Impacts of a non-native fish species (*Notropis stramineus*) on a native trophic system in the upper Colorado River basin

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Abstract

The introduction of a non-native species into an ecosystem often displaces native species through competition for similar resources. As non-native fish species exploit resources, they can trigger a trophic cascade. Trophic cascades can have devastating effects on ecosystems; the alteration of river flow attributes can affect native species abundance and community structure and is able to subsequently influence the ecosystem's biodiversity. The speckled dace (*Rhinichthys osculus*), a native species in the upper Colorado River basin, and the sand shiner (*Notropis stramineus*), a widespread non-native, both prey on macroinvertebrates within the orders of Trichoptera, Plecoptera, and Ephemeroptera. We hypothesized that the introduction of the non-native species, *N. stramineus*, to the Yampa River would negatively affect the populations of macroinvertebrates and subsequently the recruitment of the native *R. osculus*. More specifically, we predicted that the Trichoptera abundance would be greater at sites where *N. stramineus* were absent than at sites where they were present. Due to a high demand for the same prey, we suspected *R. osculus* would have a greater percentage of first-year recruitment in areas with a lower presence of *N. stramineus*. In some preliminary sampling efforts of four sites along the Yampa and Little Snake Rivers in Routt and Moffat counties, Colorado, USA, we utilized backpack electrofishers with dip and seine nets to sample fish and Surber samplers to collect macroinvertebrates. However, the data considering the relationship between the native and non-native fish species were inconclusive and the role of macroinvertebrate abundance remains unclear. The data provided us with information about the study sites and the river's microhabitats and presented continued evidence that more research is needed to better understand the role non-native fish play in the Colorado River basin. Being relatively untouched by human development, the predominantly

free-flowing Yampa and Little Snake Rivers provide suitable habitats for native fish species and have allowed these native populations to thrive. This study should contribute to a better understanding of how trophic relationships are being affected by the invasive *N. stramineus* in the Colorado River basin. The goal of our research is to contribute to the ecological significance and impact of invasive species on native species and their relatively undisturbed waterways**.**

Introduction

In recent years, much more light has been shed on the field of invasion biology and the consequences of these foreign introductions on native ecosystems. Invasion biology addresses the concerns and impacts of the introduction of a non-native species to an ecosystem in which the species has not been historically present (Crooks 2002). Invaders have the potential to change ecosystems by altering energy flows and modifying habitat (Crooks 2002). Generally, impacts from invasions include the non-native species either preying on or competing for the same prey as native species and the consequences of altering genetic effects when invaders hybridize or otherwise alter the gene flow of native species (Crooks 2002). The interactions between invading and native species can trigger a trophic cascade that can reshape the surrounding habitat and alter resource availability within the ecosystem. This study continues to investigate the role invaders have on native ecosystems by examining the potential impact a non-native fish species has on a native fish species and its macroinvertebrate prey.

A trophic cascade, the triggering of reciprocal changes throughout an ecosystem after a disturbance, for example by an invading species, can have widespread consequences. Energy shifts can be altered as generalist non-natives capitalize on resources that more specialized natives depend on for survival (Koel et al. 2019). Non-native fish predators can alter the prey assemblage structure by killing or changing the behavior of their prey (Harvey et al. 2004). The cascading effects caused by an invasion can quickly reduce native populations. Furthermore, introduced fish species that feed at levels lower in the trophic system can exploit resources that native fish rely on (Flecker and Townsend 1994) and thus displace native species that have to compete for these similar resources (Taniguchi et al. 2002). Because of the profound potential negative impacts of niche overlap between introduced and native fish species in an ecosystem, it is critical to investigate how alien introductions affect multiple native trophic levels.

The sand shiner (*Notropis stramineus*) is an extremely widespread Cyprinid species present in freshwater ecosystems. They are found in open clear water streams with sandy bottoms, where they are omnivorous and feed on a variety of aquatic and terrestrial insects and diatoms. *N. stramineus* is a non-native species in the upper Colorado River basin, and it was believed to have been introduced to the system in the late 1960s, perhaps as baitfish, where it quickly became an established population (Nico et al. 2019). While numbers of *N. stramineus* have been rapidly increasing, the impacts of this introduction on the native ecosystems remain largely unknown. A study conducted by Whitmore (1997) concluded that the species was reported to be “a problem” in the Yampa River in Colorado, whereas another study by Muth and Snyder (1995) showed that *N. stramineus* had a low to moderate degree of dietary overlap with young-of-year of a native species, the Colorado pikeminnow (*Ptychocheilus lucius*). Effects of *N. stramineus* on other species of native fish in the Colorado River basin, specifically in the tributaries of the Little Snake and Yampa Rivers, are yet to be determined.

