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University of Nebraska-Lincoln, kturek@huskers.unl.edu

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INFLUENCE OF NON-NATIVE TROUT ON NATIVE NON-GAME FISH IN NEBRASKA HEADWATER STREAMS

By

Kelly C. Turek

A THESIS

Presented to the Faculty of

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Under the Supervision of Professors Mark A. Pegg and Kevin L. Pope

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INFLUENCE OF NON-NATIVE TROUT ON NATIVE NON-GAME FISH IN NEBRASKA HEADWATER STREAMS

Kelly C. Turek, M.S.

University of Nebraska, 2014

Advisors: Mark A. Pegg and Kevin L. Pope

Introduced, non-native trout may have detrimental competitive or predatory interactions with native fishes. However, few studies have experimentally examined interactions between introduced trout and native non-game species. Therefore, the objectives of this study were to determine 1) if non-native rainbow trout *Oncorhynchus mykiss* influence survival, behavior, movement, or distribution of native longnose dace *Rhinichthys cataractae* under laboratory conditions, 2) if non-native rainbow trout influence survival of native longnose dace under *in-situ* conditions using in-stream enclosures, and 3) if native fish populations or communities differ in the presence and absence of non-native trout under natural conditions.

Rainbow trout preyed on longnose dace at low rates in both laboratory and in-stream enclosure experiments suggesting that if rainbow trout and longnose dace overlap in microhabitat use, some predation is likely to occur. Therefore, it is not recommended that non-native trout be stocked in streams containing at-risk species. Size structures of longnose dace and white sucker *Catostomus commersonii* were larger in the presence of brown trout *Salmo trutta*, and size structure of longnose dace was smaller in the presence of rainbow trout under natural conditions suggesting that non-native trout presence may influence some native populations. However, creek chub *Semotilus atromaculatus* and

fathead minnow *Pimephales promelas* size structures did not differ in the presence and absence of non-native trout. Greater non-native trout abundances resulted in greater distinction in native community composition and structure between sites with trout and sites without trout suggesting there may be increased risk to native communities in sites with high abundances of trout. Therefore, species-specific, as well as community-wide effects of non-native trout should be considered prior to any introductions.

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CHAPTER 1. GENERAL INTRODUCTION & STUDY OBJECTIVES

INTRODUCTION

Salmonids are highly desired for their recreational value; as a result, they have been introduced extensively outside of their native ranges (Krueger and May 1991). Non-native salmonids were first introduced into the United States of America in 1883 (Mather 1887). However, intra-continental transfers of salmonids beyond their native ranges began as early as the 1870s (MacCrimmon and Campbell 1969). Today, non-native salmonids have been introduced into nearly every state in the United States of America (NatureServe 2004, George *et al.* 2013) and every continent except Antarctica (Krueger and May 1991).

Salmonids were among the first non-native species introduced into Nebraska (Jones 1963). Efforts by the United States Fisheries Commission to stock Nebraska waters with desirable game fish began in the mid to late 1800s with the introduction of several salmonid species (*e.g.*, Atlantic salmon *Salmo salar*, brook trout *Salvelinus fontinalis*, brown trout *Salmo trutta*, chinook salmon *Oncorhynchus tshawytscha*, cisco *Coregonus artedii*, lake trout *Salvelinus namaycush*, lake whitefish *Coregonus clupeaformis*, and rainbow trout *Oncorhynchus mykiss*), yet few species managed to become established (Jones 1963). Additional salmonid species, including Arctic grayling *Thymallus arcticus*, Kokanee salmon *Oncorhynchus nerka*, and cutthroat trout *Oncorhynchus clarki*, were introduced throughout the early 1900s with little success (Jones 1963). Today, three main species of non-native salmonids, brook trout, brown trout, and rainbow trout, persist in Nebraska due in large part to routine stockings. In the

last 80 years, over 8.7 million trout have been stocked into Nebraska streams (NGPC unpublished data), greatly enhancing the recreational capacity of many of these systems.

BALANCING RECREATIONAL OPPORTUNITIES WITH ECOLOGICAL INTEGRITY

Trout fishing offers a unique experience for Nebraska anglers who would otherwise have to travel long distances for trout-angling opportunities. Cool, spring-fed streams, deep reservoirs, and reservoir tailwaters provide enough temperature refuge for these species to survive, and in some cases, reproduce. Trout are stocked seasonally in other areas to create put-and-take fisheries that can be of significant recreational value.

Although introduced trout can provide important recreational opportunities, they are also not native to Nebraska. Non-native species are considered one of the major causes of widespread declines in freshwater biodiversity (Dudgeon *et al.* 2006). Further, 68% of all North American fish extinctions have been attributed in part to introduced species (Miller *et al.* 1989), over two thirds of which were intentional introductions (Lassuy 1995). Similarly, introduced species were cited as causing a decline in or being a continuing threat to native species in 70% of Endangered Species Act (ESA) listings, 73% of which involved sportfish introductions (Lassuy 1995).

Balancing the desire for recreational opportunities with the desire for maintaining ecological integrity has put introduced trout at the forefront of fisheries management concerns. Managers continue to face pressure from private citizens, sports groups, and other organizations to stock non-native trout for recreation. These fish are often considered more desirable than native non-game fish, as introduced sportfish offer added

angling opportunities that can contribute considerably to local economies. However, introduced trout may have potentially harmful influences on surrounding biological communities (*e.g.*, Turek *et al.* 2013); thus, there is a need for better understanding of the ecological effects of stocking non-native trout.

RESEARCH NEEDS

The purpose of this project is to gain a better understanding of the interactions between non-native trout and native species of concern in Nebraska headwater streams, to better predict the potential outcomes of future trout stockings. Suitable trout habitat is thought to overlap with preferred habitat of native cyprinid species of concern, yet trout are rarely found coexisting with these species in Nebraska. A considerable amount of literature suggests non-native trout may have detrimental competitive or predatory interactions with native fishes (Turek *et al.* 2013). Therefore, the Nebraska Game and Parks Commission currently prohibits stocking trout in waters containing species of concern. However, few studies have experimentally examined interactions between introduced trout and species of concern, and there are similarly no known empirically based studies examining such interactions in Nebraska. Examining interactions between trout and native cyprinids will provide new insights to the potential consequences of trout introductions within Nebraska. Further, a better understanding of these interactions should enable biologists to better quantify suitable areas for trout introductions, with a goal of increasing recreational opportunities for trout anglers, while minimizing deleterious effects on native species.

Non-native species, as used throughout this thesis, are defined as species that occur outside of their native range. Introduced species is a term used to define the mechanism by which a species first arrived, in this case, purposeful stocking by governmental agencies. Species of concern are defined as threatened or endangered species listed under the ESA; state listed as threatened, endangered, imperiled or vulnerable; or endemic.

STUDY SPECIES

Longnose dace *Rhinichthys cataractae* are an abundant native cyprinid species found throughout Nebraska. Longnose dace was used as a study species throughout this thesis because they are functionally the closest, non-threatened fish to many species of concern found in streams with introduced trout (*e.g.*, blacknose dace *Rhinichthys obtusus*). Species of concern were not used because purposeful take of these species is prohibited, and there is currently no hatchery production of these species in Nebraska (with the notable exception of plains topminnow *Fundulus sciadicus*). Examining the influence of non-native trout on longnose dace will not only provide insight into responses in species of concern, but will also answer several broader ecological questions pertaining to the influence of introduced non-native trout on abundant native species.

Although parts of this thesis examine the influences of brook trout, brown trout, and rainbow trout on native species, as well as their combined effects, only rainbow trout were used in experimental manipulations. Rainbow trout are the most commonly stocked non-native trout species in Nebraska, and also the most readily available. Their abundance and distribution suggest that implications of stocking rainbow trout will likely

have the greatest effect on future management recommendations, and thus, they were used as a study species.

STUDY OBJECTIVES

The main goal of this project was to determine if non-native trout have an influence on native cyprinids. Specific objectives were to:

1. Determine if non-native rainbow trout influence survival, behavior, movement, or distribution of longnose dace under laboratory conditions (Chapter 2).
2. Determine if non-native rainbow trout influence survival of native longnose dace using in-stream enclosures (Chapter 3)
3. Determine if native fish populations or communities differ in the presence and absence of non-native trout under natural conditions (Chapter 4).

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CHAPTER 2. LONGNOSE DACE *RHINICHTHYS CATARACTAE* RESPONSE TO INTRODUCED RAINBOW TROUT *ONCORHYNCHUS MYKISS* UNDER LABORATORY CONDITIONS

ABSTRACT

Little is known about the ecological interactions between rainbow trout *Oncorhynchus mykiss* and longnose dace *Rhinichthys cataractae*, and there have been no known attempts to experimentally examine these interactions. Therefore, we used laboratory experiments to determine if rainbow trout influence survival, behavior, movement, or distribution of longnose dace. Adult rainbow trout preyed on longnose dace in 44% of trials and juvenile rainbow trout did not prey on longnose dace. We were unable to detect a difference in longnose dace habitat selection or movement pre and post rainbow trout introduction. Additionally, we observed no differences in longnose dace responses to adult and juvenile rainbow trout, or between adult rainbow trout previously fed pellet feed only and those fed a mixed diet of pellet feed and minnows. More research is needed to determine how these interactions will change in natural environments, under differing amounts of habitat and food resources, and in the context of whole assemblages. However, if rainbow trout are introduced into the habitat of longnose dace, some predation on longnose dace and behavioral changes may be expected, even when rainbow trout have no previous experience with active prey.

INTRODUCTION

Predation is one of the principal forces shaping stream-fish communities (Hoeinghaus and Pelicice 2010). Predators can alter prey survival or behavior (*e.g.*, movement and distribution) which can result in cascading population (*e.g.*, changes in abundance) and community-wide effects (*e.g.*, altered competitive interactions and changes in composition; Hoeinghaus and Pelicice 2010). Likewise, prey can alter predator survival, behavior, and abundance resulting in cascading effects (Matthews 1998). Environment (*e.g.*, habitat availability and complexity), resource availability, previous status of populations, and other organisms (*e.g.*, other predators, other competitors, or humans) can all influence the relative strengths of these interactions (*e.g.*, Hoeinghaus and Pelicice 2010). Introduced species, in particular, can influence predator-prey dynamics (Ross 1991), especially in systems in which they are not native and did not evolve with the present fish community (*e.g.*, Blinn *et al.* 1993).

Rainbow trout *Oncorhynchus mykiss* are highly desired as a sport fish and as a result have been introduced widely outside of their native range (Krueger and May 1991, Welcomme 1992). Although rainbow trout are often thought of as invertivorous, they are opportunistic generalists and can be piscivorous (*e.g.*, Marsh and Douglas 1997, Fenner *et al.* 2004, Yard *et al.* 2011). However, the direct and indirect predatory threat of rainbow trout to stream-fish communities has rarely been examined. Understanding the underlying ecological interactions among rainbow trout and native species will help provide the information necessary to ensure proper management of native species in the future.

Rainbow trout often co-occur with longnose dace *Rhinichthys cataractae* in small cool- to cold-headwater streams throughout North America, although historically their ranges overlapped only in the Pacific Northwest. Little is known about the ecological interactions between rainbow trout and longnose dace, and there have been no known attempts to experimentally examine these interactions. Therefore, the objective of this study was to use laboratory experiments to determine if rainbow trout influence survival, behavior, movement, or distribution of longnose dace. Additionally, differences in responses between adult and juvenile rainbow trout, and adult rainbow trout previously exposed to different diets were examined.

METHODS

Laboratory Methods

Longnose dace (59 ± 9 mm, mean ± stdev) were collected from Gracie Creek, Loup County, Nebraska using a pulsed-DC backpack electrofisher. Juvenile rainbow trout were obtained from Calamus State Fish Hatchery, Loup County, Nebraska. Adult rainbow trout were obtained from Grove Trout Rearing Station, Antelope County, Nebraska. Longnose dace and rainbow trout were held in separate round fiberglass recirculating tanks (1.2-m diameter x 0.9-m depth) and allowed to acclimate to laboratory conditions (minimum of 16 days for rainbow trout and 34 days for longnose dace).

Longnose dace were introduced into 1.6-m x 0.5-m rectangular tanks (water temperature = 15 ± 1 ˚C), acclimated for 20 minutes, and then monitored for 30 minutes in the absence of rainbow trout. Rainbow trout were then introduced and movement and

behavior of both species was recorded for an additional 30 minutes. A grid of 10.2-cm x 10.2-cm squares on the bottom of each tank was used to record positions of longnose dace using Go Pro® HERO 3 cameras set to take videos at a resolution of 1080 p and a frame rate of 30 fps. A single longnose dace and a single rainbow trout were used in each trial and individual fish were not used in multiple trials. Three treatments of rainbow trout were used: 1) juvenile rainbow trout (121 ± 10 mm) fed pellet feed only (hereafter referred to as pellet-fed juveniles), 2) adult rainbow trout (288 ± 17 mm) fed pellet feed only (hereafter referred to as pellet-fed adults), and 3) adult rainbow trout (288 ± 23 mm) fed a mixture of pellet feed and minnows (hereafter referred to as mixed-diet-fed adults). The study included 18 trials of pellet-fed juvenile rainbow trout, 13 trials of pellet-fed adult rainbow trout, and 6 trials of mixed-diet-fed adult rainbow trout.

Video Analysis

Types of interactions between rainbow trout and longnose dace were recorded immediately following rainbow trout introduction for 30 minutes with the exception of predation, which was monitored until camera batteries died (approximately 6 to 7 hours following introduction). Interactions fell into four categories based on rainbow trout aggression and the corresponding response of the longnose dace: 1) rainbow trout showed aggression, longnose dace reacted, 2) rainbow trout showed aggression, longnose dace did not react, 3) rainbow trout did not show aggression, longnose dace reacted, and 4) rainbow trout did not show aggression, longnose dace did not react (when both were within the same 10.2-cm x 10.2-cm square). Rainbow trout aggression was defined as an obvious increase in swimming speed toward longnose dace. Aggressive behavior was

classified as a chase, attack, or predation. A chase was defined as an aggressive act in which the predator did not make contact with the prey. An attack was defined as an aggressive act in which the predator made contact with the prey, but did not result in consumption of the prey. Predation was defined as an aggressive act in which the predator made contact with the prey and which resulted in consumption of the prey. Handling time for predation events (from moment of first contact until consumption) was also determined from video footage.

Locations of longnose dace within the grid were marked every 30 seconds for 30 minutes pre and post rainbow trout introduction (*i.e.*, 60 observation points per trial both before and after rainbow trout introduction). If an individual longnose dace was in more than one square at an interval, its position was marked in the square in which the majority of its body was located. If a longnose dace was exactly half way between two squares, its position was marked in the square in which its head was located. Similarly, if an individual juvenile rainbow trout was in more than one square at an interval, its position was marked in the square in which the majority of its body was located. If a juvenile rainbow trout was exactly half way between two squares, its position was marked in the square in which its head was located. The position of adult rainbow trout was marked in the square in which its head was located because rainbow trout occupied two to three squares at any given time. Longnose dace locations in the water column were also noted as either “bottom” – defined as the lower half of the water column, or “surface” – defined as the upper half of the water column at each observation point.

Data Analysis

Longnose Dace Survival and Behavioral Interactions

Differences in longnose dace survival between rainbow trout treatments was evaluated using generalized linear models (binomial distribution). The influence of longnose dace length and rainbow trout length on longnose dace survival was also evaluated using generalized linear models for only adult rainbow trout treatments (*i.e.*, the juvenile rainbow trout treatment was excluded because regardless of juvenile rainbow trout length or longnose dace length, juvenile rainbow trout were unlikely to prey on longnose dace). Similarly, the influence of behavioral interactions that occurred in the first 30 min following introduction (*e.g.*, number of chases and attacks) on longnose dace survival was tested using generalized linear models (binomial distribution). Differences in time to predation between treatments using only trials in which predation occurred on camera were assessed using linear models. The number of aggressive interactions between rainbow trout and longnose dace (*i.e.*, chase or attack), the number of non-aggressive rainbow trout interactions resulting in longnose dace reaction, and the number of non-aggressive rainbow trout interactions resulting in no longnose dace reaction were also compared between all treatments using generalized linear models (Poisson distribution). The proportion of non-aggressive rainbow trout interactions that resulted in a reaction (or, consequently, no reaction) in longnose dace was compared between treatments using generalized linear models (binomial distribution). All survival and behavioral interaction analyses were conducted using the GLIMMIX procedure in SAS V.9.2.

Longnose Dace Movement

Two longnose dace responses, total distance moved (2-dimensional space) and proportion of time spent at surface, were used to determine if longnose dace movement differed in the presence and absence of rainbow trout. To determine distances moved, the grid of 10.2-cm x 10.2-cm squares was converted into a plane of *x* and *y* coordinates (assuming fish were located at the center of the squares). All distances, *d*, were then calculated using a basic distance formula, $d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$. Longnose dace total distance moved in the presence and absence of rainbow trout for each treatment was compared using linear mixed models with fixed effects of presence and treatment, and random effects of individual. The proportion of time longnose dace spent at the surface in the presence and absence of rainbow trout for each treatment was compared using a generalized linear mixed model with fixed effects of presence and treatment, random effect of individual, and assuming a binomial distribution. Additionally, a linear model was used to compare average distance between predator and prey (calculated using the distance formula) for each treatment to determine if longnose dace avoidance differed between the three treatments. All movement analyses were conducted using the GLIMMIX procedure in SAS v.9.2.

Longnose Dace Distribution

Longnose dace spatial distributions pre and post trout introductions were analyzed based on positions within post-hoc classified “habitat types” within tanks (corner, edge, and open water; Figure 2-1). Longnose dace distributions, based on positions taken every 30 seconds, were compared for each individual pre and post rainbow trout introduction

using chi-square tests (PROC FREQ, SAS v.9.2). Selection ratios for each habitat type were calculated for each treatment as $\widehat{w}_i = \mu_{i+}/(\pi_i \mu_{++})$, where μ_{i+} is the amount of habitat type *i* used by all fish of a particular treatment, π_i is the proportion of available resource units that are in category *i*, and u_{++} is the total number of habitat units used by all fish of a particular treatment (Rogers and White 2007). Therefore, selection ratios greater than one indicate selection for a particular habitat and selection ratios less than one indicate selection against a particular habitat. Standard errors and 95% confidence intervals of selection ratios were calculated according to Rogers and White (2007) as

$$\mathrm{SE}(\widehat{w}_i) = \sqrt{\frac{n}{(n-1)(\mu_{++})^2} \sum_{j=1}^{n} \left(\frac{\mu_{ij}}{\pi_i} - \widehat{w}_i(u_{+j}) \right)^2} \quad \text{and}$$

$$\mathrm{CI}(\widehat{w}_i) = z_{\alpha/2I} \mathrm{SE}(\widehat{w}_i),$$

where *n* is the number of individual longnose dace for each treatment, u_{++} is the total number of habitat units used by all fish of a particular treatment, μ_{ij} is the amount of habitat type *i* used by fish *j*, π_i is the proportion of available resource units that are in category *i*, $\widehat{w}_i$ is the selection ratio for habitat type *i*, μ_{+j} is the total amount of habitat units used by fish *j*, *z* is the z-score corresponding to an upper tail probability of α/2*I*, and *I* is the number of confidence intervals being constructed (one for each habitat type).

RESULTS

Longnose Dace Survival and Behavioral Interactions

Longnose dace survival did not differ between rainbow trout treatments ($F_{2,34}$ = 0.14, P = 0.87), and no juvenile rainbow trout preyed on longnose dace. Time to predation ($F_{1,5}$ = 0.36, P = 0.57) did not differ between pellet-fed adult rainbow trout and mixed-diet-fed adult rainbow trout. Overall, 44% percent of adult rainbow trout (278-307 mm) preyed on longnose dace and took an average of 144 min before predation occurred (range: 37 - 339 min). Handling time for predation events ranged from immediate consumption to 4 seconds and averaged 2 ± 1 s (mean ± stdev). Neither longnose dace length (range: 41-78 mm; $F_{1,17}$ = 2.34, P = 0.14) nor rainbow trout length (range: 261-330 mm; $F_{1,17}$ = 1.72, P = 0.20) significantly influenced the probability that longnose dace survived in adult rainbow trout treatments. No behavioral interactions between rainbow trout and longnose dace within the first 30 minutes were significantly correlated with longnose dace survival in adult rainbow trout treatments (Table 2-1).

In all cases where rainbow trout showed aggression, longnose dace either reacted or were consumed. The number of chases ($F_{2,34}$ = 0.64, P = 0.53) and attacks ($F_{2,34}$ = 2.55, P = 0.09) within the first 30 minutes after rainbow trout introduction did not differ between treatments (Figure 2-2). However, the smallest rainbow trout (105 mm) showed the greatest amount of aggression of all rainbow trout used (including adults), chasing the longnose dace twice and attacking it 7 times within the first 30 minutes.

Seventy-eight percent of pellet-fed juvenile rainbow trout, 85% of pellet-fed adult rainbow trout, and 83% of mixed-diet-fed adult rainbow trout showed no aggression toward longnose dace within the first 30 minutes after introduction. Longnose dace

reacted to rainbow trout even though rainbow trout showed no aggression more times in trials with mixed-diet-fed rainbow trout than either trials with pellet-fed juvenile rainbow trout or pellet-fed adult rainbow trout ($F_{2,34}$ = 11.60, P = <0.01; Figure 2-2). Similarly, close proximity resulting in no apparent reaction from either the rainbow trout or the longnose dace occurred more times in trials with pellet-fed adult rainbow trout than both trials with pellet-fed juvenile rainbow trout and mixed-diet-fed adult rainbow trout ($F_{2,34}$ = 4.95, P = 0.01; Figure 2-2). However, the proportion of non-aggressive trout interactions resulting in a longnose dace reaction did not differ among treatments ($F_{2,29}$ = 2.40, P = 0.11).

Longnose Dace Movement

Longnose dace total distance traveled did not differ in the presence or absence of rainbow trout ($F_{1,36}$ = 0.52, P = 0.48) or between treatments ($F_{2,36}$ = 0.47, P = 0.63; Figure 2-3). The proportion of time spent at surface did not differ in the presence or absence of rainbow trout ($F_{1,36}$ = 0.99, P = 0.33) or between treatments ($F_{2,36}$ = 0.64, P = 0.53; Figure 2-4) and longnose dace spent the majority of their time in the bottom half of the water column. Average distance between rainbow trout and longnose dace did not differ between treatments ($F_{2,34}$ = 2.02, P = 0.15; Figure 2-5).

Longnose Dace Distribution

Longnose dace spatial distributions, based on habitat type (corner, edge, open water), differed before and after rainbow trout introduction for 8 of the 18 trials of pellet-fed juvenile rainbow trout, 5 of the 13 trials of pellet-fed adult rainbow trout, and 3 of the

6 trials of mixed-diet-fed adult rainbow trout (Table 2-2). Longnose dace selected for corners and against open water, but used edges in proportion to their availability for all treatments both before and after rainbow trout introduction (Figure 2-6).

DISCUSSION

Adult rainbow trout preyed on longnose dace in laboratory tanks suggesting that if rainbow trout and longnose dace are isolated to the same habitats, some predation is likely. However, even in small tanks and under forced interactions, predation rates were not exceptionally high. A number of factors including experience of both predator and prey, and food and habitat availability may influence these predation rates under natural conditions.

