Characterizing the flow regime in Brook Trout (*Salvelinus fontinalis*) incubation habitats and the implications for management in a hydro-regulated river

by

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Abstract

Hydropower accounts for more than one third of Ontario Power Generation's electrical production. Hydroelectric development often occurs on rivers that also support recreational fisheries. The construction and operation of dams, diversions and generating facilities unavoidably influence the ecological function of rivers. The Aguasabon River is a northern Canadian Shield river with major developments for water diversion, storage, and power generation. This river offers opportunity to examine the importance of vertical flows through the substrate at a Brook Trout (*Salvelinus fontinalis*) spawning area. The vertical and horizontal hydraulic gradients and subsequent water temperature changes are the subject of this study. Piezometers were used to monitor the river and subsurface water levels near Brook Trout redds during the spawning and incubation period under normal and increasing discharge conditions. The Brook Trout spawning area in the Aguasabon River experienced upwelling conditions for the entire monitoring period (Oct 28th, 2016 – Jan 13th, 2017) before water release at the Long Lake Control Dam (LLCD). Hyporheic temperatures declined gradually, remaining >3.7 °C. The river temperature in the winter before water release was 1.5 °C. Rapid increase in water level after discharge from above the LLCD resulted in the reversal of flow in the hyporheic zone. Negatives values of vertical and horizontal gradients occurred for up to 30 h between surface water and hyporheic water up to 1.8 m below the river substrate. The water temperature in all piezometers decreased in unison with water release. Shallow inshore piezometers showed the greatest change, >53 h at <1 °C, compared to both shallow and deep offshore piezometers, which never fell <1 °C.

A controlled experiment was used to monitor time to hatching, larval emergence and survival of Brook Trout in a lab setting. Historical data show redd temperature during the Brook

Trout incubation period (October-April) on the Aguasabon River was maximal after a 2013 discharge, which lowered the water temperature of Brook Trout redds to 0.3 °C, a drop of 5.3 °C. Recovery to pre-discharge temperatures took 72 h, after 40 h at <1°C. This worst-case cold treatment was selected for an experiment at the Dorion Fish Culture Station, Ontario. The treatment had no effect on survival of incubating Brook Trout eggs ($n = 1020$) compared to control ($n = 1020$). Survival from fertilization to hatching was high for both treatment and control replicates (>90%), but was considerably lower for fertilization to emergence (55%). The cold treatment did not decrease development time from fertilization to larval emergence. The hatchery study implies that the redd temperature change experienced on the Aguasabon River in 2013 neither decreased the survival nor delayed hatching and emergence of Brook Trout. An overall recommendation is that a staged discharge at the LLCD would lessen the reversal of flow in the hyporheic zone and the magnitude of temperature changes monitored at Brook Trout redds during 2013 and 2017.

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General Introduction

Hydropower is an environmentally clean, reliable, and renewable energy resource. The production of hydropower, however, does influence the ecological function of rivers due to channel fragmentation and abrupt changes in river flow (Renöfält et al. 2010). Hydropeaking and streamflow regulation constitute the practises used in altering the flow of rivers for creating hydroelectricity. These practises can affect fish populations due to unstable and rapidly changing water levels, along with other changes to aquatic habitats (Murchie et al. 2008).

The Aguasabon River of Northwestern Ontario, Canada is both a significant source of hydroelectricity for the province of Ontario (OPG 2013) and supports a recreational fishery that includes Walleye (*Sander vitreus*), Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*) and Brook Trout (*Salvelinus fontinalis*). Hydroelectricity on the Aguasabon River is produced by regulating streamflow in a winter reservoir system. Such a system generally involves the storage of water behind a control structure during high flows (e.g., spring freshet) and controlled or scheduled releases during periods of low flows (e.g., midwinter) to deliver water to generating facilities for hydropower in times of high energy demand (Haxton et al. 2015). Water release or storage behind the control structure may result in an immediate change from low-river to high-river stage (and *vice versa*) at various times of the year. This kind of change has potential to alter physical and chemical characteristics of aquatic habitats, such as the hyporheic environments required by fish to maintain suitable incubation environments. The spawning and incubation success of Brook Trout in the Aguasabon River is of special concern due to the species-specific spawning requirements, limited spawning habitat in the river, and the vulnerability of that habitat to upstream water management. Because of a diversion at a river meander, the Aguasabon offers a unique opportunity to understand vertical and horizontal head gradients that may be responsible for creating Brook Trout spawning habitat, and how discharges at the LLCD change the quality of that habitat.

Brook Trout Spawning and Redd Site Selection

Brook Trout require cold, clean, and well oxygenated water to survive (Wenger et al. 2011). Brook Trout select spawning sites and in them they construct redds, where they lay eggs 5-10 cm into the substrate of rivers or lakes. The hyporheic zone is the connecting ecotone beneath and adjacent to a streambed, where mixing occurs between infiltrating stream water and shallow groundwater (Boulton et al. 1998, Bencala 2000). Brook Trout spawn during the fall (October-November), their hatching occurs later in February, and their larvae emerge from the substrate in spring (April-May). In Canadian Shield waters, Brook Trout spawning areas are usually found close to shorelines, in areas associated with discharging groundwater (Curry et al. 1995a, Blanchfield 1996). Groundwater upwellings, or springs, are the result of groundwater under pressure in an aquifer that seeps through the permeable layers of porous substrate below a lake or river (Brunke and Gonser 1997). Groundwater–surface water interactions, including hyporheic flow, constitute an important factor in determining spawning locations for Brook Trout, as well as hatching success and emergence after incubation. The most commonly supported hypothesis for this association is that the upwelling groundwater (maintained at 4–6 °C) stabilizes the thermal environment where incubating eggs develop, and prevents eggs from freezing conditions during winter (Snucins et al. 1992, Curry et al. 1995a). Constant groundwater discharge through redds also helps to prevent their dewatering (Brick 1986). Selection of redd sites often occurs over coarse sediments that yield greater hydraulic conductivity, which in turn creates stronger hyporheic flow onto the spawning redds (Sear et al. 2014).

Threats of Flow Regulation to Brook Trout Spawning and Incubation

Interstitial water quality and hyporheic condition such as temperature and groundwater flow are sensitive to hydropeaking regimes and are directly influenced by the frequency, amplitude and duration of flow alteration (Casas-Mulet et al. 2015). One study analyzing dynamics of surface water and groundwater at an Atlantic Salmon redd found that during periods of low flow, hydrochemistry of the hyporheic zone was dominated by groundwater inputs, while surface water inputs dominated during high flows (Malcolm et al. 2004). Another study of a northern Canadian Shield river examined regulated flow effects on groundwater discharge at Brook Trout spawning redds (Curry et al. 1994). Changing flows altered shallow groundwater pathways at Brook Trout redds. Rising flows allowed surface water and groundwater mixing at the river bank, whereas river level recession increased potentials for groundwater flow horizontally in an offshore direction. The study concluded that hydropeaking regimes involving rapid increases in river level alter hyporheic flow in Brook Trout redds and have potentially negative impacts on Brook Trout recruitment, with changes to interstitial water conditions a main concern.

The Current Study

There is only one known Brook Trout spawning location in the main channel of the Aguasabon River. This site was found by the Ontario Ministry of Natural Resources and Forestry in 2006, when 11 Brook Trout were implanted with radio transmitters, and one was tracked at the end of September to a location in the main channel 1 km south of the Harvey Creek confluence, approximately 20 km north of Terrace Bay, Ontario (Figure 1.1). This fish was observed in the area with other spawning Brook Trout; active redd construction occurred until the end of November (OMNRF unpublished 2006, 2007). Previously, Brook Trout spawning had only been observed in Harvey Creek, a small spring-fed tributary of the Aguasabon River. Water flow at the spawning location in the main channel is dominated at certain periods by the discharging regime of the Long Lake Control Dam (LLCD), 12 km above the spawning location (Figure 1.2). The site also receives water from natural sources, including Harvey Creek and the Little Aguasabon River (OMNRF unpublished 2007). The location of the Brook Trout spawning area is along an artificial bank that was created with existing gravel at the river site, by excavating part of the river channel in order to straighten the flow and redirect it from what is now an oxbow (Figure 1.1). This channel manipulation was done to allow better passage of timber during log drive operations. It is unknown if Brook Trout used this site for spawning prior to the channel manipulation.

After discovery of spawning in the main channel, the Ontario Ministry of Natural Resources and Forestry installed a temperature logger to monitor temperature in the substrate at an active redd. Flow increases during spawning and incubation were found to decrease redd temperature with unknown consequences for incubating Brook Trout eggs. Further investigation was needed to quantify and describe surface hyporheic flow in relation to these discharge events. The importance of maintaining natural flow and temperature regimes of spawning areas on a regulated river is paramount to ensure annual recruitment and long-term success for Brook Trout (Curry et al. 1994). These recommendations led to the current study.

Ecologically determined limitations on minimum and maximum flows related to discharges at dams have been implemented on many river systems and are a critical component of river management (OPG 2013). On the Aguasabon River, there are critical flow and reservoir levels that must be maintained above and below the LLCD as part of the commitments made in water management plan. Currently, flows over LLCD should not fall less than 5 m^3/s for benthic invertebrate protection. However, the current water management plan lacks site-specific advice regarding river regulation and how it the vertical and horizontal flow through Brook Trout spawning redds. Filling the knowledge gap including hyporheic monitoring through various stages of river flow will build better models of minimal flow over control structures that will protect Brook Trout habitat and other ecological processes in porous substrates.

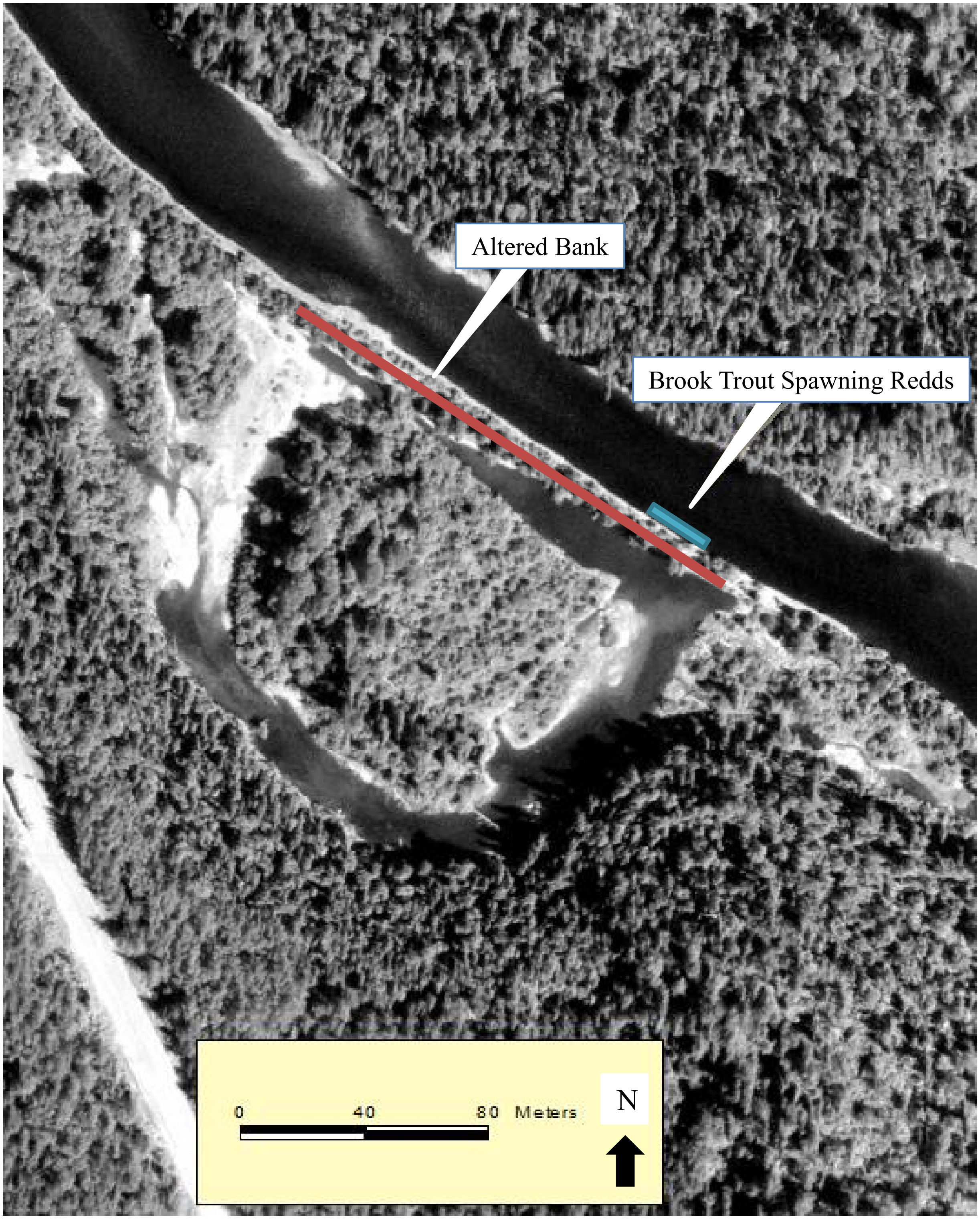


Figure 1. 1 Brook Trout Spawning Area on Aguasabon River. River flows north to south.

Objectives

The primary objectives of this study were to:

- describe surface flow through the substrate during the spawning and incubation periods of Brook Trout at the Aguasabon River's only known spawning location under (a) normal and (b) increasing discharge conditions;
- describe patterns of river discharge and site temperatures on the annual patterns of Brook Trout emergence; and
- simulate and monitor the effect of a midwinter water release during the incubation period by exposing incubating Brook Trout to colder water temperatures experienced in the spawning site in a controlled experiment, monitoring time to hatching, larval emergence and survival at the Dorion Fish Culture Station.

The results are organized into two chapters: Chapter 2, Effect of River Regulation on the Hyporheic Flow Regime of Brook Trout Spawning Habitat on a Canadian Shield River; and Chapter 3, Effects of a Prolonged Cold Treatment on Brook Trout Hatching, Emergence, and Survival.

Historical Background

The Aguasabon River flows south from Long Lake and in its last 609 m drops 63 m at Aguasabon Falls, a natural barrier to fish populations (from Lake Superior), before draining into Lake Superior. The Aguasabon River contains multiple artificial structures for water diversion, storage, and power generation, which were constructed as part of the Long Lake Diversion Project completed in 1939 (Peet and Day 1980). The diversion was originally for transporting logs from the otherwise inaccessible forests around Long Lake, and for providing a means to maintain water levels in Lake Superior waterways during drought periods. Ontario also realized the hydroelectric power production potential of the diversion, both locally and downstream in the Great Lakes (i.e., at Niagara Falls; Peet 1978). The Kenogami River Control Dam was subsequently constructed 16 km north of Long Lake to prevent northward flow and increase water moving through the diversion (Peet 1978). The LLCD was constructed at the south end of Long Lake and controls the southward flow of water through the Aguasabon River to the Great Lakes. A second phase of the project from 1945–1948 saw the construction of the Hays Lake Dam and the Aguasabon Generating Station near the mouth of the Aguasabon River (Peet 1978). During the same time, a pulp and paper mill was constructed near the mouth of the Aguasabon River upstream from the Falls and used the impounded Hays Lake and Aguasabon River as a booming ground for timber. In 1989, log booming ceased on the Aguasabon River system. The Aguasabon Generating Station was developed to supply power to the pulp and paper mill (now operated by AV Birla), the Town of Terrace Bay and the Province of Ontario through its connection to the provincial power grid, and is operated by Ontario Power Generation (OPG 2013).

