Summer 2014

Effects of fine sediment on juvenile brook trout habitat use and social interactions

Kyle Snow
James Madison University

Follow this and additional works at: https://commons.lib.jmu.edu/master201019

Part of the Biology Commons

Recommended Citation
https://commons.lib.jmu.edu/master201019/336

This Thesis is brought to you for free and open access by the The Graduate School at JMU Scholarly Commons. It has been accepted for inclusion in Masters Theses by an authorized administrator of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.
Effects of fine sediment on juvenile brook trout habitat use and social interactions

Kyle Snow

A thesis submitted to the Graduate Faculty of
JAMES MADISON UNIVERSITY
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

Department of Biology

August 2014
Dedication

I dedicate this work to my loving wife Mary, as it would not have been possible without her never ending support and encouragement. No matter how many hours I was away “playing with fish,” there was always a home cooked meal waiting for me at home. Thank you for waiting for me, Mary.
Acknowledgements

I first want to acknowledge my advisor Dr. Christine May, without whose guidance, support, and encouragement this project would not have been possible. I want to thank my thesis committee members, Dr. Patrice Ludwig, Dr. Thomas Benzing, and Mr. Mark Hudy, for their suggestions and advice throughout this project. Thank to Mr. Hopper, for throwing up an enormously welcoming wave to the passing JMU van and subsequently allowing access to his land for fish collection. I would also like to recognize Dr. Lihua Chen for her assistance with statistical analyses, and Stephen Fisher for his assistance during long hours in the trout room. Finally, thank you to DMF Bait Company for their generous donations of invertebrate prey, with which I have trained the fish to say please and thank you.
# Table of Contents:

Dedication .................................................................................................................. ii
Acknowledgements ........................................................................................................ iii
List of Tables .................................................................................................................. v
List of Figures ................................................................................................................ vi
Abstract ........................................................................................................................ vii
Introduction ................................................................................................................... 1
Objectives ....................................................................................................................... 9

Methods .......................................................................................................................... 10
  * Artificial Stream Channel System Description ......................................................... 10
  * Fish Holding Rack System Description .................................................................. 12
  * Substrate Collection ................................................................................................. 12
  * Fish Collection ........................................................................................................ 13
  * Fish Acclimation and Feeding ................................................................................... 14
  * Swim Test .................................................................................................................. 15
  * Fork Length and Photographs .................................................................................. 17
  * Water Velocity Measurement .................................................................................. 17
  * Objective 1 - Winter Flood Simulation Trials ........................................................... 18
  * Objective 2 - Low Flow Cohort Trials ..................................................................... 21
  * Data Analysis .......................................................................................................... 25

Results ............................................................................................................................. 26
  * Rock Collection ........................................................................................................ 26
  * Swim Test and Fork Length ..................................................................................... 27
  * Objective 1 - Winter Flood Simulation Trials ........................................................... 27
  * Objective 2 - Low Flow Cohort Experiments ............................................................ 29

Discussion ....................................................................................................................... 34
  * Objective 1 - Winter Flood Simulation Trials ........................................................... 34
  * Objective 2 - Low Flow Cohort Experiments ............................................................ 35

Conclusion ....................................................................................................................... 40

Appendix A. Cards used for fish and cohort identification throughout experiments ...... 41
List of References .......................................................................................................... 44
List of Tables:

Table 1. Water quality parameter settings and alarm points held constant during experiments ................................................................. 13

Table 2. Definitions of swimming positions used during winter flood simulation trials ........................................................................ 19

Table 3. Definitions of aggressive interactions and swimming positions observed during low flow cohort trial observations ...................... 24

Table 4. Grain size distribution of coarse substrate in each treatment channel by number of particles and total mass (kg) in each grain size class .......... 26

Table 5. Swimming positions recorded for observations of individual fish during winter flood simulations ................................................. 28

Table 6. Cover use generalized linear model summary of coefficient parameters including all sedimentation levels ................................... 32

Table 7. Cover use generalized linear model summary of coefficient parameters including 0% and 15% fine sediment conditions only .............. 32

Table 8. Aggressiveness generalized linear mixed model summary of coefficient parameters ................................................................. 33
List of Figures:

Figure 1. Diagram of four replicate experimental channels in two tanks as viewed from above........................................................................................................................................10

Figure 2. Rocks and fish collections sites in the North Fork Tye River in Nelson County, Virginia........................................................................................................................................13

Figure 3. Disassembled swim tube components ..............................................................................................................................16

Figure 4. Flow diagram of winter flood simulation trials with individual fish............19

Figure 5. Flow diagram of winter flood simulation trials with cohorts of 6 fish........21

Figure 6. Aerial photographs of streambeds showing the full width and ~0.5 m length of each treatment.........................................................................................................................22

Figure 7. Frequency distribution for grain size class of coarse grains at the rock collection site (n=200)........................................................................................................................................25

Figure 8. Length distribution of fish as measured by fork length (n = 23).................26

Figure 9. Length distributions four cohorts of fish used in the low flow cohort trials.........................................................................................................................................................28

Figure 10. Mean number of aggressive interactions per hours by type for all cohorts, times, and temperature........................................................................................................................................30

Figure 11. Summary of low flow cohort experimental data, organized for visual assessment of trends........................................................................................................................................31
Abstract:

Streambed sedimentation is a persistent cause of impairment to the ecological condition of streams. The objectives of this study were to investigate 1) the effects of sedimentation and temperature on the use of streambed cover as a velocity refuge by juvenile brook trout in simulated winter flood conditions and 2) the effects of sedimentation, temperature, and time of day on cover use and social interactions of brook trout cohorts in low flow summer water conditions. Using artificial stream channels, experiments were conducted with wild juvenile brook trout collected from the North Fork Tye River, Nelson County, VA. Brook trout did not use streambed cover as a velocity refuge during winter flood simulations. Generalized linear mixed models were used to analyze cohort low flow conditions with sedimentation, temperature, and time as fixed effects and cohort as a random effect. Streambed cover use decreased significantly as temperature and sedimentation increased (p < 0.01). Aggression increased significantly as sedimentation increased (p < 0.01), decreased as temperature increased (p = 0.079), and decreased significantly throughout the day (p < 0.001). These results indicate streambed fine sediment deposition has adverse effects on salmonid carrying capacity through a reduction of juvenile rearing habitat availability and increased social stress.
Introduction:

According to the United States Environmental Protection Agency’s Wadeable Streams Assessment (2006), 42% of streams in the nation are in poor ecological condition based on biological, chemical, and physical evaluations. The primary contributors to biological impairment of streams are excess nitrogen and phosphorous, riparian disturbance, and increased sedimentation. While the most common source of impairment is excess nutrients, sedimentation presents the greatest relative risk to stream health due to the persistence of legacy sediments that results from the time and energy required for removal from the system.

Stream sediment is commonly divided into two categories: suspended load and bed load. Suspended load is carried in the water column while bed load consists of larger particles that typically slide, roll, or tumble along the streambed. Eleven particle size classes are commonly used by stream ecologists as defined by Cummins (1962), ranging from clay (< 4 μm) to boulders (> 256 mm). The smallest particle categories, silt and clay (< 63 μm), contribute the majority of suspended load. Sand particles (63 μm - 2 mm) are an intermediate category, moving as suspended load at times and as bed load at other times. Bed load consists mostly of particles > 2 mm, categorized as gravel, pebbles, cobbles, and boulders. The two largest sediment particle classes, cobble (64 - 256 mm) and boulders (> 256 mm), are typically beneficial to stream organisms and are therefore not considered pollutants by biologists (Waters, 1995). Transport of sediment is controlled by particle size and channel flow. Sedimentation, or the deposition of sediment on the streambed, occurs when sediment input exceeds transport capacity (Yager et al., 2007).
Some natural sedimentation is expected and essential for habitat heterogeneity (Yarnell et al., 2006); however, the quantities of anthropogenic inputs overwhelm the assimilative capacity of streams (Cairns, 1977). Human activities such as: agriculture, forestry, mining, and urban development, increase the delivery of fine sediment to streams (Wood & Armitage, 1997; Nietch et al., 2005). Row crop cultivation on floodplains and livestock grazing in riparian zones are the two primary sources of agricultural streambed sedimentation (Waters, 1995). Logging roads cause soil compaction that reduces infiltration and increases overland flow and surface erosion (Anderson et al., 1976; Furniss et al., 1991; Waters, 1995) and have been shown to initiate larger landslides and longer debris flows than occur natural or even disturbed forests (May, 2002). Mining can directly disrupt streambed and alluvial sediments and generate large volumes of tailings that are subject to erosion (Waters, 1995).