Experience of both predator and prey can influence predation rates. Non-native rainbow trout have been suggested to pose increased risk to native populations because of a lack of co-evolutionary history and thus, a reduction in the ability of prey to recognize predators as a threat (Townsend and Crowl 1991, Blinn *et al.* 1993, Bryan *et al.* 2002, Nannini and Belk 2006). This is a potential reason introduced rainbow trout were able to easily prey on Little Colorado spinedace even in the presence of increased cover in stream enclosure experiments (Blinn *et al.* 1993). However predator experience and perception of novel prey may also influence predation rates. In this study, rainbow trout previously exposed to minnows did not pose a greater predatory threat to longnose dace and did not prey on longnose dace sooner than those previously fed only pellet food suggesting that predator experience was not an important factor in predation rates in our experiments.

The amount of time from the introduction of rainbow trout until predation occurred (for those that preyed on longnose dace on camera) was also much faster for both groups of adult rainbow trout than previously documented. In a laboratory study of the effect of experience on predation, naïve rainbow trout required 4 days of experience before approaching unfamiliar, palatable food (Ware 1971), whereas rainbow trout in our study required 144 minutes on average. Differences in these times could be due to a number of factors including size of experimental tanks, size of predators, and type of food. In particular, the mobility of prey in our experiments, as opposed to the immobility of the food (chicken liver) used in Ware's (1971) experiments may have increased the number of encounters with the predator allowing them to recognize dace as a food item faster. The apparent irrelevance of previous experience on rainbow trout predation rates suggests that stocking hatchery rainbow trout (*i.e.*, those fed only pellets) may result in predation risk to native stream fishes relatively quickly.

The overall influence of rainbow trout on longnose dace populations, given predation on individuals, is unknown. Previous studies on other small-bodied fishes have determined that low predation rates likely had little influence at the population level. For example, rainbow trout predation on native fishes in an Oklahoma Ozark stream was low and probably did not constitute a significant influence on the population (Walsh and Winkelman 2004). However, other studies have highlighted that the magnitude of the influence of predation may be amplified for some fish species, particularly threatened fishes (*e.g.*, Knight and Gido 2005). Even if a predator randomly selects prey, the overall effect on a population will be greater for threatened or endangered species due to their already low numbers. For this reason, the possibility of predation by introduced rainbow

trout should be of concern for threatened and endangered species. Others have suggested that the apparent lack of a population-level effect of predation may be due to the scale of observations, as much more evidence exists of predator effects at the mesohabitat scale than at the reach scale (*e.g.*, Matthews 1998). Similarly, we found that rainbow trout influenced longnose dace survival in small in-stream enclosures (Chapter 3), however, changes in the relative abundance of longnose dace between sites with and without rainbow trout were not evident at the reach scale (Chapter 4). These differences may be due to a number of factors including low non-native trout predation rates, native fish movement or dispersal from habitats containing non-native trout, differences in mesohabitat or microhabitat selection within stream reaches, or some mixture of these factors.

Much research has focused on behavioral decisions of stream fishes related to foraging, predator avoidance, and resource availability, as well as the interactions among these factors (*e.g.*, Fraser and Cerri 1982, Cerri and Fraser 1983, Gilliam and Fraser 1987, Lima and Dill 1990). In this study, we isolated the predatory interactions of rainbow trout on longnose dace (*i.e.*, held food availability and habitat constant), greatly simplifying the complex interactions that may occur in a stream environment. Therefore, we would expect longnose dace distribution and habitat use to be more complex in natural environments.

Although adult rainbow trout may influence longnose dace through predation, juvenile rainbow trout may influence longnose dace through different mechanisms. No juvenile rainbow trout fed on longnose dace during this short-term study, however, juvenile fish did show aggression toward longnose dace, and the most aggressive rainbow

trout observed was a juvenile. The increased stress on longnose dace could still have detrimental effects on the overall fitness of individual longnose dace even if interactions do not result in predation.

Rainbow trout influenced longnose dace survival, and the distribution and behavior of some individuals; however, we were unable to detect an overall difference in longnose dace movement pre and post rainbow trout introduction. More research is needed to determine how these interactions will change, if at all, in natural environments, under differing amounts of habitat and food resources, and in the context of whole fish community assemblages. We also observed no differences in longnose dace responses to adult and juvenile rainbow trout, or between adult rainbow trout fed pellet feed only and those fed a mixed diet of pellet feed and minnows. Therefore, stocked adult rainbow trout may pose a predatory threat to small-bodied fishes even with no previous experience.

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Table 2-1. Test of the influence of behavioral interaction variables (within the first 30 minutes after rainbow trout introduction) on the probability of longnose dace survival (within the first 6-7 hours after rainbow trout introduction) in adult rainbow trout treatments (binomial distribution, SAS PROC GLIMMIX v.9.2; RBT = rainbow trout, df_{Num} = numerator degrees of freedom, df_{Den} = denominator degrees of freedom).

Variable	df_{Num}	df_{Den}	F_{stat}	*P*-value
Chases	1	17	0.45	0.51
Attacks	1	17	0.06	0.81
Total Aggressive Interactions	1	17	0.45	0.51
No RBT aggression, dace reacted	1	17	0.29	0.60
No RBT aggression, dace did not react	1	17	0.29	0.59

Table 2-2. Chi-square test of differences in individual longnose dace spatial distributions (based on habitat types) before and after rainbow trout (RBT) introduction (bold indicates significance at α = 0.001).

Treatment	Trial	*df*	X^2	*P*-value
Adult RBT fed pellets & minnows	5	2	4.39	0.11
	6	2	2.6	0.27
	7	2	35.66	**<0.0001**
	8	2	4.41	0.11
	9	2	31.58	**<0.0001**
	10	1	28.1	**<0.001**
Juvenile RBT fed pellets only	26	2	10.26	0.006
	27	1	112.26	**<0.0001**
	28	2	3.08	0.21
	29	2	1.27	0.53
	30	2	41.8	**<0.0001**
	32	2	7.65	0.0218
	33	2	6.79	0.0335
	34	2	80	**<0.0001**
	36	1	11.58	**0.0007**
	37	1	0.0625	0.8
	38	2	1.67	0.43
	39	2	25.75	**<0.0001**
	40	2	1.68	0.43
	41	2	20.27	**<0.0001**
	42	2	34.1	**<0.0001**
	43	2	3.08	0.21
	44	1	1.77	0.18
	45	1	25.21	**<0.0001**
Adult RBT fed pellets only	47	1	0.7	0.4
	48	2	37.06	**<0.0001**
	49	2	7.22	0.027
	50	2	72	**<0.0001**
	51	1	0.036	0.85
	52	2	0.86	0.65
	53	2	2.09	0.35
	54	2	28.45	**<0.0001**
	55	2	5.26	0.0723
	56	1	0.7018	0.4
	57	2	7.83	0.02
	58	1	108.33	**<0.0001**
	59	2	35.04	**<0.0001**

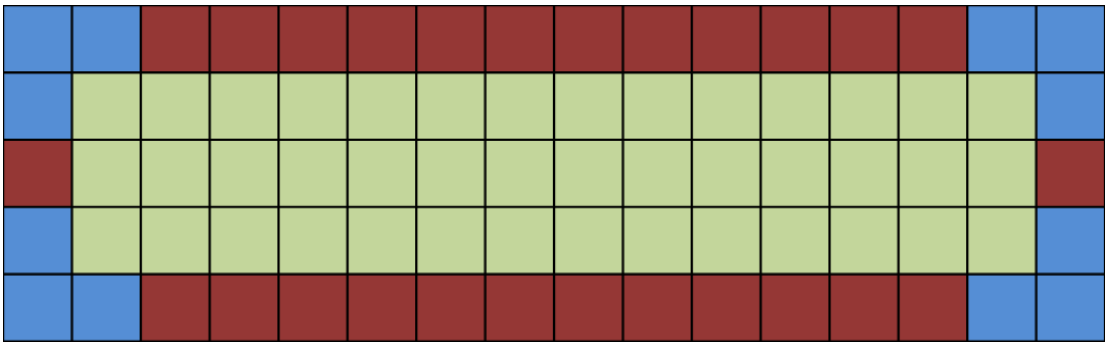

Figure 2-1. Post-hoc classification of habitat types used for chi-square tests (blue = corner, red = edge, green = open water).

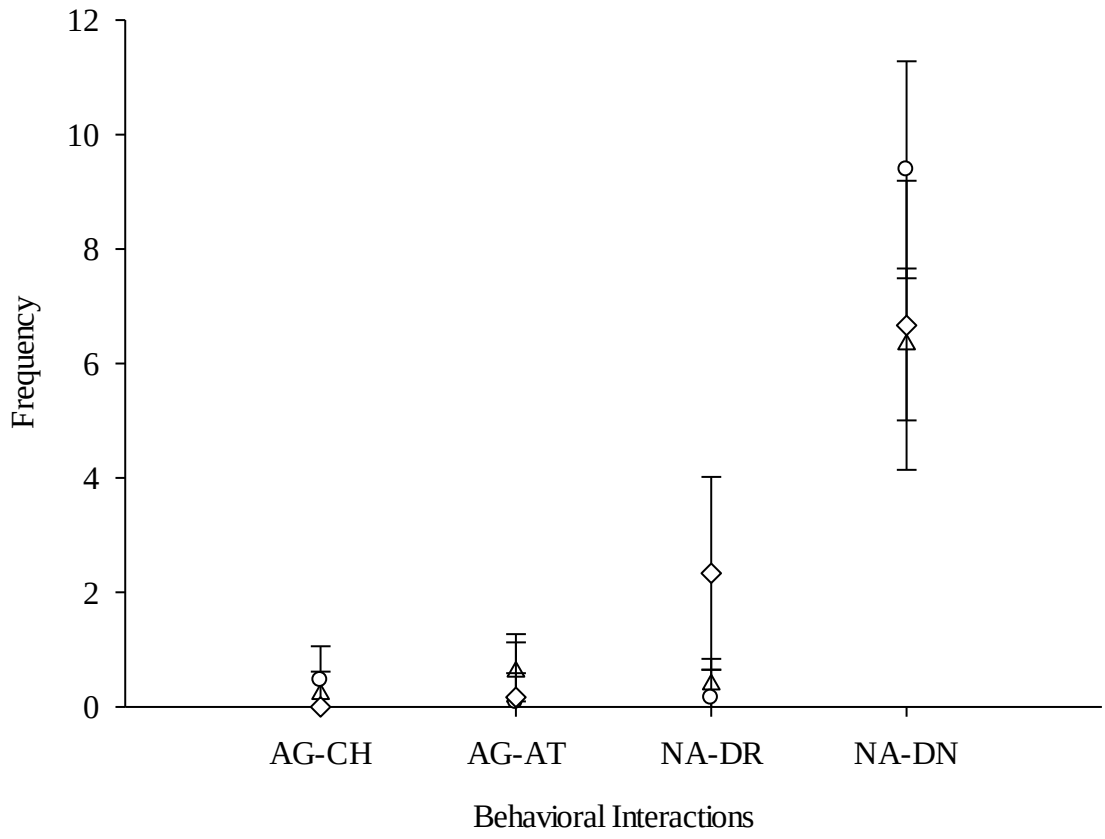

Figure 2-2. Frequency of occurrence (± 95% CI) of rainbow trout aggressive interactions classified as chases (AG-CH) and attacks (AG-AT), and non-aggressive interactions resulting in longnose dace reaction (NA-DR) and no longnose dace reaction (NA-DN) for pellet-fed juvenile rainbow trout (triangle), pellet-fed adult rainbow trout (circle), and mixed-diet-fed adult rainbow trout (diamond).

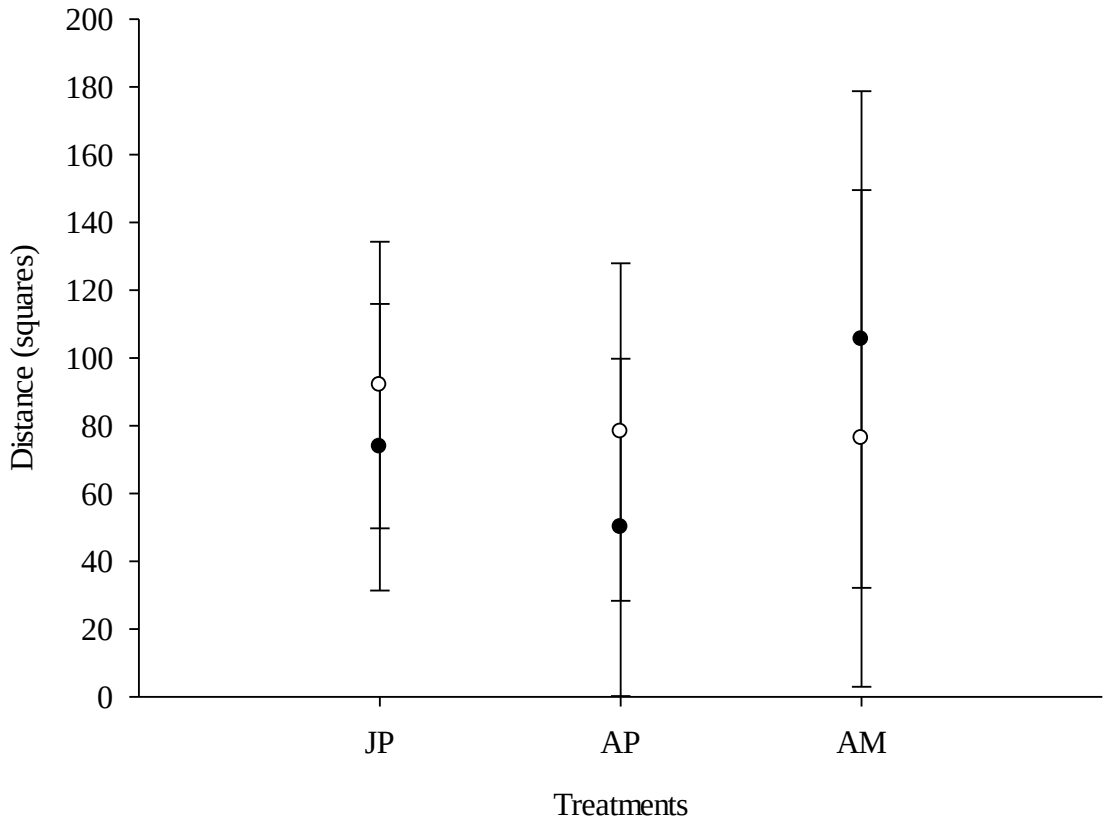

Figure 2-3. Total distance moved (± 95% CI) by longnose dace in the absence (open circles) and presence (closed circles) of rainbow trout for pellet-fed juvenile rainbow trout trials (JP), pellet-fed adult rainbow trout trials (AP), and mixed-diet-fed adult rainbow trout trials (AM).

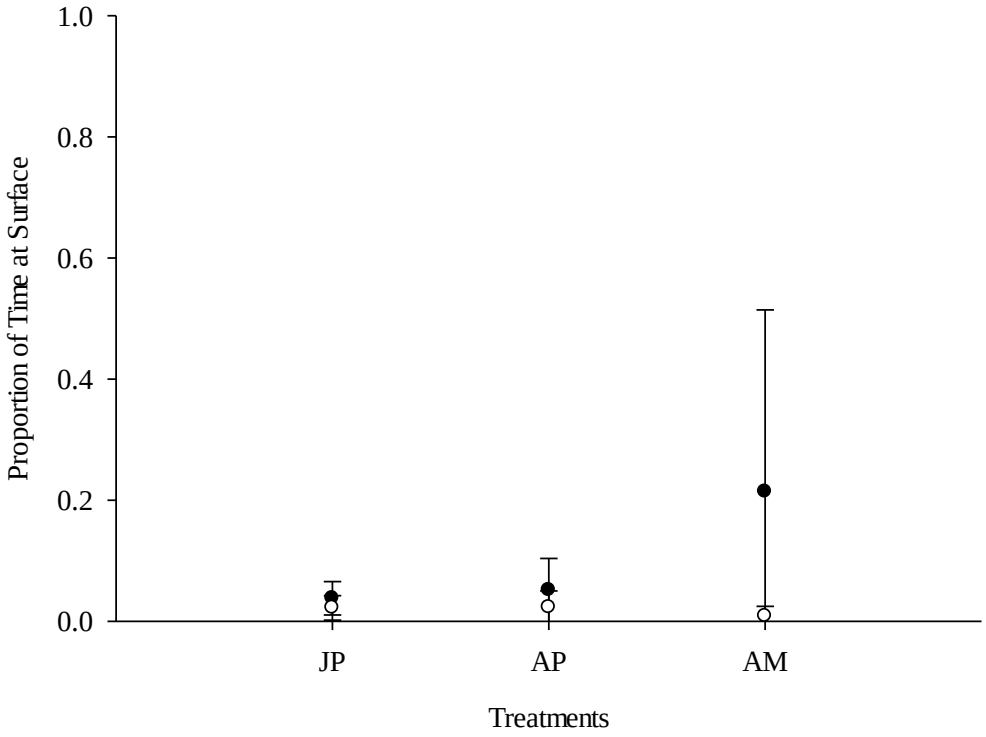

Figure 2-4. Proportion of time spent at surface (± 95% CI) by longnose dace in the absence (open circles) and presence (closed circles) of rainbow trout for pellet-fed juvenile rainbow trout trials (JP), pellet-fed adult rainbow trout trials (AP), and mixed-diet-fed adult rainbow trout trials (AM).

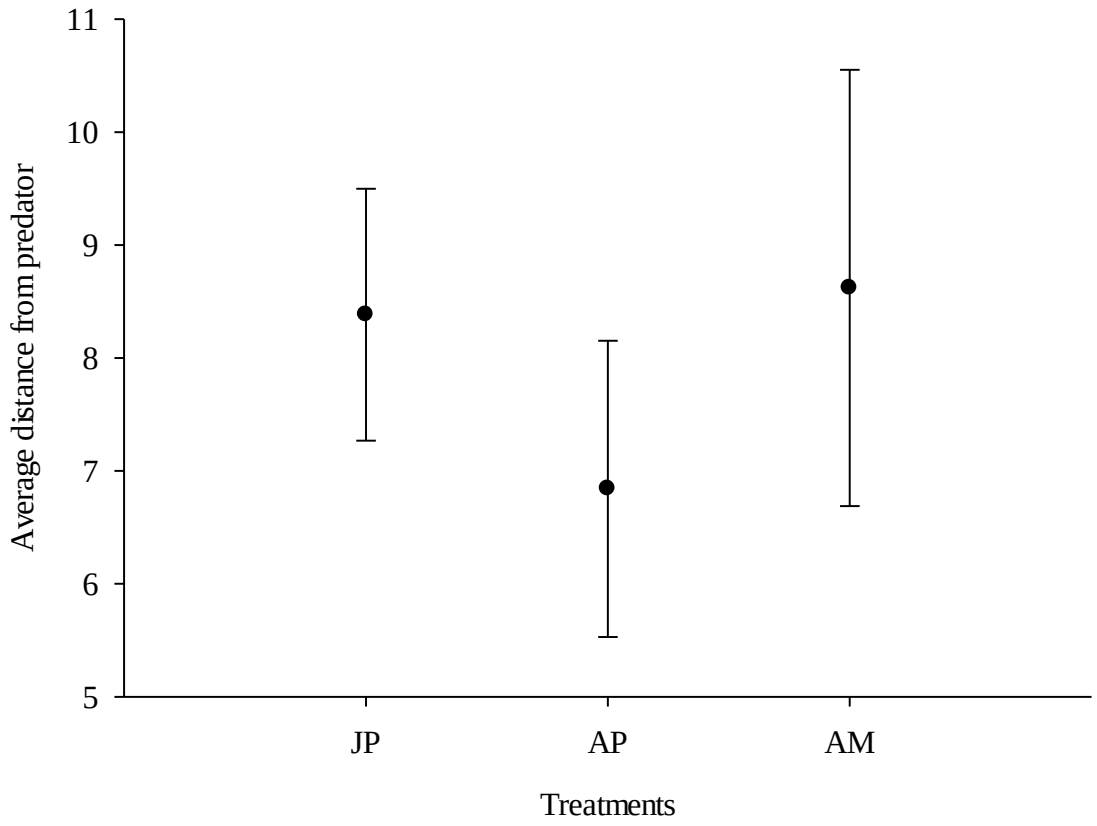

Figure 2-5. Average distance between rainbow trout and longnose dace (± 95% CI) for pellet-fed juvenile rainbow trout trials (JP), pellet-fed adult rainbow trout trials (AP), and mixed-diet-fed adult rainbow trout trials (AM).

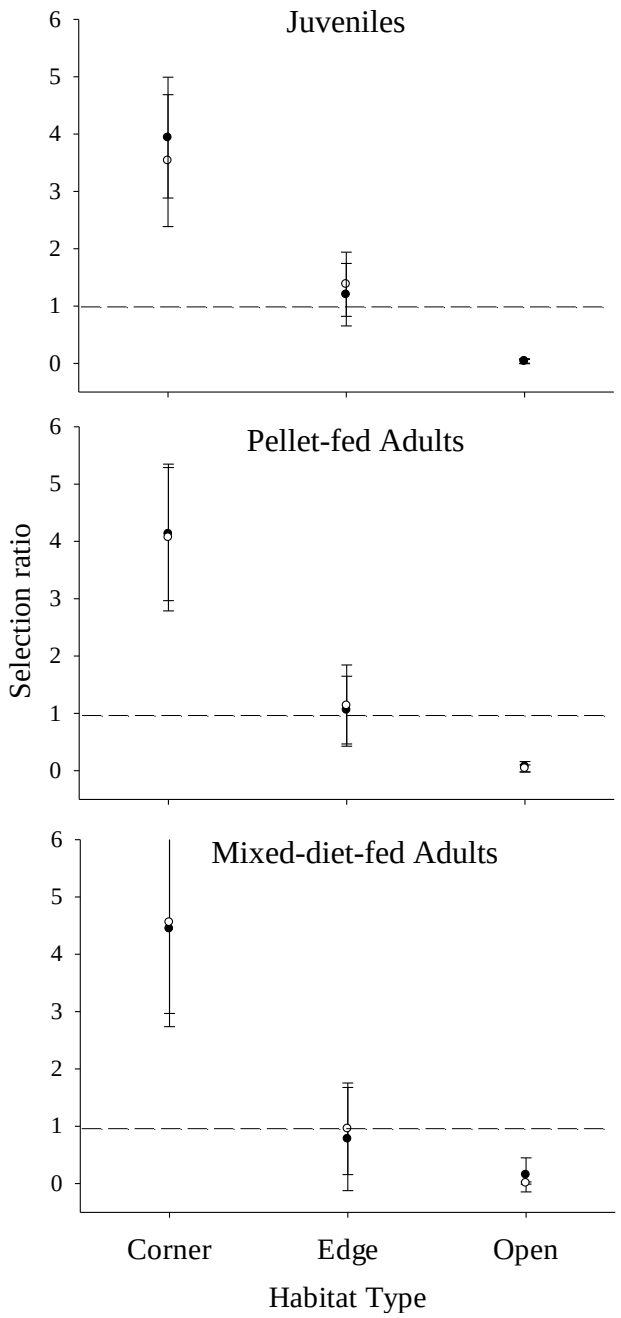

Figure 2-6. Longnose dace habitat selection ratios (± 95% CI) before (open circles) and after (closed circles) rainbow trout introduction for each habitat type (corner, edge, open water) and each treatment (juvenile rainbow trout, pellet-fed adult rainbow trout, and mixed-diet-fed adult rainbow trout). Selection ratios above one indicate selection for a particular habitat and below one indicate selection against a particular habitat.

