Experimental evaluation of rainbow trout *Oncorhynchus mykiss* predation on longnose dace *Rhinichthys cataractae*

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Abstract – Laboratory and in-stream enclosure experiments were used to determine whether rainbow trout *Oncorhynchus mykiss* influence survival of longnose dace *Rhinichthys cataractae*. In the laboratory, adult rainbow trout preyed on longnose dace in 42% of trials and juvenile rainbow trout did not prey on longnose dace during the first 6 h after rainbow trout introduction. Survival of longnose dace did not differ in the presence of adult rainbow trout previously exposed to active prey and those not previously exposed to active prey ($\chi^2 = 0.28, P = 0.60$). In field enclosures, the number of longnose dace decreased at a faster rate in the presence of rainbow trout relative to controls within the first 72 h, but did not differ between moderate and high densities of rainbow trout ($F_{2,258.9} = 3.73, P = 0.03$). Additionally, longnose dace were found in 7% of rainbow trout stomachs after 72 h in enclosures. 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 regardless of rainbow trout density treatment ($F_{4,148.5} = 2.50, P = 0.04$). More research is needed to determine how predation rates 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 is expected, even when rainbow trout have no previous experience with active prey.

Key words: predation; enclosure; laboratory; diet

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; however, rainbow trout have since been introduced across nearly the entire native range of longnose dace. With these introductions, and with such common co-occurrence, it is surprising that no studies have directly examined interactions between rainbow trout and longnose dace. Further, rainbow trout predation on morphologically similar species to longnose dace such as speckled dace *Rhinichthys osculus* has been documented (Spurgeon et al. 2014), and rainbow trout have been shown to negatively influence native species in several systems (Turek et al. 2013) suggesting potentially negative outcomes where these two species overlap. Understanding the underlying ecological interactions between rainbow trout and longnose dace will provide the information necessary to ensure proper management and survival of both species in the future.

Rainbow trout are opportunistic, generalist feeders that consume a variety of aquatic and terrestrial invertebrates (Metcalf et al. 1997; Fenner et al. 2004), as well as some small fish (e.g. Blinn et al. 1993). 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 & Crossman 1973). Longnose dace prey on a variety of invertebrates including Diptera (e.g. Simuliidae, Chironomidae), Ephemeroptera (e.g. Baetidae, Siphlonuridae) and Trichoptera (e.g. Hydropsychidae; Reed 1959; Gee & Northcote 1963; Gerald 1966; Gibbons & Gee 1972; Pappantoniou & Dale 1982 and Mullen 1991). Longnose dace shift microhabitat use with age, but are most often found in moderately fast to fast current with cobble or boulder substrate (Gee & Northcote 1963; Gee 1966; Gibbons & Gee 1972; Mullen & Burton 1995).

Both rainbow trout and longnose dace are found in streams throughout Nebraska. These streams are somewhat unique compared with others in which these two species coexist. 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 between rainbow trout and longnose dace compared with 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 are known to prey on fish in Nebraska streams (J.A. Klammer, unpublished data). Further, longnose dace may be at an increased risk of predation in headwater streams because large, native, piscivorous fish 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 & Great Blue Herons), mammals (e.g. Raccoons & Mink) and macroinvertebrates (e.g. Odonates & Belostomatids). Creek chub and grass pickerel are both native to these systems and likely prey on cyprinids to some extent (e.g. Scott & Crossman 1973; Schlosser 1988). However, relative to other predators, they probably have little overall impact on native fish populations due to 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 potential negative influences 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 between 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 between these two species is to determine whether there is a direct predatory threat of non-native rainbow trout to longnose dace, and how that threat changes over time. Two experimental studies, one under laboratory conditions and one under in situ conditions using in-stream enclosures, were used to examine the influence of rainbow trout on longnose dace survival.

Materials and methods

Laboratory

Longnose dace (59 ± 9 mm, mean ± SD) 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 × 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 × 0.5-m rectangular tanks (water temperature = 15 ± 1 °C) and acclimated for 50 min. Rainbow trout were then introduced. 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: (i) juvenile rainbow trout (121 ± 10 mm) fed pellet feed only (hereafter referred to as pellet-fed juveniles), (ii) adult rainbow trout (288 ± 17 mm) fed pellet feed only (hereafter referred to as pellet-fed adults) and (iii) 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 six trials of mixed-diet-fed adult rainbow trout.