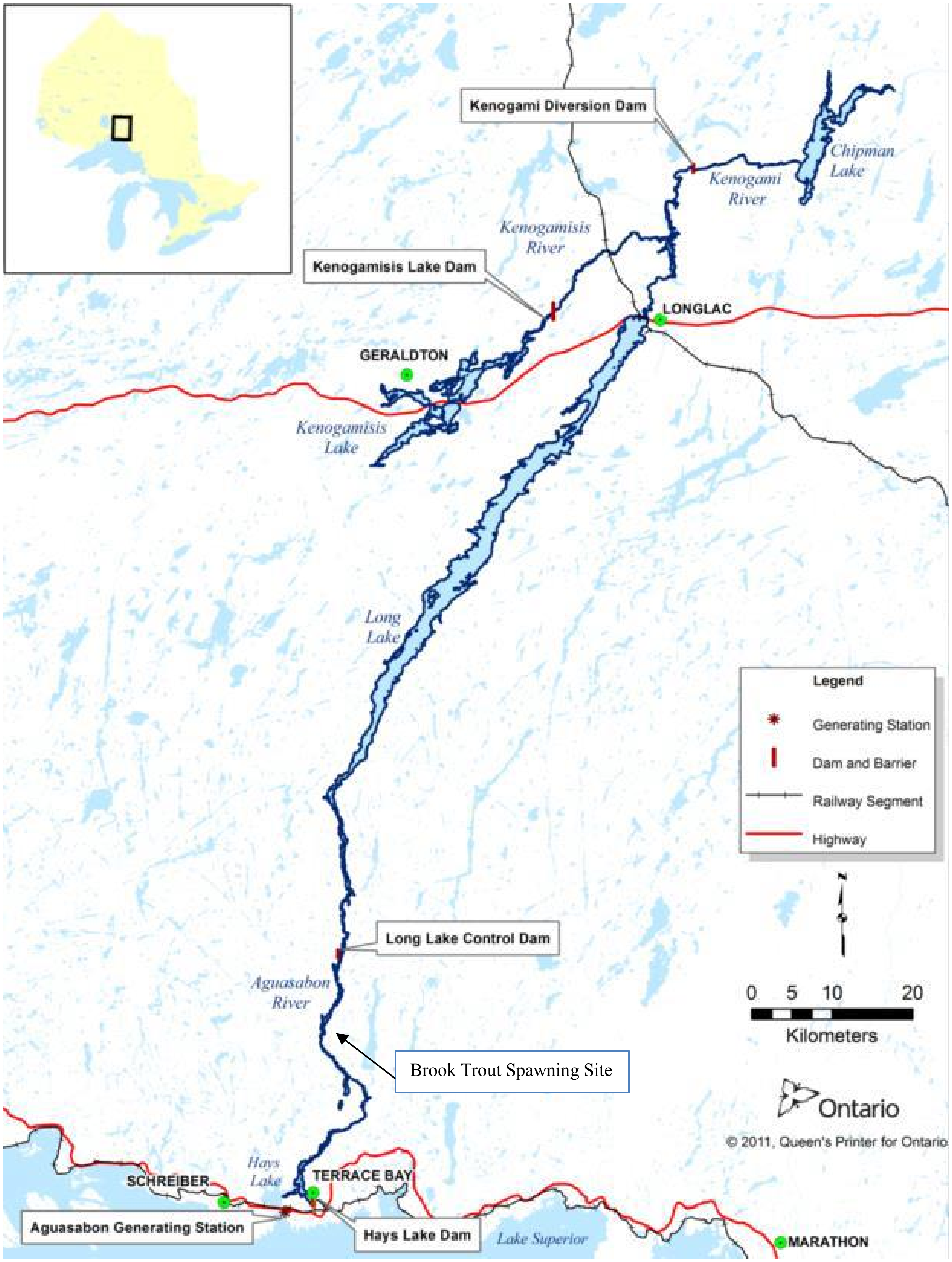


Figure 1. 2 Map of Long Lake Diversion system

Chapter 2. Effect of River Regulation on the Hyporheic Flow Regime of Brook Trout Spawning Habitat on a Canadian Shield River

2.1 Abstract

In this study, piezometers were used to monitor hyporheic flow at the Brook Trout spawning area during their spawning and incubation period on the Aguasabon River, under normal and increasing discharge conditions, to investigate the effects of upstream water management. The Brook Trout spawning area in the Aguasabon River maintained upwelling hyporheic flow for the entire 2016-17 monitoring period (Oct 28th – Jan 11th) before the water release event occurred at LLCD on Jan 11th, 2017. Vertical and horizontal gradients and vertical flux remained positive and stable during this period. Water temperatures declined gradually over the monitoring period (remaining >3.7 °C), but were higher than the river temperature (1.5 °C) in the winter before water release. Rapid increase in water levels on January 11th, 2017 resulted in the reversal of hyporheic flow through the substrate. Negatives values of flux, vertical and horizontal gradients were measured up to 1.8 m below the river substrate. The spawning area changed from positive (upwelling) to negative vertical gradients (downwelling) in the hyporheic zone. Hyporheic water temperature measured in piezometers before water release was >3.7°C, then decreased as discharge increased during the water release event at LLCD. The temperature of hyporheic water in shallow inshore piezometers had the greatest decrease (<1 °C for >53 h) compared to deeper offshore piezometers, which never fell <1 °C and returned more quickly to ambient hyporheic temperature. Because LLCD discharge increased midway through the Brook Trout spawning period, it resulted in substantially different hyporheic redd conditions, relative to conditions that existed at the time of site selection and spawning, a change that Brook Trout do not anticipate.

2.2 Introduction

Brook Trout (*Salvelinus fontinalis*) likely assess stream depth, substrate type, water velocity, and the presence of discharging groundwater to select spawning and incubation habitats (Reiser 1976, Curry et al. 1995a). The surface water–groundwater exchange in the hyporheic zone, where they construct redds to hold and incubate eggs and early larval stages, is mostly controlled by channel morphology, pressure head of overlying surface water, and the permeability of riverbed sediments (Arntzen et al. 2006, Brown and Pasternack 2008). The physical and chemical properties of discharging groundwater in the hyporheic zone are likely the most important factors in the selection of redd sites (Webster and Eiriksdottir 1976, Witzel and Maccrimmon 1983a, Curry and Noakes 1995). Maintaining the stability of these properties throughout the spawning and the egg and larval incubation periods is necessary for successful Brook Trout recruitment.

Manipulation of water flow in rivers for hydroelectric power generation has been shown to alter the chemical and physical nature of the hyporheic zone (Curry et al. 1994, Malcolm et al. 2004). Alterations to interstitial water quality may have direct impacts on Brook Trout recruitment (McCullough 1999). The development of control dams and hydroelectric facilities on rivers often leads to large fluctuations of river stage. These fluctuations in river stage alter the surface water–groundwater interactions and have lasting effects downstream of control structures on hyporheic habitats required by fish (Curry et al. 1994, Sawyer et al. 2009, Casas-Mulet et al. 2015). In the Colorado River, for example, water chemistry, temperature, and hyporheic exchange volume fluctuated in response to flow oscillations 15 km downstream of the Longhorn Dam (Sawyer et al. 2009). Dam induced hyporheic exchange penetrated several metres into the riparian aquifer. On the Nipigon River (Ontario, Canada), rising river levels for power management reversed groundwater flow at downstream locations and introduced surface water into the river bank, where various degrees of mixing with groundwater occurred, and the recession of river levels increased groundwater flow potentials in an offshore direction (Curry et al. 1994). In the absence of hydroelectric control and generating structures, groundwater would normally flow through riparian aquifers towards a river, ultimately stabilizing water chemistry, temperature, and groundwater flux into the hyporheic zone.

The Aguasabon River is located north of Terrace Bay, Ontario, east of the Nipigon River. Fish populations in this river, including the Brook Trout, are isolated between the Aguasabon Power Generating Station on Hays Lake, where a waterfall forms a natural barrier, and the LLCD, part of the Long Lake Diversion Project, a major re-routing of the northward flowing water from the Kenogami River into Lake Superior (Peet and Day 1980). The native Brook Trout population is specifically considered in the Aguasabon River Water Management Plan (OPG unpublished 2016), due to its recreational importance, its limited spawning habitat in the main channel, and the vulnerability of this habitat to water regulation at LLCD. Thus far, Brook Trout have been found to spawn in only one location in the main channel approximately 12 km downstream of LLCD. The spawning location is subject to changes in river stage and discharge as result of water management upstream at the LLCD. Ontario Power Generation manages the Aguasabon system as a winter reservoir intended to store water during high flow periods and release it during low flow periods. Historically, during the incubation period of Brook Trout (October–April), the hyporheic environment is subject to one major increase in discharge from the LLCD typically in January of each year, a change that the Brook Trout do not anticipate when they select spawning redds in the fall. The effects of annual discharge on water temperature at identified Brook Trout redds have been previously investigated using a single temperature logger buried in a Brook Trout redd, which has shown temperature declines during the incubation period after the eye-up stage and prior to hatching.

River regulation, including water release events at control structures, has the potential to alter surface water–groundwater interactions at downstream locations. These locations may include spawning habitat. For this thesis, I hypothesize that increasing discharge conditions are associated with the reversal of flow and decreasing temperature in the hyporheic zone. I further hypothesize that the effect of temperature on Brook Trout development continues to the larval emergence stage. The objectives of this study were (1) to describe flow through the substrate during the spawning and incubation periods of Brook Trout on the Aguasabon River's known spawning location under (a) normal and (b) increasing discharge conditions; and (2) describe patterns of river discharge and site temperatures on annual patterns of Brook Trout emergence.

2.3 Methodology

2.3.1 Hyporheic Flow Monitoring

Standpipe piezometers were used to measure hydraulic pressure head at 11 points at the previously recorded Brook Trout spawning location in the main channel of the Aguasabon River, which is approximately 5 m from a high-water mark on a bank. Originally, 12 piezometers were installed during the summer of 2016, but the data from one of the piezometers was discarded due to a faulty sensor. Piezometers were cut from 2.5 cm diameter galvanized steel piping and attached to a stainless-steel well screen of 1.9 × 15.2 cm dimension. Piezometers were driven directly into the river substrate, which was dominated by sand and gravel mixes, using a small, manual pile driver from a boat. Each piezometer was equipped with a Solinst EDGE water level/temperature monitor (datalogger) that hung at the bottom of the piezometer from a length of aircraft cable. A separate steel rebar was inserted into the river and equipped with a similar datalogger (referred to as a river level station). A Solinst barometric pressure and temperature datalogger was hung in the nearest tree on shore to the piezometer site. All piezometers and the river level station were marked with a line of equal elevation using transit survey equipment, to relate river level and hydraulic head measurements to a common overhead datum.

Two transects with six piezometers in each were originally installed perpendicular to the shoreline at the spawning site (Figure 2.1). Hereafter, they are labelled north (N) and south (S) transects. Each transect consisted of three piezometer nests: a shoreline nest that would be inundated during high water conditions and exposed during low conditions (Inshore, I), a nest in deeper water (~ 6 m offshore) further from shore than the observed Brook Trout redds (Offshore, O), and a nest halfway between (Middle, M). Each nest consisted of a shallow (S; 0.9 m) and deep (D; 1.8 m) piezometer. Hereafter, any individual piezometer is referenced using acronyms, e.g., “NIS,” which would refer to the piezometer on the north transect, inshore (near the shoreline), and in shallow water. The SMS piezometer data was discarded due to a faulty logger sensor. Monitoring began on October 28th, 2016 and ended January 13th, 2017, when ice flows disturbed the piezometers. On January 11th, 2017, Ontario Power Generation increased discharge at the LLCD upstream from the spawning site from 18 to 68 m^3/s, a change that encompassed minimum to maximum flow conditions for the 2016-17 winter season (Figure 2.1). These flows were in addition to natural contributions, such as from Harvey Creek and the Little Aguasabon River.

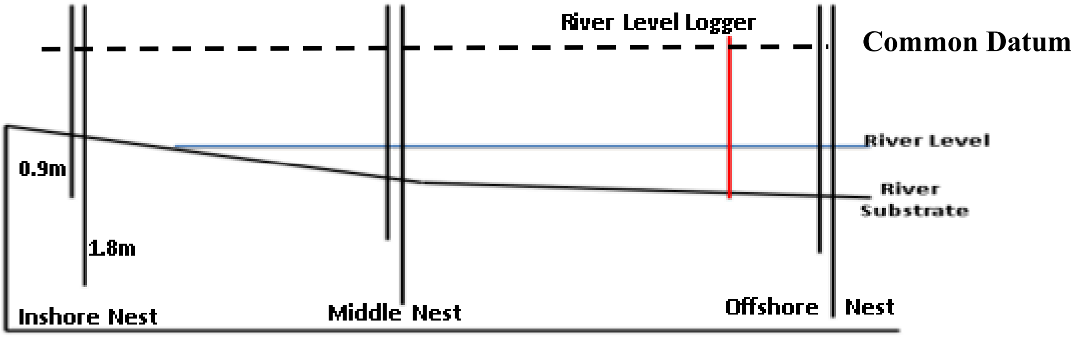


Figure 2. 1 Arrangement of piezometers in a single transect at the Aguasabon River study site, Orientation is in cross section facing upstream.

Hydraulic pressure head in each piezometer was measured at half-hour intervals. Subtracting barometric pressure measured at the same time allowed total pressure above the dataloggers to be converted to height of water above the dataloggers using the Solinst software. Manually employing a Solinst 101 P1 water level meter and clock allowed both substrate and river levels to be calibrated to depth below the common datum line. River and hyporheic temperatures were recorded by all dataloggers associated with the piezometers, and air temperature was recorded with the barometric pressure, again at half-hour intervals.

2.3.2 Vertical and Horizontal Head Gradients

Three different vertical head gradients (VHG) were calculated: first, between the shallow piezometer and the river; second, between the deep piezometer and the river; third, between the deep piezometer and the shallow piezometer. These calculations were repeated for each nest and for both transects. Vertical gradients were measured from the deepest location to the surface water, where positive VHG indicated discharging flux from the substrate to the river. Negative VHG indicate discharging flux from the river to the substrate. Horizontal head gradients (HHG) were calculated between the shallow and deep piezometers for adjacent nests within the same perpendicular transect (i.e., north nearshore - north middle, north middle - north offshore). VHG and HHG were graphed together and were calculated using the following equation:

Eq. 1: Hydraulic Gradient = dh/dl,

where dh is the difference in hydraulic head between piezometers, and dl is the vertical or horizontal distance between piezometer screens.

2.3.3 Hydraulic Conductivity

Hydraulic conductivity of the spawning area substrate was measured using a slug test. A solid bar steel slug (1.3 cm × 61 cm) was inserted into the piezometer, and water level dataloggers were set to record measurements at half-second intervals. Once water levels reached equilibrium after initial insertion, the slug was removed from the piezometer and the recovery time was recorded. These rising head slug tests were analyzed with AQETSOLV software for Windows, using the Hvorslev (1951) method to determine hydraulic conductivity.

2.3.4 Hyporheic Flux

The vertical component of flux was examined between each shallow piezometer and river combination, and between each deep piezometer and shallow piezometer combination, using the basic Darcy equation (Freeze and Cherry 1979):

Eq. 2: Flux = (vertical hydraulic gradient) × (hydraulic conductivity).

It was assumed the substrate between the piezometer tips and the river was homogenous and isotropic.

2.3.5 Contour Mapping the Hyporheic Zone

A river cross-section at each of the two transects was constructed to estimate the two-dimensional direction of hyporheic flow, using SurferV14 software and mapping isolines (contour lines of equal potential). Isolines in cross-section beneath the substrate were drawn from water elevations measured in piezometers. Contour maps were created at three specific times: late October during Brook Trout spawning (October 28th, 2017), during a period before water release (January 10th, 2017), and during the peak water level after the release (January 12th, 2017).

2.3.6 Monitoring of Brook Trout Emergence

Drift netting to capture Brook Trout fry was conducted for four days per week during April through May, 2010-2016, with two drift nets approximately 15−20 m downstream of the spawning redds. Larval drift nets (76 cm wide and 53 cm high with 0.15 cm mesh and a 1000-micron basket) were active for 18–24 h. The daily percentage of total Brook Trout Fry caught during a season was used to display annual trends in emergence, and was calculated by the following equation:

Eq. 3: (Brook Trout larvae caught each day) / (Total number of Brook Trout in the year).

Redd temperature was recorded by the Ontario Ministry of Natural Resources and Forestry personnel from the Nipigon District office from a HOBO pendant temperature data logger (UA-001-08) set 5–10 cm into the substrate of a Brook Trout redd. River temperature was similarly recorded at the substrate surface of the river on the opposite bank of the river. Estimated discharge at the Brook Trout spawning site was obtained from discharge data collected by Ontario Power Generation at Hays Lake Dam using the following equation:

Eq. 4 (Appendix 1.8): Brook Trout Site Discharge = (Daily Total Inflow, Hays Lake) – (Daily Local Inflow, Hays Lake) × (0.52),

where 0.52 represents the estimated proportional volume of water that drains into Aguasabon above the Brook Trout spawning area (OPG unpublished, 2017).

2.4 Results

During October 28th, 2016 to January 10th, 2017, no discharge events occurred at Long Lake Control Dam. During this period, the mean estimated mean daily discharge at the spawning site was 17 m^3s^{-1} and ranged from 12 m^3s^{-1} (November 16th, 2016) to 25 m^3s^{-1} (November 30th, 2016). On January 11th, 2017, Ontario Power Generation released water at the LLCD, increasing flows downstream on the Aguasabon River, including at the reach where Brook Trout spawn. Before water release, discharge at the spawning area was 18 m^3s^{-1}, and after release it increased to 67 m^3s^{-1} (OPG unpublished, 2017). This change translated to a 0.9 m increase in river depth over the spawning redds in <24 h (Figure 2.2). Monitoring occurred until January 13th, 2017, when ice flows disrupted the piezometers. A substantial increase in flow occurred after the January water release when compared to the median value for flows: ~35 m^3s^{-1} during the spawning season in late October, and ~45 m^3s^{-1} at the median peak in mid January.