CHAPTER 3. DIRECT PREDATORY EFFECTS OF NON-NATIVE RAINBOW TROUT *ONCORHYNCHUS MYKISS* ON NATIVE LONGNOSE DACE *RHINICHTHYS CATARACTAE* IN HEADWATER STREAM ENCLOSURES

ABSTRACT

The direct predatory effects of introduced rainbow trout *Oncorhynchus mykiss* on native longnose dace *Rhinichthys cataractae* were examined using a series of in-stream enclosures to determine if presence, density, or acclimation period of rainbow trout influenced survival of longnose dace. The number of longnose dace remaining in enclosures over the first 72 hours after rainbow trout introduction differed in the presence and absence of rainbow trout, but did not differ between average and high densities of rainbow trout ($F_{2,258.9} = 3.73$, $P = 0.03$). Rainbow trout acclimated to the stream for longer periods had a greater initial influence on the number of longnose dace remaining in enclosures relative to those acclimated for shorter periods ($F_{4,148.5} = 2.50$, $P = 0.04$). Several factors likely influenced rainbow trout predation rates including predator experience, prey body length, and habitat availability. Future research should focus on both direct and indirect predatory interactions between rainbow trout and longnose dace in the context of whole assemblages.

INTRODUCTION

Rainbow trout *Oncorhynchus mykiss* and longnose dace *Rhinichthys cataractae* are two species that often co-occur in small cool- to cold-headwater streams. The native ranges of rainbow trout and longnose dace historically overlapped only in the Pacific Northwest. Today, rainbow trout have been introduced across nearly the entire native range of longnose dace. With these introductions, and with such common co-occurrence which could result in negative competitive or predatory interactions, it is surprising that no studies have directly examined interactions between rainbow trout and longnose dace. Understanding the underlying ecological interactions among these two species will provide the information necessary to ensure proper management and survival of both species in the future.

Rainbow trout are opportunistic, generalist feeders, and consume a variety of aquatic and terrestrial invertebrates (Fenner *et al.* 2004, Metcalf *et al.* 1997), as well as some small fish (*e.g.*, Blinn *et al.* 1993, Klammer 1984). In general, rainbow trout are found in cool- to cold-water streams with distinct riffle-pool complexes and a variety of substrate. Microhabitat use within streams often shifts with both age and season (*e.g.*, Baltz *et al.* 1991).

Longnose dace are a small-bodied, riffle-dwelling cyprinid species. Longnose dace are abundant throughout their native range, which spans most of the North American continent (Scott and Crossman 1973a). Longnose dace prey on a variety of invertebrates including Diptera (*e.g.*, Simuliidae, Chironomidae), Ephemeroptera (*e.g.*, Baetidae, Siphlonuridae), and Tricopetera (*e.g.*, Hydropsychidae; Reed 1959, Gee and Northcote 1963, Gerald 1966, Gibbons and Gee 1972, Pappantoniou and Dale 1982, and

Mullen 1991). Longnose dace shift microhabitat use with age, but are most often found in moderately fast to fast current and cobble or boulder substrate (Mullen and Burton 1995, Gee 1968, Gee and Northcote 1963, Gibbons and Gee 1972).

Both rainbow trout and longnose dace are found in streams throughout Nebraska. These streams are somewhat unique compared to others in which these two species co-exist. Nebraska streams are typically low gradient, consist of grassy riparian vegetation, and have little in-stream woody debris. Undercut banks and overhanging vegetation make up the majority of cover for fish in these streams. There is often little distinction in pool and riffle habitat, and little substrate complexity (largely dominated by sands). The lack of distinct pools and riffles may cause greater habitat overlap among rainbow trout and longnose dace compared to other systems, and the lack of cover for small-bodied species such as longnose dace suggests that there may be increased predatory risk from rainbow trout in Nebraska streams.

Introductions of rainbow trout have negatively influenced several native species and have been implicated in the decline of many at-risk species in other systems (Turek *et al.* 2013). Competition and hybridization are the two most commonly cited mechanisms by which non-native rainbow trout influence native populations, yet rainbow trout are piscivorous and have been shown to feed on forage fish in Nebraska streams (Klammer 1984). Further, longnose dace may be at an increased risk of predation in headwater streams because large, native, piscivorous fish predators are not common in systems with longnose dace (Nebraska Game and Parks Commission unpublished data). Native predators of longnose dace in Nebraska streams consist almost entirely of birds (*e.g.*, Belted Kingfishers and Great Blue Herons), mammals (*e.g.*, Raccoons and Mink),

and macroinvertebrates (*e.g.*, Odonates and Belostomids). Creek chub and grass pickerel are both native to these systems and likely prey on cyprinids to some extent (*e.g.*, Schlosser 1988, Scott and Crossman 1973b). However, relative to other predators, they probably have little overall impact on native fish populations due the low abundance of large piscivorous individuals.

Longnose dace populations are thought to be secure throughout their range, yet some populations have responded negatively to the introduction of non-native species. Introduction of non-native fishes was cited as a factor in the decline of the now extinct Banff longnose dace *Rhinichthys cataractae smithi*, once found only in a single marsh in Alberta, Canada (Miller *et al.* 1989). Similarly, rainbow trout are cited as a major factor in the extinction of Grass Valley Speckled dace *Rhinichthys osculus reliquus*, a closely related species to longnose dace, in Nevada (Miller *et al.* 1989).

Concern over the potential negative influence of stocking non-native trout in Nebraska streams has led resource managers to prohibit stocking any trout species in streams containing species of concern, or at-risk species, until more is known about these interactions. Rainbow trout are still routinely stocked in streams with abundant native species. Examining interactions in these systems will lead to a better understanding of the ecological interactions between rainbow trout and longnose dace. Equally important, examining these interactions may also provide insight into the potential interactions among introduced trout and species of concern, and thus inform future management decisions involving stocking trout into streams with species of concern.

The first step in understanding the complex interactions among these two species is to determine if there is a direct predatory threat of non-native rainbow trout to longnose

dace, and how that threat changes over time. Therefore, a series of in-stream enclosures were used to determine if the presence, density, or acclimation period of rainbow trout influenced the number of longnose dace remaining in enclosures. Prey size also likely affects escapement and predation rates. Therefore, the influence of longnose dace length on the probability that longnose dace escaped from control enclosures, and the probability that longnose dace survived in treatment enclosures was examined. We also examined the stomachs of rainbow trout three days after introduction into enclosures with longnose dace to determine if rainbow trout consumed longnose dace, and if rainbow trout density or length explained the presence of longnose dace in the stomachs of rainbow trout.

METHODS

Study Site

Long Pine Creek is a second order, cold-water tributary to the Niobrara River, located on the edge of the Nebraska Sandhills and Northwestern Great Plains Ecoregions (Level III, US EPA). Long Pine Creek is Nebraska's longest self-sustaining trout stream with approximately 30 km of trout-supporting water. It has historically been stocked with brook trout *Salvelinus fontinalis*, brown trout *Salmo trutta*, and rainbow trout. The study site was on private land approximately 5 km south of Long Pine, Nebraska. Brown trout and rainbow trout were prevalent during 2012 in the stream reach containing enclosures. Natural reproduction of both brown trout and rainbow trout was evident, as smaller than stocked size fish were collected.

Longnose dace are native to Long Pine Creek and were first recorded in the stream in 1939 (NGPC unpublished data). No longnose dace were collected from the study reach during 2012, although longnose dace were abundant just upstream of the study site during 2011 and records indicate they historically occupied the study site.

Field Methods

Twelve enclosures (1.5-m width x 3.0-m length x 0.9-m height) constructed of 2.54-cm PVC pipe and 0.6-cm hardware wire (Figure 3-1) were placed in Long Pine Creek during July and August 2013. Enclosures also included a fake undercut bank (0.6-m width x 2.4-m length) made of landscaping fabric and PVC pipe that floated at the surface of the water and was anchored to one side of the enclosure. Longnose dace were collected from Plum Creek, Fairfield Creek, and Bone Creek, Brown County, Nebraska (Table 3-1) using a pulsed-DC backpack electrofisher and allowed to acclimate to Long Pine Creek in a temporary enclosure for 1 to 2 days. Five longnose dace (0.9 fish/m^2) were then weighed, measured, and randomly assigned to each enclosure. Longnose dace densities in enclosures were similar to previously reported natural densities of forage fish in Long Pine Creek (0.7 fish/m^2, Klammer 1984). Abundance of longnose dace was monitored daily following introduction to ensure escapement was not possible. Additional longnose dace were added until all enclosures successfully held 5 longnose dace for 24 hours. All longnose dace in each round were from the same source stream.

Following longnose dace introduction, a randomized complete block design (blocked by longitudinal position along the stream) was used to randomly assign rainbow trout density treatments to enclosures. Enclosures were blocked by longitudinal position

along the stream to account for any potential confounding effects based on spatial position along the stream. Rainbow trout density treatments were chosen to reflect a range of natural densities in Nebraska streams. Rainbow trout density treatments were 1) control (0 rainbow trout/enclosure), 2) average density (2 rainbow trout/enclosure), and 3) high density (4 rainbow trout/enclosure). The experiment was repeated four times (rounds) for a total of 16 replicates per treatment (4 within each round x 4 rounds; Figure 3-2).

Rainbow trout were transported from Grove Trout Rearing Station, Antelope County, Nebraska and temporarily placed into two extra enclosures. Rainbow trout were allowed to acclimate for 3 to 22 days before being introduced into enclosures with longnose dace (Table 3-1). Following rainbow trout introduction, abundance of all fish was checked every 12 hours at approximately 0530 and 1730 (optimal light conditions) for 72 hours. Abundance was checked by quickly lifting enclosures from the stream and counting individuals. Dead, unconsumed longnose dace were immediately removed from enclosures and were not replaced during the experiment. All fish were removed from enclosures and euthanized after 72 hours. Rainbow trout stomachs were immediately checked for the presence of longnose dace and macroinvertebrates (with the exception that for round 1, only rainbow trout in enclosures with longnose dace missing were examined). Macroinvertebrates and other food items were recorded to determine if rainbow trout were feeding on alternative food sources.

Water temperature, dissolved oxygen, and conductivity were measured in each enclosure once for each replicate. Depth and velocity was measured at three evenly spaced points along a transect positioned approximately 3.0 m upstream of enclosures, as

well as at 3 transects within the enclosures (front, middle, back) to determine if flows within enclosures were similar to natural conditions.

Data Analysis

Longnose Dace Mortality

Generalized linear mixed models (PROC GLIMMIX, SAS v.9.2) were used to evaluate differences in the number of dace remaining in enclosures (Poisson distribution) between rainbow trout density treatments and number of days rainbow trout were allowed to acclimate to the stream prior to introduction into enclosures. Any change in the number of longnose dace remaining, relative to control enclosures, was assumed to be the direct result of rainbow trout treatments. Therefore, the number of longnose dace remaining in enclosures, relative to control enclosures, was assumed to be inversely correlated with mortality of longnose dace (*i.e.*, a decrease in the number of dace remaining is representative of an increase in longnose dace mortality). Models included fixed effects of *treatment* (*i.e.*, control, average, and high rainbow trout density), *time* (*e.g.*, 12, 24, and 36 hours since stocking rainbow trout into enclosures), and rainbow trout *acclimation period* (*i.e.*, 3, 4, 5, 11, and 22 days). Random effects included *round*, *block*, *enclosure*, *enclosure* (*round*block*), and *time* by *enclosure* (*round*block*). However, blocking by *round*, *block*, *enclosure, and enclosure* (*round*block*) accounted for very little variation and so were removed from the models. A first-order autoregressive covariance structure (*AR1*) and Kenward-Roger degrees of freedom correction were used to account for repeated measures in order to reduce the risk of a type I error.

Generalized linear models (PROC GLIMMIX, SAS v.9.2) were used to assess the influence of longnose dace length on the probability that longnose dace escaped (using only fish in control enclosures; binomial distribution), and the influence of longnose dace length on the probability that longnose dace survived in treatment enclosures (binomial distribution). The lengths of longnose dace present at the end of the experiment were measured and matched to pre-stocking lengths. Lengths of longnose dace that were not accounted for were determined via process of elimination. Generalized linear models were also used to evaluate the influence of rainbow trout density treatments on the number of unconsumed dead dace (Poisson distribution) removed from enclosures.

Rainbow Trout Diet

The percentages of rainbow trout stomachs containing macroinvertebrates and longnose dace, as well as percent empty stomachs were calculated. Ninety-five percent confidence intervals (95% CI) were calculated following methods in Fleiss *et al.* (2003) for proportions assuming a binomial distribution. A generalized linear model (PROC GLIMMIX, SAS v.9.2) was used to determine if rainbow trout density treatment or rainbow trout length explained the presence or absence of longnose dace (binomial distribution) in the stomachs of rainbow trout.

Enclosures and Water Quality

Linear mixed models (PROC GLIMMIX, SAS v 9.2) were used to evaluate differences in water temperature, dissolved oxygen, and conductivity between treatments (blocked by round). Linear mixed models were also used to determine if water depths

and velocities within enclosures were similar to natural conditions (measurements taken 3.0 m upstream of enclosures; blocked by *round*enclosure*).

RESULTS

Longnose Dace Mortality

Five of the 80 longnose dace present in control enclosures escaped during the experiment (two in round 1 and three in round 2). However, the number of longnose dace remaining in enclosures decreased at a faster rate in average and high rainbow trout density treatments than in control treatments ($F_{2,258.9} = 3.73$, $P = 0.03$; Figure 3-3). The number of longnose dace remaining in enclosures also decreased at a faster rate in enclosures in which rainbow trout were acclimated to the stream for more days regardless of treatment ($F_{4,148.5} = 2.50$, $P = 0.04$; Figure 3-4). There was no rainbow trout acclimation period by treatment interaction ($F_{4,131.4} = 1.09$, $P = 0.37$).

Longnose dace in three enclosures were excluded from length analyses because accurate assignments of lengths could not be determined for all fish in those enclosures. Longnose dace length did not influence the probability that fish escaped in control enclosures ($F_{1,58} = 2.63$, $P = 0.11$) for the last three rounds. However, longer longnose dace had a greater probability of survival in enclosures with rainbow trout ($F_{1,103} = 3.73$, $P = 0.06$) for the last three rounds. Post-experiment longnose dace lengths were not available for the first round, and thus could not be matched to pre-stocking lengths to evaluate the influence of length on the probability of escapement or survival. The number of dead, unconsumed longnose dace removed from enclosures also did not differ between trout density treatments ($F_{2,45} = 0.36$, $P = 0.70$).

Rainbow Trout Diet

In the first round, where rainbow trout stomachs were examined only for enclosures where longnose dace were missing, 58 ± 29% (mean ± 95% CI; n = 7) of rainbow trout stomachs contained macroinvertebrates, 42 ± 29% were empty (n = 5), and 8 ± 16% contained longnose dace (n = 1). In the last three rounds (where all rainbow trout stomachs were examined regardless of longnose dace presence or absence in enclosures), 72 ± 11% of rainbow trout stomachs contained macroinvertebrates (n = 51), 28 ± 11% were empty (n = 20), and 7 ± 6% contained longnose dace (n = 5). The percent of rainbow trout stomachs that contained longnose dace and macroinvertebrates increased, and the number of rainbow trout stomachs that were empty decreased, with increased rainbow trout acclimation time (Figure 3-5). All stomachs (n = 6) that contained longnose dace also contained macroinvertebrates. Neither rainbow trout density ($F_{1,68}$ = 1.44, P = 0.23) nor rainbow trout length ($F_{1,68}$ = 3.42, P = 0.07) influenced the presence of longnose dace in stomachs for the last three rounds. The first round was excluded because only stomachs in enclosures with longnose dace missing were examined, whereas all stomachs were examined in subsequent rounds.

Enclosures and Water Quality

Water temperature ($F_{2,31}$ = 0.27, P = 0.77), dissolved oxygen ($F_{2,31}$ = 0.64, P = 0.53), and conductivity ($F_{2,31}$ = 0.05, P = 0.96) in enclosures did not differ between treatments in the last three rounds. The first round was omitted because equipment malfunction did not allow for measurements in all enclosures. Depth and velocity within

enclosures was similar to depth and velocity approximately 3.0 m upstream of enclosures ($F_{1,47}$ = 3.47, P = 0.07) for all enclosures.

DISCUSSION

This study confirms that rainbow trout do prey on longnose dace, but at a low rate over the relatively short duration of our experiment. Both presence of rainbow trout and acclimation period of rainbow trout to the stream prior to introduction into enclosures had significant influences on the number of longnose dace remaining in enclosures. Other factors such as predator experience, prey length, and habitat availability may also influence predation rates.

The number of longnose dace remaining in enclosures decreased at a faster rate in enclosures with rainbow trout compared to enclosures without rainbow trout, suggesting that some predation occurred. Rainbow trout are not widely recognized as piscivores, yet several studies have shown rainbow trout pose a predatory threat to native fishes, and that threat can be amplified in areas with high abundance of rainbow trout (*e.g.*, Crowl *et al.* 1992, Yard *et al.* 2011).

The density of rainbow trout did not influence the number of longnose dace remaining, however, the presence of rainbow trout did. Predator interference (*i.e.*, among multiple rainbow trout) or altered prey behavior (*i.e.*, in the presence of more predators) may explain the absence of greater predation rates in enclosures with high rainbow trout densities relative to average trout densities. Predator interference can lead to a non-linear relation between per capita predation rates and prey densities (*e.g.*, DeAngelis *et al.* 1975, Beddington 1975), and may reduce predation risk for longnose dace at high rainbow trout

densities. Longnose dace may also alter their behavior (*e.g.*, foraging behavior and timing) to decrease predation risk at high rainbow trout densities (*e.g.*, Lima and Dill 1990). Alternatively, rainbow trout densities may have been too similar to detect a difference in prey responses (*i.e.*, longnose dace may perceive 2 trout and 4 trout as the same predation risk).

Spatial scale may also play a role in the relative importance of trout density and trout presence on longnose dace survival. Based on data from previous Nebraska stream surveys (reach scale) we determined that there may be a threshold of trout density above which trout have an impact on native fish communities, but below which trout have little influence on native fish communities (Chapter 4). In enclosures, rainbow trout presence influenced longnose dace survival, but density did not. Therefore, trout *presence* may play a greater role at smaller scales and trout *density* may play a greater role at the reach scale.

The number of longnose dace remaining in enclosures also decreased at a faster rate in enclosures with trout acclimated for 11 days or 22 days compared to enclosures with trout acclimated for 3, 4, or 5 days regardless of trout density suggesting that longnose dace mortality rate was greater with longer predator acclimation periods. Similarly, examination of rainbow trout stomachs revealed that the percent of stomachs with longnose dace and macroinvertebrates increased, and the percent empty stomachs of rainbow trout decreased with increased rainbow trout acclimation time (Figure 3-5). Increased acclimation time may be a surrogate measurement for decreased stress or increased hunger in this study. Stress from handling or transportation can increase metabolic rates, impair immune function and lead to decreased disease resistance, and

thus decrease growth, condition, and fitness (*e.g.*, Wedemeyer *et al.* 1990). Increased acclimation periods after stocking may allow rainbow trout to fully recover and thus lead to higher predation rates or more aggression. In a study of stocking stress in fingerling rainbow trout, plasma cortisol levels (an indicator of stress) did not return to basal levels until 8 days post stocking (Barton *et al.* 1980). Other studies have reported much more rapid recovery after stressful events, however, fish were generally stressed for much shorter periods (Flors *et al.* 1988). The greater predation rates in enclosures where trout were acclimated for 11 or 22 days, compared to enclosures where trout were acclimated for 3, 4, or 5 days may indicate trout had a chance to recover to an unstressed condition with longer acclimation periods before being introduced into enclosures.

The increased acclimation period of rainbow trout may have also resulted in increased hunger because rainbow trout were not fed while acclimating, and thus, increased acclimation period may have artificially increased risk of predation for longnose dace. Alternative food sources were available for rainbow trout although they were not quantified. The rigid structure of the enclosures allowed caddisfly colonization, and enclosures were cleaned every 12 hours allowing drift to enter the enclosure. Observations of large numbers of macroinvertebrates on or in enclosures combined with the abundance of macroinvertebrates, particularly caddisflies, in the diet of rainbow trout suggest that alternative food sources were plentiful and may have actually been artificially inflated in the diet (especially caddisfly abundance). If this were the case, the predation rates on longnose dace would be considered conservative relative to natural conditions. Conversely, it is also possible that other macroinvertebrates constitute large

portions of natural rainbow trout diets in these systems, and would reduce the predatory risk to longnose dace as the caddisflies may have in enclosures.

Predator experience is also known to influence predation rates (*e.g.*, Ware 1971). Predation rates are likely to increase with increased experience, suggesting that initial predation rates may underestimate overall predation rates throughout the life of a rainbow trout. In laboratory experiments testing the effect of experience on predation, rainbow trout required an average of 4 days of experience (ranged from 1 to 11 days) with novel prey prior to approaching the prey (Ware 1971). In this experiment, rainbow trout were not previously exposed to longnose dace, and predation rates in enclosures were only monitored for 3 days. Predation may increase with increased exposure to longnose dace.

Predation rates are also often dependent on prey size (*e.g.*, Werner and Gilliam 1984). In this study, longer longnose dace had a greater probability of survival in enclosures with rainbow trout. This may be due to decreased susceptibility to predation (*i.e.*, rainbow trout gape limitation) or increased ability to escape predation. The largest longnose dace assumed to have been consumed was 87 mm (95th percentile of all longnose dace lengths), suggesting that gape limitation was probably not a factor. Similarly, trout do not appear to be gape limited by longnose dace under natural conditions (Chapter 4), suggesting something other than size alone (*e.g.*, increased swimming velocities with size) must explain the survival advantage of larger longnose dace.

Habitat availability and complexity can also significantly influence predatory interactions. Habitat availability and complexity in enclosures were equivalent across treatments. No artificial habitat was added within enclosures, however, longnose dace

were observed under the PVC pipe that made up the frame of the enclosure and between the PVC pipe and hardware wire, where they were inaccessible to rainbow trout. These areas provided some habitat complexity, however, how that translates to the natural availability of habitat is unknown. Further research is needed to determine if, in fact, rainbow trout and longnose dace occupy the same habitat in these systems along with what influence habitat complexity and availability have on these interactions. However, if rainbow trout and longnose dace do overlap in habitat (as was forced in this situation) then some rainbow trout predation on longnose dace is likely.

Summary

We observed that stock-size rainbow trout in Nebraska are physically capable of consuming longnose dace, and if rainbow trout and longnose dace overlap in microhabitat use, some predation is likely to occur. The impact of "some predation" could be substantial for species of concern, as any loss of individuals could have a detrimental impact on the entire population. Conversely, "some predation" may have little influence on the overall population abundance of other species (Chapter 4). Therefore, the potential overall impact of non-native trout predation on native populations should be considered prior to stocking non-native trout. Additionally, even if non-native trout predation is unlikely to influence the abundance of native fishes, managers should consider the potential indirect predatory (*e.g.*, changes in behavior) and competitive (*e.g.*, changes in food resource availability) threats to native species.

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Table 3-1. Study details for each round of the experiment including rainbow trout (RBT) and longnose dace (LND) lengths and weights, streams from which LND were collected (LND Source), and acclimation time of RBT to stream where experiments were conducted.

	Date RBT Stocked	RBT Length (mm)	RBT Weight (g)	LND Length (mm)	LND Weight (g)	LND Source	Acclimation Time RBT
Round 1	7/19/2013	289 ± 17	254 ± 38	71 ± 8	3 ± 1	Plum Creek	4
Round 2	7/29/2013	290 ± 17	281 ± 48	69 ± 7	3 ± 1	Plum Creek	3
Round 3	8/06/2013	290 ± 19	258 ± 59	70 ± 6	2 ± 1	Fairfield Creek	*11/22
Round 4	8/21/2013	294 ± 14	282 ± 42	79 ± 8	4 ± 1	Bone Creek	5

*Half of the enclosures were stocked with RBT that had an 11 day acclimation period and half with a 22 day acclimation period.

