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EVALUATING EMBRYO SURVIVAL OF SURROGATE FISH SPECIES USING MULTIPLE INCUBATORS: CONTINUING EFFORTS TOWARDS REINTRODUCING ARCTIC GRAYLING (THYMALLUS ARCTICUS) INTO LOWER MICHIGAN STREAMS

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By

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ABSTRACT

EVALUATING EMBRYO SURVIVAL OF SURROGATE FISH SPECIES USING MULTIPLE INCUBATORS: CONTINUING EFFORTS TOWARDS REINTRODUCING ARCTIC GRAYLING (*THYMALLUS ARCTICUS*) INTO LOWER MICHIGAN STREAMS

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Arctic Grayling *Thymallus arcticus* have been successfully reintroduced to the Upper Missouri River Basin of Montana using Remote Site Incubators (RSI). Widespread use of RSIs as part of reintroduction efforts in Michigan is challenged by low gradient streams, which limit deployment success. In this study, I evaluated the utility of an alternative instream rearing device, the Floating Basket Incubator (FBI), by directly comparing the survival of Rainbow Trout *Oncorhynchus mykiss*, Brook Trout *Salvelinus fontinalis*, and Walleye *Sander vitreus* (as Arctic Grayling surrogates) between RSI and FBI in stream and hatchery environments. In addition, I assessed how abiotic and biotic factors influenced survival for rainbow trout within FBIs among three natural streams. Overall, I found that mean survival of rainbow trout was higher than that of brook trout and walleye. My results suggested that FBIs performed similarly to RSIs in most experiments. Linear mixed effect model results suggested that survival of surrogate species was best explained by incubator type and stream location. The proportion of variation explained by the incubator type was low, relative to the random effect of stream location. My results suggest that although surrogates of Arctic Grayling sometimes had lower survival in FBI, the magnitude of this difference was small enough that FBIs should be considered as a tool when reintroducing Arctic Grayling.

DEDICATION

I’m grateful for my mother for her love and support. I’m grateful for my youngest sister for sharing common ground and understanding the pursuit of this career. I’m grateful for my oldest sister for setting the bar for success. I’m grateful for my aunties up north, your love and support has carried me through my entire life. I’m grateful for my father who instilled passion for the natural world. Most importantly, I’m grateful for my grandpa Jack for his love, patience and encouragement to pursue my education.

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This thesis follows the format prescribed by the North American Journal of Fisheries Management

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LIST OF ABBREVIATIONS

AIC: Akaike Information Criterion

ANOVA: Analysis of variance

ATU: Accumulated thermal units

df: Degrees of freedom

ESA: Environmental Species ACT

FBI: Floating Basket Incubator

LMM: Linear mixed-effects model

MAGI: Michigan Arctic Grayling Initiative

MIDNR: Michigan Department of Natural Resources

MSFH: Marquette State Fish Hatchery

LRBOI: Little River Band of Ottawa Indian

RSI: Remote Site Incubator

TSFH: Thompson State Fish Hatchery

Introduction

Salmonid populations native to North America have been impacted by anthropogenic stressors including habitat alteration, overfishing, and the introduction of non-native species (Beechie et al., 2001; Fausch, 2008; Hicks, 1991). Of North America's 80 salmonid species, including several subspecies of genus *Oncorhynchus*, 35 are listed as either threatened, endangered or extirpated under the Endangered Species Act (ESA) of 1973 (NANFA, 2022). Among the 35 salmonid species impacted is Arctic Grayling *Thymallus arcticus*, a species that currently exists within less than 5% of its native range in the United States (Kaya, 1992, Steed et al., 2010).

The historic native range of Arctic Grayling within North America included drainages of the Arctic and Pacific Ocean, upper portions of the Missouri River Basin, and the northern region of the Lower Peninsula of Michigan (Goble et al., 2018; Peterson and Arden, 2009; Vincent, 1962). Arctic Grayling populations from the Upper Missouri River and Lower Peninsula of Michigan were both glacial relics from the last Wisconsin glaciation (Peterson and Arden, 2009). Historical reports described Arctic Grayling in most cold-water streams of the Lower Peninsula of Michigan, encompassing a region north of the Rifle River to the east, and north of the White River to the west (Nuhfer, 1992). In addition, the Otter River within the Upper Peninsula of Michigan was known to support a small population of Grayling (Nuhfer, 1992; Vincent, 1962), although the origin of this population is uncertain and may have been stocked (G. Whelan, Michigan Department of Natural Resources, unpublished records, January 2023).

Various management strategies including stocking, translocation, and artificial rearing have been developed to support declining fish populations or reintroduce extirpated species

(Hayes and Banish, 2017; Kaeding and Boltz 2004; Nuhfer, 1992). For example, Nuhfer (1992) describes numerous stocking attempts to reintroduce Arctic Grayling to Michigan, which resulted in no evidence of natural reproduction and Grayling populations were not restored in Michigan. The lack of stocking success may be due to the disruption of olfactory imprinting occurring in hatchery environments (Keefer and Caudill, 2014; Stable,1984), which may lead to higher rates of emigration and lower probabilities of survival (Stable, 1984; Sundell et al. 1998). As a response, efforts to restore populations through instream rearing in natural streams have successfully been used to augment declining populations or reestablish formerly extirpated salmonids (Johnson et al., 2020; Kaeding and Boltz 2004).

Arctic Grayling in Michigan

The State of Michigan attempted multiple reintroduction efforts to rehabilitate Arctic Grayling populations, through stocking, from 1934-1941 (76,100 yearlings) and again in 1958-1960 (200,000 embryos and fry; Nuhfer, 1992). The largest stocking effort undertaken by the Michigan Department of Natural Resources (MIDNR) was between 1987 and 1991 where 250,000 Arctic Grayling yearlings and fry were stocked into lakes and streams throughout Michigan (Nuhfer, 1992). Reintroduction efforts failed to reestablish reproducing populations likely due to high mortality rates linked to predation, migration barriers, and no observed retention of spawning adults (Nuhfer,1992).

Despite failed stocking attempts recent interest among state, federal, and tribal agencies to re-establish Arctic Grayling to Lower Michigan rivers prompted research investigating alternative methods for reintroduction. A collaborative effort to reintroduce Arctic Grayling to Lower Michigan tributaries began in 2016, with the organization of the Michigan Arctic Grayling Initiative (MAGI). The MAGI consists of founding partners, the Little River Band of

Ottawa Indians (LRBOI) and Michigan Department of Natural Resources (MIDNR), and 45 additional organizations representing tribal, state and federal agencies (LRBOI, 2020). The Arctic Grayling was a culturally significant species for the LRBOI and their interest in reintroducing Grayling to the Manistee River watershed has led to further investigations of habitat within Big Manistee River tributaries and using remote site incubators (RSIs) as a tool for reintroduction (Gobel et al., 2021; LRBOI, 2020; Mock et al., 2021; Wilson, 2017). Moreover, because Grayling appear to exhibit a preference for familiar water sources (Watson 2024), development of incubators that enhance the ability for embryos to develop in stream environments targeted for reintroduction may enhance reintroduction efforts. Nufher (1992) speculated that rapid immigration from stocking locations by Grayling may have resulted from imprinting to hatchery water rather than natural stream waters.

A remote site incubator is a modified bucket that incubates fish embryos at the stream site by supplying continuous flow of stream water and reducing sedimentation (Kaeding and Boltz, 2004; Mock et al., 2021; Wilson, 2017). RSIs typically use a stream's natural gradient to generate sufficient head pressure to force water entering near the bottom of the bucket to flow up through a medium supporting the incubating embryos and exit near the top of the bucket (Kaeding and Boltz, 2004). Efforts to reestablish Arctic Grayling using RSIs in other native regions within the United States, including the Upper Missouri River Basin in Montana, have been successful (Kaeding and Boltz, 2004). RSI-based reintroductions of Arctic Grayling fry later resulted in observed spawning in Elk Spring Creek of Montana, the first successful spawning event there since the 1960's (Kaeding and Boltz, 2004). These results led to multiple investigations of the potential use of RSIs to raise Arctic Grayling and surrogate salmonid species (Mock et al., 2021; Wilson, 2017).

Stream Habitat in Michigan

Deployment of RSIs in Michigan waters is complicated by the low gradient of many groundwater-fed streams proposed for Grayling reintroduction (Goble et al., 2018) and a need for sufficient stream gradient to generate enough head pressure to ensure adequate and continuous flow through each RSI. Selecting sites with enough gradient for deployment is challenging throughout the northern Lower Peninsula of Michigan. Generally, stream gradients of 0.75-1.89 m per km were described where Grayling were typically found in Michigan streams, compared to stream gradients of up to 3.79 m per km in Montana streams inhabited by Grayling (Vincent, 1962). For example, a stream gradient of 0.55 m per km was described by Rozich (1998) for the mainstem of the Big Manistee River, where an abundant Grayling population formerly occurred. Comparatively, stream gradient measurements of 2.5-2.9 m per km were recorded for segments of the Big Hole River in MT, where Arctic Grayling have recently been reestablished (Liknes and Gould, 1987; McMichael, 1990). Although artificial dams or sand bag barriers were used to increase head pressure and reduce plumbing to supply RSIs in Montana (Kaeding & Boltz, 2004), this practice may not be authorized in streams of the Lower Peninsula of Michigan (MIDNR, personal communication).

I evaluated an alternative incubator known as a floating basket incubator (FBI), which was designed to alleviate potential issues associated with using RSIs in low gradient streams of the Lower Peninsula of Michigan. The FBI design is a flow-through system in which surface water supplies the embryos with dissolved oxygen, a potentially important design improvement for maintaining sufficient water supply in low gradient sections of streams. I suggest that the FBI

design will benefit incubation in low gradient habitats, removing channel gradient (i.e., hydraulic head) constraints required for successful incubation with RSIs.

Objectives

My research aims to inform fisheries managers of the potential utility of multiple types of instream incubators by evaluating the survival of multiple surrogate species in incubators to gain insight in their potential utility for the reintroductions of Arctic Grayling into streams of Michigan.

My first objective was to compare survival of Walleye, Rainbow Trout, and Brook Trout from eyed embryos to hatch between RSIs and FBIs. I predicted that there would be no significant difference in survival rates between the RSIs and FBIs when evaluating each species at each site.