One common species of native fish present in the Colorado River basin that is likely affected by the introduction of *N. stramineus* is the speckled dace (*Rhinichthys osculus*). This species, another member of the Cyprinidae family, is bottom-dwelling specialist found in rocky riffles, runs, and pools of headwaters, creeks, and small to medium rivers in temperate freshwater ecosystems (McClane 1974). Like *N. stramineus*, *R. osculus* is omnivorous and feeds upon both plant material and zooplankton as well as bottom-dwelling aquatic macroinvertebrates. Thus, *R. osculus* serves an important ecological role and can influence ecosystem structure and function of a stream through foraging on producers and affecting nutrient pathways (Baxter et al. 2004), in addition to affecting the macroinvertebrate prey assemblage. Moreover, since there is dietary overlap between *N. stramineus* and *R. osculus*, the preexisting ecological roles of *R. osculus* are likely to be altered in areas with a higher abundance of *N. stramineus*.

An important source of macroinvertebrate prey of both the native *R. osculus* and the non-native *N. stramineus* is the aquatic larvae of caddisflies (order Trichoptera). Previous studies have shown that the impacts of non-native fish species on the native system extend beyond the replacement of native fish; one study by Flecker and Townsend (1994) found large differences in the insect assemblages present in experimental channels depending on the fish species inhabiting the area with insect densities and biomass being lowest in channels containing an introduced species of trout. While few previous studies have focused specifically on the impacts of alien fish species on the abundance of important prey, we believe that the introduction of *N. stramineus* may directly affect the abundance of macroinvertebrate prey that many species of native fish rely on in the upper Colorado River basin.

Using data spanning 15 years (2004-2019), we explored the relationships among the non-native *N. stramineus* and two other tightly linked components of the aquatic food web in four sites of the Yampa and Little Snake Rivers. These ecosystems are in a state of change, as many new environmental stressors have been imposed in the past few decades, such as dam formation and the introduction of many new invasive species. First-year recruitment, the number of juveniles which join a population each year, is one way to measure the health of a population. First-year recruitment indicates the age-class structure of a population (if the population tends to have more older or younger members, for instance), which can help predict whether a population is growing or shrinking (Bradford and Cabana 1997). Fish have particularly high mortality rates while in the juvenile stage, largely because of predation and environmental risks due to their smaller size (Bradford and Cabana 1997; Gutreuter and Anderson 1985; Hurst and Conover 1998). Low first-year recruitment within a fish population, therefore, indicates that juvenile survival is low. This can be due to abiotic factors such as harsh winters (Hurst and Conover 1998) or hydrographic variation (Nunn et al. 2007). However, because juvenile mortality and first-year recruitment is strongly correlated to the growth rate of the juvenile fish (Ludsin and Devries 1997), low first-year recruitment can be an indication of low prey abundance (Gutreuter and Anderson 1985).

Preliminary sampling collected in September 2019 from four sites on the Yampa and Little Snake Rivers in Routt and Moffat counties, Colorado, USA, provided us with information about the study sites in the upper Colorado River basin, including particulars on base flow rates and microhabitats. Using backpack electrofishers with dip and seine nets to sample fish and Surber samplers to collect macroinvertebrates, we considered the relationship between native and non-native fish species and how the non-native species was potentially affecting macroinvertebrate abundance. The regression analysis of the *R. osculus* and *N. stramineus* populations at our four study sites yielded a p-value of 0.7, which is not significant (*Fig. 1)*. This suggests that other factors are influential in affecting the first-year recruitment of *R. osculus*, rather than competition from this invasive species. However, further study is needed, as the two study sites show almost complete exclusivity for one species over the other. If other sites on the Yampa and White Rivers show this exclusivity, it may provide evidence that these species are in competition with each other for food or habitat. The regression analysis of the *N. stramineus* and Trichoptera populations at the four sites produced a p-value of 0.6 (*Fig. 2*). Thus, this result is not significant and suggests that *N. stramineus* predation is not the main factor in determining population density of Trichoptera in riffle habitat. We believe that perhaps habitat quality is a better indicator, as in the sites that contain the most contiguous riffle habitat and the least sand (Hayden and the Upper Little Snake sites), Trichoptera populations were much higher. Further sampling of more riffle habitat is crucial in order to fully understand the relationships between these species and the trophic system in the Colorado River basin.

