Evaluating Brook Trout Egg and Alevin Survival at Different Temperatures in Simulated Karst Environments with Marl Sedimentation

John Davidson, *The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443*

Clayton Raines, *U.S. Geological Survey, Eastern Ecological Science Center, Leetown Research Laboratory, 11649 Leetown Road, Kearneysville, WV 25430*

Curtis Crouse, *The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443*

Christopher Good, *The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443*

Brandon Keplinger[1], *West Virginia Division of Natural Resources, 1 Depot Street, Romney, WV 26757*

Abstract: Brook trout (*Salvelinus fontinalis*) have been extirpated from many karst-geology streams in West Virginia; however, the causes are not fully understood. Specifically, the impact of calcareous precipitate (marl), which is common in hard-water environments, has not been evaluated as an impediment to juvenile survival. Accordingly, two lab-based studies were conducted to determine if brook trout egg and alevin survival is inhibited by marl. In the first study, three aeration treatments were applied to water from a limestone spring source (13–14 C; ~300 mg L^{-1} hardness), resulting in different pH levels and an increasing degree of marl precipitate. Treatments included raw/untreated (RU; no marl), once-aerated (OA; limited marl), and continuously aerated (CA; significant marl) water. Brook trout eggs obtained from a local hatchery were fertilized and stocked among gravel-filled trays receiving each water type. Mortality occurred faster in CA water where marl coated egg surfaces, but cumulative survival was negligible for all water types. After 53 days, no surviving alevins remained in RU or CA, and 1% survival was observed in OA water. However, extra eggs maintained in a marl-producing system at 8 C without gravel demonstrated >50% survival. A second study was carried out to investigate this discrepancy. Survival was evaluated at three temperatures with and without gravel while producing a thin coating of marl. Increased prevalence of alevin deformities and significantly lower survival were observed at 13.7 C versus 8.1 and 11.2 C, but gravel inclusion did not affect these variables. Potentially harmful effects of marl were observed; however, juvenile brook trout survival was higher during Study 2. This research suggests that brook trout reintroduction efforts in karst-geology streams should be focused on microhabitats with limited marl production and adequate water temperatures for juvenile survival.

Key words: reintroduction, *Salvelinus fontinalis*, sediment, water quality, West Virginia

Journal of the Southeastern Association of Fish and Wildlife Agencies 10:27–35

Many indigenous brook trout (*Salvelinus fontinalis*) populations have been reduced or extirpated across their historic range in the southeastern United States (EBTJV 2006). In response, state agencies and interested conservation groups have endeavored to restore brook trout in this region (EBTJV 2018). Restoration strategies require knowledge of factors that negatively impact brook trout recruitment and survival, including loss of riparian habitat, detrimental land use, increasing water temperature (Wehrly et al. 2007, Stranko et al. 2008, Albertson et al. 2017), non-native species (Huntsman et al. 2022), and habitat fragmentation (Letcher et al. 2007), among others (EBTJV 2006, Hudy et al. 2008). In some cases, an understanding of local geochemical factors including aquifer geology (Briggs et al. 2018) and stream water chemistry (Cleveland et al. 1991, Baldigo and Murdoch 2011, Teears et al. 2020) are also important. For example, the Great Valley Region of West Virginia, which lies in the Potomac River watershed, has unique underlying geology (Dean et al. 1987, Kozar et al. 1992) and supports many streams where brook trout have been extirpated (Clingerman 2008). Numerous streams in this region originate from an aquifer dominated by limestone structures (Dean et al. 1987, McCoy and Kozar 2008). Subsurface springs, which feed many first- and second-order streams, dissolve calcite from these formations creating elevated water hardness (Kozar et al. 1992). Upon emergence, water from these springs releases carbon dioxide (CO_2), and subsequent degassing across riffles and waterfalls increases stream pH. These effects cause a shift in solute equilibrium and dispersal of calcareous precipitate (Langelier 1946, Herman and Hubbard 1990). Herman and Hubbard (1990) broadly define

1. E-mail: Brandon.j.keplinger@wv.gov

this precipitate as marl: soft, loose, earthy material consisting of a mixture of clay and calcium carbonate that creates unstable sediment in receiving streams.

Juvenile brook trout are negatively impacted by fine sediment loads (Curry and MacNeill 2004, Hartman and Hakala 2006, Franssen et al. 2012), implying that marl precipitates could have adverse effects. Furthermore, anthropogenic activities such as adverse land use and management (Kaushal et al. 2013) and mineral weathering caused by acid precipitation (Johnson et al. 1972) have accelerated conditions that support alkalinization and precipitation of calcareous sediments. There also are potential but undetermined interactive effects of increasing water temperature resulting from climate change. Additionally, Sear et al. (2016) found that sediment-specific attributes, including source, type, and mass load or quantity, affected brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) eggs differently, suggesting that effects on trout recruitment should be studied with specificity to sediment characteristics, fish species, and local stream conditions.

