Evaluation of Remote Site Incubators in Three Tributaries of the Manistee River

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Abstract

The Arctic Grayling (*Thymallus arcticus*) was extirpated from Michigan in the 1930s. Subsequent efforts to reintroduce the species to Michigan have failed, but more recent efforts to restore the species in Montana have been successful through the use of remote site incubators (RSIs), which have not been tested in Michigan streams. RSIs allow fish eggs to be reared and fry to be stocked at the site of introduction. Due to a renewed interest in reestablishing Arctic Grayling in Michigan, we conducted a pilot study using Rainbow Trout (*Oncorhynchus mykiss*) eggs to test RSI designs in tributaries of the Manistee River (Cedar, Hinton, and Peterson creeks) to support the reintroduction effort of Arctic Grayling to Michigan streams. We evaluated three RSI designs: a single-tank RSI (single 5-gallon bucket), a triple-tank RSI (three 5-gallon buckets in parallel), and a stock-tank RSI (70-gallon trough). We installed three single-tank RSIs and one triple-tank RSI in each study stream, and one stock-tank RSI in Peterson Creek. Overall hatching success was 65%, ranging from 55 to 79% among the three study streams. Hatching success in single-tank RSIs was 1-4% greater than triple-tank RSIs, and the stock-tank RSI at Peterson Creek was 10-11% greater than single and triple-tank RSIs in the same stream. Our pilot study suggests that RSIs can be used successfully in Michigan streams.

Introduction

The Arctic Grayling (*Thymallus arcticus*) is a coldwater salmonid that inhabits streams and lakes in the northern regions of the world (Scott and Crossman 1973). In North America, outside of its main distribution (roughly Alaska to the west coast of Hudson Bay), two disjunct southern populations historically existed in Montana and Michigan (Scott and Crossman 1973). The Arctic Grayling was extirpated from Michigan by 1936 (Scott and Crossman 1973), which was likely due to logging practices, introduction of non-native salmonids, and overharvest (Creaser and Creaser 1935). In Montana, Arctic Grayling currently inhabit a small portion of their historic range in the upper Missouri River drainage (Kaya 1992) with conservation efforts targeted at reversing this declining trend (e.g., Kaeding and Boltz 2004; Lamothe and Magee 2004; Arnold et al. 2017).

Remote-site incubators (RSIs) have been used to introduce fertilized Arctic Grayling eggs into streams by Montana Fish, Wildlife and Parks since 2003 (Lamothe and Magee 2004). RSIs are small, self-contained systems that allow rearing and hatching of eggs directly at the site of introduction (Kaeding and Boltz 2004). Advantages of RSIs include allowing fish to hatch in the stream under local environmental conditions and facilitating early life-stage imprinting on the stream's water (Arnold et al. 2017). The use of RSIs to supplement natural reproduction and to reestablish fluvial Arctic Grayling populations in Montana has proven more effective than traditional stocking practices (MFWP 2013).

Attempts to reintroduce Arctic Grayling in Michigan have been unsuccessful with the most recent in the late 1980s (Nuhfer 1992). The most recent effort likely failed in rivers because of a combination of factors, including competition with and predation by other fishes, high water temperatures in some streams, use of a lake-strain stock of Arctic Graying, and hatchery disease issues (Nuhfer 1992). In concluding his report, Nuhfer (1992) noted that the Arctic Grayling may be unlikely to survive well in contemporary Michigan rivers because of the perceived need to make long seasonal migrations, which would necessitate that conservation efforts be targeted toward large, non-fragmented rivers. The use of RSIs could help to meet this challenge by allowing fry to imprint on stream water at stocking sites (Kaeding and Boltz 2004; Arnold et al. 2017), which could have played a role in the rapid dispersal of Arctic Grayling from stocking sites in Michigan rivers that was observed by Nuhfer (1992).

Although previous attempts to reintroduce Arctic Grayling to Michigan were unsuccessful (Nuhfer 1992), there is renewed interest in reestablishing the species in Michigan waters (MAGI 2017). A landscape-scale study identified and ranked Michigan streams that could potentially support Arctic Grayling (Tingley 2010), and assessment of abiotic conditions in the Manistee River tributaries suggests there is suitable Arctic Grayling habitat in portions of the watershed with ongoing efforts to evaluate abiotic and biotic conditions (Danhoff et al. 2017). Thus, recent and ongoing research in Michigan to identify the "best" candidate waters for reintroduction of Arctic Grayling and the advancements of using RSIs to reestablish Arctic Grayling in Montana streams provide the beginnings of a blueprint for establishing self-sustaining populations of Arctic Grayling in Michigan streams.

Our overall goal was to identify functional and practical RSI designs for reestablishing Arctic Grayling in Michigan streams. In this pilot study, we evaluated three RSI designs under natural stream conditions to improve our overall understanding of optimal design and operation of RSIs. Our specific objectives were to: (1) provide a realistic representation of the construction and operation of RSIs in Michigan streams, and (2) compare the hatching success of Rainbow Trout (*Onchorhynchus mykiss*) eggs (used as a surrogate for Arctic Grayling) in three types of RSIs (single tank, triple tank, and stock tank) in three tributaries of the Manistee River.

Methods

Study Site.—We conducted field trials at three tributaries to Michigan's Manistee River: Cedar Creek (N 44.304792°, W 85.820534°) on the border of Manistee and Wexford counties, Hinton Creek (N 44.276201°, W 85.815835°) in Wexford County, and Peterson Creek (N 44.263353°, W 85.846907°) in Manistee County. These streams were reported to have abiotic conditions that were within ranges consistent with those associated with Arctic Grayling populations in North America (Danhoff et al. 2017). Specific locations of RSIs at each site were selected based on consideration related to habitat (e.g., nearby juvenile habitat, stream gradient, distance from main-stem) and accessibility. Cedar Creek is a small, groundwater-fed stream; during our study, mean water temperature was 8.09 °C and mean discharge was 0.06 m^3/s (Table 1). The main branch of Hinton Creek is larger than Cedar Creek with a mean discharge of 0.16 m^3/s and had less canopy cover and likely a smaller percentage of groundwater input than Cedar Creek, resulting in a slightly higher mean water temperature of 9.21 °C in Hinton Creek (Table 1). At Hinton Creek, RSIs also were located at a "large" unnamed tributary that had a mean discharge of 0.07 m^3/s (Table 1) and a "small" unnamed tributary (discharge and water temperature were not measured in the "small" tributary, but discharge was less than the "large" unnamed tributary). Peterson Creek was the largest stream that we studied with a mean discharge of 0.68 m^3/s; mean water temperature (9.54 °C) was similar to Hinton Creek (Table 1). All three streams exhibited diel temperature variation, with hourly water temperatures ranging from about 5 °C to 15°C during the field study (Appendix A).

