FINAL REPORT

Sediment effects on Arctic grayling (*Thymallus arcticus*) early life history in Montana – a field experiment and literature review

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EXECUTIVE SUMMARY

In order to assess impacts of different fine sediment levels on Arctic grayling early life history, an experimental field manipulation of spawning gravels at Mussigbrod Lake was combined with a literature review of fine sediment effects on the ontogeny of salmonids in general and Arctic grayling in particular. The field experiment exposed fertilized grayling eggs to treatments of 0%, 10%, 25% or 50% fine sediment:gravel spawning substrate and measured a response through egg survival and percentage of egg-to-fry emergence. No significant difference in emergence was detected between sediment treatments, nor did treatments significantly differ in environmental covariates of water depth and velocity. A trend towards lower emergence did appear between the high (50% fines) sediment treatment and other treatment and control levels, but significance was precluded by high variability between sample units in emergence at the high treatment level and uneven fine sediment settlement within sample units across all treatment levels. Based on findings in this and other published studies of egg-to-fry and young-of-the year survival in salmonids, additional investigation of threshold effects of suspended sediment loads and 25 – 50% spawning substrate fines on Arctic grayling early life history stages is recommended.

INTRODUCTION

Native fluvial Arctic grayling, historically quite common in the Upper Missouri River (UMR) basin of Montana, have been extirpated from 90% of their previous distribution (Kaya 1990, 1992, USFWS 2014). Adfluvial Arctic grayling are more widespread across a number of UMR drainages, and include 4 native and 14 introduced lake-dwelling populations (Kaya 1992, USFWS 1990). Efforts at protection and restoration of grayling populations have focused on a mix of strategies, including hatchery rearing and the use of remote site incubators (RSI's) for artificial propagation and reintroduction of fish within both native and introduced waterbodies, riparian restoration, reduction of non-native fish populations to counteract potential competition and predation risk, and a suite of instream habitat manipulations linked to improved water management to address dewatering, thermal stress, entrainment, connectivity and degraded habitat condition, particularly in spawning areas (Kaya 1992, USFWS 2014, Warren et al. 2017).

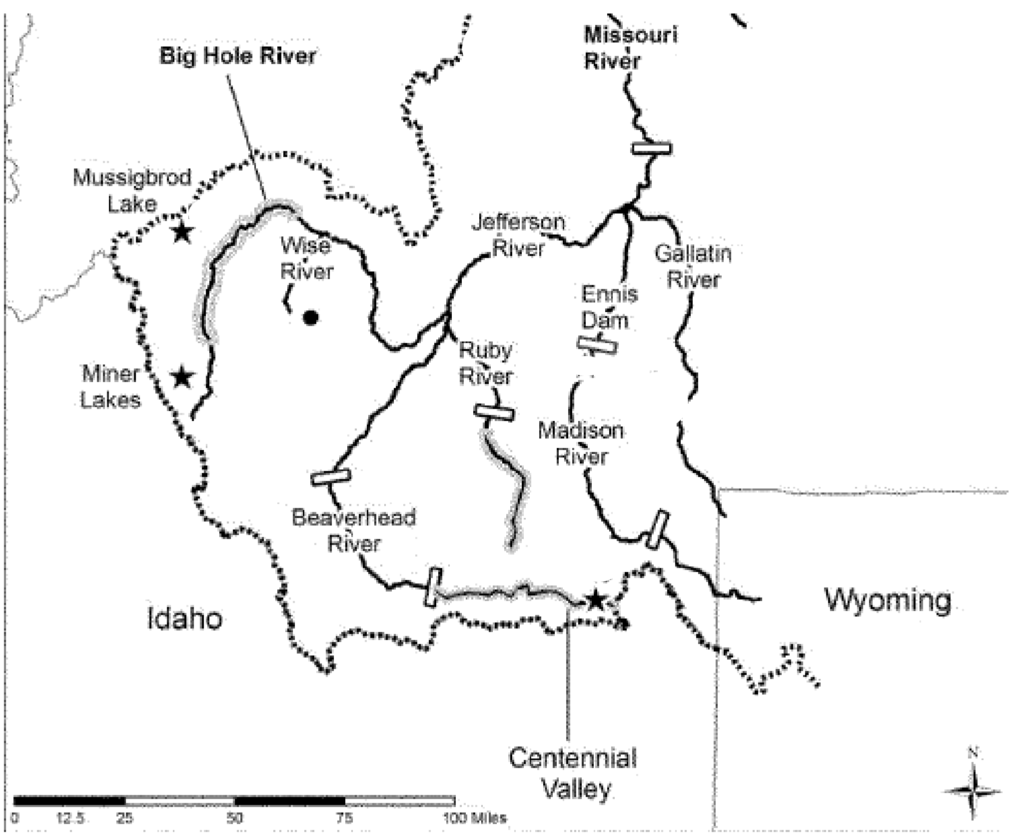
Spawning habitat and the environment encountered by Arctic grayling in early life history stages are the focus of the research described herein. Arctic grayling across North America typically spawn late April – June, a period coinciding with ice-off and a subsequent spring runoff-driven hydrograph (Northcote 1995). Spawning site conditions for Arctic grayling in Montana exhibit a range of characteristic water temperatures (migration at 0 – 4° C, spawning at 2 – 10° C), velocities (0.17 – 1.46 m/sec), and depths (15 – 100 cm) (Brown 1938, Tryon 1947, Nelson 1954, Hubert 1985, Barndt and Kaya 2000). While Arctic grayling in North America deposit eggs on a wide range of substrate sizes, the majority of studies observe spawning occurring over gravels (2 – 64 mm diameter) (Northcote 1995, Stewart et al. 2007). Most observations of Arctic grayling spawning in Montana rivers have been in clean gravels exhibiting 25% or less fine sand and silt (<2 mm diameter) at the surface (Nelson 1954, Shepard and Oswald 1989, Kaya 1990, Mogen 1996). Montana grayling reproduction in substrates with a higher proportion of fines is more common at lake inlet or outlet streams (Brown 1938, Tyron 1947, Peterman 1972, Kaya 1990). Once deposited, adhesive grayling eggs (2.5 – 4 mm diameter) either stick to sand and gravel at the spawning site or drift some distance downstream before settling into the shallow substrate. Natural mortality during the egg and embryo stage varies widely across sites, and can exceed 90% (Kruse 1959, Lund 1974) Even small percentages of fine sediment deposition in spawning areas has been shown to impact egg-to-fry survival among a broad suite of salmonids (O'Connor and Andrew 1998, Argent and Flebbe 1999, Kondolf 2000, Jensen et al. 2009). In addition, prolonged exposure of newly emerged Arctic grayling to elevated suspended sediment loads likely decreases feeding and growth rates (McLeahy et al. 1984).