My second objective was to determine the impact of abiotic factors on survival for Rainbow Trout. For this objective I examined how survival in FBIs varied across three natural streams and directly assessed how sediment accumulation, embryo movement, embryo distribution throughout the incubator (i.e., even distribution or embryo clumping), fungal coverage, surface velocity, and embryo stocking density influenced survival. I predicted that the percentage of survival for each species will decrease as sediment accumulation increases, as embryo movement increases, as fungal coverage increases, when embryo distribution was most even (or least clumped) and as velocity varies among incubators.

Methods

Study area

Hatchery experiments were conducted inside the Thompson State Fish Hatchery (TSFH) during the spring of 2022 and the Marquette State Fish Hatchery (MSFH) in Marquette, MI during the winter of 2022. Stream experiments were conducted at multiple locations during the spring of 2022 and 2023 including Cherry Creek, Cedar Creek and Big Creek, which are all tributaries within the Chocolay River watershed located near Marquette, MI (Table 1). These sites shared physical characteristics (e.g., seasonally stable groundwater-fed flows, suitable temperatures for salmonids, modest gradient, etc.) similar to other Michigan river systems considered suitable for reintroduction of Arctic Grayling (Auer et al., 2013; Danhoff, 2014, Goble et al., 2021). Although the natural channel of Cherry Creek at the experimental site was modified by the presence of the MSFH, this site was selected for the assessment of FBIs and RSIs based on accessibility and suitable flows for incubator deployment. In addition, channel modifications at MSFH (i.e., using dam boards at a stream diversion and at pond outlet) facilitated RSI installation and by limiting the amount of PVC plumbing needed to achieve adequate flow through RSIs.

Incubator Construction

Remote site incubators (RSI) and FBIs were designed and fabricated by the Michigan Department of Natural Resources (MIDNR) Marquette State Fish Hatchery and Marquette Fisheries Research Station staff. The construction of the FBIs included two PVC tubes (pontoons) that support a mesh lined basket that holds the embryos. Each pontoon was made with 10 cm (4 in) PVC pipe 104 cm (41 in) long, with each end capped to create a water tight

seal (Figure 1). The egg basket was constructed from thick grated aluminum sheeting that formed the framing to support the mesh insert. The aluminum sheeting was 0.15 cm (0.06 in) thick with slots that were 0.32 cm (0.125 in) wide and 1.2 cm (0.05 in) long. The mesh inserts of the egg basket were composed of commercial window screen mesh with an approximate mesh size of 0.08 cm (0.035 in). The dimensions for the baskets were 76 cm (30 in) in length, 25 cm (10 in) wide and 15 cm (6 in) deep. Two different FBI types were created to determine whether providing more dividers to the mesh baskets would embryo survival by limiting movement of embryos within the FBI. Separate embryo chambers were constructed for FBIs (3-cell and 12-cell) by installing sections of grated aluminum 6 cm (2.5 in) tall and 25cm (10 in) in length, perpendicular to the long-axis of the FBI (Figure 1). Three chamber baskets (FBI 3-cell) were created by inserting grated aluminum separators at approximately 1/3 and 2/3 of the bottom plane of the basket (Figure 1). Twelve chamber baskets (FBI 12-cell) were constructed by inserting additional grated aluminum barriers to the 3-chamber basket, diving each cell into fourths (Figure 1). The floating basket was assembled by fastening the PVC pontoons to the basket using 13 cm (5 in) hose clamps per PVC pontoon (Figure 1).

The RSIs were constructed following designs described by Kaeding and Boltz (2004) and Mock et al. (2021), but a finer mesh was used to create the egg tray to accommodate for walleye eggs. Individual RSIs were constructed from two 19 L (5-gal) buckets and internal piping to allow a continuous flow through system (Figure 2). The upstream bucket, described as the holding bucket, was a standard 19 L (5-gal) bucket equipped with an embryo tray and internal piping. Embryo trays were constructed of stainless-steel mesh which was fastened to the embryo tray frame (i.e., cylinder created from the bottom of a 19 L bucket) and inserted into the holding bucket. The holding bucket was modified to allow for water to percolate from underneath the

embryo tray, up through the holding bucket to the outflow pipe. The percolating apparatus (e.g. diffuser) was constructed from a cross-shaped PVC pipe capped on three ends, including 0.3 cm (1/8 in) holes along the top plane of the cross shaped section (Figure 1). An outflow pipe was installed to allow larval fish to swim out of the holding bucket into the "overflow" bucket, to avoid accidental release of larval fish. Overflow buckets were modified by cutting out four vertical sections 13 cm x 5cm (5 in x 2 in) of the side wall of the overflow bucket and replaced with mesh screen, to supply larval fish with fresh water. In addition, Jordan-Scotty incubators (commercially available) were also examined in this study but were deemed infeasible and results were not reported.

Experimental Design

Surrogate Species of Arctic Grayling

I conducted the experiment using surrogate species of Arctic Grayling to inform management of the efficacy of incubator types prior to reintroduction efforts including Arctic Grayling (Table 2). I chose surrogate species that were readily available and possessed life history characteristics (e.g., egg size, embryo size, spawning season) with varying degrees of similarity to those of Arctic grayling (Table 2). Embryos from Rainbow Trout *Oncorhynchus mykiss*, Brook Trout *Salvelinus fontinalis* and Walleye *Sander vitreus* were used as surrogate species to determine species-specific survival among incubator types (Table 2). Surrogate species are often used to obtain inference for species that are rare or imperiled (Amaral et al., 2015; Gloss and Wahl, 1983). Therefore, I believe that the selection of surrogate species to Arctic Grayling provided useful insight to the utility of incubators and the biological factors potentially impacting embryo survival. Use of surrogates can be appropriate because it reduces

the demand of species already imperiled and allows research to continue towards rehabilitation efforts for the species of interest.

Arctic Grayling spawning time varies among populations, but generally occurs in April through June as water temperatures reach 2-11°C, coinciding with spring run-off events (Armstrong, 1986; Stewart et al, 2007). Arctic Grayling egg size was reported to range 3-4 mm (post-fertilization) in diameter (de Bruyn and McCart, 1974; Northcote, 1993) and were reported to hatch at 130 accumulative thermal units (ATU) at 4 °C. Rainbow Trout spawning occurs in April through June as water temperatures reach 4-12°C, similar to Grayling, and embryo size range from 5.8 – 6.4 mm (Beacham et al. 2003; Dubois et al. 2011). Rainbow Trout were selected as a surrogate to Grayling due to the overlap in spawn timing and water temperatures required for developing embryos of both species. Brook Trout spawning occurs in October – November as water temperatures reach 8 – 12 °C and produce embryos with a size range between 5.2 – 5.5 mm that were described to hatch at 150 ATU at 3.3°C (Huddy and Wagner 2004; Torrans and Smith, 1970). Brook Trout were selected as a surrogate to Grayling based on similarities in ATU at similar water temperatures. Walleye spawning occurs in April – May as water temperatures reach 3.6 – 6.7 °C and produce embryos with a size range between 2 – 2.8 mm that were described to hatch at 145.8 ATU at 5.4 °C (Allen et al. 1997; Ivan et al., 2010; Malison and Held 1996). Walleye were selected as a surrogate to Grayling based on similarities in spawning time and temperate, embryo size and ATU at similar temperatures.

Embryo Request and Stocking

Walleye embryos were acquired in May of 2022 from Thompson State Fish Hatchery (TSFH) in Manistique, MI. Embryos were fertilized on April 22nd, in which gametes from 53 male and 53 female walleyes captured from Little Bay de Noc, Lake Michigan were mixed and

then pooled into egg jars and were incubated at 7.8 °C until the eyed-up stage (R. Espinoza, MIDNR, personal communication). Rainbow Trout embryos were acquired in May of 2022 and 2023 from TSFH. Embryos were fertilized on April 21 in 2022 and April 22 in 2023, in which gametes from feral males and females (1:1 ratio) captured from the Little Manistee River (a Lake Michigan tributary) were mixed and pooled within egg trays and incubated at 13.3 °C inside TSFH (R. Espinoza, MIDNR, personal communication). Brook Trout embryos were acquired in November of 2022 from Marquette State Fish Hatchery (MSFH) located in Harvey, MI. Brook Trout used in this experiment represent offspring from a captive Assinica strain broodstock originally sourced from Assinica Lake in Canada. Eggs were fertilized on November 1st, in which gametes from 87 males and 87 females were mixed and then pooled within egg trays inside the MSFH (T. Beck, MIDNR, personal communication).

For all experiments, embryos at the eyed stage were used based on embryo survival results from previous RSI research involving either Rainbow Trout or Arctic Grayling (Kaeding and Boltz 2004; Mock et al., 2021). Embryo densities for FBIs were determined by replicating densities previously used in RSI's as described by Mock et al. (2021), in which Rainbow Trout embryo density of 2.86 embryo/cm^2 was estimated to be approximately 1542 embryos per a 19-L RSI. The surface area of an FBI was approximately four times greater than that of an RSI. Embryo densities were consistent for Rainbow Trout among incubator types in 2022, but in 2023 six additional FBIs using reduced density of Rainbow Trout were used Cherry Creek experiments (Table 3)**.** Walleye embryo density of 25.38 embryo/cm^2 was used in RSIs and a density of 25.84 embryo/cm^2 was used in FBIs which was estimated to be 13,500 embryos per 19-L RSI and 54,000 embryos per FBIs (Table 3). Embryo densities for incubators including

Brook Trout in hatchery and stream experiments were reduced due to limited availability of embryos from the MSFH (Table 3).

Experiments in Hatcheries (Spring and Winter Trials)

In May of 2022 hatchery experiments were conducted at the Thompson State Fish Hatchery (TSFH). Three replicates of RSIs, FBI (3-cell) and FBI (12- cell) for Rainbow Trout and Walleye were deployed in separate raceways within the hatchery in which a random selection of FBI type per raceway was assigned (Table 3). In December of 2022 hatchery experiments were conducted at the Marquette State Fish Hatchery (MSFH) in Marquette, MI which involved only Brook Trout (Table 3). Three FBI (3-cell) were deployed in one raceway and 3 RSI's were deployed near the heath stacks within MSFH.