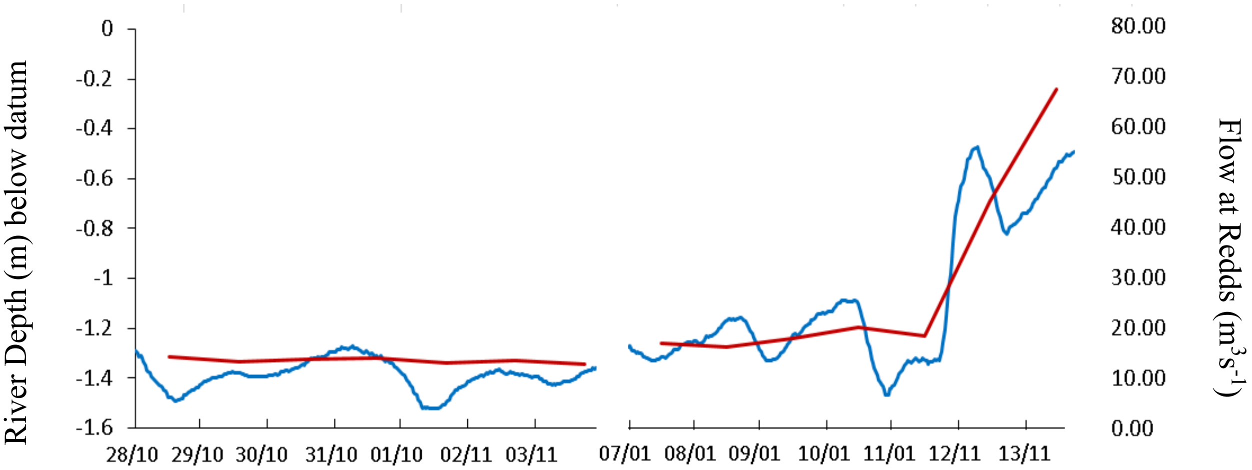


Figure 2. 2 River surface measured below the common datum (blue, left axis), and the approximate average daily discharge (OPG unpublished 2017) at the spawning area (red, right axis) for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan 7th – Jan 13th, 2017)

2.4.1 Vertical Head Gradients and Vertical Flux

VHG in both transects remained positive for the entire spawning and incubation period before the water release event occurred (Appendixes 1.2 and 1.3). As river levels rose on Jan 11th, 2017, all VHG declined into negative values on both transects (Table 2.1). VHG remained negative for a range of 5-30 h following water release. Vertical flux measured between the river and piezometers in the north and south transects followed the same pattern as VHG (Appendixes 1.4 and 1.5). Negative VHG and vertical flux only occurred during and after water release at the LLCD. Before the water release event occurred, natural fluctuations of the river measured by the river level logging station (Figure 2.2, blue line) did not cause a change to VHG and vertical flux in the way that the release event did.

Table 2. 1 Vertical head gradients (positive values indicate vertical flow from substrate to river), and vertical flux (mm s^{-1}) calculated for north and south transect piezometers inserted at Brook Trout spawning area pre-and post-LLCD water release, the difference and the time spent negative (h).

Transect	Nest	Piezometer	Vertical Gradient			Vertical Flux			Duration
			Pre	Post	Difference	Pre	Post	Difference	
North	Inshore	Shallow-River	5.32 E02	-3.76 E02	9.08 E02	2.24 E02	-1.59 E02	3.83 E02	14
		Deep-River	4.33 E02	-3.91 E02	8.25 E02	NA	NA	NA	16
		Deep-Shallow	3.36 E02	-4.11 E02	7.47 E02	9.76 E04	-1.19 E03	2.16 E03	20
	Middle	Shallow-River	9.94 E02	-6.52 E02	1.64 E01	4.16 E03	-2.72 E02	6.88 E03	18
		Deep-River	6.02 E02	-4.54 E02	1.05 E01	NA	NA	NA	19
		Deep-Shallow	2.45 E02	-2.84 E02	5.29 E02	1.81 E02	-2.19 E02	4.01 E02	21
	Offshore	Shallow-River	8.61 E02	-9.70 E02	1.83 E01	2.05 E02	-2.32 E02	4.37 E02	20
		Deep-River	5.30 E02	-5.23 E02	1.05 E01	NA	NA	NA	19
		Deep-Shallow	1.56 E02	-1.70 E03	1.73 E02	1.32 E02	-1.67 E03	1.49 E02	5
South	Inshore	Shallow-River	3.51 E02	-5.90 E02	9.40 E02	9.47 E03	-1.59 E02	2.53 E02	30
		Deep-River	4.43 E02	-5.34 E02	9.80 E02	NA	NA	NA	22
		Deep-Shallow	5.47 E02	-4.71 E02	1.01 E01	7.60 E04	-6.54 E02	1.41 E03	18
	Middle	Shallow-River	6.90 E02	-1.22 E0	1.91 E01	1.47 E02	-2.60 E02	4.08 E02	26
		Deep-River	NA	NA	NA	NA	NA	NA	NA
		Deep-Shallow	NA	NA	NA	NA	NA	NA	NA
	Offshore	Shallow-River	7.06 E02	-1.08 E01	1.78 E01	2.53 E02	-3.87 E02	6.40 E02	24
		Deep-River	4.05 E02	-5.67 E02	9.62 E02	NA	NA	NA	23
		Deep-Shallow	7.90 E03	-2.00 E04	8.11 E03	1.87 E04	-5.27 E06	1.92 E04	0.5

2.4.2 Horizontal Head Gradient

All HHGs measured between nests in the north and south transects remained positive for the entire incubation period before water release (Appendixes 1.6 and 1.7). As river levels rose on January 11th, 2017, HHGs declined into negative values, indicating flow changes to the opposite horizontal direction, towards the shoreline. The exception was the SOS-SMS pair, where HHG began to gradually decline at the time of water release, but did not reach negative values during the sampling period. HHGs remained negative for a range of 22–50 h (Table 2.2). HHG between piezometers NOD and NMD took 10 h longer to reach negative values after water release relative to the other piezometers, and did not return to positive values during the sampling period. As was the case for VHG and vertical flux, the LLCD water release was the only factor likely to have altered the horizontal flow through the substrate at the spawning area during the period of observation.

Table 2. 2 North and south transect horizontal head gradients (positive values indicate horizontal flow from deeper nests toward inshore nest) for piezometers at Brook Trout spawning area, pre- and post-water release at LLCD, time spent negative (h) and maximum recorded change in HHG at time of water release.

Transect	Nest	Piezometer	Pre-Release	Post-Release	Difference	Time Spent Negative
North	Middle - Inshore	Shallow-Shallow	6.29 E03	-1.22 E02	1.85 E02	22
		Deep-Deep	3.79 E03	-8.62 E03	1.24 E02	23
	Offshore - Middle	Shallow-Shallow	4.24 E03	-8.52 E03	1.27 E02	50
		Deep-Deep	1.54 E03	-2.98 E03	4.53 E03	40
South	Middle - Inshore	Shallow-Shallow	6.45 E03	-2.00 E02	2.71 E02	27
		Deep-Deep	NA	NA	NA	NA
	Offshore - Middle	Shallow-Shallow	6.38 E03	1.00 E03	5.52 E03	0
		Deep-Deep	NA	NA	NA	NA

2.4.4 Hyporheic Flow Contour Maps

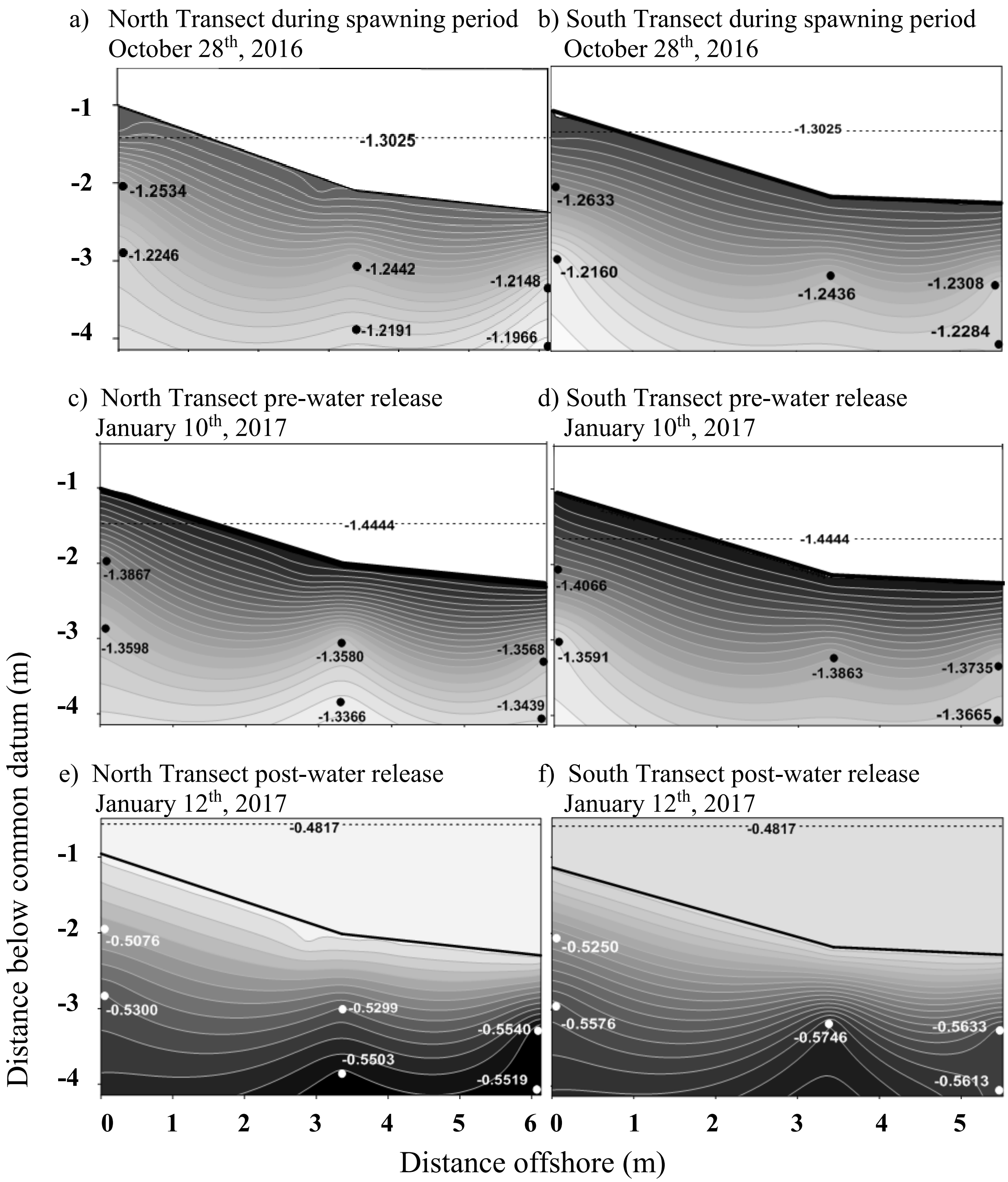
During the spawning season, the overall direction of water flow in the hyporheic zone was directed upwards to the river for both north and south transects (Figures 2.3 a-d). Tight contours around the shallow piezometers in the offshore nests display stronger horizontal movement directed inshore. Similarly, contours surrounding the inshore piezometers indicated flow in an offshore direction. Post-water release contour maps of both transects display downward vertical flow of stream water into the river substrate. During both high- and low-water conditions, direction of water flow was predominantly vertical (Figures 2.3 e, f).

2.4.5 Changes to Temperature with Water Release

At the start of the monitoring period, the average daily temperature of water in all 11 piezometers was >6.0 °C on October 28th, 2016, while temperature of water measured in the river level logger (just above the substrate over the spawning area) was 7.4 °C (Table 2.3). Water temperatures in all piezometers declined slowly from October to January, consistent with decreasing air and river temperatures, but did not exhibit the diel fluctuations typical above the river substrate. All temperatures remained above 3.7 °C before the water release event. Water temperature recorded at the bottom of the river exhibited sharp diel fluctuations related to changes in air temperature, and reached a lowest temperature ~1.5 °C before the water release event on January 10th, 2017. All temperatures then declined during the water release event and were impacted for periods that differed based on the position and depth of the piezometer (Table 2.3). Temperature reductions were less severe and shorter in duration for the piezometers that were deeper and furthest from shore. For example, the NOD piezometer decreased in temperature from 5.2 to 4.6 °C during the event, while at NIS it decreased from 4.1 to 0.3 °C, and the change lasted >53 h at <1 °C (temperatures were still <1 °C at time when piezometers were disrupted by ice flows). Similarly, temperature at SOD decreased from 5.4 to 5.0 °C, while at SIS it decreased from 5.0 to 0.1 °C, and the change lasted 48 h at <1 °C. Temperature at the bottom of the river also decreased from 1.5 to 0.2 °C during the water release event.

Table 2. 3 Temperature (°C) in piezometers and the river level station at the Brook Trout spawning area, pre-and post water release and duration (h) <1°C.

Transect	Nest	Piezometer	Temperature (°C) during spawning season	pre-release	post-release	Duration (h)
		River Station	7.4	1.5	0.2	>53
North	Inshore	Shallow	7.4	4.1	0.3	>53
		Deep	7.0	5.1	0.9	15
	Middle	Shallow	6.1	5.2	0.9	20
		Deep	6.1	5.2	3.5	0
	Offshore	Shallow	6.1	5.3	1.8	0
		Deep	5.7	5.2	4.6	0
South	Inshore	Shallow	7.2	3.7	0.5	48
		Deep	6.4	5.0	0.1	35
	Middle	Shallow	6.1	5.4	0.4	25
		Deep	NA	NA	NA	NA
	Offshore	Shallow	6.1	5.1	2.0	0
		Deep	6.1	5.4	5.0	0



a) North Transect during spawning period October 28th, 2016

b) South Transect during spawning period October 28th, 2016

c) North Transect pre-water release January 10th, 2017

d) South Transect pre-water release January 10th, 2017

e) North Transect post-water release January 12th, 2017

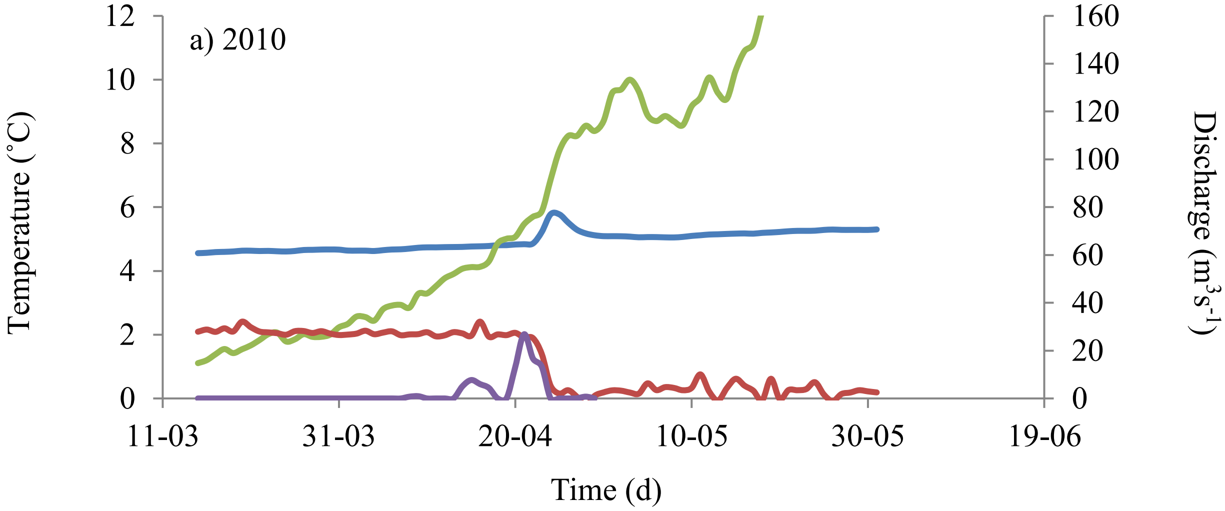
f) South Transect post-water release January 12th, 2017

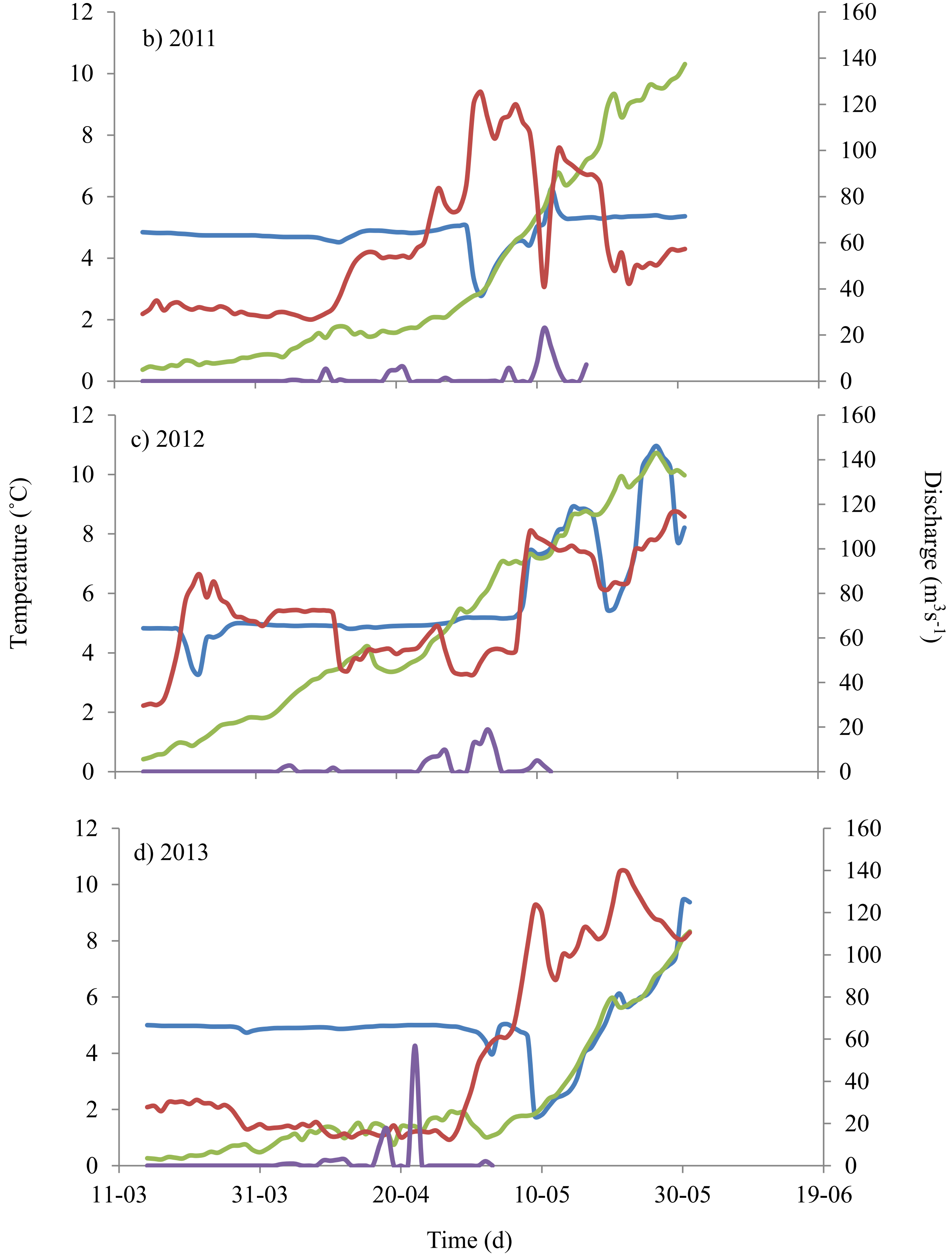
Figure 2. 3(a-f). Cross-sections of north and south transects through the Brook Trout spawning area, facing upstream during spawning season (October 28th, 2016), pre-water release (January 10th) and post water release (January 12th). Solid circles indicate the location of piezometers in the river channel. River levels (m below common datum, dotted line), estimated isolines, and general direction of flow. (Water flows light towards dark.) The South Middle Deep piezometer data was discarded due to a faulty sensor.