The Environmental Protection Agency identified the Eastern Highlands as the most impaired ecoregion in the United States, with 52% of streams in poor condition (USEPA, 2006). The brook trout (Salvelinus fontinalis) is an iconic and charismatic species as the only extant salmonid native to the Eastern Highlands, and its entire historic native range is within this ecoregion (USEPA, 2006; Hudy et al., 2008). Their distribution is limited to cold water streams high in dissolved oxygen. They are sensitive to changes in water conditions and often used as an indicator of the overall health of the streams they inhabit. Strong brook trout populations indicate a healthy ecosystem, and reduced brook trout populations can indicate decline of the ecosystem. In the United States, potential brook trout habitat has been reduced to 70% of their predicted historic native range. Intact, self-sustaining, wild populations of brook trout exist in only 66% of this reduced range (Hudy
Increases in acidification, water temperature, habitat fragmentation, and sedimentation are all factors that negatively affect the persistence of wild populations. Sedimentation has reduced or extirpated populations of many gravel-spawning fishes, such as salmonids, through the limitation of available spawning habitat, while other fishes with different reproductive strategies within the same communities are less affected.

The impacts of sedimentation on a pristine brook trout stream were studied in a 15-year experiment by the Michigan Department of Natural Resources at the Hunt Creek Fisheries Research Station (Alexander & Hansen, 1986). In this long term experiment, sediment was added daily for one year to a stream at a fixed point. A sediment removal basin was placed in the channel one mile downstream. Stream morphological and biological data were collected for five years before and after the addition of sediment, with one mile reach directly upstream as a control. A “wave” of sand bed load moved slowly downstream and accumulated in deep pools, first mid-channel and then out to the banks. Increased water surface height, water velocity, and channel width were seen in the treatment reach. Additionally, there were lower macroinvertebrate and brook trout densities in the experimental treatments relative to the control. Negative effects persisted after sand deposition ceased. The center of the channel began to scour and degrade towards its natural state, while legacy sediments remained along channel margins past the five years of data collection.

The effects of sedimentation persist through time and across trophic levels of the lotic environment. Primary productivity reduces light available for photosynthesis thus decreasing primary productivity. Disruption and abrasion by sediment in transport
reduces primary producers that attach to substrate surfaces, such as periphyton, algae, and macrophytes. Extreme cases of reduced macrophyte and periphyton growth can alter channel morphology through the reduction of heterogeneity and channel roughness (Wood & Armitage, 1997). Sedimentation also reduces macroinvertebrate habitat, abundance, and diversity (Alexander & Hansen, 1986; Richards & Bacon, 1994). These effects impact apex predators (e.g. salmonid species) through the trophic cascade.

Berkman and Rabeni (1987) found that common feeding guilds are similarly affected by sedimentation, and benthic feeders are the most vulnerable.

A reduction of macroinvertebrate prey negatively affects the growth rate of juvenile salmonids (Crouse et al., 1981; Suttle et al., 2004). Increased fine sediment can lead to a decrease in relative abundance of macroinvertebrates available as prey for insectivorous fish and an increase relative abundance of burrowing taxa (Suttle et al., 2004; Cover et al., 2008). In a controlled laboratory environment, Crouse et al. (1981) simulated a natural stream ecosystem and invertebrate community and varied the extent to which sediment covered a cobble streambed. Six treatments were established at 20% intervals from 0-100% embeddedness. Juvenile coho salmon were introduced, and their growth rates monitored for several months. A negative correlation was found between sedimentation and growth rate, which was significantly reduced in 80 and 100% embeddedness treatments. This outcome might result from decreased invertebrate abundance, although this cause may have been masked as invertebrates were stocked equally in all treatments throughout the experiment to simulate natural drift. Increased sedimentation may have also limited the foraging ability of the fish. Invertebrate abundance and survival was not measured so the direct cause cannot be assessed. In a
field experiment, Suttle et al. (2004) confined juvenile steelhead salmon (*Oncorhynchus mykiss*) to artificially embedded sections of their native stream environment and allowed natural drift of invertebrate food sources. As fine sediment increased, invertebrate prey availability and fish growth decreased. Confinement of the fish greatly restricted available foraging area and may have exaggerated the reduction in fish growth. An increase in swimming activity and aggressive interactions between fish was observed with increased sedimentation, and fish confinement may also have exaggerated this response.

Sedimentation has been shown to reduce egg and alevin survival. Suitable spawning habitat is a critical factor that controls salmonid population density (Bjornn & Reiser, 1991; Louhi et al., 2011). Berkman and Rabeni (1987) found that fish within common reproductive guilds show similar responses to sedimentation and that gravel spawners are the most susceptible to increased amounts of fine sediment. Salmonid spawning, egg incubation, and alevin rearing habitat is the subsurface area within which groundwater and surface water mix, called the hyporheic zone. In spawning, adults excavate a bed in the gravel substrate, lay and fertilize their eggs in the bed, and cover the eggs with a layer of gravel. The resulting subsurface egg nest is called a redd. As the eggs incubate, fine sediment deposition can occur above or within the redd (Lisle, 1989). Deposition of organic fine sediment within the egg pocket can result in a reduction of dissolved oxygen due to metabolic decomposition. Deposition of inorganic fine sediment over the redd can reduce hyporheic flow resulting in reduced oxygen delivery to the redd and reduced removal of metabolic waste from the redd (Malcolm et al., 2004; Greig et al., 2007).
Entombment occurs when fry are unable to emerge from the gravel due to fine sediment obstruction of the interstitial voids between larger particles (Sternecker & Geist, 2010).

Many salmonids use side channels and small tributaries as spawning sites, although juveniles will disperse to inhabit any available suitable habitat (Bjornn & Reiser, 1991). This behavior implies that suitable spawning habitat alone is insufficient to ensure the reproductive success of a population. In general, salmonids select stream positions to maximize energy intake in the summer, and to limit energy expenditure in the winter (Cunjak & Power, 1986). Suitable habitat may be the greatest factor in limiting a stream’s salmonid carrying capacity in the winter, while available energy from food sources may be more limiting during the summer (Chapman, 1966).

Interstitial spaces of a cobble and boulder streambed are a common habitat for daytime concealment cover of overwintering juvenile salmonids (Gibson, 1978; Griffith & Smith, 1993; Meyer & Griffith, 1997; Thurow, 2006). Fewer juvenile rainbow trout emigrated when substrate was layered relative to being evenly distributed in a single layer because stacked substrate provided interstitial cover space between particles (Meyer & Griffith, 1997). Cutthroat trout (*Oncorhynchus clarki*) and brown trout (*Salmo trutta*), showed an increase in juvenile trout density as substrate particle size increased for observations during winter (Griffith & Smith, 1993). In the same survey, nearly all fish were concealed under cover during the day but emerged into the channel at night. In a similar study, Thurow (2006) observed 17 juvenile bull trout (*Salvelinus confluentus*) concealed beneath substrate during the day; no individuals were visible in the water column. Forty individuals were observed swimming in the water column at the same site the following night. In experiments of gradual reduction in water temperature, Gibson
(1978) observed that some Atlantic salmon (Salmo salar) began to use streambed cover at 11°C, and nearly all disappeared into cover at 9°C. Cunjak (1988) observed overwintering juvenile Atlantic salmon resting under cover stones where water velocity was estimated near zero. Most of these resting locations were found mid channel, where sediment deposition was lowest.