Stream Experiments

In May of 2022, Cherry Creek experiments consisted of a total of 18 incubators consisting of three replicates of the FBI (3-cell), FBI (12-cell), and RSI (Table 2) per Rainbow Trout and Walleye. In the December of 2022 experiments involving Brook Trout consisted of three replicates of the FBI (3-cell) and three RSI's. In the spring of 2023 experiments were conducted at three different locations including Big Creek, Cedar Creek and Cherry Creek with only Rainbow Trout (Table 3). A similar approach to the array of FBIs was used at all creeks, except the addition of 3 more replicates of FBI (3-cell) and FBI (12-cell) at Cherry Creek which included a lower density of Rainbow Trout embryos (Table 3).

In May of 2022, RSIs and FBIs were deployed in Cherry Creek. All FBIs were distributed downstream of the MSFH, in a large pool that was directly downstream from the outlet of the outdoor holding ponds of the hatchery. The position for each FBI was randomly

selected within the study site within a 3m X 10m section of stream. Incubators were adjusted by 0.5 m – 1.0 m to avoid interference with other incubators**.** FBIs were secured by tying the upstream end of the incubator to a metal stake inserted into the streambed. At each deployment location, I recorded the velocity (m/sec) using a flow meter at the upstream end of each FBI, at 60% of the water column depth, to determine the mean water column velocity. I recorded depth (cm) at 3 locations (front, middle, rear) adjacent to each FBI. RSIs were installed in the back-water channel where the overflow from the hatchery entrance diverts excess water**.** Approximately 140 meters of PVC pipe was installed from a head gate and run along the channel to supply an optimal discharge of 1- 3 L/min per RSI (Mock et al. 2021). In addition, three RSIs were placed at the outflow of the hatchery outdoor holding ponds. Approximately 10 m of PVC pipe was used to supply an optimal discharge of 1-3 L/min (Mock et al. 2021) to each of the RSIs. Discharge of flow through the RSI (L/min) was measured for each RSI at the start of each experiment.

Field Data Collection

During each experiment, I recorded a series of daily observations of each incubator including embryo distribution, percentage of dead embryos, percentage of embryos that contained fungus, embryo movement and sediment coverage. Each daily assessment was conducted by capturing photographs and recording visual estimates. I conducted visual estimates by visually estimating the proportion of the area within the incubator that included the factor of interest (i.e., 50% sedimented) for each cell. I then averaged the proportion of the three chambers or twelve chambers to determine the percent of coverage for the entire FBI. I visually estimated the proportion of the embryo basket of the FBI that was covered with embryos. I visually estimated the proportion of dead embryos to live embryos within each FBI. I visually estimated

the percentage of embryos moving from water current within each incubator and typical distance moved (in mm), and assigned an egg movement code using a 1–5 scale, with score and movement values as 1 (0 mm), 2 (>0 and <2 mm), 3 (2-5 mm), 4 (6-10 mm), and 5 indicates high movement (>10 mm). I visually estimated the proportion of embryos with fungus to the total embryos within each incubator. I visually estimated the proportion of embryos covered by organic matter and/or sediment to the total embryos within each incubator. In addition, 3 photos per FBIs (each third of basket), and 1 photo of each RSI were collected to quantitively determine percentages of sedimentation. Photos were consistently taken from same distance by stretching an 18" string from the camera to the incubator.

Image analysis

Photos collected during the field experiments were used in comparison with visual observations to determine the accuracy of visual estimates and address potential bias in estimation. Images of FBIs including rainbow trout from stream sites including Cherry Creek from 2022, Big Creek, Cedar Creek and Cherry Creek from 2023, at the midpoint of each experiment, were analyzed to determine percent of sedimentation within each incubator. I chose to analyze images at the midpoint of the experiment to capture the extent of sedimentation on the developing embryos prior to hatch. Each image represented one third of the FBI which was proportioned into fourths and randomly assigned a number (1-4) by a random number generator. The assigned fourth was then analyzed by digitizing the area of sedimentation using ImageJ software (v.1.54) (Rounds et al. 2023). Percent sedimentation was calculated by the area of the FBI that was covered with sediment in proportion to the total area of the FBI and multiplied by 100. Linear regression was used to determine the relationship between visual estimates and image analyses estimates, and a strong relationship occurred between estimates ($p<0.001$, $r^2 =$

0.77; Figure 3). Given the correspondence between the approaches I used the visual observation for evaluation of covariate effects on survival.

Continuous water temperature data was collected using HOBO temperature loggers for all study sites throughout the duration of the project. Temperature data was used to estimate accumulated temperature units (ATUs). ATUs were calculated as the sum of mean daily water temperatures from the date of fertilization to the end of the experiments (Danner, 2008). Mean daily water temperatures from electronic thermometers installed in hatchery raceways at TSFH and MSFH were used in addition to hobo temp logger data for calculating ATUs throughout the development of embryos for each species. Stream discharge (m^3/s) was measured at all stream study sites near the start of the experiments. Multiple measurements of discharge (m^3/s) were collected at Cherry Creek to determine a reference between stream discharge and gauge height (m), which was deployed by MIDNR staff at the MSFH study site.

Duration of experiment and ATUs

The duration of development from fertilization to the eyed stage of embryos varied among species and years. Walleye embryos were fertilized on 4/22/2022 and developed within TSFH to eyed embryos after 13 days at a constant hatchery temperature of 7.8 °C (ATU=101.5) (Table 6). I received and stocked Walleye embryos on 5/5/2022, after which Walleye embryos continued to develop in Cherry Creek for an additional 26 days (ATU=240) based on an average daily stream temperature of 8.8 °C.

Rainbow trout embryos were fertilized on 4/21/2022 and developed at TSFH to eyed embryos after 21 days at a constant hatchery temperature of 13.3 °C(ATU=279.3) (Table 6). I received and stocked Rainbow Trout embryos on 5/11/2022, after which Rainbow Trout eyed

embryos continued to develop in Cherry Creek for an additional 21 days (ATU=181.7) based on an average daily stream temperature of 8.9°C (Table 6).

In 2023, Rainbow Trout embryos were fertilized on 4/22/2023, and developed at TSFH to eyed embryos after 12 days at a constant hatchery temperature of 13.3(ATU=156). I received and stocked Rainbow Trout embryos on 5/5/2023, after which Rainbow Trout embryos continued to develop in Cherry Creek (18 days, ATU=143.7), Cedar Creek (24 days, ATU=202) and Big Creek (24 days, ATU=211) (Table 6.).

Brook Trout embryos were fertilized on 11/1/2022 and developed at MSFH to eyed embryos after 50 days at an average hatchery temperature of 5.0°C (ATU =251.5). I received and stocked Brook Trout embryos on 12/20/2022, after which Brook Trout embryos continued to develop in Cherry Creek until 1/15/2023 (27 days, ATU=135.8) (Table 6).

At the conclusion of each experiment, larval fish were euthanized using tricaine methanesulfonate (MS-222) and preserved in 95% ethanol (Mock et al., 2021). All fish handling and preserving methods followed the IACUC protocol (IACUC # 428).

Larval Fish Sampling Methods

To determine embryo to hatch survival, I counted all hatched larva individually and developed taxa specific methods to prepare samples. For larval Rainbow Trout and Brook Trout samples, I drained off excess 95% ethanol through a sieve stack, removed debris and counted hatched individuals to determine survival estimates. Walleye samples were prepared for counting by draining off excess 95% ethanol through a sieve stack and removing debris. Then, I prepared a sugar-based solution by mixing 200g (~4 cups) of granulated sugar per 3.78 L (~1 gallon) of distilled water, to facilitate the separation of larval walleye from other organic material (Foth et

al., 2012). After the larval sample was mixed with the sugar solution, the suspended portion of the solution containing Walleye larvae and other organisms was collected. This process was repeated several times for each sample to ensure all larval Walleye were included in the counting process.

Statistical Analysis

Embryo survival was calculated by taking the proportion of the total number of hatched larvae by the initial embryo count, and multiplying that ratio by 100 to calculate survival as a percent (Kaeding and Boltz, 2004). Embryo survival was reported as the mean percent of survival $\pm$ 95% confidence intervals. I used a one-way analysis of variance (ANOVA) to test whether mean embryo survival for each species differed among incubator types ($\alpha \leq .05$) (Kaeding and Boltz, 2004). An arc-sine square root transformation was used to normalize the survival data to meet the assumptions of normality and variance for analyses (Ivan et al., 2009; Nagler et al., 1999; Zorn et al., 2020). A Shapiro-Wilk test was used to assess the assumption of normality. A Leven's test was used to assess the assumption of equal variance. All statistical analyses were performed with the R programming language and computing environment (R Core Team 2022).

I modeled the effect of abiotic factors including embryo density, velocity and average depth of incubator and biotic factors including embryo fungus and sedimentation using a linear mixed model (Gerig et al., 2018; Kallio-Nyberg et al., 2007). Only FBIs including Rainbow Trout from 2022 and 2023 experiments at Cherry Creek, Big Creek and Cedar Creek were used for determining the effect of covariates on survival because this group provided the most replicates per site per species. I adopted an information theoretic approach to assess which

covariates best fit the data (Gerig et al., 2018; Kallio-Nyberg et al., 2007). The statistical model of the linear mixed effects model (LMM) was:

$$Y_{ij}=\beta_0+\beta_1 X_{ij}+b_{0i}+\varepsilon_{ij}$$

in which Y_{ij} is the response variable (i.e., survival) for the *i*-th observation in the *j*-th group, β0 is the fixed intercept, β1 is the fixed effect coefficient for the predictor X (i.e., sedimentation), X_{ij} is the value of the predictor X for the *i*-th observation in the *j*-th group, b_{0i} is the random intercept for the *i*-th group, and ε_{ij} is the error term which represents the unexplained variability. All models were fit using the lme4 package in R (version 4.2.1)

Results

Survival of Surrogate Species

Survival of Rainbow Trout 2022

In 2022, survival of Rainbow Trout was similar between RSIs and FBIs at Cherry Creek and FBIs performed better than RSIs at Thompson State Fish Hatchery (TSFH). (Figure 4). At Cherry Creek, mean survival of Rainbow Trout was not significantly different among incubator types ($F_{(2,6)}$ = 4.166 , p = 0.073) (Figure 4). Overall, mean percent survival (± CI) was 78.9±9.2% for FBI (3-cell), 76.0 ± 4.6% for FBI (12-cell), and 86.3 ± 16.4 % for RSI (Table 4). At TFSH, mean survival differed among incubator types ($F_{(2,6)}$ = 7.643, p = 0.022). Mean percent survival (± CI) was 95.8 ± 3.8 % for FBI (3-cell) which was higher than observed for FBI (12-cell) (p = 0.562) or RSI (p=0.02). Overall, mean percent survival (± CI) was for 95.8 ± 3.8 % FBI (3-cell), 93.3 ± 1.6% for FBI (12-cell), and 86.3 ± 20.6 % for RSI (Table 4).

