.1	0		34	44	39	27														
25.4	0	0	54	75	77	68														
25.9	0		47	59	54	48														
26.5	0		62	79	60	52														
26.9	0	89	89	105	99	92														
28.6	0	2	126	174	164	153														
30.3	0		110	71																

Appendix 3

Twenty-four Hour Lake Temperature Data

Methods

Twenty-four hour water temperature was used to estimate hatching times in the wild using the lower developmental temperature and degree days estimated from Experiment 1a (Kocourek et al. 1994). Unfortunately, the 24 hour temperature loggers in our study lakes were not recovered at the end of the year and only one temperature was collected during daily transect surveys. To estimate the daily water temperatures of our study lakes, we used 24 hour temperature data collected in two nearby lakes (Goose Lake, Carver County, and Lake Cynthia, Scott County, MN; Bajer, unpublished) to estimate the maximum and minimum temperatures of our study lakes. We selected Lakes Goose and Cynthia because they have similar depths to our study lakes and showed a similar temperature pattern (Table A3-1). We tested lake temperature similarity between the lakes by finding the mean water temperature between Lakes Goose and Cynthia at 1200 hours each day and performing a t-test on this value versus the water temperature measured in Lake Keller during transect surveys. We chose to use temperatures at noon because transect surveys were carried out between 1000 and 1500 hours.

Results

No significant difference was found between the mean temperature of Lakes Goose and Cynthia and Lake Keller ($p = 0.785$, $df = 15$).

Table A3-1

Daily water temperatures (°C) measured at 1200 hours in Lakes Cynthia, Goose, and Keller.

Date	Cynthia	Goose	Keller	Date	Cynthia	Goose	Keller
5/20/2009	19.1	19.8		6/10/2009	15.6	16.9	
5/21/2009	19.2	19.1	20.7	6/11/2009	18.8	19.5	
5/22/2009	19.7	19.8	19.1	6/12/2009	19.5	20.6	20.3
5/23/2009	19.7	20.5	19.3	6/13/2009	20.2	21.7	
5/24/2009	20.9	21.9	25.8	6/14/2009	22.4	24.1	
5/25/2009	20.8	19.8	20.3	6/15/2009	21.5	24.4	19.4
5/26/2009	19.5	19.1	19.7	6/16/2009	21.6	22.0	22.3
5/27/2009	17.4	17.3	18.6	6/17/2009	21.5	21.3	
5/28/2009	19.8	20.1	18.1	6/18/2009	22.5	23.2	
5/29/2009	21.4	22.0	19.8	6/19/2009	24.4	24.8	
5/30/2009	20.1	20.1		6/20/2009	25.5	26.4	26.0
5/31/2009	19.6	20.7	19.4	6/21/2009	25.0	24.8	
6/1/2009	20.9	21.6	19.3	6/22/2009	25.6	28.2	
6/2/2009	21.6	22.0		6/23/2009	28.0	30.0	
6/3/2009	22.1	23.1		6/24/2009	26.2	26.6	27.1
6/4/2009	21.3	22.4		6/25/2009	28.5	30.2	
6/5/2009	22.0	22.6		6/26/2009	28.3	28.8	
6/6/2009	18.6	17.3		6/27/2009	26.8	27.4	
6/7/2009	16.6	16.0		6/28/2009	24.8	24.9	
6/8/2009	15.6	14.8		6/29/2009	22.3	22.8	
6/9/2009	16.3	17.5		6/30/2009	20.7	20.7	

Appendix 4

Spawning Surveys of Lakes Casey and Keller

Table A4-1

Spawning events in Lakes Casey and Keller during summer 2009. Table shows spawning activity, size, whether eggs were found during sampling, and the estimated time to hatch for all carp spawning events observed in Lakes Casey and Keller in 2009.

Date	Lake	Events / 5 min	Area (m ²)	Eggs Present	Estimated Days to Hatch
5/21/2009	Casey		1888	Y	4
5/21/2009	Keller			N	4
5/23/2009	Keller		14616	N	4
5/24/2009	Keller		10528	N	4
5/25/2009	Keller		5324	N	5
5/26/2009	Keller	10	4860	Y	5
5/29/2009	Keller	13	3052	Y	4
5/29/2009	Keller	7	4855	Y	4
6/4/2009	Keller	352	9120	Y	5
6/5/2009	Keller	99	5376	Y	6
6/15/2009	Keller	89	1728	N	3
6/16/2009	Keller	3	1425	N	3
6/24/2009	Keller	5	1309	N	2

Appendix 5

Diet of Fishes Found in Carp Spawning Areas

Methods

A survey of fish diets in wild carp spawning areas was carried out in two model lakes to identify potential predators of carp eggs (Experiment 1b, Chapter 2). Both study lakes were surveyed for spawning activity during spring and summer, 2009, and each spawning area was sampled daily to quantify predation on carp eggs by fishes within the spawning area. Fish were caught using a beach seine or electro-fishing boat. Fish diets were sampled using the gastric lavage technique, and the percent volume of each individual's stomach contents was recorded as one of four categories (vegetation, invertebrates, fish, or carp eggs). All fish were immediately released back into the spawning area after sampling.

