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Understanding summertime thermal refuge use by adult Atlantic salmon using remote sensing, river temperature monitoring and acoustic telemetry

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Abstract

Adult Atlantic salmon return to natal rivers several months before spawning, and during summer, can be subjected to temperatures that exceed their upper temperature tolerance limits. Salmon use thermal refuges to minimize exposure to high temperatures, but little information exists regarding behavioral thermoregulation by adult Atlantic salmon. We examined behavioral thermoregulation by Atlantic salmon during summer in-river residence in a Quebec river with a novel combination of thermal infrared (TIR) remote sensing, river temperature monitoring, and acoustic telemetry. Adults engaged in behavioural thermoregulation at cooler ambient river temperatures (17-19°C) than previously recorded for this species and maintained body temperature within a narrow range (16-20°C) via use of cool and warm refuges. Adults used large, stable, stratified pools as refuges, allowing multiple individuals to thermoregulate simultaneously without leaving the pool. Low river discharge and high temperatures can be physical barriers to salmon migration, preventing them from accessing suitable refuges (e.g. pools). Identifying and maintaining connectivity to thermal refuges may be critical for persistence of Atlantic salmon populations as climate changes and rivers warm.

Introduction

Salmonid species have narrow temperature tolerance ranges, making them highly susceptible to environmental warming resulting from climate change (Brett 1956; Pörtner and Farrell 2008; Jonsson and Jonsson 2009). Outside their range of optimal temperatures (where aerobic scope is greatest and growth and metabolism are maximized), capacity for aerobic activity decreases to the point where aerobic scope is zero and metabolism depends on anaerobic processes (Pörtner and Farrell 2008; Jonsson and Jonsson 2009). Above the incipient lethal temperature, fish become thermally stressed and long-term survival is not possible unless access to cooler water is available (Elliott and Elliott 2010). Although the upper incipient lethal temperature varies among species and populations of salmonids, it generally falls between 20° and 28°C (Goinea et al. 2006; Jonsson and Jonsson 2009; Elliott and Elliott 2010).

Salmonids cope with high temperatures using behavioural thermoregulation, whereby individuals actively seek out and use cold-water refuges, i.e. discrete patches of water that are cooler than the surrounding ambient river temperature (Kaya 1977; Berman and Quinn 1991; Torgersen et al. 1999). Such cold water patches may be formed by cool tributaries and their associated confluence plumes, groundwater seeps, thermal stratification of deep pools or alcoves, or hyporheic exchange (Bilby 1984; Ebersole et al. 2003; Torgersen et al. 2012). Behavioural thermoregulation has been observed in multiple species of salmonids during all phases of the life cycle (Gibson 1966; Berman and Quinn 1991; Tanaka 2000) and records of Atlantic salmon (*Salmo salar*) using cool water refuges during high temperature events were described as early as 1939 (Huntsman 1942).

Atlantic salmon return to natal rivers during summer, many months before the onset of spawning. While over-summering in rivers, Atlantic salmon can be subject to temperatures that approach or surpass the upper incipient lethal level. Although the optimal and critical temperatures are not explicitly known for adult Atlantic salmon, temperatures of 26° to 27°C have been associated with mortality of migrating adult Atlantic salmon and there is consensus in the literature that temperatures between 20° and 23°C are stressful (Shepard 1995; Wilke et al. 1995; Breau 2013). High summer temperatures, therefore, may reduce the number of adult salmon that survive to spawn during the autumn, with associated negative impacts on population productivity. Because salmon metabolism increases with water temperature, high temperatures also reduce the energy available for individuals to engage in costly reproductive efforts, e.g. gamete production, mate selection, male-male competition, and nest construction by females (Gilhausen 1980; Berman and Quinn 1991; Hasler et al. 2012). Such non-lethal effects of high temperature on reproductive capacity can negatively impact productivity, even in the absence of heat-induced mortality. In order to retain sufficient energy stores for spawning, therefore, some adult salmon require access to cool thermal refuges during their long, in-river pre-spawning residence (Berman and Quinn 1991; Newell and Quinn 2005; Hasler et al. 2012).

Given the importance of thermal refuges to persistence and management of salmon populations, it is surprising that to date, there has been little research directed at understanding the temperatures that trigger refuge use by adult Atlantic salmon or the specific refuge types used. Studies of thermal refuge use by juvenile Atlantic salmon (e.g. Cunjak et al. 2005; Breau et al. 2007; Dugdale et al. 2016) have been widely conducted, whereas records of refuge use by adult salmonids largely have been restricted to species of Pacific salmon (e.g. Goniea et al. 2006; Donaldson et al. 2009; Keefer et al. 2009).

Several tools are now available for monitoring river temperature and tracking fish movements in rivers across a range of temporal and spatial scales. Remote sensing of river temperature via aerial thermal infrared (TIR) imagery permits estimation of river temperature at a single time point over large spatial scales, whereas monitoring via temperature loggers can provide a long-term record of temperature at specific locations (Torgersen et al. 2001; Torgersen et al. 2012; Dugdale 2016). Temperature sensors built into very high frequency (VHF) and ultrasonic (acoustic) transmitters allow direct measurement of internal body temperature of tagged fish (Brewitt and Danner 2014; Gotkowsky 2017). We used a unique combination of TIR imagery, river temperature monitoring, and acoustic telemetry to 1) characterize the types of thermal refuges used by adult Atlantic salmon during the pre-spawning in-river residence and 2) identify the temperatures at which adults initiated thermal refuge use in a gravel bed river in Quebec, Canada. We expected that adult Atlantic salmon would engage in behavioural thermoregulation to remain within a narrow temperature range (Berman and Quinn 1991; Newell and Quinn 2005; Gotkowsky et al. 2017). Specifically, we expected that adults would use cool water patches when river temperatures reached the range thought to be stressful to adult Atlantic salmon (i.e. 20° to 23°C, Shepard 1995; Wilke et al. 1995; Breau 2013). We further expected that patterns in behavioural thermoregulation would follow the diurnal cycle of heating and cooling patterns, with adults using cool refuges during the warmest hours of the day (Ebersole et al. 2001).

Materials and methods

Study area

The Rivière Sainte-Marguerite Nord-Est (hereafter, Nord-Est) is a salmon river in Quebec, Canada, approximately 190 km northeast of the city of Quebec. The Nord-Est drains a catchment of ~1000 km^2, and joins the Rivière Sainte-Marguerite 5 km upstream from its confluence with the Saguenay River (Fig. 1). During summer, river temperature routinely exceeds 22°C and during some years (2005, 2007, 2012, 2014), river temperatures greater than 26°C were recorded in the lower seven kilometres of the river (Boyer et al. 2016).

A natural waterfall (Chute Blanche) at river kilometre (rkm) 7 blocked upstream migration of returning adult salmon until 1981, when a fish ladder was installed to allow adult salmon to bypass the waterfall. The installation of the fish ladder opened approximately 18 km of additional river habitat to salmon for spawning and juvenile rearing. All adult Atlantic salmon that return to the fish ladder are captured in an entry cage for counting and measurement before being allowed to pass into the main fish ladder. A pair of impassable waterfalls at rkm 33.7 (Chute du 16 Miles) and rkm 36.2 (Chute du 18 Miles) currently prevent further upstream movement by returning adults.

In 2014, a translocation program was initiated to trap a subset of the adults returning to the fish ladder at Chute Blanche for transport upstream of the pair of impassable falls. Transported adults are able to access approximately 13 km of river habitat, before further upstream passage is blocked by another natural barrier. As part of the evaluation of the success of the translocation program, all salmon transported between 2014 and 2016 were tagged with acoustic transmitters to assess post-transport movement patterns. Our study leveraged the existing infrastructure and tagging program on the Nord-Est to assess thermal refuge use by adult Atlantic salmon during summer 2016, by tagging a subset of transported salmon with temperature sensing acoustic tags (V13T–H, Vemco, Halifax, NS). We conducted our study in two river reaches; the river reach between Chute Blanche and Chute du 16 Miles (hereafter, the downstream sector) and upstream of Chute du 18 Miles (hereafter, the upstream sector). Due to challenging river access, we were unable to deploy acoustic receivers throughout the entirety of the downstream sector, so we focused our efforts on the seven kilometres immediately downstream of Chute du 16 Miles, which are accessible by canoe (Fig. 1).