Research evaluating the impact of marl sediments on juvenile brook trout is lacking. To address this knowledge gap, we conducted two lab-based studies to evaluate the effects of continuous marl production in hard-water environments on brook trout egg and alevin survival. During the first study, three water types with varying CO_2, pH, and probability for marl were evaluated. These treatments represented an expected range of conditions in karst-geology streams as influenced by hydraulic agitation and CO_2 release along the flow path (Herman and Hubbard 1990). Following the results of the first study, we conducted a second study to assess the effect of water temperature and gravel inclusion in marl-producing systems. Findings from these studies were expected to direct local conservation efforts, including identification of streams and microhabitats best suited for brook trout reintroduction.

Methods

Research Site and Egg Source

Experiments were conducted at The Conservation Fund Freshwater Institute's aquaculture research facility in Shepherdstown, West Virginia in the fall of 2018 and 2019. Water used for these studies was pumped from a karst-geology spring, and existing aeration systems produced the necessary water chemistry, including marl. Eggs and milt from broodstock kept at the Paint Bank Fish Hatchery in Virginia were transported on ice to the study site. This stock was selected due to disease-free certification, general availability, and confirmed reproductive success in natural settings (MacAvoy and Bulger 1995, Humston et al. 2012). Use of this hatchery strain as a conspecific surrogate for native West Virginia brook trout was further justified by the documented introgression and complexity of brook trout populations in the Southern Appalachia region (Sherrill et al. 2001, Kazyak et al. 2021). Additionally, field-spawned eggs from heritage brook trout, i.e., genetically distinct populations native to West Virginia streams, could not be sacrificed due to limited availability and lacked disease-free certification. Upon arrival, eggs were fertilized, treated with a 10-min povidone-iodine bath, and acclimated to hatching system temperatures.

Experimental Design

During Study 1, five independent hatching trays per treatment received flows (1.9 L min^{-1}) from one of three water types (Figure 1), each of which originated from a karst-geology spring. Treatments included: raw/untreated (RU), once-aerated (OA), and continuously aerated (CA) water. Water for the RU treatment was pumped directly from the spring. The OA water passed through a gas conditioning tower containing packed plastic media to remove CO_2 (Summerfelt et al. 2003) and increase oxygen, and CA water subsequently was pumped through a 1300-L tank and a separate aeration column (Figure 1) to remove additional CO_2 and increase pH, thereby creating conditions that favored marl production. Treatments primarily differed by mean pH, CO_2 concentration, and likelihood to produce marl. Hatching trays included an inlet valve, barbed fitting, attached tubing positioned to facilitate laminar flow, and a 1-cm layer of pea gravel to mimic preferred spawning substrate (Magoulick and Wilzbach 1997). An opaque flap was also placed over trays to reduce light. Effluent from replicate trays positioned above respective tanks flowed through and discharged from these vessels (Figure 1). The selected temperature range (13.6–15.1 C) simulated conditions at a West Virginia Division of Natural Resources (WVDNR) facility where heritage brook trout eggs are hatched for reintroduction purposes (i.e., mean = 12.9 C, max = 14.9 C, SD = 0.65). Additionally, a separate chilled water (8 C) heath tray system void of gravel was used to maintain extra eggs (Figure 2) to match typical salmonid egg hatching procedures employed at the research site.

Dramatically different brook trout survival rates were observed in this system at a lower water temperature and without gravel substrate; thus, Study 2 evaluated the effects of water temperature and gravel inclusion on brook trout egg and alevin survival in systems with constant marl production. Three hatching systems with independent water chillers, pump sumps, and stacked heath trays (Figure 2) were used to maintain target temperatures of 8, 11, and 14 C. Spring water entering each system was continuously recycled resulting in pH of at least 7.9, CO_2 no more than 6 mg L^{-1}, and low marl production. Six hatching trays, three with and three without gravel, were used per temperature treatment.

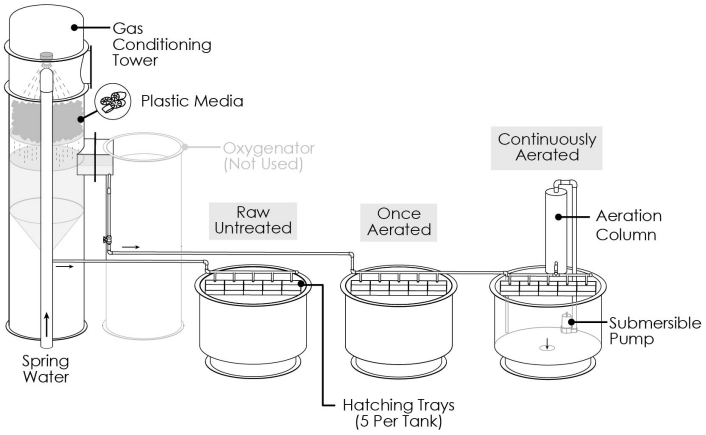

Figure 1. Water treatment processes, origin of tested water types, and experimental systems utilized during Study 1 of brook trout egg and alevin survival.