The Yampa River, the largest tributary to the Green River, is located in northwestern Colorado and southern Wyoming and is a highly productive area of the upper Colorado River basin. Downstream from its confluence with the Little Snake River, the Yampa River crosses Dinosaur National Monument where its moderate gradient greatly increases through a large section of rocky runs and riffles that provide crucial habitats to native fish species such as *R. osculus*. The average annual discharge of the Yampa is 60.5 m^3/s, however, only approximately 28% of this discharge is contributed by the Little Snake (Tyus and Karp 1989). Despite being a relatively small contributor in terms of flow to the Yampa River, the Little Snake provides 70% of the sediment sources that make up key habitat features in the Yampa (Tyus and Saunders 2001). Furthermore, the Yampa River is characterized by a natural flow regime which rises in late March as a result of spring runoff and remains high through July. However, flows decline toward a monthly baseflow of about 14.15 m^3/s between August and March with occasional large fluctuations in river level. Therefore, native fish species have had to evolve in this unique system with flows fluctuating both seasonally and annually (Tyus and Karp 1989) and may be more susceptible to changes resulting from the introduction of non-native species.

Invasive species such as *N. stramineus* can have negative impacts on native species like *R. osculus* through a trophic cascade. Thus, to analyze the relationship between *N. stramineus*, *R. osculus*, and their shared prey source, we posed the question, how does the presence of *N. stramineus* affect the percentage of first-year recruitment of *R. osculus* and the abundance of macroinvertebrate prey (order Trichoptera) in the Little Snake and Yampa River basins? We proposed two main hypotheses. First, we predicted that the macroinvertebrate abundance would be greater at sites where non-native predators were absent than at sites where they were present, since both native and non-native predators prey on the same macroinvertebrates in the order Trichoptera. Second, we predicted that *R. osculus* would have a greater percentage of first-year recruitment in areas with a lower presence of *N. stramineus* because there would be a higher prey abundance to sustain the population. Because the Yampa River is the only large river in the Colorado River basin in which flow patterns have not been substantially altered by water development projects (Tyus and Karp 1989) and is also a regional hotspot of native fish diversity (Loppnow et al. 2013), it presents a unique opportunity to examine our hypotheses in sites where the native species predominate versus other sites that contain a higher proportion of non-native species.

Identifying the impact non-native species have on the health of native species has wide-reaching implications for fisheries management within the Colorado River basin. Analyzing the effect *N. stramineus* have on first-year recruitment in *R. osculus* will allow fisheries managers to identify *N. stramineus* as either an invasive species of concern or as a non-native species with little effect on the ecosystem, and will thus allow them to focus their management of non-native species in these waterways accordingly.

Research Methods

Hypothesis 1: Since both native and non-native predators are preying on the same macroinvertebrates in the order Trichoptera, the macroinvertebrate abundance will be greater at sites where non-native predators are absent than at sites where they are present.

Hypothesis 2: *R. osculus* will have a greater first-year recruitment in areas with a lower presence of *N. stramineus* because there will be a higher prey abundance to sustain the population.

Our study took place along the Little Snake, Yampa, and White Rivers. Four sites on the Little Snake and Yampa Rivers were identified and classified as Hayden (UTM: 13N 305222E 4484665N), Upper Cross Mountain (UTM: 12N 726804E 4485168N), Lower Little Snake (UTM: 12N 718065E 4491850N), and Upper Little Snake (UTM: 12N 748440E 4537262N). These sites all contained sizeable riffle habitats which we defined as areas with rocky substrate and water depth of less than 0.5 m. These sites were also easily accessed from roads, and contain populations of either *R. osculus* and/or *N. stramineus*. Going forward, we will identify an additional six sites that meet these qualifications on the White and Yampa Rivers to sample for *R. osculus* and *N. stramineus* abundance.