This study sought to answer the question: Do increasing levels of fine sediment deposition have negative impacts on adfluvial Arctic grayling egg survival and egg-to-fry emergence? A field experiment was designed to test the hypothesis that increased levels of fine sediment in a spawning substrate matrix would inhibit grayling egg-to-fry development, compared to the null hypothesis that differing levels of fine sediment would have no effect on development. We predicted treatment levels highest in fine sediment would have the lowest egg survival and egg-to-fry emergence, with increasingly high egg survival and egg-to-fry emergence as levels of fines were reduced to 0%. A secondary objective was to compare the experimental results with additional sediment survey data from Centennial Valley grayling streams and published literature on fine sediment impacts on Arctic grayling spawning and early ontogeny.

STUDY AREA

Lakes with Arctic grayling populations in the Upper Missouri River drainage – Several Upper Missouri River drainage lakes with inlet or outlet stream spawning by adfluvial Arctic grayling were considered for this experiment (Fig. 1). A previous sediment manipulation and egg rearing experiment on Red Rock Creek, an inlet stream to Upper Red Rock Lake in the Centennial Valley, failed largely due to high flow variability and sedimentation impacts on the experimental units (Anderson 2016). Further experimentation in this system posed a risk due to the small size of the grayling spawning population in the last several years (150 – 700 individuals; Warren et al. 2018) and still considerable potential for uncontrollable environmental variation. Instead, Lake Agnes in the Rock Creek drainage, and Mussigbrod Lake and Miner Lake in the Big Hole drainage were surveyed from November 2016 – May 2017.

Figure 1. Study area in the UMR showing Mussigbrod Lake (star), Miner Lakes (star), Lake Agnes (black circle near Wise River) and Upper Red Rock Lake/Red Rock Creek in the Centennial Valley (star). Rectangles denote mainstem river dams. (Adapted from USFWS 2014).



Lake Agnes (N 45.5127 W -112.8444), while hosting a robust Arctic grayling population size, was determined to be unsuitable given that Arctic grayling spawning would have occurred later and egg development taken longer than most other UMR populations (mid-June to early July) given prolonged ice coverage in 2017 and the expectation of continued cold water conditions late into the summer (Peterman 1972, USFWS 2014). The location of this site also presents some logistical access challenges for frequent sampling to check for fry emergence. Similar issues of timing constraints arose at Miner Lake (N 45.3248, W –113.56747) when fish had not spawned by the second week in June, 2017 (direct observation and J. Olsen, personal communication), coupled with a census population estimate that was the lowest of all three lakes at 1230 – 4090 fish (USFWS 2014). In addition, the spawning site at Miner Lake receives heavy recreational fishing pressure during spawning, making it a necessity to place any experimental sediment manipulations downstream away from the major recreational fishing pressure. The potential downstream experimental sites had highly variable water depths and flows, making placement and sampling of sample units difficult.