RSI Construction.—We evaluated three RSI designs: single tank, triple tank, and stock tank. Single-tank RSIs followed the design of Kaeding and Boltz (2004) using 5-gallon buckets with a 1” diameter water intake line (Fig. 1A). Triple-tank RSIs were three single-tank RSIs connected with a common 2” diameter water intake line that directed water to each individual RSI using a PVC manifold (Fig. 1B). The stock-tank RSI was constructed from a 70-gallon Rubbermaid stock tank with a rectangular egg tray situated inside the tank and a 2” diameter water intake line (Fig. 1C and 1E).

Detailed instructions for constructing single-tank and triple-tank RSIs are reported in Appendix B. Briefly, single-tank and triple-tank RSIs were constructed using black 5-gallon buckets (Fig. 1A and 1B). Egg trays inside each RSI were made by cutting off the lower portion of a 5-gallon bucket (Fig. B1A) and melting stainless steel mesh to the bottom (Fig. B1B). Egg tray mesh was T-304 stainless steel wire mesh (0.9-mm diameter wire, 1.65-mm opening width). Each egg tray was placed inside a RSI that had a 1” diameter water intake at the bottom of the bucket (Fig. B1D), a “+” shaped PVC diffuser inside the RSI (Fig. B1J), and a 1” diameter water outflow at the top of the bucket (Fig. B1D). Each RSI had a black snap-on lid (e.g., Fig. 1B). For the triple-tank RSI, a PVC manifold was constructed to divert the flow from the 2” diameter water intake line into the 1” diameter water intake lines for each of the three RSIs (Fig. 1B).

The stock-tank RSI was constructed of a 70-gallon black tank (Fig. 1C). In the bottom of the stock tank, we installed a 2” diameter water intake line and 2” diameter diffuser (made from 2” PVC pipe and fittings; see Fig. B1L). A rectangular egg tray, made of T-304 stainless steel wire mesh (0.9 mm diameter wire, 1.65 mm opening width, Fig. 1E) was installed near the top of the stock tank, resting on galvanized steel cross pieces (Fig. B1L). The stock tank had a 2” diameter water outflow line near the top of the tank and a plywood lid (Fig. 1C).

RSI Installation.—We installed three single-tank RSIs and one triple RSI at Cedar Creek on 10-11 April 2017. At Hinton Creek, we installed one single-tank RSI at the “small” unnamed tributary, two single-tank RSIs at the “large” unnamed tributary, and one triple-tank RSI at the main branch on 12 April 2017. We installed three single-tank RSIs, one triple-tank RSI, and one stock-tank RSI at Peterson Creek on 13 April 2017. Stock-tank RSIs were not installed at Cedar or Hinton creeks due to the lack of sufficient stream gradient at those sites. Water intake lines were constructed of 1” pipe (10’ pieces of gray schedule 40 PVC conduit with bell ends) for single-tank RSIs, whereas 2” diameter pipe was used for the water intake lines of triple-tank RSIs and stock-tank RSIs. At Cedar Creek, we built an impoundment out of about 40 sand bags to achieve adequate flow because of the lack of stream gradient at this site (Fig. B1M). Building an impoundment at Hinton or Peterson creeks was not needed to achieve adequate flow through the RSIs.

The outflow from each RSI went into a 5-gallon collection bucket with screened sides that was placed in a 19-gallon blue utility tub with a screened outflow that delivered the water back to the stream. The collection buckets prevented Rainbow Trout eggs or fry from escaping into the stream and allowed us to count swim-up fry (Fig. 1A-C). Single-tank RSIs and the stock-tank RSI had one collection bucket per RSI (Fig 1A and 1C), whereas triple-tank RSIs had a single combined collection bucket (Fig. 1B). RSIs and collection buckets were secured in place using fence posts and fencing around the RSI (e.g., Fig. 1B). Most RSIs had a small stand pipe in the water intake line near the RSI (e.g., Fig 1A-B). The stand pipes were at least as tall as the top of the RSI and had a cap with a small hole drilled in it.

Fertilized Rainbow Trout eggs (at the eyed stage) were stocked into RSIs on 14 April 2017. We received an estimated 31,578 Rainbow Trout eggs (total volume = 1.5 L) from the Michigan Department of Natural Resources (MDNR; Oden State Fish Hatchery). MDNR estimated that 500 eggs were dead when the eggs were transferred to us. About 3,000 eggs were stocked into the stock-tank RSI at Peterson Creek, whereas about 1,500 eggs were stocked into each "tank" in the single-tank and triple-tank RSIs. Thus, for the triple-tank RSIs, 1,500 eggs were stocked into each "tank," meaning that the triple-tank RSI received 4,500 eggs in total.

Monitoring and Maintenance.—Single-tank, triple-tank, and stock-tank RSIs were all inspected and maintained 3-4 days per week during the study. Dead eggs and fungus were picked from egg trays 2-3 times per week; all dead eggs were preserved in 95% ethanol and brought back to the laboratory for enumeration. We visually estimated fungus (percentage of coverage) and sedimentation (on a scale of 1 to 10, with 10 being complete coverage) in each egg tray about 3 times per week. If a significant amount of sediment was present at the bottom of a single-tank or triple-tank RSI, then we disassembled (i.e., removed egg tray and disconnect water intake line) and rinsed the RSI in the stream. To clean sediment out of the stock-tank RSI (which was rarely necessary because of the high flow rate), we removed the egg tray and rinsed the tank in the stream. Collection buckets were examined and cleaned 3-4 times a week; the number of fry in each collection bucket was usually recorded and preserved.