At each location, we used backpack electrofishers (Smith-Root, Inc., LR24) and dip nets to sample 100-300 m^2 of riffle habitat. One person with an electrofisher walked across the riffle habitat in a grid pattern, while two people with dip nets collected the shocked fish and deposited them in buckets. Seine nets were used downstream to prevent the loss of any shocked fish that were missed by the dip nets. We then identified the fish collected in buckets, and if identified as *R. osculus* or *N. stramineus*, we measured them as well. Any *R. osculus* measured as less than 50 mm was classified as young of the year. We used first-year recruitment as an indicator of *R. osculus* population health. All fish were released back into the river in the same riffle they were collected from.

At each location we used Surber samplers (WildCo, Inc) to sample populations of Trichoptera macroinvertebrates. We measured the widest portion of the riffle and assigned one sample site in the center of that area. Next, we identified two more riffle sites in the same horizontal cross-section of the river that were equidistant from the center site and the edges of the riffle. At each site, we placed the Surber sampler as flat as possible on the riverbed and disturbed the substrate for 30 seconds. Then, we emptied the contents collected in the Surber sampler onto a white metal tray and counted the total amount of Trichoptera macroinvertebrates present. These macroinvertebrates were placed back into the riffle at the sample site once counted.

We compared the abundance counts of *N. stramineus* and first-year recruits of *R. osculus* using regression analysis. Any p-value less than 0.05 suggested a significant negative correlation between the amount of *N. stramineus* and *R. osculus* in riffle habitat. We used the same test to compare numbers of *N. stramineus* and Trichoptera. We also used a t-test comparing the populations of *R. osculus* in habitats with a significant population of *N. stramineus* and in habitats without it. These analyses gave us an idea of the significance of the relationship between these species on their abundance.

Budget

The itemized list below represents the minimum expenses necessary to complete field work for one 3-month season on the Little Snake, Yampa, and White Rivers. Surber samplers will be used to measure macroinvertebrate density, while the backpack electrofisher will be used to measure populations of *N. stramineus* and *R. osculus*. Dip nets, buckets, and the seine net are needed to accompany the electrofishing, while waders are necessary to protect technicians while in the water. Five technicians are needed to conduct the sampling; one person will use the backpack electrofisher while two people will catch fish with dip nets and two people will hold the seine net. All technicians will participate in the macroinvertebrate sampling.

Total Cost: ***$49,100***

- **5 Waders: $200**
    - Needed for safe sampling in the riffle habitat while using the electrofishing backpack
    - Price obtained from Amazon.com
- **5 Wading Boots: $165**
    - Needed for safe sampling in the riffle habitat while using the electrofishing backpack
    - Price obtained from Amazon.com
- **5 Hand Lenses: $75**
    - Needed to accurately count the number of Trichoptera collected with the Surber Sampler
    - Price obtained from Amazon.com
- **2 Forceps: $6**
    - Needed to accurately count the number of Trichoptera collected with the Surber Sampler

    - Price obtained from Amazon.com
- **2 Surber Samplers: $790**
    - Needed to sample the Trichoptera density in riffle habitat
    - Price obtained from Sciencefirst.com
- **2 Metal counting boards: $20**
    - Needed to accurately count the number of Trichoptera collected with the Surber Sampler
    - Price obtained from Amazon.com
- **1 Measuring Tape: $29**
    - Needed to measure riffle habitat width for accurate sampling
    - Price obtained from Amazon.com
- **Car Rental for 3 months: $1,800**
    - Needed for transportation to study sites
    - Price obtained from Enterprise Rent-A-Car website
- **Gas for 3 months: $900**
    - Needed for transportation to study sites
    - Price based on current gas prices (~$2.50/gallon), average gas efficiency of mid-range rental cars (~25 mpg), and estimated mileage (~100 miles/day).
- **5 Salaries: $31,200**
    - Needed to pay technicians for three-month period; skilled technicians are essential to successful completion of the research
    - Price based on Colorado minimum wage for the summer of 2020 ($12/hr), for five technicians working 8-hour days for three months (a total of 2,600 people-hours)
- **Accomodation cost: $4,500**
    - Needed to house technicians during the field season. Based on the remoteness of some of the study sites, technicians will often be required to camp, but will require some form of housing to store their belongings at during these periods in the field.
    - Price obtained from Homefinder.com (Rental for 5-bed house ~ $1,500/month in Craig, Colorado).
- **2 dip nets: $24**
    - Needed to catch stunned fish with while backpack electrofishing
    - Price obtained from Sportsman's Warehouse
- **3 5-gallon buckets: $75**
    - Needed to store fish in while sampling and measuring fish
    - Price obtained from ULINE
- **1 seine net: $40**
    - Needed to act as a backstop while electrofishing
    - Price obtained from Amazon.com