TIR imagery and acoustic telemetry array

We used TIR imagery to locate cold patches at the riverscape scale, which we subsequently used to inform placement of an acoustic receiver array designed to monitor movements and body temperatures of tagged Atlantic salmon. Airborne thermal and optical imagery (resolution of approximately 2.6 cm and 18.7 cm respectively from ~300 m AGL) was obtained from an aerial survey of the Nord-Est conducted on 25 August 2014 using the same method as Dugdale et al. (2013). We processed images and identified cool patches using the custom MATLAB graphical user interface and methods described by Dugdale et al. (2013). Briefly, thermal images were used to identify surface temperature anomalies, which were verified against the corresponding optical images to distinguish true water temperature anomalies from landscape features (e.g. shading on bedrock or vegetation). True surface anomalies that were ≥ 0.5°C cooler than ambient river temperature were classified as cool thermal refuges (Dugdale et al. 2013). Identified refuges were classified by type according to the definitions of Ebersole et al. (2003) and Torgersen et al. (2012), which are described in detail by Dugdale et al. (2013).

We deployed acoustic receivers in TIR-identified cool patches that were ≥ 0.5 m depth, the minimum depth required for receiver deployment. This depth requirement was deemed unlikely to bias the results of the present study because refuges shallower than this threshold are unlikely to provide thermal habitat suitable for long-term use by adult salmonids (Torgersen et al. 1999; Baird and Krueger 2003; Torgersen et al. 2012). Because only cool water patches that intersect the river surface can be identified by TIR (Dugdale 2016), acoustic receivers were also deployed in five deep pools known to be used by adult salmon for holding during in-river residence (Frechette, unpublished data). Deployment of acoustic receivers in deep pools enabled identification of subsurface cool refuges that otherwise would have been missed via evaluation of TIR imagery. Receivers were deployed in late June before tagging commenced and were recovered towards the end of the spawning period in late October. Raw telemetry data were downloaded from receivers using VUE software (Vemco, Halifax, NS) and processed using the VTrack package for R (Campbell et al. 2012).

River temperature monitoring

Water temperature within the Nord-Est was recorded at 15-min intervals using temperature loggers (HOBO UA-002-64, Onset, Bourne, MA) installed at a number of sites (Fig. 1a). Before deployment, we cross-calibrated temperature loggers and temperature-sensing transmitters in an ice-water bath to develop a correction factor. Temperature responses varied among individual loggers (0.010 – 0.678˚C, mean = 0.2˚C) so logger-specific correction factors were applied to each temperature logger prior to use in subsequent analyses. Temperature loggers (N = 7) were housed in white PVC tubes to shield them from direct sunlight and shifting river sediment, and were either attached to the anchoring blocks used to deploy acoustic receivers or attached to rock-filled plastic sacks. Temperature loggers were deployed in well-mixed areas of the river mainstem (one in the upstream sector at rkm 45 and one in the downstream sector, at rkm 30), in the two confluence tributary plumes identified in the upstream sector (U1 and U5), and in two deep pools known to be used by salmon during in river residence (U8 in the upstream sector and D3 in the downstream sector). We suspected that the upstream pool (U8) might be thermally stratified, so we deployed a second temperature logger c. 10 cm below the surface float that was attached to the anchor for the acoustic receiver. The difference between the temperatures measured 10 cm below the surface and 10 cm above the substrate allowed us to test for the presence of thermal stratification in pool U8.

Salmon capture and tagging

Salmon included in this study were part of the translocation program detailed in the 'study area' section. Twenty individuals were captured at the fish ladder and transported to upstream release sites (Fig. 1a) where they were tagged with acoustic transmitters equipped with temperature sensors (Vemco V13T–H), allowed to recover in-river, and released. The temperature-sensing transmitters allowed us to obtain measurements of instantaneous internal fish temperature whenever tagged salmon were detected by an acoustic receiver. Sixteen salmon were released in the upstream sector, c. 3 km upstream of Chute du 18 Miles (adjacent to pool U3) and four salmon were released in the downstream sector, c. 3 km downstream of Chute du 16 Miles (adjacent to pool D4).

Fish were transported in a 1 600-L tank with a water recirculation system and supplied with oxygen. No transport occurred when water temperatures exceeded 20°C and a maximum of four fish were transported at the same time. Tagging was accomplished within one hour of arrival at the release site. Before tagging, salmon were placed in an aerated anaesthetic bath containing 30 mg L^{-1} acetyleugenol. Dosage time varied depending on water temperature. Once the salmon was adequately anesthetized (determined by gill respiration rate and strength of the reflex response), it was placed on a V-shaped surgery table. During the surgical procedure, salmon received a maintenance dose of acetyleugenol (15 mg L^{-1}) and water administered over the gills using a custom-designed reservoir. The acoustic tag was inserted into the abdominal cavity anterior to the pelvic girdle through an incision using aseptic technique. The incision was closed with three or four simple interrupted sutures. During handling, fish length (fork and total length) and maxillary length were measured. Established regression relationships between maxillary length and fork length permitted sex identification for individuals that had not yet developed evident external sexual characteristics (Maisse et al. 1988; Prévost et al. 1991). Following surgery, fish were placed in an aerated recovery tank to be weighed before transfer to a water-filled stretcher for transport to the in-river recovery cage.

Salmon were held in the recovery cage and checked every 15 minutes until they regained equilibrium and were swimming freely. The recovery cage in the upstream sector was equipped with a door that was opened once salmon were swimming freely, allowing individuals to volitionally exit the cage. If an individual remained in the cage one hour after regaining equilibrium, it was released by hand. The recovery cage in the downstream sector did not have a door so individuals were released by hand one hour after they regained equilibrium.

Seasonality and location of refuge use

We obtained consistent position and temperature recordings from 15 of the 20 tagged salmon during July and August 2016 (Table 1). Four salmon tagged in the upstream sector and one salmon tagged in the downstream sector left the study area within two days of tagging and were not included in analyses. Two of the salmon tagged in the upstream sector (Fish D-01 and Fish D-02) moved downstream over the impassable falls within six days of release (termed fallback) and were detected in the downstream sector. As these fish were present in the downstream sector for the majority of the study period, they were grouped with the individuals released in the downstream sector for all analyses. Statistical analyses were conducted using R version 3.3.0 (R Core Team 2014) within R Studio version 1.0.136 (R Studio Team 2016).

To identify when and where salmon used thermal refuges, we computed the difference (ΔT) between salmon body temperature (T_B) recorded by acoustic receivers and mainstem river temperature (T_R) measured by temperature loggers. Because fish temperature was logged intermittently (only when a fish was in proximity of the receiver), while ambient temperature was logged at 15-minute intervals, we used cubic spline interpolation to estimate mainstem river temperature at the exact moment that fish temperature was measured and logged by the acoustic receiver, which allowed us to calculate the instantaneous difference (ΔT_I) between interpolated mainstem river temperature (T_{RI}) and fish temperature (T_B). We generated plots of ΔT_I by day for each individual, and color-coded the location (receiver identity) where the detections were recorded (Fig.2).

In the literature, cool refuges have been defined either using a specific temperature differential between the mainstem and cool patches, e.g. 2˚C (Torgersen et al. 2012) or 3˚C (Ebersole et al. 2001; 2003), or as simply water that is cooler than the mainstem (Baird and Krueger 2003). Donaldson et al. (2009), however, found evidence of behavioural thermoregulation in sockeye salmon (*Oncorhynchus nerka*) when the differential between body and river temperatures was 1˚C. Given the inconsistency in the literature regarding definitions of thermal refuges and/or behavioural thermoregulation, we chose to use a threshold of 1˚C to identify the location and timing of refuge use. We classified fish as using cool refuges when $\Delta T_I \leq -1$˚C. Conversely, values of $\Delta T_I \geq 1$˚C indicated that fish were located in water that was warmer than the ambient mainstem river temperature.

Identification of behavioural thermoregulation

We assessed trends in behavioural thermoregulation over the course of the summer using the difference (ΔT_H) between mean hourly internal fish temperature (T_{BH}) and mean hourly ambient mainstem river temperature (T_{RH}). We used mean hourly measurements because river temperature did not change substantially within the hour. To determine whether salmon exhibited behavioural thermoregulation, we regressed T_{BH} against T_{RH} for each individual (Berman and Quinn 1991). If salmon did not behaviourally thermoregulate (i.e., fish temperature depended on mainstem river temperature), we would expect a 1:1 relationship between T_{BH} and T_{RH}. If a fish did behaviourally thermoregulate (i.e., salmon actively selected water that was warmer or cooler than ambient mainstem river temperature), we would expect a significant departure from the 1:1 relationship (Hillyard and Keeley 2012). We accounted for temporal autocorrelation in the data by applying weighted stratified sampling to the time series of T_{BH} and T_{RH}. Specifically, the observations of each individual salmon were divided into four strata, with each stratum representing one week during the month of August (each stratum consisted of 8 days, except for the fourth stratum, which was 7 days). One half of the total observations for each fish were randomly sampled, with the number of samples taken from each stratum weighted by the proportion of observations in the stratum relative to total number of observations during the month of August. The subsampled data were used in the linear regression model and a bootstrapping routine (1000 replicates) was used to confirm that estimates of model coefficients remained constant. Regression assumptions of normality and homogeneity were assessed by visually examining residual plots and the assumption of independence was assessed using the autocorrelation function (Zuur et al. 2009). We used a Student's t-test to test the null hypothesis that the slope of the regression coefficient did not differ from unity.