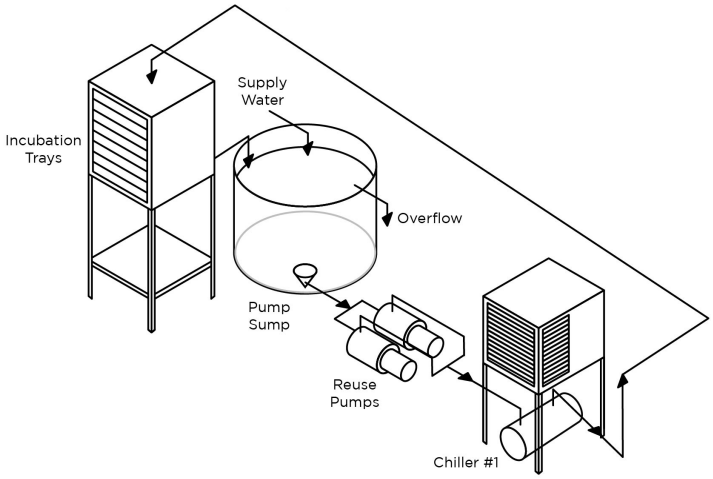

Figure 2. Individual hatching system design used to maintain chilled water with high pH, low CO_2, and affinity for marl in two studies of brook trout egg and alevin survival: Study 1 (extra brook trout eggs) and Study 2 (one of three replicate systems).

Brook Trout Eggs

A volumetric method was used to divide eggs into each tray. During Study 1, 5700 eggs were divided among treatments (~380 eggs per tray) and approximately 200 extra eggs were stocked in a separate marl-producing hatching system. During Study 2, 11,500 eggs were divided among treatments (~638 eggs per tray). Egg density across trays was 0.5–1.0 eggs cm^{-2} surface area. Dead eggs and fry were removed daily, and mortalities were counted to assess cumulative survival. When eggs reached 170–180 accumulated thermal units (ATU; i.e., cumulative temperature after egg fertilization), ten eggs per tray were removed and placed in a formalin-based solution which provided enhanced detail of developing embryos to affirm fertilization. At the end of each study, surviving fry were enumerated, and the prevalence of spinal deformities was visually assessed. Studies concluded when alevins had absorbed most of their yolk sac.

Water Quality Analyses

Carbon dioxide, pH, and observations of marl precipitate were collected before each study to validate target conditions. Water samples were gathered weekly during each study and tested for alkalinity, CO_2, total suspended solids (TSS), and total hardness. Nitrate-nitrogen (NO_3-N) was also measured during Study 1. Standard methods described by Hach Company (2015) and the American Public Health Association (2012) were followed for these analyses. Dissolved oxygen (DO), temperature, and pH were measured using a Hach HQ40d meter with LDO101 and PHC101 probes (Hach Company, Loveland, Colorado), and specific conductance and total gas pressure (TGP) were measured using a YSI Pro 30 device (Yellow Springs Instruments, Inc., Yellow Springs, Ohio) and Tensiometer 300E (In Situ, Fort Collins, Colorado), respectively. During Study 2, continuous temperature data was collected with Hobo loggers (Onset Computer Corporation, Bourne, Massachusetts) placed in each hatching system. Lastly, the Langelier Saturation Index, an indicator of calcium carbonate saturation, was used to project expected marl production (Langelier 1946). Presence of marl was also confirmed observationally, via photography, and quantitatively through pre- and post-study weights of plastic media placed in each hatching tray.

Fungal Infection: qPCR and Quantification

Saprolegniasis, commonly known as fungal infection, is a disease caused by a ubiquitous oomycete (Willoughby 1986) that periodically affects early life stage salmonids (Good et al. 2020). Because saprolegniasis was observed during Study 1, water samples were collected from each treatment during Study 2 to understand its potential impact on survival. Triplicate samples were collected from each hatching system and the supply water, 6 and 28 days after study commencement, and during a third sampling event after eggs from each treatment had hatched. Samples were processed according to procedures described by Rocchi et al. (2017) and shipped to Bowling Green State University (BGSU) for quantification of *Saprolegnia* spp. DNA. Procedures used for *Saprolegnia* spp. quantification and qPCR primers were devised and optimized at BGSU (Ghosh et al. 2021). Additionally, several eggs with suspected fungus were viewed microscopically to verify infection.