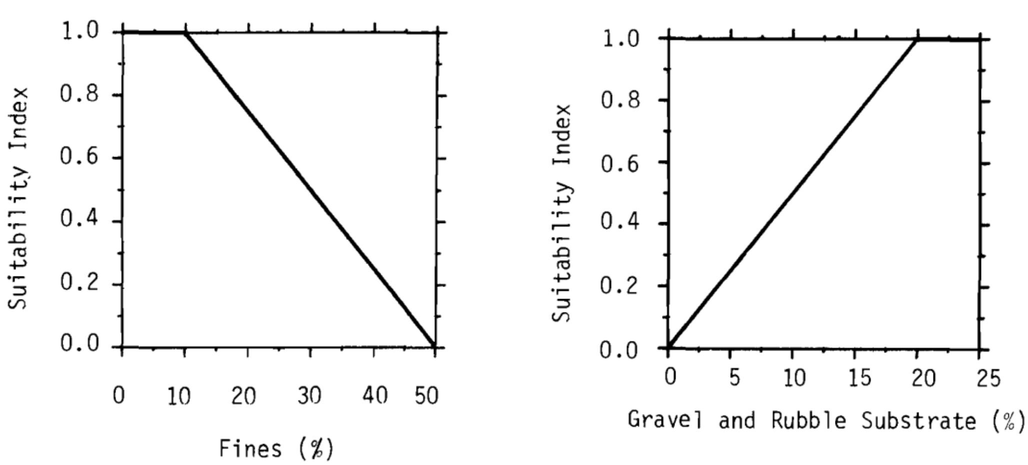
The Mussigbrod Lake (N 45.79078, W –113.61149) outlet stream was chosen for the experiment due to accessibility, early availability of eggs from a large grayling spawning population, and low stream flow variability, sedimentation, and temperature. Mussigbrod is a 42.5-hectare lake at 2009 m elevation with a fish community comprised of native Arctic grayling (*Thymallus arcticus*), burbot (*Lota lota*), longnose sucker (Catostomus *catostomus*), white sucker (*C. commersoni*), and non-native brook trout (*Salvelinus fontinalis*) (FISHMT 2018). The adfluvial Arctic grayling population of the lake is both native and genetically distinct, with an estimated effective population size of nearly 1500 fish and a census population that varies from 6,000 – 21,000 fish (Peterson and Ardren 2009, USFWS 2014). Grayling eggs are frequently collected from spawning grayling at this site for culture at either the Washoe Park Hatchery in Anaconda, Montana (MTFWP 2005) or the Yellowstone River Trout Hatchery in Big Timber, Montana. Eggs and fry where then re-stocking into Mussigbrod and other lakes in the region, a process which occurred in 2017 and facilitated the take and fertilization of eggs for this experiment.

METHODS

Experimental design – The experimental design was chosen to test the validity of the spawning substrate relationship proposed in Hubert's (1985) Habitat Suitability Index (HSI) for Arctic grayling. Hubert's HSI model conjectured that an increased percent of fine sediment (<3 mm diameter) would produce a steep decline in suitable spawning habitat for grayling (Fig. 2a) while an increased percent of substrate composed of gravel and rubble (1 – 20 cm diameter) would have the opposite effect (Fig. 2b). In the current experiment 4 treatment levels were established using an optimal percent gravel substrate (≥50%) mixed with 0% (1.0 HSI control), 10% (1.0 HSI), 25% (0.625 HSI) or 50% fine sediment (0.0 HSI). HSI predictions were based on the linear model $y = -0.025x + 1.25$ applied to Hubert's data ($R^2 = 1.0$). The current study utilized a more restrictive sediment diameter range based on the Wentworth (1922) scale for sand and gravel as these size fractions are most common at the Mussigbrod Lake spawning reach and are typical of many grayling spawning sites in Montana. An equal mix of course sand (0.5 – 1 mm), medium sand (0.25 – 0.5 mm), and fine sand (0.125 – 0.25 mm) was used for the % fines treatments mixed into a matrix of 5 – 20 mm gravel (within the range of observed grayling spawning in gravel, Hubert et al. 1985). Sediment used in treatments was collected, sieved, and mixed on site from shoreline substrate at Mussigbrod Lake. All treatments were assumed to be +/-1% based on loss or gain in fine sediment when filling experiment units with the sediment matrix and subsequent emergence checks requiring multiple opening and closing of units in the field.

Figure 2. Predicted riverine Arctic grayling spawning HSI based on a) percent fines (<3 mm diameter) and b) percent gravel and rubble (1.0 – 20.0 cm diameter) (from Hubert et al. 1985).

(a) (b)



Once sediment treatment levels were established, inexpensive incubation chambers for housing the substrate and grayling eggs instream for the duration of the experiment were needed. Incubation units needed to be large enough to host 40 - 50 developing eggs without overcrowding yet small enough to deploy 5 replicates per treatment in a 3 x 5 m stream area. In addition, incubation unit enclosures needed to prevent egg, sediment, and predator gains and losses yet maintain consistent in situ water flow and temperatures once instream. Use of RSI's was considered and ruled out due to likelihood of disturbance and frequent maintenance required with the high amount of human recreation at the Mussigbrod Lake. Given that recent efforts at Red Rock Creek employed an incubation chamber design based on Haugen (2000) proved unreliable (Anderson 2016), the choice was made to use two-compartment Whitlock-Vibert (WV) boxes (Vibert 1949, Whitlock 1979) enclosed in 30-micron nylon woven mesh (Fig. 3). The larger, lower chamber of each W-V box was filled with a randomly assigned sediment treatment to within 2.5 cm of the upper chamber floor, leaving room to deposit fertilized eggs.

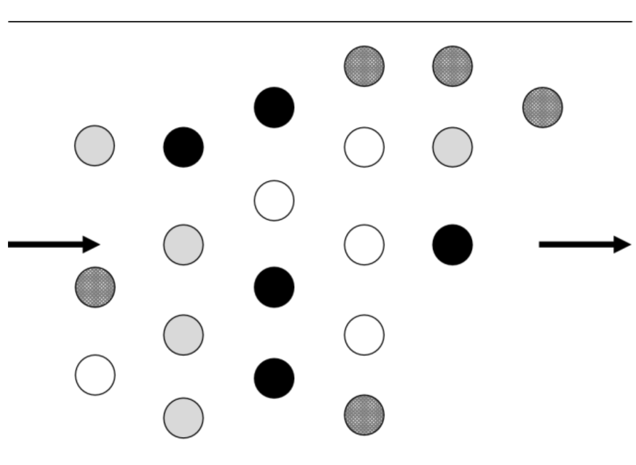
Figure 3. Example of a Whitlock-Vibert box (a) completely enclosed in nylon mesh, (b) with mesh opened but the upper chamber closed, and (c) with the upper chamber open to reveal the substrate surface and 2 cm space below the upper chamber floor.