Results

Fish captured in wild carp spawning areas had a diverse diet (Table A5-1). The abundance of carp eggs in fish diets declined as carp eggs in the environment declined (Figure A5-1).

Table A5-1

Mean number of fish per transect, mean number of fish with empty stomachs, and mean composition of fish diets by percent volume in four categories. Standard deviation shown in parenthesis. Green sunfish were only found in Lake Casey. All other species were found in Lake Keller. See Table 1 for number of fish eating carp eggs and number of carp eggs consumed per fish. (n_{Lake Keller} = 5; n_{Lake Casey} = 1)

Species	Fish / Transect	Empty	Veget ation	Invert ebrate	Fish	Carp Eggs
Black Bullhead	2.2 (2.7)	0.4 (0.5)	20.0 (20.0)	6.9 (10.5)	66.7 (30.6)	6.1 (10.6)
Black Crappie	0.6 (0.5)	0 (0)	100.0 (na)	0 (na)	0 (na)	0 (na)
Bluegill Sunfish	23.2 (15.3)	1.8 (1.8)	19.3 (6.1)	48.7 (14.6)	0 (0)	28.5 (11.6)
Common Carp	16.0 (6.9)	16 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)
Largemouth Bass	6.4 (4.8)	2.4 (1.8)	10.2 (18.3)	32.7 (45.1)	57.1 (43.1)	0 (0)
Pumpkinseed Sunfish	0.8 (0.4)	0.2 (0.4)	1.7 (2.9)	68.3 (54.8)	0 (0)	30.0 (52.0)
Walleye	0.2 (0.4)	0 (0)	10.0 (na)	0 (na)	90.0 (na)	0 (na)
Yellow Perch	0.6 (0.9)	0.2 (0.4)	15.0 (21.2)	35.0 (49.5)	50.0 (70.7)	0 (0)
Green Sunfish	15.0 (na)	10.0 (na)	8.7 (25.9)	18.0 (37.5)	0 (0)	0 (0)

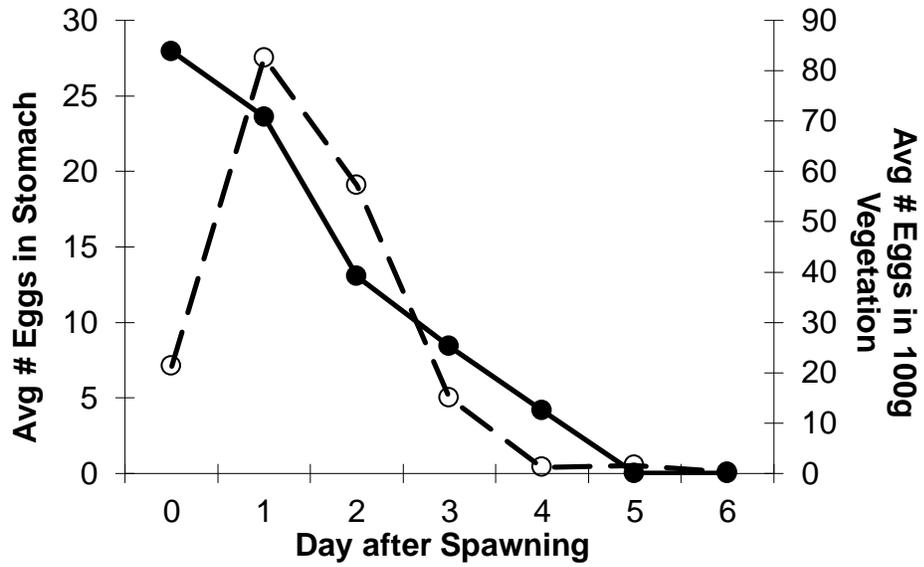


Figure A5-1

Mean number of carp eggs found in fish stomachs (solid line) and mean number of carp eggs found in the vegetation (dashed line). The number of carp eggs found in fish stomachs declined as carp eggs in the environment declined. (n = 5)

Appendix 6

The Effect of Black Bullhead on the Survival of Carp Larvae in a Simulated Carp Spawning Habitat

Methods

Bluegill sunfish and black bullhead were collected from Lake Keller using beach seines (50 m long, 15 m wide, 10 mm mesh) during May 2010. Collected fish were placed into a 500 L flow through tank with a photoperiod of 16 hours light to eight hours dark and allowed to acclimate to a diet of flake food. After 10 days, all fish had switched to a diet of flake food.