Water quality (i.e., temperature, dissolved oxygen, specific conductivity, and pH) was measured weekly using either a Hydrolab DS6 or YSI 6600 v2 sonde. Water velocity (measured with a Marsh-McBirney flow meter) and depth were measured weekly across fixed transects at each stream to calculate discharge. Hourly water temperature was monitored in each stream (main branch at Hinton Creek) with a Hobo data logger. Additionally, a Hobo water temperature data logger was deployed in a single-tank RSI at Cedar Creek, a triple-tank RSI at Hinton Creek, and the stock-tank RSI at Peterson Creek to investigate whether water warmed in the RSIs.

We concluded the field study on 5 May 2017 when almost all of the eggs had either hatched or died. Fry (Fig. 1F) were euthanized with MS-222, and all eggs and fry were preserved in 95% ethanol. In the laboratory, we counted the preserved eggs and fry. Thirty preserved fry from each RSI were randomly selected and measured for total length using dial calipers. We calculated hatching success (s) as the percentage of eggs that hatched:

$$s_i = h_i/t_i \times 100,$$

where h_i is the number of fry (i.e., eggs that hatched) in a RSI, t_i is the total number of eggs stocked in a RSI, and i denotes the seven combinations of RSI types and streams (i.e., stream = Cedar, Hinton, or Peterson creek and RSI type = single tank, triple tank, or stock tank, recalling that the stock-tank design only was tested in Peterson Creek). We calculated the overall hatching success (s_T) by summing over the seven combinations of RSI types and streams:

$$s_T = \frac{\sum_{i=1}^{7} h_i}{\sum_{i=1}^{7} t_i} \times 100,$$

where the variables are defined above and $\sum_{i=1}^{7} t_i$ = 31,578 eggs. Thus, the overall hatching success we calculated is likely conservative because it does not account for the 500 eggs that were dead when the eggs where transferred to us from the hatchery as well as any other eggs that died either in transit to the study sites or as a result of handling during the stocking of RSIs. However, when calculating s_i we estimated t_i as the number of preserved eggs plus the number of fry collected in that RSI.

Results and Discussion

Our pilot study suggests that RSIs can be used successfully in Michigan streams. Overall, 64.6% of Rainbow Trout eggs hatched in RSIs across the three study streams, which was higher than the 44.8% hatching success reported for Arctic Grayling using single-tank RSIs in Montana (Kaeding and Boltz 2004). However, in the Montana study, hatching success of Arctic Grayling eggs varied among sites and years, with the cause of the inter-annual variation unclear (Kaeding and Boltz 2004). How such inter-annual variation will translate to Michigan streams remains unknown. The percent coverage of fungus on eggs and sedimentation in egg trays was reasonably low in our field study (Table 2), which was consistent with the high overall hatching success in RSIs. Of the 19 RSIs that we used in this field study, only one completely failed—a single-tank RSI in Hinton Creek that was likely caused by a high discharge event at the beginning of the study. Given that the failure of this single-tank RSI was not related to its design (the cause is discussed below), we excluded the single-tank RSI that failed in Hinton Creek when calculating hatching success in all of the following comparisons. Hatching success was not markedly different across the three types of RSIs, although it tended to be 1-4% higher in single-tank RSIs compared with triple-tank RSIs in each of the three streams (Fig. 2). In Peterson Creek (i.e., the only stream we tested the stock-tank design), hatching success was, on average, 10-11% greater than either the triple-tank or single-tank RSIs (Fig. 2). Therefore, all three of the RSI designs that we tested have the potential for use in Michigan streams.

Several advantages and disadvantages should be considered when selecting the design of RSIs for use in Michigan streams. Common challenges to using RSIs are their susceptibility to failure from clogging with debris (detritus or sediment), disturbance by wildlife, or vandalism (Arnold et al. 2017). The main advantage of the single-tank RSI is that this design will require the use of multiple RSIs at a site to achieve the management goal of reestablishing Arctic Grayling. Thus, risk of total failure is distributed among several RSIs, which should reduce the risk of failure similar to how diversification in a financial portfolio reduces risk (e.g., Schindler et al. 2015). However, a disadvantage of using single-tank RSIs is that each RSI will need its own water intake line (the length of which can be considerable given the gradient of many Michigan streams); thus, increasing effort for set-up and maintenance as well as the cost of materials. This disadvantage is addressed by the triple-tank RSI, where three RSIs share a common water intake line. However, the trade-off is that the failure of the intake line (e.g., the water intake line becomes clogged with debris or disconnected) would essentially result in the failure of three RSIs, meaning the risk of complete failure for one triple-tank RSIs is greater than the risk of complete failure of three single-tank RSIs. Also, adjusting the flow rate in water intake lines to individual RSIs for the triple-tank design can be difficult. Similar to the triple-tank RSI, the stock-tank RSI has the advantage of incubating a substantial number of eggs in a single RSI, which minimizes the number of RSIs that need to be constructed and maintained. However, the stock-tank design magnifies the risks of using fewer RSIs. When compared with the other two RSI designs we tested, the stock-tank RSI needs a greater differential in elevation between the water outflow line (at the top of the RSI) and the intake at the end of the water line, which could prove challenging in some streams (assuming a gravity flow system for the water inflow line).

As mentioned above, one single-tank RSI at the "small" unnamed tributary of Hinton Creek filled with mostly organic debris (and some sediment) following a rain event, resulting in high egg mortality and fungus colonization (Table 2). We suspect that the failure of this single-tank RSI illustrates the challenge posed by high flow events to the successful implementation of RSIs in Michigan streams. We concluded the failure of this RSI was due to its location rather than design. Due to the catastrophic failure of this single-tank RSI (i.e., nearly 100% egg mortality) early on in the field study (i.e., by day 3 of the study), we ceased operation of this RSI, and dead eggs were not collected from this RSI.

Of the three study streams, Cedar Creek had the most stable flow regime during our field study and the highest hatching success in RSIs. Cedar Creek had the least variation in stream discharge (Fig. 3), turbidity (an index of the suspended sediments), and water temperature among the three streams (Table 1). The stable flow regime and minimal load of suspended sediments in Cedar Creek minimized the need for frequent cleaning of RSIs relative to the other streams. Hatching success was higher in Cedar Creek (78%) than Peterson Creek (62%) or Hinton Creek (57%; Fig. 2). Additionally, the total length of hatched Rainbow Trout fry at the end of the field study was largest in Peterson Creek and smallest in Cedar Creek (Fig. 4), which seemed to be positively associated with water temperature (i.e., warmer water temperature was associated with greater size of fry; Table 1).