(a) (b) (c)

In order to collect and spawn Arctic grayling from Mussigbrod Lake, Montana Fish, Wildlife, and Parks (MTFWP) personnel set up a metal picket fence weir trap across the spillway outlet in mid-May, 2017. The trap was monitored for 2 weeks until grayling arrived, and over several subsequent days trapped fish were removed and placed in nearby holding pens. On May 24, 2017 MTFWP personnel sampled, spawned, and released 90 female and 90 male fish from the pens, collecting and fertilizing 89,000 eggs. The first 50 fish spawned were measured and a genetic tissue sample collected from each. After hardening at the site for several hours, approximately 1000 of these eggs were reserved for the field experiment while the remaining eggs were transported to the Yellowstone River Trout Hatchery. A random sample of 40 grayling eggs were placed on the sediment surface within each W-V box, after which the upper chamber of the box was secured shut and the entire box enclosed in a nylon mesh bag sealed closed with a zip tie. The nylon mesh prevented eggs, fry, or sediment from treatments escaping the W-V boxes in situ. This process was repeated until 5 replicate W-V box sample units of each of the 4 treatment levels was created. Excess eggs left over after filling W-V boxes were released instream outside the study area.

The W-V box sample units were randomly assigned to evenly spaced locations on a 3 x 5 m grid within the spawning reach of the lake outlet stream (Fig. 4). The grid was established along the right bank in initial water depths of 0.2 – 1.0 m and water velocities of 0.17 – 1.46 m/sec, representing a balance between preferred grayling spawning conditions and locations that were easy to access and did not wash sample units downstream. The W-V boxes were buried in the streambed such that the gravel surface inside the basket was flush bed surface (+/- 5 cm).

Figure 4. Lake outlet stream study site illustrating random placement of the different fine sediment treatment (light gray = 10%, dark gray = 25%, black = 50%, and white = 0% control) sample units (n=5 replicates per treatment level).



Egg survival and mortality, approximate hatching date, percent larval emergence, and size at emergence of grayling were measured in each W-V box, given that estimates were contingent on successfully observing egg hatching and fry emergence. Eggs from Montana grayling cultured either at a hatchery or in RSI's in 8 – 13° C water hatch in approximately 110 – 220 degree days (10 – 23 calendar days, average of 16 – 17 days) and swim-up from the gravel at 140 – 230 degree days (3 – 7 calendar days) after hatching (Henshall 1907, Tryon 1947, Nelson 1954, Kaya 1989, Kaeding and Boltz 2004). In colder waters (2 – 6°C), hatching and swim-up may occur 5 – 10 days later at each stage (Kratt and Smith 1977, Kaeding and Boltz 2004). Newly hatched larval grayling range in size from 0.7 to 1.1 cm in length (Watling and Brown 1955). Given anticipated water temperatures at the site (5 – 15° C), we predicted grayling would hatch and emerge in June at 20 – 30 days after fertilization. Eggs reared at the hatchery took 10 days to reach the eyed stage.

A previous study by Harshbarger and Porter (1971) rearing brown trout egg in two-compartment W-V boxes indicated fungal growth could significantly reduce egg survival. Boxes were checked every 2 – 9 days to count and remove any dead eggs or fry (based on discoloration or presence of fungus), record egg eye-up and egg-to-fry emergence dates and rates, and qualitatively note sediment distribution within the box and overlaying mesh, for a total of 6 separate observations per box. Efforts made to minimally disturb sample units and prevent egg or fry loss when opening mesh enclosures and W-V boxes occurred by continuously submerged boxes in a stilling bucket filled with stream water. Photos of the surface substrate within each box were obtained during the box check on June 4 – 5, 2017 (Appendix 1). From June 17 – 19, fry counts and measurements determined final egg-to-fry survival and size at emergence. On-site release of all live fry followed, while W-V boxes were removed from the site and stored in a refrigerator overnight. Sediment in each sample was picked over to identify previously uncounted dead eggs and fry. Despite this attempt to determine the fate of all 40 eggs in each sample unit, nearly all W-V had one or more eggs remaining unaccounted for by the end of the experiment. Presumably, missing eggs died and disintegrated between sampling rounds at either the egg or fry stage, and were counted as mortalities. Sediment and mesh bags associated with each W-V box was air dried for a year and sieved into different size classes (>4 mm, 2-4 mm, 0.25 – 2 mm, < 0.0625 – 0.25 mm). Volume (ml) and weight (g) were measured for all W-V boxes, nylon mesh, and each of the different sediment size classes.