Data Analyses

Means and either SE or SD were calculated for water quality variables and brook trout survival for each treatment (Study 1:

$n = 5$; Study 2: $n = 3$). During Study 1, ANOVA was used to evaluate water chemistry parameters, and a Tukey's test was employed to identify treatment differences. A permutational multivariate analysis of covariance (PERMANCOVA), using 9999 permutations, was applied to determine if Study 1 daily mortality differed between treatments or as an interactive function of water quality. Total alkalinity, TSS, pH, CO_2, hardness, NO_3-N, DO, TGP, specific conductance, and temperature were included as covariates, and all interaction terms were considered. The PERMANCOVA was applied to a similarity matrix constructed from square root transformed daily mortality values and using the Euclidian distance coefficient. Multivariate analyses were conducted with the PERMANOVA+ add-on package for PRIMER-E v7 (Anderson et al. 2008, Clarke and Gorley 2015). During Study 2, a 2-way ANOVA followed by Tukey's test was used to evaluate differences in survival related to water temperature, substrate, and their potential interaction, using SYSTAT version 13 (Systat Software, Inc. 2009). All tests used a significance level of $\alpha = 0.05$.

Results

Study 1

Mean water quality concentrations were consistent within treatment as reflected by negligible SE (Table 1). As expected, differences were detected among treatments for most water chemistry parameters ($F_{2,12} = 5.19$ to 133,770, $P = < 0.001$ to 0.042), except for nitrate-nitrogen ($F_{2,12} = 0.21$, $P = 0.813$; Table 1). Specifically, CA water demonstrated lower alkalinity, CO_2, and specific conductance, and higher DO, pH, TSS, and temperature (Table 1). Further, marl was created in CA water, whereas trays receiving RU and OA water were generally free of visible precipitates. Reduced specific conductance and alkalinity levels, and higher TSS measurements reflected a tendency for ions to precipitate out of solution in the CA treatments compared to RU and OA. Continuous pumping and water recirculation slightly increased water temperatures for CA compared to RU and OA (Table 1).

In Study 1, successful fertilization was estimated for approximately 80% of eggs from each treatment based on observations of embryonic development. Proof of successful fertilization was critical, because mortality occurred rapidly across treatments, albeit at different rates (Figure 3). After three weeks, only 17% of fertilized eggs were viable in CA water (250 ATU) compared to 65% and 56% survival for RU and OA water (236 ATU), respectively. Hatching was asynchronous across treatments. Average hatch for RU, OA, and CA occurred on Days 33, 28, and 23 post-stocking, or at 457, 388, and 338 ATU, respectively. After hatching was complete, mortality in the CA treatment spiked and most alevins died over the next 10 days. No surviving alevins remained in RU and CA water after 53 days (718 and 754 ATU, respectively). Very low survival (1%) was documented for OA water (718 ATU; Figure 3). Further, most survivors from the OA treatment demonstrated spinal deformities including curled bodies and contorted tails. Based on PERMANCOVA tests, daily mortality varied with TSS ($F = 10.73$, $P = 0.001$), total alkalinity ($F = 6.42$, $P = 0.003$), and CO_2 ($F = 5.29$, $P = 0.008$). Overall, brook trout eggs and alevins did not survive at temperatures higher than 13.8 C, pH outside 7.1–8.1, with CO_2 outside 6–51 mg L^{-1}, or in the presence of visible precipitate. Beyond these water chemistry effects, saprolegniasis was observed

Table 1. Water chemistry (mean [SE]; $n = 5$) and ANOVA test results for aeration treatments evaluated during brook trout egg and alevin survival Study 1. Means with the same superscript were similar among treatments based on Tukey's Test or overall non-significant ANOVA result.

Water quality variable	Raw untreated	Once aerated	Continuously aerated	*P*
Langelier saturation index value	–0.1	0.4	1.0	n/a
Langelier saturation index outcome	Balanced	Balanced	Faint coating	n/a
Alkalinity (mg L^{-1})	285 (0.5)^a	286 (0.4)^a	280 (0.4)^b	<0.001
Carbon dioxide (mg L^{-1})	51 (0.1)^a	25 (0.1)^b	6 (0.1)^c	<0.001
Dissolved oxygen (mg L^{-1})	4.0 (<0.1)^c	8.3 (<0.1)^b	10.1 (<0.3)^a	<0.001
pH	7.1 (< 0.01)^c	7.5 (< 0.01)^b	8.1 (< 0.01)^a	<0.001
Specific conductance (uS cm^{-1})	629 (<1)^a	629 (<1)^a	611 (<1)^b	<0.001
Total suspended solids (mg L^{-1})	0.6 (<0.1)^b	0.7 (<0.1)^b	1.6 (0.4)^a	0.023
Hardness as $CaCO_3$ (mg L^{-1})	300 (2)^b	306 (1)^a	300 (1)^b	0.042
Nitrate nitrogen (mg L^{-1})	2.5 (<0.1)^a	2.5 (<0.1)^a	2.5 (<0.1)^a	0.813
Temperature (C)	13.8 (<0.01)^b	13.8 (<0.01)^b	14.5 (<0.01)^a	< 0.001
Total gas pressure (all gases [%])	98.8	100.1	100.4	n/a