Although hatching success was high in the study streams, we experienced several challenges with the maintenance of RSIs. Filling the water intake line proved challenging during the initial set-up in Cedar Creek because the stream was shallow (Fig. B1C). Another disadvantage of using RSIs in small, low-gradient streams such as Cedar Creek is a need to build an impoundment to achieve adequate flow to the RSI (Fig. B1M). Conversely, larger streams such as Peterson Creek proved challenging in that elevated water levels (Fig. 3) associated with rain events caused erosion around RSIs. This challenge could be mitigated by placing all RSIs higher on dry banks to create more stable locations for placement (but requiring longer water intake lines to achieve the necessary change in elevation between the water outflow line and the end of the water intake line) or placing RSIs on a "table" with fence posts supporting a stable, level platform for RSI placement (see Fig. B1F) that would be unaffected by moderate increases in stream discharge.

We found no evidence that using either black buckets or the black stock tank affected the water temperature inside of the RSIs during our field study. Mean hourly water temperature inside RSIs was essentially the same as temperatures measured in the stream (Fig. 5). In the small, coldwater streams that will be candidates for Arctic Grayling reintroduction in Michigan (e.g., Danhoff et al. 2017), we suspect most designs of RSIs will have little effect on water temperature inside the RSI given the stream's cold water temperature and the flow rates (Table 2) through the RSIs.

We had considerable success using RSIs to hatch Rainbow Trout eggs in three tributaries of Michigan's Manistee River in our pilot study. This bodes well for RSIs to play a critical role in the reintroduction of Arctic Grayling to Michigan's streams. Nevertheless, our results were from only 1 year, and others have reported that hatching success in RSIs can vary annually (Kaeding and Boltz 2004). We plan to continue testing and refining the use of RSIs in Michigan streams, which should help to quantify the magnitude of inter-annual variation of hatching success of fish eggs in RSIs. Moreover, we think that evaluating the importance of picking dead eggs from trays inside RSIs and whether design modifications (e.g., some Montana biologists place pea gravel in RSI egg trays to keep the eggs from clumping) affect hatching success. Finally, it is worth considering how well Rainbow Trout eggs serve as a surrogate for Arctic Grayling eggs. An obvious difference is that Rainbow Trout eggs (3-5 mm diameter) are slightly larger than Arctic Grayling eggs (2.7-mm diameter; Scott and Crossman 1973). Therefore, all RSI design considerations should ultimately take into account that the Arctic Grayling eggs used for restoration efforts will be a smaller size and may respond differently than the Rainbow Trout eggs used to refine the design of RSIs in Michigan streams, which could lead to varying results.

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Table 1. Mean (±1 SD) discharge, turbidity, specific conductivity, pH (these variables were measured weekly) and hourly water temperature at each stream. Sample sizes are reported parenthetically; samples sizes reported for turbidity are the same for specific conductivity and pH (which are not reported parenthetically in the table). Observations were recorded between 17 April and 5 May 2017.

Creek	Discharge (m^3/s)	Water Temperature (˚C)	Turbidity (NTU)	Specific Conductivity (µS/cm)	pH
Cedar	0.06±0.004 (8)	8.09±1.35 (365)	0.18±0.45 (6)	0.282±0.008	7.9±0.5
Hinton - main branch	0.16±0.040 (7)	9.21±2.39 (365)	2.75±2.48 (6)	0.272±0.023	7.9±0.4
Hinton - "large" tributary	0.07±0.042 (7)	--	1.98±3.37 (6)	0.259±0.029	7.8±0.2
Peterson	0.68±0.194 (6)	9.54±1.89 (365)	7.36±9.71 (7)	0.248±0.027	7.9±0.3

Note: environmental variables were measured on both the main branch and a "large" unnamed tributary of Hinton Creek (see *Study Site* description for further details).

Table 2. Mean (± 1 SD) flow rate for a remote site incubator (RSI), sedimentation on the egg tray (on a scale of 1 to 10, with 10 being complete coverage), and percentage of fungus covering eggs on the tray for each stream and type of RSI. Sample sizes for each mean are reported parenthetically.

Creek	RSI	Gradient[a]	Flow rate[b] (L/min)	Sedimentation	Fungus (%)
Cedar	Single tank	0.007	3.3±0.5 (7)	2.1±0.1 (12)	7.5±0.8 (6)
Hinton - "large" tributary	Single tank	0.014	3.7±0.6 (7)	1.8±0.1 (12)	8.3±1.2 (6)
Hinton – "small" tributary	Single tank	0.014	4.9±2.0 (6)	3.8±3.3 (11)	74.0±36.0 (5)
Peterson	Single tank	0.031	5.1±1.5 (7)	1.5±0.1 (11)	7.3±2.6 (6)
Cedar	Triple tank	0.005	11.0±2.0 (7)	1.7±1.7 (12)	6.1±4.6 (6)
Hinton - main branch	Triple tank	0.007	11.2±2.7 (7)	2.4±2.4 (12)	7.5±2.8 (5)
Peterson	Triple tank	0.034	9.8±1.9 (4)	1.5±0.7 (10)	5.0±1.7 (4)
Peterson	Stock tank	0.008	21.7±4.7 (7)	1.2±0.4 (10)	6.7±2.6 (6)

[a]Note that gradient was calculated by using a laser level to estimate the height from the top of the RSI to the end of the water intake line divided by the distance from the RSI to the end of water intake line; this ratio is not the same as stream gradient.

[b]The flow rate was measured at the outflow of the RSI where it flowed into the collection bucket, meaning the flow rate for the triple-tank RSIs is for three "buckets" (see Fig. 1B).

Figure 1. Pictures representing (A) a single-tank remote site incubator (RSI) at Hinton Creek, (B) a triple-tank RSI at Hinton Creek, and (C) stock-tank RSI at Peterson Creek used for rearing of Rainbow Trout eggs; egg trays (with Rainbow Trout eggs) used in (D) single-tank and triple-tank RSIs and (E) the stock-tank RSI; and (F) recently hatched Rainbow Trout fry on an egg tray.