One factor likely to influence winter habitat selection is a reduction in position holding ability at low water temperatures. Low water temperature correlates with the critical swimming and critical holding velocity (Rimmer, 1985; Hammer, 1995). Critical swimming velocity is the flow rate at which a fish can no longer maintain position in the water column and is displaced downstream. Similarly, critical holding velocity is the flow rate at which a fish can no longer hold a resting position against the streambed. Swimming ability of most fishes is reduced with water temperature, and brook trout show a strong reduction in swimming ability at winter temperatures (Beamish, 1978; Hammer, 1995). Reduced fish density observed under high flow conditions may suggest streambed cover is used as a velocity refuge (Heggenes, 2002; Finstad et al., 2007). Lower fish density and growth rates in habitats with a fine substratum may indicate the lack of such refugia (Suttle et al., 2004; Finstad et al., 2007; Finstad et al., 2009).

Swimming ability of most fishes also increases with body size and adults can maintain significantly higher swimming speeds than juveniles within species (Jones et al., 1974). This evidence suggests that brook trout are most susceptible to velocity disturbances as juveniles during their first winter. Limiting factors affecting all age classes must be alleviated in order to establish or restore self-sustaining wild populations. Establishing suitable spawning habitat is the first step towards restoring native
populations of brook trout, but adequate habitat for juvenile rearing is also required for the new age class to survive to reach sexual maturity.

When confronted with habitats of limited resources such as food and space, salmonid populations establish strong stable dominance hierarchies (Chapman, 1966; McCarthy et al., 1992). Social status among individuals is determined through agonistic interactions, leading to physiological stress that affects subordinate individuals more than dominant individuals (Currie et al., 2010; LeBlanc, 2011; Grobler & Wood, 2013). When size discrepancies exist between individuals, position within the hierarchy can be well predicted by size, with larger fish holding higher social status (Huntingford et al., 1990; Hughes, 1992a; Hughes, 1992b; Johnsson et al., 1999; Young, 2003). Effects of social status are long lasting, as dominant fish hold preferred feeding positions (Hughes, 1992a; Hughes, 1992b; Nakano, 1995) and ultimately have greater growth rates and reproductive success (Metcalfe et al., 1989; Pottinger & Pickering, 1992; Tiira, 2009; Grobler & Wood, 2013).
Objectives:

The objectives of this study are:

1.) To investigate the effects of temperature and sedimentation on streambed cover use as a velocity refuge by juvenile brook trout in simulated winter flood. If brook trout use streambed cover as a velocity refuge, they will spend more time using cover under high flow conditions than low flow conditions. If temperature affects streambed cover use, cover use will increase as temperature is decreased. If sedimentation affects streambed cover use, cover use will decrease as sedimentation is increases.

2.) To investigate the effects of sedimentation, temperature, and time of day on cover use and social interactions of brook trout cohorts in low flow summer water conditions. If sedimentation, temperature, and time of day affect cover use and social interactions, brook trout cohorts will spend less time using cover in higher sediment levels and at higher temperatures, aggressive interactions will increase in higher sediment levels and at higher temperatures, and the intensity of these effects will vary over the course of a day.
Methods:

Artificial Stream Channel System Description

Experiments were conducted in a custom designed artificial stream system (Aquatic Habitats). A total of four replicate channels were used, each measuring 1.30 x 0.36 x 0.40 m (LxWxD). Two tanks each consisted of two channels adjacent lengthwise with a headbox at each end (Figure 1). Water inlets at the head of each channel generated flow and semicircular baffles in headboxes directed current to the adjacent channel. Channel ends were blocked by aquaculture netting (1/2” square mesh) to exclude fish from the head boxes. Glass windows measuring 1.10 x 0.23 m (LxD) in the tank walls allowed direct observation of the water column. Water returned through gravel at the bottom of each headbox to a common filtration, monitoring, and pumping station serving all four channels.

![Diagram of four replicate experimental channels in two tanks as viewed from above. Dashed lines represent observation windows in the tank wall, dotted lines represent aquaculture netting, arrows represent water inlets, and circles represent water returns.](image)

Water in the channel system was continuously circulated with a variable speed jet pump (IntelliFlo® VF, Pentair Aquatic Systems). Channel system water was displaced to an overflow drain as fresh reverse osmosis water was automatically added every 15 minutes for an exchange rate of 10% per day. Inline sensors continuously monitored water pH (Signet 2724 electrode and Signet 2750 sensor electronics, Georg Fischer), conductivity (Signet 2850, Georg Fischer), temperature (TA3333, IFM Electric),
dissolved oxygen (Model 420, OxyGaurd®), and velocity (Signet 2551, Georg Fischer). Two peristaltic pumps (45M5, Stenner Pump Company) fed sodium bicarbonate or salt solutions as needed to adjust water quality to the set parameters. Water also passed a 50 µm pleated paper sediment filter, carbon, an ultraviolet disinfection chamber and a heat exchange chamber before returning to the channels. Inside the heat exchange chamber, a titanium coil was supplied on demand with a countercurrent flow of chilled water (~6°C). No transfer of water occurred within the heat exchanger, only a transfer of energy through the titanium coil from the relatively warmer channel system water to the relatively colder chilled water. Flow of chilled water inside the coil was regulated by a solenoid value, allowing chilled water to pass through the coil only when the water temperature water returning from the experimental channel system measured ≥0.1°C above the set temperature.

All monitoring and output devices were integrated through a central programmable controller (C351, Aquatic Habitats). Programming options included customizable set points as well as high and low alarm points for water quality parameters (Table 1). This controller was connected to a dial out remote monitoring system (Sensaphone Model 800) to notify researchers by phone if an alarm parameter was reached. The system control was also linked through an ethernet connection to a desktop computer running RemoteOperator (Version 1.0.36.0, Unitronics®). This desktop computer was available through any computer or smart phone with an internet connection using TeamVeiwer (Version 9.0.24951) remote access software. This series of remote access connections allowed continuous monitoring and quick response in the event of an alarm indicating a deviation from specified conditions.
**Table 1:** Water quality parameter settings and alarm points held constant during experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Set Point</th>
<th>Low Alarm</th>
<th>High Alarm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Varied</td>
<td>Set point – 0.5</td>
<td>Set point + 0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>6.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Conductivity (µs/cm)</td>
<td>160</td>
<td>145</td>
<td>175</td>
</tr>
<tr>
<td>Dissolved Oxygen (%)</td>
<td>n/a</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

*Fish Holding Rack System Description*

A second recirculation system (Z-Hab, Aquatic Habitats) provided twelve 10-l holding tanks in which fish were individually housed. Opaque plastic dividers were placed between tanks to eliminate visual interaction between tanks, and fish were provided with a gravel bed and length of 3” PVC pipe cut in half lengthwise as a hiding place. Water was pumped into each tank at a rate of 2 l/min and an overflow on each tank returned water to a common filtration, monitoring, and pumping system with capabilities similar to the channel system. The rack system was not fitted with a water velocity sensor. Water was circulated with a smaller, fixed speed jet pump (MD-70RLZT, Iwaki Company). All other monitors, controls, and filtration processes were identical to those stated for the channel system.

*Substrate Collection*

Rocks were collected from the North Fork Tye River in Nelson County, VA (Lat 37° 52’ 52” N, Long 79° 6’ 12” W, Figure 2). The grain size distribution of the collection site was measured using a Wolman (1954) pebble count (n = 200), and the most dominant grain sizes in the pebble to cobble range of 32, 45, 64, and 90 mm were chosen for collection. These four categories include rocks that measure 32-128 mm on their median axis. All rocks were pressure washed to remove invertebrates and macrophytic
growth and allowed to dry in the sun for several days to limit the risk of introducing pathogens to the laboratory system.