2.4.6 Brook Trout Drift

The daily percent of Brook Trout fry caught from the end of April to the beginning of May during 2010-2016 suggests that the earliest peak emergence occurred on April 21st, 2010 and the latest peak occurred on May 11th, 2011 (Figures 2.3 a-g). River temperature during peak emergence ranged from 1.4 °C in 2013 to 6.1 °C in 2012. River temperature was just above freezing at the beginning of each drift net season and increased through April and May. The corresponding redd temperatures ranged from 3.4 °C in 2014 to 5.2 °C in 2012. Redd temperature varied between 4.0 and 6.0 °C at the beginning of each net season. Redd temperature trended upwards or downwards towards river temperature when discharges from the LLCD occurred, and tracked river temperature when flows were high or increasing (Figures 2.4 b-f).

Before larval emergence, flow varied between 20 and 40 m^3s^{-1}. By mid to late April, flow gradually increased except in 2010 and 2016. Flow during the emergence period appeared to be influenced by discharge over LLCD and/or the spring freshet, and varied greatly from year to year, ranging from below 20 to above 100 m^3/s.




b) 2011
c) 2012
d) 2013
12
10
8
6
4
2
0
160
140
120
100
80
60
40
20
0
Temperature (°C)
Discharge (m³s⁻¹)
11-03
31-03
20-04
10-05
30-05
19-06
Time (d)

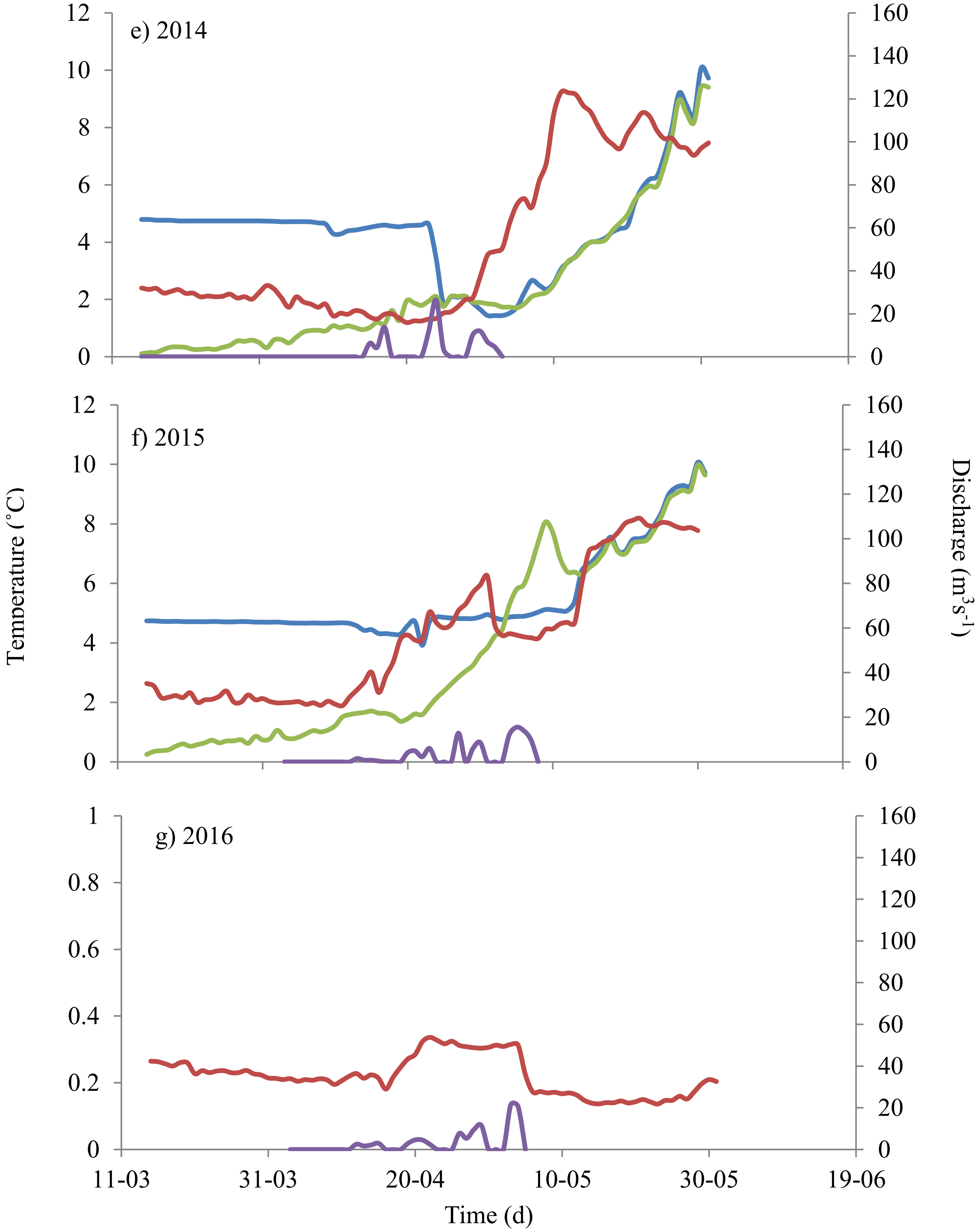


Figure 2. 4 (a-g). Results of drift netting efforts on the Aguasabon River, 2010–2016. Daily percent of fry caught each year (purple, right axis), mean daily redd temperature (blue, left axis), mean daily ambient river temperature (green, left axis), and approximate discharge at spawning site (red, right axis).

2.5 Discussion

Rapid increase in river level resulted in the reversal of vertical (VHG) and horizontal gradients (HHG) up to 1.8 m below the river substrate in the hyporheic zone. The area around Brook Trout redds changed from upwelling to downwelling conditions, likely because surface water infiltrated the substrate. Similar results were observed on the Nipigon River, a Northern Ontario river modified for hydroelectric power generation, which is also known for upwelling environments used by spawning Brook Trout and other salmonids (Curry et al. 1994). In this study, I confirmed the presence of upwelling water in the hyporheic zone at the only known Brook Trout spawning site on the Aguasabon River. Elsewhere, groundwater discharge creates stability in temperature and is essential for creating ideal spawning and incubation habitat for Brook Trout eggs (Snucins et al. 1992, Curry and Noakes 1995). Indeed, the presence of upwellings in a river's hyporheic zone is a main factor in selection of spawning sites by Brook Trout (Webster and Eiriksdottir 1976, Witzel and Maccrimmon 1983b). Constant discharge of warm (>3.7 °C) water onto Brook Trout redds is likely essential for protection against colder ambient stream temperatures (<1 °C) that occur in the winter season.

Groundwater discharge into rivers is typically maximized at the shoreline and declines with distance offshore (Winter 1974, Pfannkuch and Winter 1984, Curry et al. 1994). For the Aguasabon River, this pattern does not generally hold. Shallow water discharge in the substrate was strongest in piezometer nests furthest from shore, likely due to the straightening of the original river channel, which altered the location of the shoreline interface between surface water and the river bed. The artificial bank that exists now acts as a barrier between the flowing river and the adjacent oxbow (former river channel). The presence of the artificial bank likely decreases groundwater potential at the current shoreline–surface water interface, where it likely flows horizontally to the river, forcing groundwater to enter the river at deeper locations. Also, HHG indicated flows from further offshore were directed inshore. The position of the oxbow presents a challenge for understanding the horizontal flow of groundwater or surface water between it and the river, and for this reason further discussion of horizontal flow requires more information than what is presented here. At the same time, a unique opportunity exists to study effects of discharge over a control structure where an artificial bank downstream makes vertical flow relatively more important in determining upwelling and downwelling conditions in the hyporheic zone, a phenomenon that likely creates Book Trout spawning habitat.

Interstitial water conditions are determined by the source of water that dominates redd environments. The reversal flow in the hyporheic zone altered thermal interstitial water conditions and may have altered other physical and chemical conditions in the hyporheic zone. Such conditions include pH, dissolved oxygen and specific conductance all of which have been shown to influence the timing and success of hatching and emergence in salmonid eggs (Merz et al. 2004; Soulsby et al. 2009; Sternecker et al. 2013).

Water temperature in all piezometers in the Aguasabon River were affected by the water release at the LLCD. Depending on the distance of the redd from shore and its depth in the substrate, incubating Brook Trout eggs in a redd could experience varying degrees of cooling due to water release. Declines in temperature during the incubation period may lead to delayed hatching times or reduced hatching success depending on the stage of development (Murray and Beacham 1986, 1987, Murray and McPhail 1988, McCullough 1999a). In the Aguasabon River, historical redd temperature is consistent from hatching to larval emergence (2010–2016), at ~5 °C with small fluctuations within and between years. Consistent temperature likely leads to some consistency in the timing of peak emergence of Brook Trout (McCullough 1999).

The trigger for emergence of Brook Trout and other salmonid species may be a combination of environmental cues, including decreases or increases in dissolved oxygen, flow, temperature and sediment in redds, and/or the presence of predators (Godin 1981, Witzel and MacCrimmon 1981, Mirza et al. 2001). Increasing and peak Brook Trout emergence on the Aguasabon River follows some general trends in response to environmental conditions. A general trend among years is that it increases with increasing river temperature, but it is not clear if Brook Trout emergence is signalled by a specific river temperature. Flow estimated at the Brook Trout spawning site for the emergence periods does not follow a typical spring freshet regime. Sudden increases or decreases in flow occur over short time periods rather than a more typical increase and decline in flow over a period of several weeks. Determining emergence patterns is complicated, as the number of Brook Trout fry caught each day is likely being influenced by the cross-sectional wetted area that is sampled on a given day. For example, during high flows, drifting alevins hold a greater chance of evading capture, simply by having access to more drifting area around the opening of drift nets. During low flows, discharge may be too weak, allowing emergent Brook Trout to swim upstream. If Brook Trout emergence has any dependency on thermal, physical or chemical cues in spring, then increasing or decreasing discharge may play a role in triggering the emergence of Brook Trout on the Aguasabon River.

2.6 Conclusion

River depth over the spawning area in October and November influences the availability and locations of potential spawning redd sites for Brook Trout on the Aguasabon River. Generally, on the Aguasabon River, discharge at the LLCD is lower during spawning season when redd sites are being selected, and higher after the water release in January. Since the Aguasabon River is operated as a winter reservoir, discharge events at LLCD in January allow high water levels and flows to persist, altering the hyporheic flow conditions of downstream habitats such as Brook Trout redds for some time. This potential ecological trap involves substantially different hyporheic redd conditions beginning midway through the incubation period, relative to conditions that existed at the time of spawning site selection by brook trout. In fact, in the region, low river levels during October generally result in increased potential for groundwater flow, which may amplify the thermal and chemical gradients that Brook Trout select (Curry et al. 1994, Curry and Noakes 1995). However, emergent (drifting) Brook Trout have been seen and documented each year on the Aguasabon river since 2010, so the impacts are likely not large.

Chapter 3. Effects of a Prolonged Cold Treatment on Brook Trout Hatching, Emergence, and Survival

3.1 Abstract

Many northern Canadian Shield river systems are managed for water diversion, storage, and power generation. Regulation of river flow can alter groundwater potential in the hyporheic zone downstream of control structures, including near habitats used by Salmonid species for spawning. The effects of this altered potential on hyporheic water temperature, as well as the subsequent impact on Brook Trout incubation, are not well known generally nor site-specifically in Canadian Shield Rivers. This study simulated water temperature changes associated with a winter dam release on incubating Brook Trout embryos. A cold treatment lasting 40 h at temperatures <1 °C was imposed on incubating Brook Trout eggs, and the survival and time to hatching and emergence of Brook Trout were monitored. The severity and duration of the cold treatment re-create the most severe case of water release documented at the Long Lake Control Dam on the Aguasabon River, near Terrace Bay, Ontario. The cold treatment had no effect on mortality for incubating Brook Trout eggs (n = 1020) compared to control (n = 1020). Survival from fertilization to hatching was high for both treatment and control replicates (>90%), but was considerably lower for fertilization to emergence (55%). There was no difference in the development time from fertilization to emergence of alevins, but the cold treatment had a statistically significant impact on time from fertilization to hatching. Despite this significant result, in the context of water management on the Aguasabon River, a delay in hatching of <1 d is not likely to be ecologically significant.

3.2 Introduction

Manipulation of flow for hydroelectric power generation has been shown to alter the chemical and physical nature of water in the hyporheic zone of rivers (Curry et al. 1994, Malcolm et al. 2004, Sawyer et al. 2009). In Canada, hydropower generation often occurs on rivers where Salmonid species such as the Brook Trout (*Salvelinus fontinalis*) require the hyporheic zone for spawning and incubation during winter months. The Brook Trout is a managed sport fish in Ontario and requires cold, clean, and well oxygenated water to survive. These characteristics make it a useful indicator species of stream health in their native range (Tefft 2013).

Water temperature is an important factor driving Salmonid egg development (Tang et al. 1987), and Brook Trout are no exception. Many studies demonstrate that small changes in temperature have an effect on recruitment, development, and emergence of Salmonid alevins (McCullough 1999). Most studies that investigate this effect do so for the purpose of optimizing hatchery conditions (Beacham and Murray 1990, Marten 1992, Wagner et al. 2006). However, very few studies attempt to describe the effect on incubating eggs of varying temperature that may occur naturally or by human interference in rivers (Neitzel and Becker 1985, Tang et al. 1987). This research is necessary where hydroelectric development may alter natural stream temperature regimes (Sawyer et al. 2009).

The optimum temperature for Brook Trout incubation is 6 °C (Hokanson et al. 1973). Experiments with lower and constant incubation temperatures reveal reduced survival at 1 °C for Coho Salmon (*Oncorhynchus kisutch*) and at 4 °C for Pink (*Oncorhynchus gorbuscha*), Chinook (*Oncorhynchus tshawytscha*), Chum (*Oncorhynchus keta*), and Sockeye Salmon (*Oncorhynchus nerka*; Murray and McPhail 1988). Atlantic Salmon (*Salmo salar*) embryos and alevins are tolerant of colder temperatures (0.5 °C) for several months during their incubation period (Kazakov 1971). Brook Trout are a fall spawning species, where they must tolerate lower thermal tolerance limits during the incubation period. Elsewhere, the lower limits for hatching in Brown Trout (*Salmo trutta*), Arctic Char (*Salvelinus alpinus*) and Brook Trout have been recorded at <1 °C, with survival reduced by cold treatment (Humpesch 1985). Tang et al. (1987) demonstrated 100% egg mortality for Coho Salmon when incubated at a constant temperature of 0.6 °C, but no increase in mortality when eggs were cold treated from 3.5 to 0.1 °C for 8 h, even at early development stages. Declines in temperature from 10.0 °C to 0.1 °C for 8 h in laboratory dewatered redds did not reduce survival of eggs, embryos or alevins of Chinook Salmon (Neitzel and Becker 1985). It appears that Salmonids can withstand a threshold combination of low temperature and duration of cold before survival is affected.