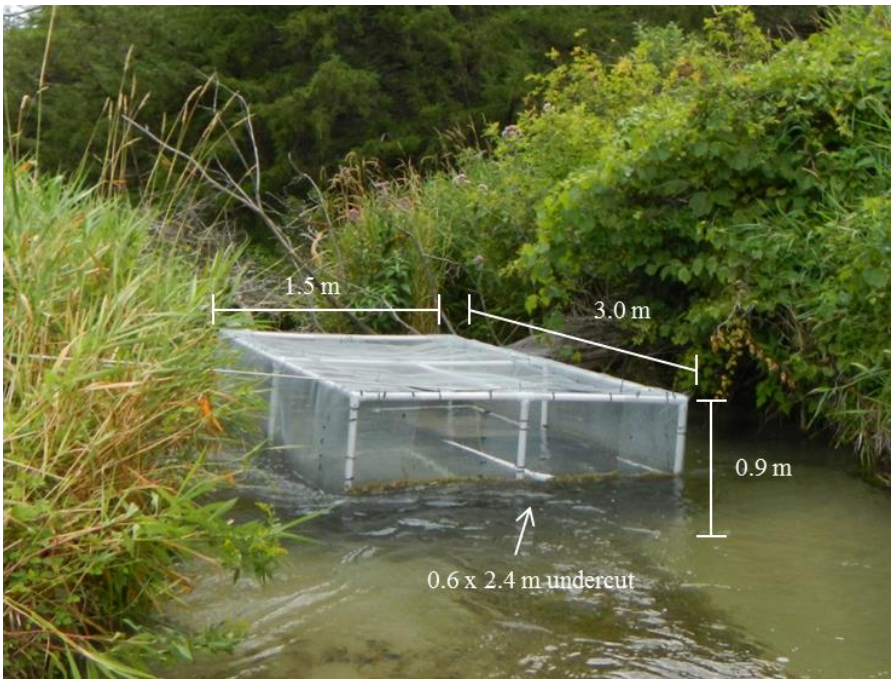

Figure 3-1. Picture of enclosure design in Long Pine Creek.

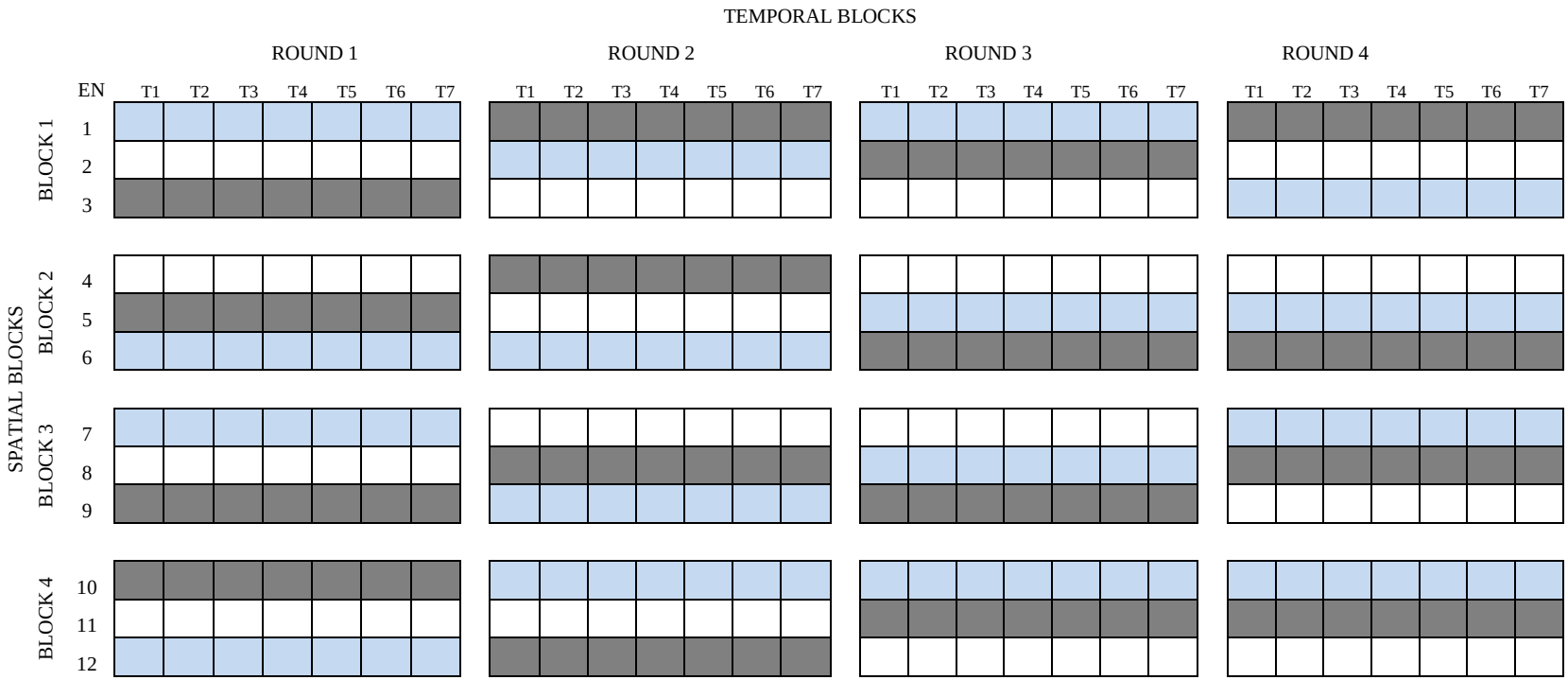

Figure 3-2. Study design for enclosure experiment. A randomized complete block design was used to assign three treatments (different colors) to enclosures (EN) within spatial blocks and rounds. The response variable was measured at 7 points in time throughout the experiment (T1-T7) for each enclosure. New fish were used and treatments were re-randomized each round.

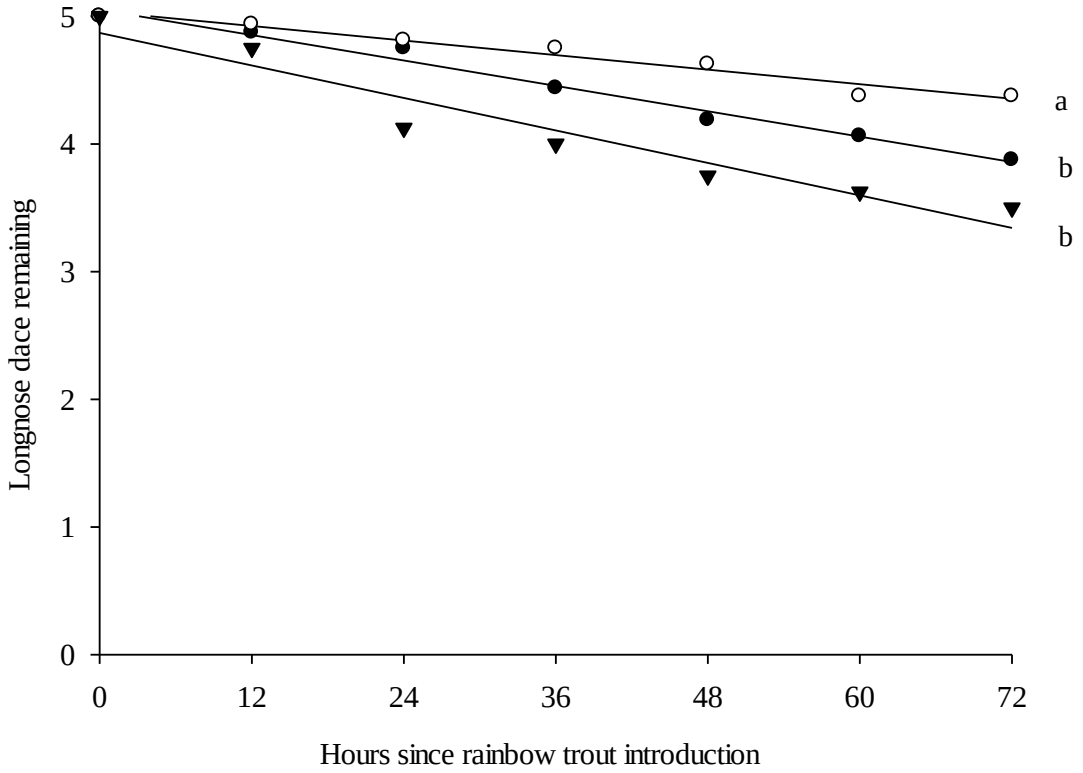

Figure 3-3. Number of longnose dace remaining in enclosures with no trout (open circle), enclosures with 2 trout (closed triangle) and enclosures with 4 trout (closed circle). Different letters indicate significant difference in slopes of lines ($\alpha = 0.05$).

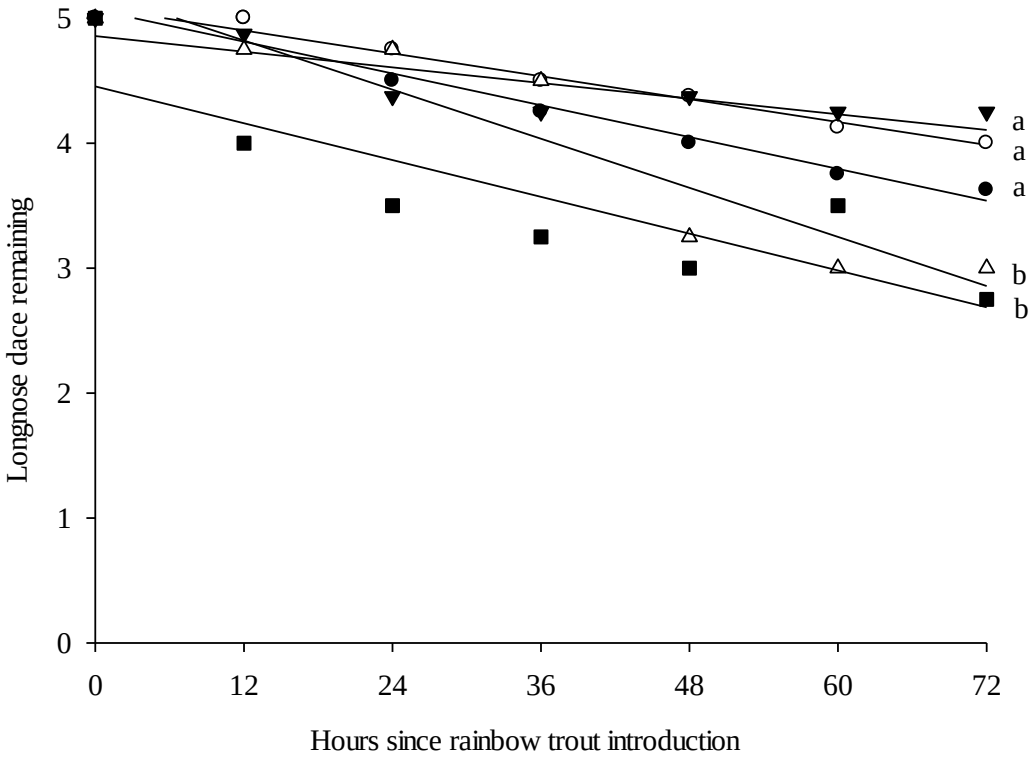

Figure 3-4. Number of longnose dace remaining in enclosures in which rainbow trout were acclimated for 3 (closed circle), 4 (open circle), 5 (closed triangle), 11 (open triangle), and 22 (closed square) days. Different letters indicate significant difference in slopes of lines ($\alpha = 0.05$).

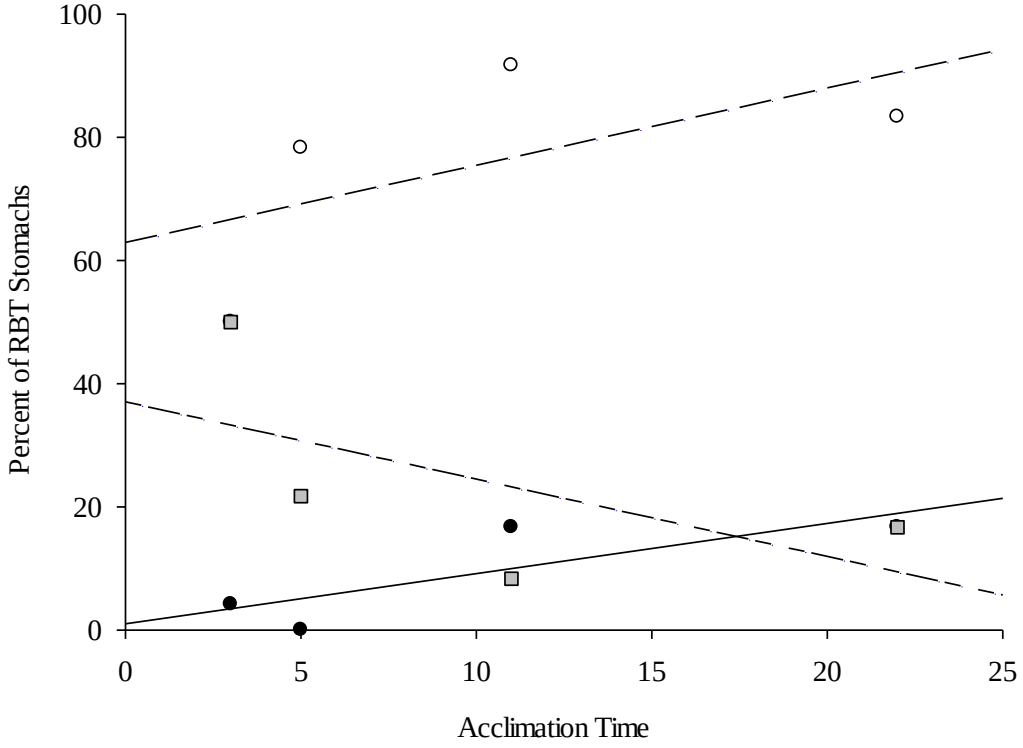

Figure 3-5. Percent of rainbow trout (RBT) stomachs that contained longnose dace (closed circle), contained macroinvertebrates (open circle), or were empty (closed square) across rainbow trout acclimation times.

CHAPTER 4. POTENTIAL POPULATION AND COMMUNITY-WIDE INFLUENCES OF NON-NATIVE TROUT ON NATIVE FISH IN NEBRASKA HEADWATER STREAMS

ABSTRACT

Non-native trout are currently stocked in headwater streams throughout Nebraska. The influence of non-native trout introductions on native fish populations and their role in structuring fish communities in these systems is unknown. The objectives of this study were to determine 1) if the size structure or relative abundance of native species differs in the presence and absence of non-native trout, 2) if native fish community composition or structure differ in the presence and absence of non-native trout, and 3) if native fish community composition or structure differ across a gradient in abundances of non-native trout in headwater streams throughout Nebraska. Size structures of longnose dace *Rhinichthys cataractae* and white sucker *Catostomus commersonii* were larger in the presence of brown trout *Salmo trutta*, and size structure of longnose dace was smaller in the presence of rainbow trout *Oncorhynchus mykiss*. Creek chub *Semotilus atromaculatus* and fathead minnow *Pimephales promelas* size structures did not differ in the presence and absence of non-native trout. Relative abundances of longnose dace, white sucker, creek chub, and fathead minnow were similar in the presence and absence of trout. There was greater distinction between native fish community composition and structure in sites with trout compared to sites without trout as trout abundances increased. There appears to be increased risk to native communities in sites with high abundances of trout, however, more research is needed to determine the role of non-native trout in structuring native fish communities in Nebraska streams, as well as the mechanisms through which introduced trout may influence native fish populations.

INTRODUCTION

Stream fish communities are formed as a result of abiotic (*e.g.*, temperature and flow regime) and biotic pressures (*e.g.*, predation and competition; Grossman and Sabo 2010). The relative importance of these pressures in structuring fish communities is frequently debated (Ross 1991; Grossman and Sabo 2010), and pressures often change through time due to anthropogenic activities such as stream impoundment, habitat alteration, and fish stockings. Non-native species in particular, can substantially alter fish communities (Ross 1991), and are considered one of the major causes of widespread declines in freshwater biodiversity (*e.g.*, Miller 1989, Lassuy 1995, Wilcove *et al.* 1998, Dudgeon *et al.* 2006).

Salmonids are currently one of the most widespread and frequently stocked non-native species in the world (Welcomme 1992, Krueger and May 1991). Introduced salmonids have directly and indirectly influenced native populations (*e.g.*, abundance, distribution, life history, and size structure) and communities (*e.g.*, composition and structure) in several systems via competition, predation, hybridization, and disease and pathogen transfer (*e.g.*, Turek *et al.* 2013). However, most studies examining the influences of non-native salmonids on native species have focused on salmonid influences on native game fish and their supporting communities. Understanding the ecological risk to native nongame fish species is studied less, but is of primary concern because many native threatened and endangered species fall into the nongame realm. Anticipating potential interactions between introduced non-native salmonids and native species will help ensure conservation of these species.

Non-native trout are currently stocked in several headwater streams throughout Nebraska. Recent reductions in abundance and distribution of some native species, along with numerous examples of negative predatory and competitive interactions between non-native trout and native species in other systems (*e.g.*, Turek *et al.* 2013), have prompted resource managers to prohibit stocking of trout in streams containing at-risk native fishes. However, the influence of non-native trout introductions on native fish populations and their role in structuring fish communities in these systems has not been examined. The relatively simple fish community structure of headwater streams, along with the lack of a widespread native predatory fish species, may exacerbate the impact of non-native trout on native species in these systems. Identifying what factors may or may not influence native fish community composition and structure will assist in efforts to conserve at-risk native fishes. Therefore, the objectives of this study were to determine 1) if the size structure or relative abundance of native species differs in the presence and absence of non-native trout, 2) if native fish community composition or structure differs in the presence and absence of non-native trout, and 3) if native fish community composition or structure differ across a gradient in abundances of non-native trout in headwater streams throughout Nebraska.

METHODS

Study Area

Fifty-six sites were surveyed in headwater streams throughout northern and western Nebraska from June 29, 2011 to August 17, 2011. These low gradient headwater streams are typically groundwater fed, with sandy substrate, herbaceous riparian

vegetation, and little in-stream habitat complexity. Sites were located mainly within three EPA defined Level III Ecoregions: Western High Plains, Nebraska Sandhills, and Northwestern Glaciated Plains. All three Ecoregions are characterized by semi-arid to arid climates, and short-grass or mixed-grass prairie. Dominant land uses include cropland agriculture and rangeland (Chapman *et al.* 2001). Native fish assemblages include catostomids (*e.g.*, *Catostomus spp.* and *Moxostoma spp.*), centrarchids (*e.g.*, *Lepomis spp.* and *Micropterus spp.*), cyprinids (*e.g.*, *Rhinichthys spp.*, *Semotilus spp.*, and *Notropis spp.*), ictalurids (*e.g.*, *Ameiurus spp.* and *Noturus spp.*), and percids (*e.g.*, *Etheostoma spp.*). Non-native species include brook trout *Salvelinus fontinalis*, brown trout *Salmo trutta*, and rainbow trout *Oncorhynchus mykiss*.

Sampling Methods

Fish were collected using a pulsed-DC backpack electrofisher with one netter. Output voltage settings ranged from 150 to 350 V and frequency settings from 40 to 80 Hz. A minimum of two 600-s reaches were sampled at each site. Additional reaches were sampled until no new species were collected in a reach for that site. If fish were too abundant to finish an entire 600-s reach due to concern over survival rates, sampling was stopped. All fishes were identified to species. Total lengths (mm) of the first 20 fish of each species were recorded, and all identified fishes were released. Unknown specimens were kept, preserved in 10% formalin, and identified later in the laboratory.

Habitat (including substrate, fish cover, and riparian cover) and human-use characteristics were also recorded because these characteristics likely alter the magnitude and nature of non-native and native species interactions. Habitat and human-use

characteristics were measured according to Nebraska Game and Parks Commission stream survey methods. Dominant substrate (≥ 50%), sub-dominant substrate (< 50%), and substrate also present were recorded for each site. However, only dominant substrate was analyzed because sub-dominant and also present categories could not be distinguished. Fish cover categories (*e.g.*, filamentous algae, macrophytes and woody debris) were classified categorically based on type and percent cover (0 – none; 1 – sparse (1-10%); 2 – moderate (11-40%); 3 – heavy (41-75%); 4 – very heavy (>75%)). Riparian cover (*e.g.*, trees, grasses, herbs and forbs) was similarly classified based on type and percent cover. Presence of human use (*e.g.*, parks, landfill, and mining activity) was categorized based on type and proximity to stream (0 – none; 1 – on bank; 2 – within 10 m; 3 – >10 m).

Native Fish Populations in the Presence and Absence of Non-native Trout

Size structures of native species were compared between sites with and without trout using Kolmogorov–Smirnov two sample tests (PROC NPAR1WAY; SAS v.9.2). Relative abundances of native species (number of fish/s) were compared between sites with and without trout using linear models (PROC GLIMMIX; SAS v.9.2). Size structures and relative abundance were examined between 1) sites where any trout species or a combination of trout species were present and sites where no trout species was present, 2) sites where brown trout were the only trout species present and sites where no trout species was present, 3) sites where rainbow trout were the only trout species present and sites where no trout species was present, and 4) sites where brook trout were the only trout species present and sites where no trout species were present.

Differences in habitat and human-use characteristics between sites with and without trout were compared using one-way analysis of similarity (similarity matrix based on Euclidean distance, ANOSIM procedure, Primer-E v.6; Clarke and Gorley 2006). Emphasis was placed on Global R values to determine differences for all ANOSIM, as suggested by Clarke and Gorley (2006), instead of *P*-values because sample sizes are known to bias *P*-values. Global R values close to zero indicate no differences among groups, whereas values close to one indicate complete separation among groups. In general, it is accepted that communities are similar if Global R values are less than 0.20 (Clarke and Gorley 2006), and are different if Global R values are greater than 0.40 (Clarke and Warwick 2001). These two guidelines were used to determine the relative abundances at which non-native trout influence native community composition and structure. Differences in habitat and human-use characteristics between sites with and without trout were also compared visually using non-metric multidimensional scaling (NMDS; MDS procedure, Primer-E v.6; Clarke and Gorley 2006). Stress values for NMDS indicate "goodness-of-fit" where lower values indicate less stress and better fit (Kruskal and Wish 1978).

Native Fish Community Composition and Structure in the Presence and Absence of Non-native Trout

Fish community assessments were made using only native species collected during 2011 stream surveys (*i.e.*, excluding non-native trout). Non-native trout were removed from the analysis because the addition of non-native trout may inherently change the fish community composition and structure in sites where trout are introduced

compared to sites where trout are not introduced (*i.e.*, the addition of a new species will, in itself, change the community). If the addition of non-native trout is having a real effect on the composition or structure of the native fish community, removing non-native trout from the analyses should still result in a change in the remaining native fish community between sites where trout were present and sites where trout were not present (*i.e.*, the change in community will not be due solely to the addition of new species).

Differences in native fish community composition (presence/absence, Kulczynski similarity matrix) and structure (relative abundance, fourth-root transformed, Bray-Curtis similarity matrix) between sites with and without trout, were compared using one-way analysis of similarity (ANOSIM procedure, Primer-E v.6; Clarke and Gorley 2006). Fish community composition and structure in these sites were also visually assessed using NMDS (MDS procedure; Primer-E v.6; Clarke and Gorley 2006). Differences in fish community composition (presence/absence, Kulczynski similarity matrix) and structure (relative abundance, fourth-root transformed, Bray-Curtis similarity matrix) between sites with and without trout nested within basins and Ecoregions were also compared independently using two-way nested analysis of similarity (ANOSIM procedure, Primer-E v.6; Clarke and Gorley 2006) to account for more variation in communities between these geographic regions. The extent to which habitat and human-use characteristics (similarity matrix based on Euclidean distance) explained fish community composition (species presence/absence, Kulczynski similarity matrix) and structure (relative abundance, fourth-root transformed, Bray-Curtis similarity matrix) was analyzed using Mantel tests (BEST procedure, Primer-E v.6; Clarke and Gorley 2006).