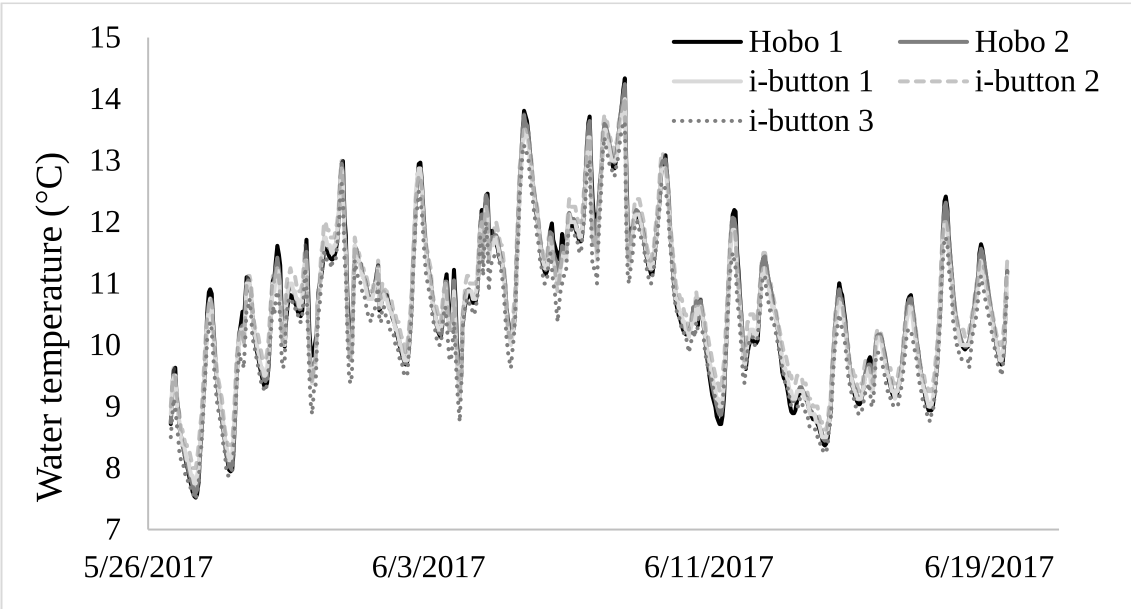
Several environmental variables potentially influencing egg survival were quantified as part of this experiment. Water depth and velocity were measured at the edge of each W-V box sample unit at the beginning, middle, and end of the experiment. Water depth from the substrate to water surface was assessed with a meter stick temperature. Water velocity was gauged using a Marsh McBirney's Model 2000 Flo-Mate. Temperatures were recorded every 10 minutes on the substrate water interface at the upstream and downstream end of the reach (HOBO Water Temperature Pro v2 Data Logger) and every hour in one W-V boxes chosen randomly from each of the four treatment groups (1-Wire Thermochron iButton). One ibutton failed during experiment and was excluded from further analyses.

Data analyses – A balanced experimental design (n = 5 observational sample units for all treatment levels) allowed for a univariate ANOVA approach to analyze grayling survival among sediment treatment groups and to detect differences in environmental variables between treatments that could confound interpretation of sediment treatment effects. Separate single factor ANOVA tests analyzed treatment effects on the following variables: number of grayling emerged, grayling size at emergence, water depth, water velocity, treatment sediment volume and weight.

RESULTS

Site physical conditions during the incubation period – The study reach at Mussigbrod Lake exhibited minimal differences in environmental variables across sample units or treatment types during the experiment. Water temperatures (Fig. 5, Appendix 1) demonstrated a predictable diel cycle (fluctuations of 1 - 3° C) and some variation over the entire sampling period (7.5° to 14.2° C), with ambient stream temperatures (Hobo 1, 2) nearly identical to temperatures in the W-V box sample units (ibuttons 1 – 3). While water depths gradually declined over time by 100 – 200 cm at all 20 sample units (Appendix 2), water velocities remained stable in the 0.10 – 0.33 m/sec range. ANOVA results indicated no significant difference between experimental sediment treatment groups in relation to mean water depths (p=0.76) or water velocities (p=0.18).

Figure 5. Water temperature data from upstream (Hobo 1) and downstream (Hobo 2) study reach locations and from i-buttons inside W-V boxes with 0% (#3), 10% (#1), and 25% (#4) sediment treatment levels.



Fine sediment effects on grayling egg survival and fry emergence – Observed percent egg-to-fry survival in individual sample units ranged between 10 – 78% (mean and standard deviation, 58% ± 0.16) across all treatment levels (Table 1). In comparison, two subsamples of 140 – 150 grayling eggs from Mussigbrod Lake reared at the Yellowstone River Trout Hatchery yielded an average egg eye-up rate of 82%. Across treatment groups, an average of 8 eggs (range 1 – 30 eggs) in each W-V sample unit remained unaccounted for by the end of the experiment. This was presumably due to death and disintegration between sampling events and missing eggs were counted as mortalities. In 25% of sample units, the number of eggs unaccounted for exceeded the number of dead eggs and fry removed during sample checks. There was no consistent pattern between sediment treatment type and number of unaccounted eggs.

Table 1. Experimental data for five replicate sample units at each treatment level (0% or control, 10%, 25% and 50% fine sediment). Data represents the number of live emergent fish observed, removed dead eggs and fish, and overall percent egg-to-fry survival by the end of experiment.