**Figure 2:** Rocks and fish collections sites in the North Fork Tye River in Nelson County, Virginia. Fish were collected by electrofishing a 450 m reach.

*Fish Collection*

Fish were collected on May 22, 2013 from the North Fork Tye River, approximately one kilometer upstream of the rock collection site (VDGIF Permit # 047054, Figure 2). Stream water pH was 6.5, conductivity was 36 µS/cm, temperature ranged from 13.5° C at the beginning of collection (~10:00 am) to 15.2° C at the end of collection (~2:00 pm). A reach 450 in length was sampled using a Smith-Root LR-20 backpack electrofisher and dip nets. Pulsed direct current was selected and minimum
duty cycle and voltage settings were used to reduce the risk of injury to fish. All individuals under 200 mm (visual estimation) were held in a downstream live well until sampling was completed. All individuals were field measured for total length (TL) to the nearest mm in order to establish a general size distribution. A bimodal length frequency distribution with reduced numbers of individuals between 140-160 mm suggested a potential separation of year classes. Fish selection was therefore restricted to individuals between 100-135 mm TL with the intention of collecting all individuals of the 1+ year class. A total of 25 fish were transported back to the laboratory in two insulated live wells with air stones for aeration.

*Fish Acclimation and Feeding*

All handling, housing, and experimental procedures were conducted in compliance with IACUC Protocol #A04-13. Upon introduction to the laboratory environment, fish were haphazardly distributed among three of the experimental channels (8-9 fish/channel). Water temperature was set at 15.0° C during hours of light and 13° C during hours of dark to simulate the diurnal temperature fluctuations of a natural stream. Over 9 days, fish were gradually weaned off of this diurnal fluctuation to a constant temperature of 13° C. Fish did not experience any subsequent diurnal temperature variations. Coarse rock substrate 20-25 cm deep provided sufficient hiding space for the high fish density. The room lights were set to a 12:12 h light-dark cycle over the course of the experiments. Despite a cover of shade cloth stretched and secured over the top of the tanks, two fish were found dead on the floor the first morning of captivity. The sudden change from darkness to full light was suspected of startling the fish, so 30-minute dawn and dusk periods were added on the second day by placing indirect LED
lighting over the channels on a 13:11 h light/dark cycle. This lighting provided a more gradual change from light to dark conditions. Clear acrylic lids were placed over the channels on the second day and maintained throughout the experiments. Headboxes remained open to atmospheric exchange.

During the acclimation period in the channels, fish were presented with several live and artificial feeds until the fish accepted a diet of larval invertebrates. Fish were initially fed butter worms (*Chilecomadia moorei*) and then switched to diet of wax worms (*Galleria mellonella*), both commercially distributed as angling bait (DMF Bait Company) at a rate of one invertebrate per fish three times per week. The feeding schedule was adjusted as necessary to ensure fish were adequately fed between experiments and were not fed during experiments. Fish were allowed to acclimate to the laboratory without handling for a minimum of 9 days before being transferred by net to individual holding tanks in the rack system for a minimum of three days prior to their swim test.

**Swim Test**

Swim tests were performed on June 6-10, 2013 at a water temperature of 13°C. A clear acrylic swim tube (10.2 cm inside diameter) was custom designed for full submersion in any of the four experimental channels (Figure 3). The inlet of the swim tube was plumbed to the discharge of the water circulation pump, and the outlet of the swim tube was fixed to the aquaculture netting at the end of the channel. At the head of the swim tube, water passed through a 20 cm long flow straightener that also served as a fish block. The fish were confined to the swim chamber 72 cm in length, allowing > 5.5 body lengths of horizontal movement. In order to place fish in the swim tube with
minimal handling stress, the inlet plumbing and flow straightener at the head of the tube were easily removed and replaced by a 90° pipe fitting. The tube made a tight connection with one end of the elbow and the other end of the elbow rose slightly above the surface of the water in the channel. After an individual fish was poured from a specimen container into the swim tube, the elbow was removed and the inlet plumbing reassembled.

**Figure 3:** Disassembled swim tube components, including A) water supply inlet, B) removable 90° elbow fitting used to load fish into swim chamber, C) flow straightener (inset gives view from an additional angle), and D) swim chamber. Yellow meter stick included for scale and in aligned with discharge end of the clear swim tube as reference.

The test fish was allowed a two-minute acclimation period within the swim tube at a water velocity of 0.1 m/s. The water velocity was gradually increased over the next three minutes to a maximum velocity of 0.75 m/s. Inlets to the other three channels were closed during the swim test, directing all flow though the swim tube, and the circulation pump was run to maximum capacity. Water velocity was monitored by the inline flow sensor within the 1-1/2” pipe supplying water to the swim tube and converted to determine the velocity within the larger swim tube given constant discharge. Maximum velocity was held for five minutes, or as long as the test fish was able to maintain swimming without impingement on the aquaculture netting at the end of the tube. The swim tube was fully visible through the viewing window in the tank wall, and the test
was terminated immediately upon displacement of the test individual. Fish unable to complete the swim test were excluded from future experiments.

Fork Length and Photographs

Immediately following the swim test each fish was transferred by netting to a clear acrylic photo box fitted with a measurement scale. This was the final time that nets were used in handling fish, and all subsequent transfers were performed by catching fish in a clear plastic specimen container to limit handling stress. Fish were measured for fork length to the nearest mm and photographed from left and right lateral perspectives. Identity, fork length (FL) and photographs were printed on laminated cards for future identification of individuals though comparison of their unique red and yellow spot patterns (Appendix A). Fish were then returned to an experimental channel with the same group structure as when they were first introduced to the lab.

Water Velocity Measurement

Water velocity was measured at acclimation flow and maximum flow conditions of winter flood simulation trials (objective 1) and at the low flow of cohort trials (objective 2). Water velocity was measured at three points (5 cm in from each wall and at the center of the channel) along five transects evenly distributed throughout the channel. Measurements were taken at 40% of channel depth with a meter and wading rod. A USGS Pygmy Meter (Model 6205, Rickly Hydrological) was used for all measurements, with the exception of the two highest velocity points under flood conditions (nearest to the water inlets). These two points exceeded the range of the Pygmy meter, and were therefore measured by an electromagnetic meter (Flo-Mate 2000, Marsh McBirney).
Objective 1 - Winter Flood Simulation Trials

An individual channel was used to conduct experiments simulating winter flood conditions. A layer of commercial filter gravel (grain size 3.4-6.3 mm, Res-Kem Corporation) 5 cm deep was added to the channel bottom to provide a flexible base to construct a streambed on. The streambed of coarse substrate (rocks of grain sizes 32-90 mm) increased from a bed depth of ~15 cm at the inlet to ~30 cm at the outlet of the channel. This sloped bed was established to reduce backwater currents at the outlet end of the channel, and no fine sediment was added to the streambed.

Fish were housed individually in the rack system for a minimum of three day prior to experiments and were not fed for 24-hours prior to trials. A randomly selected individual fish was transferred without netting from a holding tank to the experimental channel, and allowed a two minute acclimation (Figure 4) period at a pumping rate of 57 l/min (average channel velocity 0.24 m/s, minimum 0.11 m/s, maximum 0.71 m/s). Flow was then gradually increased over four minutes to a maximum pumping rate of 246 l/min (average channel velocity 0.60 m/s, minimum 0.19 m/s, maximum 2.25 m/s). Sediment and carbon filters were bypassed during trials in order to reduce flow restriction. Instantaneous observations of swimming position were made every minute for 60 minutes (Table 2). All observations were made by a single observer in order to avoid between observer variance. Observations were made through a small eyehole cut in a box shaped blind over the viewing window, to avoid disturbance of the fish by the observer’s movements. A trial was terminated immediately if the test fish was impinged on the aquaculture netting at the end of the channel. Six trials were conducted at each of two temperature treatments, and no fish was used for more than one trial.
**Figure 4:** Flow diagram of winter flood simulation trials with individual fish.