To determine mainstem river temperatures at which salmon exhibited behavioural thermoregulation, we subset the mainstem river temperature into 1°C bins for the range of ambient mainstem temperatures observed within each river sector. We then plotted the frequency histogram for values of ΔT_H within each 1°C river temperature bin. We computed the median value of ΔT_H within each 1°C river temperature bin and generated 95% confidence intervals (CI) around the estimate of median ΔT_H via bias corrected and accelerated (BCa) bootstrap resampling, which adjusts for bias and skewness using the 'boot' package in R (Efron and Tibshirani 1993; Davison and Hinkley 1997; Canty and Ripley 2017). If the 95% CI did not overlap zero, then median ΔT_H (hereafter ΔT_{Hmed}) was considered to differ significantly from zero. A negative value of ΔT_{Hmed} indicated that at a given river temperature, salmon were predominantly detected in cool patches (i.e. they used patches of water that were cooler than ambient mainstem river temperature), whereas a positive ΔT_{Hmed} indicated that salmon were predominantly detected in warm patches. No significant difference between ΔT_{Hmed} and zero indicated that there was no preferential use of cool or warm patches at the given temperature.

To examine diurnal patterns in refuge use, we merged records of ΔT_H for all fish, and for each hour of the day, summed the amount of time that fish occupied a) cool patches [$\Delta T_H \leq -1^{\circ}C$], b) the main stem [$-1^{\circ}C > \Delta T_H > 1^{\circ}C$] and c) warm patches [$\Delta T_H \geq 1^{\circ}C$]. We used Chi-square contingency table analysis to test the null hypothesis that the water type occupied (cool patch, main stem, or warm patch) was independent of the hour of the day, and graphically examined diurnal patterns in refuge use.

Within-pool behavioural thermoregulation

We hypothesized that salmon using large pools containing cool water patches would behaviourally thermoregulate within the pool to occupy cool patches, ambient pool temperature, or warm patches as needed to maintain a narrow range of body temperatures (e.g. Nielsen et al. 1994; Newell and Quinn 2005; Gutowsky et al. 2017). We tested this hypothesis using temperature data from the loggers attached to the VR2 anchors in pools. The temperature difference between internal fish temperature and ambient temperature measured in the pool allowed us to assign fish to patch type (ambient pool temperature, cool patch, or warm patch) for all detections of individuals that occurred in the pool.

We estimated instantaneous ambient pool temperature (T_{AI}) for each measurement of fish temperature (T_B) that was logged by the acoustic receiver using the cubic spline interpolation, as described previously. We then calculated instantaneous pool-specific ΔT ($\Delta T_{IP} = T_B - T_{AI}$), which we used to assign patch state (cool, ambient, or warm patch) to all detections of each individual in the pool. We again used 1˚C as a threshold to distinguish between the use of cool patches ($\Delta T_{IP} \leq$ -1˚C), ambient river temperature (-1˚C > ΔT_{IP} > 1˚C), or warm patches ($\Delta T_{IP} \geq$ 1˚C).

We employed empirical cumulative density functions to determine the temperatures at which salmon moved into cool or warm patches. To ensure that a new detection of an individual in a patch truly represented the initiation of patch use, and not an individual that was simply on the edge of a cool or warm patch (in which case it might appear to sporadically move into and out of the patch), we set a threshold of 15 min to define a patch residence. That is, for a fish to be considered resident in a warm or cool patch, it must have remained in the patch for a minimum of 15 min. The threshold of 15 min was chosen because this was the sampling resolution of our in-river temperature loggers.

Results

TIR imagery and acoustic telemetry array

Although we classified thermal refuges throughout the Nord-Est (Fig. 1b), here we present counts of only those cool refuges located in the accessible study reaches. We identified 18 cool patches in the downstream sector and 36 in the upstream sector. Three classes of cool patch were identified downstream: lateral seep (n=12), confluence plume (n=5), and cold alcove (n=1). As in the downstream sector, lateral seeps (n=16) were the most prevalent cool patch type identified in the upstream sector. The other classes identified upstream, in order of prevalence, were: cool side channel (n=6), confluence plume (n=5), hyporheic upwelling (n=5), springbrook (n=3), and cold alcove (n=1). Although we did not directly measure the size of most cool patches identified using TIR imagery, we used the optical images coupled with knowledge of the system to determine that the majority had a surface extent of less than 1 m^2 and were in water less than 0.5 m depth, making them too shallow for receiver deployment.

We deployed 13 acoustic receivers during summer 2016 (Fig. 1a). The only cool patches identified from TIR imagery that were deep enough for receiver deployment (≥ 0.5 m) were either seeps located in deep pools or confluence plumes (Fig. 1a). Maximum water depth within pools was measured on 25 July 2016 at a river discharge of 26 $m^3s\text{-}^1$ using a single beam echo sounder (Hydroball, CIDCO, Rimouski, QC). Confluence plume depth was measured with a meter stick during receiver deployment. In the downstream sector, we deployed receivers in two pools containing lateral seeps (D1 = 2.1 m deep and D2 = 5.8 m deep). In the upstream sector, lateral seeps were identified in three large pools (U3, U4, and U7), which had maximum depths ranging between 3 and 4 m. A small patch of cool hyporheic upwelling was identified in a fourth large pool (U8), which was the deepest pool in both study sectors (maximum depth = 6.2 m). Three of five confluence plumes were deep enough for receiver deployment (1 – 1.5 m at a river discharge of 21 $m^3s\text{-}^1$). We were only able to deploy receivers in two of these sites (U1 and U5) because a strong counter-current prevented receiver deployment at the third confluence plume. We deployed receivers in all remaining accessible large pools (maximum depth: 2.7 – 5.3 m) in the upstream (n=3) and downstream (n=2) sectors.

Our analyses focused on data from 15 individuals that were consistently detected during July and August (Table 1). Internal body temperature was logged whenever salmon were in proximity of a receiver, i.e., when they were in confluence plumes or pools containing receivers. Temperature records were not obtained when salmon were transiting between pools or in habitat not monitored by a receiver. Range testing conducted during the summer of 2014 indicated that under calm conditions, the detection efficiency of V13 acoustic tags by VR2W receivers deployed in pools in the Nord-Est was 95% at a distance of 40 m and 80% at 200 m (water depth ≥ 0.5 m). Reduced receiver detection range was only observed when river discharge exceeded 60 m^3s^{-1} or during discrete heavy rainfall events (Frechette, unpublished data). During the study period, median river discharge was 18.7 m^3s^{-1} (range = 8.8 – 39.4 m^3s^{-1}) and only two rain events occurred during summer 2016 (both during July) that could have interfered with tag detection over a period of less than 12 hours. In cases when multiple salmon were present within a pool, there was some evidence of tag collision, which prevented temperature records from being registered by a receiver despite the salmon being within range of the receiver. Given the high temporal frequency at which data was transmitted to the receivers (i.e. every 30 to 90 sec), data lost because of tag collisions represents only a tiny fraction of the total number of detections recorded from each individual (Table 1). Consequently, we do not expect that the loss of tag detections from rainfall or tag collisions biased our analysis of behavioural thermoregulation.

Seasonality and location of refuge use

All observed cool patch use occurred during August (Fig. 2), which was the only month when measured mainstem river temperature remained within the range considered to be stressful to adult Atlantic salmon (20-23°C, Breau 2013) for more than a few hours at a time (Fig. S1). Three high temperature events, defined as periods during which mainstem river temperature exceeded 22°C in the upstream sector and approached or exceeded 20°C in the downstream sector, occurred during August 2016. The initial onset of behavioural thermoregulation was linked to the high temperature event that occurred 4-6 August (day 217-219), when mainstem river temperature approached 23°C and remained greater than 20°C for three days. Before this event, river temperature was rarely greater than 19°C (Fig. S1) and there was no evidence of cool patch use (Fig. 2). During or immediately following the first high temperature event, all salmon moved into pools containing thermal refuges.