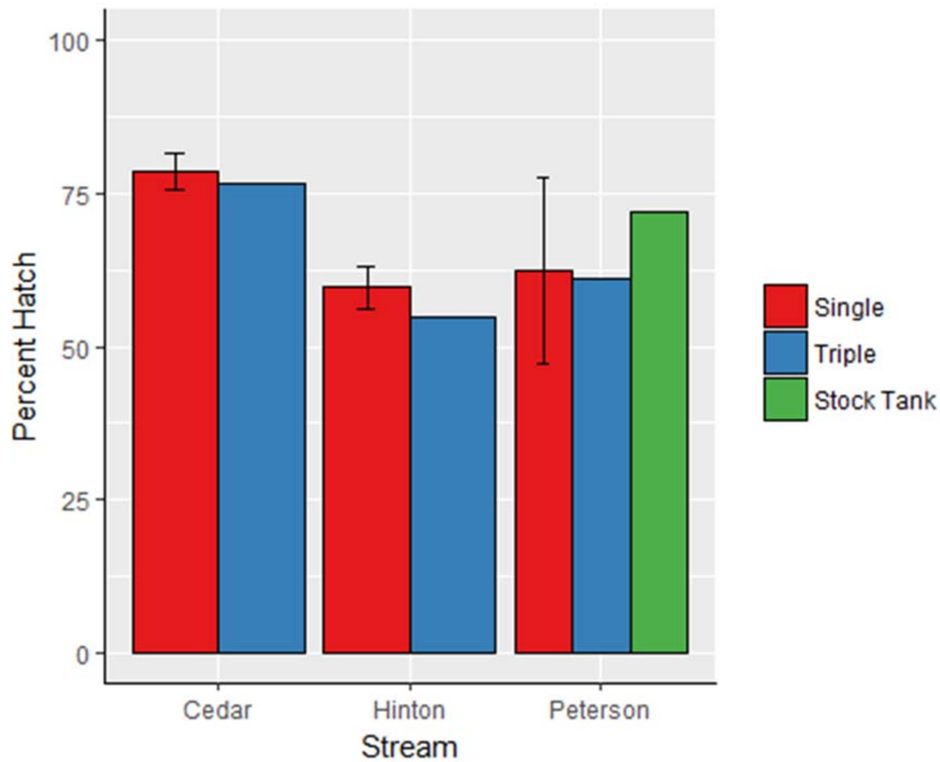

Figure 2. Hatching success (%) of Rainbow Trout eggs among three types of remote site incubators (RSIs) in three streams. Error bars are ± 1 SD, but they could not be calculated for triple-tank RSIs (because there was only one collection bucket for the three "tanks" that make the triple-tank design) or the stock-tank RSI (only one stock-tank RSI was tested in Peterson Creek and not in the other two streams). The sample size for the single-tank RSIs was n = 3 per stream. Note that the hatch success reported here excludes the single-tank RSI that failed in Hinton Creek.

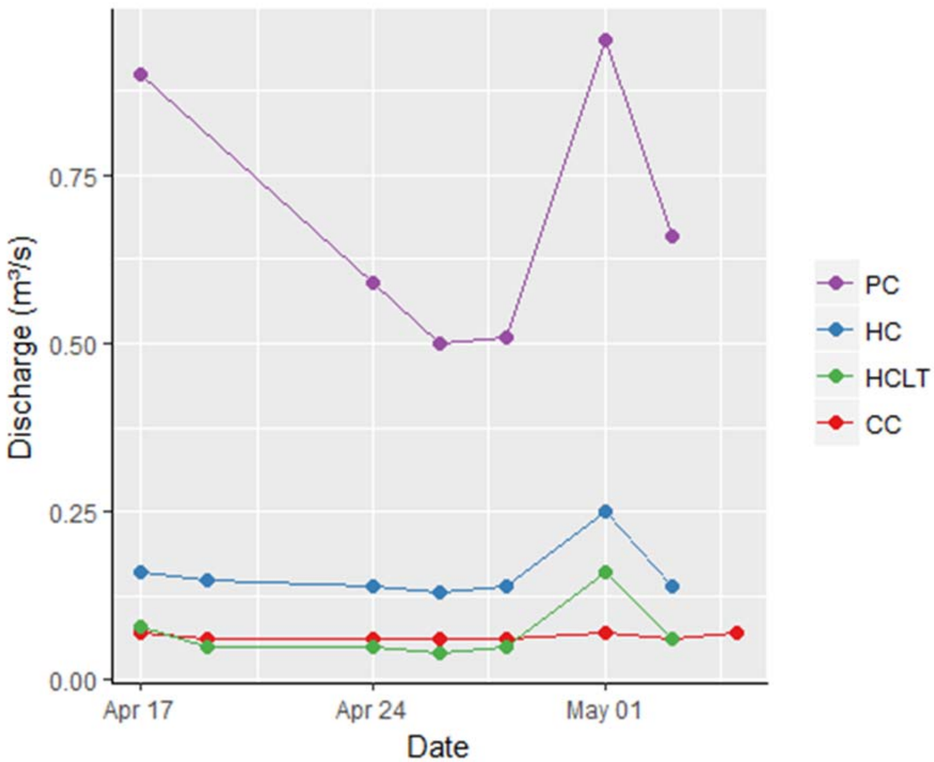

Figure 3. Discharge (m^3/s) measured once a week in the study streams during 2017. Discharge was not measured on 19 April 2017 in Peterson Creek. PC is Peterson Creek, HC is Hinton Creek, HCLT is the "large" unnamed tributary of Hinton Creek (which was the location of two single-tank remote site incubators), and CC is Cedar Creek.