Survival of Rainbow Trout 2023

Similar to 2022, survival of Rainbow Trout varied between sites and among incubator types, although survival was higher in RSIs than FBIs at Cherry Creek (Figure 5). Overall, mean percent survival (± CI) ranged from 53.1 – 57.2% at Big Creek, 71.2 – 77.7% at Cedar Creek and 66.1 – 81.4% at Cherry Creek (Table 4). At Cherry Creek, mean percent survival (± CI) of Rainbow Trout was significantly different among incubator types ($F_{(2,15)}$ = 21.3, p=<0.001). Mean survival was 82.4 ± 2.5% for RSI which was 9.7% and 16.3% higher than observed for FBI (3-cell) (p=0.002) or FBI (12-cell) (p<0.001) (Table 4). Mean survival of rainbow trout at Big Creek (BC) and Cedar Creek (CC) were not significantly different between incubator types including only FBI (3-cell) and FBI (12-cell) (BC: $F_{(1,4)}$ =0.178 , p= 0.694; CC: $F_{(1,4)}$ = 3.458 , p= 0.137).

Survival of Walleye 2022

In 2022, at TSFH, mean percent survival (± CI) of Walleye ranged from 1.2-2.2% and was not significantly different among incubator types, ($F_{(2,6)}$=1.051, p=0.406) (Table 4). At Cherry Creek, mean percent survival (± CI) of Walleye ranged from 0.3-1.2% and was not significantly different among incubator types, ($F_{(2,5)}$=3.208 , p=0.127) (Table 4). Overall, mean percent survival (± CI) of Walleye was very low (0.3±0.4%-2.2±2.1%) compared to Rainbow Trout (76.0±4.6-95.8±3.8) and Brook Trout (23.7±9.2-92.1±0.4) (Table 4), and varied between sites and among incubator types (Figure 6).

Survival of Brook Trout 2023

In 2023, mean survival of Brook Trout varied between sites and among incubator types (Table 4). At Cherry Creek, mean percent survival (± CI) ranged from 42.0-50.2% and was

significantly different between incubator types ($F_{(1,4)}$ = 15.89 , p=0.016) (Table 4). Mean percent survival (± CI) was 50.2 ± 1.9% for RSI which was 8.2% higher than observed for FBI (12-cell) (p=0.016) (Figure 7). At MSFH, Brook Trout trials were evaluated based on percent hatch due to unexplained variation in development between RSIs and FBIs. At MSFH, mean percent hatch (± CI) ranged from 23.7–92.1% and was significantly different between incubator types ($F_{(1,4)}$ = 918.7 , p<0.0001) (Table 4). Mean percent hatch (± CI) was 92.1 ± 0.40 % for FBI (12-cell) which was higher than observed for RSIs (p<0.0001) (Figure 8).

Abiotic and Biotic Covariate Results

Mean percent survival of Rainbow Trout in 2022 and 2023 stream experiments was best explained by incubator type (fixed effect) and stream location (random effect) (Table 5). This model received an AIC weight of (AIC_{wi}=0.735). The proportion of variance explained by the top ranked model included a small proportion of variance explained by incubator type (fixed effect) (marginal R^2=0.05) and a much larger proportion by stream location (random effect) (conditional R^2=0.71). The second ranked AIC_c included percent embryo coverage (fixed effect) and stream location (random effect) which received an AIC weight of (AIC_{wi}=0.173) (Table 5). Similar to the top ranked model the random effect of location explained a much higher proportion of variance (conditional R^2=0.66) than the fixed effect of percent embryo coverage (marginal R^2=0.06). Overall, models with a delta AIC_c < 10 suggested very low influence from other fixed effects, indicating other covariates we examined did not significantly affect mean percent survival of rainbow trout. A large proportion of variation was explained by the stream location in all models, conditional R^2 (0.64-0.71).

Discussion

Grayling were once a prominent species native to lower Michigan and are still considered an important cultural species of the Little River Band of Ottawa Indians of Lower Michigan and many other stakeholder groups in Michigan (LRBOI, 2022). Efforts to reintroduce Artic Grayling through the Michigan Arctic Grayling Initiative has led to a series of studies that directly inform reintroduction efforts of Arctic Grayling in Michigan (Goble et al., 2018; Goble et al. 2021; Mock et al., 2021; Watson, 2024). My research sought to inform ongoing restoration efforts by determining the efficacy of using a low-cost, easily deployed incubator known as the floating basket incubator (FBIs) as an alternative to remote site incubators (RSIs). Instream rearing via RSIs is thought to have been critical to the successful reintroduction of Grayling to the Upper Missouri River basin in Montana (Kaeding and Boltz, 2004). However, due to geomorphic differences between the Upper Missouri River and lower Michigan tributaries (Vincent,1962), RSIs may be less suitable and more vulnerable to complications (Mock et al., 2021). For instance, RSIs function best in high gradient streams that provide adequate head pressure and increased water flow which helps maintain sufficient oxygenation and reduces sedimentation for developing embryos (Kaeding and Boltz 2004). In my study, RSIs were installed to take advantage of channel modifications of Cherry Creek at the Marquette State Fish Hatchery, which provided enough gradient for sufficient head pressure to generate adequate flow through each RSI (1.8 – 2.8 L/min). Without modifications to Cherry Creek for the hatchery, considerably more PVC plumbing would have been needed to longitudinally capture enough hydraulic head to achieve target flows through an RSI. For example, within the reach of Cherry Creek immediately upstream of the hatchery having a average gradient of 2.7 m per km (a value likely higher than most Michigan streams being considered for Arctic grayling reintroduction), I estimated that 226 m of PVC pipe would have to be installed to supply an RSI with adequate

water flow. For lower gradient reaches being considered for reintroductions, plumbing RSI's using the natural gradient of the channel would require even more PVC pipe and considerable labor for set-up (e.g., an entire day for a crew to set up an RSI versus 5 minutes for one person to deploy an FBI). Even if plumbed to achieve perfect hydraulic efficiency, such RSI's would be vulnerable to high or low flow events, accidental plugging of PVC pipe, and disturbance or other events that compromise the amount and reliability of flow through the system, putting fish eggs and fry at risk.

Comparison to previous studies

My research showed that Rainbow Trout embryos reared in FBIs had comparable survival to those reared in RSIs, suggesting no clear benefit of using RSIs in low-gradient Michigan streams. The range of survival observed in my study ranged from 53-96% in FBIs. These findings are higher than previous research using RSIs in streams with relatively high gradients compared to other Lower Michigan streams (Mock et al. 2021). For example, Mock et al. (2021) found that Rainbow Trout embryo survival in RSI's was 42% and 54% from eyed embryo stage to hatch. On average survival of Rainbow Trout in FBIs was 20-32% higher than what Mock et al., (2021) reported for RSIs. Brook Trout mean percent survival in FBIs and RSIs at Cherry Creek was low (42.0-50.2%) compared to 96-98% survival described by Hutchings (1991).

Brook Trout mean percent survival at MSFH was high (23.7-92.1%) compared to Brook Trout mean percent survival at Cherry Creek (42.0-50.2%), but exhibited greater variation. One factor contributing to the variation in survival may have related to when the experiment was terminated. All incubator experiments at the Marquette State Fish Hatchery were terminated when approximately 100% of embryos of Brook Trout visibly hatched in FBIs. Although

development of Brook Trout within RSIs were visibly delayed, RSIs were terminated at the same time as FBI to maintain consistency of duration of development based on ATU's. Therefore, I evaluated survival of Brook Trout in the MSFH as percent hatched instead of percent survival. Differences in survival of Brook Trout between incubator types were not evident since embryos were selected from the same batch and were incubated at the same constant temperature in the Marquette State Fish Hatchery.

Life history characteristics influence on observed survival

Life history strategies are the approach by which organisms achieve the maximum likelihood of reproductive success while overcoming environmental, behavioral and physical constraints (Stearns, 1992). Embryo survival is influenced by multiple factors including maternal effects, origin of parental genetics (wild vs. hatchery) and environmental conditions experienced by maturing adults (Johnston et al., 2008; McDermid et al., 2010; Nagler et al., 2000). In addition, differences in life histories such as size and age at maturity, embryo size and parental fecundity were shown to influence the likelihood of embryo survival (Hutchings, 1991; Quinn et al., 2011). Variations of these life history traits of individuals used to produce embryos for this experiment were not pre-determined in this study, and were likely factors influencing survival within species specific experiments and across all experiments. Although the purpose of this study was not intended to determine the effect of pre-existing factors which likely influence embryo survival, I observed some of effects of these factors in my study.

Hatchery strains have been found to have lower survival and overall fitness throughout development compared to wild strains (McDermid et al., 2010). Rainbow Trout embryos used in my experiment were collected from a wild or naturalized population from a Lake Michigan tributary, compared to Brook Trout embryos that were collected from a hatchery brood stock.

When comparing survival of both species within Cherry Creek, I observed higher survival of Rainbow Trout than Brook Trout. Although survival differences of these species are likely due to multiple life history differences, paternal genetics were likely also influencing survival (Milot et al., 2013).

Parental fecundity and size of embryos have shown to influence embryo survival in salmonids and Walleye (Harvey and Hood, 1996; Hutchings, 1991; Quinn et al., 2011). Overall, survival of species used in this study decreased in relation to embryo size, with observed survival highest for Rainbow Trout and lowest for Walleye. These findings are in line with previous studies (Beacham et al., 2003; Hutchings, 1991; Kratt & Smith, 1977; Ivan et al., 2010; Torrans and Smith, 1970). Life history traits of Rainbow Trout (i.e., size at maturity) were described to select for larger embryos than other smaller salmonids including Brook Trout and Grayling (Quinn et al., 2011; Cooper and Wydoski 1966; Kratt and Smith 1977). In addition, average fecundity of Arctic Grayling can range from 4,077 to 14,429 with a mean fecundity of 8,968 (de Bruyn and McCart, 1974) and embryo sizes range from 3-4mm (Kratt and Smith, 1997). I hypothesize that Rainbow Trout used as a surrogate species of Arctic Grayling would result in higher mean survival to hatch than Arctic Grayling based on differing investments related to embryo size. I suggest that Arctic Grayling survival using FBI's would reflect that of previous outcomes using RSIs (average survival of 44.8%), and could potentially result in higher survival by minimizing complications of RSI operations due to sediment plugging the incubator (Kaeding and Boltz, 2004).