Cool refuge use ($\Delta T_I \leq -1^{\circ}C$) occurred in six locations (Fig. 2): D3 (downstream sector) and U2, U3, U6, U7, and U8 (upstream sector). Of the pools where cool patch use occurred, only three had cool patches that were also identified from TIR images (U3, U7 and U8). Data from temperature loggers installed at the bed and surface of U8 (cross-validated with spot measurements from a temperature probe) indicated that U8 was thermally stratified during August 2016 (Fig. S2). Temperature recorded 10 cm below the surface ranged from 0.77°C to 1.7°C warmer than the temperature recorded 10 cm above the substrate. This temperature differential is greater than the thresholds used to define thermal stratification used by Matthews et al. (1994; 0.5°C) and Gendron (2013; 0.1°C), despite the fact that the bottom logger was not placed in the coolest area of the pool. No cool patch use was identified in either of the confluence plumes (U1 and U5), nor in other pools containing lateral seeps (U4 and D1) that were identified via thermal imagery. Fish used cool patches for extended periods of time (≥ 2 weeks), in pools U8 (upstream sector) and D3 (downstream sector), which were the only pools where of $\Delta T_I \leq -2^{\circ}C$ was recorded. Minimum ΔT_I ranged from -2.97 to -3.79°C in D3 and -4.83 to -5.03°C in U8. Aside from brief forays into other pools, all tagged fish remained in D3 or U8 during the warmest part of August, with up to 90% of available individuals in the upstream sector (8 of 9) detected in pool U8 and 100% of available individuals in the downstream sector (5 of 5) detected on a given day.

Identification of behavioural thermoregulation

Although all tagged salmon engaged in behavioural thermal regulation, individuals that were in the downstream sector during August exhibited less behavioural thermoregulation than salmon in the upstream river sector and salmon tagged after the high temperature events exhibited less behavioural thermoregulation than fish tagged earlier in the summer. A linear relationship with a slope that differed from 1:1 was observed between mean hourly mainstem river temperature and mean hourly internal fish temperature for all individuals (1-tailed Student's t-test, $\alpha = 0.05$, Fig. 3) and no substantial departure from the assumptions of normality and homogeneity were evident in residual plots. In the downstream sector, greater than 70% of the variation in internal fish temperature was explained by the mainstem river temperature (measured at rkm 30). In the upstream sector, however, mainstem river temperature (measured at rkm 45) explained little of the variance in internal fish temperature for individuals that experienced the high temperature events (Fig. 3). The remaining variation in fish temperature (42 to 73%) was explained by fish using areas of the river that were cooler or warmer than the ambient mainstem temperature. Ambient mainstem river temperature explained approximately 80% of variation in fish temperature for the two individuals that were tagged during late August, after the high temperature events (Fish U-09 and Fish U-10).

In the upstream sector, ΔT_{Hmed} was significantly greater than zero when ambient mainstem river temperature was between 14.0 and 16.9°C (bootstrapped 95% CI did not overlap zero, Fig. 4a), indicating that salmon were using warm patches at these cooler temperatures. When ambient mainstem river temperature was between 17 and 18.9°C, median ΔT_{Hmed} was not significantly different than zero (bootstrapped 95% CI contain zero), indicating that salmon did not seek out warm or cool patches. Once ambient mainstem river temperature reached 19˚C, however, ΔT_{Hmed} began to skew significantly negative, indicating that fish were actively using cool patches. At water temperatures ≥ 21.0˚C, nearly 100% of detections of tagged salmon were in cool refuges, and ΔT_{Hmed} was approximately -2˚C.

Fish in the downstream sector showed no evidence of warm patch use, and cool patch use began at lower ambient mainstem river temperatures than in the upstream sector (Fig. 4b). When mainstem river temperature was between 14 and 16.9˚C, ΔT_{Hmed} was not significantly different than zero (bootstrapped 95% CI contain zero). Once mainstem river temperature reached 17˚C, ΔT_{Hmed} began to skew significantly negative, a threshold that was two degrees cooler than in the upstream habitat. At water temperatures ≥ 19.0˚C, nearly 100% of detections of tagged salmon were in cool refuges and ΔT_{Hmed} was greater than -2˚C.

There was a clear diurnal pattern in behavioural thermoregulation in the upstream sector and the thermal habitat type occupied (cool patch, ambient mainstem temperature, or warm patch) was not independent of the hour of the day (Fig. 5a; $\chi^2_{(46,\, N = 4526)}$, $P < 0.05$). Cool patch use increased throughout the afternoon, when river temperature generally increases due to solar heating, and was greatest at 16:00 before decreasing again. Warm patch use increased during the early morning, and was greatest at 06:00. There was no clear diurnal pattern in behavioural thermoregulation in the downstream sector and no warm patch use was observed (Fig. 5b), however, water type occupied (cool patch or ambient mainstem temperature) was not independent of the hour of the day ($\chi^2_{(23,\, N = 1765)}$, $P < 0.05$).

Within-pool refuge use

We examined within-pool behavioural thermoregulation in the pools where the majority of cool patch use occurred (D3 and U8). During August 2016, three salmon (2 females, 1 male) were detected in D3 on ≥ 18 days. During the same period, seven salmon (3 females, 4 males) were detected in U8 on ≥ 19 days. Measured ambient temperature in pool D3 was always cooler than downstream ambient mainstem temperature (mean = 0.42˚C; range = 0.27˚ to 0.77˚C). Measured ambient temperature in pool U8 was 3.4˚C cooler to 2.4˚C warmer than the upstream ambient mainstem temperature (on average, U8 was 0.12˚C cooler than the mainstem). The temperature range recorded at the bottom of U8 was 15.1˚C to 21.5˚C, whereas the temperature range recorded in D3 was slightly broader (15.7˚C to 22.2˚C). The locations of thermal refuges within these pools, however, were not known before deployment of temperature loggers. Consequently, temperature loggers were not placed directly in the thermal refuges and recorded pool temperature did not represent the coolest or warmest temperatures available in each pool.

The initiation of cool patch residences in pool D3 increased steadily between 16.5˚C and 20.5˚C (Fig. 6a) and no warm patch residences were observed. In pool U8, cool patch residences increased dramatically between 18.9˚C and 19.7˚C, and reached a plateau at approximately 21˚C (Fig. 6b). Initiation of warm patch residences increased steadily between 16˚C and 19˚C, however, very few warm patch residences began when pool temperature was > 19˚C (Fig. 6c).

Discussion

All adult Atlantic salmon that were tagged with temperature-sensing transmitters engaged in behavioural thermoregulation. Although mainstem river temperature in the Nord-Est was relatively cool and did not exceed 23˚C, adult salmon used mainstem pools as cool refuges during the warmest period of the summer. Individuals exhibited behavioural thermoregulation at substantially cooler ambient river temperatures (17°C in the downstream sector and 19°C in the upstream sector), than expected from previous studies that described thermal refuge use by juvenile (Gibson 1966; Cunjak et al. 2005; Dugdale et al. 2016) and adult (Shepard 1995) Atlantic salmon. These results support the literature and metabolism-based assessment by Breau (2013) that temperatures above 20°C are stressful to adult Atlantic salmon. Adult salmon predominantly used cool refuges during the afternoon when the warmest ambient mainstem water temperatures were recorded, and during refuge use body temperatures were as much as 5°C cooler than ambient mainstem river temperature.

Our combination of TIR imagery, river temperature monitoring, and acoustic telemetry enabled us to obtain a more holistic understanding of how adult Atlantic salmon use thermal habitat than would have been possible with a single-pronged approach. Use of TIR imagery allowed us to map potential thermal refuges at the riverscape scale in a system where large sections of the river are inaccessible. The resulting refuge maps, when applied in concert with optical imagery and first-hand knowledge of the river system, helped target acoustic receiver deployment to potential cool refuges, which we were able to monitor continuously over the course of the summer using acoustic telemetry. Additional deployment of acoustic receivers in deep pools allowed us to identify subsurface cool refuges that were important refuges for adults (e.g. pool D3) but that were missed via evaluation of TIR imagery because the cool water did not extend to the river surface. Because internal salmon body temperature was recorded when salmon were in proximity of a receiver, we obtained near continuous records of salmon body temperature in potential refuge sites. When coupled with long-term measurement of ambient river temperature from temperature loggers, telemetry records allowed us to assess behavioural thermoregulation via thermal refuge use over finer temporal and larger spatial scales than would have been possible using snorkel counts or radio-telemetry, techniques that have been previously applied to study of thermal refuge use by salmonids (e.g. Torgersen et al. 1999; Ebersole et al. 2001; Brewitt and Danner 2014).

Physiological stress has been observed under laboratory conditions in adult Atlantic salmon at 23˚C (Wilkie et al 1997). Ambient mainstem river temperature in the Nord-Est first approached 23˚C during the high temperature event that occurred 4-6 August (day 217-219). We observed mass movement of individual Atlantic salmon into pools containing cool refuges during or immediately after this event. The first heat wave likely induced thermal stress, which served as trigger for individuals to search out pools containing cool refuges where they could recover physiologically (Breau 2013). Once fish entered pools containing refuges, they remained in residence for ≥ 2 weeks, similar to the residence time observed for steelhead (*Oncorhynchus mykiss*) using cold tributaries as thermal refuges in the Columbia River Basin (Keefer et al. 2009).