The Aguasabon River, north of Terrace Bay, Ontario, supports a population of Brook Trout that is constrained between the Long Lake Control Dam (LLCD) and the Aguasabon Generating Station on Hays Lake. This population receives special attention in the Aguasabon River Water Management Plan due to its species-specific spawning requirements, limited spawning habitat in the main channel of the river, and the vulnerability of that habitat to upstream water management. The flow regime of the river is influenced by controlling discharge from the LLCD approximately 12 km upstream from the only known Brook Trout spawning area in the main channel. Changes in discharge during winter are correlated with temperature at a Brook Trout spawning redd (Figure 3.1). The Ontario Ministry of Natural Resources and Forestry has been monitoring temperature 5-10 cm below the substrate in a Brook Trout spawning redd since 2006. Brook Trout alevins emerge from the gravel in April-May at the study site from redds created during spawning in October (Chapter 2). This period conforms to the incubation time of Brook Trout from fall to spring in many Lake Superior tributaries.

Increases of discharge of the LLCD are associated with long term (1-3 d) declines in temperature at the Brook Trout redds, most commonly occurring in January (Figure 3.1), when the larvae are in the stage between eye-up and hatching. I hypothesize that Brook Trout development is influenced by cold (<1 ºC) for a period >1 d if the treatment occurs during this eye-up to hatching stage. The objective of an experiment in the Dorion Fish Culture Station, Dorion, Ontario was to simulate cold treatment equivalent to a temperature change experienced near redds of the Aguasabon River. I predicted that cold treatment would not decrease the survival of Brook Trout, but would delay development to hatching and emergence compared to eggs that did not experience a cold treatment.

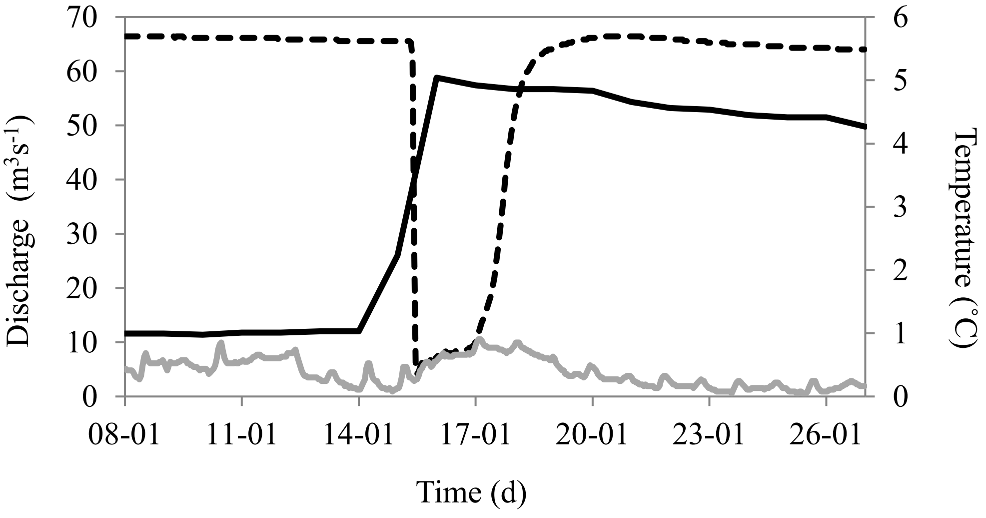


Figure 3. 1. Brook trout red temperature (dashed line) and ambient temperature (grey line) in the Aguasabon River, Ontario plotted with discharge (solid line) at the LLCD in winter 2013.

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3.3 Methodology

3.3.1 Experimental Rationale

Flow regulation at the LLCD was plotted with redd temperature during the Brook Trout incubation period (October−April) for the period 2006−2016. The goal was to show declines in temperature and recovery to ambient temperature (h) experienced at the redds. The worst-case cold treatment across years was selected for the experiment. The timing of the treatment was early January to match the development stage of Brook Trout at the time they experience temperature declines at their redds in the Aguasabon River. Because all spawning by Brook Trout on this river is completed by the end of November (Ray Tyhuis, personal communication), all eggs had reached the eye-up stage, but had not hatched, at the time of increased discharge associated with water level management each year on the Aguasabon River.

3.3.2 Experimental Design

The Dorion Fish Culture Station uses a gravity flow-through system to supply water for hatchery operations. All water to the hatchery is supplied by a spring-fed head pond. The experimental apparatus was placed on plastic stands inside an emptied tank in an isolation room (Figure 3.2). The number of replicates (incubation boxes), 12 per treatment and control, was based on a power analysis to detect a 1.5% decrease in survival based on (n = 6) observations Each replicate used 85 eggs; this was a manageable number to permit the determination of the fate of each individual egg every few days.

Brook trout eggs were obtained from the Dorion Fish Culture Station, Red Lake strain, whose origin is a relatively large watershed that should encompass genetic variation similar to what exists across Northwestern Ontario. Eggs were fertilized using the wet method, disinfected with ovodine, and water hardened. Eggs from different fish were not pooled; instead, sperm from only one male and eggs from only one female were mixed together. This was done 12 times, from 12 different pairs of fish (gametes). Each mating event was split, half going to a control replicate paired with a treatment replicate to which the other half went, to create 12 box pairs with eggs from the same broodstock pair (male and female).

After fertilization and water hardening, the eggs were placed during the same day in the incubation boxes. Each box measured 21.5 × 21.5 × 13.6 cm and contained an insert box (20.5 × 15.7 × 10.5 cm); both boxes were constructed from polyvinyl chloride (PVC), with a wire mesh screen below the insert box where the eggs were placed. The boxes were covered with lids to maintain dark conditions throughout incubation. The inflow pipe was extended with a 3-m pipe constructed from acrylonitrile butadiene styrene (ABS), to which 12 control incubation boxes were connected (Figure 3.2). Flow of unfiltered pond water originated from the main pipe, and then split to each replicate box. The inflow to each box was controlled by a turn valve. Water entered the incubation box, flowed up through the screened portion, and flowed out of the box through a standpipe. Water flow into the reservoir was controlled by an ABS valve. A second main ABS pipe was fitted to pipes connecting 12 treatment incubation boxes at the bottom of the reservoir. Water flow through each box was maintained 1.5-2.0 L/min, measured by holding a 1-L container under the outflow and recording time with a stop-watch. Adjustments to flow were made as needed.

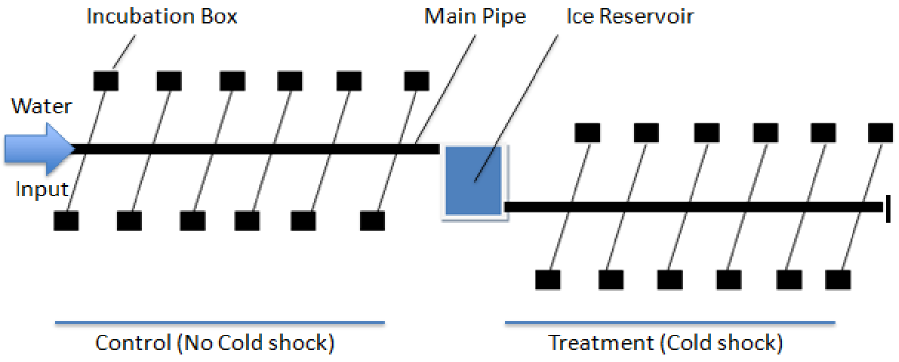

Figure 3. 2. Diagram of flow-through system for cold treatment experiment.

3.3.3 Temperature Control

Control and treatment incubation boxes shared the same water, so temperatures for all boxes were assumed to have been the same for the entire incubation period, except during the 40-h manipulation period (cold treatment). Water temperatures were monitored by the Dorion Fish Culture Station and fluctuated naturally with weather at the head pond source. The water temperature during cold treatment was logged using two HOBO loggers (Onset Computer Corporation, Bourne, MA) at the beginning of each main pipe to the control and treatment incubation boxes. These loggers were in place from December 20th, 2016 – January 24th, 2017. Temperature was reduced during the cold treatment to <1.0 °C by inserting ice blocks (frozen hatchery water) continuously into the reservoir above the treatment boxes for the 40-h period. The environment for the incubating eggs in the treatment boxes was returned to station water temperature as soon as the cold treatment was complete.

3.3.4 Hatching and Emergence

Incubation boxes were examined three times weekly to count and remove dead eggs until hatching. Once eggs reached the eye-up stage, they were briefly removed, so the boxes could be cleaned. During the cold treatment, all boxes were checked every few hours to count and remove any dead eggs. Once hatching began, daily observations were made to record the number and timing of appearance of hatched alevins. After hatching, tanks were cleaned, and newly hatched alevins were allowed to develop for 3.5 weeks. Boxes were checked two to three times per week to remove dead alevins. After 3.5 weeks, alevins were placed in a PVC ring in the center of their box, and buried in 3 cm of aquarium gravel. Alevins were counted and moved to larger rearing tanks in the Dorion Fish Culture Station daily as they emerged. Alevins were considered to have emerged if they were swimming in the box, resting on the gravel surface, or entered into gravel head first. After three days of no additional emergence, monitoring was ended, recording all non-emergent cases as mortalities.

3.3.5 Data Analysis

Mortality counts before and after the cold treatment, as well as non-emergence of alevins at the end of the monitoring, were compared between control and treatment with one-tailed, paired *t*-tests, pairing across boxes with eggs from the same broodstock pair assuming cold treatment would increase mortality. A Kaplan-Meier estimator was used to estimate mean time to hatching for each box and to model the control and treatment time to hatching, pooling across all replicates, as well as time to hatching for eggs from each broodstock pair, combining control and treatment. Time to hatching between the pooled treatment and pooled control K-M estimates was compared using a log-rank test based on the χ^2 distribution. Mean time to hatching between family pairs was compared between control and treatment with a one-tailed, paired *t*-test. A Kaplan-Meier approach was also used to estimate mean time to emergence, and time to emergence was modeled and compared in the same fashion as time to hatching. Times to hatching and emergence were converted into growing degree days (GDD) to examine the accumulated temperature unit differences between control and treatment, using a base temperature of 0 °C. SPSS Statistics for Windows, version 22.0 (IBM Corp. 2013) was the statistical software used for data analysis.

3.4 Results

Increases in discharge at the LLCD corresponding to as much as 30 m^3s^{-1} flow over the dam had little effect on temperature, but higher increases were associated with changes in temperature (Figure 3.3) The most extreme example was a water release in 2013 that raised discharge by 47 m^3/s and lowered water temperature to 0.3 °C, a drop of 5.3 °C (Figure 3.1). Following this release of water, recovery to ambient temperature at the redd site took 75 h, with 40 h at temperatures below 1.0°C.

For the experiment, the total number of control (n = 1020) and treatment (n = 1020) eggs in incubation was identical, each set of 85 eggs in one of 12 incubation boxes arising from a distinct broodstock pair. The cold treatment was initiated on January 10, 2017 at 3:50 pm and lasted until January 12, 2017 at 7:50 am, creating 40 h of temperatures <1.0 °C (mean, 0.5 °C) for the treatment incubation boxes (Figure 3.4). The initial temperature before treatment was 5.31 °C and the lowest temperature achieved was 0.2 °C, a difference of 5.1 °C. The temperature returned to ambient hatchery temperature within 5 h of the cold treatment ending. After hatching was complete, one replicate box failed, leaving emergence to be observed across 11 replicates.

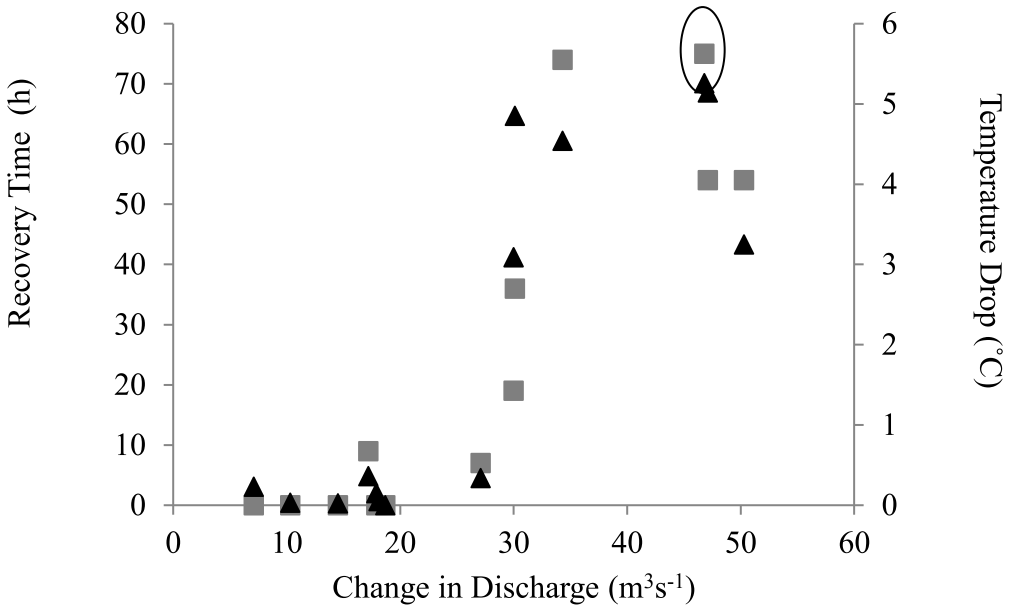

Figure 3. 3. Midwinter changes in discharge at the LLCD since 2006/07, corresponding drops in temperature (black triangles), and recovery time (grey squares) to ambient redd temperature on the Aguasabon River, Ontario. Encircled is the cold treatment with the greatest decrease in temperature, and the longest duration experienced in January 2013 used for the hatchery cold treatment experiment.

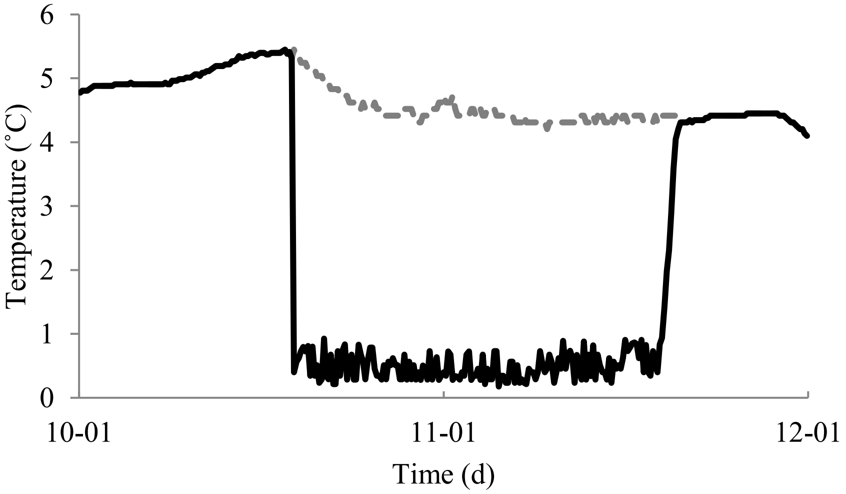

Figure 3. 2. Control (dashed line) and treatment (black line) temperature regimes at the Dorion Fish Culture Station during cold treatment experiment (January 10th–12th, 2017).

3.4.1 Mortality and Non-Emergence

The number of eggs dying before cold treatment ranged from 0−14 and 0−20, 4.6 ± 1.1 and 5.1 ± 1.4, and the corresponding total mortality was 56 and 61, for the control and treatment replicates respectively (Figure 3.5). The number of eggs dying after cold treatment to hatching ranged from 0−4 and 0−5, 1.6 ± 1.4 and 2.2 ± 2.0, and the corresponding total mortality was 19 and 26, for the control and treatment replicates respectively. Thus, there were no differences in egg mortality between control and treatment pairs, either before or after the cold treatment was administered, comparing treatment to control (before treatment: $t = 0.37$, $p = 0.33$; after treatment: $t = 1.74$, $p = 0.06$; Appendix 2.1). In total, the control replicates had a combined mortality of 75 eggs (7.3%), and the treatment 87 eggs (8.5%).

There was no difference in survival to emergence, comparing family differences between treatment to control ($t = 0.20$, $p = 0.42$; Appendix 2.2). Mortality associated with non-emergence was relatively high when compared to mortality before hatching; from the period of the experiment after cold treatment to emergence, the number of eggs dying ranged from 23−45 and 9−50, 33.0 ± 2.3 and 33.0 ± 3.7, and the corresponding total mortality was 368 and 362, for the control and treatment replicates respectively (Figure 3.5). Total survival from initial fertilization to hatching was similar for the control (945 survivors, 93%) and treatment (933 survivors, 91%). Total survival from fertilization to emergence for the control (515 survivors, 55%) and treatment (516 survivors, 55%).

3.4.2 Development Time to Hatching

Development to hatching was significantly delayed in eggs experiencing the cold treatment if results were pooled for control and treatment replicates, but with a mean of <1 d ($\chi^2 = 11.4$, $p = 0.001$; Figure 3.5). The mean times to hatching for the pooled control and treatment replicates were 97.1 ± 0.4 and 97.3 ± 0.4 d, respectively. Hatching started 84 d after fertilization and ended at 102 d for both sets of pooled replicates. Differences in time to hatching within gamete pairs were not statistically significant ($t = 0.39$, $p = 0.28$; Appendix 2.3). Among the 11 replicates, eight had longer mean times to hatching for the treatment than for the control. The K-M estimates to hatching for the control and treatment replicates ranged from 95.5–99.4 and 94.5–99.0 d, respectively. Estimates of time to hatching by broodstock pair showed greater variation than the difference between the total pooled treatment and control replicates (Figure 3.6).