Native Fish Community Composition and Structure at Differing Abundances of Non-native Trout

The number of trout collected in any one site was expected to vary widely, and the assumption that widely varying abundances have the same influence on native fish communities is not realistic. To test the effect of trout abundance on native fish community composition and structure, sites with trout were first divided into five subsets with like abundances. These subsets were based on the following percentiles when ranked by abundance: $\leq 20^{th}$ percentile (CPUE $\leq$ 0.25 trout/min), 21 – 40th percentiles (CPUE 0.31 – 0.48 trout/min), 41 – 60th percentiles (CPUE 0.50 – 0.98 trout/min), 61 – 80th percentiles (CPUE 1.22 – 1.73 trout/min), and $\geq 81^{st}$ percentile (CPUE $\geq$ 1.97 trout/min). These subsets were chosen to allow for a balance between number of subsets (n = 5) and number of sites within each subset (n = 7). Using a bootstrapping approach (1,000 iterations), native fish community composition (presence/absence, Kulczynski similarity matrix) was then compared between sites with trout (each subset) and a randomly chosen set of sites without trout (n = 7) using one-way analyses of similarity (ANOSIM function, vegan library, R v.2.15.1). Global R values (mean ± 95% CI) from the analyses of similarities (1,000 iterations) were then compared across the 5 subsets of trout CPUEs to determine if greater abundances of trout had a greater influence on native fish community composition. This process was repeated to compare native fish community structure (relative abundance, fourth-root transformed, Bray-Curtis similarity matrix) between sites with and without trout within the same five subsets of sites.

Habitat differences between subsets of sites with trout may result in differences in native community composition or structure between subsets regardless of trout

abundances. Therefore, differences in habitat and human-use characteristics (similarity matrix based on Euclidean distance) between the five subsets of sites with trout were compared using one-way analysis of similarity (ANOSIM procedure, Primer-E v.6; Clarke and Gorley 2006).

RESULTS

Native Fish Populations in the Presence and Absence of Non-native Trout

Size structure

Of the 56 sites surveyed in 2011, no fish were collected in two sites, non-native trout were collected in 35 sites, and native fish were collected but non-native trout were not collected in 19 sites. Only native species found in at least one third of sites with fish (*i.e.,* creek chub *Semotilus atromaculatus,* fathead minnow *Pimephales promelas,* longnose dace *Rhinichthys cataractae*, and white sucker *Catostomus commersonii*), as well as the two most abundant potential fish predators aside from trout (*i.e.,* green sunfish *Lepomis cyanellus*, and creek chub), were collected in sufficient numbers to assess potential responses to trout presence.

A total of 1,661 creek chubs was collected at 28 sites in 9 watersheds during 2011. The size structure of creek chub was similar in sites where brown trout were the only trout species present ($KS_a = 0.95$, $P = 0.32$), and sites where any trout species or a combination of trout species were present ($KS_a = 1.07$, $P = 0.20$) compared to sites where trout were not present. Only four creek chub were found in the presence of rainbow trout, and only one creek chub was found in the presence of brook trout. Therefore, size

structures were not compared between sites where rainbow trout or brook trout were the only non-native trout species present and sites where trout were not present.

A total of 1,738 fathead minnow was collected at 21 sites in 11 watersheds during 2011. The size structure of fathead minnow was similar in sites where any trout species or a combination of trout species were present (KS_a = 0.97, P = 0.30) compared to sites where trout were not present. The majority of sites where fathead minnows were found in the presence of trout contained multiple species of trout; therefore, size structures were not compared for individual species of trout because of insufficient sample size.

A total of 2,082 longnose dace was collected at 33 sites in 10 watersheds during 2011. The size structure of longnose dace was larger in sites where brown trout were the only trout species present (KS_a = 1.68, P = 0.01; Figure 4-1), and sites where any trout species or a combination of trout species were present (KS_a = 1.54, P = 0.02; Figure 4-1) compared to sites where trout were not present. The size structure of longnose dace was smaller in sites where rainbow trout were the only trout species present (KS_a = 1.83, P = < 0.01; Figure 4-2) compared to sites where trout were not present. The size structure of longnose dace did not differ in sites where brook trout were the only trout species present (KS_a = 0.92, P = 0.36; Figure 4-2) compared to sites where trout were not present.

A total of 682 white suckers was collected at 34 sites in 10 watersheds during 2011. The size structure of white suckers was larger in sites where brown trout were the only trout species present (KS_a = 2.88, P = <0.01; Figure 4-3), and sites where any trout species or a combination of trout species were present (KS_a = 2.82, P = <0.01; Figure 4-3) compared to sites where trout were not present. The size structure of white suckers did not differ between sites where rainbow trout were the only trout species present and sites

where trout were not present (KS_a = 1.02, P = 0.25). No white suckers were found in the presence of brook trout.

The size structure of white suckers in sites where white suckers and longnose dace occurred together was compared to examine potential competitive interactions between longnose dace and white sucker and the effects of non-native trout presence on these interactions. The size structure of white suckers was larger in longnose dace sites where brown trout were the only trout species present (KS_a = 2.78, P = <0.01; Figure 4-4), and longnose dace sites where any trout species or a combination of trout species were present (KS_a = 1.91, P = <0.01; Figure 4-4) compared to longnose dace sites where trout were not present. The size structure of white sucker did not differ between longnose dace sites where rainbow trout were the only trout species present and longnose dace sites where trout were not present (KS_a = 0.80, P = 0.54). When sample size was sufficient to make comparisons, the size structures of other native species examined (*i.e.*, creek chub, fathead minnow, and green sunfish) did not differ between longnose dace and white sucker sites with and without trout (Table 4-1).

Relative Abundance & Habitat

Relative abundances of longnose dace and white suckers did not differ in the presence or absence of trout (Table 4-2). Similarly, relative abundances of white suckers in longnose dace sites did not differ in the presence or absence of trout (Table 4-3). Relative abundances of other native species examined did not differ in the presence or absence of trout across all sites (Table 4-2), longnose dace sites (Table 4-3), or white sucker sites (Table 4-4). Habitat and human-use characteristics did not differ between

sites where trout were present and sites where trout were absent across all sites (Figure 4-5), longnose dace sites, or white sucker sites (Table 4-5).

Native Fish Community Composition and Structure in the Presence and Absence of Non-native Trout

Native fish community composition (Global R: 0.012, P = 0.398; Figure 4-6_A) and structure (Global R: 0.015, P = 0.343, Figure 4-7_A) did not differ between sites with and without trout. Native fish community composition (Global R: -0.019, P = 0.514) and structure (Global R: 0.315, P = 0.171) also did not differ between sites with and without trout nested within basins. Similarly, native fish community composition (Global R: -0.219, P = 0.857) and structure (Global R: -0.031, P = 0.600) did not differ between sites with and without trout nested within Ecoregions. Habitat and human-use characteristics had weak correlations to native fish community composition (Table 4-6) and structure (Table 4-7).

The NMDS plot of native community structure in the presence and absence of non-native trout indicated that six sites were considerably different than the remaining 48 sites (Figure 4-7_A). Further examination of the fish community in these sites showed that although non-native trout were collected, no other fish were present (Table 4-8). These 6 sites were temporarily removed to examine what influence trout may have on native fish communities in sites with native fish present at the time of sampling (n = 48). Native fish community structure still did not differ between sites with and without trout when excluding sites without any native fish (Global R: 0.115, P = 0.008, Figure 4-7_B). Native fish community composition was also evaluated for only the 48 sites with native fish

present, and again did not differ between sites with and without trout (Global R: 0.012, *P* = 0.342, Figure 4-6_B).

Native Fish Community Composition and Structure at Differing Abundances of Non-native Trout

Trout CPUE varied widely across sites surveyed during 2011 (Figure 4-8) allowing us to test the relation between trout abundance and native community composition and structure. Greater trout abundances resulted in greater separation in native fish community composition (Figure 4-9) and structure (Figure 4-10) between sites with and without trout. Community composition (Figure 4-9) and structure (Figure 4-10) was similar or differed only slightly between sites with and without trout for the three subsets of data with the lowest trout abundances (Global R values < 0.40). Clear separation in native community composition (Figure 4-9) and structure (Figure 4-10) occurred in sites with trout CPUEs greater than 1.22 trout/min (Global R values > 0.40). Separation in these communities at greater abundances of non-native trout indicates changes in the native species present in these sites or changes in their abundance; however, species-specific contributions to dissimilarity for each subset could not be determined because of the bootstrapping methodology used. Habitat and human-use characteristics did not differ across the subsets of sites with trout present (Global R: -0.052, *P* = 0.863; Figure 4-13) suggesting no relationship between habitat and separation in native fish community composition or structure.

There were six sites in which trout were present, but no native fish were collected. Five of these six sites fell into the top two subsets of trout abundances (*i.e.*, those subsets

where there was clear separation in native communities between sites with and without trout). The lack of any native species in these six sites may have caused the increased distinction in native community composition and structure as trout abundances increased. Therefore, we re-ran the analyses excluding the six sites without native fish to determine what, if any, influence these sites had on the relation between trout abundance and native fish community composition and structure. However, removal of the six sites without native fish did not change the overall relation between trout abundance and separation in native fish community composition (Figure 4-11) or structure (Figure 4-12) between sites with and without trout.

DISCUSSION

Native Fish Populations in the Presence and Absence of Non-native Trout

Creek chub, fathead minnow, and green sunfish relative abundance and size structures were similar in the presence and absence of non-native trout (Table 4-1; Table 4-2; Table 4-3; Table 4-4). Similarly, longnose dace and white sucker relative abundance was similar in the presence and absence of non-native trout (Table 4-2; Table 4-3; Table 4-4). Trout may therefore, have little influence on the relative abundance of these species at the reach scale. Alternatively, only widespread, common native species were examined, potentially biasing our results. Those species prevalent enough to test differences may be those most capable of coexisting with trout.

Although there were no changes in relative abundance of the native species we examined, there were some differences in the size structures of longnose dace and white sucker. Longnose dace (Figure 4-1) and white sucker (Figure 4-3) size structures were

larger in the presence of brown trout, and longnose dace size structure was smaller in the presence of rainbow trout (Figure 4-2). Differences in size structure could be the result of differences in growth, recruitment, or mortality of these populations. Data (*e.g.*, age structure of populations) were not available to fully evaluate the biological implications of these differences for each species, or what mechanism is responsible for these differences. However, we suggest that the size structure differences we observed could potentially be biologically significant based on what is known about the growth and reproduction of these species. Further, we discuss some of the potential mechanisms causing these shifts.

Longnose dace is a short-lived species with a maximum age of 4 - 5 years (maximum size of approximately 125 mm; Scott & Crossman 1973a). Longnose dace mature at age 1 - 2 (Scott & Crossman 1973a, and Roberts & Grossman 2001). They spawn during the spring, but can spawn multiple times within a season (Roberts & Grossman 2001). Females lay between 200 and1200 eggs in a spawning event (Scott & Crossman 1973a) and potential fecundity in a given year is thought to range from approximately 1100 to 2500 eggs (Roberts & Grossman 2001). Longnose dace growth is slow relative to other species. Reed & Moulton (1973) summarized average lengths at ages of longnose dace from four different populations (including populations from Reed 1959 and Kuehn 1949). From this summary, longnose dace growth appears to average between 11 mm and 15 mm in a given year but ranges from 6 mm to 33 mm depending on the population, age, and sex of longnose dace (Reed & Moulton 1973). We observed a 7- to 11-mm difference in longnose median length in the presence of non-native trout (Table 4-1). Based on what is known about age and growth of longnose dace, a 7- to 11-

mm difference in size structure could be the difference in an entire age group for longnose dace (either due to changes in recruitment or mortality), or could be due to changes in individual growth rates. Further, potential fecundity and clutch size of longnose dace is positively correlated with standard length and somatic mass (Roberts & Grossman 2001) suggesting that differences in size structure could potentially result in changes in fecundity. For such a short-lived species, these potential changes could have an influence on the overall fitness of an individual during its life-span either due to changes in reproductive potential in a given year or changes in the total number of spawning events throughout the life of the longnose dace.

White sucker is a longer-lived species than longnose dace with a maximum age of approximately 17 years. They reach sexually maturity between 3 and 8 years of age (Beamish 1973, Scott & Crossman 1973b), spawn in the spring, are known to spawn multiple times a year, and females produce 20,000 to 50,000 or more eggs (Scott & Crossman 1973b). Growth of white suckers is variable (Beamish 1973), but much faster than longnose dace within the first year of life. Growth is thought to slow to between 10 and 20 mm per year after age 1 and likely ceases after sexual maturity (Scott & Crossman 1973b). We observed a 58- to 123-mm difference in white sucker median length in the presence of non-native trout (Table 4-1). This could mean extreme changes in grow rates or loss of multiple year classes of white sucker. As in longnose dace, these changes could have reproductive consequences. For example, early growth in length of white sucker has been correlated with timing of maturity, in that faster early growth results in earlier maturity (Chen & Harvey 1994). Changes in the timing of maturation could then influence the overall reproductive potential of white sucker.

Aside from the reproductive consequences, changes in size of longnose dace and white sucker may also alter other predatory and competitive interactions. For example, an increase in size of white sucker (without a change in relative abundance) likely requires increased resource use, which could result in depletion of resources for other species in these systems. Based on what we know about these species from other systems, we suggest that the differences in size structure we observed for longnose dace and white sucker in the presence and absence of non-native trout could potentially have biological significance. Therefore, caution is warranted when stocking non-native trout into streams with these species even if there appears to be no initial changes in relative abundance of native species.

Although we cannot determine with any certainty what mechanisms caused the differences in size structures of longnose dace and white suckers that we observed in the presence of trout, there are many potential explanations for these differences including 1) habitat differences between sites with and without trout that may lead to increased resources, increased food supply, or increased protection in sites with trout present compared to sites without trout present, 2) the potential risk of predation in sites with brown trout may cause differences in size structures of native species, 3) brown trout may prey on or exclude longnose dace or white sucker competitors, allowing relief from interspecific competition and resulting in larger size structures of native species, 4) brown trout may prey on or exclude longnose dace or white sucker predators, allowing relief from predation and resulting in reduced mortality, or 5) rainbow trout may compete with longnose dace, causing reductions in growth or longevity. We discuss the viability of each of these below.

Differences in habitat (*e.g.*, habitat complexity, water depth, and riparian vegetation) may lead to increased resources, increased food supply, or increased protection (*e.g.*, Everett and Ruiz 1993) in sites with trout present. These habitat differences could allow longnose dace and white sucker to capitalize on increased in-stream production and attain larger size at a given age through faster growth. Similarly, increased habitat complexity could also allow for greater protection from fish, mammalian, or avian predators, allowing faster growth or increased life-span. However, there were no differences in habitat characteristics between longnose dace sites with and without trout (Table 4-5), and no habitat characteristics we measured explained the fish community composition (Table 4-6) or structure (Table 4-7) over all sites surveyed. Other habitat characteristics that were not measured in this study (*e.g.*, flow, habitat complexity, and water depth) could provide more insight into the potential mechanism of changes in longnose dace and white sucker size structures.

The introduction of predators can directly increase size structure of some native fish via relief from density-dependent limitations on growth. The introduction of northern pike into a small Nebraska Sandhill lake resulted in reduced densities and increased size structure and growth rates of largemouth bass (DeBates *et al.* 2003). Similarly, a reduction in the total number of longnose dace due to non-native trout predation could allow relief from intraspecific competition, allowing dace to attain larger sizes. However, because relative abundance of longnose dace was similar in sites with and without trout (Tables 4-2; Table 4-3; Table 4-4), this hypothesis probably does not explain the differences in longnose dace size structure. Similarly, relative abundance of

white suckers did not differ between sites with and without trout suggesting that an overall reduction in abundance did not occur (Tables 4-2; Table 4-3; Table 4-4).

The potential risk of predation in sites with brown trout may still cause a difference in size structure of longnose dace and white sucker, even if trout are not preying on these species at a high rate. Predator visual or chemical cues have been shown to cause shifts in size, morphology, and life history traits of several species of invertebrates (*e.g.*, Dodson 1989, McIntosh and Peckarsky 1996, Peckarsky *et al.* 2002), as well as morphology of some species of fish (Bronmark and Miner 1992, Bronmark and Pettersson 1994). These changes can decrease the potential risk of predation for prey species (*e.g.*, through increased swimming speed, or increased girth in the presence of gape limited predators). Longnose dace and white sucker may increase their size to reduce the risk of predation in the presence of non-native trout.

Non-native species can also benefit native species through competitive release (Rodriguez 2006). Brown trout are known piscivores (*e.g.*, Garman and Nielsen 1982, L'Abee-Lund *et al.* 1992, Yard *et al.* 2011) and high predation on longnose dace competitors could allow for a reduction in densities, and therefore, an increase in available resources for longnose dace. For example, white sucker relative abundance in sites where white sucker occurred with longnose dace did not differ between sites with and without trout (Table 4-3). The size structure of white sucker, however, did change in the presence of trout in these sites suggesting that competition may be reduced between smaller size groups of fish. There was a greater proportion of larger white sucker in longnose dace sites where brown trout were the only trout species present and sites where any trout species or a combination of trout species were present compared to longnose

dace sites where no trout species were present (Figure 4-4). Consequently, the percentage of white sucker within the observed size range of longnose dace was reduced by almost half in longnose dace sites with trout (Figure 4-4). Further, white sucker size structure showed similar patterns across all sites sampled, where there was a greater proportion of larger white sucker in the presence of trout than in the absence of trout in sites with or without longnose dace (Figure 4-2). Longnose dace and white sucker are both invertivorous allowing the potential for food competition in these systems (Gerald 1966; Carlander 1969). Less white suckers within the observed size range of longnose dace could lead to less interspecific competition between longnose dace and white sucker if white sucker diet or habitat use shifts with size. However, the increase in white sucker size structure may also increase interspecific competition for food resources if white suckers do not shift diet or habitat use with size in these systems.

The potential competitive relation between longnose dace and white sucker may be driven by size selective predation from trout. Trout have been shown to prey on the bottom feeding fish communities in these systems (Klammer 1984), and prey size is an important factor in determining predation rates (Ware 1972). Even though there were no clear differences in overall relative abundances of longnose dace and white sucker in sites with trout compared to sites without trout that might suggest relief from density-dependent interactions, size selective predation on these fishes may relieve density-dependent competition for fish of a susceptible size to predation (*i.e.*, there may be competitive release in smaller size groups of these species even though over all size groups there is no difference).

The change in size structure of longnose dace and white sucker in the presence of brown trout may also be the result of trout predation on or exclusion of other predators in these sites. It is well documented that the effects of multiple predators are often not independent (*e.g.*, McCoy *et al.* 2012). Multiple predators can interact synergistically or antagonistically resulting in either enhanced or reduced prey predation risk relative to what would be predicted based on the sums of the independent effects of each predator (*e.g.*, Sih *et al.* 1998). Trout may reduce native species predation risk (*i.e.*, decrease mortality) by either directly regulating native predator abundance or size structure, or by interfering with the ability of other predators to prey on native species (*i.e.*, behaviorally, without directly regulating native predator populations).

Competitive interactions between native and non-native species may explain the smaller longnose dace size structure in sites where rainbow trout were the only trout species present compared to sites where no trout species were present in this study. Rainbow trout (*e.g.*, Klammer 1984) and longnose dace (*e.g.*, Gerald 1966) are both invertivorous potentially allowing for increased foraging competition in these sites. Rainbow trout may have decreased availability of resources, causing reductions in longnose dace growth or longevity. However, the availability of food resources of longnose dace and rainbow trout were not quantified in this study, and therefore, the likelihood that food resources are limiting is unknown. Competitive interactions with non-native trout are often cited as possible causes of declines in abundance, species richness, and fitness, and changes in distribution, behavior, and life histories of native species (McIntosh et al. 1994; Gido and Propst 1999; Taniguchi et al. 2002; Ruetz et al. 2003; Baxter et al. 2004; Olsen and Belk 2005; Baxter et al. 2007; Kadye and Magadza

2008; Penaluna et al. 2009). Few studies, however, have been able to provide evidence of interspecific competition between non-native salmonids and native species in natural streams (Fausch 1988) due to the relatively difficult task of proving competition.

The size structure of native longnose dace and white sucker populations differed in the presence and absence of non-native trout. Differences in size structures may reflect changes in recruitment, growth, or mortality of populations and could be biologically important. However, more information (*e.g.*, age and diet data) is needed to determine the exact mechanisms behind the changes we observed. Even so, these differences in size structure may alter species interactions (*e.g.*, competition) and eventually lead to community level responses to trout presence.

Native Fish Community Composition and Structure in the Presence and Absence of Non-native Trout

Native fish community composition and structure did not differ in the presence of non-native trout suggesting that current trout presence alone may not influence native fish communities. Similarly, non-game species richness and diversity did not differ in southern Appalachian Mountain streams that had been stocked with trout relative to paired, unstocked streams (although unstocked streams contained trout as well; Weaver and Kwak 2013). Native fish assemblage structure also did not differ in an Oklahoma Ozark stream before and after rainbow trout introduction in riffles and glides (Walsh and Winkelman 2004). Assemblage structure did, however, differ in pool habitats (Walsh and Winkelman 2004).

Alternatively, fish community composition and structure in headwater streams in Nebraska may be the result of historical trout presence (*e.g.*, Thorp 1986). Emphasis in site selection was on historical trout presence, and therefore, all streams historically contained trout. The remnants of historical trout presence may still be evident, making it difficult to differentiate fish communities based on current trout presence (*e.g.*, trout may have previously homogenized fish communities prior to their disappearance in sites that currently have no trout). Examining a wider variety of streams (*i.e.*, streams with no historical trout presence) may lend further insight into the mechanisms structuring fish communities in these systems. Additionally, monitoring streams at multiple points in time may also provide insight into the potential influences of non-native trout on native fish communities.

Native Fish Community Composition and Structure at Differing Abundances of Non-native Trout

Although there were no differences in native fish community composition or structure in the presence of non-native trout, there appears to be increased separation (or more dissimilarity) in fish community composition (Figure 4-9) and structure (Figure 4-10) between sites with and without trout as trout abundances increase. There also may be some threshold of trout density (around 0.5 – 1.5 trout/min) above which non-native trout have strong influences on native fish communities, but below which non-native trout have weak or no influences on native fish communities (Figure 4-9, Figure 4-10, Figure 4-11, Figure 4-12). Therefore, stocking trout at high abundances may alter native fish community composition and structure in Nebraska headwater streams. Stocking trout at

low abundances, however, may not have direct adverse effects on native fish community composition and structure. Future research should focus on identifying the threshold in trout density, if any, which will alter native fish communities, as it may be possible to simultaneously manage for a low-density trout fishery and for native species conservation.

Conclusion

Non-native trout may have both population- and community-level influences on native species in headwater streams in Nebraska. Size structures of longnose dace and white sucker were larger in the presence of brown trout, and size structure of longnose dace was smaller in the presence of rainbow trout. These differences could potentially result in community-wide responses to non-native trout. There also appears to be increased risk to native communities in sites with high abundances of trout. Therefore, we do not recommend stocking trout at high abundances in these streams.