All observations were recorded using Go Pro® HERO 3 cameras (GoPro, Inc., San Mateo, CA, USA) set to take videos at a resolution of 1080 p and a frame rate of 30 fps. Cameras were mounted above the tanks and could be remotely controlled to minimise human interference with fish interactions. Predation was then determined from video footage, as was handling time for predation events (from moment of first contact until consumption). Cameras could record a maximum of 6–7 h of video footage (when attached directly to a power source), so our assessment of predation was limited to the first 6 h after rainbow trout introduction. Differences in longnose dace survival between rainbow trout treatments were evaluated using χ²-tests (PROC FREQ, SAS v.9.2, Cary, NC, USA).

In-stream enclosures
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, U.S. 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 (Nebraska Game and Parks Commission, 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.

Twelve enclosures (1.5 m width × 3.0 m length × 0.9 m height), constructed of 2.54-cm PVC pipe and 0.6-cm hardware wire, were placed in Long Pine Creek during July and August 2013. Enclosures also included a fake undercut bank (0.6 m width × 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 1) using a pulsed-DC backpack electrofisher and allowed to acclimate to Long Pine Creek in a temporary enclosure for 1–2 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, unpublished data). Abundance of longnose dace was monitored daily following introduction to ensure escapement was not possible. Additional longnose dace were added and enclosures were repaired until all enclosures successfully held five longnose dace for 24 h. 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.

Table 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 period of RBT to stream where experiments were conducted.

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

*Half of the enclosures were stocked with RBT that had an 11 day acclimation period and half with a 22 day acclimation period.
Rainbow trout predation on longnose dace

Rainbow trout density treatments were chosen to reflect a range of natural densities in Nebraska streams. Rainbow trout density treatments were (i) control (0 rainbow trout/enclosure), (ii) moderate density (2 rainbow trout/enclosure; \( \approx 1 \) rainbow trout \( \text{m}^{-3} \)) and (iii) high density (4 rainbow trout/enclosure; \( \approx 2 \) rainbow trout \( \text{m}^{-3} \)). The experiment was repeated four times (rounds) for a total of 16 replicates per treatment (4 within each round \( \times 4 \) rounds).

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 3–22 days before being introduced into enclosures with longnose dace (Table 1). Following rainbow trout introduction, abundance of all fish was checked every 12 h at approximately 0530 and 1730 (optimal light conditions) for 72 h. Abundance was checked in all enclosures by lifting enclosures from the stream and counting individuals. To minimise the influence of checking enclosures on predation rates, daily checks were completed as quickly as possible. Dead, unconsumed longnose dace were immediately removed from enclosures and were not replaced during the experiment. All fish were removed from enclosures and euthanised after 72 h. 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 whether 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 were measured at three evenly spaced points along a transect positioned approximately 3.0 m upstream of each enclosure, as well as at three transects within each enclosure (front, middle, back) to determine whether flows within enclosures were similar to natural conditions.

Generalised linear mixed 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 lengths at the beginning of the experiment, so that lengths of unaccounted for longnose dace could be determined via process of elimination. Generalised 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.

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

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 whether water depths and velocities within enclosures were similar to natural conditions (measurements taken 3.0 m upstream of enclosures; blocked by \( \text{round}\times\text{enclosure} \)).

Results

Laboratory

No pellet-fed juvenile rainbow trout preyed on longnose dace, 33% of pellet-fed-adult rainbow trout
preyed on longnose dace, and 46% of mixed-diet-fed adult rainbow trout preyed on longnose dace ($\chi^2 = 10.07, P = 0.007$). Survival did not differ between pellet-fed adult rainbow trout trials and mixed-diet-fed adult rainbow trout ($\chi^2 = 0.28, P = 0.60$). Overall, 42 per cent of adult rainbow trout (278–307 mm) preyed on longnose dace and average time to predation was 144 min (range: 37–339 min). Handling time for predation events ranged from immediate consumption to 4 s (2 ± 1 s, mean ± SD).

**In-stream enclosures**

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 moderate and high rainbow trout density treatments than in control treatments ($F_{2,258.9} = 3.73, P = 0.03$; Fig. 1). 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 ($F_{4,148.5} = 2.50, P = 0.04$; Fig. 2). There was no interaction between rainbow trout acclimation period and rainbow trout density treatment ($F_{4,131.4} = 1.09, P = 0.37$, Table 2).