Survival of surrogate species embryos varied among species and incubator types which was likely due to embryo condition and incubator type. Compared to Rainbow or Brook Trout, Walleye survival was extremely poor at both Cherry Creek and Thompson State Fish Hatchery.

That walleye survival was lower compared to Rainbow Trout or Brook Trout is not surprising given known differences in reproductive investment based on life history attributes (Beacham et al., 2003; Torrens and Smith, 1970; Malison and Held, 1996). Walleye have higher fecundity, lower embryo provisioning, and a shorter developmental period (Beacham et al., 2003; Torrens and Smith, 1970; Malison and Held, 1996) compared to Rainbow or Brook Trout. Compared to previous studies (Ivan et al., 2010, Suedel et al., 2012), my estimates of Walleye survival were much lower than other incubator studies. For example, Walleye survival in Ivan et al. (2010) ranged from 24- 31% at 5.4°C and 49.5% at 7.8°C. Comparatively, survival of Walleye embryos artificially fertilized from wild brood stock ranged from 27 – 95% depending on embryo quality, and was commonly aided by formalin treatments to reduce fungal infections (Harvey & Hood., 1996). Walleye egg incubating operations at TSFH involve daily formalin treatments for fungus prior to my experiments (R. Espinoza, MDNR Fisheries Division personal communication), therefore it was no surprise that fungus became an issue after experiments began without formalin treatments. Based on visual observations initial Walleye embryo mortality ranged from 35-40% in Cherry Creek and 30-60% at TSFH. This finding is within the range of expected embryo mortality post fertilization in natural settings (Johnson, 1961). However, I observed extensive fungal growth (>90% coverage of embryos) in all incubators across locations. Fungus infections of *Saprolegnia* sp. are a common cause of mortality among freshwater fish embryos in aquaculture (Gaikowski et al., 2003; Heikkinen et al., 2013; Hussein et al., 2001). Fungal infection causes embryo mortality in part by suffocating the embryo with hyphae, and mortality rates of live embryos infected from dead embryos containing fungus can occur rapidly (Gaikowski et al., 2003; Thoen et al., 2011). The widespread fungal colonization may have been facilitated by the density of Walleye embryos stocked into RSI and FBIs. Increased density of

embryos may have provided additional substrate for the fungus to spread (Heidinger et al., 1997). Given the widespread fungal infection, preventative treatments on inlet water supply using peroxide (H_20_2) and ultra violet irradiation may greatly reduce fungal infections (Heikkinen et al., 2013). Based on previous studies involving salmonids and Walleye, I would expect fungus to have less of an effect on embryo mortality of Arctic Grayling than that of Walleye (Gaikowski et al., 2003; Harvey & Hood., 1996, Mock et al., 2021). For instance, Gaikowski et al. (2003) described survival of treated Walleye embryos to be 55% and untreated to be 11%. In comparison, Mock et al, (2021) did not report a significant difference in survival among picked or unpicked incubators while raising Rainbow Trout.

Environmental controls on embryo to hatch survival

My analysis of covariate effects on Rainbow Trout survival was surprising and resulted in location explaining a much higher proportion of variation than any of the abiotic factors including sedimentation, velocity and depth in relation to FBI that were tested. In comparison, while their study did not test for additional environmental factors, Kaeding and Boltz (2004) also showed that most variation in survival was explained by year and site. While past studies have found that sedimentation increased mortality by suffocating developing embryos in gravel during the stages of eyed-embryos up to hatch (Hicks et al., 1991; Kirkland, 2012), I did not observe a consistent effect. Although sedimentation was not identified as having significant effect on survival, I suggest that qualifying organic material covering embryos as sediment may have masked the detection of the effect of sedimentation on the survival of embryos in this study. For example, I found that FBI within Cedar Creek and Big Creek had obvious accumulation of silt, typically in the upstream portion of the incubator, along with organic matter covering a portion

of the embryos. Although FBI at Cherry Creek mostly accumulated organic matter, the degree of sedimentation was equal among sites.

I did not identify significant relationships between environmental covariates and embryo to hatch survival. This was surprising because past studies suggest that survival could vary as a result of site-specific environmental differences such as stream temperature, dissolved oxygen, sedimentation, or stream velocity (Cunjak et al., 1998; Johnston et al., 2000; Scrimgeour et al., 1994). Differences in survival outcomes between my study and past research may have resulted from multiple factors. First, developing embryos incubated above the sediment surface rather than on the streambed. As a result, embryos did not directly interact with stream sediment and may have experienced less bed movement and experienced lower sediment deposition (Kirkland, 2012). Importantly, dye tests using prototypes of FBIs showed that stream flow entered the incubator from any direction, facilitating oxygen delivery to eggs, even under low streamflow conditions (T. Zorn, MIDNR, personal communication). Second, there are constraints to effective locations to deploy FBIs, including sections of streams subject to high velocities during the spring in which wading is unsafe and deployment was unfeasible. Velocity measurements recorded during deployment of each FBI showed relatively higher average velocities (m/s) at Big Creek (0.26) than Cedar Creek (0.21) or Cherry Creek (0.08, 0.18) in 2022 and 2023, respectively. Deployment of FBIs was restricted to small area of Big Creek in which incubators uniformly experienced similar velocities. Thus, my survival estimates may be more sensitive to reach scale differences than microhabitat differences associated with the location of where an incubator was deployed.

Previous studies suggest that the chemical and biological properties of river segments may have an effect on embryo survival in relation to water quality within areas of embryo

deposition (Lavery and Cunjak 2018). However, in my study substantial differences in water quality among sites was unlikely since Cherry Creek, Big Creek, and Cedar Creek all arise from a cold groundwater source associated with a large glacial outwash plain (Baker, 2006; Premo, 1999). In addition, I could not separate out site versus experiment effects. It is possible that differences in how the experiment was conducted (different condition of parentages between years, fertilization rate, embryo handling and acclimation prior to deployment) may have contributed to variation in ways that are difficult to control for and directly measure.

Comparison of FBI and RSI deployment with reference to habitat requirement of Grayling fry

I could not identify specific habitat variables that limited performance of FBIs in this study, but it is important to consider the habitat requirements of Grayling to inform incubator deployments. Habitat selection by Arctic Grayling varies throughout their development from fry to adults (Goble et al., 2021; Northcote, 1993). Grayling have been described to primarily spawn in water depths <1m and velocities less than 1.5 m/s (Stewart et al., 2007). Following hatch, emerging fry utilize back water channels and slow velocity areas for refuge and feeding until further developing into juveniles (Northcote, 1993). In addition, major flooding or scouring events have been found to be detrimental to survival of Grayling fry (de Bruyn and McCart 1974; Armstrong 1986.). Larval refuge areas are likely characterized by localized low stream velocities and gradients, areas where successful deployment of RSIs could be challenging. I suggest that the FBI is a more versatile incubator than RSI in terms of deployment location, and that areas suitable for emerging Grayling fry will be more accessible using the FBI.

Incubator deployment practices and maintenance

Previous RSI studies showed an effect on embryo survival by routinely removing dead embryos from incubators (Kaeding and Boltz, 2004; Mock et al. 2021). For example, Mock et al. (2021) found that the removal of dead embryos benefited survival when flow through RSIs was < 0.3 L/min and that highest survival was related to optimal flow (>3 L/min) through RSIs. Comparatively, Kaeding and Boltz (2004) described relatively lower survival of Arctic Grayling in RSI's that were not routinely cleaned by picking dead embryos. Although my study did not involve removal of dead embryos, survival of Rainbow Trout in FBI's was comparable to RSIs and survival may have increased further if dead embryos and fungus were removed.

Throughout this study, routine maintenance of RSIs was necessary for proper function. For example, during RSI experiments in Cherry Creek for 2022-2023 on multiple occasions water flow through the RSI was disrupted by organic material and sediment build up. I hypothesize that RSIs are more likely to fail due to sedimentation decreasing the flow through the RSI, instead of the sedimentation directly affecting the development of embryos. Both incubator designs facilitate dissolved oxygen transfer to the embryo through the bottom plane of the incubator, potentially minimizing the impact of sedimentation occurring on the surface of the embryos. In addition, another common malfunction of the RSI was the accumulation of an air pocket underneath the embryo tray mesh, which without daily removal would likely have resulted in lower survival for surrogate species involved. By comparison, in FBIs neither sediment nor trapped air seemed to be affecting performance of the incubator or embryo survival. This is likely due to the ability of the FBI to move and adjust to changes in surface current, potentially minimizing trapped air and deposition of suspended sediments. In addition, FBIs did not require extra maintenance (i.e., sediment flushing) to perform effectively.

Cost comparison of RSI and FBI

Throughout this study noticeable differences were observed in the amount of time and material involved in deployment between the RSI and the FBI. The construction of RSI's includes the instillation of PVC pipe to transport water to the incubators maintaining a suggested flow of 1-3 L/min through the system (Mock et al., 2021). In the Cherry Creek experiment sufficient flow through RSI was facilitated by taking advantage of an abnormal increases in stream gradient (i.e., using dam boards at a stream diversion and at pond outlet) due to the infrastructure of the Marquette State Fish Hatchery. As a result, this reduced the amount of PVC piping to 140 meters and the construction of the RSI assembly to approximately 1-3 hours for a 2-person crew. The initial time invested to construct one FBI was approximately 2 hours (MIDNR, Troy Zorn) and deployment occurred in less than 10 minutes per FBI. In a normal field setting (Mock et al. 2021), it took a crew of 3-4 people an 8-hour day to set up RSIs at a single field location, and crews of 6 people were needed to bring all necessary materials to start the construction of the project (i.e., sand bags to construct a dam, PVC plumbing and RSIs) (A. Mock, personal communication). I suggest the benefits to the total time invested per FBI will greatly increase as deployments increase and occur over multiple years in comparison with that of RSIs.