3.4.3 Development Time to Emergence

Emergence started 132 d after fertilization for both control and treatment replicates and ended at 142 d in control and at 144 d for treatment replicates. There was no difference in time to emergence between the pooled control and treatment replicates ($\chi^2 = 0.4$, $p = 0.52$; Figure 3.7). The mean times to emergence for the pooled replicates were 137.7 ± 0.4 and 138.4 ± 0.5 d, for control and treatment respectively. Similar to the case for hatching, functions fitting time to emergence as alevins had greater variation among broodstock pairs than the difference estimated between the pooled treatment and control replicates (Figure 3.8).

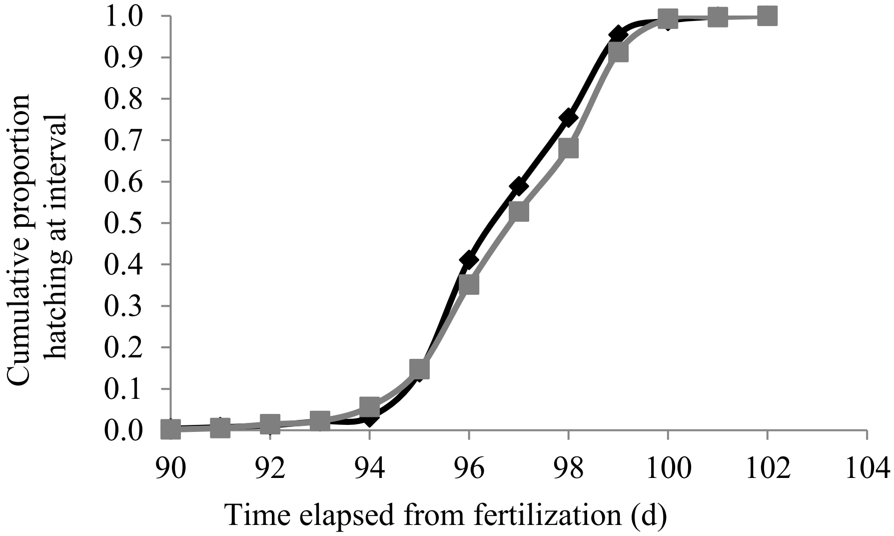

Figure 3. 5. Comparison of Kaplan-Meier functions fitted to hatching of Brook Trout alevins in a control (black line; n = 1020) and cold-treatment treatment (grey line; n = 1020) in a hatchery experiment.

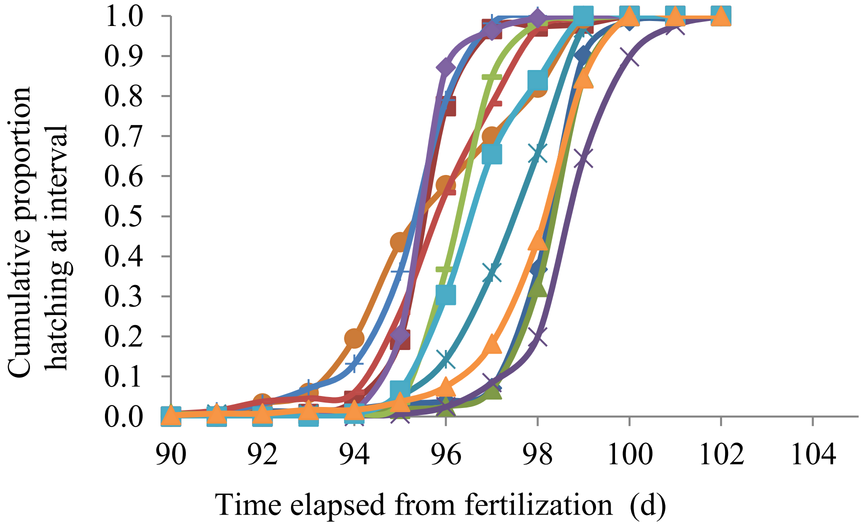

Figure 3. 6. Comparison of Kaplan-Meier functions fitted to hatching of Brook Trout alevins from 12 broodstock pairs.

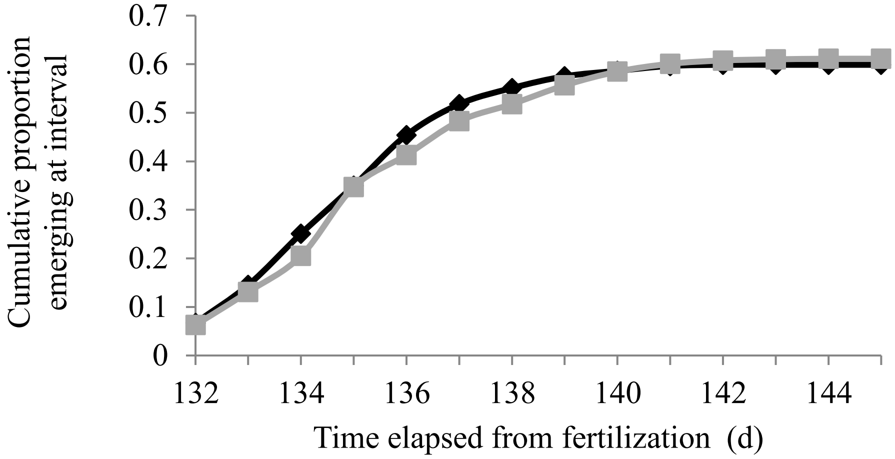

Figure 3. 7. Comparison of Kaplan-Meier functions fitted to emergence of Brook Trout alevins in a control (black line; beginning with *n* = 867 eggs) and cold-treatment treatment (grey line; beginning with *n* = 855 eggs) in a hatchery experiment.

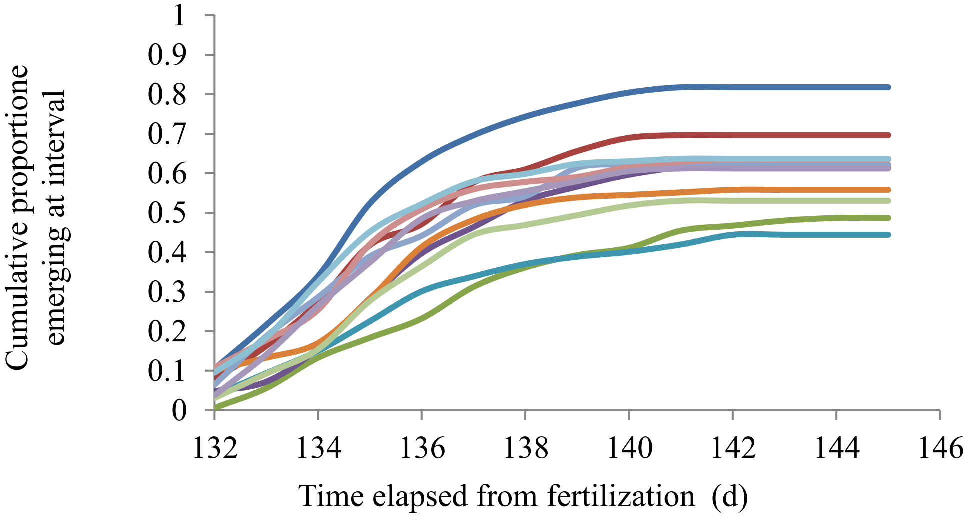

Figure 3. 8. Comparison of Kaplan-Meier functions fitted to emergence of Brook Trout alevins (beginning with n = 1722 eggs) from 11 broodstock pairs

Time from fertilization to emergence was not statistically different between treatment and control family pairs (t = 1.49*,* p = 0.14). Among the 11 replicates, seven had longer times to emergence in the treatment than in the control. The K-M estimates of time to emergence ranged from 135–140 and 136– 141 d, for the control and treatment replicates respectively. After the same number of calendar days, the cold treatment reduced the number of GDD by 6.5 temperature units in the treatment replicates. Accounting for the temperature of water in the Dorion Fish Culture Station, estimates of the mean number of GDD taken by alevins to hatch and to emerge were 765.6 for control and 764.7 for treatment replicates (Appendix 2.5). The ranges in temperature units to hatching for the control and treatment replicates were 528–549 and 521–542 GDD, respectively; to emergence, they were 750–774 and 755–783 GDD, respectively.

3.5 Discussion

The cold treatment in the Dorion Fish Culture Station experiment had no effect on survival of incubating Brook Trout eggs. This result is similar to what has been reported from other cold treatment experiments on other Salmonids (Peterson et al. 1977, Neitzel and Becker 1985, Murray and Beacham 1986, Tang et al. 1987). For example, Tang et al. (1987) assessed the effects of abrupt temperature reductions of as much as 6.1 °C for 8 h on Coho Salmon survival. Eggs at four different developmental stages, including a “just before hatching” stage, were transferred from constant temperatures ranging 1.5–10.2 °C; these shorter-term temperature reductions did not reduce egg survival, except when temperature changed from 10.2 to 4.1 °C (the maximum tested). Murray and Beacham (1987) tested effects of temperature reductions from both 8 and 12 °C down to 1 °C until hatching on Pink Salmon eggs at various development stages, and found that cold treatment had no effect on survival to hatching, but that the larger temperature reduction at the pre-hatch stage did reduce survival of hatched alevins. It is possible, then, that cold treatments occurring just before hatching do not affect Salmonid egg survival, except when initially incubated at relatively high temperatures (10–12 °C). In the Aguasabon River, neither spawning nor midwinter redd temperatures were monitored at >6 °C (Chapter 2), so it would be unlikely that incubating eggs experience initial temperatures high enough to cause mortality during even the most extreme cold treatment recorded in winter.

In the cold treatment, survival to hatching was high for both treatment and control replicates (>90%), but was considerably lower to emergence (55%). The methodology used to determine emergence involved burying the alevins, requiring them to “emerge” from the gravel. The resulting survival rates were similar to those of other experiments using similar emergence monitoring. For example, Hausle and Coble (1976) recorded survival from egg deposition to emergence of 59% in Brook Trout. In another study, eggs buried in homogeneous gravels of 4.2 mm achieved 14% and 20% survival to emergence, in Brook Trout and Brown Trout respectively; and in gravels of 9.2 mm, survival was 79% and 61% to emergence, respectively (Witzel and MacCrimmon 1983a). Different mixes of sand and gravel where eggs are buried (e.g., in natural redds) likely affect survival to emergence more than the cold treatment delivered by the Dorion Fish Culture Station experiment.

Cold treatments may negatively impact survival of Salmonids if they occur during fertilization, or up to 72 h after fertilization, if this period includes the water hardening stage (Wagner et al. 2006). This is a critical period, because the eggs are in the blastula stage and are more sensitive to temperature changes (Vernier 1977). In the Aguasabon River, Brook Trout spawn between October and November, and water hardening would occur as soon as the eggs are laid and fertilized in redds. Cold treatment induced by river regulation typically occurs in January, and at this time Brook Trout eggs will have completed the eye-up stage, and are more resilient to changes in temperature. If increases in discharge at the LLCD occur during spawning, when ambient river temperature is typically above temperature at the Brook Trout redds, a cold treatment induced by an increase in discharge would be unlikely at the water hardening stage.

The Dorion Fish Culture Station experiment reinforces the idea that Brook Trout are resilient to long-term cold treatments (up to 40 h) that occur later in development between the eye-up stage and hatching.

Temperature is a major factor influencing development from fertilization to hatching and emergence in salmonids (Tang et al. 1987, Murray and McPhail 1988, McCullough 1999b). The cold treatment tested at the Dorion Fish Culture Station had a statistically significant impact on time from fertilization to hatching. In a biological sense, a delay in hatching of <1 d does not warrant concern for recruitment by water managers. The variation in timing to hatching and emergence among progeny from different broodstock pairs, when compared to control and treatment differences, indicates that genetic differences must influence development timing more than a cold treatment of the magnitude tested (Appendixes 2.4, 2.5). Elsewhere, it has been shown that greater variation in time to hatching between different strains of Brook Trout can occur at both cold and warm incubation regimes (Baird et al. 2002).

The Aguasabon River typically receives a single cold treatment during winter if a cold treatment occurs at all, a result of the system being managed as a winter reservoir. Water is stored behind dams during periods of high flow and is released during periods of low flow (i.e., during winter months) for power generation. Other managed river systems may experience differences in the magnitude, frequency, duration, and timing of flow releases. For example, in peaking systems power is produced during periods of peak power demand, resulting in highly variable daily flows (Haxton et al. 2015). Constant ramping of flow is likely to impact the temperature regime of upwelling spawning habitat during winter, and may have greater implications for recruitment of Brook Trout (Curry et al 1994).

3.6 Conclusion

In the context of water management on the Aguasabon River, the results of the reporting of historical data and the experiment in the Dorion Fish Culture Station indicate that current practices of water discharge do affect the temperature of Brook Trout spawning habitat downstream of the LLCD control structure, but perhaps not incubating Brook Trout. These results may translate to other river systems with fall spawning in upwelling environments. Hydropeaking regimes on other rivers, such as the Nipigon River, may receive many more cold treatment events than the Aguasabon River, which may have greater impacts on the timing of hatching and/or emergence of Brook Trout or other Salmonids.

Chapter 4. Thesis Conclusions

This thesis demonstrated how river regulation alters hyporheic flow onto Brook Trout redds in a Canadian Shield river. Vertical flow was reversed in the hyporheic zone, switching the river from upwelling to downwelling conditions. In the Aguasabon River, severity and duration of vertical flow alterations were more exaggerated in nearshore and shallow piezometer locations near redds than at offshore and deeper locations. The reversal of hyporheic flow caused colder surface water to infiltrate the substrate at the redds, and reduced the redd temperatures as well as substrate temperatures at 1.8 m below the river bottom. Historical analysis of the relationship between redd temperature and water release during Brook Trout incubation periods reveals that temperature changes had increasing severity and duration when larger midwinter water releases occurred at the Long Lake Control Dam. The results of an experiment at the Dorion Fish Culture Station demonstrate the resilience of Brook Trout eggs to cold water treatment after the eye-up stage, and gives confidence to Ontario Power Generation that current water management practises likely have minimal impacts on Brook Trout eggs in the Aguasabon River. However, a significant delay in the development of cold treated hatchery eggs suggests caution to water management planners on other regulated river systems, for example those that use peaking regimes, where more frequent and severe water releases may induce chronic effects on ecological processes in the hyporheic zone.

Water management planning on the Aguasabon River should proceed cautiously, because there is still only one known spawning area in the main channel of the river. Further research should focus on determining if the spawning site is in fact the only one in the main channel. Telemetry studies should continue with a focus on tracking Brook Trout during the months of September to November. One knowledge gap that still exists is the effect of water release on the interstitial water quality of redds in terms of dissolved oxygen, pH and conductivity, since these are also important factors influencing Brook Trout recruitment from fertilized eggs (Curry et al. 1994). With the knowledge gained from this thesis on the direction of hyporheic flow under normal and increasing water conditions, inferences can be made regarding various interstitial water conditions and their impacts on incubating Brook Trout eggs. Another gap is the relationship between discharge at the Long Lake Control Dam and the water level over the known spawning area. Long-term monitoring of water level at the spawning area should continue in order to produce a rating curve for the spawning site.