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 ($F_{1,58} = 2.63, P = 0.11$, Table 2) for the last three rounds. However, longer longnose dace had a greater probability of survival in enclosures with rainbow trout ($F_{1,103} = 3.73, P = 0.06$, Table 2) for the last three rounds. Postexperiment longnose dace lengths were not available for the first round, and thus could not be matched to prestocking 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 rainbow trout density treatments ($F_{2,45} = 0.36, P = 0.70$, Table 2).

In the first round, where rainbow trout stomachs were examined only for enclosures where longnose dace were missing, 58% (95% CI = 29–87%; $N = 7$) of rainbow trout stomachs contained macroinvertebrates, 42% (95% CI = 13–71%; $N = 5$) were empty, and 8% (95% CI = 0–24%; $N = 1$) contained longnose dace. In the last three rounds (where all rainbow trout stomachs were examined regardless of longnose dace presence or absence in enclosures), 72% of rainbow trout stomachs contained macroinvertebrates (95% CI = 61–83%; $N = 51$), 28% were empty (95% CI = 17–39%; $N = 20$) and 7% contained longnose dace (95% CI = 1–13%; $N = 5$). The per cent 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. 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 (Table 2). The first round was excluded because only stomachs in enclosures with longnose dace missing were examined, whereas all stomachs were examined in subsequent rounds.
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 were 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 will prey on longnose dace, and if rainbow trout and longnose dace are isolated to the same habitats, some predation is likely. 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. Additionally, predation was documented via rainbow trout stomach content analyses in enclosures and via direct observation under laboratory conditions. Rainbow trout are not widely recognised 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). Predator density and experience, as well as prey length, and habitat availability will likely influence predation rates under natural conditions.

In this study, the density of rainbow trout did not influence the number of longnose dace remaining in enclosures. Predator interference (i.e. among multiple rainbow trout) or altered prey behaviour (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 moderate rainbow trout densities. Predator interference can lead to a nonlinear relation between predation rates and prey densities (e.g. Beddington 1975; DeAngelis et al. 1975) and may reduce predation risk for longnose dace at high rainbow trout densities. Longnose dace may also alter their behaviour (e.g. foraging behav-
ior and timing) to decrease predation risk at high rainbow trout densities (e.g. Lima & 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).

Experience of both predator and prey can also influence predation rates. Nonnative rainbow trout have been suggested to pose increased risk to native populations because of a lack of coevolutionary history and thus, a reduction in the ability of prey to recognize predators as a threat (Townsend & Crowl 1991; Blinn et al. 1993; Bryan et al. 2002; Nannini & 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 laboratory experiments, 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 immediate predation rates (within the first 6 h after introduction). These results suggest that stocking naïve hatchery rainbow trout (i.e. those fed only pellets) may result in predation risk to native stream fishes relatively quickly.

Predation rates often depend on prey size (e.g. Werner & Gilliam 1984). Prey length appeared to be an important factor in longnose dace survival in enclosures. 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 (K.C. Turek, unpublished data), suggesting something other than size alone (e.g. increased swimming velocities with size) must explain the survival advantage of larger longnose dace.

Much research has focused on behavioural decisions of stream fishes related to foraging, predator avoidance, and resource availability, as well as the interactions among these factors (e.g. Fraser & Cerri 1982; Cerri & Fraser 1983; Gilliam & Fraser 1987; Lima & Dill 1990). In this study, we isolated the predatory interactions of rainbow trout on longnose dace (i.e. held food availability and habitat constant within each study), greatly simplifying the complex interactions that may occur in stream environments. However, habitat complexity was likely greater in enclosures relative to laboratory conditions and may explain the reduction in predation rates observed. No artificial habitat was added to laboratory tanks, and no artificial habitat was added within enclosures (except the fake undercut bank), but 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 and may explain the lower predation rates observed in enclosures compared with laboratory tanks. How this habitat availability translates to the natural availability of habitat is unknown. Further research is needed to determine whether, 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 our study), then some rainbow trout predation on longnose dace is likely, even when rainbow trout have no previous experience with active prey. This should be of concern in areas with morphologically similar native species, in particular at-risk species, as some rainbow trout predation is likely. Therefore, the potential negative influences of non-native trout predation on native populations should be considered prior to stocking nonnative trout in areas with longnose dace or similar species.

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