Carrying capacity differences between FBI and RSI

In 2023 experiments high and low densities of Rainbow Trout were tested in FBIs, which resulted in no significant difference in survival. The maximum number of Rainbow Trout embryos deployed (6,300 per FBI), was equivalent to approximately 3 RSIs. Although Arctic Grayling embryos were unavailable for this study, I suggest that the number of Arctic Grayling embryos (1,490- 10,520) per RSI used by Kaeding and Boltz (2004) would be suitable. In relation to FBI's, I suggest that an upper limit of 45,000 Arctic Grayling embryos be used per

FBI. In addition, I deployed a maximum of 12 FBI's within a 3m x 10m area in Cherry Creek, which in comparison hypothetically equated to 36 RSI's.

My study suggests that FBIs could be a useful tool for the reintroduction of Arctic Grayling in Michigan streams. My recommendations are based on comparisons of previous incubator studies conducted in Michigan (Mock et al. 2021) and Montana (Kaeding and Boltz, 2004). Through this study I determined that FBIs perform comparably to RSIs in hatchery and stream environments using three different fish species. I found stream-specific characteristics were more likely influencing survival of Rainbow Trout, which suggests that further investigations of FBI deployment in varying stream environments could help determine optimal reintroduction locations. Given the generally low gradient nature of streams identified as potential reintroduction areas in the Lower Peninsula of Michigan (Zorn et al., 2023), FBIs are a practical tool to deploy and facilitate the incubation of Arctic Grayling in such environments. As the magnitude of reintroduction efforts of Arctic Grayling increases, the utility of FBI will increase efficiency by reducing cost of materials and labor, in addition to increasing the opportunity to deploy incubators in optimal locations for the development of Arctic Grayling. Given the simplicity and reliability of the FBI, this incubator design may prove useful for instream incubation and reintroductions of other species in low-velocity stream environments.

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Table 1. Stream characteristics of experiment sites within tributaries of the Chocolay River

Site	Latitude (°N)	Longitude (°W)	Year	Mean Daily Temp (°C)	Mean Velocity (m/s)	Mean Depth (cm)
Cherry Creek	46.469109	-87.357476	2022	8.78 ± 0.81	0.18 ± 0.10	45.91 ± 12.38
			2023	8.51 ± 0.52	0.08 ± 0.04	59.08 ± 8.54
Big Creek	46.462587	-87.317239	2023	9.91 ± 0.89	0.26 ± 0.08	56.2 ± 9.93
Cedar Creek	46.462727	-87.34776	2023	8.83± 0.77	0.21 ± 0.06	38.8 ± 7.04

Table 2. Life history characteristics of surrogate species for comparison to Arctic Grayling.

Species	Spawn Timing @ Temperature ˚C	Incubation Period (ATU)	Embryo Size (Post fertilization)	Fecundity Embryos /Female	Reference
Arctic Grayling (*Thymallus* arcticus**)**	Apr.-Jun. 2-11˚C	130 @ 4˚C 250 @ 7˚C	3 – 4 mm	4,077-14,429	Kratt & Smith (1977), de Bruyn and McCart (1974)
Rainbow Trout (*Oncorhynchus* mykiss)	Apr.-Jun. 4-12˚C	213 @ 4˚C 440 @ 10˚C	5.8-6.4mm	3,000-6,900	Beacham et al. (2003), Pearson and Lantis (1978), Dubois et al. (2011)
Brook Trout (*Salvelinus* fontinalis)	Oct. – Nov. 8-12˚C	150-280 @ 3.3˚C	5.2-5.5mm	1,277-2,376	Torrans and Smith (1970), Hudy and Wagner (2004), Johnston and Mckenna (1976)
Walleye (*Sander* vitrues)	Apr. - May 3.6-6.7 ˚C	145.8 @ 5.4˚C 179.4 @ 7.8 ˚C	2-2.8mm	160,000-200,000	Ivan et al. (2010), Malison and Held (1996)

Table 3. Total embryos for each species used at each study site per incubator type during 2022 and 2023 experiments. Species include: walleye (WAE), rainbow trout (RBT) and brook trout (BKT). Incubator types include: Remote Site Incubator (RSI), Floating Basket Incubator (FBI) and Jordan-Scotty Incubator (JSI). Locations include: Thompson State Fish Hatchery (TSFH), Cherry Creek (downstream of MSFH) and Marquette State Fish Hatchery (MSFH).

Location	Year	Species	Incubator Type	# Reps	# Embryos	Density (embryo/cm^2)
TSFH	2022	WAE	RSI	3	13,500	25.38
			FBI (3-cell)	3	54,000	25.84
			FBI (12-cell)	3	54,000	25.84
	2022	RBT	RSI	3	1,600	3.00
			FBI (3-cell)	3	6,300	3.00
			FBI (12-cell)	3	6,300	3.00
Cherry Creek	2022	WAE	RSI	3	13,500	25.38
			FBI (3-cell)	3	54,000	25.84
			FBI (12-cell)	3	54,000	25.84
	2022	BKT	RSI	3	200	0.38
			FBI (12-cell)	3	800	0.38
	2022	RBT	RSI	3	1,600	3.00
			FBI (3-cell)	3	6,300	3.00
			FBI (12-cell)	3	6,300	3.00
	2023	RBT	RSI	6	1100	2.07
			FBI (3-cell)	3	4200	2.01
			FBI (12-cell)	3	4200	2.01
			FBI (3-cell)	3	1400	0.67
			FBI (12-cell)	3	1400	0.67
MSFH	2022	BKT	RSI	3	600	1.13
			FBI (12-cell)	3	2300	1.10

Cedar Creek	2023	RBT	FBI (3-cell) FBI (12-cell)	3 3	4,200 4,200	2.01 2.01
Big Creek	2023	RBT	FBI (3-cell) FBI (12-cell)	3 3	4,200 4,200	2.01 2.01

Table 4. Mean percent survival of individual species at each site including experiments from 2022 and 2023. Survival was reported as the mean percent based on the (N) number of incubators including the 95% confidence interval (CI). Abbreviated locations include Thompson State Fish Hatchery (TSFH) and Marquette State Fish Hatchery (MSFH). Abbreviated species include walleye (WAE), rainbow trout (RBT) and brook trout (BKT). The experiment assigned with (*) marks the occurrence of an FBI being removed from the study due to complications.

Location	Year	Species	Incubator	Mean % Survival ± CI	N
TSFH	2022	RBT	FBI (12-cell)	93.3±1.6	3
			FBI (3-cell)	95.8±3.8	3
			RSI	83.6±20.6	3
Cherry Cr.	2022	RBT	FBI (12-cell)	76.0±4.6	3
			FBI (3-cell)	78.9±9.2	3
			RSI	86.3±16.4	3
Cherry Cr.	2023	RBT	FBI (12-cell)	66.1±8.0	6
			FBI (3-cell)	72.7±1.4	6
			RSI	82.4±2.5	3
Big Cr.	2023	RBT	FBI (12-cell)	53.1±35.1	3
			FBI (3-cell)	57.2±22.1	3
Cedar Cr.	2023	RBT	FBI (12-cell)	71.2±15.0	3
			FBI (3-cell)	77.7±3.9	3
TSFH	2022	WAE	FBI (12-cell)	1.2±1.2	3
			FBI (3-cell)	2.2±2.1	3
			RSI	2.0±3.1	3
Cherry Cr.	2022	WAE	FBI (12-cell)	0.3±0.4	2*
			FBI (3-cell)	1.2±1.2	3
			RSI	1.0±1.3	3
MSFH	2022	BKT	FBI (12-cell)	92.1±0.4	3
			RSI	23.7±9.2	3
Cherry Cr.	2022	BKT	FBI (12-cell)	42.0±8.5	3
			RSI	50.2±1.9	3

Table 5. AICc model selection table for linear mixed-effects model (LMM) used to explain abiotic and biotic (fixed) effects on survival of rainbow trout within FBIs for stream experiments (random effect). K = number of parameters in model. Marginal (mR^2) represents the proportion of variance explained by the fixed effect and conditional (cR^2) represents the proportion of variance explained by the random effect within the LMM

Fixed Effect	Model	K	ΔAIC	AICw_i	mR^2	cR^2
Incubator type	Survival~ Incubator+(1|stream)	4	0.00	0.735	0.05	0.71
Percent Embryo Coverage	Survival~ % embryo coverage+(1|stream)	4	2.89	0.173	0.06	0.66
Velocity + Depth	Survival~ Velocity+Depth+(1|stream)	5	5.48	0.047	0.02	0.64
Null	Survival~+1+(1|stream)	3	5.93	0.038	0	0.66
Percent Fungus	Survival~ % fungus+(1|stream)	4	10.95	0.003	0.01	0.68
Percent Sedimentation	Survival~ % sedimentation+(1|stream)	4	11.15	0.003	0.01	0.65
Global	Survival~ (All fixed effects)+(1|stream)	10	13.89	0.001	0.26	0.70

Table 6. Days of embryo development within the hatchery up to eyed up stage and ATUs acquired in the hatchery and stream per species for 2022 and 2023 experiments. Total ATU represents the ATUs acquired in the hatchery and the stream. Values containing (*) were estimated based on daily average temperatures provided by the Marquette State Fish hatchery for the period of (1/10/23 -1/17/23)

Site	Year	Species	Development in Hatchery (Days)	ATU (Hatchery)	Development in Stream (Days)	Mean Daily Temp (°C)	ATU (stream)	ATU (total)
Cherry Creek	2022	WAE	13	101.5	26	8.78 ± 0.81	240	341.5
		RBT	21	279.3	21	8.87 ± 0.97	181.7	461
		BKT	50	251.1*	27	5.03 ± 0.59*	135.8	386.9*
	2023	RBT	12	156	18	8.5 ± 0.5	143.7	299.7
Big Creek	2023	RBT	12	156	24	9.91 ± 0.89	211	367
Cedar Creek	2023	RBT	12	156	24	8.8 ± 0.77	202	358

Figure 1. General design of a floating basket incubator (FBI). FBI 3-cell (left) and FBI 12-cell (right). Surface current supplies water to embryos resting in the floating basket which is supported by two pontoons constructed from PVC piping.

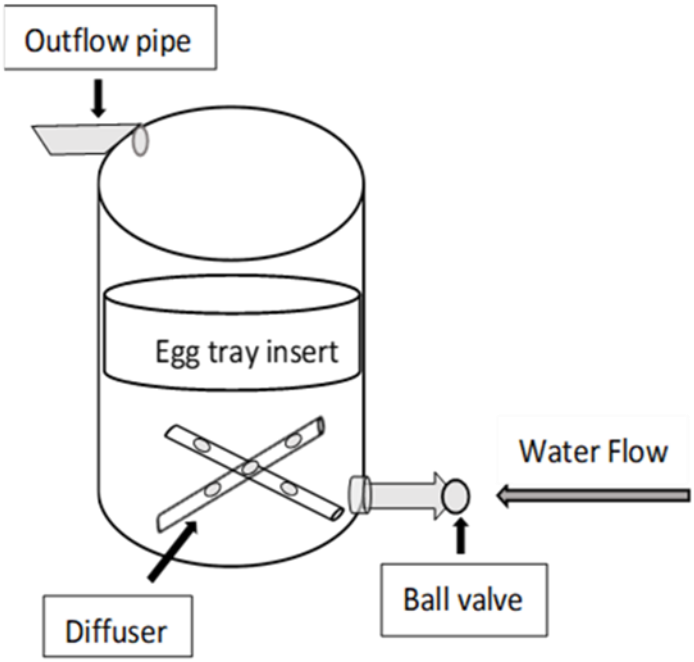

Figure 2. General design of a remote site incubator (RSI). Water enters from the bottom of the RSI is controlled by a ball valve and then passes through the diffuser. Embryos rest in the embryo tray and are exposed to water passing through the embryo tray from the diffuser. The RSIs were composed of standard 19-L buckets and all piping materials were composed of PVC. This design was replicated from Mock et al. (2021).

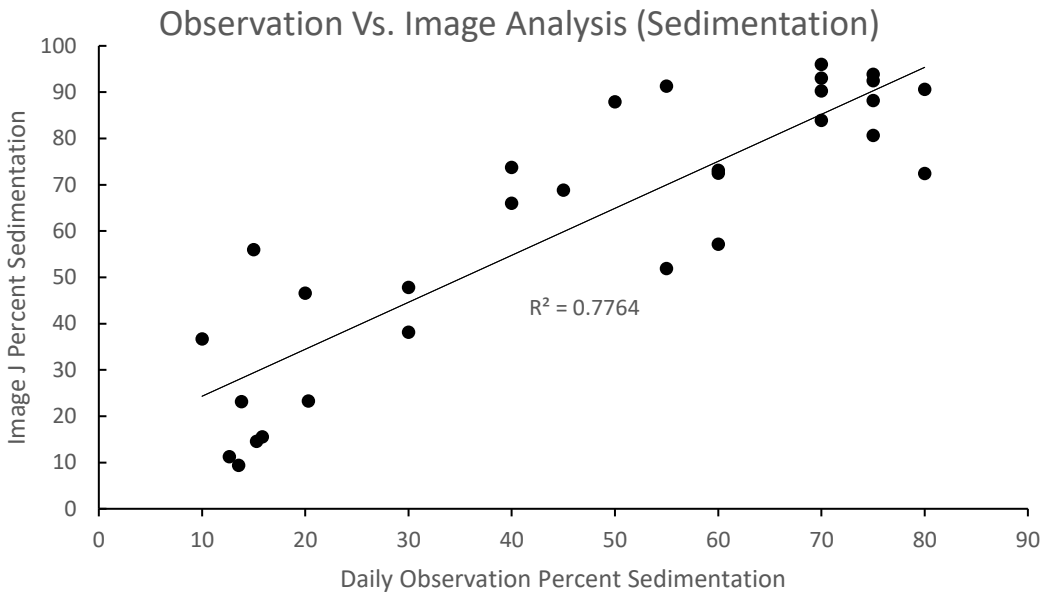

Figure 3. Relationship between daily observation estimates and digitized imagery estimates in ImageJ of the percent of sedimentation within FBIs including rainbow trout throughout the 2022 and 2023 stream experiments

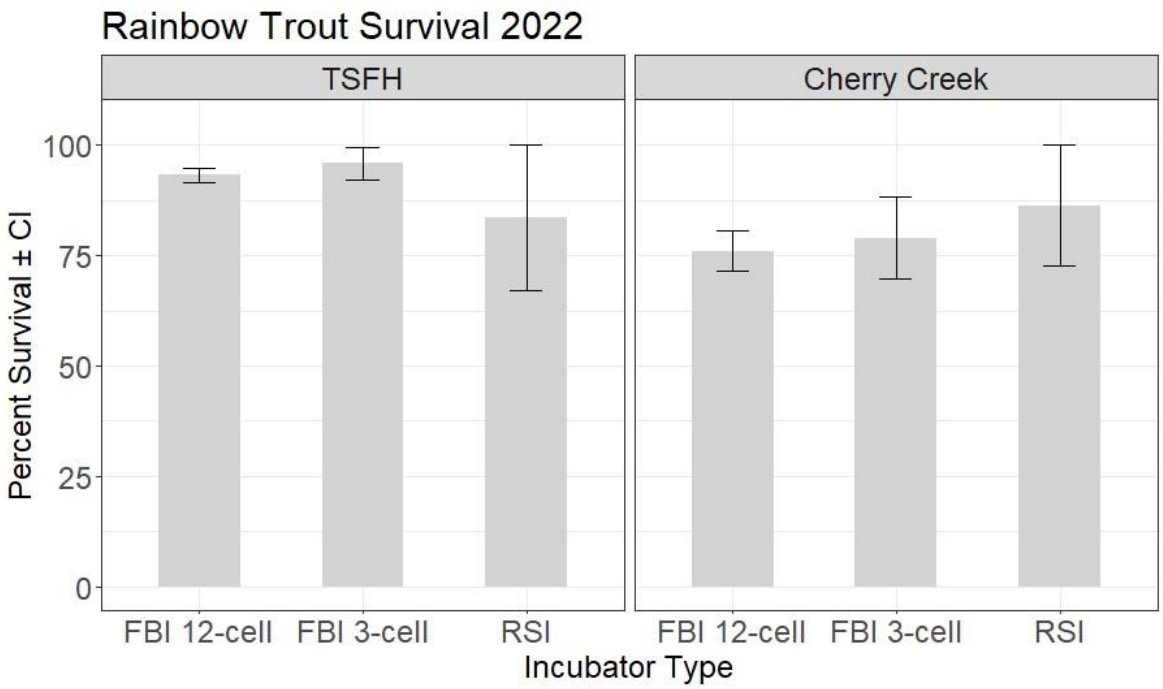

Figure 4. Mean percent survival for Rainbow Trout at Thompson State Fish Hatchery (TSFH) and Cherry Creek in 2022. All trials per incubator type included replications of (*N*=3). Error bars are ± confidence intervals (CI).

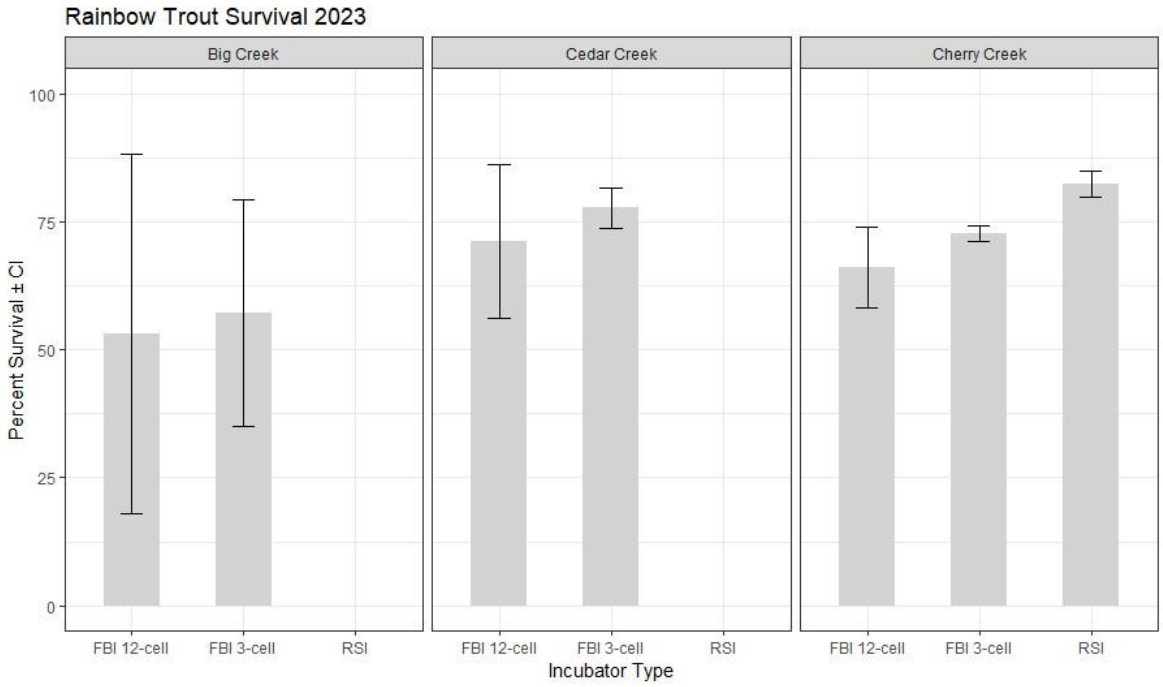

Figure 5. Mean percent survival for Rainbow Trout in 2023. All trials per incubator type included replications of (*N*=3) except for and all incubator types for rainbow trout at Cherry Creek in 2023 (*N*=6). Error bars are ± confidence intervals (CI).