After all fish had acclimated to lab conditions, four 1,600 L tanks (2.5 m x 2.5 m x 0.25 m) were each stocked with five individuals of a single species (two tanks with bluegill sunfish, two tanks with black bullhead). To replicate wild carp spawning habitat, natural vegetation (curly leaf pondweed and coontail) was collected from Lake Keller and placed in all tanks until it covered 25% of the surface. Fish were allowed to acclimate to the new tanks over the course of one week. An additional two tanks were treated the same way, except no fish were introduced (i.e. no fish control). This test group of six tanks was replicated twice for a total of four replicates for each treatment (bluegill sunfish, black bullhead, or no fish control).

Carp eggs were collected and fertilized and treated as in Experiment 3 to maximize hatching success. Eggs were treated with a solution of 3 g urea and 4 g NaCl per L of water after fertilization to remove the adhesiveness of the eggs (Schoonbee and Brandt 1982). Treated eggs were then placed in six L Zoug jars that kept the eggs

suspended in the water column through the use of a bubbler at the bottom of the jar (Billard 1999). After hatching, larvae were collected in 75 L tanks and fed a diet of brine shrimp until they were introduced to the test tanks and the experiment began.

One thousand larvae ($SD = 74$) were distributed evenly throughout each of the six test tanks to begin the experiment. This density was used to ensure that larval carp could be sampled during the experiment. Larvae were introduced at 2400 hours because sunfish are visual predators and we wanted to limit predation until the larvae were able to acclimate to the test tank (artificial sunrise was at 0600 hours). Larvae were sampled using a dip net (35 cm x 25 cm; 350 μ m mesh) at 8 evenly spaced locations throughout the tank. The dip net was placed parallel to the surface of the water and swept through the water column in a “U” shape that touched the bottom of the tank and ended back at the surface next to where the net started. Larvae caught in each net sweep were immediately counted and released back into the tank at the same place they were captured. Initial larval sampling took place two hours after larvae were introduced to the tank, and regular sampling was conducted at 1000 and 2100 hours for three days after the introduction of larvae. A generalized linear model (R 2.13.0) was used to analyze the interaction between time and treatment type (bluegill sunfish, black bullhead, or no fish) on carp larval densities within tanks.

Results

There was a significant difference between bluegill sunfish and the control ($p < 0.001$, $df = 558$; Figure A5-1). However, black bullhead did not show a significant difference from the control at any time period (minimum $p = 0.829$ at 45 hours). This suggests that larval carp are not preyed on by black bullhead.

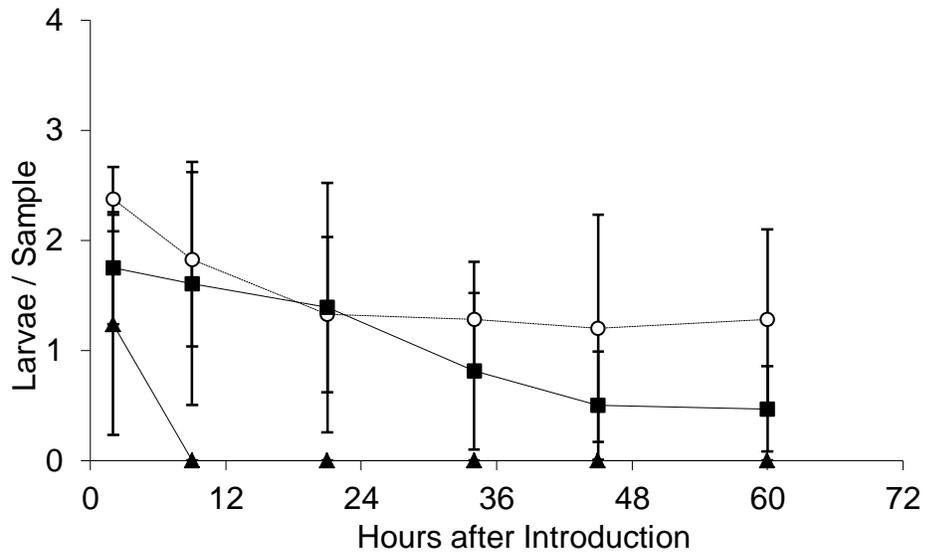


Figure A6-1

Mean number of carp larvae present sampled per dip net sweep in the presence and absence of fish predators. Open circles represent control (no fish), squares represent black bullhead, and triangles represent bluegill sunfish. Bars represent standard deviation. (n = 4)