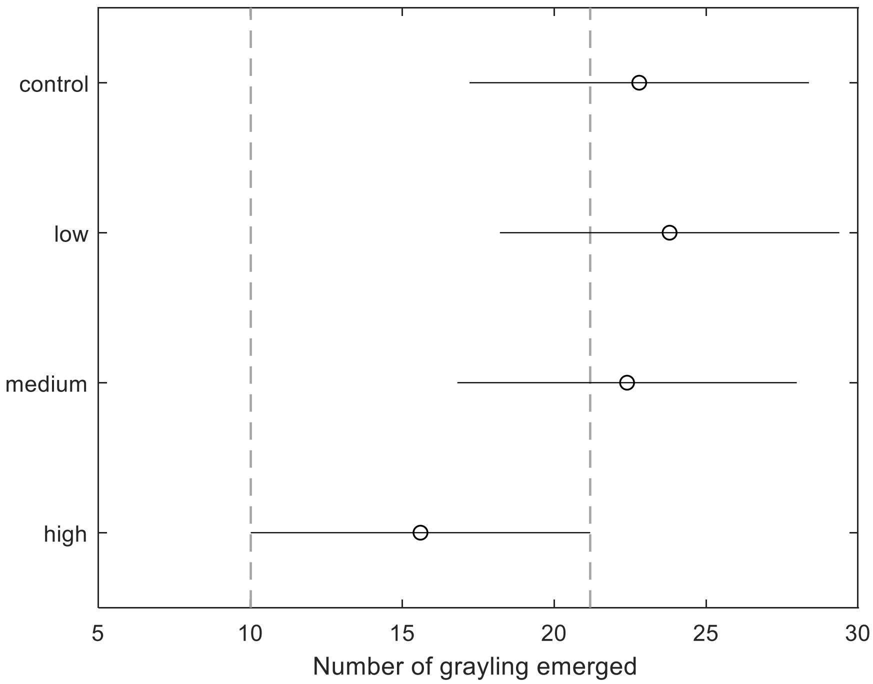
	0%			10%			25%			50%		
Units	Live	Dead	% Survive	Live	Dead	% Survive	Live	Dead	% Survive	Live	Dead	% Survive
1	26	5	0.65	19	15	0.48	22	11	0.55	10	18	0.25
2	24	8	0.60	29	10	0.73	24	11	0.60	4	6	0.10
3	17	13	0.43	26	10	0.65	24	7	0.60	31	9	0.78
4	28	11	0.70	19	8	0.48	22	13	0.55	13	12	0.33
5	19	11	0.48	26	10	0.65	20	14	0.50	20	13	0.50
Mean	*22.8*	*9.6*	*0.57*	*23.8*	*10.6*	*0.60*	*22.4*	*11.2*	*0.56*	*15.6*	*11.6*	*0.39*
Var	*21.7*	*9.8*	*0.01*	*20.7*	*6.8*	*0.01*	*2.8*	*7.2*	*0.00*	*107.3*	*20.3*	*0.07*
StDev	*4.7*	*3.1*	*0.12*	*4.5*	*2.6*	*0.11*	*1.7*	*2.7*	*0.04*	*10.4*	*4.5*	*0.26*

Single factor ANOVA results comparing the number of emerging larval fish among sediment treatment groups did not produce a statistically significant difference (overall model p = 0.18, all pairwise comparisons p>0.19) between treatments (Table 2, Fig. 6). There was also no statistically significant difference (p = 0.62) in larval size at emergence across treatment groups. Univariate ANOVA run on sediment size fraction weights and volumes among treatment levels showed that the 4 treatment levels were quite significantly different (p <0.001).

Table 2. Single factor ANOVA results showing no significant differences in emergence between the fine sediment treatment groups in the experiment.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	210.55	3	70.183	1.841	0.180	3.239
Within Groups	610	16	38.125			
Total	820.55	19				

Figure 6. Mean larval emergence across control (0%), low (10%), medium (25%) and high (50%) treatments groups, with 95% confidence interval bars. The 95% confidence interval for the mean of the high sediment treatment is indicated by dashed lines.



A single sample unit with a large number of emerged larval fish (n = 31) in the 50% sediment treatment represented an outlier. Field notes indicated that a large amount of the fine sediment in this sample unit had piped down below the egg layer and out of the hatch box to settle in the bottom of the containment mesh. However, this same settling of fine sediments out of box sides into mesh occurred in other sample units with lower emergence rates across treatment classes. Removal of the outlier high emergence sample unit prior to statistical analysis yielded a significant difference between low egg-to-fry emergence in the 50% fines group compared to higher emergence at all other treatments levels (Appendix 3, 4). Unfortunately, after considering field notes on random grid layout and site disturbance of units early in the experiment, photos of sediment distribution within different treatment levels of in situ hatch boxes, and determining sediment fraction weights and volumes in different sediment treatment classes post-experiment, we were unable to determine a single definitive cause for any outlier effects. Univariate ANOVA of sediment size fractions indicated experimental treatment levels differed distinctly as intended. As such, there was no viable reason to drop individual sample units from the analyses.

CONCLUSIONS AND RECOMMENDATIONS

Statistical analyses of experimental results described here fail to reject the null hypothesis that different levels of fine sediments do not influence egg-to-fry survival and emergence. Experimental results demonstrated a mix of 25% fines to 75% gravel spawning substrate yielded fry emergence rates comparable to treatments with 0-10% fines to 90-100% gravel. While the 50% fine sediment to gravel treatment did not produce a statistically significant drop in fry emergence due to a single outlier sample unit with high emergence, there was a general trend towards reduced emergence in the 50% fines group. This lends support to the conclusion by Hubert et al. (1985) and others that ≥ 50% fines may be unsuitable for grayling emergence.