**Table 2:** Definitions of swimming positions used during winter flood simulation trials.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Searching</td>
<td>Moving from place to place within the water column while maintaining a controlled swimming posture</td>
</tr>
<tr>
<td>Flailing</td>
<td>Moving about the water column by current and turbulence, lacking a controlled swimming posture</td>
</tr>
<tr>
<td>Maintaining</td>
<td>Maintaining swimming position over the streambed one body length by controlled swimming</td>
</tr>
<tr>
<td>Partial Cover</td>
<td>Resting in the interstices of the streambed with 50-90% of the body concealed</td>
</tr>
<tr>
<td>Complete Cover</td>
<td>Resting in the interstices of the streambed with &gt;90% of the body concealed</td>
</tr>
</tbody>
</table>

Six trials were conducted at 13° C (+/- 0.3° C) before the water temperature of the rack system was gradually reduced at a rate of ≤ 1° C/day to a constant temperature of 7.0° C (+/- 0.3° C). Fish were held for a minimum of 3 days at this temperature prior to trials. The heat exchanger on the channel system was unable to reduce the water temperature below 8.5° C therefore, ice was required before each trial to reduce the water temperature of the channel system further. This was the only time during all experiments that the water temperature was inconsistent between the rack and channel systems.

System water was removed from the channels and frozen in the days leading up to the trials in order to eliminate effects on water chemistry as ice melted in the channels.

Batches of 24 ice cube trays were frozen to -85° C. Before each trail, the chilled water
loop to the heat exchanger was disabled and one 5-gallon bucket of ice cubes was split between the headboxes. This reduced the water temperature in the channel system to a minimum temperature of ~6.5° C. Once the majority of ice had melted and the water temperature rebounded to the desired 7.0° C, a randomly selected individual fish was transferred without netting to the experimental channel to begin the trial. The water temperature gradually increased during the trial, and chilled water flow to the heat exchanger was restored 45 minutes into each trial in order to prevent the water temperature from rising above 8.5° C.

Sedimentation was predicted to reduce streambed cover use in simulated winter flood conditions. Cover use was not observed in simulated winter flood conditions in the absence of fine sediment, therefore the affect of fine sediment was not investigated during winter flood simulations. Exploratory winter flood experiments were conducted with six fish per trail due to the lack of streambed cover use during winter flood trial in trials with individual fish. The same channel and substrate configuration as described for winter flood trials of individuals was used, and six test fish were simultaneously introduced and allowed approximately 24 hours acclimation before observations. Trials lasted 140 minutes with extended low flow observations before and after simulated flood flows (Figure 5). One cohort was observed at 13° C and another at 7-9° C. Trials were conducted with each cohort during the day as well as at night under red lights for observation.
**Figure 5:** Flow diagram of winter flood simulation trials and cohorts of six fish.

**Objective 2 - Low Flow Cohort Trials**

Four cohorts of three fish each were established and maintained throughout the remaining experiments. Cohorts were not established randomly but rather individuals were selected to ensure variation in size within each cohort. Low flow pool conditions were simulated, with an average water velocity of 0.10 m/s (minimum 0.05 m/s, maximum 0.30 m/s) cohort trials were conducted at two treatment temperatures, 18\(^\circ\) C and 12\(^\circ\) C. In order to reduce the physiological stress caused by repeated fluctuations in water temperature, the order of experience for temperature treatments was not randomized. Water temperature was gradually increased at a rate of \(\leq 1\,^\circ\) C/day, and fish were held at a constant temperature of 18\(^\circ\) C (\(\pm 0.3\,^\circ\) C) for a minimum of 3 days prior to trials. Each cohort experienced all sediment treatments in a randomly established order. After 18\(^\circ\) C trials were complete, the water temperature was gradually reduced at a rate of \(\leq 1\,^\circ\) C/day until reaching a constant 12\(^\circ\) C (\(\pm 0.3\,^\circ\) C) for a minimum of 3 days prior to trials. Each cohort again experienced each sediment treatment in a randomly established order at 12\(^\circ\) C.
All four channels were used in low flow cohort trials, and sediment treatments of 0, 15, 30, and 45% fine sediment were randomly assigned to channels and assignments were maintained throughout the experiment (Figure 6). A layer of commercial filter gravel (grain size 3.4-6.3 mm, Res-Kem Corporation) 5 cm deep was added to the channel bottoms to provide a flexible base on which to construct the streambeds. Rocks were haphazardly distributed among the four channels, resulting in approximately equivalent grain size distributions. Added to each channel in descending size order, rocks were pieced together tightly to create a stable streambed of 15-20 cm depth. Fine sediment in the form of silica pool filter sand (Southern Products & Silica Company) was sieved to 0.35-1 mm and distributed over each of the treatment channels. The initial amount of sand added to each channel was measured by volume, with 4, 8, and 12 liters added to the 15, 30, and 45% fine sediment treatments, respectively. No sand was added to the control channel (0% fine sediment).

**Figure 6**: Aerial photographs of streambeds showing the full width and ~0.5 m length of each treatment. Percentages imbedded in each photograph indicate sedimentation treatment levels, measured by surface expression.

After hydraulically settling the sand into the interstices between rocks, the surface expression of fine sediment for each streambed was quantified by defining the substrate type at 120 points evenly distributed on a grid pattern over the entire streambed. A proportional error range of +/- 10% of the target percentage was allowed (i.e., the acceptable range for the 15% fine sediment treatment was +/- 1.5%, or 13.5-16.5%). For channels measuring under their acceptable range, more fine sediment was added and the
surface expression measurement was repeated until it did fall within the acceptable range. Evenly distributing additional fine sediment was much more feasible than evenly removing excess, therefore the initial volume of sand added was intentionally underestimated. In some cases, the process of adding sand and measuring surface expression was repeated up to four times, but no surface expression measurement ever exceeded the acceptable range and therefore no fine sediment ever had to be removed from a channel to compensate.

Due to the requirements of a concurrent study, all streambed materials (including the gravel under bedding) were completely removed following each observation day during 18°C trials. Gravel and sand were mechanically separated through a sieve, and the beds were reconstructed as described above. In order to maintain streambed uniformity across trials, the distribution of rocks among the channels was conserved and a single individual constructed all streambeds throughout the experiment. Although streambeds were not removed and reconstructed during the 12°C trials, a cohort experienced all sediment treatments only once at each temperature, therefore all cohorts experience a novel streambed for every trial.

All fish of a single cohort were transferred without netting from their individual tanks and introduced to a common experimental channel between 9:15-10:15 am the day prior to observations, allowing 21-22 hours of acclimation to their new environment. Fish were not fed for a minimum of 24-hours prior to trials. Observations were conducted during three two-hour time blocks over the course of a single day; morning (7:15-9:15 am), midday (12:00-2:00 pm) and evening (4:45-6:45 pm). A single cohort was observed for five consecutive minutes out of every 20 minutes in each time block, for a total of 30
minutes of observation per cohort per time block. This allowed for concurrent trials by a
single observer moving about the room to observe multiple cohorts during a time block.
All observations were made by a single observer in order to avoid between observer
variance. Observations were made through a small eyehole cut in a box shaped blind over
the viewing window, to avoid disturbance of the fish by the observer’s movements.

During each minute a tally of aggressive interactions was maintained. At end of
each minute, an instantaneous observation of each individual’s swimming positions was
made. Aggressive interactions were classified as nip, chase, and push/display. Swimming
positions were classified as active, maintaining, resting, partial cover, and complete cover
(Table 3).

**Table 3:** Definitions of aggressive interactions and swimming positions observed
during low flow cohort trial observations.