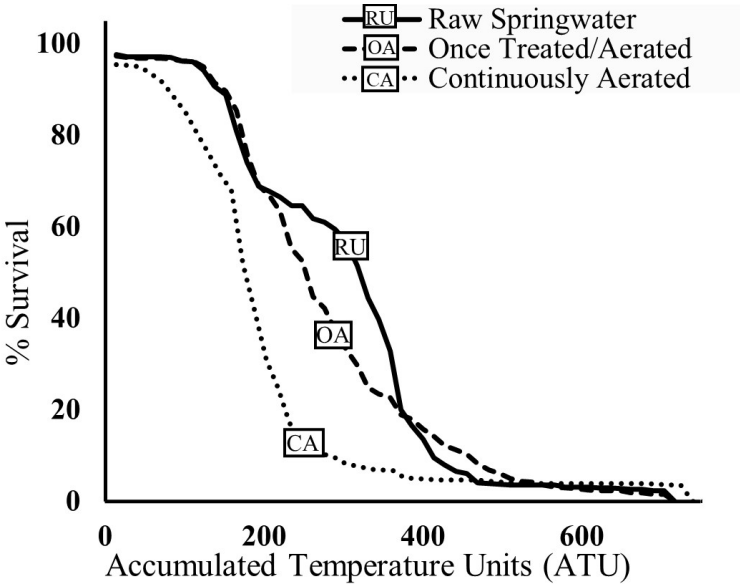

Figure 3. Mean brook trout survival from egg fertilization and stocking to yolk sac absorption for three aeration treatments during Study 1.

within each treatment. As noted previously, a much higher survival rate (>50%) was simultaneously observed in a non-replicated, marl-producing system maintained at 8 C without gravel, leading to Study 2.

Study 2

Mean water-quality concentrations were consistent among treatments during Study 2, except for temperature (Table 2). Langelier Saturation Index calculations also predicted that all treatments were expected to produce marl (Table 2), and general observations provided confirmation. Initial and end of study weights of installed plastic media demonstrated a weight increase of 63–97%, providing further evidence of marl deposition and adherence. Water temperatures were generally maintained within 0.3–0.5 C of the mean, except for short-term chiller failures in the 8 and 14 C treatments, which were quickly resolved.

One day following egg fertilization and stocking in Study 1, a mortality spike occurred resulting in 29–46% loss per hatching tray. This mortality event happened across treatments and subsided after one day; thus, mortalities were likely due to external causes including egg transport and handling. Day 1 mortalities were therefore excluded from cumulative survival calculations and a new baseline was established. Fertilization was confirmed in >90% of eggs for each treatment at 169–176 ATUs. Hatching occurred at approximately 511, 491, and 445 ATU at 8.1, 11.2, and 13.7 C, respectively. Eggs maintained at 13.7 C exhibited a faster mortality rate than those held at 8.1 and 11.2 C ($F_{2, 12} = 368$, $P < 0.001$; Figure 4). By the end of the study, juvenile brook trout maintained at 8.1 and 11.2 C demonstrated at least 50% survival, whereas survival of those kept at 13.7 C was no higher than 22% (Figure 4). No significant effect of gravel substrate was detected ($F_{1, 12} = 0.16$, $P = 0.694$); an interaction between temperature and gravel was observed ($F_{2, 12} = 6.12$, $P = 0.015$) but was not consistent across temperature × substrate treatments (Figure 4). A significant difference in deformity percentage was also detected between treatments related to temperature ($F_{2, 12} = 392.00$, $P < 0.001$) when the alevins reached 556–563 ATU, where more than half of surviving fish kept at 13.7 C exhibited curved spines (Figure 5). Further, marl was observed as a coating on egg membranes (Figure 6).

The qPCR analysis showed that *Saprolegnia* spp. DNA was consistently detected in the supply water and recirculating water of each Study 2 treatment, but saprolegniasis minimally affected eggs. Statistical differences in *Saprolegnia* spp. DNA concentration were detected based on temperature ($F_{2, 12} = 6.38$ to 68.20, $P \leq 0.013$); however, the effect shifted from lower *Saprolegnia* spp. DNA concentrations at 13.7 C during the first sampling to lower DNA concentrations for the 11.2 C treatment for the second and third sampling events. Overall, the magnitude of reported values and

Table 2. Water chemistry data (mean [SD]; $n = 3$) for water temperature × substrate treatments evaluated during brook trout egg and alevin survival Study 2. Concentrations are mg L^{-1}.