Several factors in this experiment limit the scope of inference to grayling populations in Montana. The artificial conditions of this experiment preclude exposure of eggs to predation, flow, and sediment related disturbances expected to depress emergence rates in open systems. The average Arctic grayling survival to emergence of 53% across experimental treatment groups observed in this experiment is well above that expected under natural stream conditions (Kruse 1959, Lund 1974). Experimental field conditions occurred in a single year under declining flows in a lake outlet with minimal observed bedload transport or streambed disturbance and sample units were protected from additional fine sediment infiltration by a mesh bag. The choice of sand fraction for fine sediment treatments also precludes inferences about impacts of silt and smaller grain sizes on egg-to-fry emergence. Exposure to large suspended sediment loads (≥1000 mg/L) for several weeks to months is likely to cause impaired feeding performance and growth of newly emerged grayling (McLeahy et al. 1984). Arctic grayling eggs and swim up fry may be particularly sensitive to moderate suspended sediment loads for even short durations, with mortality rates >25% in one trial after just 48 hours at concentrations as low as 142 mg/L (Newcombe and Jensen 1996). Suspended sediment loads of 100 – 1000 mg/L are observable on the Beaverhead and Gallatin Rivers (Lambing and Cleasby 2006), drainages within the historic range of Arctic grayling distribution.

Additionally, many eggs were not visible at the substrate surface within the W-V boxes when doing repeated checks of egg survival and fry emergence, further complicated by unequal fine sediment settling with W-V boxes (Appendix 1). This is not surprising given the complexities of fine sediment transport, which does not simply deposit on gravel surfaces but infiltrates, exfiltrates, and is longitudinally transported through the bed sediment matrix (Casas-Mulet et al. 2017). This could have created highly variable egg pocket conditions within and among sample units and likely added to survival to emergence variation across sample units within treatment levels. It is quite likely that eggs unaccounted for at the end of the experiment may have settled and died within fine sediments in or below W-V boxes. It must be acknowledged that this complicates unbiased estimation of accurate egg-to-fry survival rates in relation to sediment treatment levels, which is contingent upon precise observation of egg hatch and fate.

Finally, long-term genetic isolation as observed at several Arctic grayling sites in Montana (Peterson and Ardren 2009) may have resulted in rapid local adaptation as observed in some European grayling populations (Haugen 2000). If rapid local adaptation occurred, it could limit inferences of this study to other grayling populations in Montana shaped by different locally adaptive forces.

Despite the lack of statistically significant results and limitations in experimental design, the results do not provide a basis to discard current use in Montana of Arctic grayling optimal suitability spawning habitat criteria as ≤ 10% fines and ≥ 20% gravel and rubble (Warren and Jaeger 2017). This criteria represent a conservative benchmark but one that adheres to a precautionary principle approach to quantifying suitable spawning habitat. In larger stream networks occupied by Arctic grayling, much higher spatial and temporal variation in fine sediment transport and infiltration are expected. Repeat sediment surveys on Red Rock Creek and Elk Springs Creek in the Centennial Valley have yielded weighted estimates of suitable spawning habitat for grayling that vary from a low of 0.38 ha in 2016 followed by a rebound to a high of 3.97 ha in 2016 – 2017 (Warren et al. 2019). Modeling of sediment transport in gravel-bed streams indicates salmonid embryo survival is variable in space and time, but can be less than 10% under conditions of high bedload flux (Lisle and Lewis 1992). Survival of eggs and fry also declines rapidly with 10 – 30% exposure to ultrafine sediments less than 0.85 mm (Jensen et al. 2009). Given variability in fine sediment distribution and abundance in space and time in streams occupied by grayling, and small population size and recruitment potential of grayling present in most Montana stream systems, the criteria adopted by Warren and Jaeger seems appropriate at this time.

While several field studies in Montana have noted habitat preferences of young-of-the-year grayling (Nelson 1954, Skaar 1989, McMichael 1990, Deleray 1991, Levine 2007), studies of field conditions or laboratory manipulations of environmental conditions experienced by grayling eggs of swim up fry are almost nonexistent (Kaya 1989). Coupled laboratory and field studies may help ameliorate issues of uncertainty by allowing more control over the structure the spawning sediment matrix and impacts of other environmental variables such as water velocities, temperatures, and dissolved oxygen, providing greater confidence in survival-to-emergence estimates for individual eggs in a sample chamber (Chapman 1988, Kondolf 2000). It would be worth considering and perhaps testing alternate egg incubation chambers (Bowerman et al. 2014) in the laboratory to help address sediment matrix stability and egg exposure to expected treatment conditions prior to field experimentation. Laboratory study would also facilitate manipulation of suspended sediment source material, concentration, and exposure duration, as well as observations of grayling survival and behavior. In some circumstances, underyearling grayling preferentially seek out low velocity habitats (McClure and Gould 1991, Mogen 1996), which may provide some protection from exposure to suspended sediment. Surface water and groundwater dynamics may also influence egg to fry survival, as downwelling can counteract the impacts of fine sediment and significantly influence early life-history success in related salmonid species like bull trout (Bowerman et al. 2014). Coupled laboratory and field study of eggs and fry interacting with fine sediments infiltrated in the streambed and suspended sediment would be a fascinating, if challenging, direction for research (Newcombe and Jensen 1996, Haugen 2000, Sear et al. 2016). Finally, the ongoing hypothesis testing of grayling population dynamics in Centennial Valley streams involves several actions to increase fish passage and access to suitable spawning habitat. An attempt to measure if and how fine sediment mobilizes as suspended sediment and redistributes into the streambed is warranted. Continuation of existing sediment surveys would be enhanced by additional work to quantify suspended sediment loads and percent fraction of sand and silt (<2 mm) in the streambed. This could help inform further restoration actions in this and other stream systems occupied by Arctic grayling in Montana.