The degree of behavioural thermoregulation observed varied among individuals, and appeared tied to location and timing of release. Individuals in the upstream sector exhibited a greater degree of behavioural thermoregulation than salmon in the downstream sector, which may be explained by the difference in ambient mainstem temperature between the two river sectors. In the upstream sector, measured ambient mainstem temperature exceeded 20˚C and approached 23˚C during all three high temperature events, whereas in the downstream sector, ambient mainstem river temperature only approached 23˚C during the first event. Salmon that were present in the upstream sector before at least one of the three high temperature events that occurred during August 2016 exhibited a greater degree of behavioural thermoregulation than salmon that were tagged near the end of August, when water temperature was cooler (Fig. 3).

Use of cool refuges has also been linked to run timing in Columbia River steelhead. Steelhead that migrated during the warmest part of the summer had the greatest rates of refuge use, whereas fish that migrated either early or late in the season experienced lower mainstem temperatures and had lower rates of refuge use (Keefer et al. 2009). Salmonids that migrate during periods when heat stress-inducing warm water events occur seem to have a greater need to use cool water refuges than salmonids migrating later in the summer when water is cooler.

In our study, tagged adult Atlantic salmon routinely used cool patches when river temperature in the Nord-Est was less than 19˚C, which is substantially cooler than previously reported for Atlantic salmon. Juvenile Atlantic salmon moved into cold water refuges in eastern Canadian rivers when water temperature was 22-24˚C (Gibson 1966; Cunjak et al. 2005; Dugdale et al. 2016), and adults stopped migrating and congregated in cold water tributaries in the Penobscot River in Maine at 23˚C (Shepard 1995). In the upstream sector, adult salmon used thermal refuges when ambient mainstem river temperature was as cool as 19˚C, and nearly 100% of detections were in thermal refuges when ambient mainstem temperature was ≥ 21˚C. These results are similar to the patterns of thermal refuge use exhibited by summer steelhead in the Columbia River Basin, where steelhead began using cold water tributaries as thermal refuges when mainstem river temperature reached 19˚C, and 70% of tagged fish were detected in refuges when river temperature exceeded 21˚C (Keefer et al. 2009). In the downstream sector, however, adults used thermal refuges at ambient mainstem river temperatures as low as 17˚C, with 100% of detections occurring in cold refuges when river temperature was ≥ 19˚C.

Interestingly, in the upstream sector, adult Atlantic salmon used warm water patches when river temperature was < 17˚C. Although use of warm water patches by salmonids during winter has been documented (Craig and Poulin 1975; Cunjak and Power 1986; Brown and

McKay 1995), use of warm water patches during summer by migrating adult salmon has not been previously reported in the literature. Temperatures are more stable in thermal refuges created by thermal stratification or groundwater inputs than in surrounding river habitat that is more susceptible to diurnal heating and cooling cycles. Warm patch use could result from salmon remaining in refuges during periods when ambient river temperature decreases relative to refuge temperature. Alternatively, adults may actively seek out warm water patches when ambient river temperature is low. Regardless of the mechanism, warm patch use could enable adults to remain within an optimal temperature range, allowing them to balance energy conservation with gamete maturation, which can be slowed if temperature is too low (Berman and Quinn 1991; Newell and Quin 2005; Hasler et al. 2012). Taken together, the temperatures at which cool and warm patch use occurred in the Nord-Est suggest that adults may have a narrow optimal temperature range similar to that previously described to optimize growth in juvenile Atlantic salmon, i.e. approximately 16-20˚C (Elliott 1991; Jonsson and Jonsson 2009). Bull trout (*Salvelinus confluentus*) tagged with temperature-sensing V13 acoustic tags were found to occupy a narrow temperature range comparable to laboratory-derived optimal temperatures for growth and metabolism, despite a much broader range of temperatures available to free-swimming individuals in a British Columbia reservoir (Gutowsky et al. 2017). Sockeye salmon have also been observed using a narrow temperature range (9-11˚C) while over-summering in a stratified lake, despite the availability of warmer and cooler temperatures (Newell and Quinn 2005).

We observed a diurnal pattern in refuge use in the upstream sector of the Nord-Est. Cool refuge use was greatest in the afternoon and warm patch use was greatest in the early morning, corresponding to daily maximum and minimum water temperatures. The absence of a clear diurnal pattern in behavioural thermoregulation in the downstream sector may be because the sample size was smaller downstream (5 individuals) than upstream (10 individuals). Peak refuge use by rainbow trout (*O. mykiss*) in the Snake River (Oregon) drainage also occurred in the late afternoon when ambient temperature was greatest (Ebersole et al. 2001). Observations by Breau et al. 2007, however, indicated that aggregation of juvenile Atlantic salmon in cool refuges was influenced more by water temperature than by time of day. Observation of cool refuge use by Breau et al. (2007) was based on snorkel counts in one study reach, whereas our observations were based on near-continuous records of adult body temperature and river temperature, and spanned multiple potential refuges, which could explain the discrepancy between our findings and those of Breau et al. (2007).

Although cold water tributaries and confluence plumes served as important thermal refuges for adult salmonids in the much larger Columbia and Penobscot river systems, (Shepard 1995; Goinea et al. 2006; Keefer et al. 2009), the two confluence plumes in Nord-Est that were equipped with acoustic receivers did not serve as thermal refuges for adult Atlantic salmon. Mainstem discharge greatly influences confluence plume volume, which subsequently influences refuge carrying capacity (Gendron 2013). The monitored confluence plumes in the Nord-Est were shallow (≤ 1.5 m deep at river discharge of 21 m^3s^{-1} but ≤ 0.75 m deep when river discharge was 9 m^3s^{-1}). Discrete measurements made during August 2016 indicated that the cooling influence of confluence plumes did not extend more than 3 m from the mouth of each tributary, despite temperature differences between tributaries and the mainstem of 10-12°C (Frechette, personal observation). Thus it is unlikely that shallower unmonitored confluence plumes were used as refuges. Our findings are similar to those of Beguin et al. (2000), who found that adult steelhead over-summering in Steamboat Creek (a mid-order stream in Oregon) did not use shallow (< 0.8 m) confluence plumes. Cool water confluence plumes in the Nord-Est, therefore, were likely too shallow to be used by adult Atlantic salmon for long-term behavioural thermoregulation (Torgersen et al. 1999; Beguin et al. 2000; Ebersole et al. 2001).

The most important thermal refuges used by over-summering Atlantic salmon adults in the Nord-Est were stratified alluvial pools (D3 and U8). Thermal stratification may form in pools where groundwater seeps or hyporheic flow is present, or in areas where channel morphology causes water velocity to slow sufficiently to prevent mixing throughout the water column (Nielsen et al. 1994). In the absence of mixing, surface water heats up during the day while bottom water maintains a relatively constant temperature. During the night, surface water cools and sinks to the bottom, maintaining a temperature gradient (Nielsen et al. 1994). Although mainstem alluvial pools are rarely stratified (Nielsen et al. 1994; Gendron 2013), pool U8 is unusually large relative to other pools in the Nord-Est. The pool inlet is shallower than the outlet, the maximum depth exceeds 6 m, and a gravel bar island in the centre of the pool may entrain cool bottom water, characteristics that are ideal for formation of thermal stratification via solar heating (Matthews et al. 1994; Nielsen et al. 1994; Gendron 2013).

We identified hyporheic flow associated with a small gravel bar near the head of pool U8 from the TIR imagery, which may enhance thermal stratification (Gendron 2013). At present, it is not possible to identify solar heating or hyporheic flow as the main mechanism producing thermal stratification in pool U8, however the temperature at the substrate was as much as 1.7°C cooler than at the surface, which provides evidence of strong thermal stratification (Matthews et al. 1994; Gendron 2013). The source of thermal stratification in pool D3 is still under investigation, but is thought to result from resurgence of cool water originating in a cool tributary located upstream of the pool. Although the confluence plume was clearly evident in TIR images, surface evidence of the plume did not extend into pool D3. Regardless of the source of thermal stratification, pools D3 and U8 were extremely important to over-summering Atlantic salmon, a finding which corresponds with previous studies of thermal refuge use by steelhead and Chinook salmon (*Oncorhynchus tshawytscha*). Thermally stratified pools were the most heavily used pool type by summer run steelhead in the Middle Fork Eel River, a river system in California that is comparable in size to the Nord-Est (Nielsen et al. 1994), and both over-summering spring run Chinook and summer run steelhead disproportionately used pools as cool refuge habitat, relative to their availability in Oregon rivers (Torgersen et al. 1999; Baigun et al. 2000).