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Table 4-1. Tests of differences in size structures of native species between sites where any trout species or a combination of trout species were present (All Trout), sites where brown trout were the only trout species present (BNT only), and sites where rainbow trout were the only trout species present (RBT only) compared to sites where no trout species were present (No Trout; n_Y = sample size in sites with trout, n_N = sample size in sites without trout, $\bar{X}_Y$ = mean length in sites with trout, $\bar{X}_N$ = mean length in sites without trout, $\bar{X}_{diff} = \bar{X}_Y - \bar{X}_N$, Med_Y = Median length in sites with trout, Med_N = Median length in sites without trout, $Med_{diff} = Med_Y - Med_N$, KS_a = asymptotic Kolmogorov-Smirnov statistic, P_{KS}-value = asymptotic P-value of KS_a).

Sites	Comparison	n_Y	n_N	$\bar{X}_Y$	$\bar{X}_N$	$\bar{X}_{diff}$	Med_Y	Med_N	Med_{diff}	KS_a	P_{KS}-value
Longnose dace sites	*All Trout vs. No Trout*										
	Creek Chub	97	156	101	96	5	100	94	6	1.09	0.19
	Fathead Minnow	23	156	57	52	5	56	54	2	1.29	0.07
	Green sunfish	26	43	71	64	7	70	62	8	0.92	0.37
	Longnose dace	222	233	71	67	**4**	73	66	**7**	1.54	**0.02**
	White Sucker	96	161	190	144	**46**	183	132	**51**	1.91	**<0.01**
	BNT only vs. No Trout										
	Creek Chub	69	156	96	96	0	90	94	-4	1.13	0.16
	Green sunfish	26	43	71	64	7	70	62	8	0.92	0.37
	Longnose dace	117	233	74	67	**7**	74	66	**8**	1.68	**0.01**
	White sucker	51	161	230	144	**86**	265	132	**133**	2.78	**<0.01**
	RBT only vs No Trout										
	Longnose dace	40	233	55	67	**-12**	55	66	**-11**	1.83	**<0.01**
	White sucker	24	161	124	144	-20	102	132	-30	0.80	0.54
White sucker sites	*All Trout vs. No Trout*										
	Creek chub	74	178	107	100	7	105	97	8	1.21	0.11
	Green sunfish	33	67	71	68	3	70	64	6	0.88	0.42
	Longnose dace	130	190	68	65	3	70	66	4	1.17	0.13
	White sucker	179	185	208	149	**59**	200	142	**58**	2.82	**<0.01**
	BNT only vs. No Trout										
	Creek chub	29	178	119	100	19	115	97	18	1.38	0.05
	Green sunfish	26	67	71	68	3	70	64	6	0.82	0.52
	Longnose dace	79	190	74	65	**9**	73	66	**7**	1.58	**0.01**
	White sucker	51	185	230	149	**81**	265	142	**123**	2.88	**<0.01**
	RBT only vs No Trout										
	Longnose dace	40	190	55	65	**-11**	55	66	**-12**	1.55	**0.02**
	White sucker	37	185	124	149	-25	112	142	-30	1.02	0.25

Table 4-2. Tests of differences in relative abundance (PROC GLIMMIX, SAS v.9.2) of select native species across all sites surveyed during 2011 in sites where any trout species or a combination of trout species were present (ATP), sites where brook trout were the only trout species present (BKT), sites where brown trout were the only trout species present (BNT), and sites where rainbow trout were the only trout species present (RBT) compared to sites where no trout species were present.

Native Species	Trout Species	Num DF	Den DF	*F*-value	*P*-value
Creek Chub	ATP	1	51	1.92	0.17
	BKT	1	51	0.17	0.68
	BNT	1	51	0.02	0.88
	RBT	1	51	0.24	0.62
Fathead Minnow	ATP	1	51	0.94	0.34
	BKT	1	51	0.19	0.66
	BNT	1	51	0.13	0.72
	RBT	1	51	0.00	0.98
Green Sunfish	ATP	1	51	0.61	0.44
	BKT	1	51	0.15	0.70
	BNT	1	51	0.00	0.95
	RBT	1	51	0.29	0.59
Longnose Dace	ATP	1	51	0.28	0.60
	BKT	1	51	0.05	0.82
	BNT	1	51	0.04	0.84
	RBT	1	51	0.21	0.65
White Sucker	ATP	1	51	0.00	0.94
	BKT	1	51	1.33	0.25
	BNT	1	51	0.52	0.47
	RBT	1	51	0.02	0.90

Table 4-3. Tests of differences in relative abundance (PROC GLIMMIX, SAS v.9.2) of select native species across longnose dace sites surveyed during 2011 in sites where any trout species or a combination of trout species were present (ATP), sites where brook trout were the only trout species present (BKT), sites where brown trout were the only trout species present (BNT), and sites where rainbow trout were the only trout species present (RBT) compared to sites where no trout species were present.

Native Species	Trout Species	Num DF	Den DF	*F*-value	*P*-value
Creek Chub	ATP	1	29	0.85	0.36
	BKT	1	29	0.12	0.73
	BNT	1	29	0.01	0.93
	RBT	1	29	0.20	0.66
Fathead Minnow	ATP	1	29	0.48	0.49
	BKT	1	29	0.06	0.81
	BNT	1	29	0.00	0.96
	RBT	1	29	0.24	0.62
Green Sunfish	ATP	1	29	0.05	0.83
	BKT	1	29	0.21	0.65
	BNT	1	29	0.00	1.00
	RBT	1	29	0.41	0.53
Longnose Dace	ATP	1	29	0.22	0.65
	BKT	1	29	0.02	0.89
	BNT	1	29	0.03	0.85
	RBT	1	29	0.07	0.80
White Sucker	ATP	1	29	0.11	0.74
	BKT	1	29	0.52	0.48
	BNT	1	29	0.11	0.74
	RBT	1	29	0.03	0.87

Table 4-4. Tests of differences in relative abundance (PROC GLIMMIX, SAS v.9.2) of select native species across white sucker sites surveyed during 2011 in sites where any trout species or a combination of trout species were present (ATP), sites where brook trout were the only trout species present (BKT), sites where brown trout were the only trout species present (BNT), and sites where rainbow trout were the only trout species present (RBT) compared to sites where no trout species were present.

Native Species	Trout Species	Num DF	Den DF	*F*-value	*P*-value
Creek Chub	ATP	1	31	2.42	0.13
	BNT	1	31	0.03	0.86
	RBT	1	31	0.01	0.92
Fathead Minnow	ATP	1	31	2.28	0.14
	BNT	1	31	0.01	0.94
	RBT	1	31	0.03	0.86
Green Sunfish	ATP	1	31	0.47	0.50
	BNT	1	31	0.00	0.95
	RBT	1	31	0.14	0.71
Longnose Dace	ATP	1	31	0.22	0.64
	BNT	1	31	0.05	0.83
	RBT	1	31	0.05	0.83
White Sucker	ATP	1	31	0.05	0.82
	BNT	1	31	0.63	0.43
	RBT	1	31	0.09	0.76

Table 4-5. One way analysis of similarity (ANOSIM procedure, Primer-E v.6) of habitat and human-use characteristics (resemblance matrix based on Euclidean distance) between sites with species present and absent for all sites, longnose dace sites, and white sucker sites.

Sites	Presence/Absence	Global R	*P*-value
All (*n* = 54)	Any trout species or a combination of trout species	0.083	0.052
	Brown Trout	0.103	0.003
	Rainbow Trout	0.010	0.405
	Longnose dace	0.016	0.304
	White sucker	0.039	0.146
Longnose Dace (*n* = 33)	Any trout species or a combination of trout species	0.118	0.036
	Brown Trout	0.091	0.040
	Rainbow Trout	0.011	0.412
White Sucker (*n* = 34)	Any trout species or a combination of trout species	0.029	0.239
	Brown Trout	0.100	0.027
	Rainbow Trout	0.057	0.185

Table 4-6. Top correlations between habitat and human-use characteristics (similarity matrix based on Euclidean distance) and native community composition (presence/absence, Kulczynski similarity matrix; BEST Procedure, Primer-E v.6).

No. Vars	Correlation	Selections
5	0.122	Undercuts, boulders, landfill/trash, road/railroad, riprap/dike/levee
5	0.121	Undercuts, sand, landfill/trash, road/railroad, riprap/dike/levee
5	0.120	Undercuts, landfill/trash, road/railroad, buildings/pavement, riprap/dike/levee
4	0.118	Undercuts, landfill/trash, road/railroad, riprap/dike/levee
5	0.118	Undercuts, other, landfill/trash, road/railroad, riprap/dike/levee

Table 4-7. Top correlations between habitat and human-use characteristics (similarity matrix based on Euclidean distance) and native community structure (relative abundance, fourth-root transformed, Bray Curtis similarity matrix; BEST Procedure, Primer-E v.6).

No. Vars	Correlation	Selections
5	0.241	Undercuts, sand, fines, park, road/railroad
5	0.241	Undercuts, sand, fines, park, buildings/pavement
4	0.234	Artificial structures, sand, feedlot, park
5	0.234	Artificial structures, sand, other, feedlot, park
5	0.234	Artificial structures, sand, mining activity, feedlot, park

Table 4-8. Sites sampled in 2011 where non-native trout were present, but no native fish were collected.

Site	Latitude	Longitude	Trout Species Present
Alliance Drain	41.90144	103.4979	RBT
Little Sand Creek	42.82534	98.88818	BNT
Monroe Creek	42.76646	103.92779	BKT
Ninemile Creek (Upper)	41.88848	103.4339	BNT, RBT
Sowbelly Creek	42.73632	103.83487	BNT
West Ash Creek	42.62959	103.24926	BKT

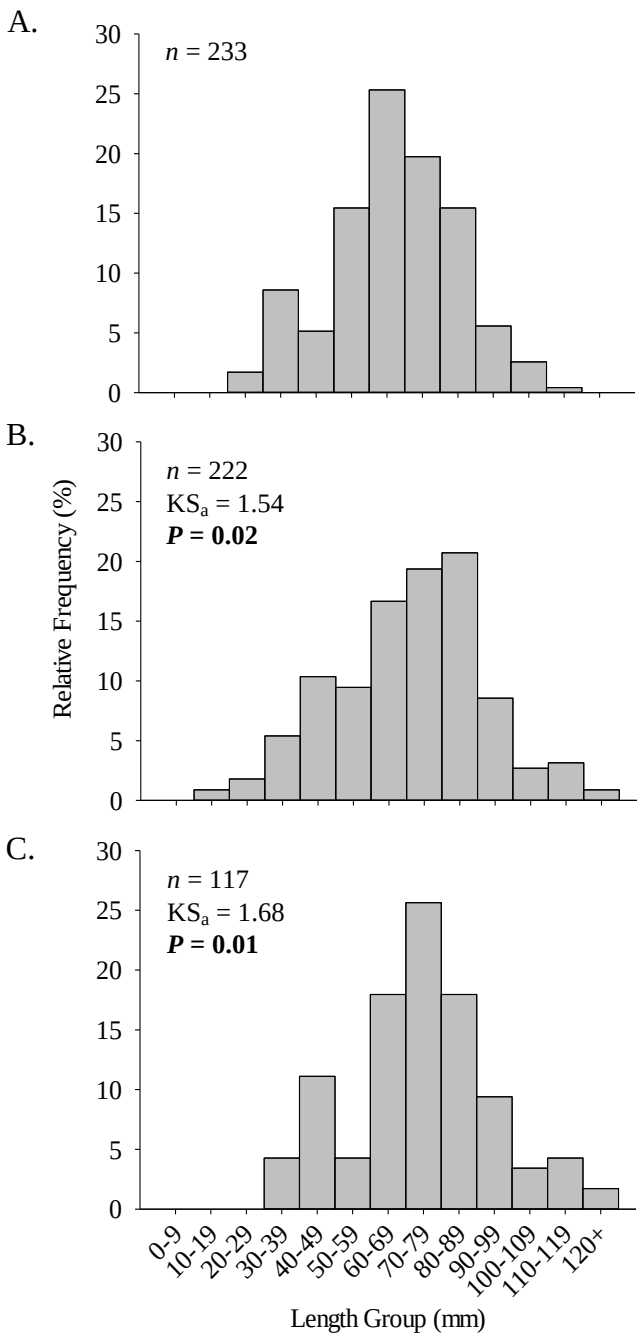


Figure 4-1. Longnose dace size structures for fish collected during 2011 at A. sites where no trout species were collected, B. sites where any trout species or a combination of trout species were collected (includes brook trout, brown trout, and rainbow trout), C. sites where brown trout were the only trout species collected (KS_a = asymptotic Kolmogorov-Smirnov statistic, and P = asymptotic P-value of KS_a for comparisons of sites with trout to sites without trout).

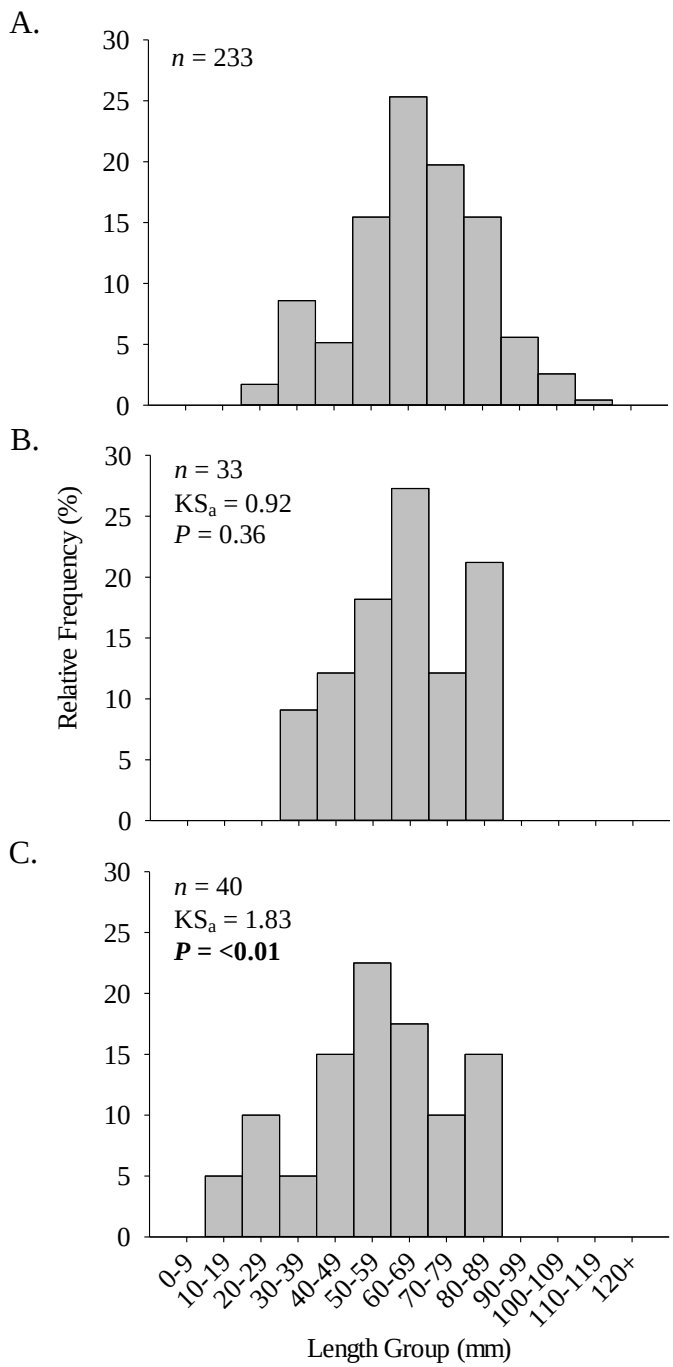


Figure 4-2. Longnose dace size structures for fish collected during 2011 at A. sites where no trout species were collected, B. sites where brook trout were the only trout species collected, and C. sites where rainbow trout were the only trout species collected (KS_a = asymptotic Kolmogorov-Smirnov statistic, and P = asymptotic P-value of KS_a for comparisons of sites with trout to sites without trout).

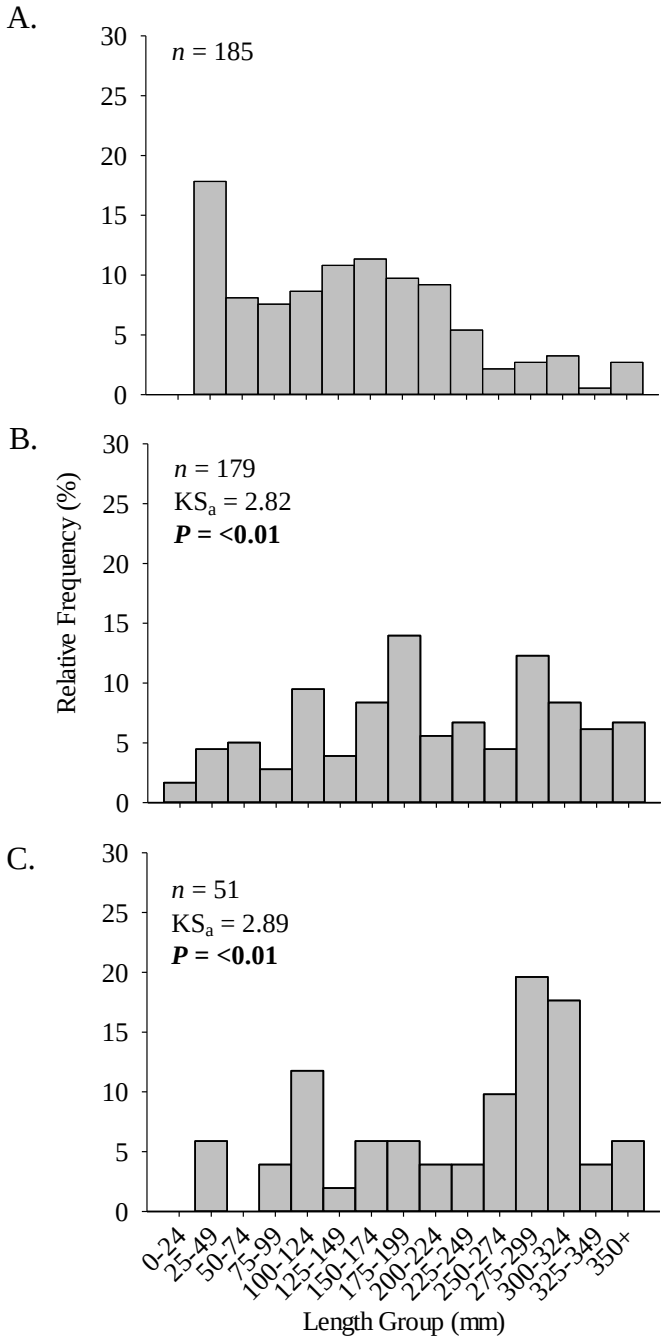


Figure 4-3. White sucker size structures for fish collected during 2011 at A. sites where no trout species were collected, B. sites where any trout species or a combination of trout species were collected (includes brook trout, brown trout, and rainbow trout), and C. sites where brown trout were the only trout species collected (KS_a = asymptotic Kolmogorov-Smirnov statistic, and P = asymptotic P-value of KS_a for comparisons of sites with trout to sites without trout).

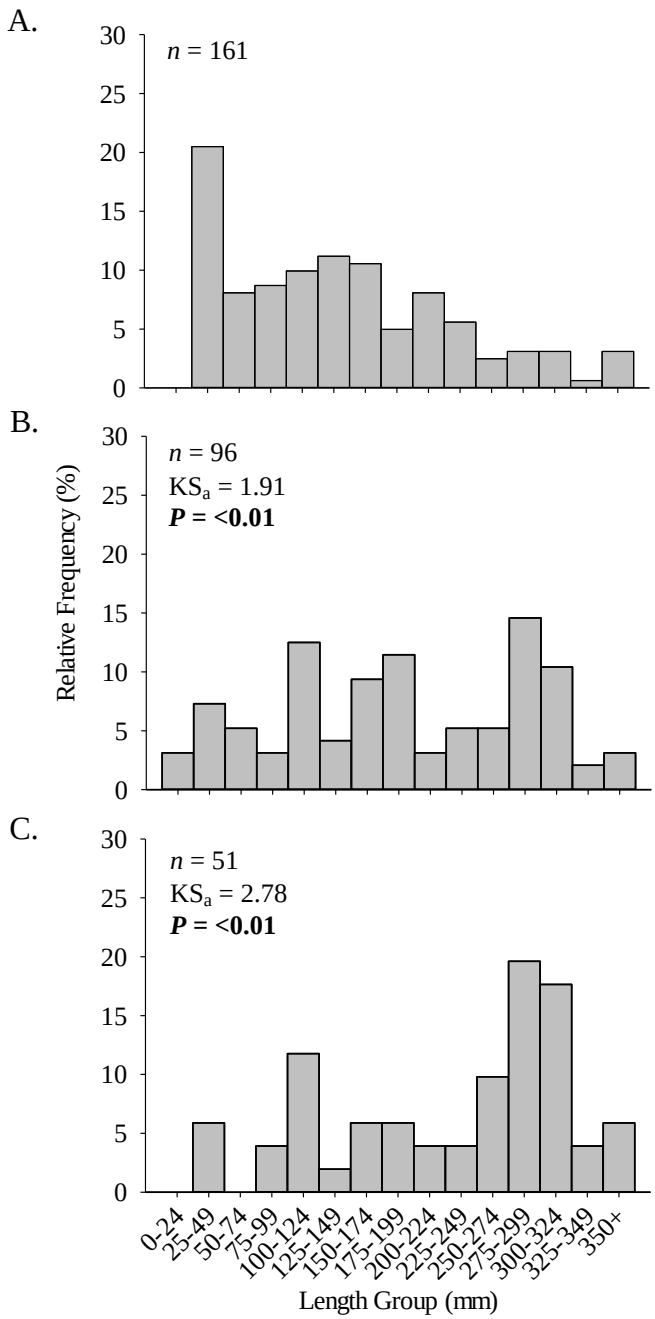


Figure 4-4. White sucker size structures for fish collected during 2011 at A. longnose dace sites where no trout species were collected, B. longnose dace sites where any trout species or a combination of trout species were collected (includes brook trout, brown trout, and rainbow trout), and C. longnose dace sites where brown trout were the only trout species collected (KS_a = asymptotic Kolmogorov-Smirnov statistic, and P = asymptotic P-value of KS_a for comparisons of sites with trout to sites without trout).