- **Backpack Electrofisher: $9,276**
    - Needed to humanely sample fish in riffle habitats at sites
    - Price obtained from Smith-Root website

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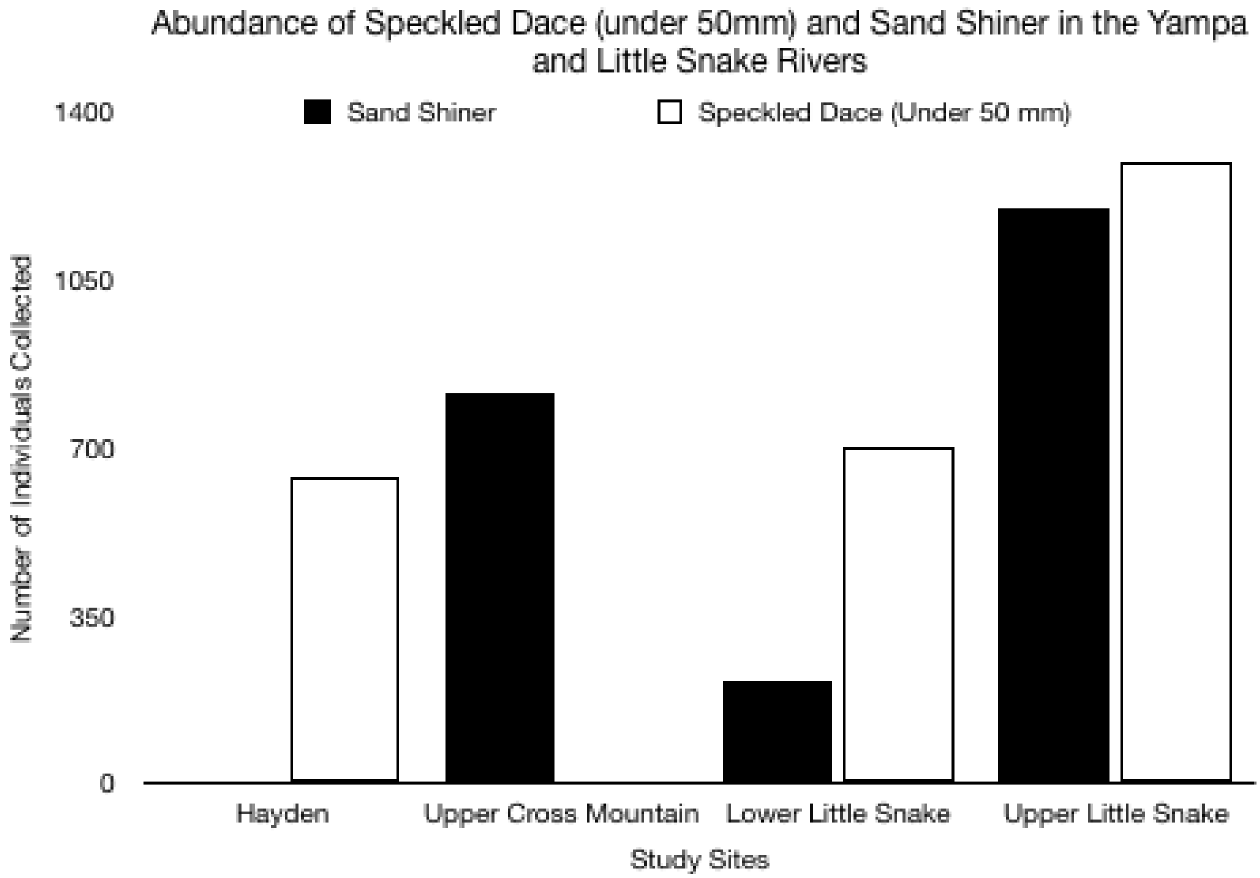