Target C	Gravel present?	Handheld meter C	Hobo logger mean C	Hobo logger max C	pH	Carbon dioxide	Total alkalinity	Total hardness
14	Yes	13.5 (0.5)	13.7	15.9	8.0 (0.1)	3.0 (1.2)	224 (24)	268 (32)
14	No	13.5 (0.5)	13.7	15.9	8.0 (0.1)	2.8 (1.1)	220 (29)	262 (27)
11	Yes	11.2 (0.2)	11.2	11.9	7.9 (0.1)	5.7 (1.6)	261 (10)	294 (14)
11	No	11.2 (0.2)	11.2	11.9	7.9 (0.1)	6.0 (1.7)	256 (14)	296 (13)
8	Yes	8.1 (0.2)	8.1	13.0	8.2 (0.1)	2.6 (0.9)	210 (20)	267 (13)
8	No	8.1 (0.2)	8.1	13.0	8.2 (0.1)	2.7 (0.9)	208 (20)	263 (15)

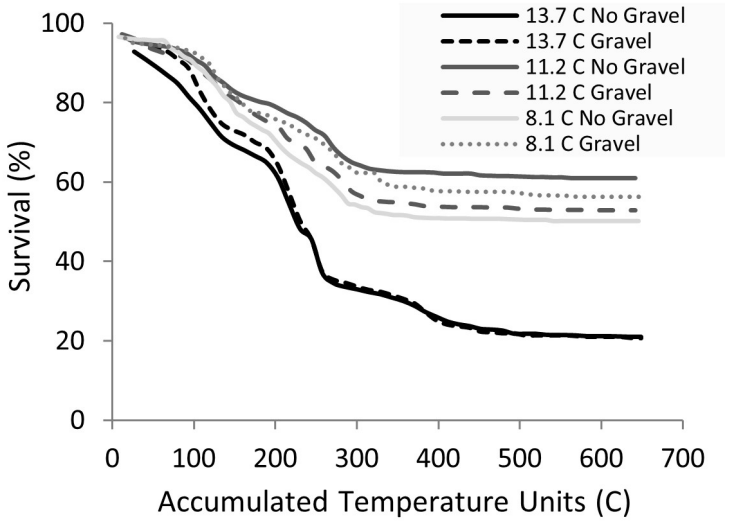

Figure 4. Brook trout survival within each temperature × substrate treatment during Study 2.

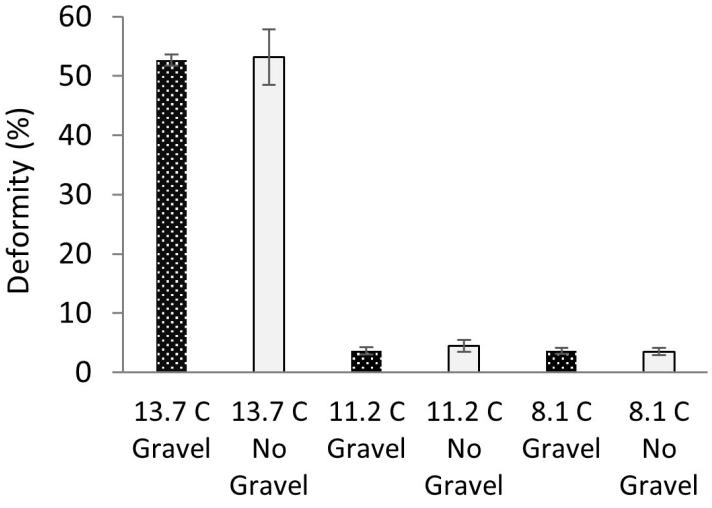

Figure 5. Percentage of surviving brook trout alevins exhibiting spinal deformities (mean ± SE; $n = 3$) for temperature × substrate treatments during Study 2.

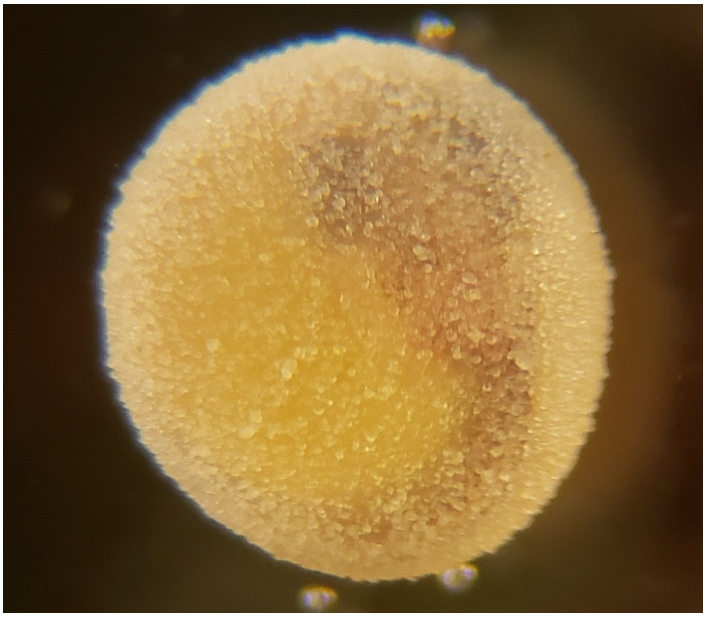

Figure 6. Microscope photograph of a brook trout egg covered with marl precipitate (Study 2).