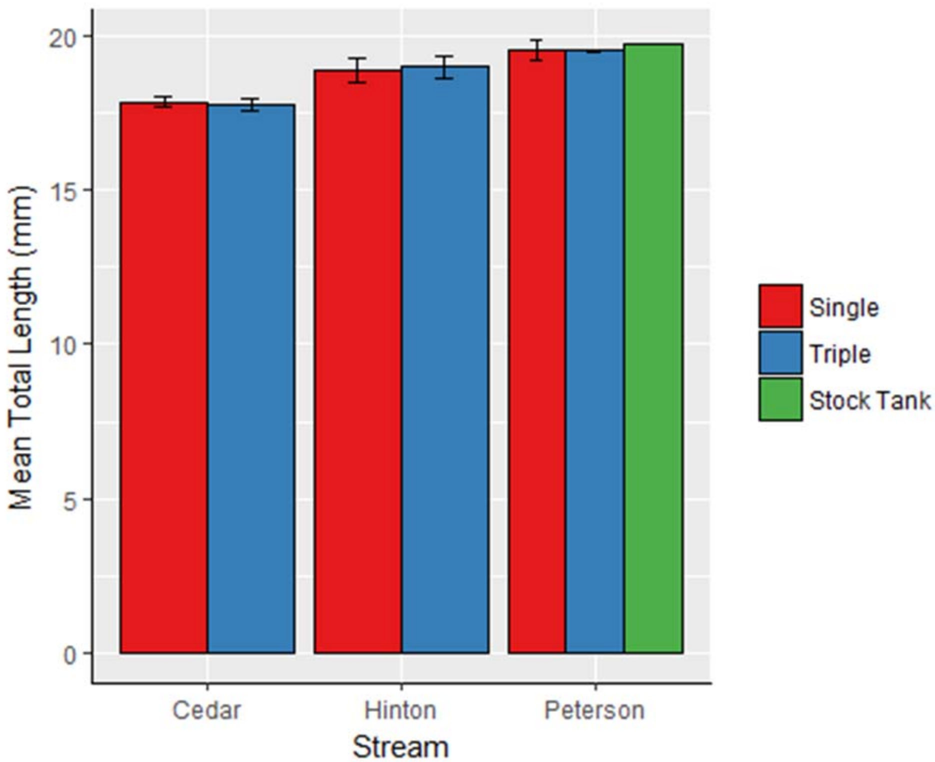

Figure 4. Mean total length (mm) of Rainbow Trout fry collected from three types of remote site incubators (RSIs) in three streams. Thirty Rainbow Trout were randomly selected from each RSI at the conclusion of the study and measured for total length. Error bars are ±1 SD.

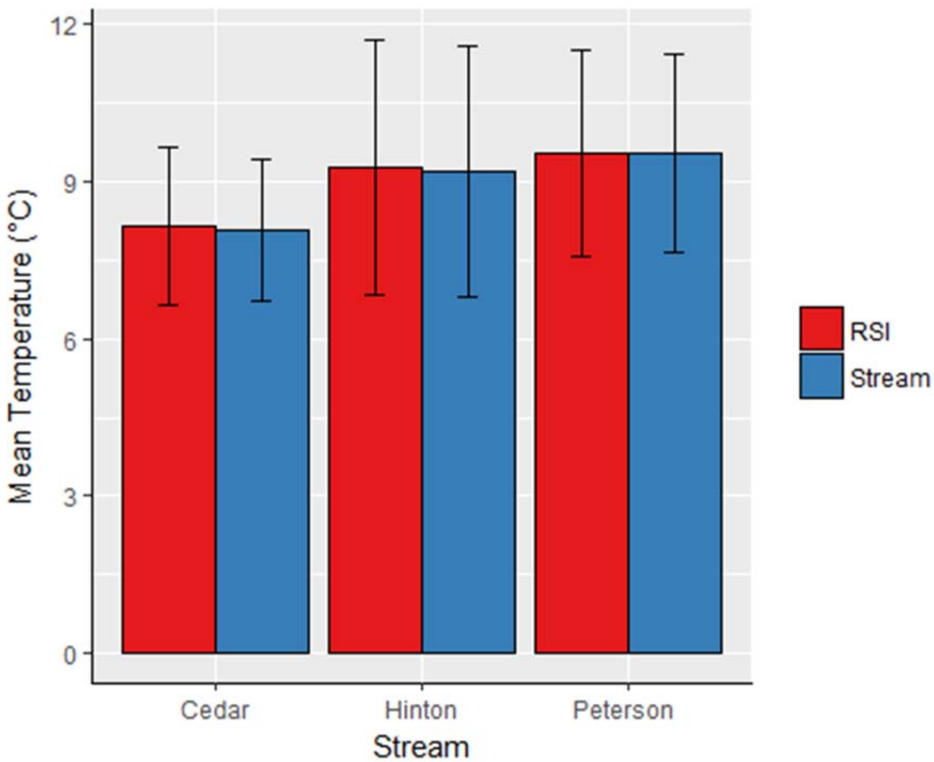

Figure 5. Comparison of mean hourly water temperature (˚C) inside a remote site incubator (RSI) to the stream. The water temperatures for RSIs were measured inside one single-tank RSI at Cedar Creek (*n* = 356 hourly measurements), one triple-tank RSI at Hinton Creek (*n* = 356; both stream and RSI measurements were made on the main branch), and the stock-tank RSI at Peterson Creek (*n* = 356). Error bars are ±1 SD.

Appendix A

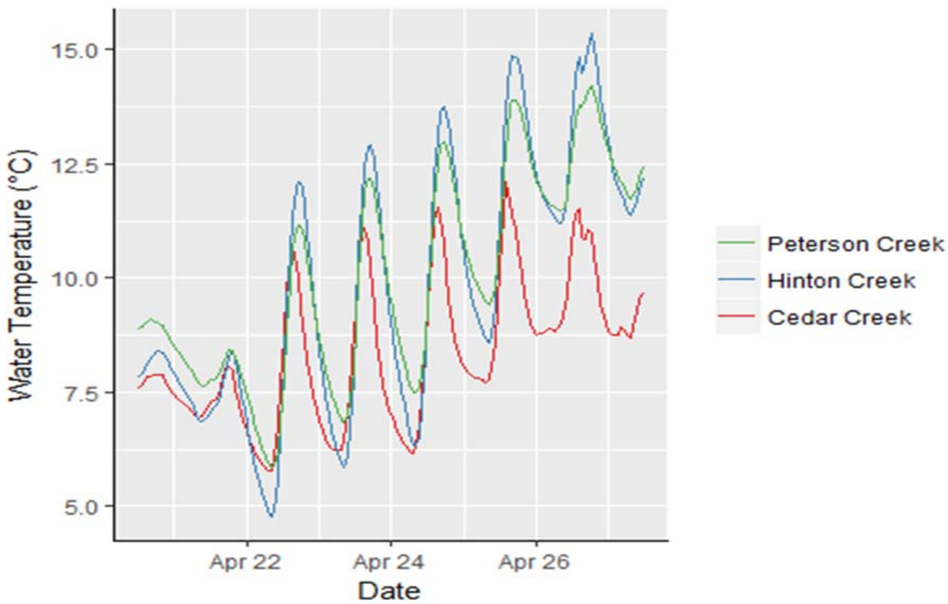

Figure A1. Hourly water temperatures measured in Cedar Creek, Hinton Creek (main branch), and Peterson Creek (20-27 April 2017).