Deep pools with stable thermal refuges should allow individuals to thermoregulate with relatively little expenditure of energy (Berman and Quinn 1991). Adults holding in large pools may maintain a stable body temperature either by changing position in the pool or by remaining in a stable thermal refuge while ambient water temperature around the refuge changes throughout the day. Such behaviour would buffer adults from large temperature fluctuations associated with daily warming and cooling cycles in the river and allow them to retain valuable energy stores for spawning (Sutton et al. 2007; Hasler et al 2012). It must be noted, however, that temperature is not the only factor driving habitat selection during the in-river pre-spawning period. River discharge, proximity to spawning sites, season, and density of conspecifics may also influence choice of holding habitat. For example, large pools likely serve not only as thermal refuges, but also as hydraulic refuges, allowing fish to hold and maintain optimal temperature while waiting for discharge to increase and permit resumption of migration (Gendron 2013). Such pools are likely to be particularly important in shallow, gravel-bed rivers that are prone to low river discharge during the warmest periods of the summer when adult Atlantic salmon are present, and their abundance and location along a riverscape may influence migratory patterns of adult Atlantic salmon. Although we measured the maximum depths of the pools containing receivers, we did not make detailed measurements of physical habitat. A detailed characterization of available pools, confluence plumes, and intervening habitat would permit the effects of temperature to be disentangled from other factors that influence habitat choice by adults during in-river holding, and is the subject of on-going research in the Nord-Est.

Limitations inherent in our study give rise to potential improvements for future research. We based evidence of thermal refuge use on the difference between ambient river temperature and internal fish temperature. We selected river temperature monitoring sites in well-mixed areas of the river that were representative of mainstem temperature, therefore, choice of temperature monitoring sites was unlikely to overly influence our identification of the timing, location, and temperatures at which fish initiated behavioural thermoregulation. It is possible, however, that choice of temperature monitoring sites introduced a minor bias into our results (Torgersen et al. 1999). Such a bias could be mitigated by deploying multiple mainstem temperature loggers from which an average reach-specific ambient mainstem temperature could be calculated and used for identifying behavioural thermoregulation. Our study also represents a limited number of individuals tagged during one year in a medium-sized river that is relatively cool compared with more southerly rivers within the range of Atlantic salmon. Since thermal tolerance in salmon is related to the temperature at which they are acclimated (Elliott 1991), salmon populations in rivers that are warmer (or cooler) than the Nord-Est likely have different thresholds for initiation of thermal refuge use. Inter-annual differences in temperature regimes might also produce differences in thermal habitat use within a given river system. Additionally, refuge types that are important in large river systems (e.g. confluence pools) may be less important in smaller systems. Application of our methods in catchments of varying sizes across the range of Atlantic salmon and over multiple years would facilitate identification of refuge types and thresholds for behavioral thermoregulation under different hydrological and temperature conditions. This would further improve our understating of thermal habitat requirements of Atlantic salmon, which could prove invaluable in predicting responses of Atlantic salmon populations to climate change.

River temperatures are rising in northern latitude rivers within the range of Atlantic salmon, a trend that is expected to continue under current climate change projections (Ferrari et al. 2007; Webb and Nobilis 2007; van Vliet et al.2013). Although optimal temperature for adults likely differ among river systems due to different thermal regimes and acclimation temperatures, it is clear that adult salmon used a narrow temperature range and required access to cool refuges at much lower temperatures than are regularly measured in salmon rivers (Shepard 1995; Breau et al. 2007; Dugdale et al. 2016). Increased temperatures and reduced river discharge can serve as physical barriers to salmon migration, preventing adults from reaching the thermal refuges that are crucial to survival and energy maintenance when river temperatures become stressful (Torgersen et al. 1999; Hasler et al. 2012). Thus, the ability for adult salmon to access suitable holding pools and cool refuges will become even more important for maintaining populations in a warming climate because of the negative impacts of high temperature on survival and reproductive success. It is therefore imperative to identify, protect, and maintain connectivity among suitable thermal refuges in salmon rivers to ensure population persistence. Such protections may include controlling releases of water from dams on regulated rivers, restricting fishing and other human activities in refuges, and limiting groundwater extraction or other land use practices that reduce riverbed shading or groundwater inputs (Sutton et al. 2007; Breau and Caissie 2013; Kurylyk et al. 2015). In certain cases, enhancing existing thermal refuges or creating new refuges via channel modification or groundwater pumping may be warranted (Kurylyk et al. 2015). Presence of suitable adult thermal habitat is also essential for enhancement programs like the habitat expansion initiative currently in place on the Rivière Sainte-Marguerite Nord-Est, where adult salmon are introduced to previously inaccessible habitat via fish ladders or transport. Inventory of potential thermal refuges should therefore be included in feasibility studies before the implementation of such conservation translocation programs to ensure their success.

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Table 1. Size and sex of Atlantic salmon implanted with temperature sensing acoustic transmitters (Vemco V13T–H) during summer 2016, by river sector, site of release, and dates individuals were first available for detection in each river sector, and number of times each individual was detected (number of records). Tagging data for fish removed from analyses are included for reference.

River Sector	Fish ID	FL (mm)	Sex	Release site	Date Available Upstream	Date Available Downstream	Number of Records
Upstream	U-01	820	M	Upstream	29-Jun	NA	17 248
	U-02	720	F	Upstream	10-Jul	NA	14 270
	U-03	740	F	Upstream	12-Jul	NA	13 416
	U-04	820	M	Upstream	12-Jul	NA	22 760
	U-05	770	M	Upstream	12-Jul	NA	14 196
	U-06	770	F	Upstream	13-Jul	NA	14 596
	U-07	830	M	Upstream	11-Aug	NA	4 048
	U-08	680	M	Upstream	11-Aug	NA	8 080
	U-09	750	M	Upstream	18-Aug	NA	8 016
	U-10	630	M	Upstream	22-Aug	NA	4 005
Downstream	D-01	790	M	Upstream	13-Jul	16-Jul	31 406
	D-02	780	F	Upstream	13-Jul	23-Jul	14 926
	D-03	770	F	Downstream	NA	30-Jul	14 019
	D-04	775	F	Downstream	NA	31-Jul	12 124
	D-05	760	F	Downstream	NA	31-Jul	4 560
Removed	R-01	720	F	Upstream	29-Jun	NA	1 611
	R-02	975	F	Upstream	1-Jul	NA	269
	R-03	920	F	Upstream	3-Aug	NA	1 494
	R-04	780	F	Upstream	18-Aug	NA	174
	R-05	665	M	Downstream	NA	2-Aug	1 353

Figure Captions

Figure 1. Map of study area. Panel A indicates the fish ladder where adult salmon were captured, sites where salmon were released after transport, and placement of acoustic receivers and temperature loggers. Impassable waterfalls bounding the upstream and downstream river sectors are indicated with an X. Receiver positions are indicated with solid grey circles. Receivers in the upstream sector of the river are labelled U1-U9 and receivers in the downstream sector are labelled D1-D4. Temperature loggers are indicated with small dark black circles and were deployed in the river mainstem (at rkm 30 and rkm 45), or in association with acoustic receivers in confluence plumes (U1 and U5) or deep pools (1 logger in D3; 2 loggers in U8 to test for thermal stratification). Panel B depicts the location of all cool patches identified from TIR imagery. Boundaries delineating each study sector are identified with solid black lines. The map was created using ArcGIS software by ESRI.