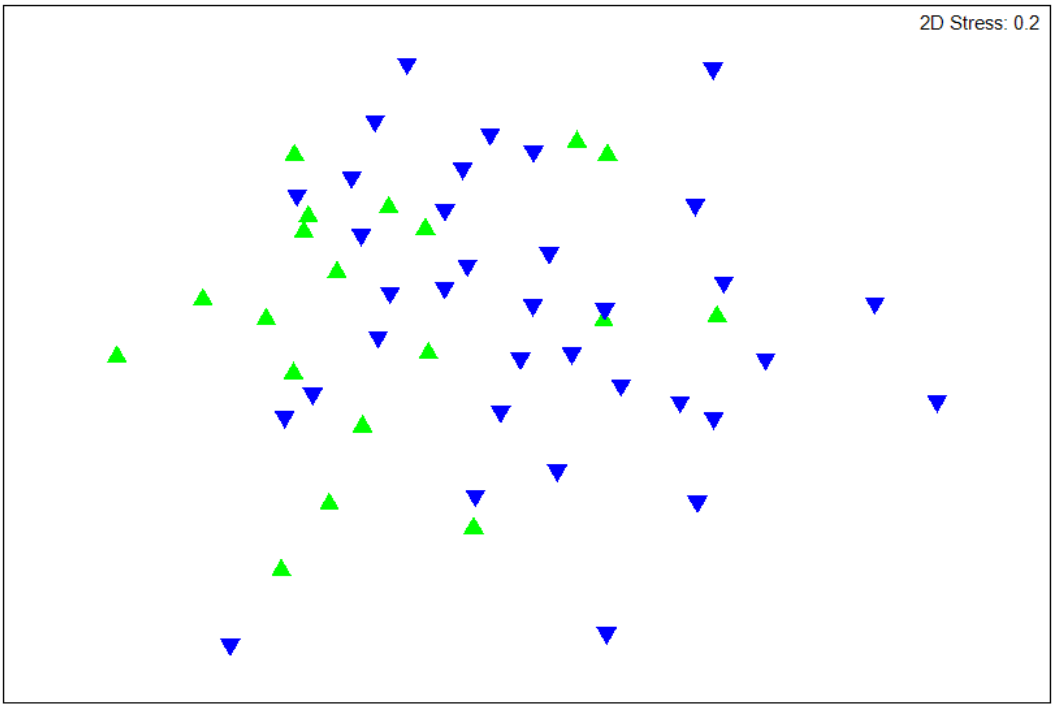
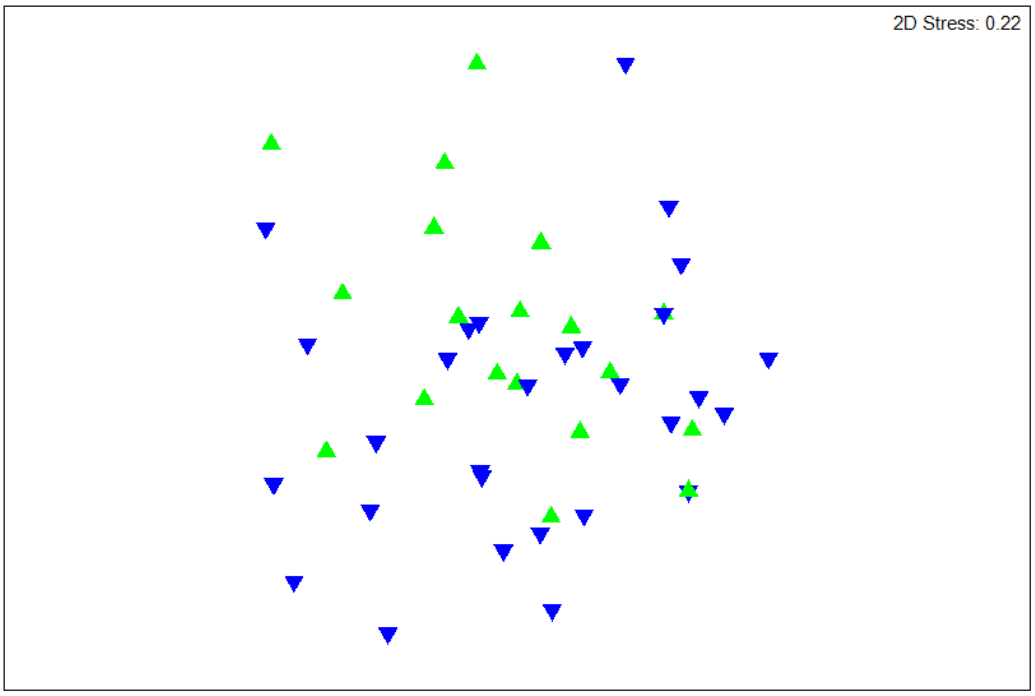


Figure 4-5. Non-metric multidimensional scaling plot of habitat and human-use characteristics (similarity matrix based on Euclidean distance) in sites where any trout species or a combination of trout species were present (blue) and sites where no trout species were present (green).

A.



B.

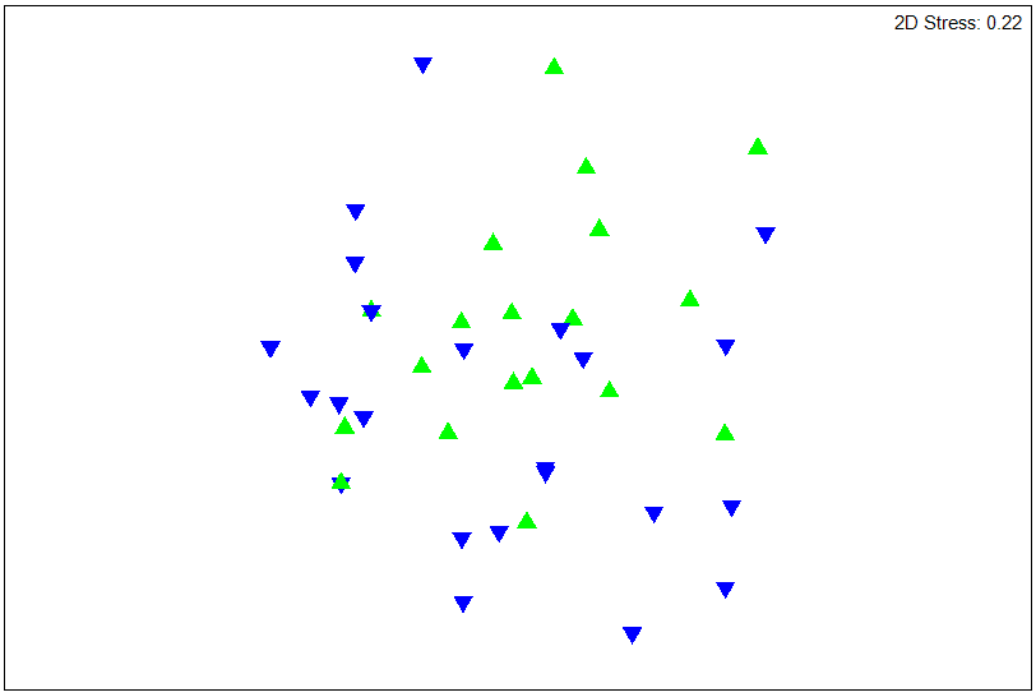
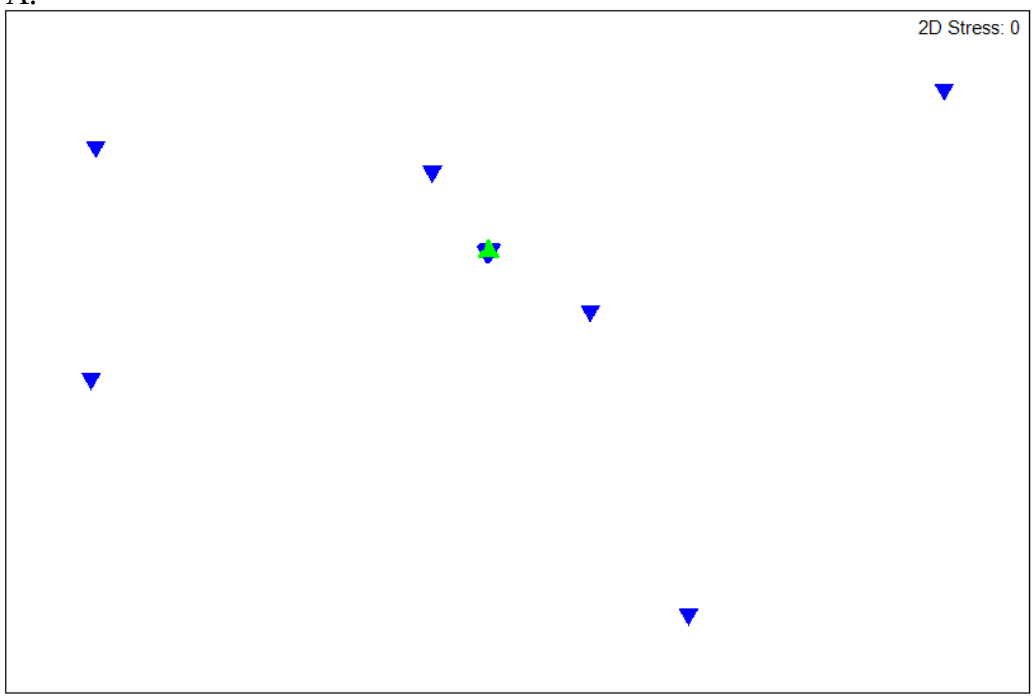


Figure 4-6. Non-metric multidimensional scaling plot of native fish community composition (presence/absence, Kulczynski similarity matrix) in sites where any trout species or a combination of trout species were present (blue) and sites where no trout species were present (green) for A. all sites (n = 54), and B. excluding 6 sites with no native fish (n = 48).

A.



B.

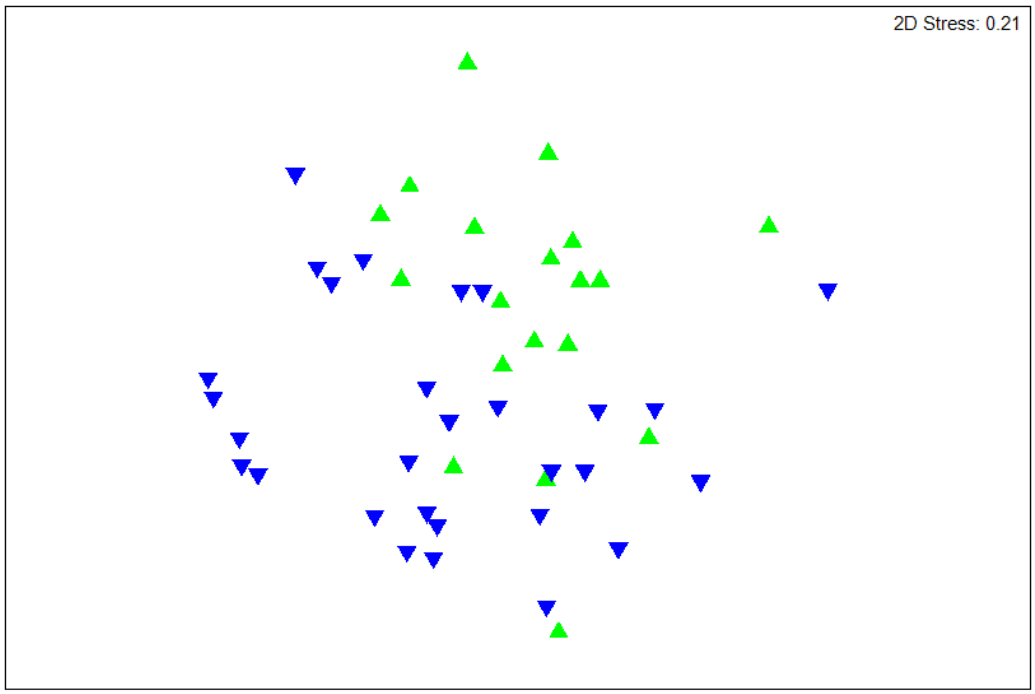


Figure 4-7. Non-metric multidimensional scaling plot of native fish community structure (relative abundance, fourth-root transformed, Bray Curtis similarity matrix) in sites where any trout species or a combination of trout species were present (blue) and sites where no trout species were present (green) for A. all sites (n = 54), and B. excluding 6 sites with no native fish (n = 48).

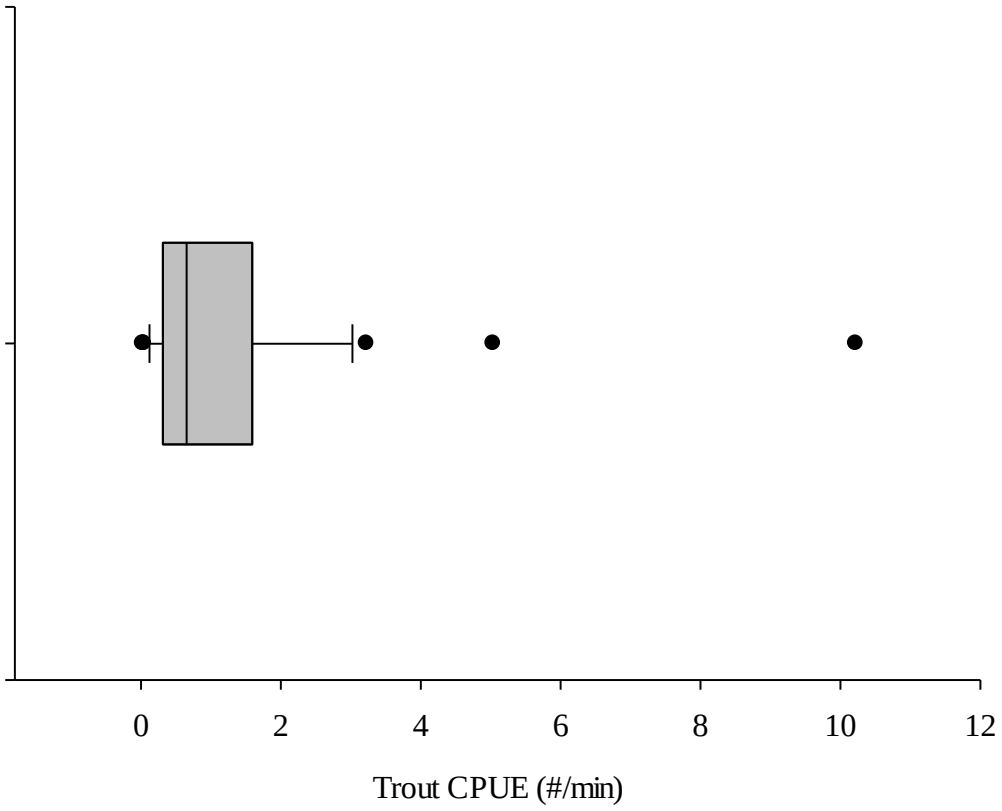


Figure 4-8. Boxplot of trout abundance (#/min) by site. Box represents 25th, 50th, 75th percentiles, whiskers extend from the box to the 10th and 90th percentiles, and points represent outliers.

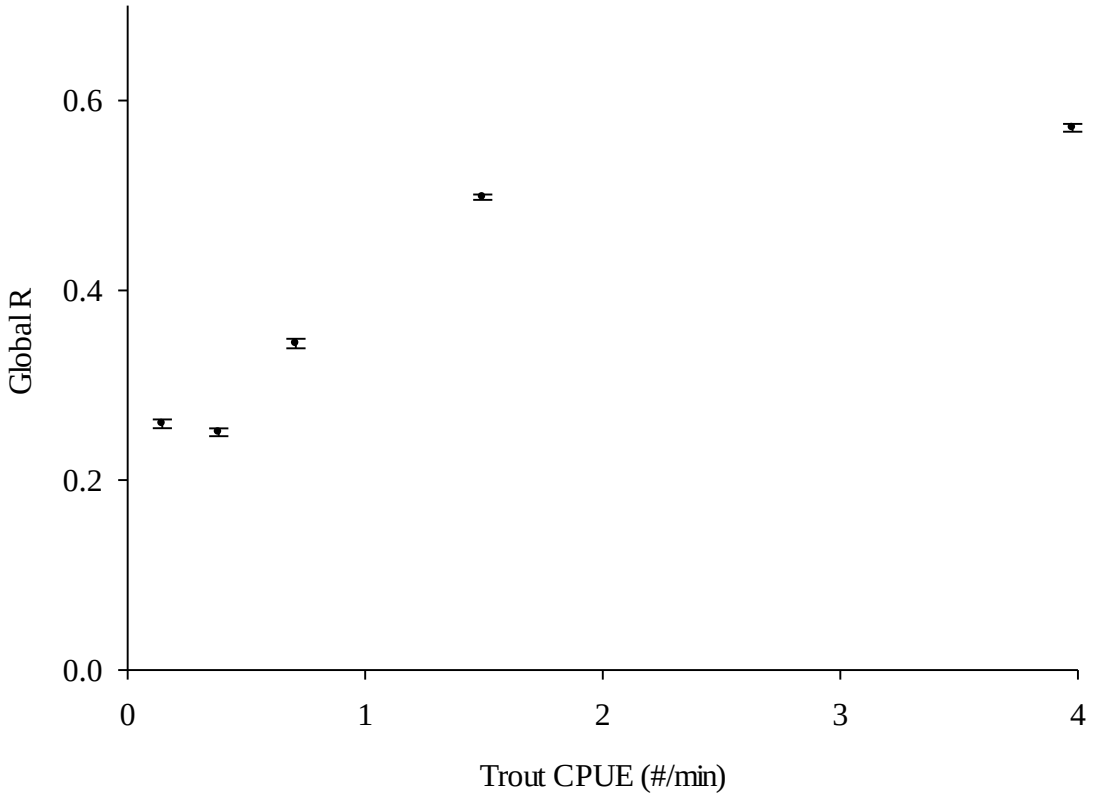


Figure 4-9. Mean Global R value ± 95% CI of analysis of similarities (1,000 iterations) for native fish community composition (presence/absence, Kulczynski similarity matrix; Primer-E v.6) between sites with and without trout with increased relative abundance of trout (CPUE, trout/min). Subsets were based on the following percentiles when sites were ranked by trout abundance: ≤ 20th percentile (CPUE ≤ 0.25 trout/min), 21 – 40th percentiles (CPUE 0.31 – 0.48 trout/min), 41 – 60th percentiles (CPUE 0.50 – 0.98 trout/min), 61 – 80th percentiles (CPUE 1.22 – 1.73 trout/min), and ≥ 81st percentile (CPUE ≥ 1.97 trout/min). Points are plotted at the average trout CPUE for each subset.

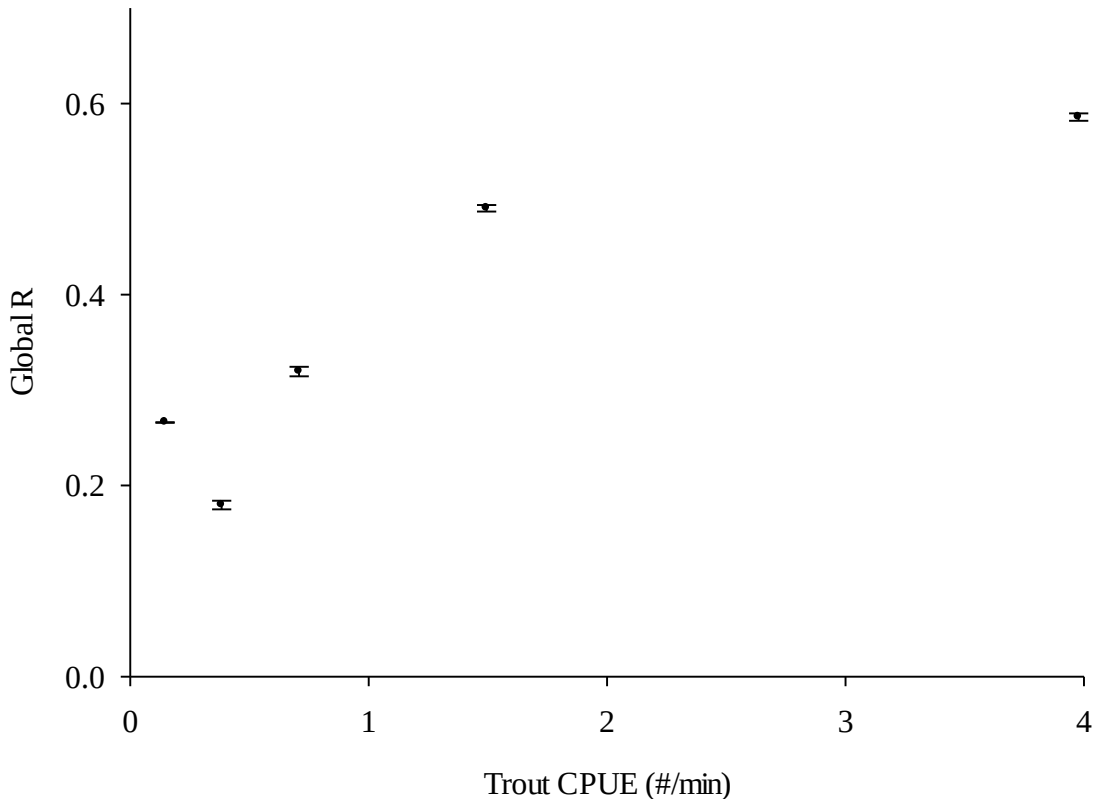


Figure 4-10. Mean Global R value ± 95% CI of analysis of similarities (1,000 iterations) for native fish community structure (fourth-root transformed, Bray-Curtis similarity matrix; Primer-E v.6) between sites with and without trout with increased relative abundance of trout (CPUE, trout/min). Subsets were based on the following percentiles when sites were ranked by trout abundance: ≤ 20th percentile (CPUE ≤ 0.25 trout/min), 21 – 40th percentiles (CPUE 0.31 – 0.48 trout/min), 41 – 60th percentiles (CPUE 0.50 – 0.98 trout/min), 61 – 80th percentiles (CPUE 1.22 – 1.73 trout/min), and ≥ 81st percentile (CPUE ≥ 1.97 trout/min). Points are plotted at the average trout CPUE for each subset.

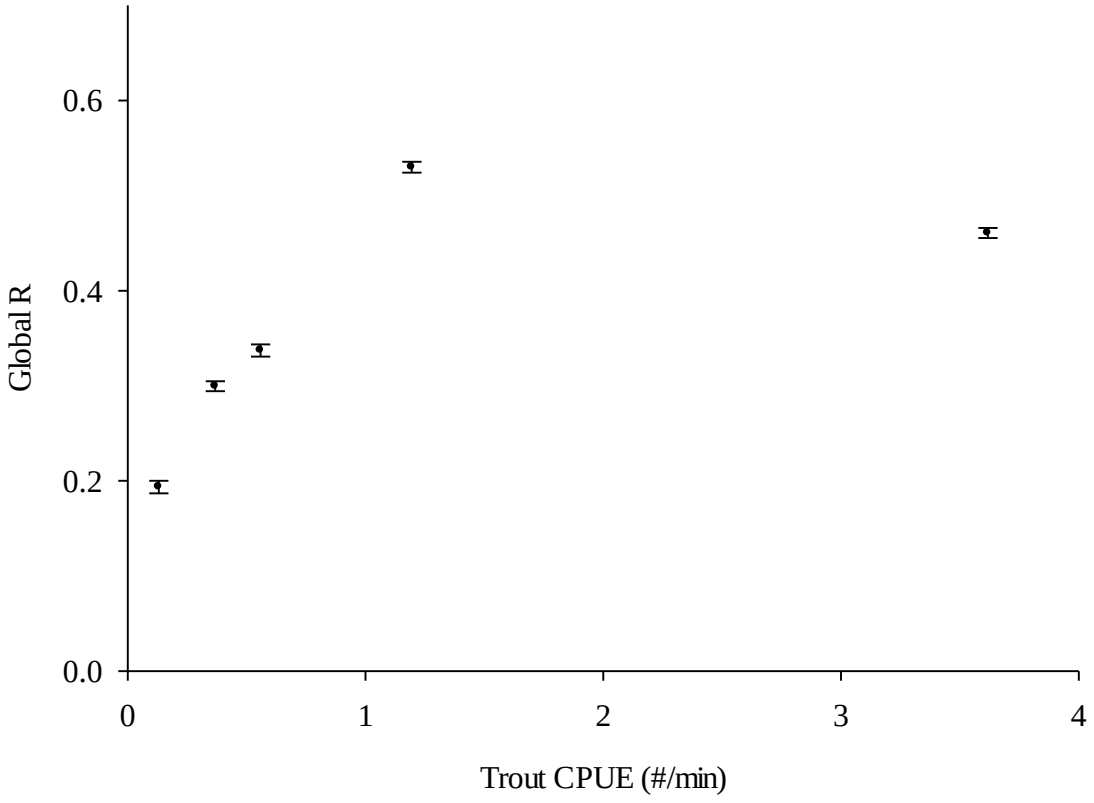


Figure 4-11. Mean Global R value ± 95% CI of analysis of similarities (1,000 iterations) for native fish community composition (presence/absence, Kulczynski similarity matrix; Primer-E v.6) between sites with and without trout, but excluding sites with trout and no native fish (n = 6),with increased relative abundance of trout (CPUE, trout/min). Subsets were based on the following percentiles when sites were ranked by trout abundance: ≤ 20th percentile (CPUE ≤ 0.25 trout/min), 21 – 40th percentiles (CPUE 0.31 – 0.47 trout/min), 41 – 60th percentiles (CPUE 0.48 – 0.78 trout/min), 61 – 80th percentiles (CPUE 0.89 – 1.57 trout/min), and ≥ 81st percentile (CPUE ≥ 1.59 trout/min). Points are plotted at the average trout CPUE for each subset.