differences between treatments was relatively small and similar to *Saprolegnia* spp. DNA concentration in the supply water. No effects of substrate or interactive effect of temperature × substrate were detected for *Saprolegnia* spp. DNA concentration ($F_{1-2,\ 12} = 0.23$ to 3.45, $P \geq 0.065$).

Discussion

Effects of Marl Precipitate

This research sought to understand the effect of karst conditions, particularly marl precipitate, on early life stage brook trout. However, influence of other factors, namely water temperature, generally masked hypothesized effects. Nevertheless, potentially harmful effects of marl deposition were observed. For example, the mortality rate for the CA condition that produced visible marl was steeper than other treatments suggesting that this water type was initially more detrimental to embryo survival. Differences in Study 1 daily mortality were partly related to TSS; higher TSS in CA water was likely due to marl precipitate (Langelier 1946). In addition, hatching and subsequent alevin mortality occurred sooner in CA water during Study 1 (338 ATU), suggesting that hatching may have occurred prematurely and/or embryonic development was not sufficient for survival. Premature hatching occurring at 338 ATU is supported by timing of hatch during Study 2 (445–511 ATU from 8.1–13.7 C), which coincided with higher survival rates. Moreover, Baird et al. (2002) reported brook trout hatching at 457–672 ATUs from 5.1–9.4 C under natural stream conditions.

Although the reason for differences in Study 1 hatching rates cannot be explained definitively, these findings could, in part, be related to the propensity for brook trout to select spawning locations that are associated with upwelling water (Webster and Eiriksdottir 1976, Curry and Noakes 1995, Alberto et al. 2017) that is thermally and chemically similar to RU or OA water, but unlike CA water. Alberto et al. (2017) reported increased early life-stage survival of brook trout that selected spawning sites with upwelling groundwater and observed preference of these locations vs. areas with fine sediments. Evidence of an adverse effect of marl was also implied by microscope imagery of an egg from Study 2 that was coated with calcareous crystals (Figure 6). This condition may have impeded oxygen and nutrient transfer across the egg membrane. Franssen et al. (2012) described egg entombment and asphyxiation effects caused by fines-rich substrates as a cause for brook trout embryo mortality in artificially constructed redds.

Although detrimental effects of marl precipitate were observed during these trials, Study 2 findings imply that these sediments alone do not cause catastrophic mortality of juvenile brook trout. Observational, analytical, and photographic evidence confirmed the creation of marl during Study 2. Unlike Study 1, however, surviving alevins were observed within all treatments including at least 50% survival at 8.1 and 11.2 C despite marl deposition on hatching trays, gravel, and egg surfaces. Conversely, eggs kept at 13.7 C exhibited low survival and most surviving alevins demonstrated spinal deformities, suggesting that a finite temperature threshold was exceeded. Study 2 findings match those of Alberto et al. (2017) who reported that fine sediment loads did not negatively affect the survival of brook trout embryos at relatively low temperatures.

Temperature Implications

The present studies demonstrated hatching success and substantial survival at 8 C and 11 C and catastrophic mortality and/or a significant decline in survival at 13.7–14.5 C. Of the surviving alevins observed at warmer temperatures, most demonstrated spinal deformities. The upper temperature threshold for adult brook trout subsistence in natural waters is reportedly near 22 C (Wehrly et al. 2007, Petty and Merriam 2010); however, temperature thresholds for eggs and alevins have been sparsely reported, particularly with specificity to hard-water streams that are prone to marl precipitate. Raleigh (1982) indicated that brook trout generally spawn at temperatures ranging from 4.5–10 C, and Cook et al. (2018) found that survival of brook trout fry increased linearly from 2–9 C. Further, Hokanson et al. (1973) reported that the upper median temperature limit for brook trout hatching was 12.7 C during a study assessing embryo survival from 6–18 C. The same authors noted increased incidence of deformed alevins at 15 C vs. 9 C.