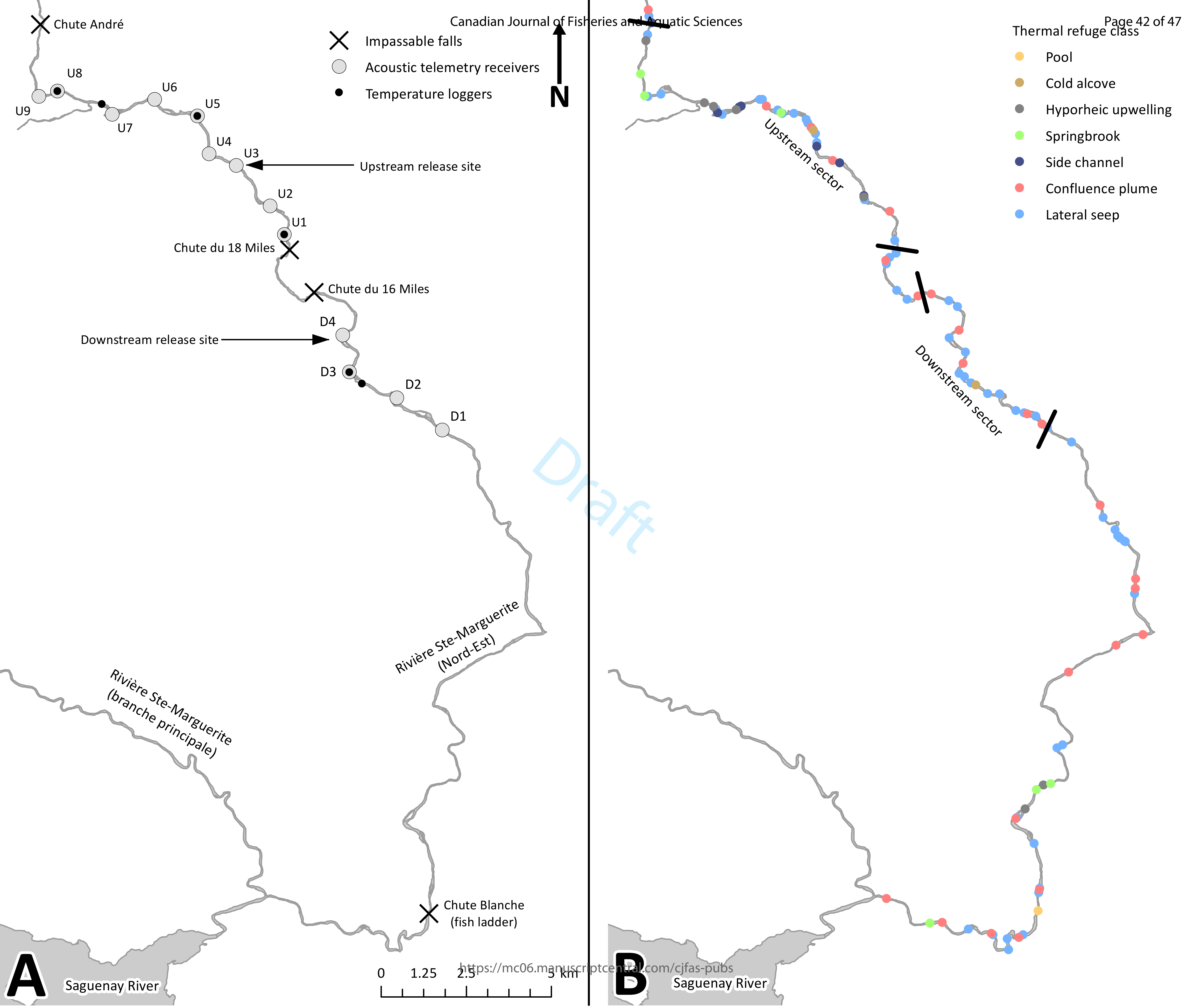
Figure 2. Temporal variation in behavioural thermoregulation by individual adult Atlantic salmon tagged with temperature-sensing acoustic tags during summer 2016 from June 29 (day 181) to August 31 (day 244). Behavioral thermoregulation is represented as the difference between body temperature and instantaneous mainstem temperature (ΔT_I, where mainstem river temperature was measured at rkm 45 (upstream sector) and rkm 30 (downstream sector). Fish identity corresponds to Table 1 and is presented in the gray box above each individual plot along with sex (M = male, F = female). Each point marks a detection of an individual by a given receiver, with receiver location coded by color. Habitat type is specified for each receiver (DP = deep pool; GW = groundwater seep; CP = confluence plume).

Figure 3. Slope plots depicting the median slope and median intercept obtained via bootstrapping (1000 replicates) for the linear relationship between mean mean hourly internal fish temperature (T_{BH}) and mean hourly ambient mainstem river temperature (T_{RH}) in the upstream and downstream river sectors for all fish included in analyses. The solid black line represents a 1:1 relationship between T_{BH} and T_{RH}. Males are represented by dashed lines in shades of blue, females by solid lines in shades of red. Fish identity corresponds to Table 1.

Figure 4. Frequency histograms of the difference (ΔT_H) between mean hourly internal fish temperature and mean hourly mainstem river temperature for the A) upstream sector (n = 10 salmon) and B) downstream sector (n = 6 salmon), binned by mainstem river temperature (measured at rkm 45 for the upstream sector and at rkm 30 for the downstream sector), for August 2016. Solid red vertical lines denote the median (ΔT_{Hmed}) and dashed black vertical lines denote the 95% CI, generated using 5,000 Bca bootstrap replicates. Note different y-axes between panel A and panel B, and in the 14-15.9°C bin in Panel B.

Figure 5. Percent of total hours that salmon used cool patches ($\Delta T_H \leq -1^{\circ}C$; black bars) mainstem river temperature ($-1^{\circ}C > \Delta T_H > 1^{\circ}C$; light gray bars), and warm patches as ($\Delta T_H \geq 1^{\circ}C$; dark gray bars) in the upstream (a) and downstream (b) sectors.

Figure 6. Empirical cumulative density curves for river temperature (measured at the bottom of pool U8 in the upstream sector and at the bottom of pool D3 in the downstream sector) at the start of each patch residence (use of cool or warm patches ≥ 15 min).


Chute André
Impassable falls
Acoustic telemetry receivers
Temperature loggers
N
U8
U9
U7
U6
U5
U4
U3
Upstream release site
U2
U1
Chute du 18 Miles
Chute du 16 Miles
D4
Downstream release site
D3
D2
D1
Rivière Ste-Marguerite (Nord-Est)
Rivière Ste-Marguerite (branche principale)
Chute Blanche (fish ladder)
A
Saguenay River
0
1.25
2.5
5 km
Thermal refuge class
Pool
Cold alcove
Hyporheic upwelling
Springbrook
Side channel
Confluence plume
Lateral seep
Upstream sector
Downstream sector
B
Saguenay River

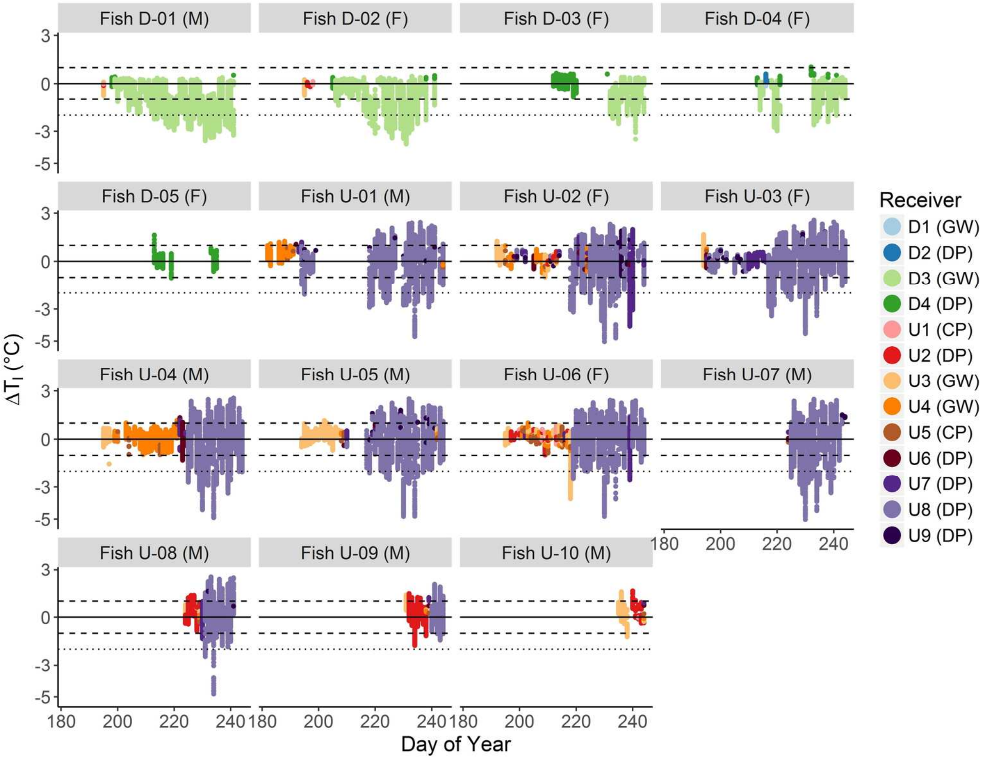


Figure 2

181x139mm (300 x 300 DPI)