<table>
<thead>
<tr>
<th>Aggressive Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nip</td>
</tr>
<tr>
<td>Snapping movement towards the head, body, or tail of another individual, not requiring actual physical contact.</td>
</tr>
<tr>
<td>Chase</td>
</tr>
<tr>
<td>Pursuit of another individual for at least two body lengths.</td>
</tr>
<tr>
<td>Push/Display</td>
</tr>
<tr>
<td>Erection and fluttering of fins, extension of vertebral column, flaring of opercula, and/or gradually moving into the space occupied by another individual.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Swimming Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
</tr>
<tr>
<td>Movement within the water column</td>
</tr>
<tr>
<td>Maintaining</td>
</tr>
<tr>
<td>Exerting effort through caudal movement to hold a position against the current in the water column</td>
</tr>
<tr>
<td>Resting</td>
</tr>
<tr>
<td>Laying on top of the streambed in the absence of caudal movement or within the interstices of the streambed with &lt;50% of the body concealed</td>
</tr>
<tr>
<td>Partial Cover</td>
</tr>
<tr>
<td>Resting in the interstices of the streambed with 50-90% of the body concealed</td>
</tr>
<tr>
<td>Complete Cover</td>
</tr>
<tr>
<td>Resting in the interstices of the streambed with &gt;90% of the body concealed</td>
</tr>
</tbody>
</table>
Data Analysis

Habitat use data were recorded for all three fish at the end of each minute of observation, for a total of 90 data points per trial (30 observations times 3 fish). For analysis, cover use was defined as partial or complete cover, therefore cover use was analyzed as a proportional binary response in a logit model. Sediment level, temperature, and time of day were included in the model as fixed effects. Small sample size and non-normal data distribution required a non-parametric test for differences in cover use among cohorts, and a Kruskal-Wallis ANOVA showed no significant difference; therefore cohort was not included as a random factor in the cover use model. Cover use was not observed in 30-45% fine sediment trials, resulting in a change from a finite value to zero. This is an infinite change at the logit level, resulting in very large negative coefficient parameters with corresponding large error estimates. Eliminating these two sediment levels, at which cover was not used, allowed for a more stable model to analyze the effects of time and temperature on cover use. For aggressiveness, a Kruskal-Wallis ANOVA showed there was a significant difference in aggression among cohorts, therefore a generalized linear mixed model was used to include cohort as a random variable. Figures were generated and ANOVAs performed using IBM SPSS (Version 21). Statistical modeling was performed using R (Version 3.1.0).
Results

Rock Collection

A Wolman (1954) pebble count (n = 200) at the rock collection site showed four grain size classes, 32-90 mm, were inclusive of 73.5% of the all coarse grains on the streambed (Figure 7). A total of 625 grains (~ 370 kg total) were collected and used in the experimental channels. Although distribution of grains among treatment channels was not measured until after the experiments were completed, visual assessment for evenness among channels during initial allocation did result in consistent coarse sediment distributions (Table 4).

**Figure 7:** Frequency distribution for grain size class of coarse grains at the rock collection site (n=200). Shaded bars indicate grain size classes collected.

**Table 4:** Grain size distribution of coarse substrate in each treatment channel by number of particles and total mass (kg) of each grain size class.

<table>
<thead>
<tr>
<th>GRAIN SIZE</th>
<th>CONTROL</th>
<th>15%</th>
<th>30%</th>
<th>45%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>Mass (kg)</td>
<td>#</td>
<td>Mass (kg)</td>
</tr>
<tr>
<td>32</td>
<td>34</td>
<td>4.2</td>
<td>30</td>
<td>3.6</td>
</tr>
<tr>
<td>45</td>
<td>51</td>
<td>15.4</td>
<td>56</td>
<td>16.7</td>
</tr>
<tr>
<td>64</td>
<td>50</td>
<td>37.0</td>
<td>51</td>
<td>35.8</td>
</tr>
<tr>
<td>90</td>
<td>21</td>
<td>36.7</td>
<td>20</td>
<td>33.2</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>93.3</td>
<td>157</td>
<td>89.3</td>
</tr>
</tbody>
</table>
Swim Test and Fork Length

Only the smallest individual (FL = 96 mm) failed to complete the swim test and was therefore excluded from subsequent experiments. Three of the remaining six smallest fish, all ≤ 110 mm, were also noted to spend a majority of the swim test near the back of the swim chamber, suggesting that the swim test flow rate of 0.75 m/s is near the critical swimming velocity of smaller individuals tested. Fork length measurements were normally distributed, with a mean of 113 ± 7 mm and a range of 96-128 mm (Figure 8).

![Size distribution of fish as measured by fork length (n = 23).](image)

**FIGURE 8.** Length distribution of fish as measured by fork length (n = 23).

Objective 1 - Winter Flood Simulation Trials

Cover use was not observed during any of the winter flood simulation trials of either temperature treatment (Table 5). All individuals were observed to struggle with the water velocity and turbulence, moving about the water column throughout the channel apparently searching for calmer conditions. Displacement ended the trial early for fish M at 8° C and fish F at 12° C. During simulated floods fish did not appear to investigate the substrate for velocity refuges, which were readily available in the interstices of the streambed.
Table 5. Swimming positions recorded for observations of individual fish during winter flood simulations. Trials were ended early in the event of displacement of the test fish against the aquaculture netting, resulting in fewer observations for fish M and F.

<table>
<thead>
<tr>
<th>Fish ID</th>
<th>Maintaining</th>
<th>Searching</th>
<th>Flailing</th>
<th>Partial Cover</th>
<th>Complete Cover</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8°C C</td>
<td>13°C C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>38</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>41</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>55</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>49</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>49</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>31</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>G</td>
<td>26</td>
<td>17</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>H</td>
<td>107</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>I</td>
<td>16</td>
<td>17</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>J</td>
<td>41</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>K</td>
<td>49</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>11</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
</tbody>
</table>

Due to the small sample size data useful in analysis was not gathered during exploratory cohort flood simulation trials, although many informative observations were made. In the day at low flow before and after flood conditions, only the largest individual of each cohort was observed in the water column while the remaining five individuals took partial or cover in the streambed. On occasions when fish emerged from cover at low flow, the largest fish previously established in the water column aggressively nipped and chased the second individual, eventually causing the smaller fish to return to a position of cover in the streambed. As flow was increased during these trials, several individuals emerged from the streambed to maintain position in the water column and remained in the water column, receiving minimal aggression at high flows. As flow was gradually reduced, the largest individual once again acted aggressively towards the other fish, eventually resulting in their return to cover in the streambed. During night at low flow before flood conditions, several individuals (including the largest) were observed in maintaining positions in the water column in the absence of aggression. As observed during the day, increasing water velocity resulted in the emergence of additional fish from positions of cover in the streambed. At night, poor visibility of fish in turbulent conditions.
flood conditions under red lights precluded the completion of trials without unnecessary risk to the fish due to undetected displacement.

**Objective 2 - Low Flow Cohort Experiments**

The 12 fish placed in cohort trials ranged in FL from 107-128 mm, the difference in FL between two consecutive dominance positions within a cohort ranged from 3-9 mm, and the difference in FL within a cohort varied from 10-18 mm. There were no significant differences in average FL among the four cohorts (Kruskal-Wallis one-way ANOVA, $H = 1.729$, d.f. = 3, $p = 0.631$, Figure 9).

![Figure 9](image)

**Figure 9.** Length distributions four cohorts of fish used in the low flow cohort trials. Circle fill indicates hierarchy position (solid = dominant, shaded = intermediate, open = subordinate). Differences in fork length among cohorts are non-significant (Kruskal-Wallis ANOVA, $H = 0.733$, d.f. = 3, $p = 0.631$).