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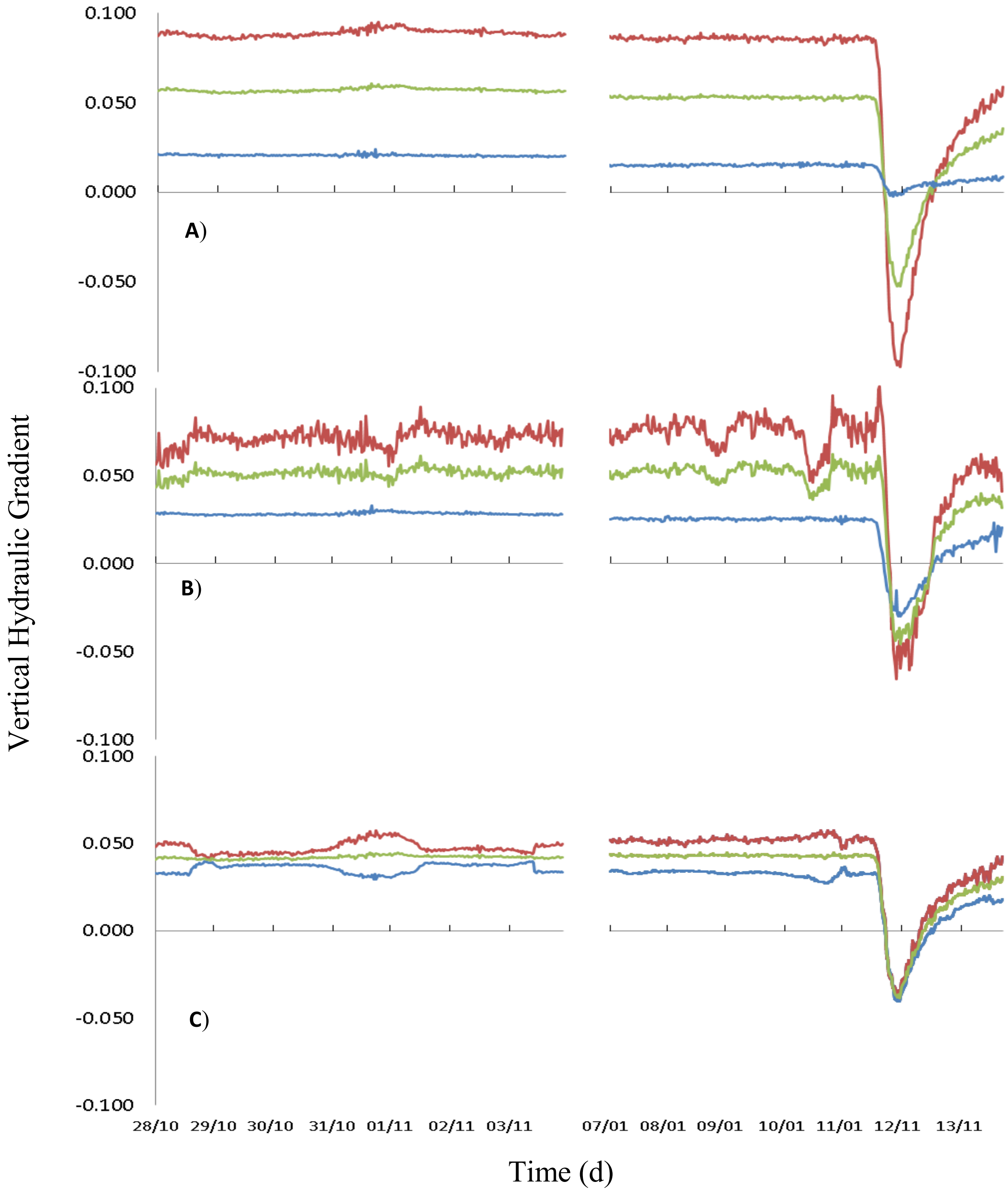
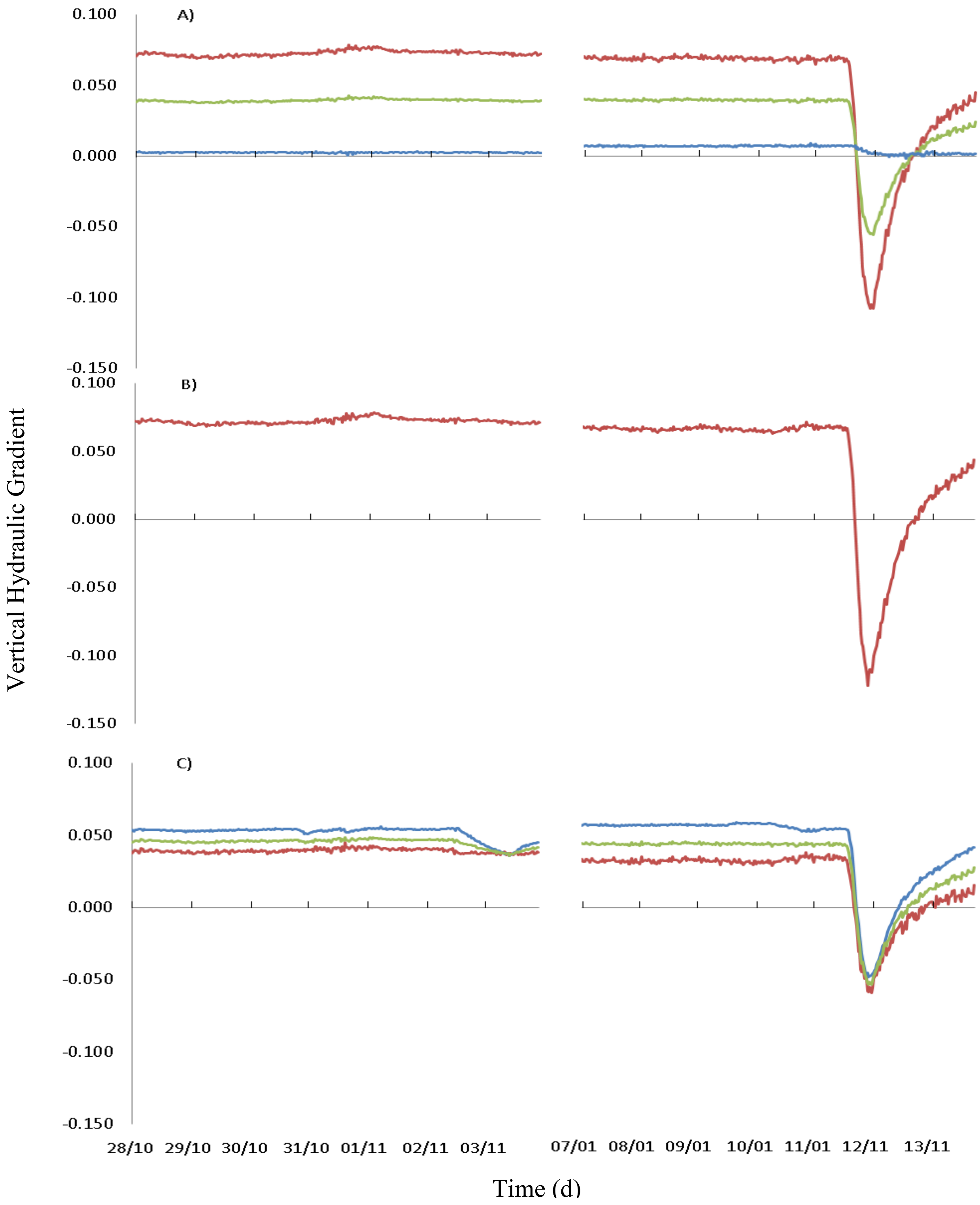
Appendix 1

Appendix 1. 1 Mean hydraulic conductivity (mm s^{-1}) of substrate at shallow and deep piezometers for north and south transects inserted at the Brook Trout Spawning area on the Aguasabon River, August 2017, and expected substrate (Halford, Kuniansky 2004).

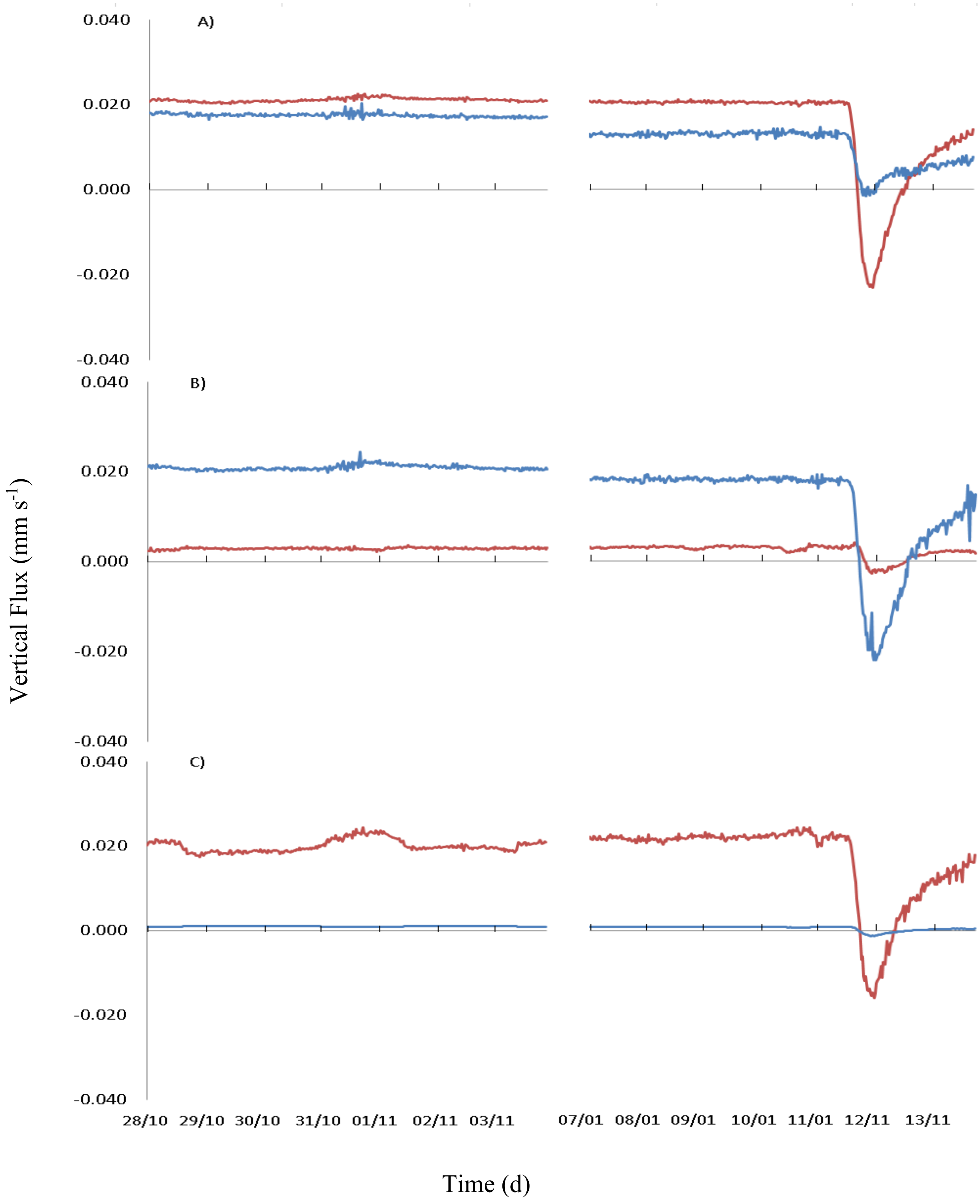
Piezometer	Conductivity	Expected Substrate
NIS	4.2 E01	Coarse Sand
NID	2.9 E02	Fine Sand
NMS	4.1 E02	Fine Sand
NMD	7.3 E01	Coarse Sand
NOS	2.3 E01	Medium Sand
NOD	8.4 E01	Coarse Sand
SIS	2.6 E01	Coarse Sand
SID	1.3 E02	Fine Sand
SMS	2.1 E01	Medium Sand
SMD	5.5 E01	Coarse Sand
SOS	3.5 E01	Coarse Sand
SOD	2.3 E02	Fine Sand



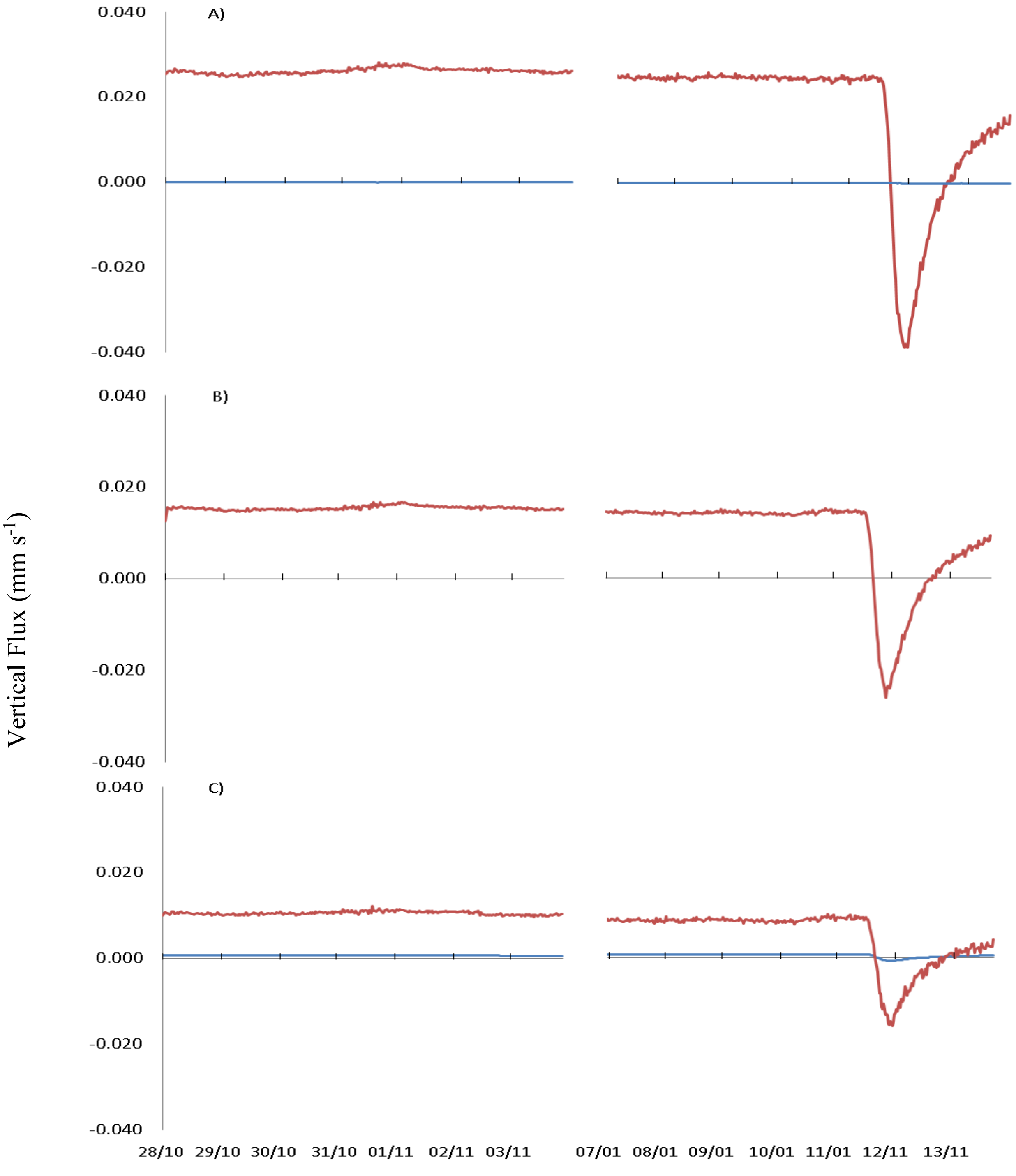
Appendix 1. 2 North Transect vertical gradients between the shallow - river (red), deep – river (green), deep - shallow (blue) piezometers in the offshore (A), middle (B), inshore (C), nests for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan7th – Jan 13, 2017)



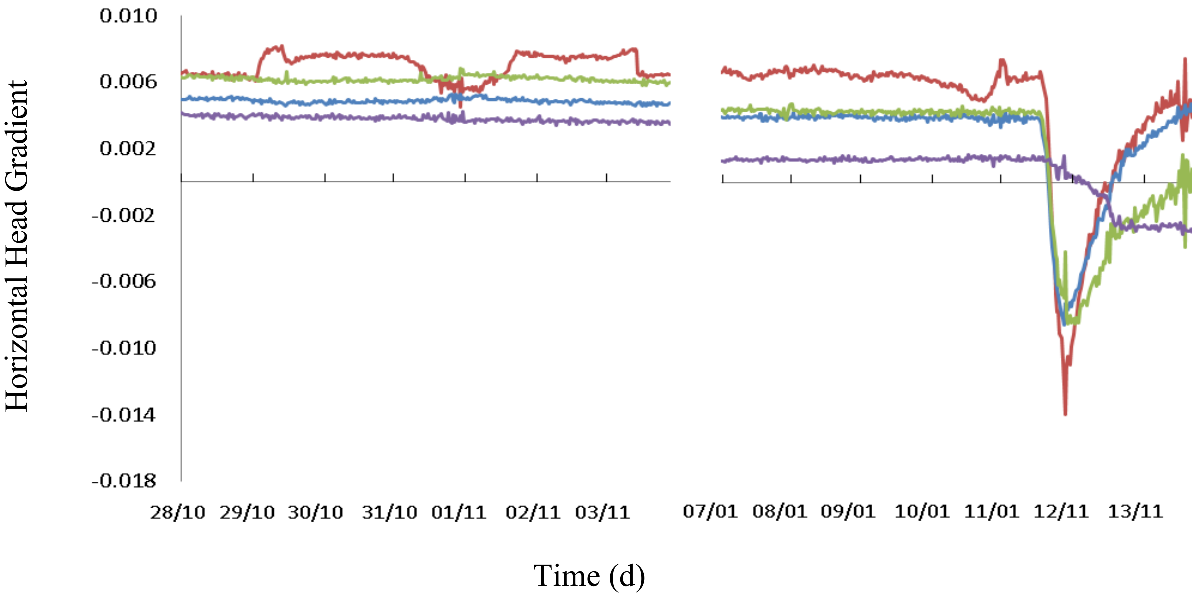
Appendix 1. 3. South transect vertical gradients between the shallow - river (red), deep – river (green), deep - shallow (blue) piezometers in the offshore (A), middle (B), inshore (C), nests for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan 7^{th} – Jan 13^{th}, 2017)