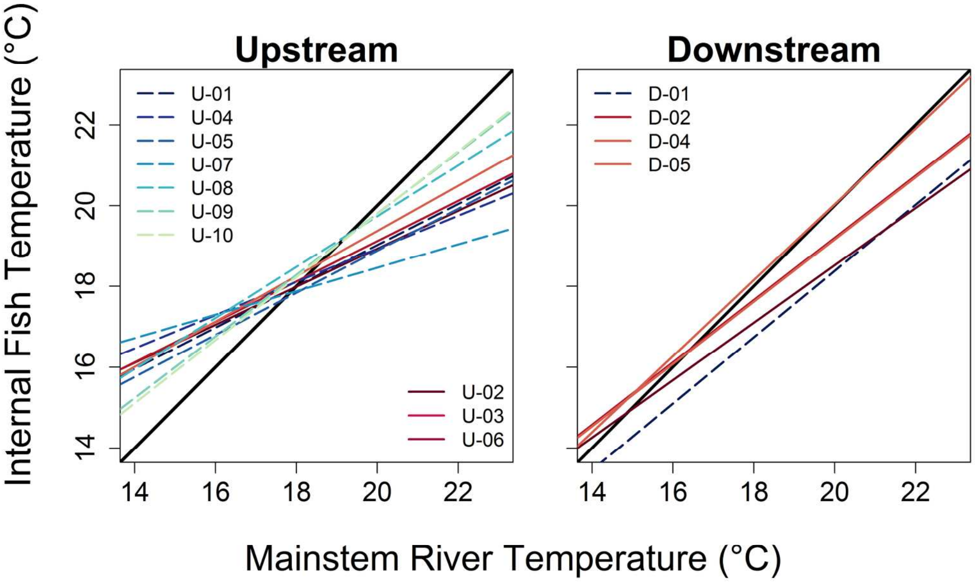
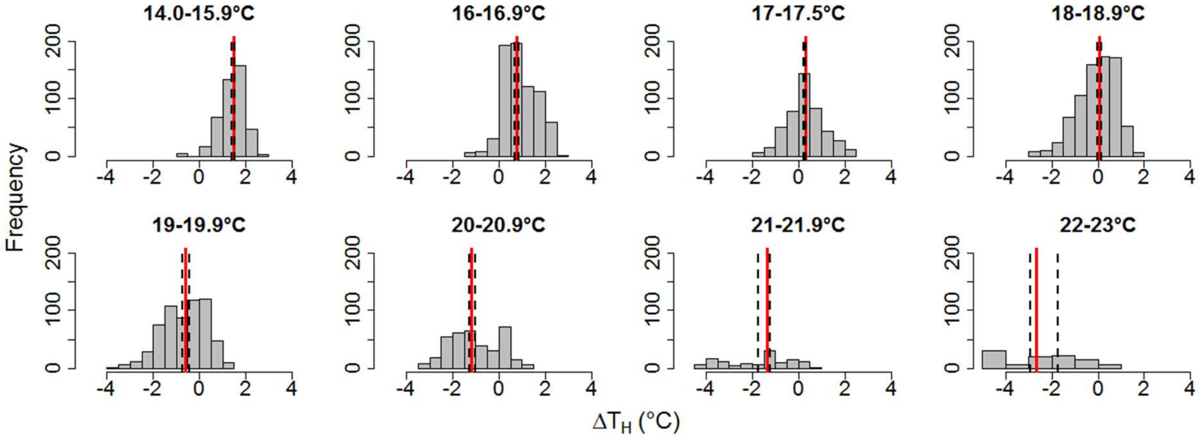


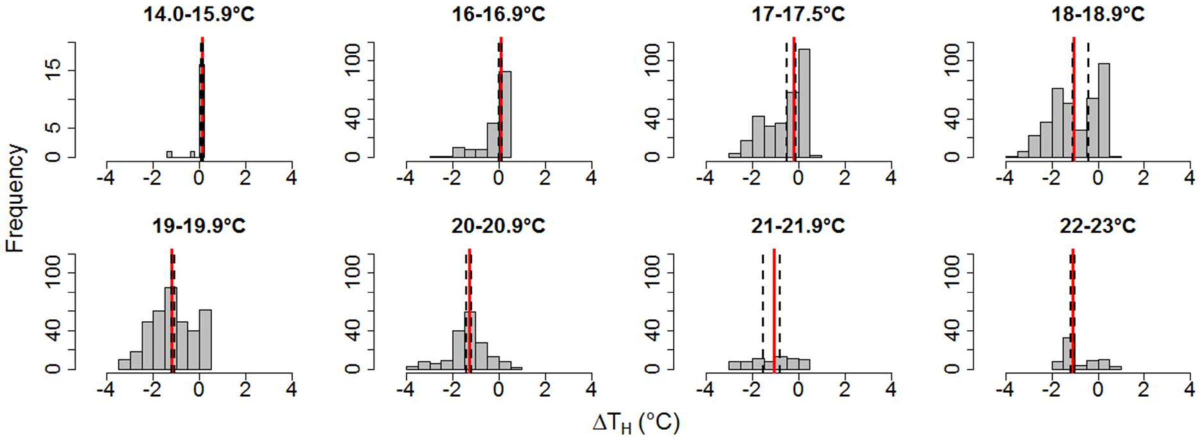
Figure 3

181x139mm (300 x 300 DPI)

A) Upstream Sector



B) Downstream Sector



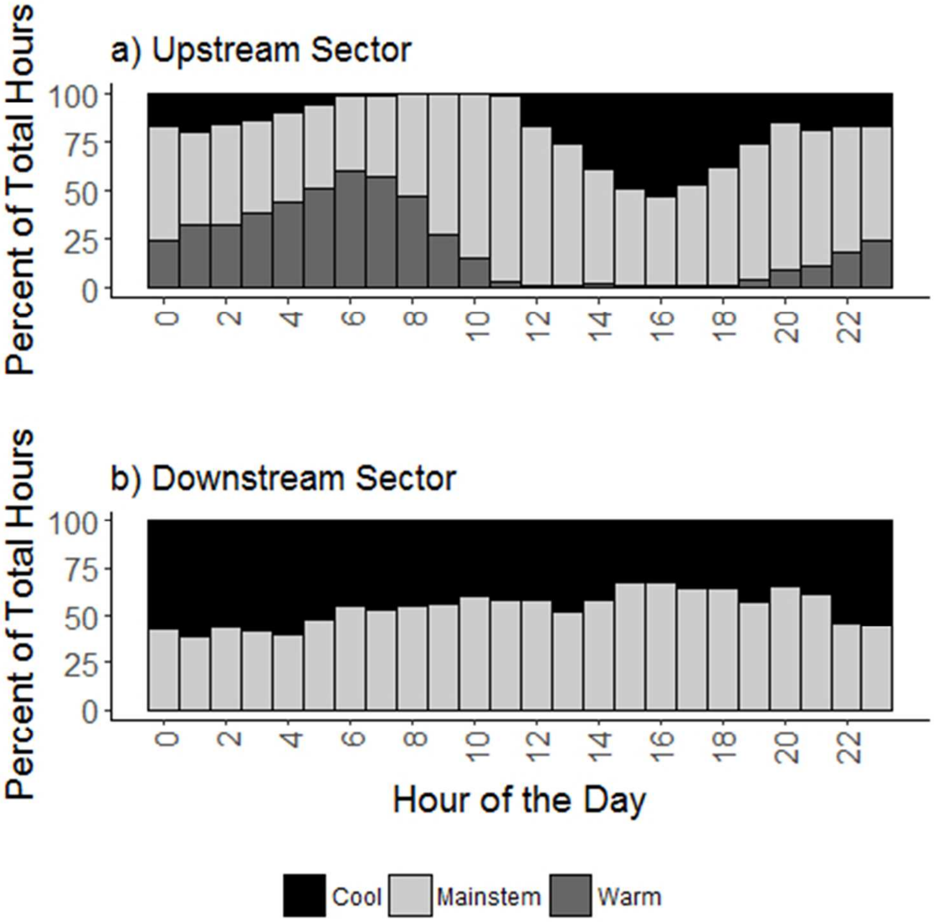


Figure 5

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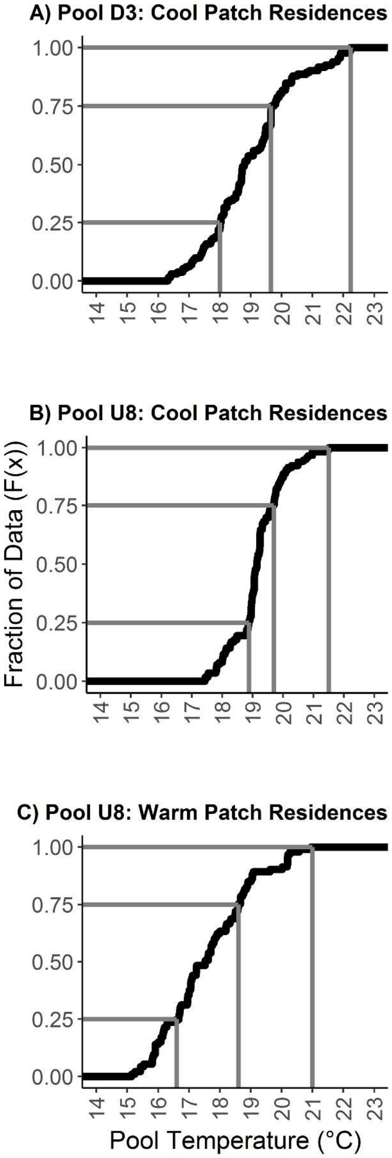

Figure 6

236x653mm (300 x 300 DPI)