In order to determine if the effect of cohort should be included in statistical models, an analysis of variance (ANOVA) was performed to establish the significance of variation among cohorts for either of the response variables, cover use and aggression. Due to a non-normal distribution of the response variables, a non-parametric analysis was performed. The proportion of time spent using cover was not significantly different among cohorts (Kruskal-Wallis one-way ANOVA, $H = 0.289$, d.f. = 3, $p = 0.962$, Figure 10A), therefore no cohort effect was included in the generalized linear model for cover use. There was a significant difference in aggressiveness among cohorts (Kruskal-Wallis
one-way ANOVA, $H = 18.137$, d.f. = 3, $p < 0.001$, Figure 10D), therefore cohort was included as a random variable in the generalized linear mixed model of aggression. All interaction types (push, chase, and nip) were combined for analysis (Figure 10).

![Figure 10](image)

**Figure 10.** Mean number of aggressive interactions per hour by type for all cohorts, times, and temperature. (Error bars ± 2 S.D.)

The effects of variables are best visualized independently, although statistical analyses of individual parameters were not necessary as parameters were analyzed simultaneously in generalized linear models. There is a decreasing trend in cover use as fine sediment increases (Figure 11B). In control and 15% fine sediment treatments, streambed cover was available and one or both of the subordinate fish in each cohort were regularly observed using cover. In treatments of 30 & 45% fine sediment, all interstitial spaces within the streambed large enough to hold a fish were filled with sand. The dominant fish in each cohort was rarely observed using cover under any conditions, the time fish spent using cover rarely exceeded 60% as a result. No trends are seen in cover use over the course of a day (Figure 11C). A decreasing trend in cover use as temperature increased is seen in all data displays (Figure 11A-C). Aggressiveness increases as fine sediment level increases (Figure 11E) and decreases over the course of a day (Figure 11F). Aggressiveness decreased as temperature increased, as seen in all data displays (Figure 11D-F).
FIGURE 11. Summary of low flow cohort experimental data, organized for visual assessment of trends. Data are the same for all cover use (left column) and aggression (right column) plots, clustered by cohort (A&B), fine sediment level (C&D), and time of day (E&F). In each plot, open bars represent 12° C trials and shaded bars represent 18° C trials. Percent time spent using cover and number of aggressive interactions per hour are relative to cohorts as a whole.
While these figures allow visualization of trends in response variables for individual independent variables, statistical models of the response variables evaluate significance of independent variables simultaneously. Cover use was treated as a binary response in a generalized linear model including fixed effects of fine sediment, temperature, and time of day (Table 6). Fine sediment levels of 30 & 45% clearly affect cover use, as no cover was available for use. When included in the model, these upper sediment levels showed large negative coefficient estimates with large standard errors, therefore data entered into the model was restricted to those collected in control and 15% fine sediment treatments (Table 7).

**Table 6.** Cover use generalized linear model summary of coefficient parameters including all sedimentation levels. Coefficients and significance are relative to 0% fine sediment, 12°C, morning conditions.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.31</td>
<td>0.08</td>
<td>-3.79</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sediment 15%</td>
<td>-2.03</td>
<td>0.10</td>
<td>-20.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sediment 30%</td>
<td>-21.58</td>
<td>844.59</td>
<td>-0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Sediment 45%</td>
<td>-21.61</td>
<td>882.09</td>
<td>-0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Temp 18°C</td>
<td>-0.83</td>
<td>0.08</td>
<td>-10.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time Mid Day</td>
<td>0.01</td>
<td>0.10</td>
<td>0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>Time Evening</td>
<td>0.18</td>
<td>0.10</td>
<td>1.83</td>
<td>0.068</td>
</tr>
</tbody>
</table>

**Table 7.** Cover use generalized linear model summary of coefficient parameters including 0% and 15% fine sediment conditions only. Coefficients and significance are relative to 0% fine sediment, 12°C, morning conditions.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.31</td>
<td>0.08</td>
<td>-3.79</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sediment 15%</td>
<td>-2.02</td>
<td>0.10</td>
<td>-20.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Temp 18°C</td>
<td>-0.83</td>
<td>0.08</td>
<td>-10.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time Mid Day</td>
<td>0.01</td>
<td>0.10</td>
<td>0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>Time Evening</td>
<td>0.24</td>
<td>0.12</td>
<td>2.13</td>
<td>0.068</td>
</tr>
</tbody>
</table>
Removal of high sediment treatments (30 & 45%) does not affect statistical interpretations of remaining parameters. The greatest factor affecting cover use was fine sediment level, and at 15% fine sediment cover use was significantly reduced relative to control conditions. The effect of temperature on cover use was also significant, with less time spent using cover at 18° C relative to 12° C. Although cover use does not vary significantly with time of day, there is a nearly significant increase in cover use in the evening relative to the morning.

In order to incorporate difference in aggressiveness among cohorts, generalized linear mixed model was used with fixed effects of sedimentation, temperature, and time and a random effect of cohort (Table 8). Aggression decreased slightly as temperature increased, although this effect is non-significant (p = 0.079). Aggressive interactions increased with sedimentation, and a significant increase in aggression is seen even at 15% fine sediment relative to control conditions. The greatest factor effecting aggressiveness is time of day, with a significant decrease in aggressiveness over the course of a day.

**Table 8.** Aggressiveness generalized linear mixed model summary of coefficient parameters. Coefficients and significance are relative to 0% fine sediment, 12° C, morning conditions.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.31</td>
<td>0.08</td>
<td>-3.79</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sediment 15%</td>
<td>-2.03</td>
<td>0.10</td>
<td>-20.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sediment 30%</td>
<td>-21.58</td>
<td>844.59</td>
<td>-0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Sediment 45%</td>
<td>-21.61</td>
<td>882.09</td>
<td>-0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Temp 18 °C</td>
<td>-0.83</td>
<td>0.08</td>
<td>-10.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time Mid Day</td>
<td>0.01</td>
<td>0.10</td>
<td>0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>Time Evening</td>
<td>0.18</td>
<td>0.10</td>
<td>1.83</td>
<td>0.068</td>
</tr>
</tbody>
</table>
**Discussion:**

*Objective 1 - Winter Flood Experiments*

Streambed cover use as a velocity refuge was not observed during experiments. It was sought to verify that fish could access cover in flood conditions to eliminate habitat unfamiliarity as a contributing factor and verify the acclimation period and gradual flow increase were sufficient. Following the final three flood simulation trials, which did not result in displacement of the fish, high flow conditions were maintained and fish were presented an overhead threat stimulus (a hand waved over the channel). Each of the three fish responded by immediately darting into the interstices to take cover in the streambed. This behavior demonstrates the ability of brook trout to access streambed cover under turbulent conditions. Rocks in the channel were carefully positioned to remain in place even in the turbulence of the highest velocity. Although particles were not able to roll or tumble, some were able to wobble in place slightly. Fish may have perceived a risk of bed mobility that outweighed the risk associated with downstream displacement, as loose rocks could potentially crush a fish, but impingement as a result of downstream displacement is not likely in a natural flood. Many prior published investigations of winter streambed cover use account only for non-flood conditions that lack the potential for bed mobility (Gibson, 1978; Griffith & Smith, 1993; Meyer & Griffith, 1997; Thurow, 2006).

The seasonality of this experiment may have affected behavioral response as well because as winter flood simulations were performed during summer months. Fish were collected in late spring maintained in the lab at temperature of 13° C, simulating an extended cool season rather than a thermal reversal. If strategy shifts from energy
conservation in winter months to energy intake during summer months, fish may be more likely to seek velocity refuge during winter floods and more likely to remain in the water column to take advantage of increase macroinvertebrate drift during summer floods. Although water temperature was maintained below summer conditions, fish may still have expressed inherent summer behaviors, seeking to take advantage of feeding opportunities typically associated with flood conditions rather than conserving energy as expected during winter spates. Limitations of the closed loop circulation and filtration equipment prohibited the introduction of turbidity typically associated with natural flood events that may act as a cue for behavioral responses.