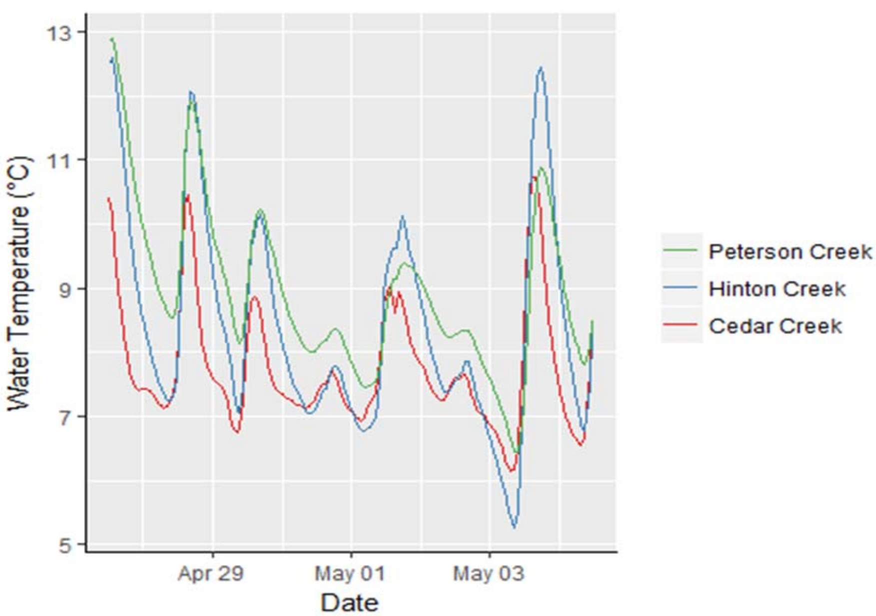

Figure A2. Hourly water temperature measured in Cedar Creek, Hinton Creek (main branch), and Peterson Creek (27 March-4 May 2017).

Appendix B

Constructing 5-gallon "bucket" remote site incubators (RSIs)

1. Cut bottom off a 5-gallon bucket (~1" from bottom of bucket) using a table saw.
2. Cut 12" × 12" sheet of stainless steel wire mesh.
3. Use propane torch to melt the T-304 stainless steel wire mesh (0.9 mm diameter wire, 1.65 mm opening width) onto the bottom of the bucket. Melt the screen onto the bottom of the bucket before cutting off the top of the bucket; leaving the top of the bucket on makes it rigid, so the egg tray is perfectly round and fits properly inside a 5-gallon bucket.
4. Use an angle grinder with metal cut off disc to remove the excess wire mesh on the edges of the egg tray.
5. Use a table saw to cut off the top of bucket so that the egg tray is the desired height (we used 4").
6. With a second 5-gallon bucket, make the hole for the water inflow 1.25-1.5" from the bottom of the bucket using a 1.25" hole saw.
7. Make the water outflow 1" from the top of the bucket opposite from the water inflow using a 1.25" hole saw. Make sure that the bucket cover can be attached to the bucket with the male and female fittings attached at the water outflow.
8. Cut 4 pieces of 1" PVC pipe to 3.5" and attach to a 1" cross (4 way) PVC fitting to construct the diffuser in the bottom of the RSI. Place end caps on three of the four pieces of PVC pipe in the 4-way PVC fitting. Drill desired number of holes in diffusor (Fig. B1J; we used 3/8" drill bit and drilled 7 holes total).
9. At the water outflow on the inside of the bucket, cut and grind (close to flush) one male PVC fitting so that the egg tray can slide past the outflow fitting (Fig. B1D, B1I, and B1J).

Note that we initially tried to form the bottom of egg trays with aluminum screen. However, the aluminum screen melted when using a propane torch to adhere the screen to the bucket. We switched to stainless steel mesh (even though it was significantly more expensive) because it had a higher heat resistance (i.e., unlike the aluminum screen the stainless steel mesh did not melt), resulting in a better egg tray.

For the construction of all RSIs and their components, the only PVC parts that were glued together were the manifold for the water inflow on the triple-tank RSIs. Throughout our field study, we had no issues with RSI components coming undone that were not glued.

Problems and solutions during the field study

We encountered a number of challenges during this pilot study. Below we detail a few of the many issues we encountered and offer solutions that we implemented or propose for future use.

When the triple-tank RSI was installed at the main branch of Hinton Creek, we assumed the stream was at base-flow because of high water clarity and no recent rain events. We placed the end of the water intake pipe close to the surface, assuming the stream was near base-flow.

Halfway through the pilot study we needed to move the end of the water intake pipe further upstream because of lower water levels, which resulted in reduced flow into the RSI. The middle "tank" of the triple-tank RSI had visually appeared to have high egg mortality, likely due to low flow and difficulties adjusting the flow to each "tank" attached to the manifold. Thus, adjustments to the water intake line may be necessary during the use of RSIs to maintain adequate flow through the RSIs depending on stream discharge.

We used both 1" and 2" diameter conduit for the water intake lines to RSIs. Overall, the 1" diameter conduit was easier to work with than the 2" diameter conduit; however, the 2" diameter conduit delivered a significant increase in flow (~4× given that the cross-sectional area of a pipe is $\pi \cdot r^2$, where $\pi \approx 3.14$ and r is the radius). At each of the three sites, the triple-tank RSI (which used a 2" water intake line reduced to 1" for each "tank") had more than adequate flow, requiring the valve at each "tank" to be partially closed to reduce flow. Single-tank RSIs (which used 1" water intake lines) had adequate flow at Cedar and Hinton creeks when the valves for the water intake lines were completely open. In Peterson Creek, some of the RSI valves needed to be partially closed due to higher stream gradient. The 2" diameter conduit is approximately twice as expensive (per linear foot) as the 1" diameter conduit (1" conduit was $3.42/10' piece vs. $6.71/10' piece for 2" conduit) and much more rigid than 1" diameter conduit, making 2" diameter conduit difficult to use when installing RSIs in stream sections that had many bends in the channel. If using 2" conduit as the water intake line, then it is very important to select a stream section where the channel is relatively straight. Additionally, carrying 2" diameter conduit is more cumbersome due to its diameter and increased weight compared to 1" diameter conduit. Thus, the benefit of increased flow in the water intake line using 2" diameter conduit has the disadvantages of increased cost, limited site selection (requiring relatively straight stream reaches), and increased effort to transport to the study site (when access requires hiking to the site).

Stand pipes usually were used at the valve on most of the RSIs (Fig. B1D, B1H, and B1K). For the single-tank and triple-tank RSIs, the stand pipes did not offer any great benefit during our study. However, on the stock-tank RSI, we were able to slowly drain the stock tank for cleaning by lowering the stand pipe, but this was only a small advantage because we could easily disconnect the water intake and open the valve on the bottom of the stock tank.

When installing water intake lines using 1" diameter conduit, air pockets often formed in the line, reducing the flow to the RSI. To avoid this problem, the 1" diameter conduit was fully submerged in the stream during the installation process, keeping the conduit full of water without any air bubbles. Additionally, we installed a stand pipe in the water intake line approximately every 40', which consisted on a PVC "T" fitting, 10-12" of 1" diameter PVC pipe, and an end cap with a hole drilled in it (Fig. B1C). Periodically during the installation we would cover the end of the conduit so that flowing water through the line would drive any air pockets out of the water intake line into the stand pipes.

Triple-tank RSIs were hard to keep level so that flow in and out of the RSIs was consistent among the three "tanks" installed in-parallel. This was often due to erosion under the buckets and settling. We struggled with adjusting the flow for all three "tanks" when they were not level.

In the future, we suggest using a level "table" or stand for the triple-tank RSIs (e.g., Fig. B1F), which would keep the RSIs level and at the same height as well as protect against erosion.

The manifold on the triple-tank RSIs made it difficult to adjust flow to each individual "tank" (Fig. B1H). When flow was increased to one "tank," the flow would decrease to another "tank." As sediment built up in the manifold, flow could become low for one "tank" among the three. This low flow likely reduced hatching success in the middle "tank" in the triple-tank RSI at Hinton Creek. A possible solution would be to use gate values on the manifold instead of ball valves (we used ball valves; see Fig. B1H). Gate valves might give more fine adjustment of flow for each "tank" in the RSI. However, we do not think there is a perfect solution to this problem with triple-tank RSIs, or any RSIs installed in-parallel using a manifold. Therefore, we see an advantage to using single-tank RSIs with a dedicated water intake line for each RSI. The advantage is being able to more easily adjust the flow to each single-tank RSI compared to each "tank" in a triple-tank RSI with a common intake manifold.

Fry that swam out of a RSI and into the collection bucket were often caught on the screens of the collection bucket (Fig. 1A-C). Screen surface area was too small on the collection buckets and would become clogged with sediment and debris, causing rapid flows through the areas of the screen that remained open. The rapid flow through the screens caused fry to become impinged on the screens in the collection buckets. To alleviate this challenge, we recommend increasing the surface area of screens on the collection buckets.

We recommend modifying the egg tray design for the stock tank to minimize the number of fry that can swim to the bottom of the RSI. In this study, we used a rectangular egg try (see Fig. 1E) in the stock tank (see Fig. B1K), which allowed Rainbow Trout fry to swim to the bottom of the RSI. Increasing the surface area of the egg tray in the stock-tank RSI should minimize this issue.

Our study site at Cedar Creek had very low stream gradient because it was so close to the headwaters. To achieve enough differential in height (from the water outflow on the RSI to the end of the water intake line) for adequate flow to each RSI, we built an impoundment in the stream to draw water for the intake lines to RSIs (Fig. B1M). This impoundment held up well and required minimal maintenance during the study because of relatively low discharge in Cedar Creek. However, there was a significant time commitment to construct and remove the impoundment as well as risk to the success of RSIs in the event that the impoundment failed.

Figure B1. Construction of egg trays: (A) 5-gallon buckets with bottom cut off (foreground) and buckets with stainless steel mesh melted on the bottom prior to trimming excess mesh (background), and (B) 5-gallon buckets with the stainless steel mesh trimmed around the edges; the final step (not shown) is to cut-off top bucket (about 4-5” above mesh bottom). (C) Stand pipes (lower right corner) in 1” diameter water intake line at Cedar Creek were used to remove air from the line; note that 2” diameter water intake line for triple-tank remote site incubators (RSIs) is shown on left. (D) A completed 5-gallon bucket RSI with egg train installed and standpipe on water intake line. (E) All hardware for single-tank and triple-tank RSIs, including RSI “tanks” with eggs trays (center), PVC manifold for triple-tank RSIs (left), and standpipes for each RSI (right). (F) RSIs in-parallel in Montana illustrating the use of a “table” that could be used for triple-tank RSIs (MFWP 2013).

Figure B1 (cont.). (H) Triple-tank RSI with egg trays at Hinton Creek. (I) Single-tank RSI with egg tray in Hinton Creek. (J) Single-tank RSI with egg tray removed to show “+” shaped diffusor made from 1” PVC pipe and fittings with 3/8” holes. Stock-tank RSI with (K) rectangular egg tray present and (L) with the egg tray removed to highlight diffusor made from 2” PVC pipe and fittings with 3/8” holes. (M) An impoundment was required at Cedar Creek in order to increase the difference in elevation between the end of the water intake and the RSI to establish sufficient flow.