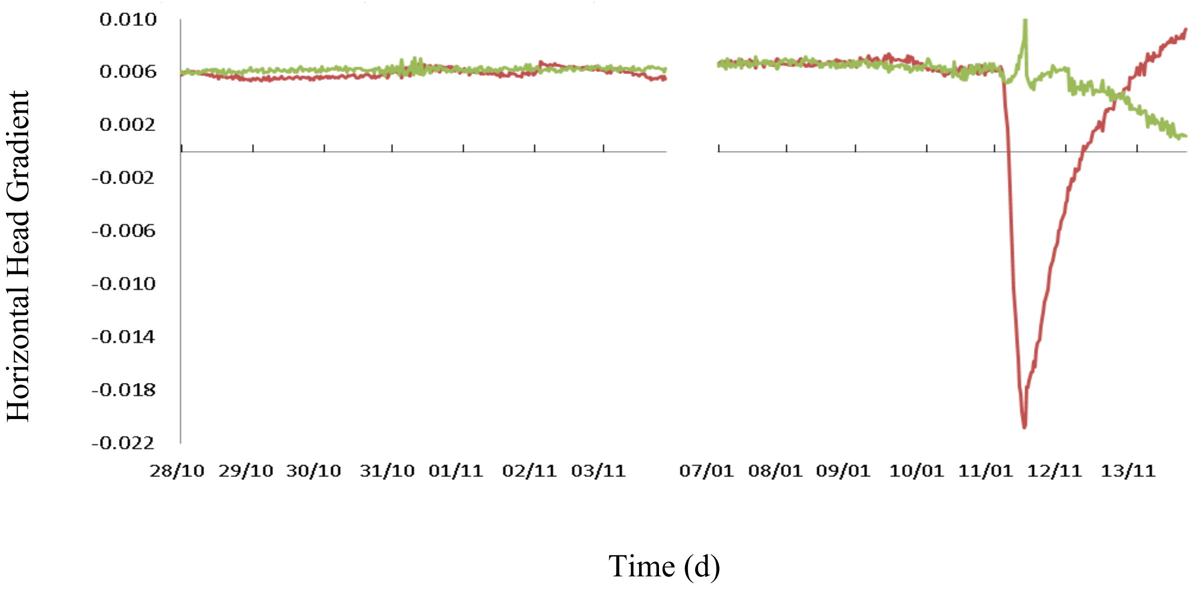
Appendix 1. 4. North transect vertical flux between the shallow – riv8er (red) and deep – shallow (blue) piezometers in the offshore (A), middle (B), inshore (C), nests for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan 7th – Jan 13, 2017)



Appendix 1. 5 South transect vertical flux between the shallow – river (red) and deep – shallow (blue) piezometers in the nearshore, middle and offshore nests for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan7th – Jan 13, 2017)



Appendix 1. 6. North transect horizontal gradients between the nearshore shallow – middle shallow (red), nearshore deep – middle deep (blue), middle shallow – offshore shallow (green), middle deep – offshore deep (purple), piezometers for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan7th – Jan 13, 2017)



Appendix 1.7 South transect horizontal gradients between the nearshore shallow – middle shallow (red), middle shallow – offshore shallow (green), piezometers for a portion of the spawning period (Oct 28th – Nov 3rd, 2016) and the water release event at the LLCD during the Brook Trout Incubation period (Jan7th – Jan 13, 2017)

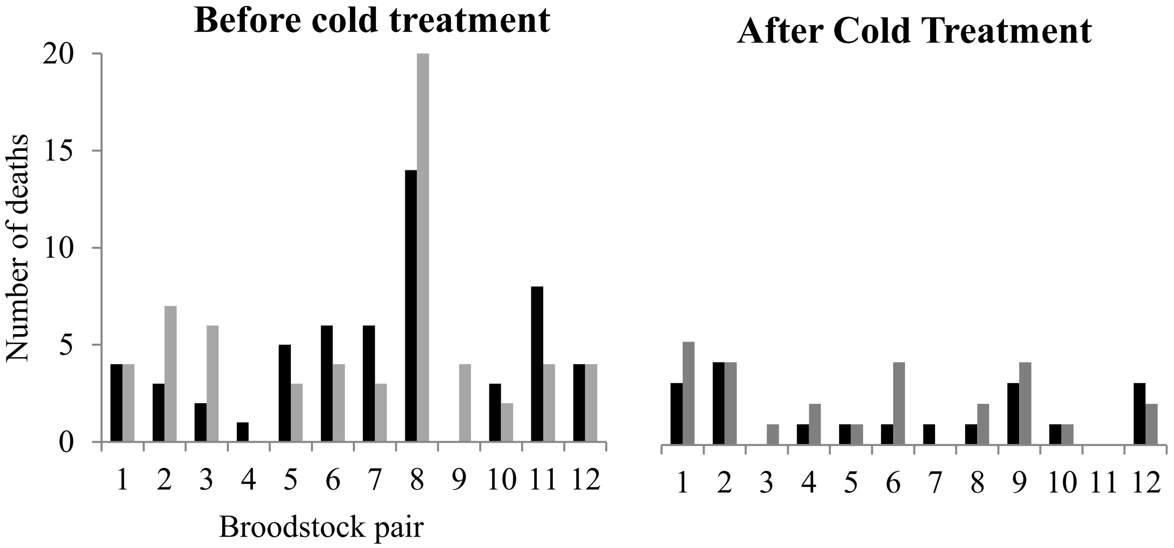
Appendix 1. 8 Hays Lake Level (m), average daily total inflow at Hays Lake(m3s-1), average local daily inflow (m3s-1) at Hays Lake, and the approximate flow (m3s-1) at Brook Trout spawning area. (OPG unpublished 2017)

Date	Hays Lake Level	Total Inflow	Local Daily Inflow	Approximate flow at spawning area
01-Oct-16	273.49	17.2	0.3	17.0
02-Oct-16	273.40	17.9	1.3	17.2
03-Oct-16	273.54	24.0	7.4	20.2
04-Oct-16	273.67	22.0	5.9	18.9
05-Oct-16	273.67	22.7	7.0	19.1
06-Oct-16	273.60	17.8	2.4	16.6
07-Oct-16	273.54	29.1	16.2	20.7
08-Oct-16	273.56	25.7	15.0	17.9
09-Oct-16	273.54	27.0	16.1	18.6
10-Oct-16	273.48	22.7	12.0	16.5
11-Oct-16	273.45	29.7	19.3	19.7
12-Oct-16	273.44	28.2	16.8	19.5
13-Oct-16	273.40	29.7	18.3	20.2
14-Oct-16	273.29	23.5	12.6	16.9
15-Oct-16	273.17	24.4	13.8	17.2
16-Oct-16	273.05	23.6	12.9	16.9
17-Oct-16	273.13	29.3	17.7	20.1
18-Oct-16	273.32	34.0	22.4	22.4
19-Oct-16	273.51	32.0	20.6	21.3
20-Oct-16	273.68	27.0	15.6	18.9
21-Oct-16	273.71	21.6	10.0	16.4
22-Oct-16	273.67	18.8	7.2	15.1
23-Oct-16	273.63	18.0	6.4	14.7
24-Oct-16	273.60	17.3	5.5	14.4
25-Oct-16	273.55	17.5	5.9	14.4
26-Oct-16	273.51	15.8	4.2	13.6
27-Oct-16	273.51	16.4	5.0	13.8
28-Oct-16	273.54	17.4	6.2	14.2
29-Oct-16	273.56	14.9	3.3	13.2
30-Oct-16	273.57	16.1	4.5	13.8
31-Oct-16	273.59	16.4	4.8	13.9
01-Nov-16	273.60	15.7	5.1	13.0
02-Nov-16	273.61	15.8	4.2	13.6
03-Nov-16	273.62	13.8	2.2	12.7
04-Nov-16	273.62	15.0	3.2	13.3
05-Nov-16	273.62	14.0	2.4	12.8

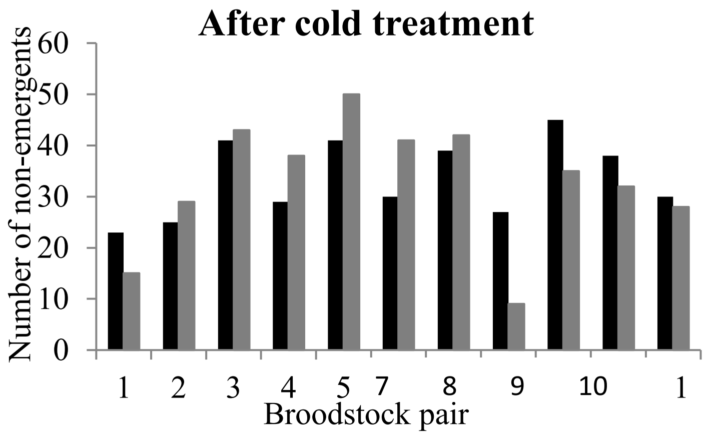
06-Nov-16	273.62	14.0	2.4	12.8
07-Nov-16	273.62	14.0	2.4	12.8
08-Nov-16	273.61	13.0	1.4	12.3
09-Nov-16	273.61	13.8	1.8	12.9
10-Nov-16	273.61	13.8	2.2	12.7
11-Nov-16	273.60	11.7	-0.7	12.1
12-Nov-16	273.60	13.7	1.9	12.7
13-Nov-16	273.59	11.7	-0.1	11.8
14-Nov-16	273.58	12.5	0.5	12.2
15-Nov-16	273.58	13.4	1.4	12.7
16-Nov-16	273.57	11.4	-0.6	11.7
17-Nov-16	273.58	16.4	4.6	14.0
18-Nov-16	273.60	15.7	2.9	14.2
19-Nov-16	273.60	13.8	-0.5	14.1
20-Nov-16	273.60	13.7	0.5	13.4
21-Nov-16	273.64	15.1	2.3	13.9
22-Nov-16	273.75	19.0	6.2	15.8
23-Nov-16	273.86	20.0	7.2	16.3
24-Nov-16	273.96	18.0	5.0	15.4
25-Nov-16	273.91	11.9	-0.9	12.4
26-Nov-16	273.86	13.5	0.7	13.1
27-Nov-16	273.82	12.3	-0.5	12.6
28-Nov-16	273.79	16.6	3.8	14.6
29-Nov-16	273.84	27.4	14.4	19.9
30-Nov-16	273.95	37.7	24.2	25.1
01-Dec-16	273.95	29.5	15.4	21.5
02-Dec-16	273.88	30.0	16.1	21.6
03-Dec-16	273.86	30.2	16.3	21.7
04-Dec-16	273.88	30.9	16.8	22.2
05-Dec-16	273.87	29.6	15.3	21.6
06-Dec-16	273.88	29.8	15.5	21.7
07-Dec-16	273.92	31.4	16.6	22.8
08-Dec-16	273.86	24.5	9.9	19.4
09-Dec-16	273.84	27.5	12.3	21.1
10-Dec-16	273.77	21.4	6.4	18.1
11-Dec-16	273.63	19.8	4.8	17.3
12-Dec-16	273.62	24.6	9.1	19.9
13-Dec-16	273.62	24.1	9.1	19.4
14-Dec-16	273.57	20.7	5.9	17.6
15-Dec-16	273.56	18.7	3.9	16.7
16-Dec-16	273.54	23.2	8.2	18.9
17-Dec-16	273.55	19.6	4.1	17.5
18-Dec-16	273.54	19.0	3.8	17.0

19-Dec-16	273.54	21.6	6.6	18.2
20-Dec-16	273.60	25.1	9.9	20.0
21-Dec-16	273.60	20.3	4.8	17.8
22-Dec-16	273.62	20.9	5.2	18.2
23-Dec-16	273.64	27.2	11.7	21.1
24-Dec-16	273.66	19.5	4.0	17.4
25-Dec-16	273.65	20.7	4.8	18.2
26-Dec-16	273.81	30.3	14.4	22.8
27-Dec-16	273.90	24.2	8.5	19.8
28-Dec-16	273.79	17.5	1.6	16.7
29-Dec-16	273.84	21.5	5.3	18.7
30-Dec-16	273.93	23.2	7.0	19.6
31-Dec-16	273.97	22.0	6.1	18.8
01-Jan-17	273.89	16.0	0.1	15.9
02-Jan-17	273.86	19.2	3.3	17.5
03-Jan-17	273.82	21.8	5.4	19.0
04-Jan-17	273.85	21.0	4.6	18.6
05-Jan-17	273.75	13.0	-3.4	14.8
06-Jan-17	273.71	20.6	4.4	18.3
07-Jan-17	273.69	17.6	1.4	16.9
08-Jan-17	273.59	16.0	-0.2	16.1
09-Jan-17	273.55	19.8	3.6	17.9
10-Jan-17	273.54	24.5	8.3	20.2
11-Jan-17	273.52	20.0	3.3	18.3
12-Jan-17	273.59	58.0	24.5	45.3
13-Jan-17	273.64	68.9	2.9	67.4
14-Jan-17	273.70	66.0	1.1	65.4

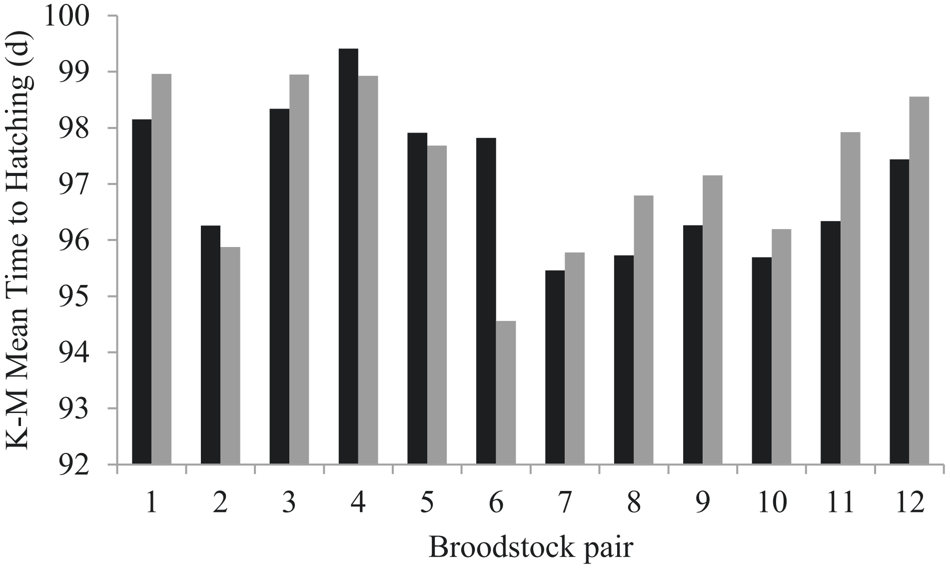
Appendix 2



Appendix 2. 1 Summary of mortality in Brook Trout eggs (n = 2040) from 12 broodstock pairs in a controlled (black bars) and a cold-treatment treatment (grey bars) in a hatchery experiment, before (1-70 d) and after (71-104 d) the 40-h treatment was administered on January 10, 2017.



Appendix 2. 2 Summary of non-emergence in Brook Trout alevins (n = 1743) from 11 broodstock pairs in a controlled (black bars) and a cold-treatment treatment (grey bars) in a hatchery experiment, after (70-144 d) the 40-h treatment was administered on January 10, 2017



Appendix 2. 3 Summary of Kaplan-Meier estimates of mean time to hatching for Brook Trout alevins (starting with n = 2040 eggs) from 12 broodstock pairs in a controlled (black bars) and a cold-treatment treatment (grey bars) in a hatchery experiment.

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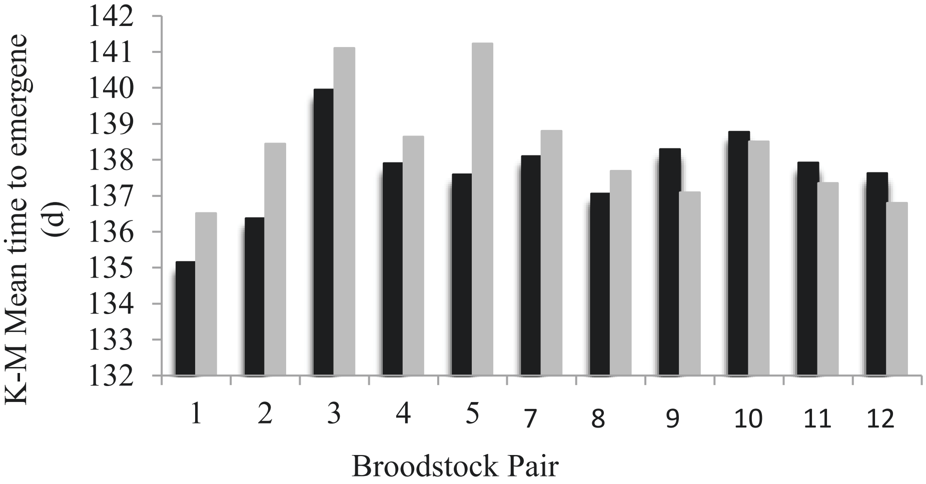

Appendix 2. 4 Summary of Kaplan-Meier estimates of mean time to emergence from fertilization for Brook Trout alevins (from n = 1722 eggs) from 11 broodstock pairs in a controlled (black bars) and a cold-treatment treatment (grey bars) in a hatchery experiment

Appendix 2. 5 Kaplan-Meier estimates of hatching and emergence expressed as accumulated temperature units (growing degree days: GDD).

	Hatching GDD		Emergence GDD	
Family	Control	treatment	control	Treatment
1	543.4	542.3	749.9	755.0
2	532.6	526.1	755.5	761.1
3	543.4	542.3	774.4	782.9
4	548.8	542.3	767.6	767.9
5	543.4	531.3	767.6	782.9
6	543.4	521.3	NA	NA
7	527.8	526.1	767.6	767.9
8	532.6	521.3	761.5	761.1
9	532.6	531.1	767.6	755.0
10	532.6	526.1	774.4	767.9
11	532.6	536.9	767.6	755.0
12	537.9	542.3	767.67	755.0
Mean	537.6	532.5	765.6	764.7
Standard Error	(+/- 1.9)	(+/- 2.5)	(+/-2.2)	(+/- 3.15)