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APPENDICES

Appendix 1: Experimental sample unit photos, June 4 – 5, 2017.

Basket #3 (50% fine sediment treatment)

Basket #9 (50% fine sediment treatment)

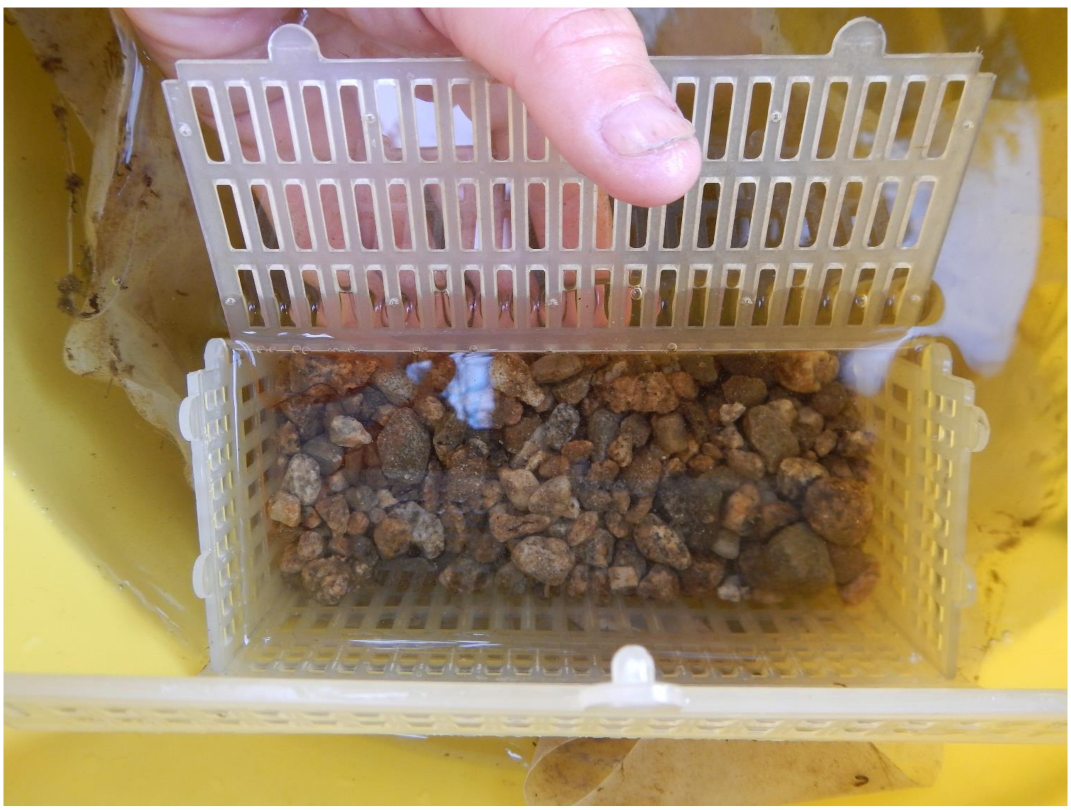
Basket #11 (50% fine sediment treatment)

Basket #12 (50% fine sediment treatment)

Basket #17 (50% fine sediment treatment)

Basket #1 (25% fine sediment treatment)

Basket #4 (25% fine sediment treatment)



Basket #10 (25% fine sediment treatment)

Basket #15 (25% fine sediment treatment)

Basket #19 (25% fine sediment treatment)


Basket #2 (10% fine sediment treatment)

Basket #13 (10% fine sediment treatment)

Basket #14 (10% fine sediment treatment)

Basket #18 (10% fine sediment treatment)

Basket #20 (10% fine sediment treatment)

Basket #5 (control 0% fine sediment treatment)

Basket #6 (control 0% fine sediment treatment)

Basket #7 (control 0% fine sediment treatment)

Basket #8 (control 0% fine sediment treatment)

Basket 16 (control 0% fine sediment treatment) – photo not available

Appendix 2: Thermograph downloaded temperature data (external Excel file spreadsheets).

Appendix 3: Experimental fish and habitat field data (external Excel file spreadsheets).

Appendix 4: Alternate ANOVA results and means plot for grayling emergence from experimental sediment treatments, 1 outlier sample unit removed from the high treatment group.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*StDev*
Control	5	114	22.8	21.7	4.7
Low	5	119	23.8	20.7	4.5
Medium	5	112	22.4	2.8	1.7
High	4	47	11.8	44.3	6.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	404.8711	3	134.957	6.456244	0.005062	3.287382
Within Groups	313.55	15	20.90333			
Total	718.4211	18				

Comparisons	**P-value**
control v. low	0.9852
control v. medium	0.9990
control v. high	0.0124
low v. medium	0.9614
low v. high	0.0065
medium v. high	0.0161