Introduction

Invasive alien species are a major threat to global biodiversity. Removal of aquatic invasive alien species is often costly and have low long-term success rates. This may be due to the method of removal being biased towards certain phenotypic traits inducing unexpected phenotypic trait changes in the remaining individuals, which could counterproductively cause the impact of invasion to be more severe. Brook trout and brown trout are two widely introduced salmonid species originating from eastern North America and Eurasia, respectively, that tend to displace each other along the longitudinal gradient, and are deemed invasive in much of their non-native range. The two species cause negative effects in areas they have been introduced by displacing local salmonids and altering local food webs. In this project the aim is to further understand how the presence of the invasive brook trout in Sweden affects the dynamics of the invaded ecosystems and evaluate methods to effectively remove or reduce this species to restore and preserve natural, pre-invasion ecosystem functions. The initial work started in the fall of 2021 and will conclude with a PhD thesis that is set to be completed in 2025. The project is divided into four interconnected work packages outlined in the following pages:

Project scope

Work Package 1: Establish control populations and develop novel removal methods for brook trout.

In WP1 we will initially assess the allopatric and sympatric populations. These populations inhabit eight different natural stream sections we have selected in the Borås area of western Sweden. We will sample approximately 50 fish form each section (in sympatric sections we will aim for 25 of each species): two streams have allopatric brown trout and sympatric brown and brook trout populations at different sections (Ringsbäcken and Lindåsabäcken). Hökabäcken has sympatric populations upstream and downstream, and Sävbäcken has allopatric upstream and downstream. The ratio of species density in the sympatric stretches differ which will give further insight into how the effects of the invasion may be dependent on how the system is more or less affected by brook trout (**Fig. 1**).

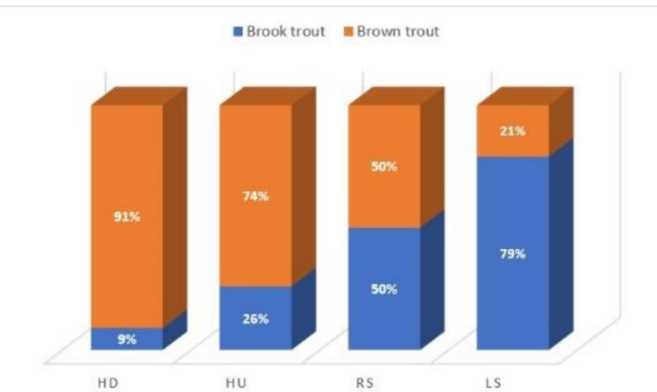

Figure 1 The achieved ratios of brook and brown trout when fishing the stretches twice and aiming for 50% of each species

We will catch fish using electro fishing and traps. We will tag each fish with Passive Integrated Transponders (PIT) tags, weigh and measure fork length and photograph each fish. We will be assessing the social hierarchy by utilizing a modified open field freeze test and habitat use by monitoring their movement over time using portable antenna. We will also assess their diets by using stable isotope analyses of carbon and nitrogen through sampling of pelvic fin clips (isotope content in fins correlate with that of muscle tissue and allows for non-lethal sampling) and potential prey, and growth by recapturing the tagged individuals later in the season (Cucherousset et al., 2020). These will form our “control” populations where we have data on growth, diets and boldness. We will also examine some fundamental properties of each section including temperature, pH, productivity and rate of decomposition and nutrient cycling, in addition to examining the benthic invertebrate compositions of our stream sections using kick sampling.

In the second part of this work package, we will test methods of removal. These methods include electrofishing, traps and nets. We will vary our electrofishing temporally and spatially in order to target more brook trout. Temporally this will be done at different times throughout the day as well as the season where we expect to find differences in capture rates and phenotypic traits depending on when we electro fish. We will also attempt to target brook trout where and when they spawn to reduce reproductive success. Different traps will be evaluated; "social" traps and traps baited with pheromones. In total, this work package will give use the comparable baseline information and the efficiency of several methods of removal.

Work Package 2: Study behavioral changes of both the invasive brook trout and the sympatric brown trout when exposed to removal/nearly eradicated brook trout population.

Brown trout tend to alter diet and habitat use in sympatry with brook trout. In this WP will assess to what extent these effects remain after removal. We will also investigate whether these effects are equally impacted by brook trout, or whether a few more dominant individuals affect brown trout behavior to a larger extent. This will be done by collecting the same type of data from the same populations of our chosen stream sections after removal of brook trout.

Work Package 3: Study of ecosystem functioning following removal

In WP3 the focus will be on evaluating the ecosystem after removal. Again, we will use the same stream sections as in WP1 allowing us to assess the composition of benthic invertebrates in the stream both in allopatric stretches and sympatric stretches as well as the composition of polyunsaturated fatty acids in brook and brown trout in sympatric and allopatric populations. The flux of emerging invertebrates will be quantified using emergence traps and their contribution to terrestrial consumers will be quantified using stable isotope analyses. We will also assess the stream section as they relate to functions including primary production, recycling of organic matter and nutrient cycling.

Work Package 4: Study the effects of brook trout invasion and eradication on the interaction between freshwater pearl mussel (FPM) and brown trout.

Similar to WP3, this WP will focus on ecosystem effects but on the interaction between Freshwater pearl mussel (FPM), brown trout and the invasive brook trout since all species often share habitats.