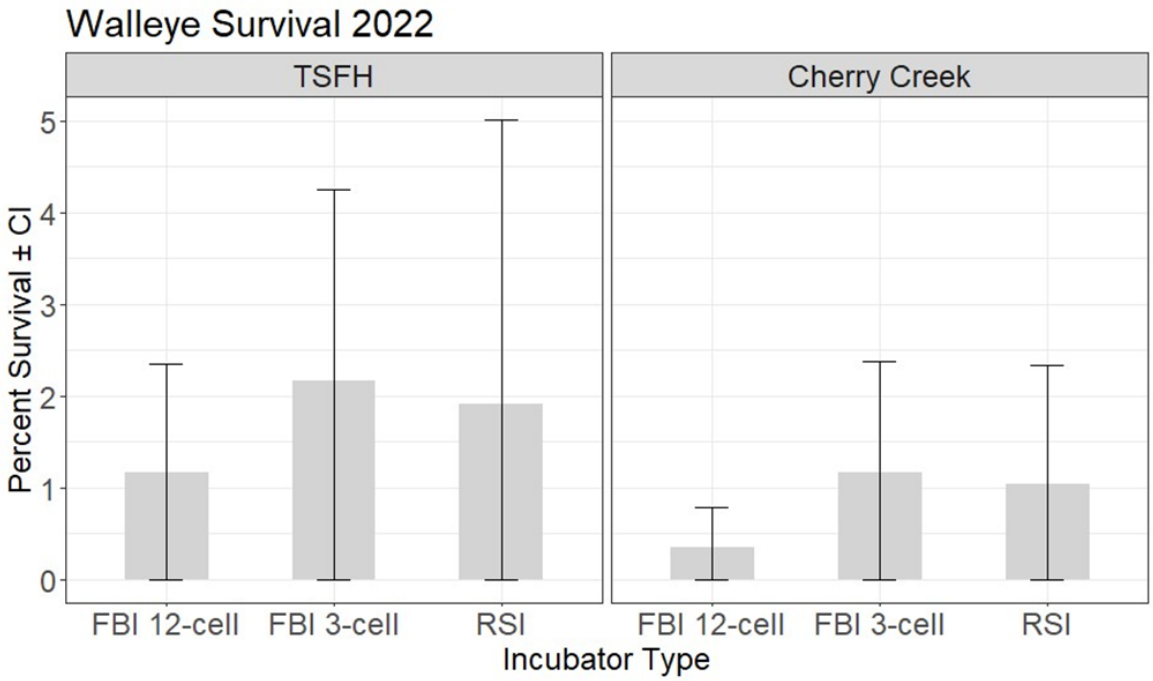

Figure 6. Mean percent survival for Walleye at Thompson State Fish Hatchery (TSFH) and Cherry Creek in 2022. All trials per incubator type included replications of (*N*=3) except for walleye FBI 12-cell trials at Cherry Creek (*N*=2). Error bars are ± confidence intervals (CI).

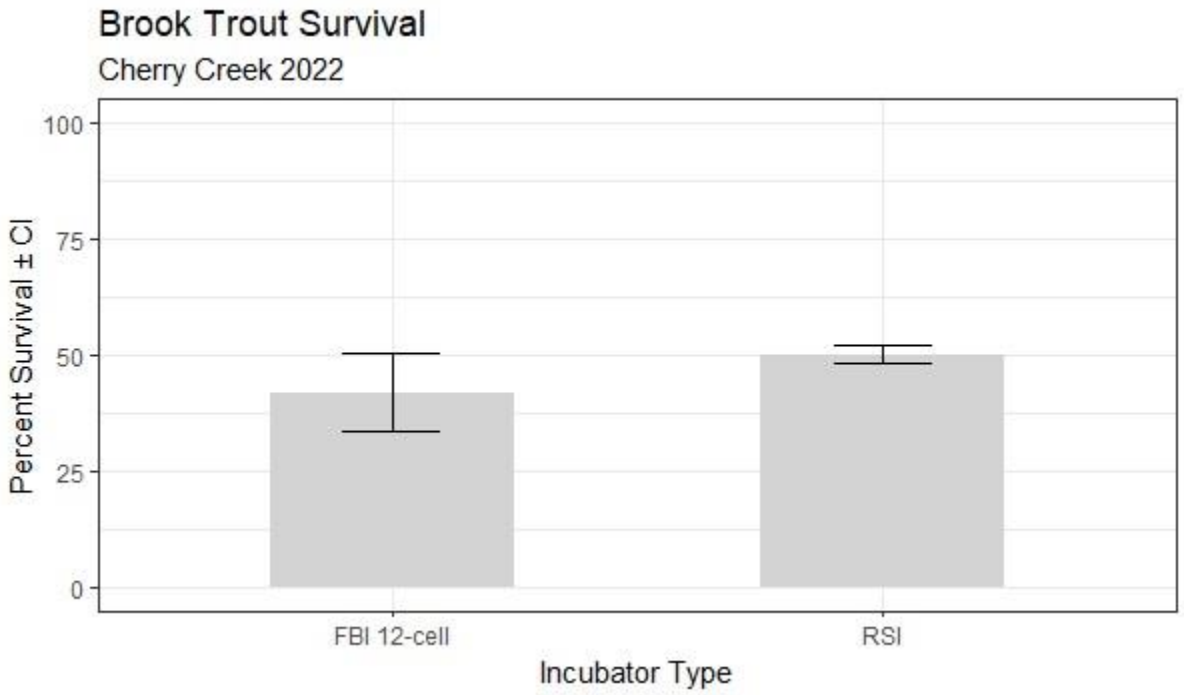

Figure 7. Mean percent survival for Brook Trout at Cherry Creek in 2022. All trials per incubator type included replications of (*N*=3). Error bars are ± confidence intervals (CI).

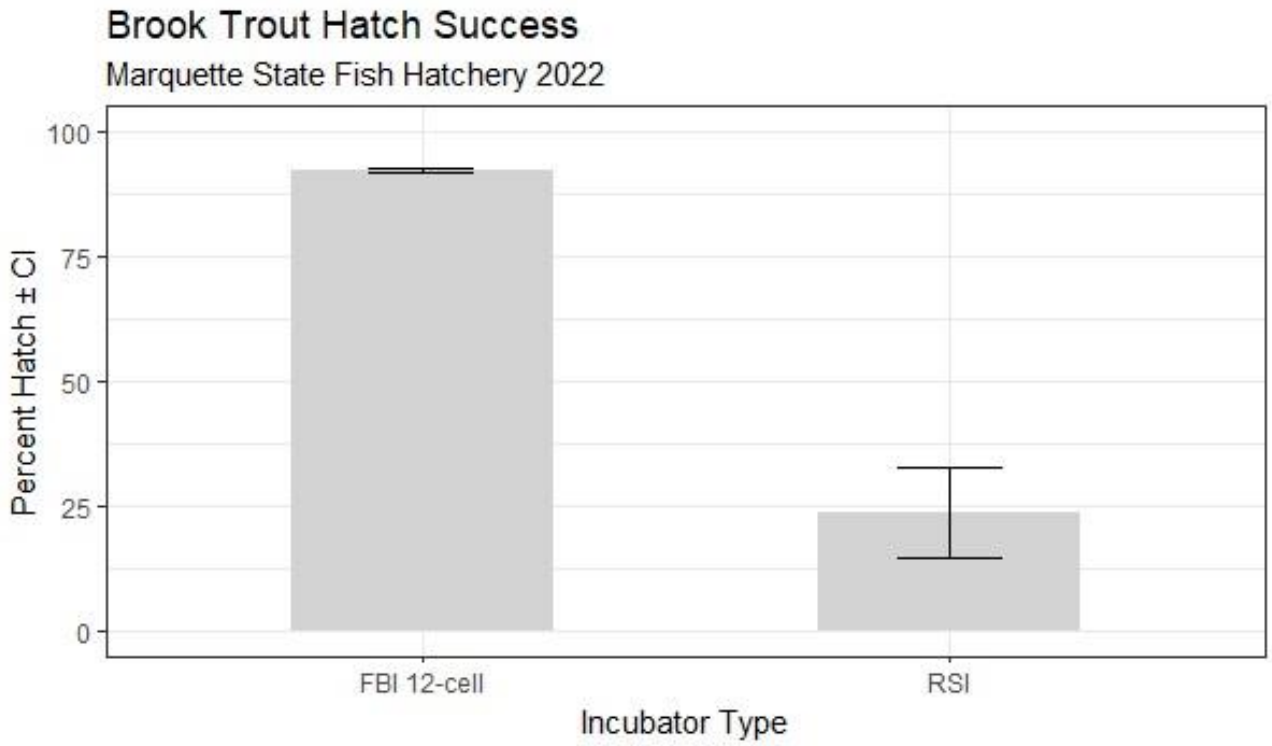

Figure 8. Mean percent hatch of Brook Trout at Marquette State Fish Hatchery in 2022. All trials per incubator type included replications of (*N*=3). Error bars are ± confidence intervals (CI).

Appendix A

Application to Use Vertebrate Animals in Research, Testing or Instruction

NORTHERN MICHIGAN UNIVERSITY

Project Title (If using external funds, enter the title used on the grant application): Identifying best options for instream reintroductions of Arctic Grayling in Michigan streams

Shaded area for IACUC use only.

Application Number: 428 Modification
Date Application Received: 4/19/2023
☒ Approved ☐ Denied on April 27, 2023

General Instructions
Please check the IACUC website to ensure you are using the current version of the form. All parts of this form *must be submitted electronically* to the Institutional Animal Care and Use Committee (email: IACUC@nmu.edu) and the relevant Department Head or other departmental designee. Review of this application will commence upon receiving the electronic application, but the project may not begin until all required approval signatures are obtained via Right Signature. Please contact the IACUC chair (email: IACUCChr@nmu.edu) if you have any questions.

Review Dates:
Designated Member Review of applications (appropriate for USDA Use Categories B and C) will be completed within two weeks after receipt of the electronic application.

Full Committee Review of applications will take place on the last Friday of every month. Applications for Full Committee Review must be electronically received by the first Friday of the month. Full Committee Review is required for applications that fall under USDA Use Categories D and E. Applications that fall under USDA Use Categories B and C will receive Full Committee Review if requested by an IACUC member. Detailed procedures on the IACUC review processes are located at the IACUC website.

I. Principal Investigator (Must be a faculty member or Department Head): Dr. Jill Leonard

Co- Investigator: Dr. Brandon Gerig (non-biology member)

Department: Biology

Phone number: 227-2302

II. Funding Sources/Course Information and Dates

If the proposed work is for a course, please include the number of the course and title of the course
NA

Funding Sources (External & Internal, if applicable) Consumers Energy Foundation

Additional Funding Pending (click on the correct box)? ☐Yes ☒No

Project/Course Start Date: September 8, 2022
End Date (three year maximum): September 7, 2025

This application is (check one) ☐New ☒ Modification of an application currently approved by the Institutional Animal Care and Use Committee (a **new** protocol must be submitted after three years)