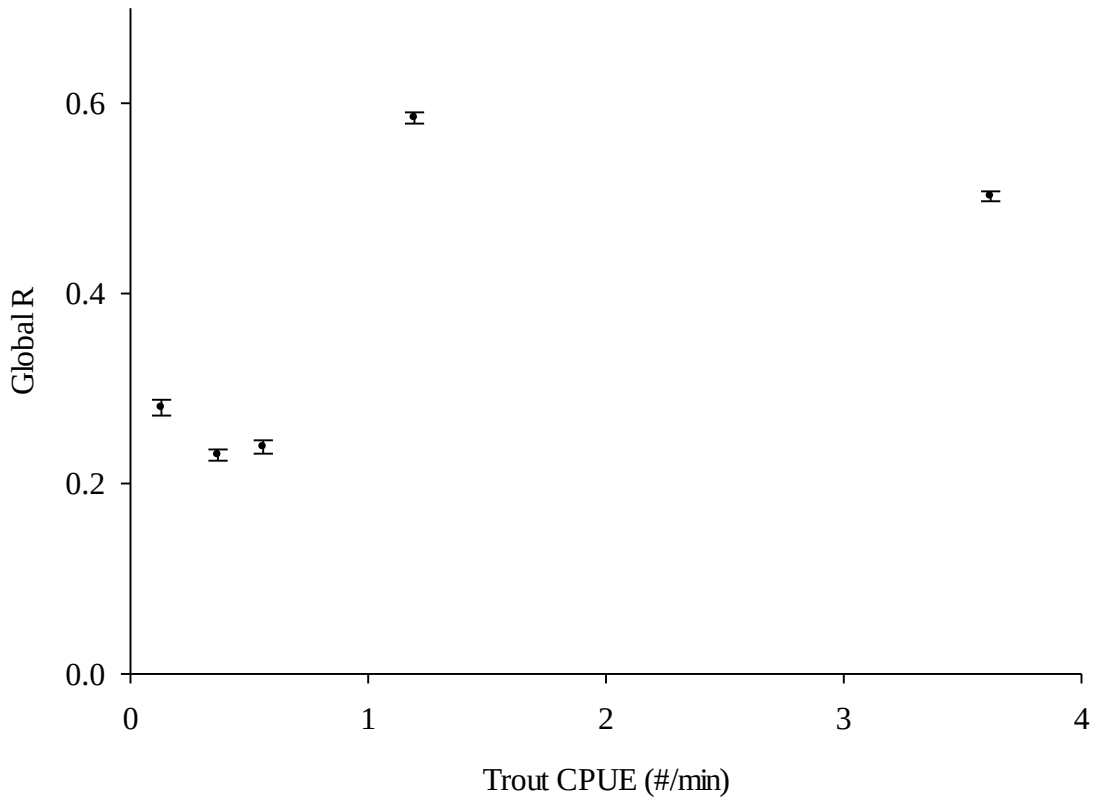


Figure 4-12. Mean Global R value ± 95% CI of analysis of similarities (1,000 iterations) for native fish community structure (fourth-root transformed, Bray-Curtis similarity matrix; Primer-E v.6) between sites with and without trout, but excluding sites with trout and no native fish (n = 6), with increased relative abundance of trout (CPUE, trout/min). Subsets were based on the following percentiles when sites were ranked by trout abundance: ≤ 20th percentile (CPUE ≤ 0.25 trout/min), 21 – 40th percentiles (CPUE 0.31 – 0.47 trout/min), 41 – 60th percentiles (CPUE 0.48 – 0.78 trout/min), 61 – 80th percentiles (CPUE 0.89 – 1.57 trout/min), and ≥ 81st percentile (CPUE ≥ 1.59 trout/min). Points are plotted at the average trout CPUE for each subset.

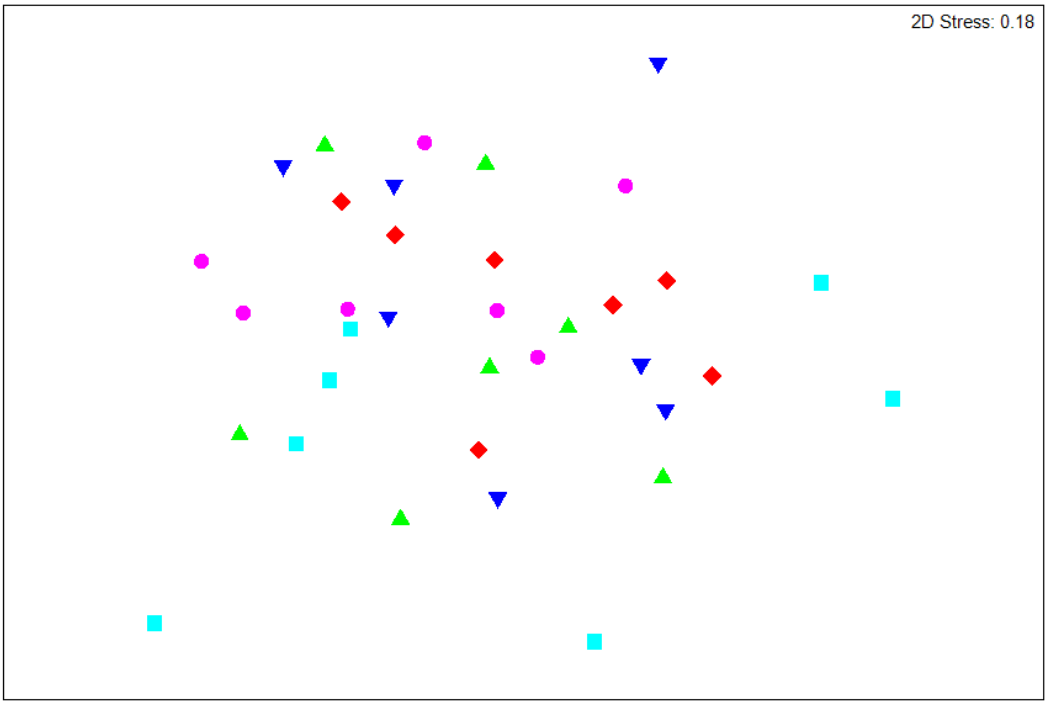


Figure 4-13. Non-metric multidimensional scaling plot of habitat and human-use characteristics (similarity matrix based on Euclidean distance) for subsets of sites with trout based on abundance. Subset 1 = blue triangle (CPUE ≤ 0.25 trout/min), subset 2 = red diamond (CPUE 0.31 – 0.48 trout/min), subset 3 = green triangle (CPUE 0.50 – 0.98 trout/min), subset 4 = blue square (CPUE 1.22 – 1.73 trout/min), and subset 5 = pink circle (CPUE ≥ 1.97 trout/min).

CHAPTER 5. MANAGEMENT AND RESEARCH RECOMMENDATIONS

GENERAL RECOMMENDATIONS

The introduction of non-native trout has provided a unique recreational opportunity for anglers, and has greatly enhanced the recreational capacity of many streams. However, the potential ecological costs of these introductions on native species need additional consideration. This study determined that non-native trout may influence some native fish at the individual (Chapter 2 and Chapter 3), and potentially population (Chapter 4), and community (Chapter 4) levels. Therefore, I make the following recommendations.

Non-native trout should not be stocked into headwater streams if competition or predation is likely to result in a threat to native species. Although, I did not evaluate competition, non-native trout are physically capable of consuming small-bodied cyprinids (Chapter 2 and 3), and some predation is likely to occur if non-native trout and native cyprinids overlap in habitat use under natural conditions. The effect of predation (regardless of degree) could be detrimental for some species of concern if abundances are already low. Therefore, I recommend a conservative approach of not stocking non-native trout into streams known to have species of concern. However, predation may have little influence on the overall population abundance of common and abundant species (Chapter 4) and stocking may be acceptable under certain circumstances. In those instances, non-native trout should only be stocked if it is determined that other changes in native populations (*e.g.*, size structure) and communities (*e.g.*, composition) are acceptable (Figure 5-1). Additionally, the process used to determine whether to stock non-native

trout or not should be continually modified based on the best available scientific knowledge at the time. Identification of new factors, as well as the relative importance of factors I identified as likely to influence native fish, may change as systems change and we gain more information about non-native trout, native species, their interactions, and the ecological effects of their interactions.

SPECIFIC RECOMMENDATIONS FOR NEBRASKA HEADWATER STREAMS

Changes in management practices and policies, along with hatchery closings and other restraints, have changed stream trout stocking practices in Nebraska considerably over the last 80 years (Nebraska Game and Parks Commission, unpublished data). There have been drastic reductions in the number (Figure 5-2) and changes in the species of trout stocked. Over 8.7 million trout have been stocked into Nebraska streams since 1930, nearly half of which were stocked in the 1940s and 1950s (~200,000 trout per year). In contrast, recent estimates approximate only 30,000 trout per year. There has also been a noticeable shift in the species of trout stocked in Nebraska. From 1950 to 1990, approximately two brown trout were stocked into Nebraska waters for every one rainbow trout. This ratio began to even out in the 1990s due to reductions in the number of brown trout stocked, and from 2000 to 2013 approximately nine rainbow trout were stocked for every one brown trout. The number of streams stocked with non-native trout has also declined recently (Figure 5-3). On average, twenty-five streams per year were stocked with trout throughout the 1940s and 1950s, and only 10 streams per year were stocked with trout from 2000 to 2013 (Nebraska Game and Parks Commission,

unpublished data). These reductions in the number of trout stocked and number of streams stocked have likely benefited some native fishes. However, I make the following recommendations to ensure continued conservation of native species in Nebraska headwater streams.

1. Continue to prohibit stocking non-native trout into Nebraska streams known to contain species of concern if take (*i.e.*, predation) is an issue.
    - I determined that 1) if non-native trout and species of concern overlap in habitat, some predation is likely to occur, and 2) the presence of non-native trout may alter population- and community-level characteristics of native species (*e.g.*, size structure and community structure). Therefore, stocking non-native trout is not recommended in sites known to contain species of concern because any take of species of concern could potentially be detrimental to the population.
    - Non-native trout are likely not the sole reason for declines in populations of species of concern, however many of the other threats to species of concern (*e.g.*, habitat degradation and anthropogenic flow regime alterations) are beyond our immediate control. Adding an additional stressor such as non-native trout to these populations, especially one that can be easily controlled, would be illogical.

2. Account for potential movement of non-native trout when stocking stream reaches where there are no barriers to fish movement.

- Several rivers and streams have been stocked with non-native trout upstream and downstream of reaches and tributaries known to have species of concern. Long-range movements of brook trout (*e.g.*, Gowan and Fausch 1996), brown trout (*e.g.*, Young 1994), and rainbow trout (*e.g.*, Quinn and Kwak 2011) are relatively common, yet are not considered when stocking these species. Determination of a minimally acceptable stocking distance from populations of species of concern, based on what is known about movements of both native and non-native species, changes in environmental conditions or habitat, and physical barriers to movement, should reduce potential unintended consequences of trout introductions.

3. Determine the value of native non-game species.
    - The ecological function of many native non-game species has not been fully evaluated, and the value (*e.g.*, ecosystem services) of these species is unknown in most areas. Determination of the functions, and associated values, that non-game species serve would allow for a better understanding of the consequences of extirpating these species.

4. Determine the recreational value (*e.g.*, angling effort and economic value) of non-native trout in headwater streams, and identify key streams for recreation.
    - The recreational value of stream trout fishing in Nebraska has not been thoroughly evaluated. A recreational assessment would allow for cost-

benefit (*i.e.*, production costs-recreational benefit) analysis of trout introductions (regardless of potential ecological costs). Such assessment would also allow for identification of key streams for recreational use.

5. Reduce the total number of streams stocked with trout and isolate potential ecological impacts.
    - Concentrating recreational activities to a few streams may be beneficial both economically and ecologically. Nebraska is unique in that we can have a recreational stream fishery for trout and protect native species of concern. Designation of a few trout streams, not thought to contain species of concern, per district or region would still provide a unique opportunity to fish for trout, while reducing the impact of non-native trout introductions. Additionally, concentrating trout stockings to a few streams could increase management efficiency and efficacy, which could result in higher quality trout fisheries.

6. Examine the roles of habitat use, availability, and complexity on interactions between non-native trout and species of concern.
    - If non-native trout and native species overlap in habitat use, some predation is likely to occur. However, we have not yet determined the habitat preferences of these species in Nebraska, and do not know the extent of habitat overlap between non-native trout and native species. We

also do not know how habitat availability will influence potential competitive or predatory interactions among these species.

7. Study the ecology of species of concern.
    - Little information exists on the ecology of many of the cyprinid species of concern found in Nebraska. Additionally, the majority of the information that is known about these species comes from studies in regions where they are common and abundant. Further examination of microhabitat preferences, reproductive ecology, and population dynamics of species of concern in Nebraska will aid in efforts to conserve species of concern. Additionally, a better understanding of these species will assist with hatchery production should it become necessary in the future.

8. Develop a hatchery-production protocol for species of concern.
    - Currently, little is known about how to artificially produce many of the species of concern in Nebraska. Development of a hatchery-production protocol for species of concern to assist with research needs would enable experimental evaluation of the influence of non-native trout on species of concern, as well as further other research on species of concern, without jeopardizing natural populations.
    - Hatchery production of species of concern may also allow for supplemental stockings in natural systems should they become necessary in the future. However, it is not recommended that a production protocol

be developed with the intent of ameliorating the effects of stocking non-native trout. Nor is it recommended that artificial propagation be used as a substitute for mitigating other stressors to species of concern. Artificial propagation should only be considered to supplement natural stocks if all other attempts to alleviate threats to species of concern have failed.

9. Monitor common, abundant native species.
    - I documented that non-native trout may be capable of altering common, abundant native fish populations. Therefore, common native species should be monitored in conjunction with non-native trout and species of concern to ensure the conservation of all native species and their ecological functions. Additionally, non-native trout should not be stocked in streams containing common abundant species if changes in size structure or changes in community composition or structure are unacceptable.

10. Mitigate other stressors to native species and species of concern.
    - The absence of non-native trout alone is unlikely to lead to the recovery of at-risk populations in many of these streams. Mitigating all stressors (*e.g.*, habitat degradation and anthropogenic flow alterations) is essential to the protection and recovery of these species. Where feasible, habitat, in-stream flows, temperatures, and other limiting factors should be restored to conditions favoring populations of concern.

REFERENCES

Gowan, C., and K.D. Fausch. 1996. Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. Canadian Journal of Fisheries and Aquatic Sciences 53:1370-1381.

Quinn, J.W., and T.J. Kwak. 2011. Movement and survival of brown trout and rainbow trout in an Ozark tailwater river. North American Journal of Fisheries Management 31:299-304.

Young, M. 1994. Mobility of brown trout in south-central Wyoming streams. Canadian Journal of Zoology 72:2078-2083.

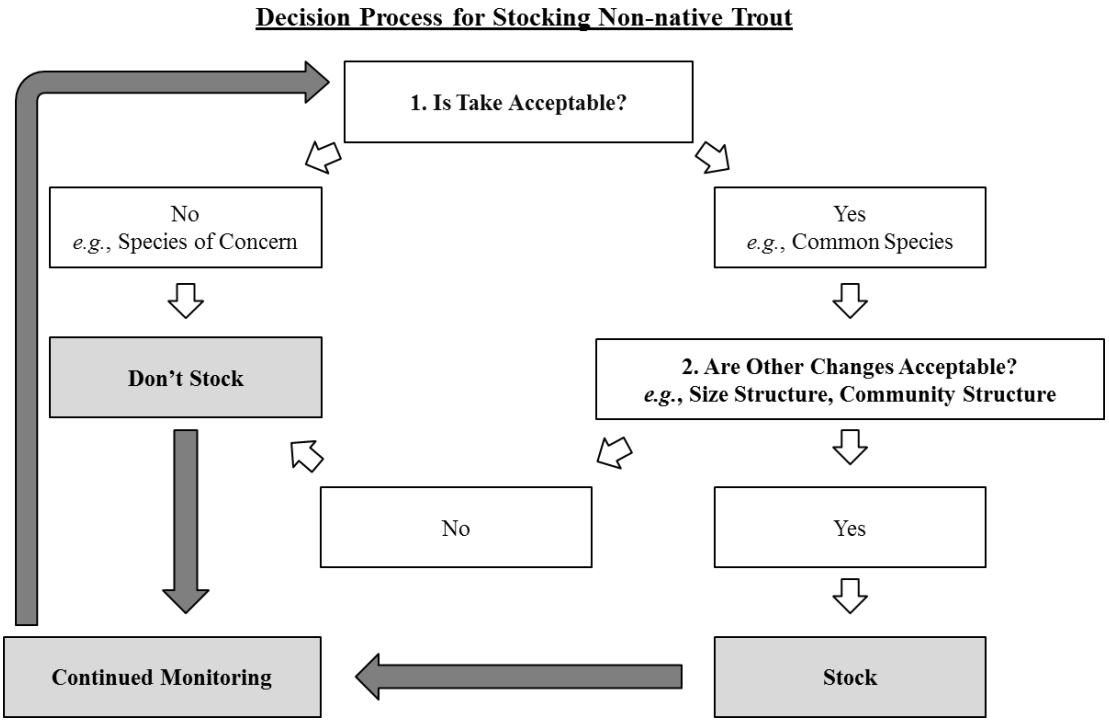


Figure 5-1. Recommended decision process for stocking non-native trout into headwater Nebraska streams.

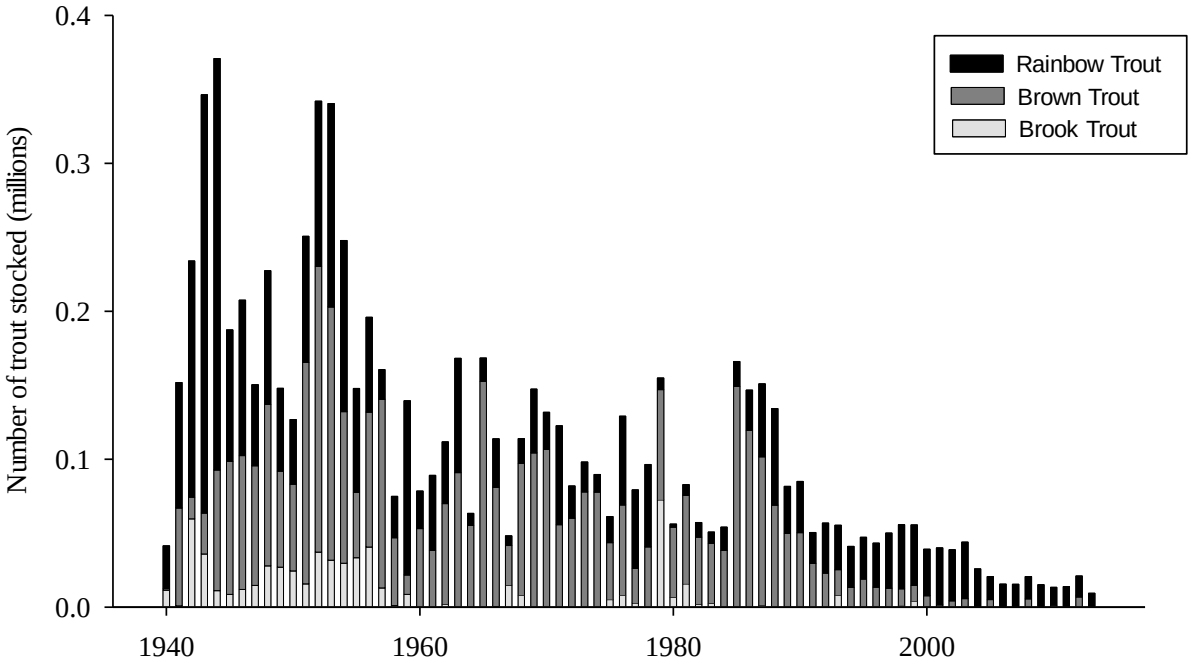


Figure 5-2. Number of rainbow trout, brown trout, and brook trout stocked into Nebraska streams since 1940 (Nebraska Game and Parks Commission, unpublished data).

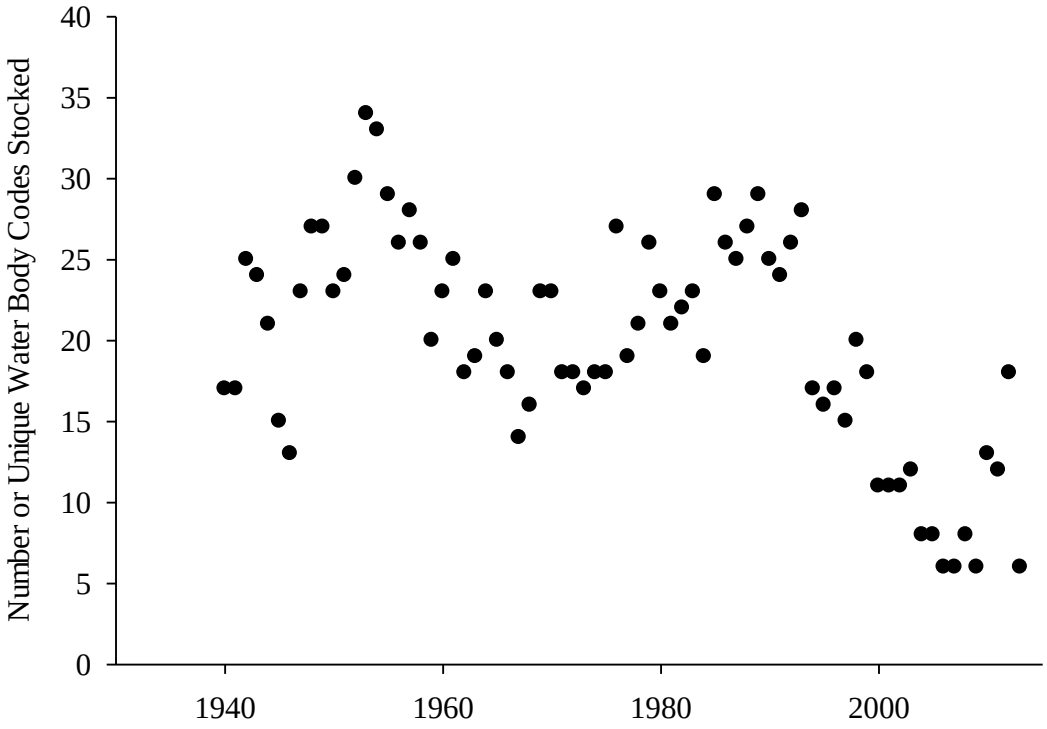


Figure 5-3. Number of unique stream water bodies stocked with non-native rainbow trout, brown trout, or brook trout since 1940 (Nebraska Game and Parks Commission, unpublished data; does not depict multiple stockings of a single stream in a given year).