We will here focus on how brook trout and its phenotypic variability induced by removal may modulate interactions between a host (brown trout) and the parasite (larva of freshwater pearl mussel). Brook trout might have a direct negative effect on this relationship if brook trout gets infested but cannot produce juvenile mussels (Salonen et al., 2016). However, there might also be indirect effects if the presence of brook trout would alter the temporal and/or spatial habitat use of brown trout resulting in fewer exposures to the glochidia larva. This WP will partly be performed in a different system than the other WP where we have a population of FPM. The aim of this WP is therefore to establish to what extent invasive brook trout can act as a functional host of FPM and to examine how this in turn affects the functionality of native brown trout as the main host for the glochidia larvae of the FPM, both direct and indirect.

Project progress

So far, the focus has been on WP1 where we have established control populations before removal. The focus has thereby been on establishing a baseline, or the "before" picture to which we will compare the "after" picture after removal in the selected experimental stream sections in Borås. More information about the methodology and some preliminary observations are discussed as follows:

Assessment of behavioral traits

Invasive species may through competition and predation induce changes in phenotypic traits (including behavior, morphology and life-history-traits) of native species responding to the new biotic interactions the are subjected to, potentially leading to a long-term downward spiraling if these changes represent costly trade-offs. The impact of brook trout invasion could be influenced by the behavioral phenotypes (i.e. bold/active or shy/inactive) of the individuals of the populations, as the development of social hierarchies in stream-dwelling salmonids is common (Sloman et al., 2000). Trout may display a difference in movement activity levels, reflecting behavioral types, which in turn can affect probability of catching same sized individuals (Näslund et al., 2018).). Additionally, circadian patterns also differ according to behavioral phenotypes (Závorka et al., 2016). The spatial and temporal activity patterns of the fish will be assessed.

Boldness scoring

In the populations of the experimental streams we have conducted boldness tests for a subset of individuals of both species in the populations of these stream sections. This scoring entails placing each individual in a novel, barren environment where it is covered for 2 minutes before the cover is removed and a timer begins timing when the fish starts moving around and exploring its new environment with five minutes as maximum time given (the fish that have been still for the duration of five minutes are categorized as “no movement” and are given the lowest boldness score) (**Fig. 2**). These fish have been electro fished and kept in in-stream boxes for approximately one hour before scoring. This is done to minimize handling time and thereby stress that may skew the result, while also giving the fish some time to recover from the electro fishing. he movement is then captured by a video camera in order to minimize human disturbance during this time. The fish that move in shorter amount of time is given a higher score for boldness. Preliminary results from the initial boldness scoring indicate that brown trout in sympatry are slightly less bold, as there was found a significant relationship between being less bold and living in sympatry for the brown trout.

Figure 2 The setup for the boldness scoring. Each box contains one fish which gets two minutes underneath a cover before the cover is swiftly removed. The boxes are then left alone without disturbance for five minutes while a camera captures how long it takes for the fish to beging moving/exploring.

Telemetry

The fish that were tagged in fall of 2021 were tracked with portable antennas weekly for seven consecutive weeks after the initial fishing was completed. Preliminary results indicate that home range, when calculated as average distance between the two furthest apart positions of detection, give no clear trends that brown trout have a smaller or larger home range in sympatry, although the data needs to be evaluated more precisely and corrected for sample sizes and extreme values that may skew the data (**Fig. 3**). Furthermore, the stream sections have been scanned weekly through late winter and early spring which will give a more detailed perspective on the dispersal through the seasons.

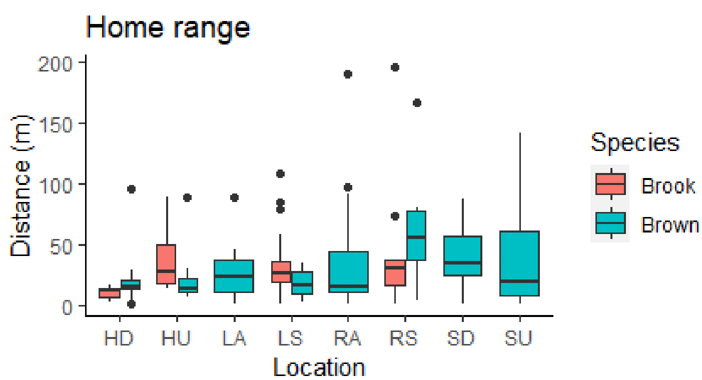

Figure 3 Visual representation of the home ranges of both species of each stream section (location).

Habitat mapping

Each stretch has been habitat mapped and been given a score for the quality of habitat for trout based on the physical characteristics of the stretches (**Fig. 4**). Examination of the quality of habitat choice of brown trout will be compared between stretches invaded by brook trout and not invaded by brook trout in order to determine whether either species is suppressed to a less suitable environment when in sympatry.

Figure 4 Each stream section has been habitat mapped and a trout habitat score has been calculated. The perimeters of this score includes depth, flow, substrate and shading.

Plans for 2022/2023

Removal

In the fall of 2023 we will begin the efforts of removing the brook trout from two of the stream sections in Borås.

Artificial stream channels

In order to get a more detailed perspective of the effects we are going to be conducting more controlled experiments in artificial stream channels in Lunz, Austria next spring and summer (**Fig. 5**). This will be conducted as a supplement to the field based approach.

Here, we will again examine the behavioral phenotypes of the fish. We will design the enclosures so as to create different subhabitats of different qualities. This will allow us to determine which fish is the most dominant one as the most dominant fish will inhabit the best habitat (i.e. the most stable water flow and best food supply), and we will subsequently remove this fish and observing the response of the remaining population. The enclosures will emulate both sympatric sections with both species, as well as allopatric sections with only brown trout.

Figure 5 The artificial stream channels in Lunz, Austria. These channels will allow us to control flow, temperature, food availability, shading and substrate, as well as allow us to observe the fish with antennas and cameras.

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