**Objective 2 - Low Flow Cohort Experiments**

Under control conditions with no fine sediment and ample cover available within the streambed for all three individuals in the cohort, both subordinate fish were commonly observed using streambed cover. Cover use has been demonstrated to be density dependent in wild populations (Armstrong & Griffiths, 2001), and available cover is expected to be a limiting factor for carrying capacity as individuals consistently unable to seek refuge are likely to be subject to predation (Gregory & Griffith, 1996a).

Although food was not available during trials, dominant fish were typically observed maintaining position in the water column where invertebrate drift is expected. This supports previous studies that found dominant fish secure superior feeding positions (Hughes, 1992a; Hughes, 1992b) and have higher growth rates (Metcalf et al., 1989; Pottinger & Pickering, 1992; Grobler & Wood, 2013). Streambed cover use was significantly reduced at 15% sedimentation presumably due to a reduction in the presence of interstices large enough for fish to inhabit, and streambed cover use was completely
eliminated at 30 & 45% sedimentation as interstices large enough for a fish to inhabit were not present.

In lower sediment conditions, the subordinate ranking individual frequently retreated into cover within the streambed following aggressive interactions with individuals of superior social ranking. When streambed cover was limited (15% sedimentation) or unavailable (30-45% sedimentation), bouts of aggression were often prolonged and involved more numerous interactions (nips and chases). This increased aggression is likely to have induced physiological stress, especially for subordinate individuals (Currie et al., 2010; LeBlanc, 2011; Grobler & Wood, 2013).

Variation in aggression among cohorts might be explained by relatedness. Salmonids in dominance hierarchies have been shown to be less aggressive towards relatives than unrelated fish (Griffiths & Armstrong, 2001), and higher levels of aggression are associated with greater heterozygosity (Tiira et al., 2003). All fish for this study were collected within < 0.5 km of stream and were assumed to be within the same year-class. Brook trout show limited dispersal following emerge from discrete red locations, and patchy aggregations of close kin indicate non-random dispersal Virginia headwater streams (Hudy et al., 2010). It is therefore likely that some individuals in this study were closely related to one another. Aggression among cohorts may have been affected if individuals within some cohorts were more closely related than individuals within other cohorts.

Aggression increased with amount of sediment but there was no change in cover use. This increase may result from the relative colors of sand and rock used in experiments relative to the fish color. Sand was lightly colored relative to rocks, so
provided little camouflage for subordinate individuals. Increasing sedimentation with lightly colored sand resulted in lighter substrate, over which subordinate movements seemed more easily recognized and less tolerated by higher ranking individuals. Movement of subordinate fish agitated higher ranking individuals and initiated many bouts of aggression. Sand more closely matching the color of coarse substrate in a natural setting and would be recommended in subsequent experiments.

The trade off between feeding and cover can be viewed as a complicated balance between growth and survival, with behaviors of dominant fish demonstrating the most efficient combination. Although prey items were not available during trials, fish were only fed during the day over the course of this study.

Cover use was significantly higher at 12° C than at 18° C, suggesting a greater drive towards feeding activity resulting from increased metabolic demands and at higher temperatures. A confounding factor of order of experience is also possible, as all 18° C trials were completed prior to 12° C trials to reduce the physiological stress of repeated temperature fluctuations necessary to randomize the order of temperature experience. The increase in cover use at the lower temperature may be partially attributed to increased acceptance of social rank by subordinates and increase habitat familiarity.

Change in cover use was not significant between morning and midday trials (p = 0.92), but there was a trend for more cover use in the evening compared to morning trials (p = 0.068). Aggression was significantly affected by time of day; the greatest level of aggression occurring in the morning as compared to either afternoon or evening observations (p < 0.001 for both comparisons). Time of day was included as a variable in this study based on preliminary observations of changes in behavior over the course of a
day. Most studies that address daily temporal behavioral changes address diel fluctuations, characterizing daytime feeding and nighttime sheltering in summer (Young et al., 1997; Bradford & Higgins, 2001) and the reverse in winter (Gregory & Griffith, 1996b; Bremset, 2000; Meyer & Gregory, 2000). Although winter observations result in a contrasting behavioral shift at dawn, Gregory & Griffith (1996a) observed a peak in aggressive interactions at dawn as fish established concealment positions. Likewise in the current study, aggression may have been escalated in the morning due to competition to establish feeding positions.

The greatest findings of this study are the significance of sedimentation on social interactions among juvenile brook trout. These results suggest that sediment levels even as low as 15% can impair habitat for juvenile salmonid rearing, demonstrating the necessity for maintenance of currently intact habitats over restoration of those already degraded. This is a critical issue considering that only 50% of streams in the United States have healthy sediment compositions (USEPA, 2006). The USEPA calculates the amount of fine sediment necessary to cause impairment by factoring channel roughness and gradient at the reach level. Although sediments are assessed nationally, there is no national regulatory standard for sedimentation. Sedimentation is not considered in the Virginia Stream Condition Index used to evaluate impairment status, although qualitative assessments are applied to impaired streams and commonly acknowledge sedimentation as a potential stressor. Based on the low level of fine sediment that resulted in significant effects in this study, direct quantitative assessments of streambed sediments are recommended in the evaluation of stream condition.
In order to further investigate objective one of this study in the future, the effects of sedimentation on salmonid populations during winter flood conditions might be better addressed with younger fish than were presently used. Brook trout young of the year could be collected in late fall to be experimented with throughout the natural winter months and colder water temperatures than were achieved in the present study may also have better replicated natural conditions. Personal observations of massive and socially passive aggregations of brook trout confined to small isolated pools in intermittent stream systems also demand further investigation in the light of this study. While tank reared fish have been shown share streambed refuges at high densities of up to 20 individuals (Burns et al., 1997; Metcalfe et al., 1999), it stands to be discovered if local behavioral adaptations result in more passive, less socially aggressive populations.
Conclusion:

Juvenile brook trout did not demonstrate the use of streambed cover as a velocity refuge during simulated winter flood conditions. At low flows and summer water temperatures, cover used was significantly reduced at 15% fine sediment and eliminated completely at 30-45% fine sediment, implying that even moderate sedimentation can affect juvenile salmonid rearing habitat. Cover use was also significantly lower at 18°C than 12°C, which may reflect increased boldness to overcome greater metabolic demands of higher temperatures. Aggression among juvenile brook trout significantly increased above control conditions even at 15% fine sediment by surface expression, the lowest level tested and was significantly highest in the morning relative to afternoon and evening. This indicates that sedimentation, which has already been established to reduce food availability for salmonids, also increases social stress among subordinate individuals that is likely to result in reduced growth rates and survival. Compounded with the effects documented on other life stages, streambed sedimentation is likely a primary factor of the carrying capacity of salmonids.
List of References:


Appendix A. Cards used for fish and cohort identification throughout experiments. Fish were properly postured and aligned for fork length measurement for caudal photos (upper right of each card).
Appendix A (continued). Cards used for fish and cohort identification throughout experiments. Fish were properly postured and aligned for fork length measurement for caudal photos (upper right of each card).
Appendix A (continued). Cards used for fish and cohort identification throughout experiments. Fish were properly postured and aligned for fork length measurement for caudal photos (upper right of each card).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Hierarchy Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q - 112 mm</td>
</tr>
<tr>
<td>No Cohort</td>
<td>S - 103 mm</td>
</tr>
<tr>
<td>No Cohort</td>
<td>U - 110 mm</td>
</tr>
<tr>
<td>No Cohort</td>
<td>W - 109 mm</td>
</tr>
<tr>
<td>2</td>
<td>V - 111 mm</td>
</tr>
</tbody>
</table>

Right Side Photo Unavailable

Table A1. Cohort assignments and hierarchy positions for fish in low flow cohort trials (I - III = dominant - subordinate.)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>M</td>
<td>Q</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>F</td>
<td>V</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>O</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>G</td>
<td>H</td>
<td>J</td>
</tr>
</tbody>
</table>
List of References:


