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Evaluating remote site incubators to support restoration of Arctic Grayling in Michigan

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Dedication

To family and friends who have supported me during this academic journey. Thank you!

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Abstract

Remote site incubators (RSIs) have been used to rear salmonid fish eggs along streams in the Pacific Northwest since the 1980s. Recently, the successful use of RSIs for Arctic Grayling *Thymallus arcticus* restoration in Montana has sparked a renewed interest to reestablish the species in Michigan. To support future reintroduction efforts of Arctic Grayling in Michigan, I evaluated RSIs in three Michigan streams during 2018 and 2019 using Rainbow Trout *Oncorhynchus mykiss* eggs (as surrogates for Arctic Grayling). My objectives were to: (1) compare hatching success between two different RSI designs (19-L vs. 265-L RSIs), and (2) test whether the removal of dead eggs ("picking") from 19-L RSIs affected hatching success. Overall survival (i.e., hatching success of all RSIs) in 2018 and 2019 was 41.3% and 52.4%, respectively. Survival between unpicked 19-L and 265-L RSIs by stream differed from 1.5% to 14.3% (mean = 5.8%) in 2018 and 0.2% to 0.4% (mean = 0.3%) in 2019. On average, the picked 19-L RSIs had greater survival—although not always statistically significant—than unpicked 19-L RSIs during both years (2018: mean = 1.6%, P = 0.27; 2019: mean = 10.4%, P = 0.02). I documented a positive correlation between survival and RSI flow rates, and a decline in survival when RSI flow rates could not be maintained above ~0.4 L/min. My results show that both 19-L and 265-L RSIs can be used successfully in Michigan streams. Moreover, my results suggest that removing dead eggs was most likely to improve survival when RSI flow rates cannot be maintained above 0.4 L/min.

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Abbreviations

Remote site incubator – RSI

Michigan Department of Natural Resources – MDNR

Little River Band of Ottawa Indians – LRBOI

Chapter I

Introduction

The remote site incubator (RSI) is a confined flow-through system that incubates eggs stream-side and allows newly hatched fish to volitionally move into the introduction site (Wampler and Manuel 1992; Kaeding and Boltz 2004). RSIs were developed in the late 1980s for the restoration of Pacific salmon (*Oncorhynchus* spp.; Wampler and Manuel 1992) but have since been used for the restoration of other native fishes, including Cutthroat Trout (*O. clarkia*; Hoffman et al. 2002; Arnold et al. 2017) and Arctic Grayling (*Thymallus arcticus*; Kaeding and Boltz 2004, Arnold et al. 2017). Compared with natural reproduction in the stream, RSIs protect the eggs during the early life stages (i.e., from egg to fry) while still allowing the fish to acclimate to natural stream conditions (Kaeding and Boltz 2004; Kirkland 2012). Despite being used for native species restoration in the western USA (Denny and Evans 2012; Hoffman et al. 2002; Wampler and Manuel 1992; Arnold et al. 2017; Kaeding and Boltz 2004), few studies have evaluated RSIs and none have tested different RSI designs and protocols, which may help fishery managers establish protocols when using RSIs.

The Arctic Grayling is a native species to North America and Eurasia (McAllister and Harington 1969; Stamford and Taylor 2004) and historically was abundant in water bodies across the northern Lower Peninsula of Michigan (Vincent 1962; Nuhfer 1992). However, population declines in the late 1800s through habitat degradation (i.e., logging activities that removed riparian vegetation and instream habitat), overfishing, and competition with non-native fish led to their extirpation from Michigan by 1936 (Creaser and Creaser 1935; Vincent 1962; McAllister and Harington 1969). Throughout the 1900s there were a number of attempts to re-establish Arctic Grayling populations in Michigan, but these attempts failed and it was determined that suitable habitat for Arctic Grayling was lost in contemporary Michigan streams (Nuhfer 1992). Similarly, extensive stocking efforts to restore populations of Arctic Grayling in Montana during the 1900s were unsuccessful (Kaya 1992).

Rearing fish eggs directly at the site (i.e., in RSIs) may help facilitate imprinting on natal waters, increasing their chance of homing and producing a self-sustaining population (Kaeding and Boltz 2004; Kirkland 2012). Homing is when fish imprint to the unique water chemistry in a water body and allows migratory fish to return to previously successful spawning grounds, which has been observed in many salmonids (Hasler et al. 1978; Dittman and Quinn 1996). Although the degree to which Arctic Grayling imprint to natal waters is unclear (Northcote et al. 1995), RSIs may still improve upon previous reintroduction attempts by allowing Arctic Grayling to acclimate to natural stream conditions prior to release. This is supported by recent restoration efforts of Arctic Grayling in Montana, which have been successful since implementing RSIs (Cayer and McCullough 2014).

Purpose

The purpose of this study was to provide an evaluation of RSIs in Michigan streams, which typically have lower gradients and differing bedload compared with the streams in the western USA that have used RSIs previously. Moreover, I wanted to investigate factors that influence RSI success to help inform the restoration of Arctic Grayling in Michigan and more generally to other native fishes in the world.

Scope

Various stream incubation methods have been developed to assist the restoration of salmonids (Barlaup and Moen 2001; Coghlan and Ringler 2004; Kaeding and Boltz 2004; Bernier-Bourgault et al. 2005; Kirkland 2012). One method of in-stream incubation is to place the eggs directly into the gravel substrate or bury boxes with trays of eggs in the stream; however, studies have reported varying levels of success due to poor intra-gravel water chemistry and high sedimentation (Barlaup and Moen 2001; Coghlan and Ringler 2004; Bernier-Bourgault et al. 2005). RSIs reduce the amount of sediment accumulation on the eggs by keeping the eggs out of the stream substrate while still allowing the eggs to incubate in stream water (Wampler and Manuel 1992; Kaeding and Boltz 2004). Despite the importance of RSIs for the conservation of native fishes, I am only aware of one published study that has evaluated the success of RSIs (Kaeding and Boltz 2004). My study helps to fill this knowledge gap by providing an evaluation of RSI use and design in Michigan streams that differ in gradient and bedload than those previously used with RSIs. Findings from my research are applicable to anyone wanting to use RSIs for the restoration of salmonid fishes, especially in Michigan.

Assumptions

I had three main assumptions for my field experiment:

1. All Rainbow Trout (*Oncorhynchus mykiss*) eggs stocked into RSIs had an equal chance of survival among RSIs within a given year.
2. No Rainbow Trout escaped from RSIs or collection buckets.
3. Rainbow Trout eggs are a good surrogate for Arctic Grayling eggs.

Hypothesis

I tested RSI designs and protocols in Michigan streams to help inform future fisheries management decisions. Specifically, my objectives were to: 1) compare hatching success between two different RSI designs (i.e., 19-L and 265-L RSI), and 2) assess hatching success when dead eggs were removed from the incubator, a common practice that seeks to reduce the spread of fungus to developing eggs. I hypothesized that 19-L RSIs with dead eggs removed during incubation (i.e., picked 19-L RSIs) would have greater survival than RSIs without dead eggs removed (i.e., unpicked 19-L RSIs).

Significance

RSIs have been used successfully for conservation efforts to restore native fish populations in rivers (Wampler and Manuel 1992; Hoffman et al. 2002; Kaeding and Boltz 2004; Denny and Evans 2012; Arnold et al. 2017). These efforts have used RSIs in medium to high gradient streams in the western USA but have not systematically addressed factors that could limit their success. Thus, my research fills this gap by systematically evaluating two RSI designs and protocols in Michigan streams, which are lower gradient than the streams where RSIs have been used successfully. Moreover, my research will support the conservation effort in Michigan to reintroduce Arctic Grayling by providing critical information to managers regarding RSI design and use. Information gained from this study also can be applied to other stream fishes throughout the world, supporting the conservation of native fishes.

Definitions

Alevin – a newly hatched salmonid still carrying the yolk.

Homing – the ability of an animal to migrate back to a specific location later in its life.

Chapter II

Evaluating remote site incubators to support restoration of native river fishes: implications for Arctic Grayling reintroduction in Michigan

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Abstract

Remote site incubators (RSIs) have been used to rear salmonid fish eggs along streams in the Pacific Northwest since the 1980s. Recently, the successful use of RSIs for Arctic Grayling *Thymallus arcticus* restoration in Montana has sparked a renewed interest to reestablish the species in Michigan. To support future reintroduction efforts of Arctic Grayling in Michigan, we evaluated RSIs in three Michigan streams during 2018 and 2019 using Rainbow Trout *Oncorhynchus mykiss* eggs (as surrogates for Arctic Grayling). Our objectives were to: (1) compare hatching success between two different RSI designs (19-L vs. 265-L RSIs), and (2) test whether the removal of dead eggs ("picking") from 19-L RSIs affected hatching success. Overall survival (i.e., hatching success of all RSIs) in 2018 and 2019 was 41.3% and 52.4%, respectively. Survival between unpicked 19-L and 265-L RSIs by stream differed from 1.5% to 14.3% (mean = 5.8%) in 2018 and 0.2% to 0.4% (mean = 0.3%) in 2019. On average, the picked 19-L RSIs had greater survival—although not always statistically significant—than unpicked 19-L RSIs during both years (2018: mean = 1.6%, $P = 0.27$; 2019: mean = 10.4%, $P = 0.02$). We document a positive correlation between survival and RSI flow rates, and a decline in survival when RSI flow rates could not be maintained above ~0.4 L/min. Our results show that both 19-L and 265-L RSIs can be used successfully in Michigan streams. Moreover, our results suggest that removing dead eggs was most likely to improve survival when RSI flow rates cannot be maintained above 0.4 L/min.

Introduction

Hatchery rearing is a common method used to supplement wild fish stocks, especially for salmonids (Fraser 2008). However, there is a growing body of evidence that suggests hatchery reared salmonids have lower fitness (e.g., reproductive success) than wild origin fish (Brannon et al. 2004; Araki et al. 2008; Fraser 2008; Thériault et al. 2011). Factors influencing the lower fitness of hatchery reared salmonids generally include the acclimation of juveniles and adaptation of broodstocks to the hatchery environment (Olla et al. 1998; Wessel et al. 2006; Araki et al. 2008; Chittenden et al. 2010; Thériault et al. 2011; Neff et al. 2015). Incubating eggs and rearing fishes under more natural conditions has been suggested to improve the post-release survival of hatchery fishes (Olla et al. 1998; Chittenden et al. 2010). Furthermore, allowing early-life stages to imprint and acclimate to natal waters would benefit conservation practices seeking to establish self-sustaining populations (Hoffman et al. 2002; Kaeding and Boltz 2004; Al-Chokhachy et al. 2009; Kirkland 2012; Arnold et al. 2017).

Various incubation methods have been developed to rear eggs under more natural conditions (Barlaup and Moen 2001; Coghlan and Ringler 2004; Kaeding and Boltz 2004; Bernier-Bourgault et al. 2005; Bamberger 2009; Kirkland 2012). The methods typically either incubate fish eggs in the stream substrate (e.g., buried in artificial redds or in buried boxes; reviewed by Barlaup and Moen 2001; Coghlan and Ringler 2004; Bernier-Bourgault et al. 2005) or use a stream-side incubator design receiving gravity-fed water from the stream (Kaeding and Boltz 2004; Kirkland 2012). Sedimentation has been shown to reduce the hatching success of salmonid eggs (Greig et al. 2005; Julien and Bergeron 2006; Jensen et al. 2009) and is a common reason in-stream incubation devices report low hatching success (Barlaup and Moen 2001; Coghlan and Ringler 2004; Bernier-Bourgault et al. 2005; Kirkland 2012). However, hatching success greater than 85% has been reported when sedimentation is not an issue (Donaghy and Verspoor 2000). Stream-side incubators have been suggested as an improved method of incubating salmonid eggs by reducing the risks of sedimentation (Kaeding and Boltz 2004; Kirkland 2012).

The remote site incubator (RSI) is a confined, flow-through system that incubates eggs stream-side and allows newly hatched fish to volitionally move into the site of introduction (Kaeding and Boltz 2004). RSIs were developed in the western USA for the incubation of Pacific salmon (*Oncorhynchus* spp.;Wampler and Manuel 1992), and have since been used for restoration of other salmonids (Hoffman et al. 2002; Kaeding and Boltz 2004; Arnold et al. 2017). Although RSIs have been used to incubate salmonids since the late 1980s, few studies have evaluated the use of RSIs. Kaeding and Boltz (2004) used RSIs to rear green Arctic Grayling *Thymallus arcticus* eggs and documented an average hatching rate of 44.8%; however, hatching rates were highly variable between years and sites, ranging from 0 to 95%.

The Arctic Grayling has a Holarctic distribution (McAllister and Harington 1969; Stamford and Taylor 2004); however, two discrete southern populations are native to the contiguous United States: one in the upper Missouri River drainage (Kaya 1992) and another in Michigan (Vincent 1962; Nuhfer 1992). Arctic Grayling were extirpated from Michigan by 1936 (Creaser and Creaser 1935; Vincent 1962; McAllister and Harington 1969) and are currently restricted to approximately 5% of their historic range in the upper Missouri River drainage (Kaya 1992; Stamford and Taylor 2004). Before the decline of populations across the contiguous United States, Arctic Grayling were an important game fish for early European settlers and were harvested for subsistence by Native American tribes (Metcalf 1961; Nuhfer 1992). Due to their importance and value in Michigan, efforts to reestablish populations of Arctic Grayling persisted through the 1900s, with the latest effort ending in 1991 (Nuhfer 1992). Arctic Grayling used for stocking were introduced to various streams and lakes across Michigan's Lower Peninsula; however, these early attempts were unable to establish a reproductive population despite living to maturity in some lakes (Nuhfer 1992). Similarly, extensive stocking efforts to restore populations of Arctic Grayling in Montana during the 1900s were unsuccessful (Kaya 1992). Although the reasons why these stocking attempts were unsuccessful remain unclear, contributing factors are thought to include interspecific interactions (e.g., predation), use of lake-strain stocks of Arctic Grayling for reintroduction in rivers, hatchery diseases, and unsuitable habitat at some introduction sites (Kaya 1992; Nuhfer 1992).

Despite previously failed attempts, there is a renewed interest to restore Arctic Grayling populations in Michigan, and recent studies have suggested favorable abiotic and biotic conditions still persist in the state (Danhoff et al. 2017; Goble et al. 2018). RSIs have the potential to assist the reintroduction effort by allowing Arctic Grayling to acclimate to natural stream conditions and allow early life-stages to imprint on natal waters, potentially improving the chances of Arctic Grayling returning to natal streams to spawn and establish self-sustaining populations (e.g., Kaeding and Boltz 2004). The process of imprinting to unique chemical signatures in a water body allows migratory fish to return to previously successful spawning grounds, termed homing, and has been observed in many salmonid species (Hasler et al. 1978; Dittman and Quinn 1996). The degree to which homing is prevalent in Arctic Grayling populations remains unknown (Northcote 1995); however, homing and behavioral differences associated with hatchery rearing could explain why Arctic Grayling stocked in Michigan and Montana during the 1900s as part of reintroduction efforts were unable to establish populations (Kaya 1992; Nuhfer 1992). Additionally, hatchery rearing could explain why Arctic Grayling stocked in Michigan streams were observed to have rapid dispersal from introduction sites, almost exclusively downstream (Nuhfer 1992). Recent restoration efforts in Montana have used RSIs to stock Arctic Grayling to streams since 2003 and natural reproduction has been observed at introduction sites (Cayer and McCullough 2014).

Although RSIs have been widely used in the western US, they have not been tested in the Midwest, which typically has lower gradient streams that may also have greater sediment loads in some cases. Therefore, the purpose of our study was to test RSIs in Michigan streams to determine whether they are a viable option to assist future reintroduction efforts of Arctic Grayling. We evaluated RSIs using Rainbow Trout *O. mykiss* eggs (as surrogates for Arctic Grayling) in three Michigan streams. Our objectives were to: 1) compare hatching success between two different RSI designs (i.e., 19-L and 265-L RSI) and 2) assess hatching success when dead eggs were removed from the 19-L RSIs, a common practice that seeks to reduce the spread of fungus to developing eggs. We hypothesized that picked 19-L RSIs (i.e., the 19-L RSIs that had dead eggs removed) would have greater hatching success than unpicked 19-L RSIs (i.e., the 19-L RSIs without dead eggs removed).

Methods

Study Area

The Manistee River flows 373 km across the northwest portion of Michigan's Lower Peninsula and drains an area of 4,610 km^2 (Rozich 1998). We installed RSIs on three tributaries to the Manistee River: Cedar, Hinton, and Peterson creeks (Table 2.1).

To characterize basic physical conditions in the three tributaries where RSIs were installed, we measured water velocity, depth, discharge, and several water quality variables during the 2018 and 2019 study periods. Water velocity (Marsh-McBirney model 2000 flow meter) and depth were measured (2018: N = 10; 2019: N = 10) along a fixed transect at each stream to calculate discharge. Water quality (i.e., dissolved oxygen, turbidity, and specific conductivity; YSI 6600 v2 sonde) was measured (2018: N = 10; 2019: N = 10) near the fixed transects. Hourly water temperature was monitored (Hobo data logger) in each stream during the study. These data show that the three study sites differed by size, with Cedar Creek having the lowest discharge and Peterson Creek having the highest discharge throughout the study (Table 2.1). Cedar Creek had the coldest water temperatures during the study, while water temperatures were similar between Hinton and Peterson creeks (Table 2.1). Water temperatures were colder at all three sites in 2019; however, water quality parameters among the three streams were more than adequate for incubating Rainbow Trout eggs during both years (Table 2.1).

RSI Construction

Eight 19-L RSIs and one 265-L RSI were installed at each study stream (N = 27). The 19-L RSIs were constructed using black 19-L plastic buckets, and the 265-L RSIs were constructed using a black 265-L plastic stock-tank. The water delivery system for each RSI was made of PVC conduit (19-L RSIs: diameter = 25 mm; 265-L RSIs: diameter = 51 mm) and consisted of an inflow pipe, water diffuser, and outflow pipe (Figure 2.1). A ball valve made of PVC (19-L RSIs: diameter = 25 mm; 265-L RSIs: diameter = 51 mm) was connected to each RSI and was used to prevent water from entering the RSI during sampling events. Inflow pipes for all RSIs at each site were placed at the same location and secured in place with sandbags. Egg trays were made out of stainless steel mesh (0.9 mm diameter wire, 1.65 mm opening width) and were smaller in 19-L RSIs (surface area = 532 cm^2) than 265-L RSIs (surface area = 3903 cm^2). To protect the eggs from light and predation, 19-L RSIs were covered with a fitted black plastic lid and 265-L RSIs were covered with a large plywood lid. Collection buckets with fitted lids were connected to each RSI unit by the outflow pipe. The collection buckets prevented larval fish and eggs from entering the stream and allowed for enumeration of swim-out alevins.

Egg Stocking

Fertilized Rainbow Trout eggs at the eyed-stage of development were stocked into RSIs on 30 April 2018 and 15 April 2019. In 2018, the Rainbow Trout eggs were from first-year spawning adults (age = 3+) at the Michigan Department of Natural Resources (MDNR) Oden State Fish Hatchery. On average, the eggs were 3.8 mm in diameter (20,408 eggs/L). In 2019, the Rainbow Trout eggs we received were from older spawning adults (age > 3+). On average the eggs were 4.1 mm in diameter (17,342 eggs/L). In both years, egg density and diameter were estimated following von Bayer (1910).

After stocking eggs into RSIs, an image was taken of the egg tray from each 19-L RSI. The number of eggs stocked into 19-L RSIs was estimated by counting eggs from the images using the multi-point tool in ImageJ (Schneider et al. 2012). In 2018, the initial egg number in 19-L RSIs was 676 eggs/RSI (95% CI: 653 – 699). In 2019, the initial egg number in 19-L RSIs was 1,524 eggs/RSI (95% CI: 1473 – 1575).

Counting eggs from images of 265-L RSIs was infeasible because of the large surface area of the egg trays. Therefore, in 2018, the initial egg number in 265-L RSIs was estimated by taking the mean egg number in 19-L RSIs divided by the volume of eggs stocked in each 19-L RSI (i.e., 40 mL per RSI). This gave an estimate of egg density (eggs per mL), which was multiplied by the stocking volume of eggs in 265-L RSIs to estimate the initial egg number. The initial egg number in 265-L RSIs was 1,436 eggs at Cedar and Hinton creeks and 1,521 eggs at Peterson Creek. In 2019, the initial egg number in 265-L RSIs (i.e., 11,000 eggs) was estimated at the hatchery. The mean percent measurement error in 2019 between the hatchery estimated and ImageJ counted number of eggs in 19-L RSIs was 3.3% (SE = 1.6).

RSI Evaluation

All RSIs were monitored three times per week. The water flow rate through each RSI (hereafter termed RSI flow rate) was measured by the time required for the outflow pipe to fill a 1-L container. Percent fungus and sediment coverage were visually assessed by one person throughout the study on a 1-5 scale, with one being little coverage (0-20%) and five being nearly complete coverage (80-100%). RSI collection buckets were checked for swim-out alevins. Four 19-L RSIs were selected to have dead eggs removed (i.e., "picked") from each site and the time required for picking was recorded. Dead eggs were not removed from the 265-L RSIs.

To evaluate how temperatures changed as water passed through the black RSIs, hourly water temperature (Hobo data logger) was monitored inside one randomly selected 19-L RSI at each site ($N = 3$) and one randomly selected 265-L RSI ($N = 1$). The study was concluded after 23 days (in both years) when all of the Rainbow Trout had either hatched or died. All alevins were euthanized in MS-222 and preserved in 95% ethanol for enumeration in the laboratory.

Data Analysis

For each RSI, survival—our measure of hatching success that accounted for swim-out and alevins that remained in RSIs—was calculated as:

$$S = \frac{C + R}{D} \times 100,$$

where C is the number of swim-out Rainbow Trout collected in the collection bucket, R is the number of alive Rainbow Trout remaining in a RSI when the experiment concluded, D is the initial number of eggs in a RSI.

All statistical analyses were performed with the R programming language and computing environment (R Core Team 2019). A randomized complete block analysis of variance (ANOVA) was used to test for differences in survival between picked and unpicked 19-L RSIs, with the study stream as the blocking variable. We performed a logit transformation on the proportion data of survival for all statistical analyses to satisfy the assumptions of ANOVA (Warton and Hui 2011). The following statistical model was employed:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \varepsilon_{ijk},$$

where μ is the overall mean, β_i is the block effect, τ_j is the treatment effect, and ε_{ijk} is random error in replicate *k* of treatment *j* in block *i*, with $\varepsilon_{ijk} \sim N(0, \sigma^2)$. We used $\alpha = 0.05$ for assessing statistical significance. In 2018, one picked 19-L RSI at Peterson Creek underwent a dewatering event when the inflow pipe disconnected (survival = 14.7%), and one unpicked 19-L RSI at Cedar Creek did not have the water flow returned (i.e., the ball valve was closed) between sampling events (survival = 16.0%). Since the survival observed in these 19-L RSIs was not due to the picking treatment, they were removed from analyses comparing picked and unpicked 19-L RSIs. To better understand how RSI flow rates relate to survival, we estimated Spearman's rank correlation between survival and minimum RSI flow rates in picked and unpicked 19-L RSIs, where the minimum RSI flow rate was the lowest measured rate for each RSI during the incubation of eggs (2018: *n* = 10/RSI; 2019: *n* = 10/RSI).

Results

RSIs were stocked with 20,610 eggs in 2018 and 69,572 eggs in 2019. Survival across all RSIs was 42.1% and 52.3% in 2018 and 2019, respectively. In 2018, mean survival across the three study sites was 40.6% (SD = 2.7) at Hinton Creek, 41.9% (SD = 10.7; with outlier removed: mean = 45.1%; SD = 4.7) at Cedar Creek, and 43.7% (SD = 12.1; with outlier removed: mean = 47.3%; SD = 5.7) at Peterson Creek. In 2019, mean survival across the three study sites was 42.0% (SD = 16.1) at Hinton Creek, 61.7% (SD = 2.3) at Cedar Creek, and 59.4% (SD = 5.2) at Peterson Creek. Mean daily water temperatures in RSIs were slightly warmer than stream temperatures at all sites in 2018 (range of differences = 0.18-0.66 ˚C; Figure

2.2). In 2019, RSI water temperatures were nearly identical to stream temperatures at Cedar and Hinton Creeks (mean difference = 0.04 ˚C) and were slightly colder at Peterson Creek (mean difference = 0.22 ˚C; Figure 2.2).

Survival between RSI types (i.e., picked 19-L, unpicked 19-L, and 265-L RSIs) varied between years and sites (Figure 2.3). In 2018, mean survival in picked 19-L RSIs was 1.6% (range = 0.2-3.4%) greater than unpicked 19-L RSIs; however, this was not statistically significant (ANOVA: $F_{1,2} = 1.31$, $P = 0.27$; Figure 2.3). In 2019, mean survival in picked 19-L RSIs was 10.4% (range = 2.4-22.9%) greater than unpicked 19-L RSIs and was statistically significant (ANOVA: $F_{1,2} = 6.34$, $P = 0.02$; Figure 2.3). Survival between unpicked 19-L and 265-L RSIs by stream differed from 1.5 to 14.3% (mean = 5.8%) in 2018 and 0.2 to 0.4% (mean = 0.3%) in 2019 (Figure 2.3). Fungus coverage (on a scale of 1-5) was low in picked 19-L RSIs during both years (mean ± SD; 2018: 1.1 ± 0.1; 2019: 1.0 ± 0.0) and was greater in unpicked 19-L RSIs (2018: 2.9 ± 0.5; 2019: 2.1 ± 0.8) and 265-L RSIs (2018: 2.9 ± 0.5; 2019: 2.2 ± 0.4). Picking 19-L RSIs required 7.3 min/RSI/visit in 2018 and 8.4 min/RSI/visit in 2019.

In 2018, mean RSI flow rates in 19-L RSIs was 4.4 L/min (SD = 0.6) at Cedar Creek, 1.3 L/min (SD = 0.5) at Hinton Creek, and 2.5 L/min (SD = 0.5) at Peterson Creek. In 2019, mean RSI flow rates in 19-L RSIs was 4.5 L/min (SD = 0.5) at Cedar Creek, 1.2 L/min (SD = 0.5) at Hinton Creek, and 2.1 L/min (SD = 0.7) at Peterson Creek. Mean RSI flow rates in 265-L RSIs across the three study sites was 10.0 L/min (SD = 1.8) in 2018 and 8.9 L/min (SD = 0.6) in 2019. Minimum RSI flow rates (i.e., the lowest measured flow rate of each RSI during egg incubation) ranged from 0.16 to 4.61 L/min in 2018 and 0.02 to 4.65 L/min in 2019 (Figure 2.4). In picked RSIs, there was weak evidence of a correlation between minimum RSI flow rates and survival in 2018 ($r_S = 0.52$, $P = 0.11$) and 2019 ($r_S = 0.52$, $P = 0.08$). In unpicked RSIs, there was even less evidence of a correlation between minimum RSI flow rates and survival in 2018 ($r_S = 0.07$, $P = 0.84$); however, there was strong evidence of a positive correlation in 2019 ($r_S = 0.90$, $P < 0.01$). The same general relationship held when exploring mean RSI flow rates and survival.

Discussion

Our study is the first to successfully rear fish eggs in Michigan streams using RSIs, documenting an average survival of 42.1% in 2018 and 52.3% in 2019. One possible factor for the survival observed in our study was the initial egg quality stocked into RSIs. Following our first site visit (2 days after stocking eggs into RSIs), mean mortality among picked 19-L RSIs was 42.6% (SD = 15.4) in 2018 and 8.8% (SD = 0.6) in 2019. Therefore, the survival observed in our study is likely due to initial mortality (dependent on egg quality at the time of stocking) and challenges faced when using RSIs in the field (e.g., RSI inflow pipes disconnecting and maintaining RSI flow rates). However, our hatching success was similar to the 44.8% documented for Arctic Grayling in Montana using similar 19-L RSIs (Kaeding and Boltz 2004). Other in-stream incubation methods report similar hatching success for salmonids (Barlaup and Moen 2001). In-stream incubators that do poorly (i.e., < 20% hatching success) usually have high sedimentation rates or poor intra-gravel water chemistry (e.g., low dissolved oxygen; Barlaup and Moen 2001; Kirkland 2012).We documented little sedimentation on eggs in RSIs (mean sediment score among all RSIs: 2018 = 1.2; 2019 = 1.6), but poor water conditions could explain why we observed low survival in RSIs with RSI flow rates below 0.40 L/min (Figure 2.4). Continuous measurements of RSI flow rates and water chemistry properties inside RSIs could help to disentangle mechanisms resulting in egg mortality. Moreover, comparing hatching success of RSIs to other stream incubation techniques could provide useful information for managers working to restore native stream fishes.

Our study found no meaningful difference between the two RSI designs we tested (i.e., 19-L and 265-L RSIs). On average, survival in unpicked 19-L RSIs was marginally greater than 265-L RSIs (mean difference: 2018 = 5.8%; 2019 = 0.3%; Figure 2.3). Between years, egg densities between 19-L and 265-L RSIs were more similar in 2019 (19-L RSI = 2.86 eggs/cm^2; 265-L RSI = 2.82 eggs/cm^2) than 2018 (19-L RSI = 1.27 eggs/cm^2; 265-L RSI = 0.38 eggs/cm^2). This suggests that under similar egg densities, we would expect similar survival between unpicked 19-L and 265-L RSIs. Installing and establishing sufficient flow rates is easier in 19-L RSIs compared with 265-L RSIs. The time and difficulty installing either RSI design is primarily dependent on stream characteristics (i.e., stream gradient, bank height, and sinuosity), but at most sites installing one 265-L RSI required less time and space compared with seven 19-L RSIs (i.e., one 265-L RSI is equivalent to seven 19-L RSIs). Therefore, 265-L RSIs may be advantageous by reducing the number of RSIs needed at a site; however, putting most or all available eggs into a single 265-L RSI increases the risks of catastrophic failures (e.g., inflow pipe disconnecting) at a site, whereas the 19-L RSIs are able to distribute risks among multiple RSIs.

Our study documented that removing dead eggs during incubation can improve the success of RSIs; however, the magnitude of improvement varied between years and sites (Figure 2.3). There was a 1.6% increase in survival from picking 19-L RSIs in 2018 compared with the 10.4% increase in 2019. Although picking 19-L RSIs required more time in 2019 with greater egg densities (egg density = 2.86 eggs/cm^2; picking time = 8.4 min/RSI/visit) than 2018 (egg density = 1.27 eggs/cm^2; picking time = 7.7 min/RSI/visit), picking effectively reduced the spread of fungus in picked 19-L RSIs compared with unpicked 19-L RSIs. Moreover, the fungus coverage in unpicked 19-L and 265-L RSIs was similar between both years, and suggests that the higher egg densities used in 2019 did not account for picking having a greater effect on survival. Instead, the variation in survival between sites and picking seemed to be associated with RSI flow rates. Cedar Creek had the highest RSI flow rates and highest survival rates during both years of the study, with RSIs consistently having flow rates above 3 L/min (Figure 2.4). Comparatively, Hinton Creek had the lowest RSI flow rates and lowest survival rates during both years of the study, with RSIs consistently having flow rates below 1 L/min (Figure 2.4). During both years there was evidence of a weak positive correlation (although not statistically significant) between RSI flow rates and survival in picked 19-L RSIs. In comparison, survival in unpicked 19-L RSIs were not correlated with RSI flow rates in 2018, but a significant, positive correlation was present in 2019. The observed trend could be due to the range of RSI flow rates in unpicked RSIs between years, where the highest minimum RSI flow rates were similar but the lowest minimum RSI flow rates in 2019 were markedly lower than 2018 (0.02 vs 0.67 L/min; Figure 2.4). Additionally, we observed a sharp decline in survival for unpicked 19-L RSIs when minimum RSI flow rates were below 0.40 L/min (Figure 2.4). This suggests that low RSI flow rates, even for 1-2 days between sampling events, negatively affected survival in our experiments; however, this pattern was not consistent in picked 19-L RSIs. This implies that picking may be the most beneficial when RSI flow rates are low, and we recommend picking 19-L RSIs when flow rates cannot be maintained above 0.4 L/min. Similarly, picking dead eggs from 19-L RSIs may not be a good use of labor when RSI flow rates are sufficiently high (e.g., >0.4 L/min).

Although we were broadly interested in testing the designs and protocols for RSIs, we were specifically interested in applying our research to reintroduction plans of Arctic Grayling to Michigan. Thus, we acknowledge that Rainbow Trout are not a perfect surrogate species for

Arctic Grayling. Although both species are spring spawners, Arctic Grayling eggs (2.7 mm average diameter; Scott and Crossman 1973) are smaller than Rainbow Trout eggs (3.8 and 4.1 mm average diameter in our study), and Arctic Grayling require less time to hatch and swim-up compared with Rainbow Trout (Bishop 1971; Kratt and Smith 1977; Northcote 1995; Kaeding and Boltz 2004). Therefore, the reduced time required by Arctic Grayling to develop compared to Rainbow Trout will reduce the duration RSIs are needed in streams, which should reduce risks of RSI failures. However, it is still unclear if Arctic Grayling eggs will respond in a manner similar to Rainbow Trout when dead eggs are removed during incubation. Kaeding and Boltz (2004) documented high mortality rates of Arctic Grayling eggs in some RSIs despite daily picking of dead eggs. Other studies of stream incubators have not indicated any species-specific trends in hatching success, and most variation reported in hatching success are attributed to different methods and environmental conditions (Barlaup and Moen 2001; Kirkland 2012). Studies comparing hatching success of different species in RSIs and other stream incubators would be useful for assessing the degree to which experimental results are species or condition specific.

In conclusion, our study is the first evaluation of RSIs in Michigan streams, where previous research and use has predominately occurred in the western US (Kaeding and Boltz 2004; Arnold et al. 2017). We found that removing dead eggs during incubation can effectively reduce the spread of fungus and increase the hatching success in RSIs. This supports other studies that suggest mitigating fungus is an important factor of improving hatching success when incubating salmonid eggs (Barlaup and Moen 2001; Kaeding and Boltz 2004). We observed similar amounts of fungus on eggs during both years, suggesting the effect was independent of egg density in RSIs; however, our study suggests that unfavorable environmental conditions (e.g., caused by low RSI flow rates in our study) may cause higher mortality and facilitate the spread of fungus when incubating salmonid eggs. Therefore, we recommend managers remove dead eggs from RSIs when RSI flow rates cannot be maintained above 0.4 L/min. Our study highlights that RSIs (i.e., 19-L and 265-L RSIs) hold considerable promise for future restoration efforts of Arctic Grayling in Michigan and may benefit the conservation of native species in other regions of the world.

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Table 2.1. Site descriptions of the three study streams in Michigan, including location (latitude and longitude) and the following environmental variables (mean ± SD): discharge, daily water temperature (Temp), dissolved oxygen (DO), turbidity, and specific conductivity (SPC). Discharge, DO, turbidity, and SPC were measured three times a week (2018: *n* = 10; 2019: *n* = 10). Daily water temperature is the average hourly water temperature during each day of RSI deployment (2018: *n* = 17 daily comparisons; 2019: *n* = 23 daily comparisons).

Site	Latitude (˚N)	Longitude (˚W)	Year	Discharge (m^3/s)	Temp (˚C)	DO (mg/L)	Turbidity (NTU)	SPC (µS/cm)
Cedar	44.3048	85.8205	2018	0.12± 0.02	8.8± 0.6	10.7± 0.3	0.0± 0.3	302± 4
			2019	0.09± 0.02	7.4± 0.7	10.7± 0.2	0.0± 0.1	301± 4
Hinton	44.2762	85.8158	2018	0.19± 0.04	12.0± 1.5	10.7± 0.6	2.5± 1.0	287± 35
			2019	0.18± 0.03	8.4± 1.7	11.5± 0.5	4.4± 6.1	289± 12
Peterson	44.2634	85.8469	2018	0.78± 0.10	11.5± 1.2	10.8±0.5	4.0± 2.4	282± 11
			2019	0.73± 0.13	8.4± 1.5	11.4± 0.6	2.7± 2.0	271± 18

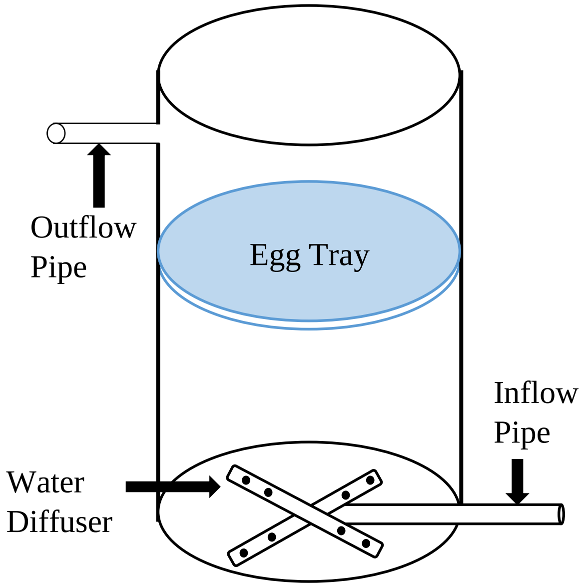

Figure 2.1. General design of a remote site incubator (RSI). Gravity-fed water is carried through the inflow pipe to the RSI, where it upwells from the water diffuser and flows out of the outflow pipe. The 19-L RSIs were constructed using black 19-L buckets, and the 265-L RSIs were constructed using a black 265-L stock-tank. The water delivery system was made of PVC conduit (19-L RSIs: diameter = 25 mm; 265-L RSIs: diameter = 51 mm). Design was modified from Kaeding and Boltz (2004).

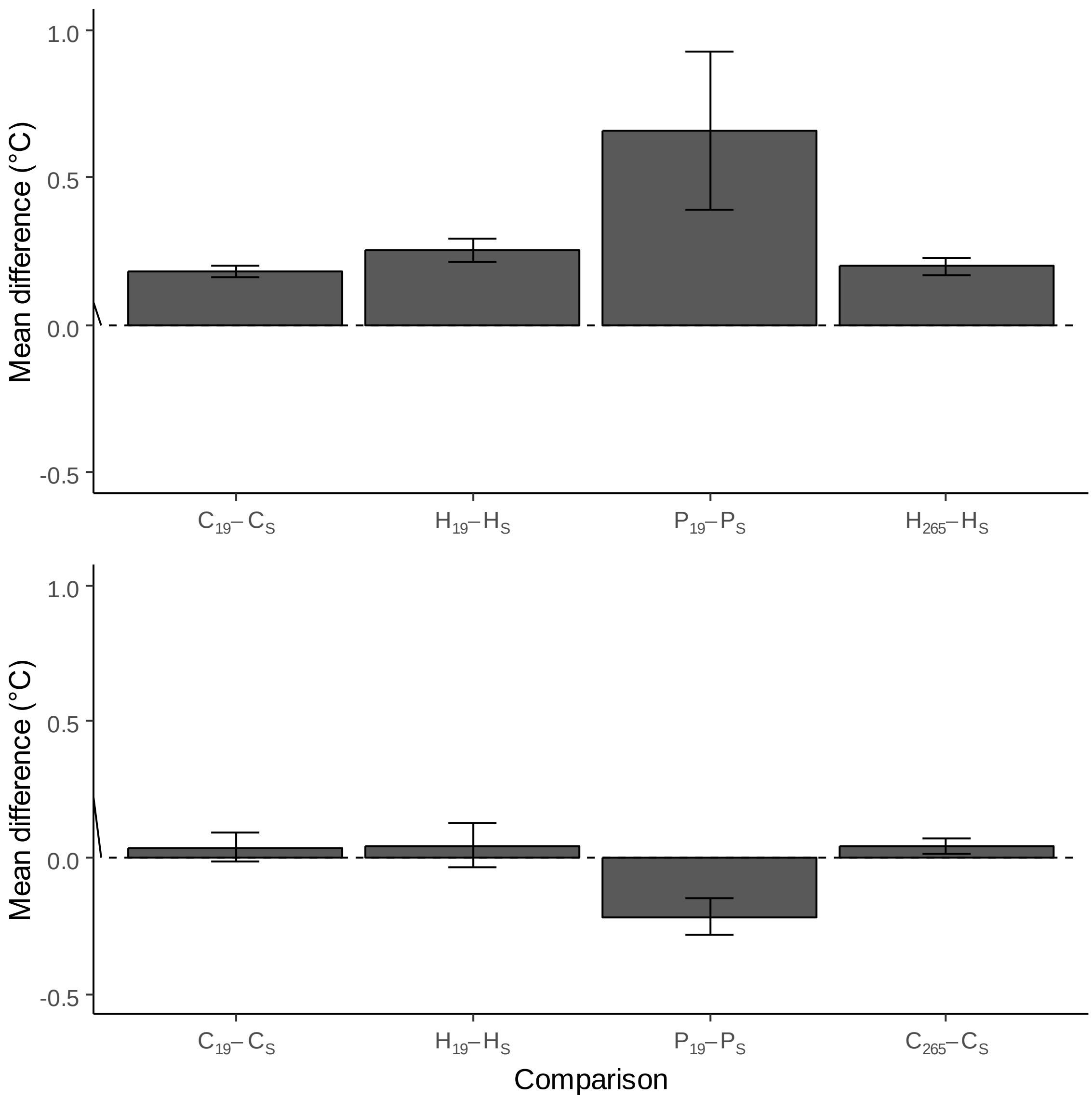

Figure 2.2. Difference in the mean daily water temperature (˚C) between a RSI and the stream in 2018 (top panel; *n* = 17 daily comparisons) and 2019 (bottom panel; *n* = 23 daily comparisons). The water temperatures for RSIs were measured inside one randomly selected 19-L RSI at each site and one randomly selected 265-L RSI. The water temperatures for each site were measured near RSI locations. Logger locations defined: C = Cedar Creek, H = Hinton Creek, P = Peterson Creek; 19 = 19-L RSI, 265 = 265-L RSI, S = stream). Error bars represent the 95% confidence interval.

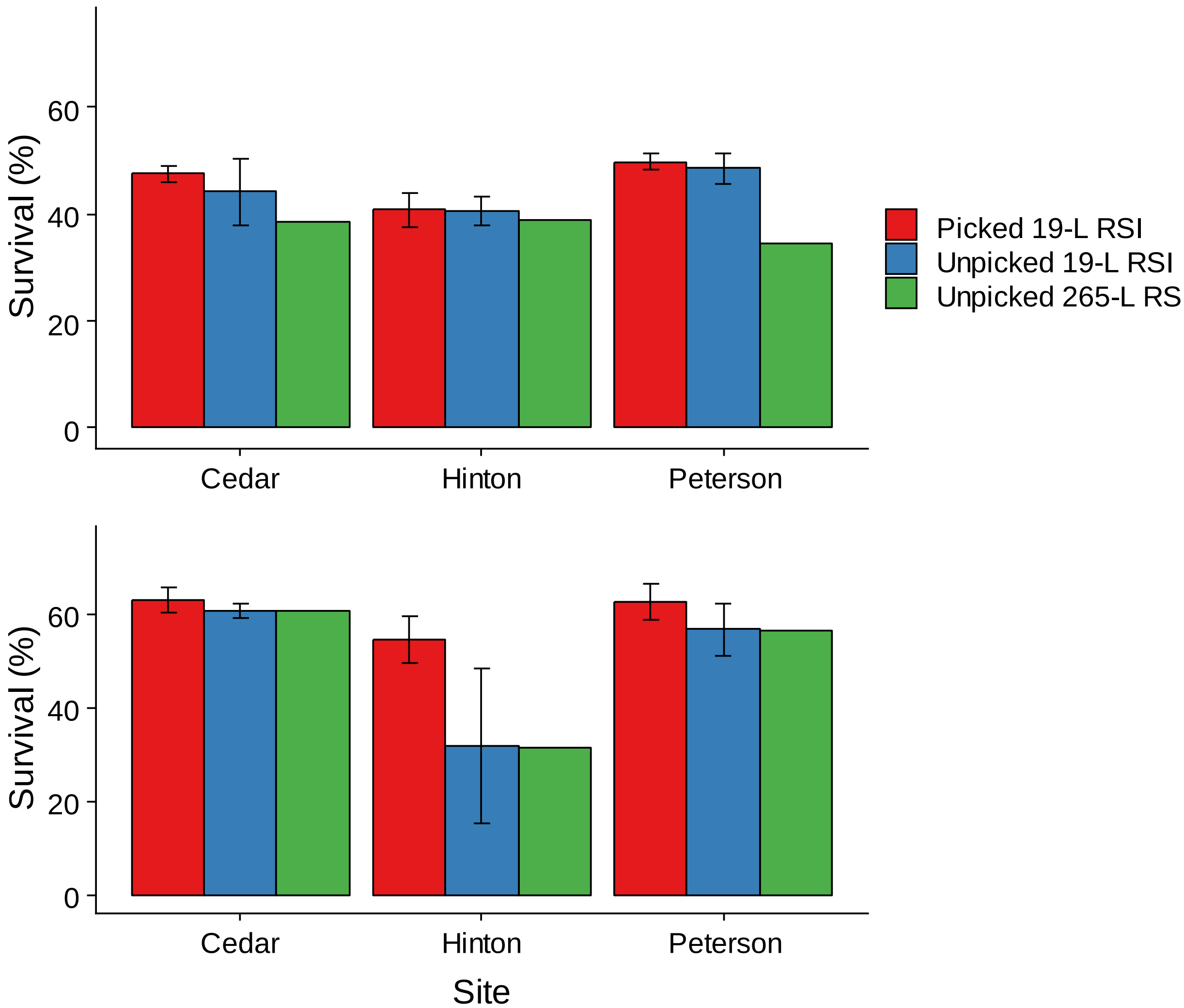

Figure 2.3. Mean percent survival of Rainbow Trout *Oncorhynchus mykiss* in 2018 (top panel) and 2019 (bottom panel) between picked and unpicked 19-L remote site incubators (RSIs) and unpicked 265-L RSIs. Eight 19-L RSIs (four picked and four unpicked) and one 265-L RSI were installed at each stream. In 2018, the failed picked 19-L RSI at Peterson Creek and failed unpicked 19-L RSI at Cedar Creek were excluded (2018: N = 25 RSIs; 2019: N = 27 RSIs). Survival between picked and unpicked 19-L RSIs was not significant in 2018 (ANOVA, P = 0.27) but was significant in 2019 (ANOVA, P = 0.02). Error bars are ± 1 SD. Note that there was only one unpicked 265-L RSI at each stream.

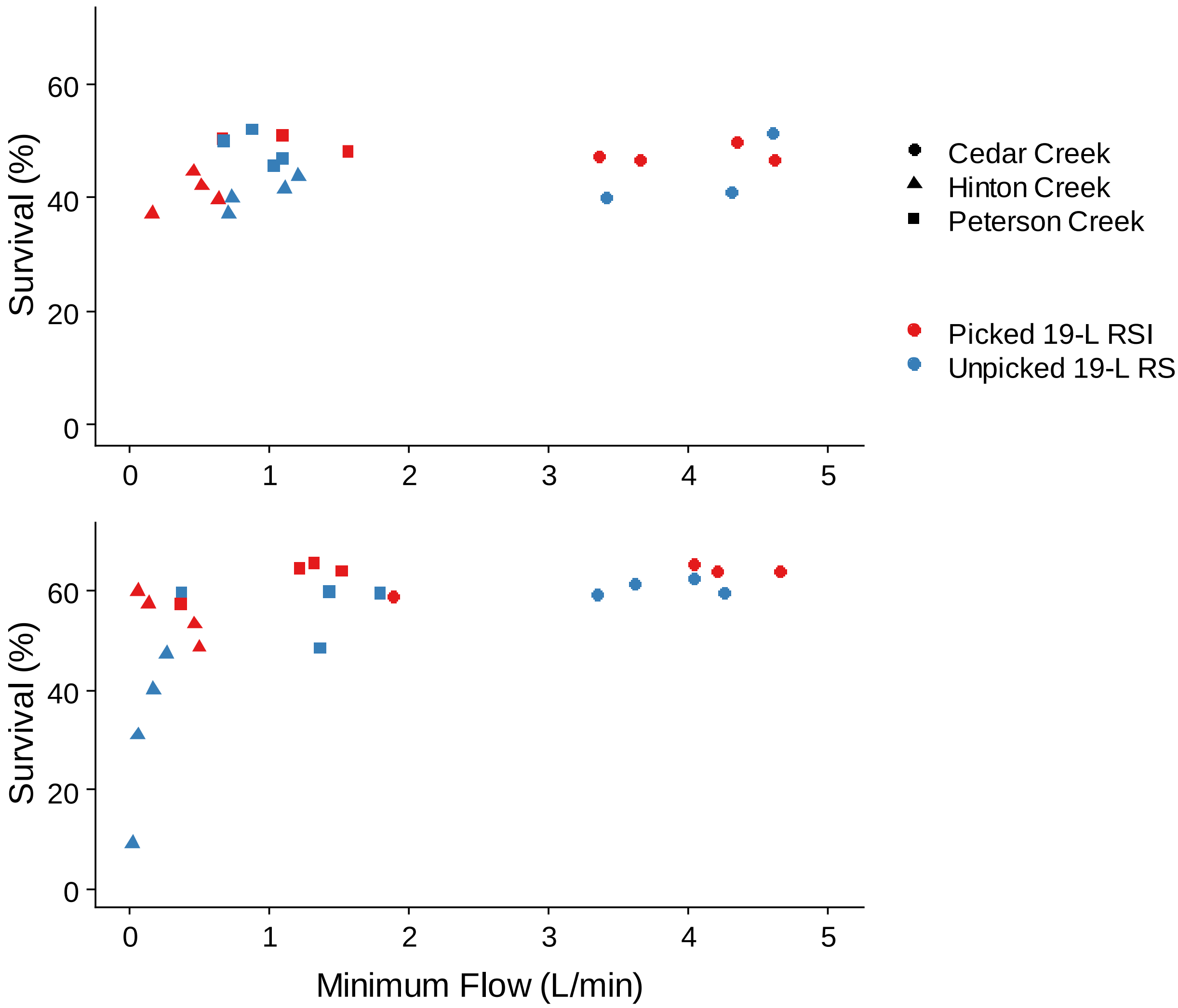

Figure 2.4. Relationship between survival in 19-L RSIs and minimum RSI flow rates in 2018 (top panel) and 2019 (bottom panel). The 2018 observations exclude the failed picked RSI at Peterson Creek and failed unpicked RSI at Cedar Creek (2018: N = 22 RSIs; 2019: N = 24 RSIs). In picked RSIs, there was weak evidence for a positive correlation between minimum RSI flow rates and survival (2018: $P = 0.11$; 2019: $P = 0.08$). In unpicked RSIs, survival was not correlated with minimum RSI flow rates in 2018 ($P = 0.84$), but a significant positive correlation was present in 2019 ($P < 0.01$).

Chapter III

This chapter explores an extended version of literature review and methodology that provides the reader a further understanding of information relevant to Chapter II. The goal of the literature review is to broadly examine peer-reviewed articles and management reports that cover methods of artificial propagation and Arctic Grayling natural history and management. The goal of the extended methodology section is to assist future work with RSIs by providing a detailed description of RSI construction, installation, and maintenance as well as how egg numbers were estimated in 19-L RSIs.

Extended Review of Literature

Methods of Artificial Propagation

Artificial propagation of fish eggs is a common practice that aims to support degraded fish stocks by supplementing natural reproduction, especially for salmonids (Olla et al. 1998; Fraser 2008; Thériault et al. 2011). This is commonly done by raising fish in a hatchery and then directly planting them at the introduction site (Olla et al. 1998). Compared to the natural fluctuations of stream environments, fish experience little environmental variation as they develop in hatcheries, and hatchery-raised fish typically have lower fitness (e.g., reproductive success) than fish from wild populations (Olla et al. 1998; Brannon et al. 2004; Fraser 2008; Thériault et al. 2011). However, the question remains unresolved whether reduced fitness of hatchery-raised fish is caused by genetic differences between hatchery and wild populations or due to the controlled environment that fish experience in hatcheries (Araki et al. 2008). As captive broodstocks are maintained for hatchery supplementation, there can be a strong selection for traits that inversely increase survival in the hatchery while decreasing survival in the wild (Olla et al. 1998; Araki et al. 2008; Thériault et al. 2011). However, the influence of genetics may be hard to differentiate from other confounding factors as the hatchery environment may be regulating the expression of genes through epigenetics (Araki et al. 2008). If the hatchery environment is regulating the expression of genes and behaviors of fish, and ultimately decreasing their post-release survival, then raising fish under more natural conditions may be advantageous (Olla et al. 1998; Bamberger 2009). This is supported from studies that have found fish raised in a more natural environment to have increased predator avoidance and improved swimming endurance despite being progeny of hatchery or wild adults (Griffiths and Armstrong 2002; Wessel et al. 2006; Chittenden et al. 2010). Furthermore, imprinting may occur during later stages of embryonic development (i.e., eyed egg) or soon after hatching (Dittman and Quinn 1996). Imprinting has been observed in many salmonid fish species and allows migratory fish to return to natal streams for spawning (Hasler et al. 1978; Dittman and Quinn 1996). This provides a reasonable argument that the rearing environment plays a crucial role in the success of artificially propagated fish, and allowing early-life stages to imprint and acclimate to natal waters could benefit conservation practices seeking to establish self-sustaining populations (Hoffman et al. 2002; Kaeding and Boltz 2004; Al-Chokhachy et al. 2009; Kirkland 2012; Arnold et al. 2017).

Stream incubation methods have been developed to improve the post-release survival of artificially propagated fish (Barlaup and Moen 2001; Coghlan and Ringler 2004; Kaeding and Boltz 2004; Bernier-Bourgault et al. 2005; Kirkland 2012). The methods are more commonly used in local conservation practices aiming to restore native fish populations (Barlaup and Moen 2001); however, recent success of such methods have supported their use at a larger scale (Kaeding and Boltz 2004; Al-Chokhachy et al. 2009; Kirkland 2012; Arnold et al. 2017). There are two main approaches to stream incubation: in-stream or stream-side rearing. In-stream incubation methods incubate the fish eggs within the stream channel by burying eggs in the stream substrate freely or in boxes (Barlaup and Moen 2001; Coghlan and Ringler 2004; Bernier-Bourgault et al. 2005). Burying the eggs into the stream substrate freely is done by creating an artificial salmonid nest, termed redd, and planting eggs in the gravel using a standpipe (Barlaup and Moen 2001). Boxes used for stream incubation have come in many different designs, but generally are buried in the substrate and protect the eggs and alevins from predation until the fish has absorbed enough of its yolk-sac to escape the box and emerge from the gravel (Barlaup and Moen 2001; Bernier-Bourgault et al. 2005; Kirkland 2012). Hatching success greater than 75% has been reported for in-stream incubators; however, results vary greatly depending on the study (Barlaup and Moen 2001; Coghlan and Ringler 2004; Bernier-Bourgault et al. 2005). Different methodologies between studies can explain some of the observed variation, as studies usually quantify hatching success by counting dead eggs periodically or capturing fry after they emerge from the gravel, but both methods can result in biased estimates of survival (Barlaup and Moen 2001). Factors influencing the success of in-stream incubators include poor intra-gravel water chemistry and the infiltration of fine sediments covering the eggs (Barlaup and Moen 2001; Kirkland 2012). Poor intra-gravel water chemistry and sedimentation are not unique to in-stream incubators, and the effects of both on natural reproduction have been the focus of many studies (Garrett and Bennett 1996; Greig et al. 2005; Geist et al. 2006; Julien and Bergeron 2006; Rombough 2007; Jensen et al. 2009; Sternecker et al. 2013). This suggests that in-stream incubation methods may imitate natural reproduction while protecting early life-stages from predation. However, there remains a lack of studies comparing the various in-stream incubation methods and confounding variables between studies, making few generalizations that can be broadly applied (Barlaup and Moen 2001).

Stream-side incubators can have advantages over in-stream incubators by keeping the eggs out of the stream substrate and reducing the risks of sedimentation while still allowing fish to acclimate to stream conditions (Kaeding and Boltz 2004; Kirkland 2012). However, stream-side incubators for salmonid restoration is rarely discussed in the literature (Kaeding and Boltz 2004; Kirkland 2012). The remote site incubator (RSI) is a type of stream-side incubator developed in the Pacific Northwest in the 1980s (Wampler and Manuel 1992). RSIs use gravity-fed water from the stream to incubate eggs and allows swim-up fry to swim out at the introduction site (Wampler and Manuel 1992; Kaeding and Boltz 2004). RSIs have been an important management tool to support restoration of Chinook Salmon (*Oncorhynchus tshawytscha*; Wampler and Manuel 1992), Cutthroat Trout (*O. clarkii*; Hoffman et al. 2002; Arnold et al. 2017), Steelhead Trout (*O. mykiss*; Denny and Evans 2012), and to reestablish populations of Arctic Grayling (*Thymallus arcticus*) in Montana (Cayer and McCullough 2014) and Wyoming (Arnold et al. 2017). Despite their importance for native species restoration, designs and protocols for using RSIs have varied among regional biologists. RSIs used in the Pacific Northwest are typically tall 208-L designs with multiple egg trays, pea gravel, and bio-saddles to incubate Pacific salmon (Wampler and Manuel 1992; Denny and Evans 2012). In comparison, other management agencies have adopted the smaller 19-L RSI designs for restoration efforts of Cutthroat Trout and Arctic Grayling (Cayer and McCullough 2014; Arnold et al. 2017). When using RSIs, removing dead eggs during incubation is thought to reduce the spread of fungus and increase hatching success (Hoffman et al. 2002; Kaeding and Boltz 2004; Denny and Evans 2012); however, this has not been tested experimentally. Wampler and Manuel (1992) documented hatching success greater than 90% using Chinook Salmon eggs with minimal site visits and no picking of dead eggs. Comparatively, Kaeding and Boltz (2004) documented highly varied hatching success (i.e., 0-95%) while removing dead Arctic Grayling eggs daily. This suggests that studies are needed to elucidate factors that are influencing the success of RSIs and other stream-side incubators (e.g., Kirkland 2012).

Arctic Grayling Natural History and Management

The Arctic Grayling is a native fish species to North America and Eurasia with a unique distribution in North America (McAllister and Harington 1969). During the Pleistocene glaciation, Arctic Grayling had two main refugia, one along the northern half of the Bering Strait and another along the southern Great Plains, possibly the upper Missouri River drainage (Redenbach and Taylor 1999). The northern refuge population is attributed to founding populations throughout Alaska, north-west Canada, and central to east Siberia (Redenbach and Taylor 1999; Stamford and Taylor 2004). The southern refuge population is attributed to founding populations in the Upper Missouri River Drainage throughout southern Montana (Kaya 1992) and northwest Wyoming (Steed et al. 2010), and possibly founded the population in northern Michigan (Creaser and Creaser 1935; Nuhfer 1992). However, inland glacial refugium have been documented in the Mackenzie River drainages in Saskatchewan, Canada (Stamford and Taylor 2004), suggesting that the Michigan population could have been founded by a separate refuge population. Regardless, the Michigan population of Arctic Grayling was extirpated during the 1930s (Nuhfer 1992), making genetic analysis of this distinct population inaccessible.

Declines in Arctic Grayling populations in Michigan were the result of overharvest, habitat degradation, and introductions of non-native species (Vincent 1962; Kaya 1992). Vincent (1962) tabulated fishing reports from the late 1800s and documented that initial angling success could be found near Grayling, Michigan, but exploitation near the city caused a decline in angling success and good catches could only be made further downstream. The loss of Arctic Grayling in upstream sections of the Au Sable River started prior to extensive logging in the area and progressed as logging practices intensified in the region (Vincent 1962). Timber during the logging era were exported through rivers to downstream mills (Vincent 1962). These log drives occurred in the spring, coinciding with the Arctic Grayling spawning season, and potentially influenced Arctic Grayling habitat in three ways: increasing water temperature from forest removal, scouring of instream habitat, and impeding fish movement by the construction of dams (Vincent 1962). During the latter half of the logging era, populations of Brown Trout (*Salmo trutta*) and Rainbow Trout became established in many Arctic Grayling streams (Vincent 1962). Evidence suggests that Arctic Grayling are able to co-exist with Brook Trout (*Salvelinus fontinalis*) but are unable to coexist with Brown Trout and Rainbow Trout (Creaser and Creaser 1935; Vincent 1962; Byorth and Magee 1998; Mccullough 2017). The degree to which Brown Trout and Rainbow Trout influenced the decline of Arctic Grayling in Michigan remains unknown; however, it is likely that initial declines were the result of overharvest and habitat degradation followed by the replacement with non-native salmonids (Vincent 1962).

Successful use of RSIs for Arctic Grayling restoration in Montana (Cayer and McCullough 2014) has prompted the reintroduction of Artic Grayling in Michigan. In 2016, the Little River Band of Ottawa Indians (LRBOI) partnered with the Michigan Department of Natural Resources (MDNR) to create the Michigan Arctic Grayling Initiative (www.migrayling.org). Now with over 45 partners, this initiative seeks to establish self-sustaining populations of Arctic Grayling within the historic range in Michigan. The Manistee River historically held a healthy population of Arctic Grayling (Vincent 1962) and has been the focus of studies to identify suitable habitat for Arctic Grayling in Michigan (Danhoff et al. 2017;

Goble et al. 2018). As the Michigan Arctic Grayling Initiative moves forward with selecting sites for reintroduction efforts, studies evaluating stream incubation methods (i.e., RSIs) are needed to improve the post-release survival of Arctic Grayling and establish self-sustaining populations.

Extended Methodology

Constructing RSIs

Detailed instructions on RSI design and construction are outlined in a report to the LROBI by Ruetz et al. (2018), which are briefly described here. The 19-L RSIs were constructed using black 19-L buckets with their own water-delivery system and egg tray. Inflow pipes were constructed of PVC conduit (25-mm diameter) and were ran upstream from each RSI until enough gradient was achieved for water to continuously flow through the RSI unit. Inflow pipes for all 19-L RSIs at a site were placed at the same location and secured in place with sand bags. A ball valve (25-mm diameter) connected the inflow pipe to the RSI unit and was used to adjust water flow rates into each 19-L RSI. A water diffuser located at the bottom of the RSI unit was constructed of PVC conduit (25-mm diameter) with seven holes (9.5-mm diameter) to allow water to upwell and fill the RSI unit. Egg trays were made by cutting off the bottom of a separate 19-L bucket and melting stainless-steel mesh (0.9-mm diameter wire, 1.65-mm opening width; surface area = 532 cm^2) to the opening. Excess mesh was trimmed off and the bucket was cut so the egg trays were 114-mm deep. Each 19-L RSI was fitted with a black lid to prevent light from affecting the eggs and protect them from predation.

The 265-L RSIs were constructed using a black 265-L stock-tank. The water delivery system followed the design of 19-L RSIs, but used larger PVC conduit (54-mm diameter). A rectangular egg tray was made of T-304 stainless steel wire mesh (0.9-mm diameter wire, 1.65-

mm opening width; surface area = 3903 cm^2), and was placed on galvanized steel cross pieces to keep the egg tray near the top. A ball-valve (54-mm diameter) was used to adjust water flow rate into each 265-L RSI. Each 265-L RSI was covered with a large plywood lid to protect eggs (see Figure 3.1).

Each RSI had its own collection bucket consisting of two screened containers to prevent fish and eggs from entering the stream and to allow for enumeration of swim-out. Collection buckets were placed near and connected to each RSI unit by the outflow pipe. The 19-L RSIs used a screened 19-L bucket inside a screened 72-L container for collection buckets. The 265-L RSIs used a screened 72-L container inside a screened 90-L container for collection buckets. The larger collection bin for each RSI design (i.e., 72-L container for 19-L RSIs and 90-L container for 265-L RSIs) was checked every 1-3 d throughout the study for any escaped Rainbow Trout from the smaller collection bucket. No Rainbow Trout were captured outside of the smaller collection bucket. Note that the collection buckets were used for this experiment but would not be needed if the goal had been to "stock" fish in each stream.

RSI Installation and Maintenance

At each site, RSIs were installed at locations with low and stable banks. This allowed the RSIs to be lower in elevation to the surface of the stream and made it easier to establish adequate water flow through each RSI. When installing RSIs, the aim was to establish a flow rate of 3.79 L/min. After identifying ideal locations for RSIs, inflow pipes were connected from an upstream to downstream direction. When connecting sections of PVC conduit for the inflow pipes, air was purged from the conduit by holding the PVC underwater before connecting concurrent pieces. Glue was not used to secure the PVC conduit together, but an effort was made to ensure a snug connection was established between pieces of PVC conduit. The only issue observed with inflow pipes during field experiments was at Peterson Creek in 2018 when one 19-L RSI and the 265-L RSI became disconnected, which was caused by high discharge due to rain.

RSI flow rates at all sites decreased during the study, which was likely caused by sediment and detritus entering the inflow pipes and restricting flow (see Figure 3.2). Hinton Creek had the lowest RSI flow rates during both years of the study (see Figure 2.4). In order to maintain RSI flow rates at Hinton Creek, I cleaned the inflow pipes of all the RSIs by disconnecting and washing out the most upstream section of PVC conduit. This was done twice in 2018 and six times in 2019, and RSI flow rates were measured for each RSI before and after cleaning (note – only the measured RSI flow rate prior to cleaning was used in statistical analyses; see Figure 2.4).

Cedar Creek was the smallest stream in our study and likely had the lowest gradient. Therefore, we built a partial impoundment where the RSI inflow pipes started upstream. This raised the water height and made it easier to establish adequate RSI flow rates. Furthermore, a pool was created behind the impoundment where the RSI inflows were located, which reduced the amount of sediment and detritus that could enter the inflow pipes. This is likely why RSI flow rates were the greatest at Cedar Creek throughout our study (see Figure 2.4).

Estimating Eggs in 19-L RSIs

I estimated the initial egg number in each 19-L RSI by analyzing photo images of the egg tray in the computer program ImageJ (Schneider et al. 2012). In ImageJ, I manually clicked on each observed egg in an egg tray from a photo. Using the multi-point tool, a symbol and the next sequential number appeared with each click on an egg. Therefore, the multi-point tool counted the number of clicks I made, and the symbol effectively reduced the likeliness of double-counting eggs. To measure the precision of my counts, I estimated the initial egg number three times for each 19-L RSI in 2018. The mean difference between my first count and the average of three counts was 2.5 eggs (SE = 0.4, n = 12), which established the high precision of the estimate of initial egg numbers in 19-L RSIs. When calculating survival in 2018 and 2019, the initial egg number was estimated from counts using ImageJ.

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Figure 3.1. A black bear (*Ursus americanus*) inspecting the 265-L RSI at Cedar Creek. All RSIs (i.e., 19-L and 265-L RSIs) were fitted with lids to protect the eggs from light and predation. No damage to RSIs was observed during the study. A game camera captured the photo on 22 April 2019.

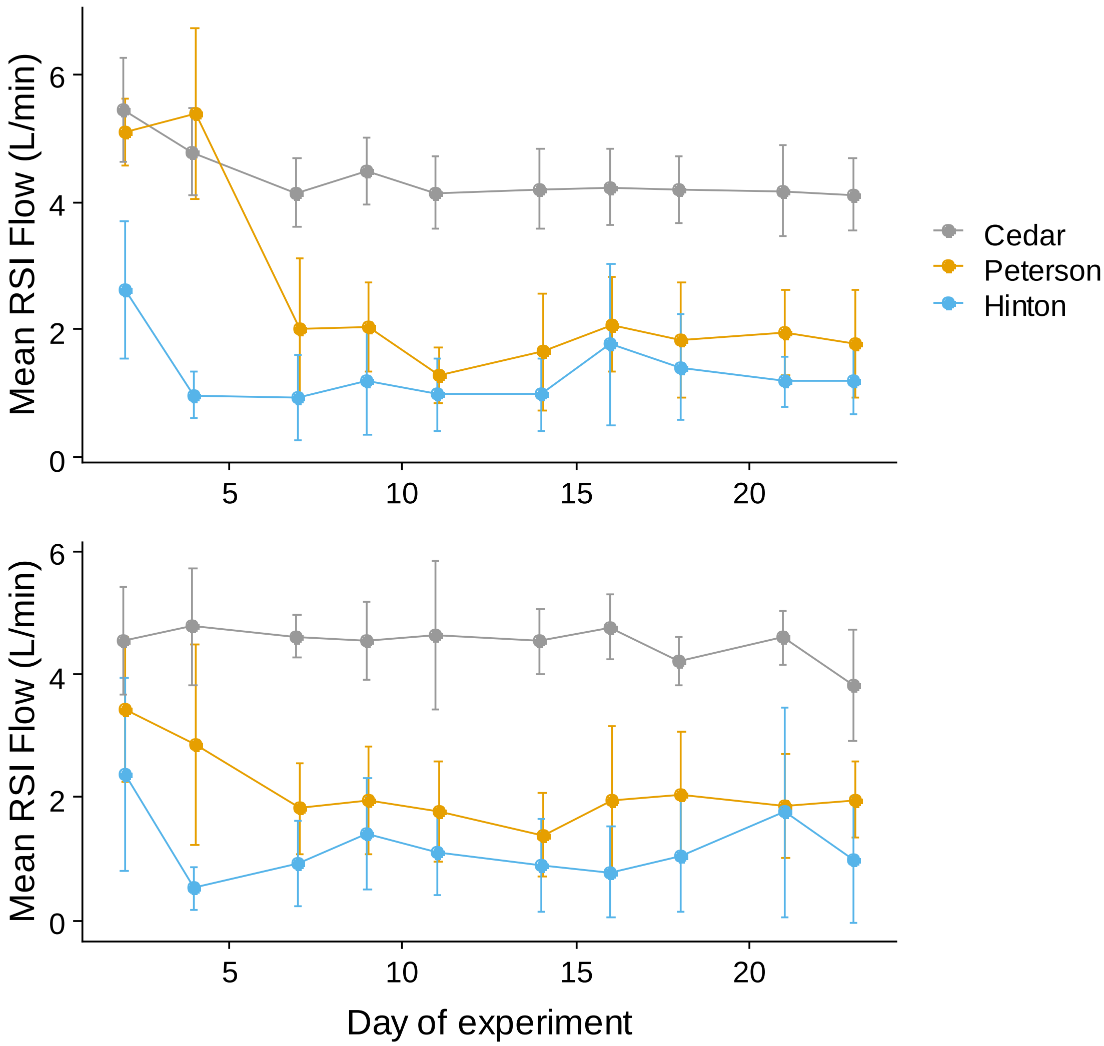

Figure 3.2. Mean RSI flow rates of 19-L RSIs at Cedar, Peterson, and Hinton creeks during 2018 (top panel) and 2019 (bottom panel). Error bars are ±1 SD. Note – Cedar Creek had an impoundment installed where RSI water intakes were located, which reduced the amount of sediment and detritus that entered the inflow pipes.