Interestingly, the WVDNR has observed approximately 60% survival in heritage strain brook trout hatched at temperatures within the upper tested range (13.7–14.5 C) of the present studies (B. Keplinger, unpublished data). Although juvenile brook trout survival was not compared with time or strain between the research site and WVDNR facilities, varying survival results are of interest. For instance, surviving populations of West Virginia heritage brook trout may have developed an adaptive tolerance to elevated temperatures, similar to what Carline and Machung (2001) found for wild brook trout strains compared to domestic strains. Brook trout strains with southern U.S. ancestry have also demonstrated greater thermal tolerance to rising temperatures than northern populations (McDermid et al. 2012, Stitt et al. 2014). However, it is important to note that hatchery-reared brook trout of northern ancestry are widespread through the Southern Appalachian region and interbreeding with wild southern populations has resulted in mixed genetic origin (Sherrill et al. 2001). Similarly, brook trout used for the present studies are suspected to have originated from a northern hatchery (Nashua, New Hampshire; B. Beers, Paint Bank Fish Hatchery, Virginia, personal communication).

Interacting Water Chemistry

Although the present studies were not designed to study the interaction of marl and water temperature, the steeper mortality rate observed in the marl-producing CA treatment during Study 1 could point to an interactive effect. Moreover, Teears et al. (2020) conducted a study with brook trout eggs from the same Virginia hatchery and found that intermediate concentrations of calcium and increased acid neutralizing (buffering) capacity of natural springs provided improved water quality for brook trout, but nitrogen gas (N_2) saturation had negative effects. However, N_2 saturation was higher than in our study and Teears et al. (2020) did not evaluate or mention effects of marl. Interestingly, brook trout eggs in that study were exposed to certain conditions that were similar to the present study, including pH levels 7.8–8.2 and temperatures ranging from 9.7 C to 14.7 C, but survival was ≥ 60% for all tested groups. Relatively high survival of brook trout from the same egg provider at temperatures up to 14.7 C (Teears et al. 2020), and assumedly without observation of marl, is interesting when considering the high mortality rate observed at ~14 C during our studies, possibly pointing to an additive effect of marl. Furthermore, WVDNR successfully hatches brook trout at temperatures reaching 14.9 C, but in relatively soft water ($CaCO_3 \leq 80$ mg L^{-1}) with no marl. Future research could provide additional clarity by hatching brook trout eggs (Paint Bank and/or West Virginia heritage strain) within a similar temperature range in soft water without marl precipitate vs. hard water conditions that produce marl.

Management Implications

This study confined brook trout eggs and alevins to specific temperatures and conditions that produced marl. In natural settings, however, brook trout have opportunities to seek out cold-water refugia and microhabitats with upwelling spring water that are preferred for spawning (Webster and Eiriksdottir 1976, Curry and Noakes 1995, Alberto et al. 2017, Briggs et al. 2018). Further, although karst-geology streams are generally dominated by long stretches with calcareous precipitate, marl-free zones exist. For example, Herman and Hubbard (1990) reported that marl deposition occurred in a Virginia stream across stretches with the greatest hydraulic agitation such as turbulent runs, cascades, or waterfalls, but marl was generally absent immediately downstream from upwelling springs until the water reached a second significant cascade. Findings from a West Virginia study also indicated that stretches of a karst-geology stream situated immediately downstream from cold-water inputs had minimal fines imparted by marl and relatively abundant gravel, thereby offering potential brook trout spawning sites (Petty and Merriam 2010). Accordingly, although the present study hypothesized and provided some evidence that marl can negatively impact brook trout eggs and alevins, calcareous sediment did not cause catastrophic juvenile mortality, particularly at lower water temperatures. Availability of potential brook trout spawning habitats in karst-geology streams may therefore be greater than initially expected. Moreover, when considering the likelihood of adaptive temperature tolerance, West Virginia heritage brook trout could be excellent candidates for reintroduction in local streams with relatively warm water. Based on the body of evidence provided by this research, we conclude that identification of microhabitats with reduced loads of marl precipitate and temperature regimes that are adequate for juvenile brook trout survival could be identified in karst-geology streams, and reintroduction efforts could be focused within these areas.

Acknowledgements

Special thanks are extended to Freshwater Institute staff who supported this work including Natalie Redman, Megan Murray, Destiny Evy, and Kevin Turner—water chemistry analysis, Travis May and Anna DiCocco—fish production, Kata Sharrer—graphics design, and Shanen Cogan and JC Stanley—maintenance and operations. We also thank Jacob Whalen (WVDNR) for egg delivery, Brian Beers (Paint Bank Fish Hatchery) for brook trout eggs, Vipaporn Phuntumart, Satyaki Ghosh, and Gayathri Udayangika Beligala (Bowling Green University) for quantifying *Saprolegnia* spp. DNA, and to Mark Scott (WVDNR) for his support of this partnership. This project was funded by the WVDNR Fisheries Management Section. Any opinion, findings, and conclusions or

recommendations expressed in this material are those of the authors and do not reflect the views of the participating agencies. The use of trade, product, or firm names does not imply endorsement.

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