Fig. 1. This figure shows the total number of individual Sand Shiners and Speckled Dace under 50mm collected at these four sites between 2004-2019. Speckled dace were present at all four of the study sites, while sand shiners were only present at three of the study sites. At both Yampa River sites, the riffle habitat contained almost exclusively one species, while in the Little Snake both were present in significant numbers. Both species were most populous at the Upper Little Snake site.

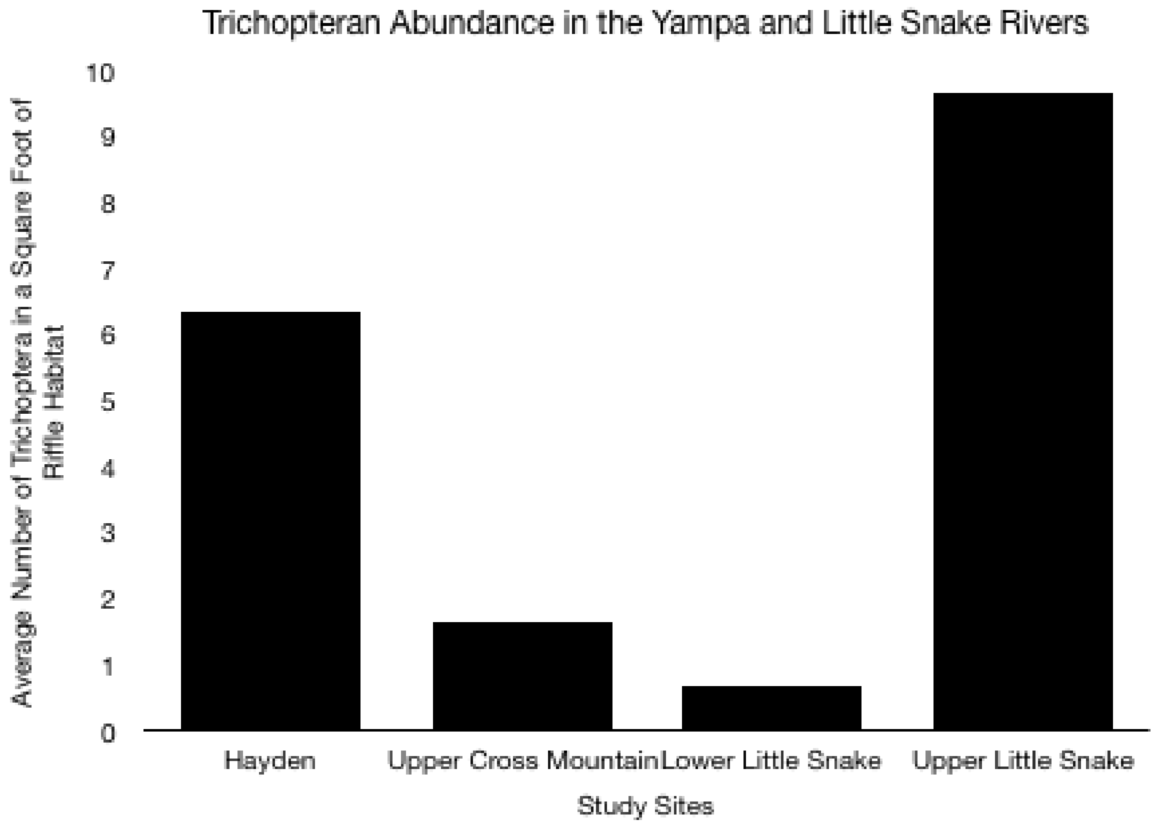

Fig. 2. This figure shows the average number of Trichopterans found in a square foot of riffle habitat at each of these sites. Trichoptera were observed at all four sites. They were most abundant at sites with more contiguous riffle habitat (Hayden and the Upper Little Snake), and were not as abundant in sandier habitats (Upper Cross Mountain and Lower Little Snake).

On our honor, we have neither given nor received unauthorized aid on this assignment.